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THE EXTRACTION OF ACETIC ACID
FROM *THERMOTOGA NEAPOLITANA*
FERMENTATION SPENT MEDIUM USING
FRESH/RECOVERED ALIQUAT 336 AND
PRODUCE CMA

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THE EXTRACTION OF ACETIC ACID FROM *THERMOTOGA NEAPOLITANA*
FERMENTATION SPENT MEDIUM USING FRESH/RECOVERED ALIQUAT 336
TO PRODUCE CMA

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Engineering
Biosystems Engineering

by
Yifan Du
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Accepted by:
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ABSTRACT

To offer an alternative pathway to produce calcium magnesium acetate (CMA) as an environmental friendly deicer to relieve the pressure on fossil fuels demand, a liquid-liquid extraction process was studied. This process includes using Aliquat 336 (tri-n-octylmethylammonium chloride) diluted in kerosene as an organic extractant to extract acetic acid from *Thermotoga neapolitana* fermentation spent medium, which contained 34 g/L of acetic acid and 10 g/L of NaCl.

The extraction efficiency of Aliquat 336 was studied under several different extraction conditions. The initial pH of the spent medium was adjusted to a pH range of 2.25 to 5.75 with a 0.5 pH interval to study the initial pH effect on Aliquat 336 extraction efficiency. Two different Aliquat 336 concentrations in kerosene (20% and 50% v/v) were made for comparison. The highest extraction efficiency of Aliquat 336 mixture was nearly 50% for fresh Aliquat 336 mixture at 50% concentration.

$\text{Na}_2\text{CO}_3/\text{Ca}(\text{OH})_2$ and $\text{Mg}(\text{OH})_2$ were used to recover the acetic acid from used Aliquat 336 mixture and produce NaCH_3COOH and CMA at the same time. It was shown that the extraction efficiency of recovered Aliquat 336 was approximately 83% as high as the fresh one. The extraction efficiency of both fresh and recovered Aliquat 336 mixtures were greatly affected by the initial pH of the fermentation spent medium. Extraction efficiency were greatest in pH range of 2.25 to 3.75. A purity of 75% CMA was produced.

DEDICATION

This is dedicated to my family.

My father Haipeng Du, and my mother Xiao Zhong.

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I would like to express my appreciation to Dr. Caye Drapcho, who spent enormous amount of time and effort on helping me accomplishing the project goals. I would also like to thank Dr. Nhuan P. Ngheim , Dr. Nishanth Tharayil and Dr. Terry Walker for their instructions during my Master's program.

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CHAPTER ONE

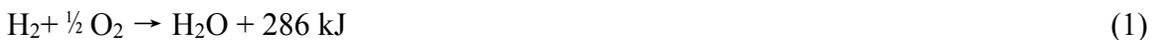
LITERATURE REVIEW

1.1.1. Global energy situation

The percentages of US energy consumption that come from fossil fuels, nuclear, and renewable energy are 81.8%, 8.5%, and 10%, respectively in 2012 (EIA, 2012). However, fossil fuel is a nonrenewable energy source. The proven crude oil and natural gas reserves are estimated to last no longer than 70 years (Klass et al, 1998; Klass et al, 2003). What is more, the exploiting, production and combustion of fossil fuels release greenhouse gases and other pollutants, causing problems such as global warming, acid rain, and health issues (Levin et al, 2004; Solomon, 2009). As a consequence, in order to avoid the crisis of both energy and environment, seeking renewable energy source is a matter of course (Benemann, 1996; Momirlan and Veziroglu, 2002).

1.1.2. The hydrogen production fermentation

Among all kinds of renewable and environment friendly energy sources, hydrogen produced from biological sources is favorable for its low environmental impact and high gravimetric energy density 143 kJ/g (Brown, 2003). Hydrogen combustion does not contribute to environmental pollution and climate change (Levin et al, 2004), because the only product of its combustion is water (da Rosa, 2005).



There are lots of processes to utilize microbes to ferment renewable substrates, including agriculture waste (Logan et al, 2002; Hussy et al, 2005), municipal waste (Wang et al. 2003), food processing waste (Van Ginkel et al. 2005). Hydrogen can be also produced by this kind of processes: *Thermotoga neapolitana* can ferment glucose to produce hydrogen, acetic acid and carbon dioxide (Yu and Drapcho, 2011).

What makes the future of bio-hydrogen more promising is that, in the industrial production of it, food processing wastes such as cull peach, which contains a high percentage of sucrose, glucose, fructose, may be used instead of pure glucose to lower the cost (Jain and Abhiney, 2008).

The summative biological reaction for *T. Maritima* is as follows (Schroder et al. 1994)



In addition to 4 mols of H₂, there are also 2 mols of acetic acid plus 2 mols of CO₂ formed in this process. Yu and Drapcho (2011) found that 32.82 mmol/L-medium H₂ was produced when glucose was used as substrates. They also found that the batch fermentations end at a pH around 4.75-5.

There are several disadvantages of the existence of acetic acid in the fermentation broth. First of all, the acetic acid can lower the pH value of fermentation broth, which will limit the growth of the microbes and inhibit the production of hydrogen, because the

best pH for the growth and production of *Thermotoga neapolitana* is around 7 (Jannasch et al, 1988). Acetic acid accumulation can also inhibit the hydrogen fermentation by product inhibition (Jones and Woods, 1986; Van Niel et al, 2003; Van Ginkel and Logan, 2005). Liu (2008) reported that the hydrogen fermentation could be affected when the acetate concentration was more than 50 mM. The duration of lag phase for the mixed culture also could be altered by acetate concentrations (Liu, 2008).

Second, the acetic acid in the fermentation broth is a wasted resource if not used, since 2/3 of the carbon source and energy contained in glucose has been converted into acetic acid. Because lowering the manufacturing cost can certainly encourage the industrialization of bio-hydrogen production, recovering and using the acetic acid could lower the costs and make the industrialization more possible.

1.1.3. CMA (Calcium Magnesium Acetate)

Acetic acid is useful for the production of a wide range of chemical compounds in the modern chemical industry. One of the most economically promising products that can be produced from acetic acid is CMA (Calcium Magnesium Acetate) (Dionysiou 2000). As shown in equation 3, $\text{CaMg}(\text{CH}_3\text{COO})_4$ can be formed by the reaction of acetic acid and dolomitic lime (calcium magnesium hydroxide).



CMA was proposed to be a deicing chemical to replace regular deicing salt (sodium chloride) in a research program started by the Federal Highway Administration (FHWA) in 1980s (Yang et al. 1992). CMA stood out from a large number of competitors in this program because it is more environmental friendly and easier to handle and spread (Robidoux, 2001). Another major advantage of CMA is that it is noncorrosive to metals, and nondestructive to concrete and other constructional materials (Robidoux, 2001). What is more, for the reason that its main ingredient is dolomitic lime, it can actually increase the amounts of calcium and magnesium in soil, which could be essential to all kinds of surface vegetation (Albright, 2005). Because dolomitic lime is abundant throughout the world, it was expected that the economical process of CMA production could be developed.

After a serial of field tests of the CMA deicing performance, it was determined that CMA is able to be a promising road deicer, although it requires more usage amount and longer time to take effect. There is a 15 to 30 min of delay between the application of CMA and the melting of snow (Current deicing practice and alternative deicing materials, 1993), and it had to be employed early during a storm cycle, otherwise its performance would be significantly impacted. CMA is especially good at preventing snowpack and pavement bonding when it was applied at the outset of a storm. Generally saying, CMA was used in the same temperature range as salt, although it was less effective at a temperature lower than -5°C .

CMA was proven to be much less harmful than regular deicing salt to water quality plus most of aquatic life and terrestrial plants (TRB Special Report 235, 1991). So when used for deicing, CMA was expected to cause less environmental damage than regular deicing salt. (Albright, 2005)

Another major application of CMA is to reduce the emission of SO₂ and NO_x from coal burning factories (Levendis, 1993; Nimmo, 2004). Two billion tons of coal are exploited in China in only one year (China Energy Outlook, 2012). The burning of it will release a huge amount of acidic gases such as SO₂ and NO_x into the atmosphere, which would be spread all over the world by global atmospheric motion, causing environmental problems like acidic rain and extinction of species globally. Some recent studies have reported that the CMA is a great sulfur dioxide sorbent for in-boiler injection; sulfur removal efficiencies as high as 90% has been achieved with a Ca/S ratio at 2 (Levendis et al. 1993).

However, although calcium and magnesium are abundant throughout much of the world, the cost of producing CMA is high compare to regular road salts (\$30/ton) (Road Salt Use in the United States), simply because of the high price of acetic acid. So, finding an economical process to produce relatively low concentration of acetic acid to produce CMA is essential for the widely use of CMA as a road deicer. Yang and his coworkers (1992) found a way to produce 40 tons CMA from a plant processing with 1.5 million lbs whey permeate per day, at a cost of \$215/ton. The total capital investment is estimated at

7 million dollars, with a return rate of less than 1.5 years at the market price of \$600/ton. Using different kinds of residue biomass as feedstock (sewage sludge, biomass, municipal solid wastes, industrial wastes etc.), Palasantzas and Wise (1994) was able to produce CMA at a price of \$325 per US ton. They also revealed that if the regular disposal fee of residue biomass was considered as savings (\$1100 per US ton), the price of CMA could be even lower.

To further reduce the cost of CMA production, we propose to make use of the acetate in the hydrogen production fermentation broth of *Thermotoga neapolitana* (Yu, Drapcho, 2011). However, the concentration of acetate in the fermentation broth is usually too low for the production of CMA.

1.1.4. Acetic acid and methods to concentrate it in water

Acetic acid is an essential chemical compound in the chemical industrial nowadays, which is widely used in the production of medicines, dyes, fragrances and pesticides. Consequently, the separation of water and acetic acid has received lots attention for a long time. Although no azeotrope will be formed between them, there is little difference of relative volatility between water (100 °C) and acetic acid (117 °C), which means that it will consume huge amount of energy to separate them by regular distillation or azeotropic distillation (Yeom, 1996). As a result, both research and industrial communities are trying to find out a more effective and efficient way to separate them especially at a low original concentration (<100 g/L).

Another distillation method to separate acetic acid from water is called extractive distillation, which is much more efficient in energy use than general distillation or azeotropic distillation (Yeom, 1996). However, the efficiency of extractive distillation will reduce significantly when the original concentration of acetic acid is below 300 g/L (Demiral, 2003).

For the mixture of water and acetic acid with low concentration of acetic acid (<100 g/L), researchers were also trying to find a way that requires little energy to achieve the goal. An organic acids permselective polymeric membrane was synthesized by using phosphate compound and polrvinyl chloride to separate water and acetic acid by pervaporization, the acetic acid concentration in the final solution can be as high as 9 times of that in the original solution with an original acetic acid concentration of 1% to 30%. (胡兴兰, 周荣琪, 2002). However, to get a satisfactory result of separation, with the technology we have right now, the price of the membrane is too high to be acceptable. Membrane technology was also used together with electrodialysis method (Weier, 2008), in which anion-exchange membrane was applied. To make this method more practical in industrial field, improvements such as enhancing the electric current, increasing production capacity and reducing energy use need to be made in future researches.

1.1.5. Choices of organic extractants

There are also varieties of extractants that can be used to extract acetic acid directly from fermentation broth, and can be recovered and produce CMA at the same time with back-extraction processes (Juang and Wu, 1999). When choosing extractants, most researches in this field had focused on extractants that incorporate a phosphoryl or amine group. Trantolo and his coworkers (1990) made the conclusion that TOPO (Trioctylphosphine oxide) was perhaps the most effective extractant for CMA production, however, their experiments are conducted without pH adjustment, and the pH they chose was different from the pH of fermentation broth generated from the hydrogen production. The price is another disadvantage of TOPO (Trioctylphosphine oxide), it is much more expensive than other extractants such as Tertiary amines (Alamine 336) and quaternary amines (Aliquat 336). Alamine 336 had a good performance at a low pH range and it can be easily back extracted and recovered because it can only combine with undissociated acetic acid. However, since the CSTR fermentation reaction of hydrogen production will be ended at a pH higher than the pKa of acetic acid, the majority of acetic acid will be at a dissociated form, which makes Alamine unacceptable. Aliquat 336, on the other hand, was able to extract acetic acid at both dissociated and undissociated form (Yang, et.al, 1991).

Katikaneni and Cheryan (2002) recovered acetic acid with Alamine 336 and Aliquat 336 from a model solution. It was proven that at lower pH, Alamine 336 had greater extraction efficiency than Aliquat 336, but Aliquat 336 could extract higher amounts than Alamine 336 at pH above acetic acid pKa (4.75). Juang and Wu (1999)

compared the extraction efficiency of four different kinds of extractants including Aliquat 336 and Alamine 336. They showed that Aliquat 336 is the best choice with simulated waste streams in the production of an antibiotic---- Chloromycetin that typically contains 15.4% acetic acid, 8.8% sodium acetate, and 4.7% (w/w) NaCl. Yang and his coworkers (1991) studied how the pH affects the extraction efficiency of Aliquat 336, and showed that higher pH of the feed solution led to a lower extraction efficiency. Further, the cost of Alamine 336 is more than double that of Aliquat 336 (VWR Scientific).

1.1.6. Extraction mechanism of Aliquat 336

Aliquat 336 can be designated as R_3N-X^- , with the counter ion X^- (Cl^- , HSO_4^- , etc.) (Santos et al, 2005) and R_3N^+ as the ammonium entity bearing long aliphatic chains, thus making the complete molecule poorly soluble in the aqueous phase (solubility 0.12% w/w, Yang et al, 1991). With properties of an anion exchanger capable of complexing conjugate acids.

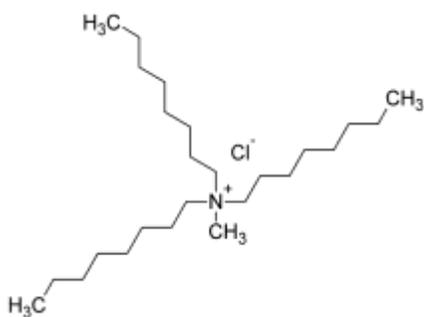
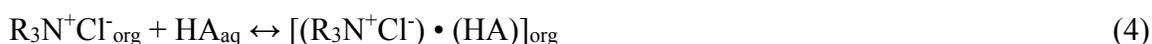


Fig 1.1 Molecular structure of Aliquat 336
(<http://chemistry.about.com/od/factsstructures/ig/Chemical-Structures---A/Aliquat-336.htm>)

Santos and his coworkers (2005) tried to extract 4-Hydroxybenzoic acid using Aliquat 336, they came up with two different mechanisms of the Aliquat 336 extraction process. The experiment results revealed that the extraction of undissociated 4-hydroxybenzoic acid (HA) with Aliquat 336 (R_3NCl) can be described by the following equation 4 (Santos, 2005):



While the extraction of anionic 4-hydroxybenzoic acid (A^-) with Aliquat 336 is described by the following equation:



This mechanism may describe why Aliquat could have a higher extractability than the Alamine 336 at the relatively high pH range.

