Improvements in Biobutanol Production: Separation and Recovery by Adsorption

Niloofar Abdehagh

Thesis submitted to the
Faculty of Graduate and Postdoctoral Studies
In partial fulfillment of the requirements
for the Doctorate in Philosophy degree in Chemical Engineering

Department of Chemical and Biological Engineering
Faculty of Engineering
University of Ottawa

Abstract

Due to environmental challenges, depleting oil resources, rising cost of oil and instability in oil-producing countries, biofuel production has attracted a lot of attention in recent decades. Biobutanol is one of the biofuels showing the most potential as an alternative for partly replacing petroleum-based fuels. Both researchers and industrialists are currently working at developing an energy-effective process to produce biobutanol at a large scale. Acetone-butanol-ethanol (ABE) fermentation is the biological process of biobutanol production and *Clostridia* are the most common bacteria used to produce biobutanol. However, there are several challenges in the butanol bioproduction process that should be addressed to make this process economically viable. The main challenge in the biobutanol production process is the low concentration of butanol in the ABE fermentation broth. It is therefore important to develop an efficient separation method. Several separation methods such as distillation, liquid-liquid extraction (LLE), pervaporation, gas stripping and adsorption have been considered to recover butanol from dilute solutions and ABE fermentation broths.

Adsorption is considered as one of the most promising methods due to its high performance and energy efficiency for butanol separation. In this study, the focus was on developing an efficient separation method for butanol recovery from dilute model solution and fermentation broth using adsorption. A comprehensive adsorbent screening was first carried out to identify the best commercially available adsorbent among a series of potentially promising adsorbents. Activated carbon (AC) F-400 was selected for further experimentation since it showed high adsorption capacity and adsorption rate in addition to high selectivity toward butanol. AC F-400 was then tested extensively in packed adsorption column experiments for binary and ABE model solutions and fermentation broths to investigate the competitive adsorption between butanol and other components present in ABE broths. The results showed that the butanol adsorption capacity was not affected by the presence of ethanol, glucose and xylose while the presence of acetone led to a slight decrease in adsorption capacity at low butanol concentrations. On the other hand, the presence of acids (acetic acid and butyric acid) in the ABE broth showed a significant effect on the butanol adsorption capacity over a wide

range of butanol concentration and this effect was more pronounced for butyric acid. At the end, different competitive adsorption isotherm models were also studied to appropriately represent the behaviour of the competitive adsorption.

Desorption of butanol was subsequently investigated to evaluate both the desorption capacity of butanol and the capability of the adsorbent particles to be used for multiple adsorption-desorption cycles. The results of this set of experiments showed that AC F-400 can retain its initial adsorption capacity after 6 adsorption/desorption cycles. The recovery of butanol from butanol-water (1.5 wt%) binary and ABE model solutions was 84 and 80% with butanol adsorption capacity of 302 and 171 mg/g, respectively.

The combination of adsorption and gas stripping techniques was also studied to investigate the performance of CO_2 gas stripping of solvents from the model solutions and fermentation broths followed by adsorption. The results showed that the butanol adsorption capacity of the overall system for binary solutions (260 mg/g for a binary butanol-water solution of 15 g/L with vapour phase concentration of 5.8 mg/L), ABE model solutions (192 mg/g for a corresponding vapour concentration of 5.2 mg/L) and ABE fermentation broths (247 mg/g for a corresponding vapour phase concentration of 2.5 mg/L) was higher than what has been published in the literature.

Finally, a model was developed and adequately validated the experimental data to predict the behaviour of the ABE compounds in a packed bed adsorption column for butanol separation from dilute solutions.

Résumé

En raison de défis environnementaux, de l'épuisement des ressources pétrolières, la grande variation des prix du pétrole et l'instabilité dans les pays producteurs de pétrole, la production de biocarburants a attiré beaucoup d'attention au cours des dernières décennies. Le biobutanol est un des biocarburants présentant le plus de potentiel comme une alternative pour remplacer en partie les combustibles liquides à base de pétrole. Les chercheurs et les industriels travaillent actuellement à l'élaboration d'un procédé efficace pour produire le biobutanol à grande échelle. La fermentation dénommée ABE (Acétone-butanol-éthanol) est le procédé biologique le plus commun pour la production de biobutanol et les bactéries de type *Clostridia* sont les plus utilisées pour produire le biobutanol. Cependant, il y a plusieurs défis rencontrés lors de la bioproduction de butanol qui doivent être résolus afin de rendre ce procédé viable économiquement. Le principal défi du procédé de production de biobutanol est la faible concentration de butanol dans le bouillon de fermentation ABE. Il est donc important de développer une méthode de séparation efficace. Plusieurs méthodes de séparation telles la distillation, l'extraction liquide-liquide (LLE), la pervaporation, l'extraction gazeuse et l'adsorption ont été envisagées pour récupérer le butanol à partir de solutions diluées et de bouillons de fermentation ABE.

L'adsorption est considérée comme l'une des méthodes les plus prometteuses en raison de ses performances et de son efficacité énergétique élevée pour la séparation du butanol. Dans cette étude, l'accent a été mis sur l'élaboration d'un procédé de séparation efficace pour la récupération du butanol à partir de solutions modèles et de bouillons de fermentation par adsorption. Une recherche a été réalisée afin d'identifier le meilleur adsorbant disponible commerciallement parmi une série d'adsorbants potentiellement prometteurs. Le charbon activé (CA) F-400 a été choisi pour une expérimentation plus poussée car il a montré une capacité d'adsorption élevée et un haut taux d'adsorption en plus d'avoir une bonne sélectivité pour le butanol. Le CA F-400 a ensuite été testé dans des colonnes d'adsorption pour des solutions modèles eau-butanol et des bouillons de fermentation ABE pour étudier l'adsorption compétitive entre le butanol et les autres composantes présentes dans les bouillons de fermentation. Les résultats ont montré que la

capacité d'adsorption butanol n'a pas été affectée par la présence de l'éthanol, de glucose et de xylose tandis que la présence de l'acétone conduit à une légère diminution de la capacité d'adsorption pour de faibles concentrations de butanol. D'autre part, la présence d'acides organiques (acide acétique et acide butyrique) dans le bouillon de fermentation a montré un effet significatif sur la capacité d'adsorber le butanol sur une large gamme de concentration en butanol et cet effet était plus prononcé par la présence de l'acide butyrique. Par la suite, différents modèles d'adsorption isotherme ont également été étudiés pour trouver le modèle qui représente le mieux le phénomène d'adsorption compétitive.

La désorption du butanol a ensuite été étudiée pour évaluer à la fois la capacité de désorption du butanol et le potential à utiliser les particules d'adsorbant pour de multiples cycles d'adsorption-désorption. Les résultats de cette série d'expériences ont montré que le CA F-400 peut conserver sa capacité d'adsorption initiale même après six cycles d'adsorption/désorption. La récupération du butanol à partir d'une solution butanol-eau (1.5% en poids) et de solutions ABE était de 84 et 80% avec une capacité d'adsorption de butanol de 302 et 171 mg/g, respectivement.

La combinaison des procédés d'adsorption et d'extraction gazeuse a également été étudiée pour examiner le potentiel de l'extraction gazeuse des solvants des solutions modèles et des bouillons de fermentation en utlisant le CO_2, suivi par l'adsorption. Les résultats ont montré que la capacité d'adsorption du butanol des solutions binaires (260 mg/g pour une solution butanol-eau binaire de 15 g/L donnant une concentration en phase vapeur de 5.8 mg/L), des solutions modèles ABE (192 mg/g pour une concentration en phase vapeur de 5.2 mg/L) et des bouillons de fermentation ABE (247 mg/g pour une concentration en phase vapeur correspondant de 2.5 mg/L) était plus élevée que celle publiée dans la littérature.

Enfin, un modèle a été développé et validé de manière adéquate pour représenter le comportement dynamique des composés d'ABE dans une colonne d'adsorption à lit fixe pour la séparation du butanol à partir de solutions diluées.

Statement of contributions of collaborators

I hereby declare that I am the sole author of this thesis**.** I was responsible for the experimental design, experimental procedures and all data analysis throughout the project. The project guidance was provided by my supervisors: Dr. Handan Tezel and Dr. Jules Thibault.

Priya Gurnani worked under my guidance on her co-op project on the desorption process, performing some parts of the experiments. Priya is the second author of the paper contained in Chapter 5 and entitled "Adsorptive separation and recovery of biobutanol from ABE model solutions".

Bo Dai was another student working under my guidance on her graduate (MEng) project. Bo assisted in performing the experiments of gas stripping-adsorption system experiments. Bo is listed as the second author of the paper contained in chapter 6 entitled "Biobutanol separation from ABE model solutions and fermentation broths using a combined adsorption-gas stripping process".

Mehran Bagheri is a colleague who assisted me in modeling of HPLC chromatograms peak deconvolution and also the modeling of the adsorption in a packed bed. Mehran developed a program to deconvolute the overlapped peaks of ABE components in the HPLC chromatograms; this program was used to detect the multicomponent solutions compositions. He also assisted in developing the model for packed bed adsorption and multicomponent adsorption isotherm.

The model used for peak deconvolution is presented in chapter 4 entitled "Simulation and validation of ethanol removal from water in an adsorption packed bed: Isotherm and mass transfer parameter determination in batch studies" in which Mehran is listed as the second author. The model for adsorption process in a packed bed column for multicomponent systems is presented in chapter 8 entitled "Adsorption packed bed simulation for butanol-water and ABE model solutions using multicomponent Freundlich isotherm" and Mehran is listed as the second author.

Acknowledgments

First and foremost I want to thank my supervisors Dr. Handan Tezel and Dr. Jules Thibault. Without their continued support over the years, this thesis would not have been possible. I was fortunate to have two supervisors to offer critical analysis of my work and moral support in my life.

I would also like to extend a thank you to the technical support team at the University of Ottawa: Louis Tremblay, Franco Ziroldo and Gérard Nina who were always there to solve any unforeseen technical issues.

Thanks to my lab mates Priya Gurnani, Bo Dai, Hoda Azimi, Muhammad Tawalbeh and Burcu Ugur for their assistance and companionship.

I gratefully acknowledge Natural Sciences and Engineering Research Council (NSREC) and Ontario Graduate Scholarship (OGS), for providing me financial support to carryout my research.

I owe my deepest gratitude towards my family especially my husband, Mehran, for his eternal support and understanding of my goals and aspirations. His infallible love and support has always been my strength. His patience and sacrifice will remain my inspiration throughout my life. Without his help, I would not have been able to complete much of what I have done and become who I am.

Contents

List of Tables

List of Figures

SECTION – I: Introduction

Chapter I

Introduction

1 Introduction

This study is conducted in the field of renewable energy, and in particular, the separation and recovery of biobutanol as one of the key unit operations in biobutanol production processes. There are environmental, political and economical factors shaping the industry and stimulating research in this developing field. Depleting oil supply, rising oil price, political instability in oil producing countries and environmental challenges are some of these factors that are motivating both related industry and researchers to develop the production of biofuels from sustainable resources (Harvey and Meylemans, 2011; Antoni et al. 2007; Dellomonaco et al., 2010; Dürre, 2007; Ezeji et al, 2004).