However, because of the high viscosity of Aliquat 336 (1450 cP at 30°C, Yang et al, 1991), it will be very difficult to be handled in the extraction processes without any diluent. Kerosene can be used for this purpose, due to a good solvating effect for the acid–amine complex and low toxicity for organisms (Yang et.al, 1991).

Whether the extraction efficiency of Aliquat 336 would be affected in this fermentation broth is unknown. The initial acetic acid concentration of hydrogen production fermentation broth can be as low as 32 g/L. The fermentation process can be conducted under both pH controlled and uncontrolled conditions, which will impact the form of acetic acid present (Equation 6). The pKa for this equation is 4.75 at 0 ionic strength.



Other than acetic acid, the fermentation spent medium also has 10 g/L of sodium chloride and small amount of other carboxylic acids (Yu, Drapcho, 2011). Consequently, the effect of pH on the Aliquat 336 extraction efficiency and the volume ratio of Aliquat 336 and fermentation broth will be studied.

1.2. Main goal the specific objectives

The overall goal of this study is to determine the feasibility of extracting acetic acid from *Thermotoga neapolitana* fermentation spent medium to produce calcium magnesium acetate. The specific objectives are as follows:

- Determine the extraction efficiency of acetic acid using Aliquat 336 mixture from spent glucose fermentation medium.
- Determine the optimal pH for extraction.

- Determine the extraction efficiency of recovered Aliquat 336 mixture to fresh Aliquat mixture.
- Develop preliminary process to recover Aliquat 336 mixtures and to produce CMA.

1.3. Chapter Two

This chapter presents the study of a liquid-liquid extraction process using Aliquat 336 (tri-n-octylmethylammonium chloride) diluted in kerosene as an organic extractant to extract acetic acid from *Thermotoga neapolitana* fermentation spent medium, which mainly contains 34 g/L of acetic acid and 10 g/L of NaCl. The extraction efficiency of Aliquat 336 was studied under several different extraction conditions. The initial pH of the spent medium was adjusted to a pH range of 2.25 to 5.75 with a 0.5 pH interval to study the initial pH effect on Aliquat 336 extraction efficiency. Two different Aliquat 336 concentrations in kerosene (20% and 50% v/v) were made for comparison. $\text{Na}_2\text{CO}_3/\text{Ca}(\text{OH})_2$ and $\text{Mg}(\text{OH})_2$ were used to recover the used Aliquat 336 mixture and produce NaCH_3COOH and CMA at the same time. Aliquat 336 mixture extraction efficiency under all conditions are reported.

1.4. Chapter Three

Chapter three presents the conclusions of this study. It also provides a direction for further investigation of Aliquat 336 mixture extraction efficiency under other

conditions. The impact of this study on commercial production of CMA is also discussed.

References

About.com Chemistry: Aliquat 336. Available from:

<http://chemistry.about.com/od/factsstructures/ig/Chemical-Structures---A/Aliquat-336.htm>

Benemann J. Hydrogen biotechnology: Progress and prospects. *Nat Biotechnol*, SEP 1996, 1996; 14(9).

Brown, R. C. *Biorenewable Resources*. Iowa State Press, Blackwell Publishing Co., Iowa. 2003.

Bruce E. Logan, Sang-Eun Oh, In S. Kim, and Steven Van Ginkel. Biological Hydrogen Production Measured in Batch Anaerobic Respirometers. *Environ. Sci. Technol.*, 2002, 36 (11): 2530–2535.

Celina M. L. Santos, M. Rosinda C. Ismaela, M. Lurdes F. Gameiroa & Jorge M.R. Carvalho. Extraction of 4 - Hydroxybenzoic Acid with Aliquat336: Equilibrium Studies, Solvent Extraction and Ion Exchange, 2005, 23 (2): 213-230.

Claassen, P. A. M., Niel, van, E.W.J and Stams, A. J. M. Substrate and product inhibition of hydrogen production by the extreme thermophile, *Caldicellulosiruptor saccharolyticus*, *Biotechnology and Bioengineering*, 2003, 81, (3): 255–262.

Clayton Ormsby, *Highway Deicing: Comparing Salt and Calcium Magnesium Acetate*, Transportation Research Board, National Research Council, Washington, D.C.; 1991.
Da Rosa, Aldo V. *Fundamentals of renewable energy processes*, Academic Press, Aug 3, 2005.

Demiral, H, Yildirim, M Ercengiz. Recovery of acetic acid from waste streams by extractive distillation, *Water Science & Technology*, 2003, 47 (10): 183–188.

D. D. Dionysiou, M. Tsianou, G. D. Botsaris. Investigation of the Conditions for the Production of Calcium Magnesium Acetate (CMA) Road Deicer in an Extractive Crystallization Process, *Cryst. Res. Technol*, 2002, 35(9): 1035–1049.

D. T. Jones, D. R. Woods. Acetone-butanol fermentation revisited, *Microbiol Rev.* 1986 December; 50(4): 484–524.

Holger W. Jannasch, Robert Huber, Shimshon Belkin, Karl O. Stetter. *Thermotoga neapolitana* sp. nov. of the extremely thermophilic, eubacterial genus *Thermotoga*, *Archives of Microbiology*, May 1988, 150(1): 103-104.

Hubert Reisinger and C. Judson King. Extraction and sorption of acetic acid at pH above pKa to form calcium magnesium acetate, *Ind. Eng. Chem. Res.* 1995, 34 (3):845–852.

Hussy, I. Continuous dark fermentative hydrogen production by mesophilic microflora: Principles and progress, February 2007, 32(2): 172–184.

Jain, A. 2009. Biohydrogen production from cull peach medium by hyperthermophilic bacterium *Thermotoga neapolitana*. MS thesis ,Clemson,South Carolina : Clemson University, Department of Biosystems Engineering.

Janet A. Tamada and C. Judson King. Extraction of carboxylic acids with amine extractants. 2. Chemical interactions and interpretation of data, *Ind. Eng. Chem. Res.*, 1990, 29 (7): 1327–1333.

Janet A. Tamada and C. Judson King. Extraction of carboxylic acids with amine extractants. 3. Effect of temperature, water coextraction, and process considerations, *Ind. Eng. Chem. Res.*, 1990, 29 (7): 1333–1338.

Janet A. Tamada, A. Steven Kertes, and C. Judson King. Extraction of carboxylic acids with amine extractants. 1. Equilibria and law of mass action modeling, *Ind. Eng. Chem. Res.*, 1990, 29 (7): 1319–1326.

Jaquet, A., Quan, L., Marison, I. W. and von Stockar, U. Factors influencing the potential use of Aliquat 336 for the in situ extraction of carboxylic acids from cultures of *Pseudomonas putida*, *Journal of Biotechnology*, 19 February 1999, 68 (2-3): 185–196.

Juang, Ruey-Shin, Wu, Rong-Tzong. Extraction of acetate from simulated waste solutions in chloromycetin production, *Separation and Purification Technology*, 6 December 1999, 17(3): 225–233.

Kawamura, Kunio, Takahashi, Keiji and Okuwaki, Akitsugu. Influence of pH and Diluent on the Ion - Pair Solvent Extraction of Aromatic Carboxylic Acids Using Quaternary Ammonium Salts, *Separation Science and Technology*, 2006, 41(12).

Kertes, A. S, King, C. J. and Harvey W. Blanch, Extraction chemistry of fermentation product carboxylic acids, *Biotechnology and Bioengineering*, February 1986, 28(2): 269–282.

Klass, Donald L. Biomass for renewable energy, fuels, and chemicals, Academic Press, Jul 6, 1998.

Klass, Donald L. A critical assessment of renewable energy usage in the USA, *Energy Policy*, March 2003, 31(4): 353–367.

Levendis, Yiannis A., Zhu, Wenqi, Wise, Donald L. and Simons, Girard A. Effectiveness of calcium magnesium acetate as an SO_x sorbent in coal combustion, *AIChE Journal*, May 1993, 39(5): 761–773.

Levin, David B., Pitt, Lawrence and Love, Murray. Biohydrogen production: prospects and limitations to practical application, *International Journal of Hydrogen Energy*, February 2004, 29(2): 173–185.

Liu, Da w. Bio-hydrogen production by dark fermentation from organic wastes and residues, *Vester Kopi Virum*, May 2008.

Lloyd Wheatley, Yiannis A. Levendis, Paul Vouros. Exploratory study on the combustion and PAH emissions of selected municipal waste plastics, *Environ. Sci. Technol.*, 1993, 27 (13): 2885–2895.

Matthew Albright. Changes in Water Quality in an Urban Stream Following the Use of Organically Derived Deicing Products, *Lake and Reservoir Management*, 2005, 21(1).

Momirlan M, Veziroglu T. Current status of hydrogen energy, *Renewable and Sustainable Energy Reviews*, 2002, 6(1–2): 141–179.

Nimmo, W., Patsias, A. A., Hampartsoumian, E., Gibbs, B. M. and Williams, P. T. Simultaneous reduction of NO_x and SO₂ emissions from coal combustion by calcium magnesium acetate, *Fuel*, January 2004, 83(2): 149–155.

Palasantzas, Ioannis A., Wise, Donald L. Preliminary economic analysis for production of calcium magnesium acetate from organic residues, *Resources, Conservation and Recycling*, 30 June 1994, 11(1–4):225–243.

Paul C, Yanrui Wu, Energy consumption in China: past trends and future directions, *Energy Economics*, January 2005, 27(1): 195–208.

Pursell, Mark R., Alcina Mendes-Tatsis, M. and Stuckey, David C. Co-Extraction during Reactive Extraction of Phenylalanine using Aliquat 336: Interfacial Mass Transfer, *Biotechnology Progress*, 2003, 19(2): 469–476.

Robidoux, Pierre Y., Delisle, Claude E. Ecotoxicological Evaluation of Three Deicers (NaCl, NaFo, CMA)—Effect on Terrestrial Organisms, *Ecotoxicology and Environmental Safety*, February 2001, 48(2): 128–139.

Sai P. R. Katikaneni and Munir Cheryan. Purification of Fermentation-Derived Acetic Acid by Liquid–Liquid Extraction and Esterification, *Ind. Eng. Chem. Res.*, 2002, 41 (11): 2745–2752.

Sarangi, K., Padhan, E., Sarma, P. V. R. B., Park, K. H. and Das, R. P. Removal/recovery of hydrochloric acid using Alamine 336, Aliquat 336, TBP and Cyanex 923, Hydrometallurgy, November 2006, 84(3–4): 125–129.

Schroder C, Selig M, Schonheit P. Glucose fermentation to acetate, CO₂ and H₂ in the anaerobic hyperthermophilic eubacterium *Thermotoga-maritima* - involvement of the embden-meyerhof pathway, Archives of Microbiology, June 1994, 161(6): 460-470.

Shang Tian Yang, Scott A. White, and Sheng Tsiung Hsu. Extraction of carboxylic acids with tertiary and quaternary amines: effect of pH, Ind. Eng. Chem. Res., 1991, 30 (6): 1335–1342.

Solomon S, Plattner G, Knutti R, Friedlingstein P. Irreversible climate change due to carbon dioxide emissions, PNAS, February 10, 2009, 106(6): 1704-1709.

Steven Van Ginkel and Bruce E. Logan. Inhibition of Biohydrogen Production by Undissociated Acetic and Butyric Acids, Environ. Sci. Technol., 2005, 39 (23): 9351–9356.

Tim Eggeman, Dan V. Recovery of organic acids from fermentation broths, Twenty-Sixth Symposium on Biotechnology for Fuels and Chemicals ABAB Symposium, 2005: 605-618.

Trantolo, Debra J., Gresser, Joseph D., Augenstein, Don C. and Wise, Donald L. The feasibility of a residue biomass bioconversion process to prepare calcium magnesium acetate deicing salt, Resources, Conservation and Recycling, September 1990, 4(3): 215–232.

USEIA: Primary Energy Consumption by Source, November 2012: 2012.

Van Ginkel, Steven, Oh, Sang-Eun and Logan, Bruce E. Hydrogen Production Using Anaerobic Wastewater Treatment Processes, Proceedings of the Water Environment Federation, WEFTEC 2002: Session 11 through Session 20: 842-848(7).

Wang, C.C., Chang, C.W., Chu, C.P., Lee, D.J., Chang, B.V., Liao, C.S., Tay, J.H. Using filtrate of waste biosolids to effectively produce bio-hydrogen by anaerobic fermentation, *Water Research*, June 2003, 37(11): 2789–2793.

Weier, Anthony J., Glatz, Bonita A. and Glatz, Charles E. Recovery of Propionic and Acetic Acids from Fermentation Broth by Electrodialysis, *Biotechnology Progress*, November/December 1992, 8(6): 479–485.

William L. Bryan, Research approaches to reduce CMA cost, *Resources, Conservation and Recycling*, October 1992, 7 (1-3): 25-42.

Yang, Shang-Tian, Zhu, Hui, Lewis, Vivian P. and Tang, I-Ching. Calcium magnesium acetate (CMA) production from whey permeate: process and economic analysis, *Resources, Conservation and Recycling*, October 1992, 7(1–3): 181–200.

Yeom, Choong-Kyun, Lee, Kew-Ho. Pervaporation separation of water-acetic acid mixtures through poly (vinyl alcohol) membranes cross linked with glutaraldehyde, *Journal of Membrane Science*, 24 January 1996, 109(2): 257–265.

Yu X, Drapcho C. Hydrogen production by the hyperthermophilic bacterium *Thermotoga neapolitana* using agricultural-based carbon and nitrogen sources. *Biological Engineering Transactions*, 2011, 4:101-112.

Yun Suk Huh, Yeon Ki Hong, Won Hi Hong, Ho Nam Chang. Selective extraction of acetic acid from the fermentation broth produced by *Mannheimia succinici* producens, *Biotechnology Letters*, October 2004, 26(20): 1581-1584.

CHAPTER TWO

THE EXTRCTION OF ACETIC ACID FROM *THERMOTOGA NEAPOLITANA* FERMENTATION SPENT MEDIUM USING FRESH/RECOVERED ALIQUAT 336 TO PRODUCE CMA

2.1. Introduction

2.1.1. Biological hydrogen production by fermentation

In order to avoid the crisis of both energy and environment, seeking renewable energy source is a matter of course, because fossil fuels are nonrenewable and environmentally harmful to use (Benemann, 1996; Momirlan and Veziroglu, 2002).

Thermotoga species were proven capable of fermenting glucose to produce one of the most promising bio renewable energy sources----hydrogen gas (H₂). The summative biological reaction for *T. Maritima* is as follows (Schroder et al. 1994):



Yu and Drapcho (2011) found that 32.82 mmol/L-medium H₂ was produced when glucose was used as substrates. They also found that the batch fermentations end at a pH around 4.75-5. In addition to 4 mols of H₂, there are also 2 mols of acetic acid plus 2 mols of CO₂ formed in this process.

There are several disadvantages of the existence of acetic acid in the fermentation broth. First of all, the acetic acid can lower the pH value of the fermentation broth, which will limit the growth of the microbes and inhibit the production of hydrogen, because the best pH for the growth and production of *Thermotoga neapolitana* is around 7 (Jannasch et al, 1988). Acetic acid accumulation can also inhibit the hydrogen fermentation by product inhibition (Jones and Woods, 1986; Van Niel et al, 2003; Van Ginkel and Logan, 2005). Liu (2008) reported that the hydrogen fermentation could be affected when acetate concentration was more than 50 mM. The duration of lag phase for the mixed culture also could be altered by acetate concentrations (Liu, 2008).

Second, the acetic acid in the fermentation broth is a wasted resource if not used, since 2/3 of the carbon source and energy contained in glucose has been converted into acetic acid. Because lowering the manufacturing cost can certainly encourage the industrialization of bio-hydrogen production, recovering and using the acetic acid could lower the costs and make it more possible.

2.1.2. CMA (Calcium Magnesium Acetate)

One of the most economically promising and environmental friendly products that can be produced from acetic acid is CMA (Calcium Magnesium Acetate) (Dionysiou 2000).

As shown in equation 2, $\text{CaMg}(\text{CH}_3\text{COO})_4$ can be formed by the reaction of acetic acid and dolomitic lime (calcium magnesium hydroxide).



CMA was proposed to be a deicing chemical to replace regular deicing salt (sodium chloride) in a research program started by the Federal Highway Administration (FHWA) in 1980s (Yang et al, 1991). CMA stood out from a large number of competitors in this program because it is more environmental friendly plus it is easier to handle and spread (Robidoux, 2001). CMA was proven to be much less harmful than NaCl to water quality plus most aquatic life and terrestrial plants (TRB Special Report 235). So, when used for deicing, CMA was expected to cause less environmental damage than regular deicing salt (Albright, 2005). CMA is noncorrosive to metals, and nondestructive to concrete and other constructional materials (Robidoux, 2001). What is more, for the reason that its main ingredient is dolomitic lime, it can actually increase the amounts of calcium and magnesium in soil, which could be essential to all kinds of surface vegetation (Albright, 2005). Because dolomitic lime is abundant throughout the world, it was expected that an economical process of CMA production could be developed.

Another major application of CMA is to reduce the emission of SO₂ and NO_x from coal burning factories (Levendis, 1993; Nimmo, 2004). Two billion tons of coal are exploited in China in only one year (Paul, 2008). The burning of it would release a huge amount of acidic gases such as SO₂ and NO_x into the atmosphere, which would be spread all over the world by global atmospheric motion, causing environmental problems like acidic rain and extinction of species globally. Some recent studies have reported that the CMA is a great sulfur dioxide sorbent for in-boiler injection; sulfur removal efficiencies as high as 90% has been achieved with a Ca/S ratio at 2 (Levendis et al. 1993).

2.1.3. Extraction of acetic acid from fermentation broth

The acetate in the spent medium of *Thermotoga neapolitana* fermentation could be used for CMA production. However, the concentration of acetic acid in the fermentation broth is usually too low for it. So concentrating the acetic acid is necessary for its production.

There are many ways to concentrate acetic acid from a diluted solution. Considering that the general distillation and azeotropic distillation are highly energy intensive because the boiling points of water and acetic acid are so close (water: 100°C, acetic acid: 117°C) (Yeom, 1996), extractive distillation is not effective enough for acetic acid solution with an original concentration lower than 300 g/L (Demiral, 2003) and membrane separation for acetic acid from water is still too costly (Weier, 2008), liquid-

liquid extraction with an organic extractant might be the best option (Katikaneni and Cheryan, 2002).

Two organic solvents, Aliquat 336 and Alamine 336 have been used to extract acetic acid from aqueous solution by Yang and his coworkers. Aliquat 336 has a relatively wider pH acceptability than Alamine 336 (Yang, et.al, 1991). Aliquat 336 is capable of extracting both dissociated and undissociated acetic acid (Yang, et.al, 1991). It has a relatively lower price than TOPO (Trioctylphosphine oxide) (Trantolo et.al, 1990).

Katikaneni and Cheryan (2002) recovered acetic acid with Alamine 336 and Aliquat 336 from a model solution. It was proven that at lower pH, Alamine 336 had greater extraction efficiency than Aliquat 336, but Aliquat 336 could extract higher amounts than Alamine336 at pH above the pKa of acetic acid (4.75). Juang and Wu (1999) compared the extraction efficiency of four different kinds of extractants including Aliquat 336 and Alamine 336. They showed that Aliquat 336 is the best choice with simulated waste streams in the production of an antibiotic---- Chloromycetin that typically contains 15.4% acetic acid, 8.8% sodium acetate, and 4.7% (w/w) NaCl. Yang and his coworkers (1991) studied how the pH affects the extraction efficiency of Aliquat 336, and showed that higher pH of the feed solution led to a lower extraction efficiency. Furthermore, the cost of Alamine 336 is more than double that of Aliquat 336 (VWR Scientific).

Aliquat 336 can be designated as R_3N-X^- , with the counter ion X^- (Cl^- , HSO_4^- , etc.) (Santos et al, 2005) and R_3N^- as the ammonium entity bearing long aliphatic chains, thus making the complete molecule poorly soluble in the aqueous phase (solubility of 0.12% w/w, Yang et al. 1991), with properties of an anion exchanger capable of complexing conjugate acids. Santos and his coworkers (2005) tried to extract 4-Hydroxybenzoic acid using Aliquat 336. They came up with two different mechanisms of the Aliquat 336 extraction process. The experimental results revealed that the extraction of undissociated 4-hydroxybenzoic acid (HA) with Aliquat 336 (R_3NCl) can be described by the following equation 3:



While the extraction of dissociated 4-hydroxybenzoic acid (A^-) with Aliquat 336 is described by the following equation:



Because of the high viscosity of Aliquat 336 (1450 cP at 30°C, Yang et al, 1991), it will be very difficult to handle in the extraction processes without any diluent. Kerosene can be used for this purpose, due to a good solvating effect for the acid–amine complex and low toxicity for organisms (Yang et.al, 1991).

Whether the extraction efficiency of Aliquat 336 would be affected by the H₂ production fermentation broth is unknown. The initial acetic acid concentration of hydrogen production fermentation broth can be as low as 32 g/L. The fermentation process can be conducted under both pH controlled and uncontrolled conditions, which will impact the form of acetic acid present (Equation 5). The pK_a for this equation is 4.75 with 0 ionic strength.



Other than acetic acid, the fermentation broth also has 10 g/L of sodium chloride and small amount of other carboxylic acids (Yu, Drapcho, 2011), which could potentially affect the extractability of Aliquat 336.

Therefore, the overall goal of this study was to determine the feasibility of extracting acetic acid from *Thermotoga neapolitana* fermentation spent medium to produce calcium magnesium acetate. The objectives of this research were:

- Determine the extraction efficiency of acetic acid using Aliquat 336 mixture from spent glucose fermentation medium.
- Determine the optimal pH for extraction.
- Determine the extraction efficiency of recovered Aliquat 336 mixture to fresh Aliquat mixture.

- Develop preliminary process to recover Aliquat 336 mixture and to produce CMA.

2.2. Materials and methods

2.2.1. Fermentation spent medium

Fermentation spent medium was obtained from the cultivation of *Thermotoga neapolitana* as described below.

2.2.1.1. Organism

Thermotoga neapolitana was obtained from DSMZ (German Collection of Microorganisms and Cell Cultures).

2.2.1.2. Medium preparation

Thermotoga neapolitana was cultivated and maintained on medium described by Van Ootegham (Van Ootegham et al. 2002): 75 g/L glucose, 1.0 g of NH₄Cl, 0.3 g of K₂HPO₄, 0.3 g of KH₂PO₄, 0.2 g of MgCl₂•2H₂O, 0.1 g CaCl₂, 10.0 g of NaCl, 0.1 g of KCl, 1.0 g of Cysteine HCl, 2.0 g of yeast extract, 2.0 g of Trypticase, 10.0 mL of vitamin solution (DSM medium 141), 10.0 mL of trace element solution (DSM medium 141), 0.121 g of trizma base per 1.0 L of distilled H₂O. The pH was adjusted to 8.0 with 5N NaOH.

2.2.1.3. Cultivation conditions

A 4L fermentation tank with 2L of the medium was used as batch reactor for the experiments. The fermentation tank headspace was sparged with nitrogen for 1 minute, then connected to a vacuumed gas collection bottle. After a period of 90 minutes, which was used to allow cysteine HCl to react with dissolved oxygen in medium, the medium was inoculated with 200 mL culture from seed bottles. The fermentation process was controlled at 200 rpm and 77°C without pH control. After 40 hours of inoculation, the fermentation spent medium was collected, filtered with 0.45 µm filter and stored at a temperature of -4°C.

2.2.2. Extraction phase preparation

2.2.2.1. Aqueous phase preparation

The acetic acid concentration in the original fermentation spent medium was measured to be approximately 2 g/L by HPLC. An additional 32 g/L of glacial acetic acid (99.3%, Fisher Chemicals) was added to the fermentation spent medium, so the total acetic acid concentration in the solution was 34 g/L. (34 g/L of acetic acid concentration is expected with the *Thermotoga neapolitana* hydrogen gas fermentation when there is 75 g/L of glucose and no product inhibition).

Then, 20 mL of spent medium with additional acetic acid was added to a 50 mL centrifuge tube for each extraction trial.

The original pH of the aqueous mixture was 2.97. It was then adjusted to a range of 2.25 to 5.75 with a 0.5 pH interval, using NaOH pellets or HCl (min 35%-max 38% w/w, Chemical Inc. for Fisher Scientific).

2.2.2.2. Aliquat 336 mixtures preparation

The extractant used in the experiments was Aliquat 336 (Tri-n-octylmethylammonium chloride, MW=442.0, density=0.88 g/cm³, Alfa Aesar). Because of high viscosity of the Aliquat 336, dilution with kerosene (Alfa Aesar) was used to lower its viscosity. The Aliquat 336 mixtures were prepared by diluting the Aliquat 336 in kerosene as shown in Table 2.1

Table 2.1 The composition of two different Aliquat 336 mixtures

	Aliquat 336 volume	Kerosene volume	Aliquat percentage (v/v)
Aliquat 336 mixture 1	10 mL	10 mL	50%
Aliquat 336 mixture 2	4 mL	16 mL	20%

2.2.3. Extraction procedure

20 mL of aqueous phase and 20 mL of Aliquat 336 mixture were added to a 50 mL centrifuge tube and placed on a rotor (RotoFlex, Model: R2000, Argos) to keep mixing for 10 min. Then, after 1 min at the speed of 4400 rpm centrifugation to separate the phases, 5 mL of the aqueous phase was sampled from each centrifuge tube, and transferred to beakers. This procedure was repeated for the Recovered Aliquat 336 mixtures. Based on the preliminary experiments not reported here, the extraction

efficiency did not change after 10 min of extraction time. It was also proven that 10 min is long enough for the extraction procedure to finish (喻新平, 2002)

Table 2.2 Extraction process treatments

Initial pH			
Fresh Aliquat 336 mixture		Recovered Aliquat 336 mixture	
20% Aliquat 336 mixture	50% Aliquat 336 mixture	20% Aliquat 336 Mixture	50% Aliquat 336 mixture
2.25	2.25	2.25	2.25
2.75	2.75	2.75	2.75
3.25	3.25	3.25	3.25
3.75	3.75	3.75	3.75
4.25	4.25	4.25	4.25
4.75	4.75	4.75	4.75
5.25	5.25	5.25	5.25
5.75	5.75	5.75	5.75

As shown in Table 2.2, both fresh and recovered Aliquat 336 mixtures have two different concentrations. Each group has 8 different initial pH.

2.2.4. Aliquat 336 mixture recovery

After the aqueous phase was sampled, the Aliquat 336 mixture in each sample was separated from the residual aqueous phase using separating funnel. Then, by combining the Aliquat 336 mixtures from 5 tubes of initial pH 2.25-3.75, a total volume of 100 mL of separated used Aliquat 336 mixture was obtained, then 17 mL of 0.6 mol/L of Na₂CO₃ (63.6 g/L) solution was added for 24 hours to complete the back-extraction process of acetic acid. After the back-extraction process, the Na₂CO₃ solution and the

recovered Aliquat 336 mixture were separated again using a separating funnel. This recovered Aliquat 336 mixture was used for acetic acid extraction.

2.2.5. Analytical tests

The pH of the separated aqueous phase liquid was measured using a digital pH meter equipped with a gel electrode after calibration with pH 4.0 and 7.0 buffers. The salinity of every sample was measured using a Master Refractometer (ATAGO).

For HPLC analysis, the aqueous samples were filtered with a 0.45 μm membrane syringe filter into HPLC vials. They were analyzed for acetic acid concentration using a high-performance liquid chromatography (HPLC) system. The detector with the HPLC is a refractive index detector. The mobile phase was 0.01 N H_2SO_4 with a flow rate at 0.6 mL/min. The column was a Bio-Rad Aminex HPX-87H at a temperature of 60°C. Acetic acid standards were made using distilled water with 10 g/L of sodium chloride, by adding 10, 20, 30, 40, and 50 g/L of glacial acetic acid (99.3% Fisher chemicals) respectively. A 20 μL portion of the aqueous sample was injected into the column.