Biorefinery and biofuel production first appeared in the media when United States President, Richard Nixon, announced the "Project Independence" due to the Arab oil embargo in 1973. The objective of the project was to reach energy self-sufficiency for United States of America by 1980. Since US is considered as the world's largest energy consumer, many other countries surfed on that wave and funded research projects to develop a biorefinery industry. Bioethanol industrial fermentation was first considered as biofuel production in 1970s using corn as the major feedstock for a few decades. The biofuel industry attracted increasing interest in 1979 and 1980s, during the Iranian revolutionary and Iraq invasion of Iran that caused significant rise in Iranian oil price and reduction in oil production in this country (Soloman et al., 2007; Dhamole et al, 2012; Ezeji et al, 2003 and 2007).

Although ethanol production helped to reduce the Greenhouse gas (GHG) emissions anywhere from 50 to 100% compared to conventional gasoline, the food versus fuel controversy arose and the first generation bioethanol production was subjected to new obstacles regarding this significant drawback of the process (Sims et al. 2010). Since 23% of the worldwide GHG emissions are attributed to transportation fuels, the efforts to produce ethanol from non-food resources led to developing second-generation processes

for bioethanol production using lignocellulosic non-food materials as the feedstock for the process (Chavez-Rodriguez and Nebra, 2010).

In second-generation biofuel production processes, instead of sugar and starch crops, lignocellulosic biomass is used. Lignocellulosic materials are not considered as food source and could also grow in a variety of climates, which favors its worldwide availability. In addition, agricultural and forestry residues can be used as a biomass source. Moreover, the cost of lignocellulosic materials is generally lower compared to corn or sugarcane (Qureshi et al., 2008; Harvey and Meylemans, 2011; Dürre, 2007).

Over several years of research and industrial efforts in bioethanol production, researchers' attention was drawn to biobutanol as a more interesting potential alternative for petroleum-based fuels. Biobutanol production was witnessed by Pasteur for the first time while performing an anaerobic fermentation in 1861. The interest in biobutanol production increased in early 20th century due to its contribution in solving the material shortage in the world. Biobutanol was used initially in the synthetic rubber industry.

Acetone-butanol-ethanol (ABE) fermentation is the anaerobic fermentation process used to produce biobutanol. Between 1912 and 1914, the first microorganism screening was carried out by Chaim Weizmann to achieve a better understanding of the fermentation process. He isolated *Clostridium acetobutylicum*, which is still considered as the most common bacteria to produce butanol in a fermentation process with higher yield than ethanol and acetone (Fouad and Feng, 2008; Shapovalov et al., 2008; http://www.biobutanol.com/Biobutanol-History.html).

Another application for ABE fermentation process was in the First World War where the British Army was in need of producing smokeless gunpowder in a large quantity to avoid importing it and acetone was a required solvent in this process. Weizmann was asked by the British Army to design a large-scale process to produce acetone via ABE fermentation. When United States joined the war, Great Britain and US built two acetone production facilities in Midwest United States, which ran for less than a year.

After the world war ended, large amount of butanol was produced and stored as the by-product of these facilities. E.I du Pont de Nemours Co. used this excess butanol as

a solvent in nitrocellulose lacquer, which is a quick-drying automobile finish. Finally, the two facilities were purchased and reopened this time for butanol production.

In 1936, Weizmann's patent expired and many plants were designed and implemented to produce butanol via fermentation using different kinds of microorganisms and feedstock. But the second spike in biobutanol production happened during the Second World War and this time more countries such as Australia, South Africa and Japan were involved in producing biobutanol too. By the end of the war, the demand for acetone and butanol was less and butanol production plants were gradually shut down by 1960s. One additional reason for the decline in biobutanol production was the wave of petrochemical production of acetone and butanol in an easier and cheaper way (Cao et al., 2015; http://www.biobutanol.com/Biobutanol-History.html).

In recent decades, the increase and oscillating price of petroleum fuels and their negative impact on the environment led to the revival of the biobutanol production industry.

Butanol has some enviable advantages over ethanol and even though bioethanol is currently produced worldwide in many industrial production plants, biobutanol production is considered more interesting to the extent that some companies have recently retrofitted their bioethanol production facilities to biobutanol production plants.

The advantages of biobutanol over ethanol include a 25% higher energy content (33 MJ/kg butanol versus 23.4 MJ/kg for ethanol while the gasoline energy content is 42 MJ/kg). In addition, butanol is safer to store and handle due to its lower volatility, corrosiveness and flammability compared to ethanol. Therefore, the existing pipelines and equipment could be used to store and transfer biobutanol. Moreover, biobutanol can be blended with gasoline in any proportion and used in existing car engines without requiring engine modifications (http://www.abercade.ru/en/materials/analytics/339.html).

However, to make the biobutanol production process economically viable, many issues need to be resolved. The main challenge is the low final butanol concentration in the fermentation broth. It is therefore required to develop an effective separation method to remove biobutanol from the dilute fermentation broth. Distillation has been the conventional method for separating bioethanol up to now. However, due to the very low concentration of butanol, the presence of other solvents in the fermentation broth and the

high-energy requirement, distillation is not considered as an efficient separation method. One of the main focuses in biobutanol production field is developing an energy-effective separation technique to make the process more economically viable (Cao et al. 2015; Faisal et al., 2014; García et al., 2009; Garcia-Chavez et al., 2009; Groot et al., 1986).

For the purpose of this research project, the focus has been placed on bench-scale experiments intended to investigate the application of adsorption to separate and recover butanol from ABE model solutions and real fermentation broth to develop an effective downstream separation process for biobutanol recovery. The motivations for using adsorption as a separation technique for butanol are presented more comprehensively in Chapter II which has been published as a review article (Abdehagh et al., 2014).

1.1 Main objectives

The main objectives of the thesis are to:

1. Identify suitable candidates among commercially available adsorbents for the selective extraction of butanol from ABE model solutions and fermentation broth.
2. Investigate the key adsorbent performance parameters in dynamic studies of the selected adsorbent:
    a. Equilibrium capacity
    b. Adsorption kinetics
    c. Column performance
    d. Butanol selectivity over residual sugars and broth medium
    e. Reversibility of adsorption for the recovery of butanol: Desorption
    f. Develop and validate a mechanistic model that can use data acquired from simple butanol adsorption performance in bench scale column to simulate the behaviour of dynamic breakthrough experiments over a range of conditions.
3. Design and implement a novel integrated packed adsorption bed combined with a gas stripping system for butanol separation.
4. Develop and verify an integrated model that can predict butanol separation through a packed bed adsorber that will consider multicomponent competitive adsorption.

Following a comprehensive adsorbent screening, the most promising adsorbent was selected for further research. From the best performing adsorbents, F-400 activated carbon (AC) was selected for further study. F-400 activated carbon performed well in terms of both butanol adsorption capacity and kinetics. AC F-400 is commercially available at a reasonable cost, which was not the case for some of the other screened adsorbents. AC F-400 was first tested extensively in bench scale packed column for binary, ternary and ABE model solutions and fermentation broths to evaluate the kinetics associated with butanol adsorption, and the ultimate uptake of butanol that could be achieved. The competitive adsorption between butanol and other components present in the ABE fermentation broths (acetone, ethanol, butyric acid, acetic acid, glucose and xylose) was also investigated in multicomponent systems. Different competitive adsorption isotherm models were studied to appropriately represent the behavior of the competitive adsorption.

In the next step, desorption of butanol was investigated following the adsorption process in the column, for different adsorption-desorption cycles to evaluate both desorption capacity of butanol in the designed system and the capability of the adsorbent particles to be used in numerous adsorption-desorption cycles.

The combination of adsorption and gas stripping techniques was also studied to investigate the performance of the stripping of solvents from model solutions and fermentation broths. The results of adsorption experiments performed in the vapour phase were compared with the results obtained for liquid phase adsorption.

Finally, with an understanding of the competitive nature of adsorption, actual fermentation runs were carried out and the cell-free fermentation broth was used to examine the adsorption performance for an actual fermentation broth. A mechanistic model was developed and validated with the experimental data, to predict the adsorption process in a packed bed for butanol separation from dilute solutions.

1.2 Structure of the thesis

The main body of the thesis is subdivided into eight sections: introduction, adsorbent screening, analytical method, desorption method, adsorption-gas stripping

method, adsorption process modeling, conclusions and Recommendations and appendix. Each section includes the particular chapters within the scope of that section. Many chapters are articles that have been accepted or are currently been considered in refereed journals. These chapters have been written using the format of the particular journals and the method for referencing may vary slightly from chapter to chapter. There are seven journal papers included in this document, covering the entire scope of the project. The focus of each section is outlined below along with a description of the contents of each chapter.

Chapter 1: "*Introduction*" presents some background on the research topic, the scope and structure of the thesis.

Chapter 2: "*A review: Separation techniques in butanol production: Challenges and developments*" is a review on butanol bioproduction and separation background and challenges. The focus of the chapter is to review the separation techniques and to compare the performances of different methods for butanol removal and recovery as presented in the literature.

Published:

N. Abdehagh, F.H. Tezel, J. Thibault, "A REVIEW: Biobutanol Production and Separation: Challenges and Developments", Biomass and Bioenergy Journal, 60, 222- 246, (2014)

Presented:

N. Abdehagh, A. Sharif, F.H. Azimi, H. Tezel, P. Mehrani, J. Thibault, "Biobutanol: A Viable Fuel", (Opening Plenary presentation), International Conference and Exhibition on Clean Energy, Ottawa, Canada, Sep 9-11, (2013)

N. Abdehagh, A. Sharif, F. H. Tezel, J. Thibault, "In situ removal of biobutanol from fermentation broth", Oral presentation in the XXVI Interamerican Congress of Chemical Engineering, Montevideo, Uruguay, November 12-14, (2012)

Chapter 3: "*Adsorbent screening for biobutanol separation by adsorption: kinetics, isotherms and competitive effect of other compounds*" presents a comprehensive study on adsorbent screening to find the best adsorbent for butanol removal from dilute model solutions with high adsorption capacity and fast kinetics in addition to high selectivity toward butanol. The effects of the presence of the other main components present in the ABE fermentation broths on butanol adsorption was studied separately and, based on the results, the best adsorbent was selected among the tested commercially-available adsorbents for further research.

Published:

N. Abdehagh, F.H. Tezel, J. Thibault, "*Adsorbent Screening for Biobutanol Separation by Adsorption: Kinetics, Isotherms and Competitive Effect of Other Compounds*", Adsorption Journal, 19, 1263-1272, (2013)

Presented:

N. Abdehagh, F.H. Tezel, J. Thibault, "Biobutanol separation by adsorption: Adsorbent screening, kinetics and equilibrium", Oral presentation in the 14th CSChE Ontario-Quebec Biotechnology Meeting, University of Ottawa, Ottawa. ON, May 30- 31 (2012)

N. Abdehagh, F. H. Tezel, J. Thibault, "Improving Biobutanol Production by Adsorption: Equilibrium and Kinetic Studies for Adsorbent Screening", Oral presentation in the 62nd Canadian Chemical Engineering Conference (CSChE), Vancouver, BC, October 14-17 (2012)

N. Abdehagh, F. H. Tezel, J. Thibault, "Improvements in Biobutanol Separation by Adsorption: Adsorbent Screening, Kinetics and Equilibrium", Oral presentation in AIChE 2012 Annual Meeting taking place in Pittsburgh, PA, USA, October 28–November 2 (2012)

N. Abdehagh, F.H. Tezel, J. Thibault, "Adsorption Separation of Biobutanol: Kinetic and Equilibrium studies and Effect of Impurities", Oral presentation in 11th International Conference on Fundamentals of Adsorption FOA11, Baltimore, Mariland, May 19-24 (2013)

N. Abdehagh, F.H. Tezel, J. Thibault, "Adsorptive Separation of Biobutanol: Adsorbent Screening, Effect of Impurities and Breakthrough Experiments", 15th CSChE Quebec-Ontario Biotechnology Meeting, Quebec, Canada, 30-31 May (2013)

H. Azimi, N. Abdehagh, P. Gurnani, A. Sharif Rohani, F.H. Tezel, Mehrani P., Thibault J., "Butanol Recovery via Pervaporation Separation from ABE Fermentation", 15th CSChE Quebec-Ontario Biotechnology Meeting, Quebec, Canada, 30-31 May (2013)

A. Sharif Rohani, N. Abdehagh, P. Mehrani, F.H. Tezel, J. Thibault, "In-situ Removal of Biobutanol During Fermentation Process", 10th World Congress on Industrial Biotechnology, Montreal, Canada, June 16-19 (2013)

Chapter 4: "*Improved Acetone-Butanol-Ethanol (ABE) Solution Analysis Using HPLC: Chromatograph Spectrum Deconvolution Using Asymmetric Gaussian Fit*" presents the analytical method developed to better analyze the overlapping peaks in the HPLC chromatograms for the detection of components in ABE solutions. A program was written to fit the raw data from HPLC chromatograms to asymmetric Gaussian equations. Achieving an excellent fit, the program was able to calculate the area of the curve and helped to detect all the components in the solution more accurately using only HPLC.