The concentration of acetic acid in the Aliquat 336 mixture could not be analyzed by HPLC due to high viscosity. The concentration of acetic acid in the organic phase was calculated by subtracting the acetic acid concentration in the post-extraction aqueous phase from the acetic acid concentration in the pre-extraction aqueous phase. The extraction efficiency (EE) of Aliquat 336 mixture can be calculated as below:

$$EE = (C2-C1) / C2 * 100\% \quad (6)$$

Where C1 is the acetic acid concentration in the post-extraction aqueous phase and C2 is the acetic acid concentration in the pre-extraction aqueous phase, g/L.

Also, distribution coefficient is another way to describe this:

$$\text{Distribution Coefficient} = [TA]_{\text{org}} / [TA]_{\text{aq}} \quad (7)$$

Where $[TA]_{\text{org}}$ was the concentration of total acetic acid in the organic phase after extraction and $[TA]_{\text{aq}}$ was the concentration of total acetic acid in the aqueous phase after extraction

2.2.6. CMA and sodium acetate production

To test whether acetic acid could be recovered from the Aliquat 336 mixture, two tests were conducted on the 50% fresh Aliquat 336 mixture at pH 2.25-3.75 after it was recovered. Test 1 used sodium carbonate and test 2 used the mixture of calcium hydroxide and magnesium hydroxide

Juang and Wu (1999) used 0.1 mol/L (10.6 g/L) of sodium carbonate as the stripping solution to recover acetic acid from Aliquat 336 mixture in 1:1 volumetric ratio. They achieved stripping efficiency from 80% to 96% (stripping efficiency was defined as the acetic acid concentration in the stripping solution after back extraction divided by the initial concentration in the loaded used Aliquat 336 mixture). Because 100 g/L of acetic

acid concentration in the stripping solution after back extraction was desired, a volume ratio of Aliquat 336 mixture to sodium carbonate solution of 6:1 (17 mL of stripping solution) was required. Therefore, a concentration of sodium carbonate of 0.6 mol/L (63.6 g/L) was used.

For test 2, as there is 0.28 mol/L (17 g/L) of acetic acid in the 100 mL Aliquat 336 mixture, 0.28 mol/L of $\text{Ca}(\text{OH})_2$ (20.72 g/L) and 0.28 mol/L of $\text{Mg}(\text{OH})_2$ (16.24 g/L) aqueous slurry was prepared in a beaker. The dose of $\text{Ca}(\text{OH})_2$ and $\text{Mg}(\text{OH})_2$ was a four-fold stoichiometric excess relative to the acetic acid concentration amount in the organic phase. The total volume of the aqueous slurry used was 17 mL to match the sodium carbonate solution.

Because higher concentration in the stripping solution resulted in higher stripping efficiency (Juang and Wu, 1999), for both two tests, the stripping solutions were overdosed to reach the highest stripping efficiency based on the methods instructed by Jung (1999) and Trantolo (1990).

For both two tests, these two phases were combined and shaken at room temperature for 4 hours. Then the two phases were separated using a separation funnel. The aqueous phase was then filtered through a 0.45 μm filter and dried at 70 °C to obtain the sodium acetate and CMA solid. The dry weight of the recovered white powdered solids was measured.

0.05g of both white powdered solids were then dissolved in 10 mL of distilled water, and acidified with HCl (min 35%-max 38% w/w, Chemical Inc. Fisher Scientific)

and then the samples were processed by the HPLC system mentioned above to test the acetate concentration.

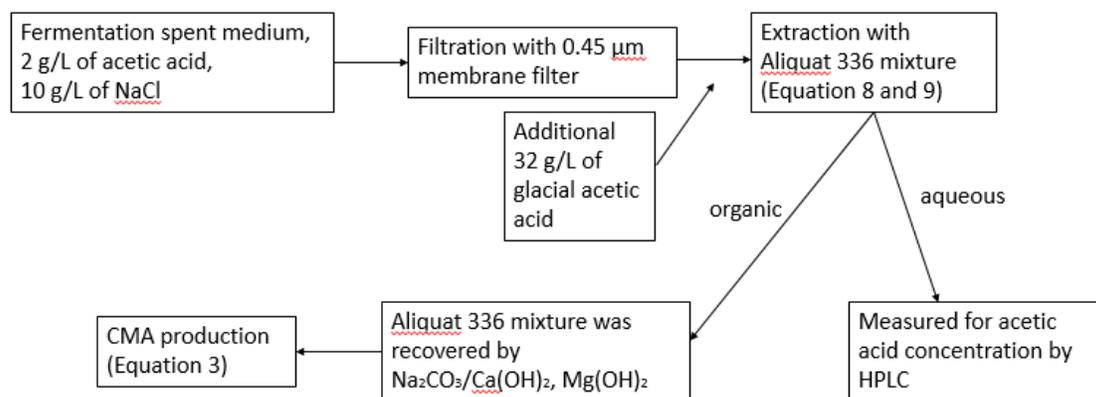


Fig 2.1 Sample Process Flow Diagram of this project.

2.2.7. Statistical analysis

The software SAS 9.3 was used to test significant difference of extraction efficiency and pH change during the extraction process for each treatment. Proc Means with a Tukey's Studentized range test was used with $\alpha = 0.05$.

2.3. Results and discussion

2.3.1. Effect of initial pH of the spent medium

As shown in Table 2.3, for 20% (v/v) fresh Aliquat 336 mixtures, there was no significant difference in extraction efficiency among pH 2.25 to 4.75. The extraction efficiency decreased as the initial pH of the spent medium increased above pH 4.75.

Table 2.3 The extraction efficiency of 20% fresh Aliquat 336 mixtures with different initial pH values^[a]

Initial pH value	Mean acetic acid concentration in aqueous phase after extraction, g/L, [SD]	Mean extraction efficiency %, [SD] ^[b]	Mean distribution coefficient
2.25	25.69 [0.87]	24.47 a [2.55]	0.32
2.75	24.75 [1.44]	27.21 a [4.24]	0.37
3.25	25.67 [0.05]	24.51 a [0.14]	0.32
3.75	26.48 [0.04]	22.13 a [0.11]	0.28
4.25	25.72 [0.11]	24.36 a [0.33]	0.32
4.75	27.34 [0.75]	19.59 a [2.21]	0.24
5.25	31.47 [0.22]	7.44 b [0.64]	0.08
5.75	34.00 [0.00]	0.00 b [0.00]	0

^[a] Means followed by different letters are significantly different using Tukey's Studentized range test ($p < 0.05$)

^[b] $n = 2$

^[c] Initial acetic acid concentration in the aqueous phase was 34 g/L

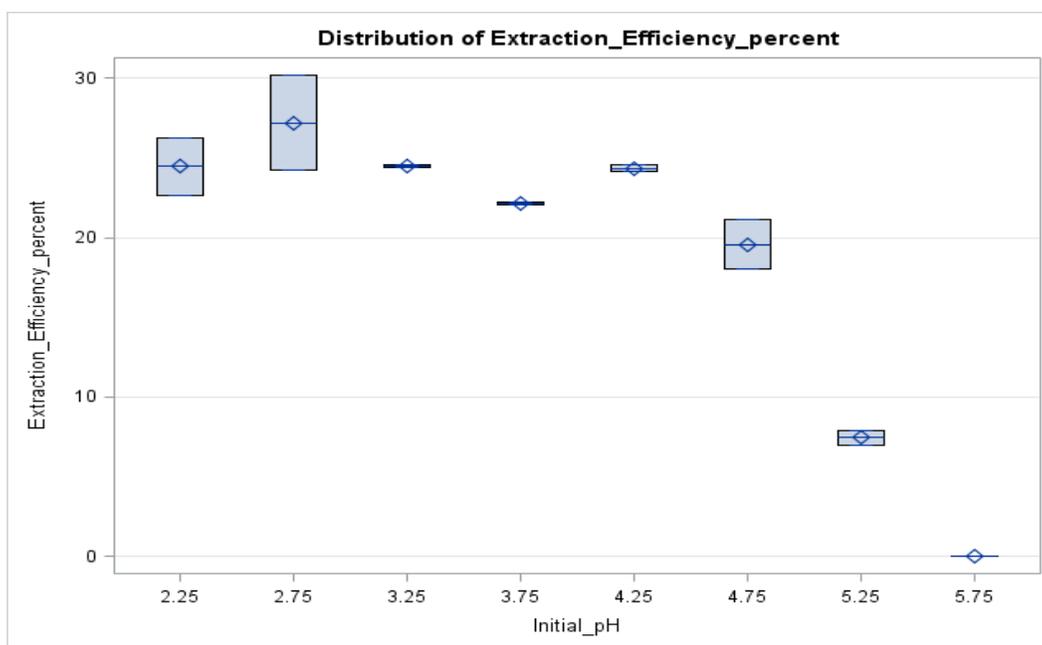


Figure 2.2 The extraction efficiency of 20% fresh Aliquat 336 mixtures with different initial pH values.

The highest extraction efficiency value achieved in this group was in the initial pH range 2.25 to 4.75.

As shown in Table 2.4, for 50% (v/v) fresh Aliquat 336 mixtures, there was no significant difference in extraction efficiency among pH 2.25-4.25. The extraction efficiency decreased as the initial pH of the spent medium increased above pH 4.25.

Table 2.4 The extraction efficiency of 50% fresh Aliquat 336 mixtures with different initial pH values^[a]

Initial pH value	Mean acetic acid concentration in aqueous phase after extraction, g/L, [SD]	Mean, extraction efficiency %, [SD] ^[b]	Mean distribution coefficient
2.25	18.26 [0.96]	46.29 a [2.82]	0.86
2.75	19.62 [0.26]	42.29 a [0.77]	0.76
3.25	18.89 [0.27]	44.44 a [0.81]	0.80
3.75	19.41 [0.17]	42.90 a [0.50]	0.75
4.25	19.91 [0.14]	41.45 a [0.42]	0.71
4.75	22.88 [0.51]	32.69 b [1.49]	0.49
5.25	26.70 [0.76]	21.48 c [2.24]	0.27
5.75	29.44 [1.40]	13.41 c [4.13]	0.15

^[a] Means followed by different letters are significantly different using Tukey's Studentized range test ($p < 0.05$)

^[b] $n = 2$

^[c] Initial acetic acid concentration in the aqueous phase was 34 g/L

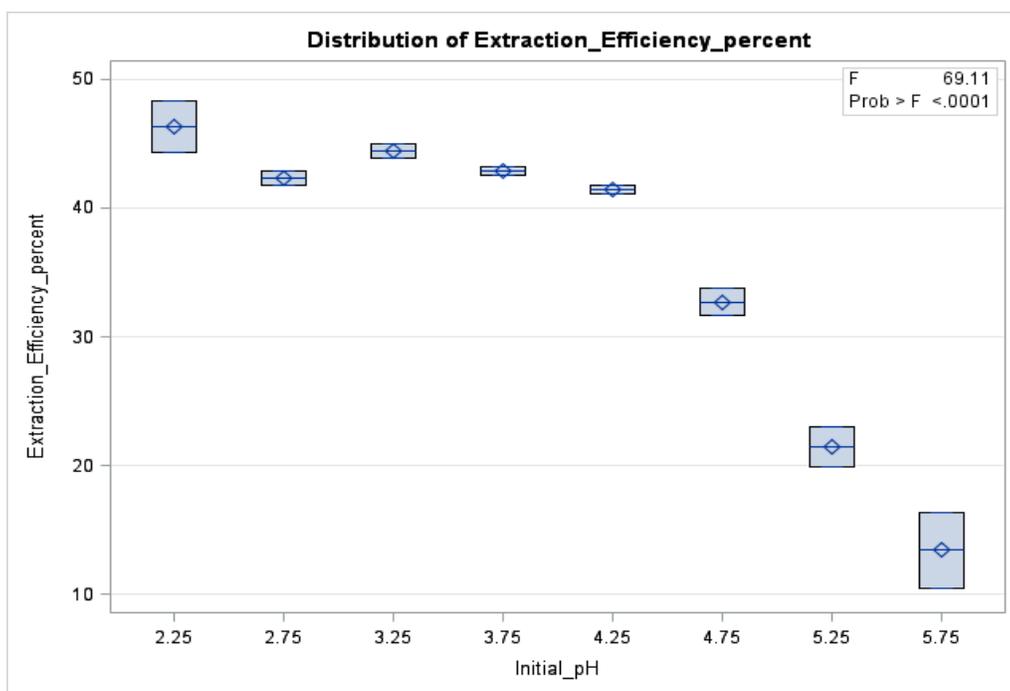


Fig 2.3. The extraction efficiency of 50% fresh Aliquat 336 mixtures with different initial pH values.

The highest extraction efficiency value achieved in this group was in the initial pH range 2.25 to 4.25. This also had the highest mean extraction efficiency achieved among all the treatments.

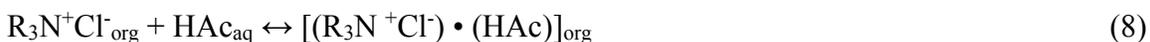
Katikaneni and Cheryan (2002) used Aliquat 336 at a 50% concentration and a 2:1 volumetric ratio of organic phase to aqueous phase, they achieved an extraction efficiency of acetic acid 55% at a pH of 3.3 for acetic acid concentration of 35 g/L. However, their aqueous phase was a simulated model instead of a real fermentation broth.

The results suggested that the extraction efficiency of Aliquat 336 mixtures was greatly affected by the initial pH of the spent medium (table 2.3-2.6). Generally speaking, the extraction efficiency decreased with the increase of initial pH of the spent medium.

The results of the Tukey's Test showed no significant difference in extraction efficiency among pH range from 2.25 to 3.75 in all four treatments.

Based on the results, although the extraction efficiency of Aliquat 336 mixture decreased when the pH was above 4.75, it was still capable of extracting acetic acid when the pH was above the pKa, which indicates that the extraction mechanism of Aliquat 336 consists of two parts. So, we can formulate equations of Aliquat 336 extraction for acetic acid under two different circumstances, similar to Aliquat 336 extraction for 4-hydroxybenzoic acid (Santos et.al 2005).

At a pH lower than the pKa of acetic acid, most acetic acid is undissociated, the reaction equation for the dominated mechanism is



At a pH higher than the pKa of acetic acid, most acid is dissociated, and the reaction equation for the dominated mechanism is



Generally saying, for all four groups, the extraction efficiency started to decrease drastically around the pH of 4.25. Although the normal pKa of acetic acid is 4.75, considering the fact that the pKa of acetic acid could be decreased by strong ionic strength in the spent medium (W.P. Jencks 1975), the pKa of acetic acid under this condition could be decreased to around 4.25, which supported the fact that the initial pH and the pKa of acetic acid played an important role in deciding the extraction efficiency. The data suggested that Aliquat 336 was still capable of extracting acetic acid when the initial pH was above the pKa, however, its extraction efficiency was still significantly affected by the initial pH of the fermentation spent medium.