Published:

N. Abdehagh, M. Bagheri, F.H. Tezel, J. Thibault, "*Improved Acetone-Butanol-Ethanol (ABE) Solution Analysis Using HPLC: Chromatograph Spectrum Deconvolution Using Asymmetric Gaussian Fit*", American Journal of Analytical Chemistry, 5(16), 1078-1089 (2014).

Chapter 5: "*Adsorptive separation and recovery of biobutanol from ABE model solutions*" presents the results of the butanol adsorption and desorption study from butanol–water and ABE model solutions in numerous subsequent adsorption–desorption cycles.

Published:

N. Abdehagh, P. Gurnani, F.H. Tezel, J. Thibault, "Adsorptive Separation and Recovery of Biobutanol from ABE Model Solution", Adsorption, 21(3), 185-194 (2015).

Presented:

N. Abdehagh, F. H. Tezel, J. Thibault, "Biobutanol Separation By Adsorption: Adsorbent Screening and Breakthrough Experiments", AIChE 2013 Annual Meeting, San Francisco, CA, USA, Nov 3-8 (2013).

N. Abdehagh, F. H. Tezel, J. Thibault, "Adsorptive biobutanol separation from ABE model solutions", 11th Annual world Congress on Industrial Biotechnology, Philadelphia, PA, USA, May 12-16 (2014).

Chapter 6: "*Biobutanol separation from ABE model solutions and fermentation broths using a combined adsorption-gas stripping process*" presents the data of the butanol separation experiments using the adsorption-gas stripping combined method. The butanol separation was studied using the vapour phase adsorption by adding a gas stripping system allowing to compare the butanol adsorption performance in liquid and vapour phases.

Publication submitted:

N. Abdehagh, B. Dai, J. Thibault, F.H. Tezel, "Biobutanol separation from ABE model solutions and fermentation broths using a combined adsorption-gas stripping process", Submitted to Journal of Chemical Technology & Biotechnology.

Presented:

N. Abdehagh, F. H. Tezel, J. Thibault, "Biobutanol Separation and Recovery by Adsorption", 12th Annual World Congress on Industrial Biotechnology July 19-22, Montreal, CA (2015).

Chapter 7: "*Multicomponent Adsorption Isotherm Modeling for ABE Model Solutions*" presents the adsorption isotherm modeling results for the prediction of the multicomponent adsorption isotherms using different models to find the best one capable of predicting the adsorption isotherms of all the components in the ABE fermentation in the presence of other components.

Publication accepted:

N. Abdehagh, F.H. Tezel, J. Thibault, "Multicomponent Adsorption Isotherm Modeling for ABE Model Solutions", Accepted for publication in Adsorption journal

Chapter 8: "*Simulation and validation of butanol separation from ABE model solutions and fermentation broth in breakthrough experiments for an adsorption packed bed*"

presents the results of breakthrough experiments and also the model developed to predict the butanol adsorption process from ABE model solution and fermentation broth in a packed bed.

Publication to be submitted:

N. Abdehagh, F.H. Tezel, J. Thibault, "Simulation and validation of butanol separation from ABE model solutions and fermentation broth in breakthrough experiments for an adsorption packed bed", in preparation to be submitted shortly.

Chapter 9: "*Conclusions*" is expanding on the specific conclusions of the respective papers. This chapter identifies the findings that we now know conclusively due to the work contained in this thesis. Some recommendations for future work are also provided.

1.3 References

Journals:

Abdehagh, N., Gurnani, P., Tezel, F.H., Thibault, J., Adsorptive Separation and Recovery of Biobutanol from ABE Model Solution. Adsorption, 21(3), 185-194 (2015).

Abdehagh, N., Tezel, F.H., Thibault, J., A REVIEW: Biobutanol Production and Separation: Challenges and Developments. Biomass and Bioenergy Journal 60, 222- 246, (2014).

Abdehagh, N., Tezel, F.H., Thibault, J., Adsorbent Screening for Biobutanol Separation by Adsorption: Kinetics, Isotherms and Competitive Effect of Other Compounds. Adsorption Journal, 19, 1263-1272, (2013).

Antoni, D., Zverlov, V.V., Schwarz W.H., Biofuels from microbes (mini review). Appl. Microbiol. Biotechnol., 77, 23-35 (2007).

Cao, Y., Wang, K., Wang, X., Gu, . Z., Gibbons, W., Vu, H., Butanol vapor adsorption behavior on active carbons and zeolite crystal. Applied Surface Science, 349, 1–7 (2015).

Chavez-Rodriguez, M.F., Nebra, S.A., Assessing GHG emissions, ecological footprint and water linkage for different fuels. Environ. Sci. Technol., 44, 9252-9257(2010).

Dellomonaco, C., Fava, F., Gonzalez, R., The path to next generation biofuels: successes and challenges in the era of synthetic biology. Microb. Cell Fact., 9, 3–17 (2010).

Dhamole, P.B., Wang, Z., Liu, Y., Wang, B., Feng, H., Extractive fermentation with non-ionic surfactants to enhance butanol production. Biomass Bioenergy, 40, 112–119 (2012).

Dürre, P.: Biobutanol: an attractive biofuel. Biotechnol., J. 2, 1525–1534 (2007).

Ezeji, T.C., Qureshi, N., Blaschek, H.P., Production of acetone, butanol and ethanol by *Clostridium beijerinckii* BA101 and in situ recovery by gas stripping. World J. Microbiol. Biotechnol., 19, 595–603 (2003).

Ezeji, T.C., Qureshi, N., Blaschek, H.P., Butanol fermentation research: upstream and downstream manipulations. Chem., Rec. 4, 305–314 (2004).

Ezeji, T.C., Qureshi, N., Blaschek, H.P., Bioproduction of butanol from biomass: from genes to bioreactors. Curr. Opin. Biotechnol., 18, 220–227 (2007).

Faisal, A., Zarebska, A., Saremi, P., Korelskiy, D., Ohlin, L., Rova, U., MFI zeolite as adsorbent for selective recovery of hydrocarbons from ABE fermentation broths. Adsorption, 20, 465–470 (2014).

Fouad, E.A., Feng, X., Use of pervaporation to separate butanol from dilutes aqueous solutions: effects of operating conditions and concentration polarization. J. Membr. Sci., 323, 428–435 (2008).

García, V., Pongracz, E., Muurinen, E., Keiski, R.L., Recovery of n-butanol from salt containing solutions by pervaporation. Desalination, 241, 201–211 (2009).

Garcia-Chavez, L.Y., Garsia, C.M., Schuur, B., de Haan, A.B., Biobutanol recovery using non-fluorinated task-specific ionic liquids. Ind. Eng. Chem. Res., 51, 8293–8301 (2012).

Groot, W.J., Luyben, K.Ch.A.M., In situ product recovery by adsorption in the butanol/isopropanol batch fermentation. Appl. Microbiol. Biotechnol. 25, 29–31 (1986).

Harvey, B.G, Meylemans, H.A., The role of butanol in the development of sustainable fuel technologies. J. Chem. Technol. Biotechnol., 86, 2-9 (2011).

Qureshi, N., Ezeji, T.C., Butanol, 'a superior biofuel' production from agricultural residues (renewable biomass): recent progress in technology. Biofuels. Bioprod. Bioref. 2, 319-330 (2008).

Shapovalov, O.I., Ashkinazi, L.A., Biobutanol: biofuel of second generation. Russian J. Appl. Chem., 81(12), 2232-2236 (2008).

Sims, R.E.H., Mabee, W., Saddler, J.N., Taylor, M., An overview of second generation biofuel technologies. Bioresour. Technol., 101, 1570-1580 (2010).

Solomon, B.D., Barnes, J.R., Halvorsen, K.E., Grain and cellulosic ethanol: History, economics and energy policy. Biomass and Bioenergy, 31, 416-425 (2007).

Websites:

1. http://www.biobutanol.com/Biobutanol-History.html
2. http://www.abercade.ru/en/materials/analytics/339.html

Chapter II

Biomass and Bioenergy Journal, 60, 222- 246, (2014)

Review

Separation techniques in butanol production: Challenges and developments

N. Abdehagh, J. Thibault, F. H. Tezel

Department of Chemical and Biological Engineering
University of Ottawa
161 Louis-Pasteur, Ottawa, ON, Canada K1N 6N5

Corresponding author: Tel: (613) 652-5800 Ext. 6107; Email: Handan.Tezel@uottawa.ca

Abstract

The rising cost of crude oil combined with the depletion of the resources, political instability in oil producing countries, and the desire to reduce the current dependence on imported oil are some of the reasons that have motivated the different waves of interest for renewable and sustainable fuels. In addition to bioethanol and biodiesel, biobutanol is attracting significant interest as a biofuel mainly due to the recent advances in biotechnology for its production. Biobutanol has lower water miscibility, flammability, and corrosiveness than ethanol, and has the enviable advantage to be able to directly replace gasoline in car engines without requiring modifications. Butanol can be produced from a wide variety of waste biomass feedstock which does not compete with food. In butanol bioproduction, the most widely used microorganisms for acetone-butanol-ethanol (ABE) fermentation are anaerobic bacteria such as the solventogenic Clostridia including *Clostridium acetobutylicum* and *Clostridium beijerinckii.* However, the production of biobutanol via fermentation is facing significant engineering challenges due to the very low final concentration and low yield due to the severe butanol toxicity to microorganisms. It is therefore important to find an efficient separation technique to

recover butanol at the end of fermentation or during the fermentation to reduce the level of toxicity and prolong the fermentation. In this article, the main butanol separation techniques such as adsorption, gas stripping, liquid-liquid extraction (LLE), perstraction, reverse osmosis (RO) and pervaporation were discussed. It was concluded that adsorption and pervaporation are the separation techniques that offer the most potential for butanol separation from dilute solutions.

Keywords: Biobutanol, Biofuel, Liquid-liquid extraction, Gas stripping, Adsorption, Pervaporation

1 Introduction

Numerous reasons have motivated the different waves of interest for renewable and sustainable fuels. The rising cost of crude oil combined with the depletion of resources, CO_2 related environmental challenges facing our planet, political instability in oil producing countries, and the desire to reduce our dependence on imported oil are some of these reasons [1-3]. The use of biofuels allows for a more carbon neutral cycle, thereby significantly reducing net carbon dioxide emissions. To achieve a greater use of biofuels, it is paramount to develop innovative techniques to convert biomass to biofuel as efficiently as possible and to recover biofuels from the fermentation broth in order to make these production processes more economically viable. Recent developments in biotechnology offer an avenue for fuels bioproduction from renewable and sustainable resources instead of petroleum-based fuels [1,3].

For liquid fuels used for transportation, the production of bioethanol and biodiesel have crystallized research efforts for few decades with a greater emphasis in the past decade due to large and unpredictable increases in the price of oil. More recently, biobutanol has also attracted significant attention as a biofuel because it can be more directly substituted for gasoline. Butanol was initially produced from fermentation, but in the 1950s, butanol produced from petroleum via the oxo alcohol process became much cheaper and the chemical route remains the only economic source of butanol today. However, the resurgence of interest in the production of butanol from fermentation is

being witnessed not only from research institutions but also from major players, including large multionational petroleum companies. The renewed interest is mostly motivated by the rising price of oil [4-6].

The fermentation route to produce biobutanol represents a significant engineering challenge because of very low final concentration, low yield and severe butanol toxicity to microorganisms. On the other hand, butanol can be produced from a wide variety of waste biomass feedstock which do not compete with food, and possesses enviable fuel properties compared to ethanol [4-6].

As a biofuel, biobutanol has several advantages over other biofuels such as ethanol [4-9]:

1. When lignocellulosic hydrolysate sugars (hexoses and pentoses) are used for the production of butanol, the use of genetically-modified microorganisms is not required [5,6]. On the other hand, these genetically-modified microorganisms are currently being developed to increase their tolerance to butanol and, as a result, to increase the final concentration of butanol in the fermentation broth.

2. The net heat of combustion (NHOC) of butanol (29.2 MJ/L) is 83% that of gasoline (32.5 MJ/L) whereas corresponding values for ethanol and methanol are 65 and 45%, respectively.

3. Butanol is very hydrophobic with a water solubility of 7.7 g/ 100 mL (at 20°C) whereas lower alcohols (methanol, ethanol and n-propanol) are miscible in water.

4. Butanol is less flammable and can replace gasoline without any modifications to car engines. It can be blended with gasoline in any proportion and it is less corrosive than ethanol. This implies that butanol, unlike ethanol, can be blended with gasoline in the refinery before storage and distribution.

5. Butanol is less hazardous to handle because of its lower vapor pressure and lower volatility. Also the higher boiling point (117.7°C) and flash point (29°C) make butanol safer than other lower alcohols. The fermentation process used for butanol production is referred to as acetone-butanol-ethanol (ABE) fermentation since a mixture of acetone,

butanol and ethanol is produced. The most widely used microorganisms for ABE fermentation are anaerobic bacteria such as the solventogenic Clostridia including *Clostridium acetobutylicum* (CA) and *Clostridium beijerinckii* (CB) which are classified as gram-positive, strictly anaerobic and spore forming bacteria. Low cost substrates such as algal biomass, soy molasses, wheat straw, corn stover, barley straw and switchgrass have been investigated for butanol bioproduction. Lignocellulosic biomass is considered to be the substrate of choice for butanol production. For this process to become economically viable, a more efficient bioconversion to butanol of fermentable sugars obtained from the hydrolysis of cellulose and hemicellulose is required [1,10-12]. Currently, the butanol:acetone:ethanol mass fraction ratio in a typical fermentation broth is approximately 6:3:1 and butanol concentration in the product varies between 1 and 2 wt% [4-6,13-18].

From the metabolic engineering aspect, it would be ideal if a microorganism could be genetically modified to increase the concentration of butanol while reducing or eliminating the formation of other co-products such as acetone and ethanol. Several studies have been undertaken to genetically modify *Escherichia coli* and *Saccharomyces cerevisiae* to produce butanol from sugars [3,10,19,20]. Table 1 presents a list of recent selected native and genetically-modified microorganisms with typical ABE concentrations of fermentation products.

Table 1 List of some native and genetically-modified clostridia microorganisms with corresponding typical fermentation broth final ABE concentrations

Microorganism	Butanol (g/L)	Acetone (g/L)	Ethanol (g/L)
Clostridium acetobutylicum (JB200)[28]	19.2	1	1.7
Clostridium acetobutylicum (CGMCC 5234)[29]	12.3	5.9	1.6
Clostridium acetobutylicum ATCC824 [30]	10.1	4.5	1.0
Clostridium acetobutylicum ATCC824 [27,31-33]	11.0	5.0	1.0
Clostridium acetobutylicum (BKM19) [34]	17.6	4.4	10.5
Clostridium acetobutylicum (BKM19) [35]	12.6	6.7	1.7
Clostridium beijerinkii 8052 [36]	10.8	3.2	0.8
Clostridium saccharobutylicum (DSM 13864)[37]	10.1	4.6	1.4
Clostridium beijerinkii BA101 [1,7,24,38,39] (Genetically modified)	19.6	4.3	0.3

In Clostridium species, ABE production is a two-phase fermentation: acidogenic and solventogenic phases. In the acidogenic phase, acetate and butyrate are produced during the exponential growth phase. This is followed by the solventogenic phase where acids are re-assimilated and converted into ABE in the late exponential and stationary phases. In the solventogenic phase, sporulation occurs and bacterial cells go into a dormant state, where butanol production stops. Fig. 1 shows the simplified fermentation phases for butanol production by Clostridium species. The mechanism of butanol fermentation is very complex and not yet fully understood. However, efforts have been done to shift the acidogenic phase to solventogenic phase by inducing solventogenic enzymes while reducing acidogenic enzyme activity. Selection of butanol producing strains depends on the feedstock material, nutrient requirement, butanol tolerance and ability of producing broths with high butanol concentration. Attempts have been made to isolate or enhance novel microorganisms with desired characteristics using different genetic engineering techniques [5,10,21-23].

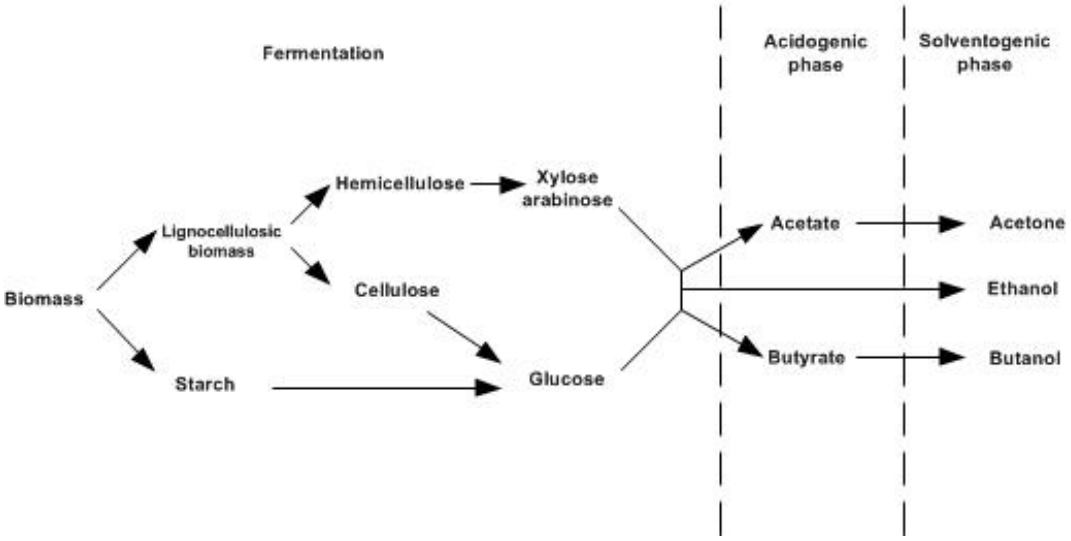


Figure 1 Simplified product formation pathway by solventogenic Clostridium species.

The toxicity of butanol causes damages to the cell membrane and makes it permeable to ADP (adenosine diphosphate) and some ions, and subsequently causes cell lysis. Some genetic modifications have been attempted to improve butanol yield, to decrease solvent toxicity, and to decrease by-product formation. All these efforts have so

far led to a butanol concentration of less than 2 wt%, a productivity of 4.46 g/L h and an increase of 25% for butanol-to-glucose yield [6,9,11,24,25].

The extensive problems in butanol bioproduction can be attributed to: 1) lack of productive butanol producing microorganisms, 2) using cost-intensive food materials instead of cost-effective non-food materials as a feedstock, 3) product inhibition and 4) cost-intensive butanol recovery techniques. Recent research efforts are mainly focused on investigating genetic and metabolic engineering techniques and strategies, utilizing renewable and cost-effective cellulosic and hemicellulosic materials as a feedstock and developing integrated fermentation processes coupled with product recovery techniques. In this review, the focus was placed on the challenges in butanol separation techniques to find the best methods to separate butanol from dilute fermentation broths.

Given the very low concentration of butanol and the presence of other fermentation products in the broth, albeit in low concentration, it is important to find an efficient separation technique to recover butanol [5,6,9]. Distillation can be used but the high energy requirement to process the low solvent concentration makes this technique non-economically viable [4]. At a concentration of 1 wt% butanol, distillation requires 1.5 times the energy contained in the resulting butanol. If fermentation could result in a butanol concentration of 40 g/L (4 wt%) this ratio would decrease to 0.25 [1]. It is therefore important to find alternatives for the separation of butanol at the end of fermentation but also for the in situ recovery during the fermentation to reduce toxicity and enhance productivity [5]. Product removal techniques include adsorption, gas stripping, liquid-liquid extraction (LLE), perstraction, reverse osmosis (RO) and pervaporation [5,26,27]. In this review, these important techniques are briefly described with the main emphasis being placed on the two most promising techniques: adsorption and pervaporation.

2 Butanol separation techniques

2.1 Gas stripping

Gas stripping is a simple technique that can be used to separate butanol from product solutions. In this method, oxygen-free nitrogen or fermentation gases (hydrogen and carbon dioxide) are bubbled through the fermentation broth to strip away acetone,

butanol and ethanol. Fig. 2 shows the schematic diagram of butanol removal by gas stripping. The stripping gas is circulated through the fermenter and exits containing acetone, butanol, ethanol and water usually close to their equilibrium partial pressure. The stripping gas is then passed through a condenser where the vapours are partly condensed. The depleted gas stream is recycled to the fermenter for another extraction cycle. Given the strong inhibition of butanol during fermentation, using gas stripping should lead to higher productivity and yield. It has been shown that circulating large amount of gas does not cause any cell damage but may lead to foam formation [1,4,5,10,15]. The performance of the gas stripping process depends on the gas flow rate, antifoam and the presence of other components in the fermentation broth [38].