Yang, and his coworkers (1991) also reported that the extraction efficiency of Aliquat 336 was significantly affected by the pH. They also had a 50% of Aliquat 336 concentration and a 1:1 volumetric ratio of organic phase to aqueous phase. In their results, in the pH range from 1.5 to 2.5, Aliquat 336 had the highest distribution coefficient of 2, which means 66% of extraction efficiency. The reason why they had higher extraction efficiency could be attributed to that they did not have Na^+ in their aqueous phase, which was claimed potentially harmful to the Aliquat 336 mixture extraction efficiency (Katikaneni and Cheryan, 2002). Yang (1991) still had a Distribution Coefficient of 0.5 (extraction efficiency 33%) when the pH ranged from 6 to 8.5. In addition to the difference of Na^+ in the feed solution, another reason to explain the lower extraction efficiency in our experiments could possibly be that the presence of Cl^- influenced the Aliquat336 extraction mechanism when the pH was high by pushing the reaction equation to the left side (Equation 9).

From the results we achieved in our experiments and the results reported in other papers, it suggested that the extraction mechanism at a pH below acetic acid's pKa is more efficient than the one at a pH above acetic acid's pKa. In addition to the effect of sodium ion, the extraction mechanism at a pH above acetic acid's pKa could also be affected by the presence of chloride ion.

The effect of the initial concentration of acetic acid was also studied by the other researchers. Katikaneni and Cheryan's (2002) results with 50% Alamine 336 and 2:1 volumetric ratio of organic phase to aqueous phase indicated that the extraction efficiency increased from 76% to 80% when the initial acetic acid concentration was increased from 51.6 g/L to 116 g/L. However, on the opposite, Yang and his coworkers (1991) claimed that extraction efficiency of 50% Aliquat 336 would decrease when the initial acetic acid concentration was increased, especially when the aqueous acid concentration was lower than 10 g/L. They also mentioned that this initial acetic acid concentration effect diminished when it is more than 10 g/L. Based on the results of our preliminary experiments, there was no extraction efficiency difference between acetic acid initial concentration of 32 g/L and 48 g/L for 50% Aliquat 336 mixture.

As shown in Table 2.5, for 20% (v/v) recovered Aliquat 336 mixtures, there was no significant difference in extraction efficiency among pH 2.25-4.25. The extraction efficiency decreased as the initial pH of the spent medium increased above pH 4.25.

Table 2.5 The extraction efficiency of 20% used Aliquat 336 mixtures with different initial pH values^[a]

Initial pH value	Mean acetic acid concentration in aqueous phase after extraction, g/L, [SD]	Mean, extraction efficiency %, [SD] ^[b]	Mean distribution coefficient
2.25	27.34 [0.24]	19.60 a [0.72]	0.24
2.75	26.99 [0.04]	20.63 a [0.12]	0.26
3.25	27.32 [0.24]	19.63 a [0.71]	0.24
3.75	26.70 [0.07]	21.47 a [0.21]	0.27
4.25	27.19 [0.09]	20.04 a [0.27]	0.25
4.75	27.90 [0.37]	17.95 b [1.08]	0.22
5.25	29.86 [0.01]	12.22 c [0.04]	0.14
5.75	21.50 [0.39]	4.42 d [1.16]	0.05

^[a] Means followed by different letters are significantly different using Tukey's Studentized range test ($p < 0.05$)

^[b] $n = 2$

^[c] Initial acetic acid concentration in the aqueous phase was 34 g/L

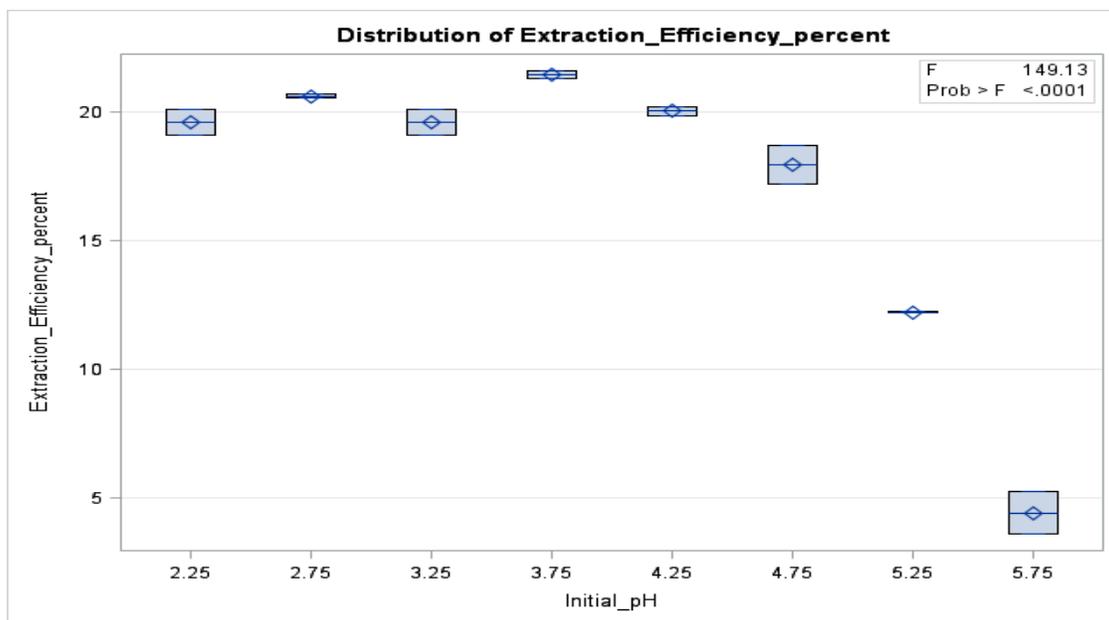


Fig 2.4 The extraction efficiency of 20% used Aliquat 336 mixtures with different initial pH values.

The highest extraction efficiency value achieved in this group was in the initial pH range 2.25 to 4.25.

As shown in Table 2.6, for 50% (v/v) recovered Aliquat 336 mixtures, there was no significant difference in extraction efficiency among pH 2.25-3.75. The extraction efficiency decreased as the initial pH of the spent medium increased pH above 3.75.

Table 2.6 The extraction efficiency of 50% used Aliquat 336 mixtures with different initial pH values^[a]

Initial pH value	Mean acetic acid concentration in aqueous phase after extraction, g/L, [SD]	Mean extraction efficiency, % [SD] ^[b]	Mean distribution coefficient
2.25	20.96 [0.72]	38.35 a [2.13]	0.62
2.75	20.54 [0.10]	39.60 a [0.29]	0.66
3.25	21.85 [0.61]	35.73 a [1.81]	0.56
3.75	22.49 [1.10]	33.86 a [3.23]	0.51
4.25	25.75 [0.48]	24.26 b [1.41]	0.32
4.75	26.80 [0.76]	21.18 b [2.25]	0.27
5.25	27.90 [0.34]	17.94 b [1.00]	0.22
5.75	29.55 [0.46]	13.09 c [1.35]	0.15

^[a] Means followed by different letters are significantly different using Tukey's Studentized range test ($p < 0.05$)

^[b] $n = 2$

^[c] Initial acetic acid concentration in the aqueous phase was 34 g/L

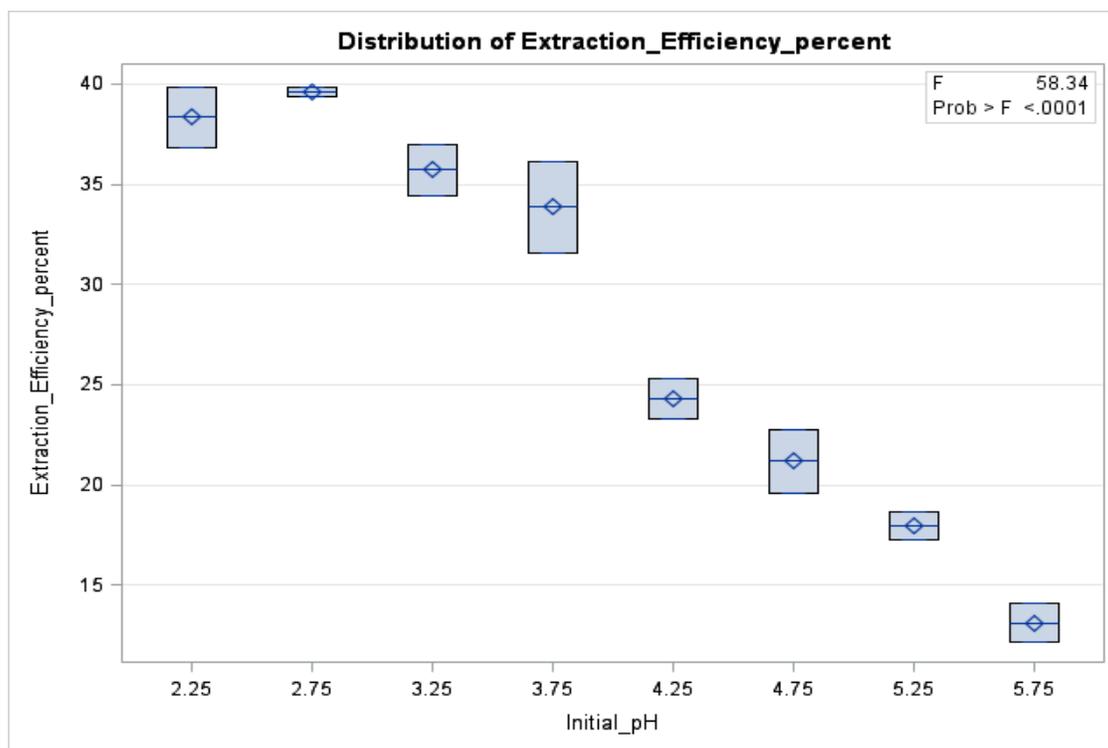


Figure 2.5 The extraction efficiency of 50% used Aliquat 336 mixtures with different initial pH values.

The highest extraction efficiency value achieved in this group was in the initial pH range 2.25 to 3.75.

2.3.2. Fresh Aliquat 336 mixtures vs. recovered Aliquat 336 mixtures

As the results shown above, there was no significant difference in extraction efficiency for Aliquat 336 mixtures to extract acetic acid from fermentation spent medium over pH range of 2.25 to 3.75 for all four treatments. So, a comparison between the fresh Aliquat 336 mixtures and recovered Aliquat 336 mixtures was performed in this pH range.

As shown in Table 2.7 and 2.8, there was a significant difference in extraction efficiency between the fresh and recovered Aliquat 336 mixtures for both the 20% (v/v) and 50% (v/v) treatments. The mean extraction efficiency of Fresh Aliquat 336 mixture is higher than the recovered Aliquat 336 mixture. The greatest extraction efficiency of 44% in these studies was attained with fresh Aliquat 336 mixture at 50% (v/v) concentration. The extraction efficiencies were approximately 17% lower for the recovered Aliquat 336 mixture.

Table 2.7 The extraction efficiency of Fresh and Recovered Aliquat 336 mixtures at 20% concentration^[a]

Aliquat 336 mixture condition	Mean extraction efficiency %, [SD] ^[b]	Mean distribution coefficient
Fresh	24.58 a [2.68]	0.33
Recovered	20.33 a [0.92]	0.26

^[a] Means followed by different letters are significantly different using Tukey's Studentized range test ($p < 0.05$)

^[b] $n = 8$

^[c] Initial acetic acid concentration in the aqueous phase was 34 g/L

Table 2.8 The extraction efficiency of Fresh and Recovered Aliquat 336 mixtures at 50% concentration^[a]

Aliquat 336 mixture condition	Mean extraction efficiency, % [SD] ^[b]	Mean distribution coefficient
Fresh	43.98 a [2.02]	0.79
Recovered	36.89 b [2.89]	0.58

^[a] Means followed by different letters are significantly different using Tukey's Studentized range test ($p < 0.05$)

^[b] $n = 8$

^[c] Initial acetic acid concentration in the aqueous phase was 34 g/L

Based on Juang and Wu's result (1999), the stripping efficiency of sodium carbonate for Aliquat 336 could not reach 100%. This means the extractability of recovered Aliquat 336 mixtures should not be as high as the fresh ones.

Other kinds of stripping solutions such as HCl, H₂SO₄, NaOH and water were also used to recover the Aliquat 336 mixture by Juang and Wu (1999). It turned out that the other stripping solutions had problems such as lower stripping efficiency and causing emulsions.

Although experiments to recover the Aliquat 336 mixture were reported, no research that test the extractability of recovered Aliquat 336 mixture was found. Our results indicated that recovered Aliquat 336 mixture was approximately 83% as efficiency as the fresh one.

2.3.3. The effect of Aliquat 336 mixture concentration.

The higher concentration of Aliquat 336 in the mixture solvent resulted in a higher extraction efficiency. The mean extraction efficiency for the 20% Aliquat 336 was 24.5%, while the mean extraction efficiency for the 50% Aliquat 336 was 44.0%.

Using simulated waste streams in chloromycetin production, which typically contains 15.4% acetic acid, 8.8% sodium acetate, and 4.7% (w/w) NaCl, Juang and Wu (1999) achieved a distribution coefficient of 0.3 (23% extraction efficiency) from the acetic acid extraction with 25% (v/v) concentration of Aliquat 336 at room temperature, which was not a very different result from ours for 20% (v/v) concentration of Aliquat 336. The reason why their extraction efficiency was a little bit lower than ours even when they had higher Aliquat 336 concentration could be that they had higher Na⁺ and Cl⁻ concentration in their simulated waste streams.

They also suggested that higher Aliquat 336 concentration could result in a higher extraction efficiency, because they had a distribution coefficient of only 0.1 (9.1% extraction efficiency) when the concentration of Aliquat 336 mixture was 10%, and even lower extraction efficiency for lower Aliquat 336 concentrations.

2.3.4. Effect of 10 g/L of NaCl in aqueous phase.

By comparing the extraction efficiency of Aliquat 336 for a sodium acetate solution and a bipolar electro-dialysis (ED) broth. Katikaneni and Cheryan (2002) suggested that the existence of sodium ions is responsible for the lower extraction efficiency by an unknown mechanism. Another disadvantage of sodium chloride is that the chloride ion could possibly affect the Aliquat 336 extraction mechanism at a relatively high pH as mentioned above.

However, in our preliminary experiments, comparing acetic acid extraction solutions prepared using distilled water and 10 g/L NaCl water, we found that NaCl might had acted as demulsifier during the Aliquat 336 mixture extraction process. Without the existence of NaCl, there was a wide emulsion layer between the aqueous phase and organic phase. The formation of this wide white emulsion layer may be because of the combination of Aliquat 336 and acetic acid molecule, which become a complex that is insoluble in both layers.

Although acetic acid was extracted out of the aqueous phase, this emulsion layer made it difficult for the separation of phases. There are other ways to avoid the emulsion

such as adding phase modifiers like alcohols or applying the developing dispersion-free hollow fiber extraction processes (Juang and Wu, 1999)

Yang and his coworkers (1991) also noticed the emulsion layer in the extraction process with Aliquat 336 concentration higher than 25% and 1:1 ratio of organic phase to aqueous phase when the equilibrium pH of the non-10 g/L of NaCl aqueous phase was lower than 3.0. However, in our experiments, we did not have the emulsion layer even when pH was 2.25.

2.3.5. pH changes during the process

After the extraction, the pH of the aqueous phase increased for most trials except for small pH decreases noted for 20% fresh Aliquat mixture treatments (Fig 2.6).

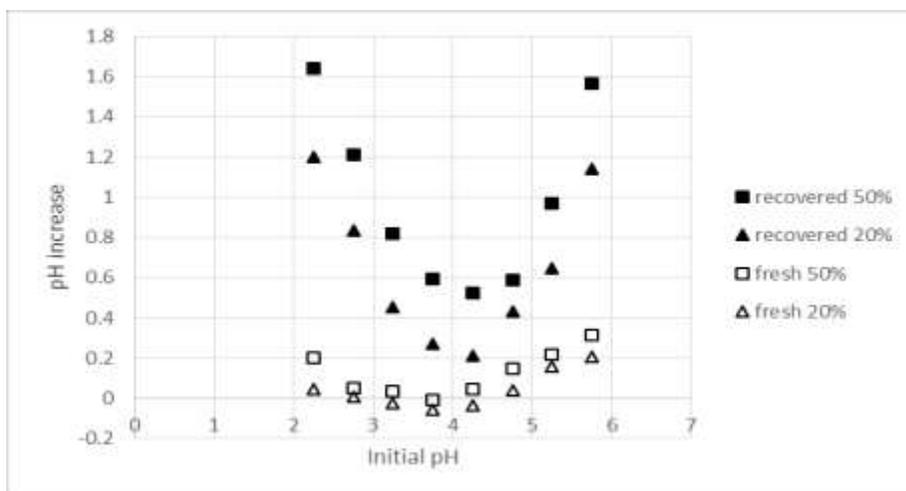


Figure 2.6 pH increase with different initial pH values (pH at the end of the extraction process minus the initial pH of the spent medium).

The pH change of the aqueous phase is the effect of the combination of two mechanisms. The first one is the association of Aliquat 336 molecule and the acetic acid molecule (equation 8). The first mechanism is decreasing the concentration of hydrogen

ion and increase the pH because it pushed equation 2 to the left side by taking the undissociated acetic acid molecules away from the aqueous phase. The second mechanism is doing the opposite, it is an anion exchange reaction (Equation 9) between chloride ion in the Aliquat 336 and acetate ion in the aqueous phase. So, in the aqueous phase a weaker acid replaced by equimolar of hydrochloric acid, a much stronger acid. As acetate ion was replaced by chloride ion, the ionization equation of acetic acid (equation 2) was pushed to the right side, and more hydrogen ion was released.

What the Fig 2.6 suggests is that for most cases, the pH of the spent medium increased after 10min of extraction process. It should be noted that the ranges of the pH increase during the extraction process were quite different from initial pH to initial pH. The pH increases are larger when the initial pH of the spent medium is at lowest and highest. The smallest pH increase occurred around the new pKa of acetic acid, W.P. Jencks and J. Regenstein (1975) found that the pKa of anionic acid could be decreased by an increasing ionic strength. This explained why the symmetric line of the U shape curve is around 4.25, which is below the normal pKa of acetic acid: 4.75.

What is more, the result also shows that the 50% Aliquat 336 mixtures caused larger pH variations than the 20% Aliquat 336 mixtures. The recovered Aliquat 336 mixtures caused larger pH variations than the fresh Aliquat 336 mixtures.

The reason why the recovered Aliquat 336 mixtures had much more pH influence on the spent medium than the fresh Aliquat 336 mixtures could be that the Na_2CO_3 stripping solution used for recovering of Aliquat 336 mixtures was dissolved into the Aliquat 336 mixture (Aliquat 336 and kerosene). When the recovered Aliquat 336

mixture was used for acetic acid extraction, the Na_2CO_3 again dissolved into the spent medium and increased the pH of it.

A small side trial was tested to prove this hypothesis. It was observed that the kerosene was able to increase the pH of the spent medium after it was mixed and then separated with a 200 g/L of Na_2CO_3 solution.

Similar pH increase phenomenon was also observed in Katikaneni and Cheryan (2002)'s result with Alamine 336. When they had 50% concentration of Alamine 336 and a 2:1 volumetric ratio of organic phase to aqueous phase, they observed a pH increase from 3.84 to 5.46 when the initial acetic acid concentration was 51.6 g/L, which is the closest initial acetic acid concentration in their experiments to ours.

The pH increase for recovered Aliquat 336 mixtures could explain the lower extraction efficiency for initial pH values below the pKa of 4.75, as addition of the recovered Aliquat 336 mixtures caused an increase in pH above pKa and resulting lower extraction efficiency (as shown in Fig 2.7).

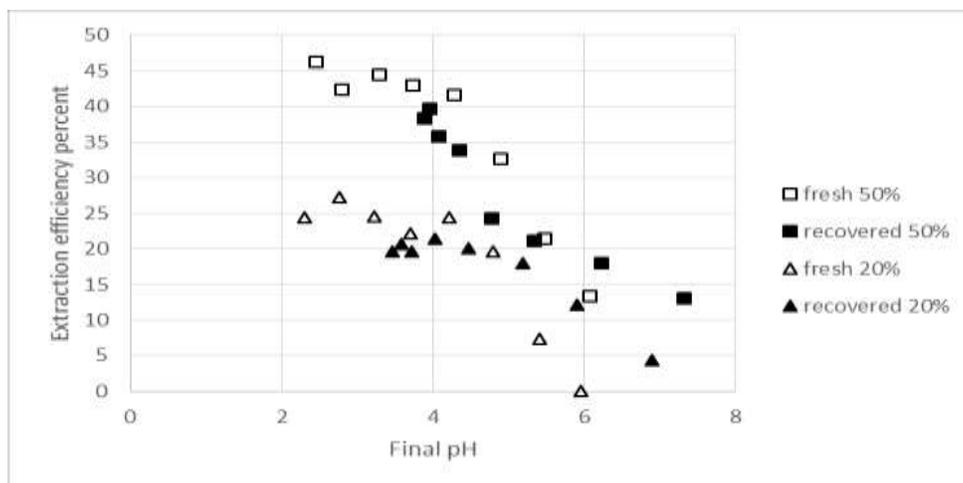


Fig 2.7 The extraction efficiency percent vs. the final pH of the spent medium

2.3.6. Salinity changes during the process

The salinity of the spent medium is greatly impacted by the volume of sodium hydroxide added to alter the initial pH. The higher the initial pH desired, the more sodium hydroxide was added, the higher the salinity (Fig 2.8).

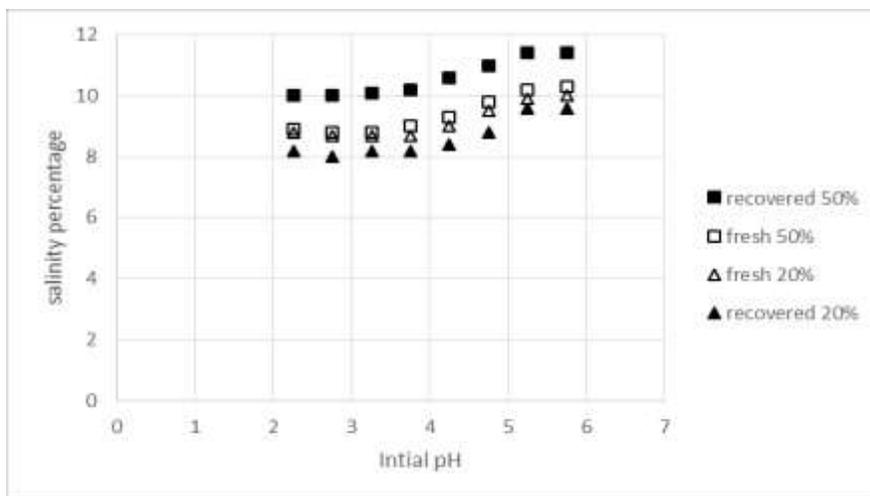


Fig 2.8 Salinity level with different initial pH values (Other media components were refracting light too).

What is more, the salinity remained the same during the whole extraction process. This could lead to the conclusion that the salinity has no obvious relation to the extraction efficiency for solution containing over 10 g/L of NaCl.

2.3.7. Acetate salt recovery.

The process to recover the acetic acid from the Aliquat 336 mixtures resulted in 1.28 g of white powdered solid precipitated from the aqueous phase of the back-extraction process when the stripping solution was Na_2CO_3 . There was 1.72 g when the

stripping solution was the slurry mixture of $\text{Ca}(\text{OH})_2$ and $\text{Mg}(\text{OH})_2$. Because both stripping solutions were overdosed, the white powdered solids would not be 100% acetate salt (sodium acetate salt and CMA respectively).

If each sample was 100% pure, for sample 1 (sodium acetate, MW= 82 g/L), 0.05 g sodium acetate dissolved in 10 mL of water should give 0.0625 mol/L of acetate concentration. For sample 2 (CMA, MW=300 g/L), 0.05 g CMA dissolved in 10 mL of water should give 0.0667 mol/L of acetate concentration.

The results from the HPLC analysis showed that the actual acetate concentration in sample 1 was 0.03 mol/L, the actual concentration in sample 2 was 0.05 mol/L. So, there is 49.2% (w/w) of sodium acetate in sample 1 salt production, and 75% (w/w) of CMA in the sample 2 salt production. Based on these results, the stripping efficiency of Na_2CO_3 in our experiments was 32%, the stripping efficiency of $\text{Ca}(\text{OH})_2$ and $\text{Mg}(\text{OH})_2$ was 69%. So, the entire extraction and back extraction process has optimum 31.74% efficiency (46% extraction efficiency and 69% stripping efficiency).

2.4. Conclusion

Aliquat 336 mixture was able to extract as high as 50% of the acetic acid out of the *Thermotoga Neapolitana* fermentation spent medium at the optimum conditions with kerosene as the diluent. Aliquat 336 was recoverable and reusable with little loss of extraction efficiency when the stripping solution was sodium carbonate or slurry solution of the mixture of calcium hydroxide and magnesium hydroxide. When the stripping solution was slurry solution of the mixture of calcium hydroxide and magnesium

hydroxide, 1.78 g of white powdered solid could be produced from 100 mL of *Thermotoga Neapolitana* fermentation spent medium and 100 mL of Aliquat 336 mixture. The purity of CMA could be as high as 75%. The optimum conditions for the extraction process in these experiments are initial pH of 2.25-4.25, 50% of fresh Aliquat 336 mixtures. The presence of sodium chloride in the fermentation spent medium could harm the extraction efficiency of the Aliquat 336 mixture, but also prevent the formation of emulsion.

References

Benemann J. Hydrogen biotechnology: Progress and prospects. *Nat Biotechnol.* 1996 SEP 1996: 14(9).

Brown, R. C. *Biorenewable Resources*. Iowa State Press, Blackwell Publishing Co., Iowa. 2003.

Bruce E. Logan, Sang-Eun Oh, In S. Kim, and Steven Van Ginkel. Biological Hydrogen Production Measured in Batch Anaerobic Respirometers. *Environ. Sci. Technol.*, 2002, 36 (11): 2530–2535.

Bryan, William L. Research approaches to reduce CMA cost, *Resources, Conservation and Recycling*, October 1992, 7 (1–3): 25–42.

Celina M. L. Santos, M. Rosinda C. Ismaela, M. Lurdes F. Gameiroa & Jorge M.R. Carvalho. Extraction of 4 - Hydroxybenzoic Acid with Aliquat336: Equilibrium Studies, Solvent Extraction and Ion Exchange, 2005, 23 (2): 213-230.

Claassen, P. A. M., Niel, van, E.W.J and Stams, A. J. M. Substrate and product inhibition of hydrogen production by the extreme thermophile, *Caldicellulosiruptor saccharolyticus*, *Biotechnology and Bioengineering*, 2003, 81, (3): 255–262.

Clayton Ormsby, *Highway Deicing: Comparing Salt and Calcium Magnesium Acetate*, Transportation Research Board, National Research Council, Washington, D.C.; 1991.
Da Rosa, Aldo V. *Fundamentals of renewable energy processes*, Academic Press, Aug 3, 2005.

Demiral, H, Yildirim, M Ercengiz. Recovery of acetic acid from waste streams by extractive distillation, *Water Science & Technology*, 2003, 47 (10): 183–188.

D. D. Dionysiou, M. Tsianou, G. D. Botsaris. Investigation of the Conditions for the Production of Calcium Magnesium Acetate (CMA) Road Deicer in an Extractive Crystallization Process, *Cryst. Res. Technol*, 2002, 35(9): 1035–1049.

D. T. Jones, D. R. Woods. Acetone-butanol fermentation revisited, *Microbiol Rev.* 1986 December; 50(4): 484–524.

Holger W. Jannasch, Robert Huber, Shimshon Belkin, Karl O. Stetter. *Thermotoga neapolitana* sp. nov. of the extremely thermophilic, eubacterial genus *Thermotoga*, *Archives of Microbiology*, May 1988, 150(1): 103-104.

Hubert Reisinger and C. Judson King. Extraction and sorption of acetic acid at pH above pKa to form calcium magnesium acetate, *Ind. Eng. Chem. Res.* 1995, 34 (3):845–852.

Hussy, I. Continuous dark fermentative hydrogen production by mesophilic microflora: Principles and progress, February 2007, 32(2): 172–184.

Jain, A. 2009. Biohydrogen production from cull peach medium by hyperthermophilic bacterium *Thermotoga neapolitana*. MS thesis ,Clemson,South Carolina : Clemson University, Department of Biosystems Engineering.