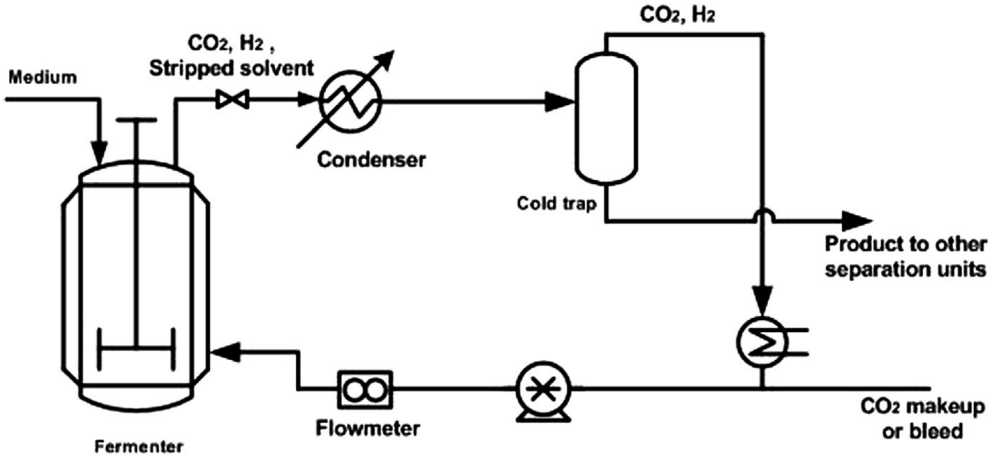


Figure 2 Schematic diagram of butanol removal from fermentation broth by gas stripping.

Tables 2 and 3 summarize the results obtained by numerous researchers for the separation of butanol and ABE from model solutions and fermentation broths using gas stripping. Park et al. (1991) investigated butanol separation from the fermentation broth by gas stripping and found that an increase in gas flow rate and temperature led to an increase in glucose conversion and butanol separation [40]. As it can be seen in Table 2, the highest butanol selectivity is obtained when gas stripping is performed at 67 °C [42]. Ezeji et al. (2005) also found that stripping rate is proportional to the gas recycle rate [38]. They stated that for a 1 L fermenter, 4.8 L/min is an optimized gas recycle flow rate to keep butanol in a concentration less than its toxic level. They also found that the presence of acetone does not affect butanol separation by gas stripping. These researchers also

studied different fermentation systems (batch, fed-batch and continuous fermentation) coupled with gas stripping and observed that the yield and productivity in fed-batch and continuous fermentations are the same and higher than for batch fermentation. However, the fed-batch fermentation failed after 201 h due to accumulation of unknown inhibitors such as salts and dead cells. Therefore, they reported that the effectiveness of gas stripping should be considered only in continuous fermentation [7,41,43]. Mollah and Stuckey (1993) also used gas stripping in a 2.5 L fluidized bed reactor with *C. acetobutylicum* immobilized in calcium alginate beads using glucose as a substrate [44]. The carrier gas was a mixture of H_2 (20%), CO_2 (10%) and N_2 (70%). They found that increasing the gas flow rate up to 1.67 L/min led to an increase in productivity but further increasing the gas flow rate caused a reduction in productivity. It was found that among the different factors, the gas flow rate and the amount of antifoam added to the gas stripping system were the most important parameters affecting system performance [38,40].

Table 2 ABE separation from model solutions and fermentation broths by gas stripping.

	Rate of ABE removal (g/L.h)	Operating temperature (°C)	Reactor Volume (L)	Gas recycle rate (L/min)	Condensation temperature (°C)	Selectivity[a]
Model butanol solutions						
Ezeji et al. (2005) [38]	-	35-36	1.0	4.8	3	6.3
Ennis et al. (1986) [45]	0.3	34	1.2	3.24	-60	19.3
Fermentation broths						
Setlhaku et al. (2013) [49]	0.02	35	1.4-3.8	4.8-6.6	-2	0.5-4.2
Richter et al. (2012)[50]	0.97	35	1	6.4	-	-
Lu et al. (2012) [48]	-	37	1	1.25	1	-
Ezeji et al. (2005) [38]	-	35-36	1.0	4.8	3	9
Ezeji et al. (2003) [7]	-	35	2.0	4.6	-	-
Mollah and Stuckey (1993) [44]	0.18	37	2.5	1.67	-	-
Maddox et al. (1995) [52]	0.32	34	2.0	1.5-3.3	-0.8	9.5-13
Park et al. (1991) [40]	-	36	0.067	15	-	0.450
Qureshi et al. (1992) [53]	0.31	35	2.0	3-3.2	0-3	6-23
Groot et al. (1989) [46]	1	30	2.0	10	-40	4
Qureshi and Maddox (1991) [42]	0.1	65-67	0.45	2.5	3-4	30.5
Ennis et al. (1986) [45]	0.45	34	1.2	2.7	-60	23.4

[a] $\boldsymbol{S = y(1-x)/x(1-y)}$ in which y and x are the butanol mass fraction in the product and the feed, respectively

Table 3 compares the results obtained during fermentation with and without butanol separation by gas stripping. Ennis et al. (1986) used *C. acetobutylicum* to convert whey permeate to butanol in a 1.2 L batch bioreactor where a 2.7 L/min nitrogen gas stream was used to strip the produced butanol [45]. Even though gas stripping led to higher productivity and sugar utilization, the ABE yield decreased from 0.39 to 0.27 g solvent/g sugar. This reduction may be due to incomplete solvent capture in the condenser. Groot et al. (1989) studied butanol separation produced in batch and continuous fermentation using a separate packed bed stripper [46]. They found that the substrate consumption increased from 37 to 126 g/L when gas stripping product recovery was implemented in batch fermentation, whereas the productivity and product yield remained fairly constant. In continuous fermentation, gas stripping led to an increase in biomass concentration, a three-fold increase in productivity and a 10% increase in substrate utilization. However, the productivity and the selectivity in their system were still too low and needed to be optimized by tuning the flow rates in the integrated system. Qureshi and Blaschek (2001) obtained a substrate utilization of 30 g/L in batch fermentation, while a sugar consumption of 199 g/L has been observed in a fed-batch butanol fermentation coupled with gas stripping for butanol separation [47].

Table 3 ABE separation from fermentation broth by gas stripping: comparison between control fermentation and integrated fermentation

	Control fermentation parameters			Integrated fermentation parameters (Using gas stripping)			Recovered ABE conc. (g/L)
	Productivity (g/L.h)	ABE yield (g/g)	Substrate utilization (g/L or %)	Productivity (g/L.h)	ABE yield (g/g)	Substrate utilization (g/L)	
Xue et al. (2013) [28]	0.48	0.32	79.7	0.66	0.4	80	420
Xue et al. (2012) [51]	0.4	0.36	86.4	0.53	0.36	474.9	113.24
Lu et al. (2012) [48]	0.62	0.39	86.2	0.41	0.32	336.6	76.4
Ezeji et al. (2007) [26]	0.27	0.4	46.4	0.58	0.4	60.5	18.6 to 24.5
Ezeji et al. (2013) [43]	0.29	0.39	47.3	0.92	0.41	1125	461.3
Ezeji et al. (2004b) [25]	0.29	0.39	47.3	1.16	0.47	500.1	232.2
Ezeji et al. (2003) [7]	0.29	0.39	47.3	0.6	0.4	161.7	75.9
Ennis et al. (1987) [54]	0.15	0.32	28.1	0.62	0.35	40.9	-
Groot et al. (1989) [46]	0.17	0.37	37	0.18	0.34	126	9.1
Maddox et al. (1995) [52]	0.07	0.26	31	0.32	0.35	199	20-120
Qureshi et al. (1992) [53]	0.07	0.32	22.6	0.26	0.38	182.5	26.7
Qureshi, Maddox (1991) [42]	4.8	0.36	30.8%	1.85	0.4	88.3%	59.2
Ennis et al. (1986) [45]	0.22	0.39	29	0.31	0.27	58.3	75.9

Based on the literature results, it can be concluded that although gas stripping may increase the productivity and sugar utilization in butanol fermentation, the selectivity for butanol separation is still low. In some recent investigations, different processes such as two-stage gas stripping or two-stage fermentation coupled with gas stripping have been studied to achieve higher performances [28,48-51]. Xue et al. (2012, 2013) studied the in situ gas stripping in butanol fermentation and were able to increase the yield from 0.36 to 4 g/g and the productivity from 0.53 to 0.66 g/L h using a two-stage gas stripping system instead of the ordinary gas stripping process [28,51]. The useful contribution of gas stripping is to prevent the butanol concentration to increase to a level that would be inhibitory. In addition, the simplicity of the process which does not use complicated equipment or chemical makes it as one of the efficient butanol separation techniques. The main concerns in butanol separation by gas stripping are mostly the selectivity and the energy requirement. The energy required in this technique is highly dependent on the energy consumed in the heater and the condenser.

In this study, a series of CO_2 gas stripping simulations have been performed to determine the final concentration of butanol recovered in the cold trap using UniSim Design R400 (Honeywell). NRTL was used as the thermodynamic fluid package. Fig. 3(a) presents the mass fraction of butanol and acetone of the product as a function of the cold trap temperature. For a simulated fermentation broth containing an ABE mass% of 0.6:1.2:0.2 at 37°C and assuming a gas stream in thermodynamic equilibrium with the fermentation broth at the exit of the fermenter, the mass fraction of butanol in the final recovered product varies from 0.24 at a cold trap temperature of -40 to about 0.32 at 10°C. It is important to observe that the quantity of butanol recovered decreased by about 20% when the cold trap temperature is increased from -40 to 10°C. The increase in butanol mass fraction and the decrease in total recovered butanol are mainly due to the decrease in the amount of acetone being condensed as the temperature of the cold trap is increased. Indeed, the acetone mass fraction decreases significantly over that temperature range as observed in Fig. 3(a). Fig. 3(b) presents the percentage recovery of the butanol and acetone contained in the stripping gas at the exit of the fermenter. Since butanol has a much lower partial pressure than acetone at a given temperature, the percentage recovery of butanol is much higher than that for acetone. This result does not mean that

uncondensed solvents will be lost since the gas exiting the cold trap, except for a tiny bleed stream corresponding to rate of CO_2 production in the fermenter, will be recycled to the fermenter for another stripping cycle. Fig. 3 also presents the results obtained under the same conditions for a simulated fermentation broth containing an ABE mass percent of 0.3:0.6:0.1. Since the mass percentage of solvents is half the mass fraction than the previous case, the final mass fraction of the recovered product has decreased. This decrease is mainly due to the higher proportion of water in the gas stream exiting the fermenter.

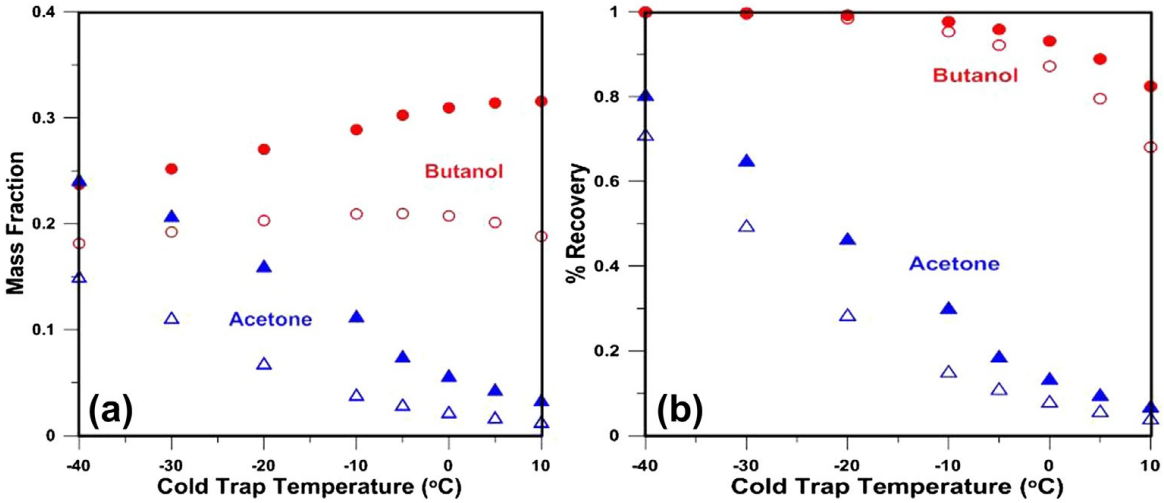


Figure 3 Mass fraction (a) and % recovery (b) of butanol and acetone of the final condensate of CO_2 gas stripping as a function of the cold trap temperature for different simulated ABE broth composition: Filled symbols: 0.6:1.2:0.2 mass% (filled symbols) and 0.3:0.6:0.1 mass% (open symbols).