Janet A. Tamada and C. Judson King. Extraction of carboxylic acids with amine extractants. 2. Chemical interactions and interpretation of data, *Ind. Eng. Chem. Res.*, 1990, 29 (7): 1327–1333.

Janet A. Tamada and C. Judson King. Extraction of carboxylic acids with amine extractants. 3. Effect of temperature, water coextraction, and process considerations, *Ind. Eng. Chem. Res.*, 1990, 29 (7): 1333–1338.

Janet A. Tamada, A. Steven Kertes, and C. Judson King. Extraction of carboxylic acids with amine extractants. 1. Equilibria and law of mass action modeling, *Ind. Eng. Chem. Res.*, 1990, 29 (7): 1319–1326.

Jaquet, A., Quan, L., Marison, I. W. and von Stockar, U. Factors influencing the potential use of Aliquat 336 for the in situ extraction of carboxylic acids from cultures of *Pseudomonas putida*, *Journal of Biotechnology*, 19 February 1999, 68 (2-3): 185–196.

Juang, Ruey-Shin, Wu, Rong-Tzong. Extraction of acetate from simulated waste solutions in chloromycetin production, *Separation and Purification Technology*, 6 December 1999, 17(3): 225–233.

Kawamura, Kunio, Takahashi, Keiji and Okuwaki, Akitsugu. Influence of pH and Diluent on the Ion - Pair Solvent Extraction of Aromatic Carboxylic Acids Using Quaternary Ammonium Salts, *Separation Science and Technology*, 2006, 41(12).

Kertes, A. S, King, C. J. and Harvey W. Blanch, Extraction chemistry of fermentation product carboxylic acids, *Biotechnology and Bioengineering*, February 1986, 28(2): 269–282.

Klass, Donald L. Biomass for renewable energy, fuels, and chemicals, Academic Press, Jul 6, 1998.

Klass, Donald L. A critical assessment of renewable energy usage in the USA, *Energy Policy*, March 2003, 31(4): 353–367.

Levendis, Yiannis A., Zhu, Wenqi, Wise, Donald L. and Simons, Girard A. Effectiveness of calcium magnesium acetate as an SO_x sorbent in coal combustion, *AIChE Journal*, May 1993, 39(5): 761–773.

Levin, David B., Pitt, Lawrence and Love, Murray. Biohydrogen production: prospects and limitations to practical application, *International Journal of Hydrogen Energy*, February 2004, 29(2): 173–185.

Liu, Da w. Bio-hydrogen production by dark fermentation from organic wastes and residues, *Vester Kopi Virum*, May 2008.

Lloyd Wheatley, Yiannis A. Levendis, Paul Vouros. Exploratory study on the combustion and PAH emissions of selected municipal waste plastics, *Environ. Sci. Technol.*, 1993, 27 (13): 2885–2895.

Logan B, Oh S, Kim I, Van Ginkel S. Biological hydrogen production measured in batch anaerobic respirometers. *Environ Sci Technol* 2002 JUN 1;36(11):2530-5.

Matthew Albright. Changes in Water Quality in an Urban Stream Following the Use of Organically Derived Deicing Products, *Lake and Reservoir Management*, 2005, 21(1).

Momirlan M, Veziroglu T. Current status of hydrogen energy, *Renewable and Sustainable Energy Reviews*, 2002, 6(1–2): 141–179.

Nimmo, W., Patsias, A. A., Hampartsoumian, E., Gibbs, B. M. and Williams, P. T. Simultaneous reduction of NO_x and SO₂ emissions from coal combustion by calcium magnesium acetate, *Fuel*, January 2004, 83(2): 149–155.

Palasantzas, Ioannis A., Wise, Donald L. Preliminary economic analysis for production of calcium magnesium acetate from organic residues, *Resources, Conservation and Recycling*, 30 June 1994, 11(1–4):225–243.

Paul C, Yanrui Wu, Energy consumption in China: past trends and future directions, *Energy Economics*, January 2005, 27(1): 195–208.

Pursell, Mark R., Alcina Mendes-Tatsis, M. and Stuckey, David C. Co-Extraction during Reactive Extraction of Phenylalanine using Aliquat 336: Interfacial Mass Transfer, *Biotechnology Progress*, 2003, 19(2): 469–476.

Robidoux, Pierre Y., Delisle, Claude E. Ecotoxicological Evaluation of Three Deicers (NaCl, NaFo, CMA)—Effect on Terrestrial Organisms, *Ecotoxicology and Environmental Safety*, February 2001, 48(2): 128–139.

Sai P. R. Katikaneni and Munir Cheryan. Purification of Fermentation-Derived Acetic Acid by Liquid–Liquid Extraction and Esterification, *Ind. Eng. Chem. Res.*, 2002, 41 (11): 2745–2752.

Sarangi, K., Padhan, E., Sarma, P. V. R. B., Park, K. H. and Das, R. P. Removal/recovery of hydrochloric acid using Alamine 336, Aliquat 336, TBP and Cyanex 923, *Hydrometallurgy*, November 2006, 84(3–4): 125–129.

Schroder C, Selig M, Schonheit P. Glucose fermentation to acetate, CO₂ and H₂ in the anaerobic hyperthermophilic eubacterium *Thermotoga-maritima* - involvement of the embden-meyerhof pathway, *Archives of Microbiology*, June 1994, 161(6): 460-470.

Shang Tian Yang, Scott A. White, and Sheng Tsiung Hsu. Extraction of carboxylic acids with tertiary and quaternary amines: effect of pH, *Ind. Eng. Chem. Res.*, 1991, 30 (6): 1335–1342.

Solomon S, Plattner G, Knutti R, Friedlingstein P. Irreversible climate change due to carbon dioxide emissions, *PNAS*, February 10, 2009, 106(6): 1704-1709.

Steven Van Ginkel and Bruce E. Logan. Inhibition of Biohydrogen Production by Undissociated Acetic and Butyric Acids, *Environ. Sci. Technol.*, 2005, 39 (23): 9351–9356.

Tim Eggeman, Dan V. Recovery of organic acids from fermentation broths, *Twenty-Sixth Symposium on Biotechnology for Fuels and Chemicals ABAB Symposium*, 2005: 605-618.

Trantolo, Debra J., Gresser, Joseph D., Augenstein, Don C. and Wise, Donald L. The feasibility of a residue biomass bioconversion process to prepare calcium magnesium acetate deicing salt, *Resources, Conservation and Recycling*, September 1990, 4(3): 215–232.

USEIA: Primary Energy Consumption by Source, November 2012: 2012.

Van Ginkel, Steven, Oh, Sang-Eun and Logan, Bruce E. Hydrogen Production Using Anaerobic Wastewater Treatment Processes, Proceedings of the Water Environment Federation, WEFTEC 2002: Session 11 through Session 20: 842-848(7).

Wang, C.C., Chang, C.W., Chu, C.P., Lee, D.J., Chang, B.V., Liao, C.S., Tay, J.H. Using filtrate of waste biosolids to effectively produce bio-hydrogen by anaerobic fermentation, Water Research, June 2003, 37(11): 2789–2793.

Weier, Anthony J., Glatz, Bonita A. and Glatz, Charles E. Recovery of Propionic and Acetic Acids from Fermentation Broth by Electrodialysis, Biotechnology Progress, November/December 1992, 8(6): 479–485.

W.P. Jencks, J. Regenstein, Handbook of Biochemistry and Molecular Biology: Physical and Chemical Data, 1975.

Yang, Shang-Tian, Zhu, Hui, Lewis, Vivian P. and Tang, I-Ching. Calcium magnesium acetate (CMA) production from whey permeate: process and economic analysis, Resources, Conservation and Recycling, October 1992, 7(1–3): 181–200.

Yeom, Choong-Kyun, Lee, Kew-Ho. Pervaporation separation of water-acetic acid mixtures through poly (vinyl alcohol) membranes cross linked with glutaraldehyde, Journal of Membrane Science, 24 January 1996, 109(2): 257–265.

Yu X, Drapcho C. Hydrogen production by the hyperthermophilic bacterium *Thermotoga neapolitana* using agricultural-based carbon and nitrogen sources. Biological Engineering Transactions, 2011, 4:101-112.

Yun Suk Huh, Yeon Ki Hong, Won Hi Hong, Ho Nam Chang. Selective extraction of acetic acid from the fermentation broth produced by *Mannheimia succinici* producens, Biotechnology Letters, October 2004, 26(20): 1581-1584.

CHAPTER THREE

CONCLUSION AND IDEAS FOR FURTHER RESEARCH

In this experiment, Aliquat 336 mixture was proven to be able to extract as high as 50% of the acetic acid out of the *Thermotoga Neapolitana* fermentation spent medium at the optimum conditions with kerosene as the diluent. The extraction efficiency of Aliquat 336 decreases with the increase of the initial pH in the fermentation spent medium.

The concentration of Aliquat 336 in the organic extractant also plays an important role in the extraction efficiency, the 50% of Aliquat 336 extracted much more acetic acid from the fermentation spent medium than the 20% Aliquat 336.

Aliquat 336 was recoverable and reusable with little loss of extraction efficiency when the stripping solution was sodium carbonate or slurry solution of the mixture of calcium hydroxide and magnesium hydroxide. The extraction efficiency of the recovered Aliquat 336 is inevitably lower than the fresh Aliquat 336 due to the imperfection of back-extraction processes, plus the back-extraction efficiency of sodium carbonate is not able to reach 100%.

There was an increase of pH in the fermentation spent medium feed solution from time zero to time 10 min in most cases during the extraction process. The salinity of the fermentation spent medium feed solution remained the same during the whole extraction process.

When the stripping solution was slurry solution of the mixture of calcium hydroxide and magnesium hydroxide, 1.78 g of white powdered solid could be produced from 100 mL of *Thermotoga Neapolitana* fermentation spent medium feed solution and 100 mL of organic extractant. The purity of CMA could be as high as 75%.

The optimum conditions for the extraction process in these experiments are initial pH of 2.25-4.25, 50% of fresh Aliquat 336 in the organic extractant.

The present of sodium chloride in the fermentation spent medium could harm the extraction efficiency of the Aliquat 336, but also prevent the formation of emulsion.

The result that the Aliquat 336 is recoverable and reusable with little extraction efficiency reduction could potentially lower the cost of this process and made the industrialization of this process more reachable, because the most costly part of the whole process was the purchase of the organic extractant. To further improve the efficiency of the process and low the cost, a lot of parameters could be further studied with appropriate experimental support.

Although the present of sodium chloride was able to prevent the formation of emulsion, it greatly affected the extraction efficiency of Aliquat 336, and the prevention of emulsion could also be achieved by adding other kinds of phase modifiers like alcohols. It could potentially increase the extraction efficiency by removing the chloride ion and sodium ion by cheap and well developed membrane and add alcohols to prevent emulsion.

Also the effect of Aliquat 336 on *Thermotoga neapolitana* remains unknown. It is possible to make the extraction process in situ if Aliquat 336 would not affect the growth

of *Thermotoga neapolitana* and the production of hydrogen gas. In A. Jaquet and coworkers' researches, the organic phase of the mixture of Aliquat 336 0.3M/octan-1-ol showed no inhibitory effect to the *Ps. Putida* microorganism. However, Aliquat 336 has the potential to influence both the pH and the concentration of counter ions X^- exist in the medium because of its role as anion exchanger.

Based on the results of our preliminary experiments, the extraction process seems to be finished in 10min. Because the extraction efficiency reached the highest point and the pH of the fermentation spent medium feed solution stopped changing. With appropriate experimental support and design, the extraction process within the first 10min could be further studied.

APPENDIX A
HPLC ANALYSIS DATA

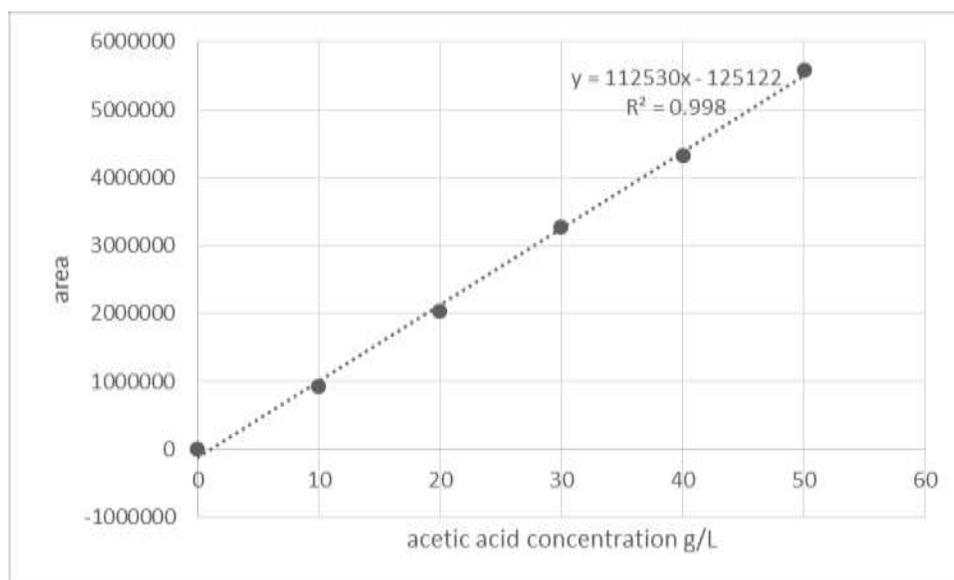


Figure A-1: The Acetic acid standard 0-50 g/L.

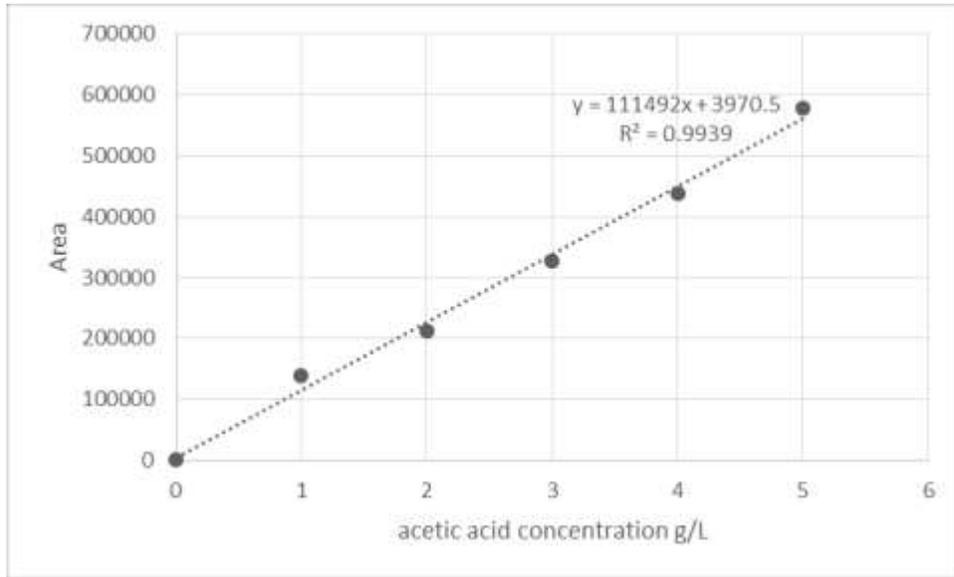


Figure A-2: The Acetic acid standard 0-5 g/L.