Results clearly show that high mass fractions for butanol are obtained for the recovered product following the gas stripping operation. The previous simulations considered conditions at only one point in time and cannot assess how the concentration of butanol will vary with time inside the fermenter. Since the recycled gas stream contains different amount of solvents being returned to the fermenter as well as more solvent being produced in the fermenter, the next step will be to perform a dynamic simulation to simulate the biochemical reactions taking place simultaneously in the

fermenter, the stripping gas process and the condensation process. The temperature of the cold trap must be based on an overall economic analysis since the cost of cooling and heating of the stripping gas must be considered in addition to all the other costs of production and separation.

2.2 Liquid-liquid extraction (LLE)

Liquid-liquid extraction (LLE) is another technique used for the removal of butanol or ABE from fermentation broths. In this process, a water-insoluble organic extractant, with a low toxicity to the microorganism, is mixed with the fermentation broth to selectively remove butanol. Due to the immiscibility of the extractant in the fermentation broth, the organic phase can be easily separated to recover a fraction of the butanol and other miscible solvents. It is desired to have an organic solvent that would preferentially separate butanol without significantly removing substrates, water and nutrients [4,15]. Until now, extractants with high butanol distribution coefficients were found to be toxic to butanol producing microorganisms. Oleyl alcohol is one of the extractants used for butanol separation because it possesses good extraction ability and a relatively low toxicity to bacteria [1,10]. In an attempt to reduce the toxicity and obtain a reasonable partition coefficient, Evans and Wang (1988) have suggested combining an extractant with a high partition coefficient, even if its toxicity is high, with an extractant having low toxicity, even if its partition coefficient is low, to give an extractant with both relatively high partition coefficient and low toxicity [55]. They used a mixture of 20% decanol in oleyl alcohol as a butanol extractant and found that using this mixture enhanced butanol separation by 72%. Results also showed that oleyl alcohol or a mixture of oleyl alcohol and benzyl benzoate had the best performance as a butanol extractant. This mixed extractant in a fed-batch butanol fermentation system increased butanol productivity by 60% compared to regular batch fermentation [1,4,15,19,56]. Roffler et al. (1988), using a mixture of oleyl alcohol and benzyl benzoate, observed that glucose conversion increased from 81 to 100 g/L and the maximum butanol volumetric productivity increased from 1.4 to over 2 g/L h in an extractive fermentation [31]. Dibutyl phthalate and kerosene were also used as extractants for butanol separation [21]. However, the main concern in making this mixture is optimizing the combination of non-

toxicity and partition coefficient to provide an extractant with improved characteristics. Other extractants used for butanol removal from fermentation broth are 2-ethyl-1-hexanol, room temperature ionic liquids (RTILs), 4-n-butylphenol, n-decanol and soybean-derived biodiesel [57-61].

Cockrem et al. (1986) used 4-n-butylphenol as an extractant for the recovery of butanol from dilute solutions [57]. Based on their results, it was calculated that the butanol mass distribution coefficient relative to water and the selectivity was approximately 25 and 200, respectively. Cascon et al. (2011) investigated selected ammonium and phosphonium cation-based room temperature ionic liquids (RTILs) such as bis(tri- fluoromethylsulfonyl)imide [Ph3t][NTF] and bis(tri-fluoromethylsulfonyl)imide [BMIM][NTF] as extractants for butanol extraction from butanol-water solutions and fermentation broths [61]. They found that the majority of RTILs are toxic to *C. acetobutylicum* and *C. beijerinckii*. They investigated RTILs and mixtures of RTIL-oleyl alcohol and found that [Ph3t][NTF] is toxic in biphasic fermentation. RTIL and oleyl alcohol are biocompatible with *C. beijerinckii* but has inhibitory effect on *C. acetobutylicum* culture. Oleyl alcohol is completely biocompatible with *C. beijerinckii* in biphasic fermentation conditions and is even stimulatory to bacterial growth. Some imidazolium-based ionic liquids (ILs) were also investigated for butanol separation by liquid-liquid extraction and it was found that bis(trifluoromethylsulfonyl)imide [Tf2N]-based ILs among all tested ILs, showed the best performance to remove butanol from dilute solutions. Butanol selectivity for these ionic liquids was around 132 which is relatively high compared to the value for other extractants and it was possible to extract 74% of the initial butanol [62]. Nonfluorinated task-specific ionic liquids (TSIL) are other types of ionic liquids used as a butanol extractant. Garcia-Chavez et al. (2012) tried to test different ionic liquids to separate butanol from butanol-water solutions and found that tetraoctylammonium 2-methyl-1-naphthoate [TOAM- Naph] has a higher distribution coefficient and selectivity (21 and 274) for butanol in comparison to oleyl alcohol and other ILs tested. They also simulated the conventional distillation and LLE processes to calculate their energy requirement and found that butanol separation with LLE using [TOAMNaph] requires 73% less energy to produce butanol at 99.9 wt%. However, the biocompatibility of this ionic liquid was not

investigated in this study which is one of the most important characteristics in the selection of an extractant [63].

Some researchers have also studied the behaviour of non-ionic surfactants as butanol extractants in an extractive fermentation process. To save on the cost of downstream processes, Dhamole et al. (2012) studied the extractive fermentation using non-ionic surfactants to remove butanol from the fermentation broth during the course of fermentation. They tested the biocompatibility of different non-ionic surfactants and found that surfactant L62 could be considered as a potential extractant since it did not affect the yield and productivity of fermentation. Butanol partition coefficient in L62-water ranged from 3 to 4 and a 6-time enrichment of butanol was obtained in the surfactant-rich phase over the control. At the end of fermentation, 95% of the butanol present in the surfactant rich phase was removed by evaporation and its concentration reached 106.8 g/L. The surfactant was used for three extraction cycles and the same butanol recovery was obtained for each cycle [64].

There are however some potential problems with using extractants. Problems such as loss of extractant, toxicity to culture, emulsion formation and sometimes accumulation of biomass in the extractant can be problematic in an LLE process. In addition, ABE extraction in external columns was suggested to solve problems such as slow mass transfer into the extractant, formation of emulsions through agitation and cell growth inhibition and damage by extractant. Using an external extraction column, it is possible to use a solvent having a high partition coefficient even if it is toxic, provided that the mutual solubility of water and the extractant is low. In addition, the extraction temperature can be different than the fermentation temperature in a way to optimize the extraction. However, for this process, it is necessary to use a large extraction column and retain the microorganisms in the fermenter via immobilization or ultrafiltration. By computer-aided molecular design (CAMD), it was suggested that mesitylene would be an excellent extractant for butanol produced in a continuous ABE fermentation and for a hybrid extraction-distillation downstream process. However, there are no experimental studies using this extractant in a hybrid extraction-distillation downstream process in the literature [65,66].

2.3 Reverse osmosis

The problem of dilute product concentration in ABE fermentation can be alleviated by using reverse osmosis (RO) to dewater the broth at the end of fermentation. High pressure is used to expel water via a semipermeable membrane [10]. Obviously, this technique where water is the permeate species cannot be used, however, for in situ solvent removal during fermentation. On the other hand, reverse osmosis could also be used to remove the solvents from the fermentation broth provided a suitable membrane for this specific process can be found. In reverse osmosis using hollow fibre ultrafiltration, the suspended solids are removed prior to separation. By dewatering the broth, the concentration of products increases and the subsequent distillation can be made more efficient since a smaller volume of more concentrated products is treated. A few researchers have investigated the performance of RO in butanol separation for both model solutions and fermentation broths. García et al. (1986) used reverse osmosis with polyamide membranes as a continuous separation system for butanol removal from fermentation broth [67]. They found that in this case, the permeate flux was only one-third of the flux obtained with model solutions. When they used an ultrafiltration unit as the pre-treatment process to separate cells and suspended solids before sending the feed stream to the RO unit, the results showed that ultrafiltration worked well since passing the cell free broth to the RO unit prevented the fouling of the membrane. On the other hand, the ultrafiltration module itself fouled very quickly and it was necessary to wash or replace the ultrafiltration membrane after short operating times. In an RO system, they observed that 90% of the butanol could be removed but the flux through the RO membrane for the fermentation broth was extremely low and it was likely due to the presence of other components. The obtained butanol-acetone rejection rate (1 minus the quo-tient of the concentrations of the permeate and retentate) was as high as 98% but the optimum rejection of butanol was obtained at recoveries of 20-45%. The recovery was defined as the permeate volume over the initial feed volume at pre-selected times. The results showed that the flux decreased from 0.6 to 0.15 L/min m^2 (at the highest pressure, 6.89 MPa) as recovery increased to 85%. The mass percent of butanol removed had also the same trend (decreasing from 98 to 70%) in the same range of recovery. Their results

pointed to the need to develop membrane technology to cope with the problems of low yield and low solvent concentration in the production of butanol by continuous fermentation.

2.4 Adsorption

Adsorption is an energy-efficient process that can be used to selectively separate butanol from fermentation broths. In this technique, butanol is adsorbed onto the surface of a suitable adsorbent and subsequently desorbed by increasing the temperature and/or using displacers to produce a concentrated butanol solution. Fig. 4 shows typical energy requirements for different techniques used in butanol separation. This figure shows that adsorption, pervaporation and extraction are the most energy-efficient techniques for butanol separation from dilute solutions [68-70].

In selecting a suitable adsorbent, many factors need to be considered: adsorption rate, adsorption capacity, ease of desorption, selectivity for the desired product and cost of the adsorbent. The adsorption kinetics directly impacts the contact time required between the solution containing the adsorbate and the adsorbent. A fast kinetic is desirable since it allows the fermentation broth to circulate more rapidly and decreases its concentration below the inhibitory range. For slow adsorption rate, a larger amount of adsorbent is needed to achieve the product removal in the desired time [71].

The adsorption capacity, selectivity and ease of desorption depend on the type of the solid adsorbent materials used. Materials such as activated carbon, polymeric resins, polyvinylpyridine (PVP), and zeolites are commonly used as butanol adsorbents for model solutions and fermentation broths. Silicalite has a zeolite structure with a very high SiO_2/Al_2O_3 ratio and hydrophobic properties that make it suitable to selectively adsorb small organic compounds (C1-C5) from dilute solutions. Activated carbon has a very high adsorption capacity but product recovery and desorption for this adsorbent has not been investigated comprehensively [10,72-74,76]. Selectivity of the adsorbent also significantly impacts the performance of the recovery process since the ABE fermentation broth contains numerous species such as substrates and nutrients [4,75]. In the case of butanol recovery, adsorption and desorption are equally important to achieve a high butanol concentration solution as a product and to reuse the adsorbent for numerous

separation cycles. However, desorption process is not as well considered in the literature as adsorption is [76,77].

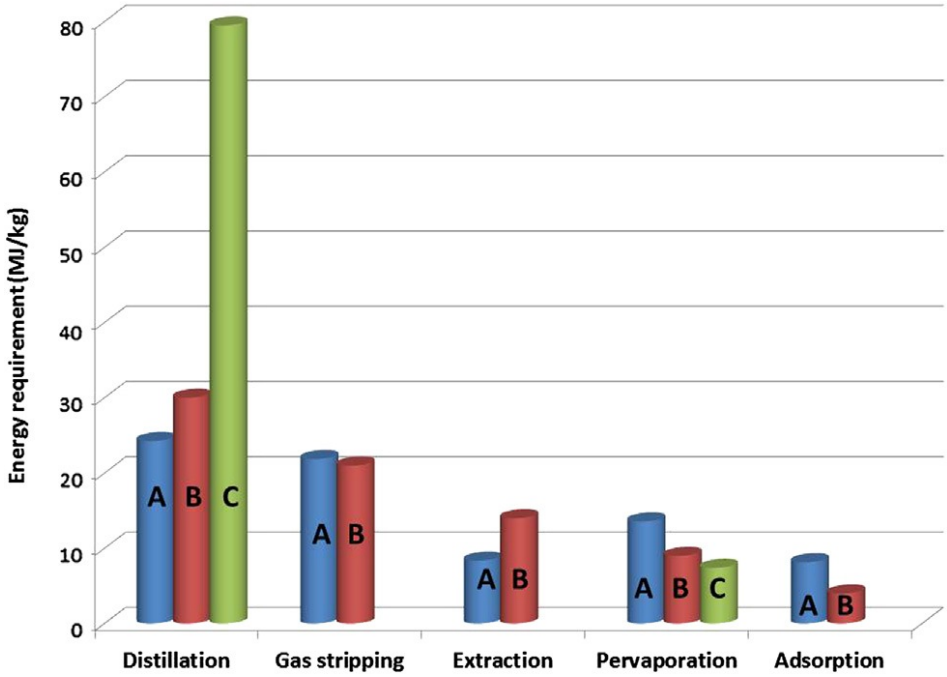


Figure 4 Energy requirements for butanol separation from ABE broth. A for distillation, gas stripping, extraction, and pervaporation are reported in Ref. [25] but the details of the process are not reported. B for distillation: to increase butanol concentration from 1 to 99.99 wt% in the solution and C for distillation: to increase butanol concentration from 0.5 to 99.9 wt% [123]; B for gas stripping: to increase the butanol concentration to 50 wt%; B for extraction: to remove 75% of butanol from the solution using oleyl alcohol as the extractant. B for pervaporation: to remove 25% of butanol from the solution using silicone rubber as the membrane and C for pervaporation: using the combination of pervaporation and distillation to increase the butanol concentration from 0.5 to 99.9 wt% [69,123]; A for adsorption: to remove butanol using silicalite as the adsorbent [25], and B for adsorption: the estimated energy required for butanol adsorption by Oudshoorn et al. [68,76].

2.4.1 Butanol adsorption from model solutions

To screen adsorbents for a specific separation, it is customary to use model solutions containing the desired species. A large number of resins have been used as butanol adsorbents in different studies [24,78-81]. In an early study, Groot and Luyben

(1986) used activated carbon and few resins as adsorbents to recover butanol from a butanol-water solution [78]. They found that activated carbon had the highest adsorption capacity (252 mg/g) for a 15 g/L equilibrium concentration of butanol compared to XAD4, XAD2, and XAD8 resins (resins supplied by Fluka) with capacities of 100, 78 and 66 mg/g, respectively (see Table 4). They reported that regenerated activated carbon has the same original adsorption capacity and could be used for multiple cycles. These researchers heated the column to 150°C for desorption. However, they have not provided the details of their desorption results [78]. Nielsen et al. (1988) also used a series of polymer resins as adsorbents: XAD-4, XAD-7, bonopore and nitrated bonopore (copolymers of divinylbenzene and styrene). They found that XAD-4 and bonopore have the best adsorption capacity for butanol among these adsorbents, with capacity of 83 and 74 mg/g, respectively, for initial butanol concentration in the aqueous phase of 20 g/L at room temperature (Table 4). No data on desorption was reported [75].

Yang et al. (1994) used polyvinylpyridine (PVP) resin to separate butanol from a butanol-water solution. They found that butanol concentration in the resin phase was directly proportional to the butanol concentration in the bulk phase. The adsorption isotherm was determined testing 0.66-7.35 g/ L of butanol in the model solution and a linear regression fit of the data showed that the equilibrium concentration of butanol in the PVP resin was 6.25 times higher than in the feed solution [71]. The adsorption of butanol and other by-products (acetone, ethanol, acetic acid and butyric acid) on PVP was characterized by fast kinetics, the equilibrium being reached within 5 min. These researchers also investigated the competitive effect of butyric acid on butanol adsorption on PVP resin. They found that the higher the initial butyric acid concentration, the smaller was the amount of butanol adsorbed by the resin. They also studied butanol desorption by methanol as a displacer because of its low boiling point and because it could be easily recovered. They stated that all the solvents adsorbed were desorbed in two bed volumes of methanol and concluded that desorption by methanol was rapid and effective [71]. Dowex Optipore L-493, a poly(styrene-co-divinylbenzene) resin, is another polymer resin used in butanol adsorption. Eom et al. (2013) investigated butanol adsorption and desorption using this polystyrene resin for in-situ and ex-situ butanol removal and obtained a maximum adsorption capacity of 133 mg/g for butanol. They also

studied desorption process using steam at 140°C and were able to recover 95% of butanol as the final product [80].

Table 4 Some results of butanol adsorption from model solutions on different adsorbents

Reference	Adsorbent	Feed solution	Initial butanol conc. in solution (g/L)	Eq. butanol conc. in solution (g/L)	Butanol adsorption capacity (mg/g)	Temp. (°C)
Abdehagh et al. (2013) [76]	Activated carbon F-400	Binary	15	1.4	185	Room temp.
	Activated carbon F-600	Binary	15	1.75	136	Room temp.
	Zeolite NaY	Binary	10	4.6	77	Room temp.
	Zeolite ZSM-5	Binary	15	4.9	104	Room temp.
	Silicalite	Binary	15	3.9	111	Room temp.
Ying et al. (2013) [81]	L15 macroporous	Binary	-	2	250	37
	polymer resin	Binary	-	2	190	30
		Binary	-	2	145	20
		Binary	-	2	130	10
Lin et al. (2013) [82]	KA-I resin	Binary	-	10	144	25
Lin et al. (2012) [79]	KA-I resin	Ternary	30	22.5	132	10
		Ternary	30	22	150	20
		Ternary	30	21	200	30
		Ternary	30	20	252	37
Cousin Saint Remi et al. (2012)[89]	ZIF-8	Binary	40	-	300	27
		Ternary	20	-	227	27
Groot, Luyben (1986) [78]	Activated Carbon	Binary	-	15	252	37
	XAD4 resin	Binary	-	15	100	37
	XAD2 resin	Binary	-	15	78	37
	XAD8 resin	Binary	-	15	66	37
Nielsen et al. (1988) [75]	XAD4 resin	Binary	20	-	83	Room temp.
	XAD7 resin	Binary	20	-	69	Room temp.
	Bonopore	Binary	20	-	74	Room temp.
	Bonopore-nitrated	Binary	20	-	55	Room temp.
Sowerby, Crittenden (1988) [83]	Silicalite	Binary	47	-	104	-
			60	-	146	
Holtzapple, Brown (1995) [94]	Silicalite	Binary	-	30	122	25
Oudshoorn et al (2009) [84]	CBV28014 (ZSM-5)	Binary	-	>10	118	25
	CBV901 (Zeolite Y)	Binary	-	>10	160	25
	CBV811 (Zeolite Beta)	Binary	-	>10	118	25
	CBV28014 (ZSM-5)	ABE model	-	5.58	111	25
Saravanan et al. (2010) [77]	Alumina-based CBV28014:					
	30-80 mesh	ABE model	10	-	95	20
	16-24 mesh	ABE model	10	-	85	20
	12-24 mesh	ABE model	10	-	74	20
	Long extrudates	ABE model	10	-	39	20
Sharma, Chung (2011)[86]	MEL type zeolite	Binary	2	0.2	222	30

A new type of resin used as a butanol adsorbent is KA-I with a cross-linked polystyrene framework used by Lin et al. (2013). These researchers' main goal was to investigate the effect of some operating parameters such as feed flow rate, feed composition and height of the packed adsorption bed for a series of breakthrough experiments. This new resin led to an adsorption capacity of 142 mg/g in equilibrium with a butanol concentration of 10 g/L [82]. In another study, they investigated the kinetic behaviour of this resin and the effect of temperature on adsorption capacity for 15 g/L butanol-water model solutions at 37, 30, 20 and 10°C. The results showed that the time needed to reach the equilibrium was decreased from 580 to 120 min with increasing the temperature from 10 to 37°C. And this could be due to the increase of the rate of diffusion of the adsorbate molecules across the external boundary layer and in the internal pores of the adsorbent particles. The same trend was observed for the adsorption capacity [79].

Zeolites are other common adsorbents investigated for butanol separation. Sowerby and Crittenden (1988) performed breakthrough experiments in a packed bed of silicalite for water-butanol model vapour solutions with feed butanol concentrations containing 4.7 and 6.0 wt% butanol. The equilibrium adsorption capacities for an initial bed temperature of 120°C were 104 and 146 mg/g, respectively. They then studied the thermal desorption of butanol using N_2 as the purge gas using a flow rate of 200 mL/min, for these 2 initial concentrations. The first hour of desorption was performed with the packed column maintained at 200 and 150°C, respectively. The temperature for both experiments was subsequently increased to 275°C, respectively for 120 and 180 min, where the desorbed product was collected. 26.2 and 31.5% of the adsorbed butanol in the adsorption column was recovered, respectively, during that time [83].

Oudshoorn et al. (2009) used three different types of zeolites, ZSM-5 (CBV28014), Beta (CBV811) and Y (CBV901) from Zeolyst International (Conshohocken, PA, USA) to investigate their adsorption capacities for butanol and water separately by performing gas phase equilibrium experiments where a known amount of adsorbent was placed in a desiccator at 25°C next to liquid butanol or water [84]. The SiO_2/Al_2O_3 ratio for ZSM-5, Beta and Y was 280, 360 and 80, respectively. Also, the surface area and the estimated pore volume of these adsorbents were 400, 620

and 700 m^2/g and 0.19, 0.24 and 0.50 cm^3/g, respectively. Results for single component adsorption (gas phase equilibrium experiments performed in a closed desiccator) showed that, although ZSM-5 did not have a very high adsorption capacity for butanol (120 mg/g), it had the lowest adsorption capacity for water (61 mg/g) compared to the other adsorbents. The butanol adsorption capacities of zeolites Beta and Y in the single component adsorption experiment were 340 and 370 mg/g, respectively, whereas they were 270 and 470 mg/g for water for the same amount of adsorbent used. The next set of experiments done was the binary adsorption experiments having both water and butanol in the model solution. Results for butanol-water binary systems showed again that ZSM-5 was far more selective than the other two zeolites and had the highest affinity for butanol at low concentrations. Indeed, the maximum butanol adsorption capacity in the binary system for ZSM-5 was reached at an equilibrium butanol concentration of 0.5 g/L. The maximum butanol adsorption capacity was the same for zeolites Beta and ZSM-5 (115 mg/g at 5 g/L butanol at equilibrium) whereas it was 160 mg/g for zeolite Y (12 g/L butanol at equilibrium) which may be due to the adsorbent higher internal volume. Butanol adsorption capacity for binary systems decreased for zeolites Beta and Y compared to the single component adsorption, whereas it remained the same for ZSM-5 (118 mg/g). Therefore, it was concluded that ZSM-5 was the best adsorbent for butanol among the three zeolites studied [84]. In a more recent paper, they investigated the desorption process for zeolites ZSM-5 (CBV28014) and Y (CBV901) to complete the study of butanol adsorption-desorption process. They studied the recovery by thermal swing and found that the desorption rate was significantly lower for ZSM-5 than Y which may be due to the mass transfer resistance caused by the difference in the pore diffusion characteristics in smaller pore channels in ZSM-5 [85].