Table A-1: HPLC data of 20% fresh Aliquat 336

Initial pH	HPLC peak area	Acetic acid concentration in aqueous phase g/L	EE %
2.25	2853981	25.06894271	26.26782
2.25	3004336	26.29669127	22.65679
2.75	2690128	23.730974	30.20302
2.75	2939626	25.76829109	24.21091
3.25	2923249	25.63456199	24.60423
3.25	2931244	25.69984649	24.41222
3.75	2857321	26.50353683	22.04842
3.75	2851236	26.44946237	22.20746
4.25	2777888	25.79765396	24.12455
4.25	2759878	25.63760775	24.59527
4.75	3011390	27.87267395	18.02155
4.75	2891720	26.80922421	21.14934
5.25	3398941	31.31665334	7.892196
5.25	3433786	31.6263041	6.981459
5.75	3726190	34	0
5.75	3763033	34	0

Table A-2: HPLC data of 50% fresh Aliquat 336

Intial pH	HPLC peak area	Acetic acid concentration in aquous phase g/L	EE %
2.25	1937502	17.58528221	48.27858
2.25	2103291	18.93905964	44.29688
2.75	2209527	19.80654723	41.74545
2.75	2164146	19.43598119	42.83535
3.25	2120932	19.08311014	43.87321
3.25	2073473	18.69557584	45.01301
3.75	1983046	19.29449319	43.25149
3.75	2008632	19.53435895	42.546
4.25	2059032	20.00685304	41.15631
4.25	2037571	19.80565868	41.74806
4.75	2404274	23.24345633	31.63689
4.75	2327773	22.52626842	33.74627
5.25	2830217	27.23662204	19.89229
5.25	2715374	26.15998238	23.05888
5.75	3171223	30.43351333	10.48967
5.75	2959317	28.44691941	16.33259

Table A-3: HPLC data of 20% recovered Aliquat 336

Intial pH	HPLC peak area	Acetic acid concentration in aquous phase g/L	EE %
2.25	3029777	27.16384209	20.10635
2.25	3070817	27.50833102	19.09314
2.75	3005208	26.9576104	20.71291
2.75	3011841	27.01328767	20.54915
3.25	3069196	27.49472438	19.13316
3.25	3028624	27.15416383	20.13481
3.75	2980619	26.75121083	21.31997
3.75	2968330	26.64805721	21.62336
4.25	3024681	27.12106637	20.23216
4.25	3039925	27.2490242	19.85581
4.75	3147856	28.15499484	17.19119
4.75	3086127	27.63684286	18.71517
5.25	3350312	29.85440642	12.19292
5.25	3348254	29.83713161	12.24373
5.75	3698455	32.77671174	3.597907
5.75	3632055	32.21935148	5.237202

Table A-4: HPLC data of 50% recovered Aliquat 336

Intial pH	HPLC peak area	Acetic acid concentration in aquous phase g/L	EE %
2.25	2434715	21.47172211	36.84788
2.25	2320632	20.44985758	39.85336
2.75	2338024	20.60564125	39.39517
2.75	2322404	20.46572974	39.80668
3.25	2428805	21.41878505	37.00357
3.25	2525793	22.2875262	34.44845
3.75	2634821	23.26411207	31.57614
3.75	2461327	21.71009118	36.14679
4.25	2874406	25.41012343	25.26434
4.25	2950343	26.09030652	23.2638
4.75	2969223	26.2594185	22.76642
4.75	3089965	27.34092904	19.5855
5.25	3179181	28.14005482	17.23513
5.25	3125432	27.65861414	18.65113
5.75	3372563	29.87221655	12.14054
5.75	3300164	29.22372405	14.04787

Table A-5: HPLC data of acetate salts

sample	HPLC peak area	acetate concentraion g/L
CMA solution	327753	2.904087289
NaAc solution	211085	1.857662433

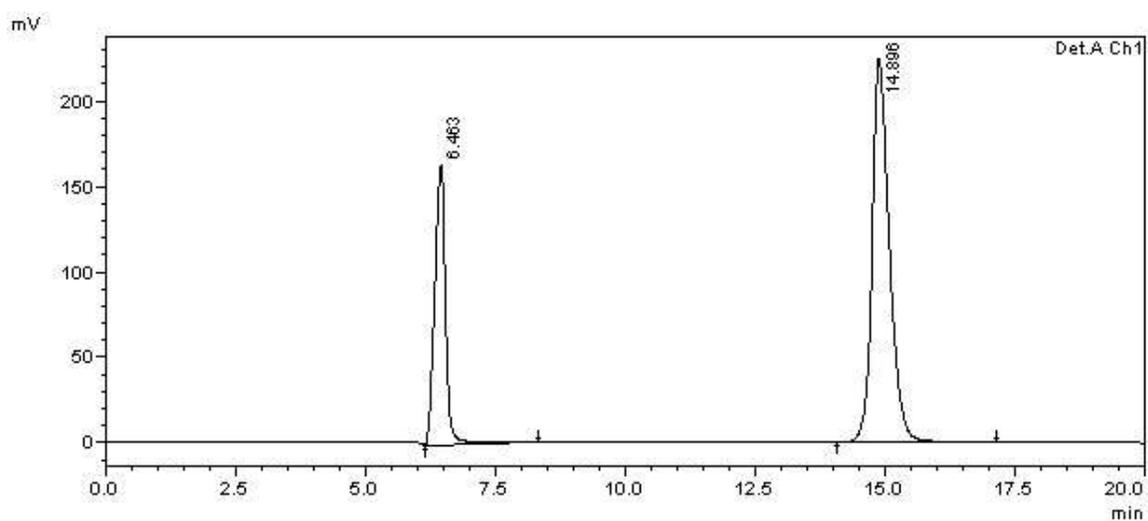


Figure A-3: HPLC peaks of the 50 g/L acetic acid standard.

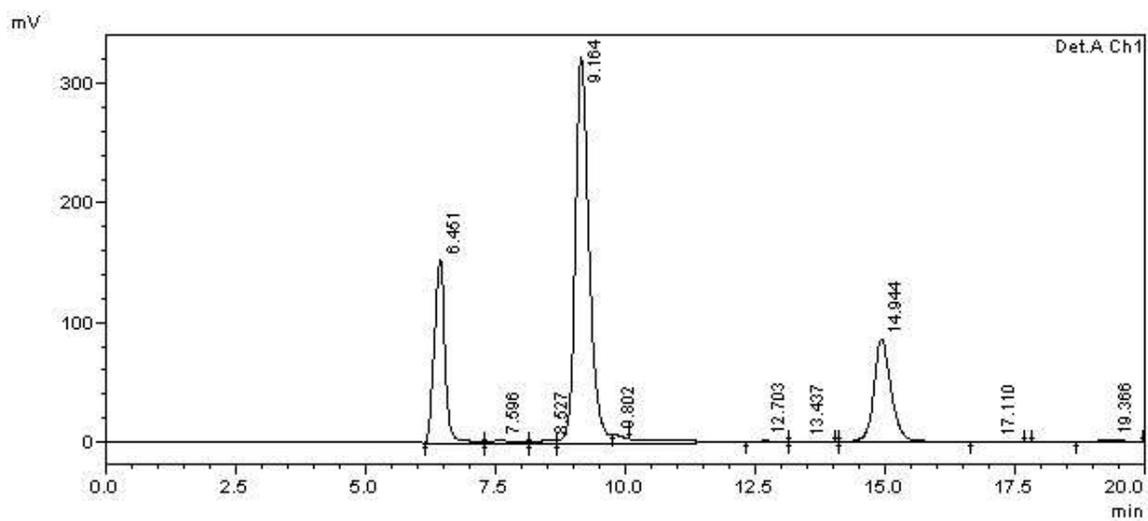


Figure A-4: HPLC peaks of post extraction aqueous phase.

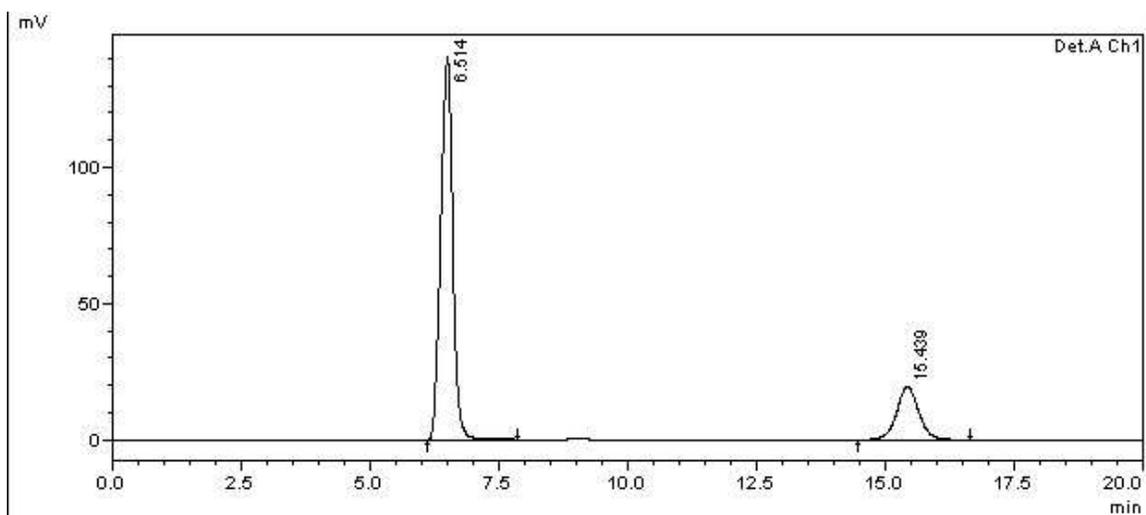


Figure A-5: HPLC peaks of the 5 g/L acetic acid standard.

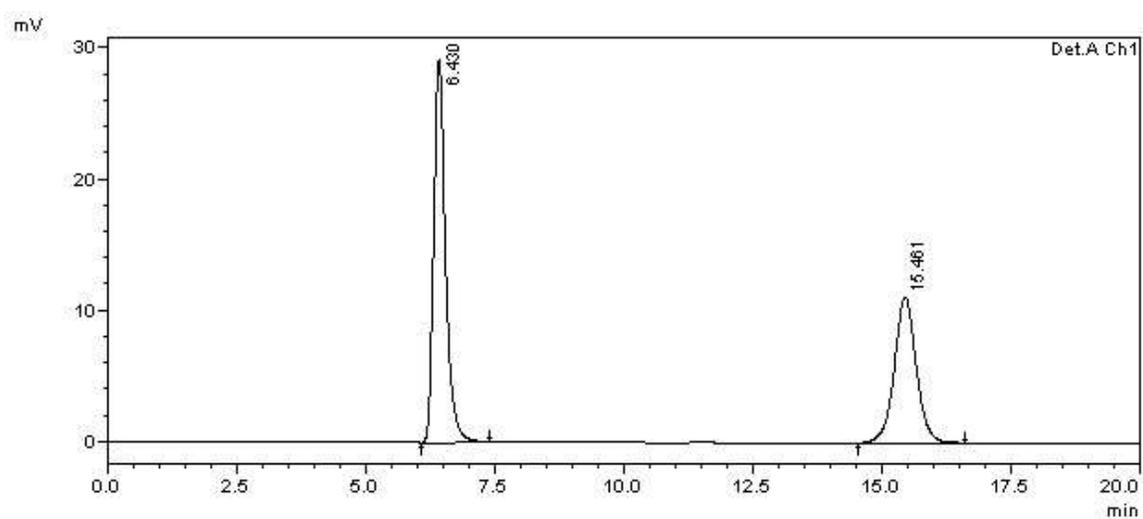


Figure A-6: HPLC peaks of the CMA solution

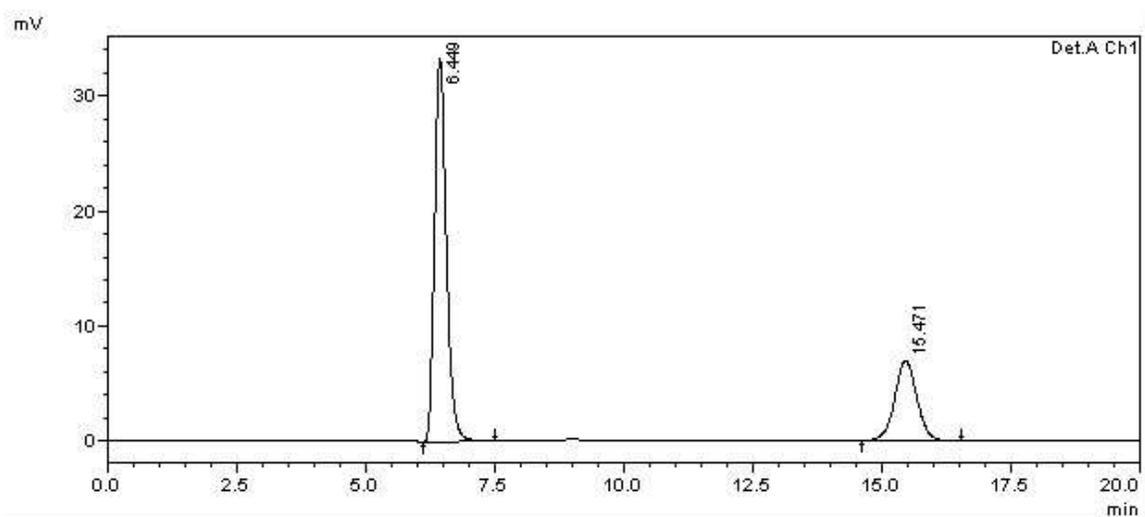


Figure A-7: HPLC peaks of the sodium acetate solution