Saravanan et al. (2010) also used zeolites ZSM-5 and Y (CBV28014 and CBV901) powders from Zeolyst International to make alumina-based (20% binding agent) and silica-based (50% binding agent) zeolite extrudates [77]. It was observed that the presence of binding agent caused higher energy requirements to heat up the column for desorption operation, so it was suggested to use the adsorbent with a minimum amount of binding agent. In addition, because the higher SiO_2/Al_2O_3 ratio makes the zeolite more

hydrophobic, it would be more effective to use an adsorbent with higher silica content which has more affinity for butanol compared to water.

To consider the effect of other components on butanol adsorption, Saravanan et al. (2010) used a model ABE solution, containing 1.28 wt% butanol, 0.4 wt% acetone and 0.03 wt% ethanol as the feed solution. In their study, the adsorption kinetics of adsorbents was also investigated. ZSM-5 powder showed the highest adsorption rate whereas larger particle extrudates showed solid phase diffusion limitation. By investigating the adsorption equilibrium for cell free fermentation broth containing 1.28 wt% butanol and model ABE broth, similar results were obtained which showed that the other components in the broth such as sugars did not have a noticeable effect on butanol adsorption [77].

Saravanan et al. (2010) also studied desorption of butanol adsorbed on zeolite ZSM-5. They incubated zeolite in an aqueous solution containing 1.28 wt% butanol. Then they carried out desorption experiment in three steps by gradually increasing the temperature. In the first step, water was desorbed almost completely at 45°C, and increasing the temperature to 150°C, butanol was desorbed. At higher temperatures (more than 250°C), butanol was released as 1-butene. In the second step (150°C), the butanol concentration in solution was 84.3% and a further 18% recovery was done in the form of butene at the final step (at temperature more than 250°C) [77].

Sharma and Chung (2011) synthesized a high silica MEL type zeolite (ZSM11) for the preparation of a mixed matrix membrane for butanol pervaporation [86]. To characterize the material, they used it as a butanol adsorbent in an aqueous solution. Care was taken to prepare zeolite material with the highest level of uniformity in pore size, shape and volume. Interestingly, a relatively high butanol adsorption of 222 mg/g was obtained at 0.2 g/L butanol concentration at equilibrium at 30 °C. However, desorption of butanol was not investigated.

One of the new processes for butanol adsorption-desorption has been investigated by Águeda et al. (2013). They performed the adsorption-drying-desorption (ADD) process using silicalite pellets to produce high butanol concentration from dilute model solutions. Their results showed that butanol 98% (w/w) could be recovered from 0.5 to 2% (w/w) dilute aqueous solutions by drying the column by air at low temperatures (50-70°C)

and desorption by heated air flow at higher temperatures (130-150°C). They have also stated that the energy requirement for this process would be significantly lower (around 3.4 MJ/kg) compared to the energy content of butanol, which is 36 MJ/kg [87].

In a recent study, Faisal et al. (2013) investigated the adsorption of butanol from model solution using a hydrophobic MFI type zeolite (ZSM-5) with the SiO_2/Al_2O_3 ratio of 230 and the surface area of 403 m^2/g. They performed the batch adsorption experiments in closed stirred glass Erlen-Meyer flasks at pH 4 for 48 h on shaking tables. They observed that MFI zeolite had a higher adsorption capacity for butanol than the other two components. The adsorption capacities for butanol, acetone and ethanol at 10 g/L of equilibrium concentration were 106, 95 and 62 mg/g, respectively. In another series of experiments they compared the adsorption capacity of MFI type zeolite for adsorption of butanol and acids (butyric acid and acetic acid) at pH 4 and 6 that are the pHs below and higher than the pKa value of the acid. It was observed that the isotherms of butyric acid and butanol were similar both showing high adsorption capacities at pH4. In fact, the adsorption capacity of butyric acid was slightly higher (120 mg/g). The researchers performed the similar experiment at pH 6 and observed that the adsorption capacity of MFI zeolite decreased significantly for butyric acid (120-25 mg/g). However, the adsorption experiments with multicomponent solutions containing all five components were not reported in this study. The researchers also studied the thermal desorption of butanol (for butanol-water model solution) and observed that most of the water desorbed at the temperature range of 55-120°C whereas the butanol desorption mainly took place at the temperature range of 120-160°C [88].

Metal organic frameworks (MOF) are new types of adsorbents used for butanol adsorption. Cousin Saint Remi et al. (2011, 2012) have investigated the adsorption of butanol from butanol-water and ABE model solutions [89,90]. These researchers have proposed a conceptual adsorption process in which three adsorption steps are used to remove butanol from a dilute solution. In their proposed process, the first adsorption step is used to adsorb butanol using an adsorbent with a high selectivity for it. Subsequently, butanol is recovered by desorption to collect the concentrated product. As the butanol concentration is high in the recovered product, phase separation occurs. This two-phase system contains a butanol rich phase containing around 80 wt% of butanol and aqueous

phase containing around 7 wt% of butanol. In the second step, the aqueous phase is recycled to the adsorption column again and the butanol rich phase is pumped to an adsorption column to adsorb water and produce a highly concentrated butanol stream. The outlet stream of this step, containing higher butanol concentration, is sent to the third adsorption to remove the side-products and water traces still present to obtain pure butanol as the final product of the process. These researchers have performed investigations to find the best adsorbents for the first and third adsorption steps. Testing silicalite, activated carbon and ZIF-8 (zeolitic imidazolate frameworks) as the adsorbents showed that silicalite had a very low adsorption capacity for butanol whereas activated carbon and ZIF had similar capacities in static adsorption experiments (around 300 mg/g for a butanol-water solution with initial concentration of 40 g/L) and ZIF-8 had also the same adsorption capacity in the breakthrough experiments. They also studied the effect of the presence of other compounds such as acids, sugar and salts on butanol adsorption. Results showed that the presence of other compounds did not affect butanol adsorption on ZIF-8. The desorption of butanol from a saturated ZIF column with an ethanol-butanol-water aqueous solution was also investigated by stepwise increase of temperature with a nitrogen gas flow. During the initial desorption time, only a small amount of butanol and ethanol was detected at the outlet of the column. After 1 h at 90°C, a concentrated mixture of the desorbed stream contained 42.2 wt% and 13.5 wt% of butanol and ethanol, respectively. The concentration of butanol in the desorbed phase did not increase when the temperature was increased to 150°C [89,90].

Popescu et al. (2003) investigated the adsorption-desorption process of volatile organic compounds (VOCs) using two activated carbons (R-CAFS and AC40). Butanol was one of the VOCs they studied and their results showed that the adsorption capacity remained constant even after seven subsequent adsorption-desorption cycles. They also observed that the used adsorbents had equal or higher BET surface area and micropore volume compared to the new adsorbents. The method these researchers used for VOCs desorption was isothermal desorption with air as a purge gas at 150°C [91].

Since one of the most important factors of a good adsorbent is its selectivity for butanol, it is essential to know the effect of the presence of each component of the ABE fermentation broth on butanol adsorption capacity and selectivity of the adsorbents.

Abdehagh et al. (2013) have studied butanol adsorption using different types of zeolites and ACs to investigate their performance as butanol adsorbents [76]. The adsorption capacity and the rate of adsorption were measured. In addition, the effect of the presence of other ABE broth components on butanol adsorption was also investigated. The results showed that activated carbons (AC F- 600 and F-400) had the fastest adsorption rate and AC F-400 had the highest butanol adsorption capacity (294 mg/g in equilibrium with 10 g/L butanol concentration in the aqueous solution). The selectivity of activated carbon F-400 for butanol was also studied to conclude that it had the highest affinity for butanol adsorption and to a lesser extent for butyric acid whereas the adsorption capacity for other fermentation broth components was very low. It was also stated that the presence of ethanol and sugars (glucose and xylose) did not have any effect on butanol adsorption and the presence of acetone slightly decreased the butanol adsorption capacity at low butanol concentrations. However, the presence of acids affected the adsorbent capacity more significantly for butanol and this effect was more pronounced for the presence of butyric acid [76].

A summary of butanol adsorption capacities for different adsorbents taken from the literature is presented in Table 4. As it reveals, the highest capacities were obtained for activated carbon and ZIF-8. Zeolites also showed high adsorption capacities [78,83,86,89]. Some other adsorbents such as PVP have high adsorption capacities for butanol but their low selectivity discounts them as good adsorbents.

For a successful adsorption process, the recovery of adsorbed butanol must be efficient and the adsorbent needs to be used for a large number of cycles. At this time, very little information is available in the literature and a comprehensive desorption study needs to be undertaken.

2.4.2. Butanol adsorption from fermentation broths

In addition to the low butanol concentration obtained in ABE fermentation, the separation is further complicated by the presence of many other components, namely acetone, ethanol, acetic acid, butyric acid, cells, nutrients and unreacted substrates. To recover butanol, it is paramount to identify an adsorbent that has a high selectivity in the presence of these other compounds. Table 5 presents the main studies that investigated

butanol adsorption from fermentation broths. Maddox, as early as 1982, used silicalite and found an adsorption capacity of 85 mg/g in equilibrium with an aqueous solution containing 2.4 g/L butanol [92]. Ennis et al. (1987) used silicalite and XAD16 resin to adsorb butanol between the two stages of a two-stage continuous fermentation and found that sugar utilization, productivity and solvent concentration further increased in the second stage [54]. The total sugar utilization increased from 28.1 g/L for the standard batch fermentation to 36 and 33.6 g/L for the two-stage fermentation using silicalite and XAD16 resin, respectively. Groot and Luyben (1986) observed medium decolourization when they used activated carbon as a butanol adsorbent from fermentation broth and concluded that nutrients and other components were also adsorbed [78].

Table 5 Selected results of butanol adsorption from fermentation broths

Reference	Adsorbent	Butanol concentration in aqueous phase (g/L)	Butanol adsorption capacity (mg/g)	Adsorption temperature (°C)
Maddox (1982) [92]	Silicalite	11.7 16.8	64 85	-
Nielsen et al. (1988) [75]	XAD4 XAD7 Bonopore Bonopore-nitrated	4 4 4 4	27 22 23 13	-
Oudshoorn et al. (2009) [84]	CBV28014 (ZSM-5)	2.73[a] 6.57[b] 6.8[b]	98 117 100	25 25 25
Nielsen and Prather (2009) [93]	Poly(styrene-co-DVB) derived resins: Dowex1 Optipore L-493 Dowex1 Optipore SD-2	 20 20	 175 152	 30-37 30-37

[a]The feed was filtered fermentation broth

[b]The feed was unfiltered fermentation broth

Nielsen et al. (1988) investigated butanol adsorption from a fermentation broth containing butanol (4.0 g/L), acetone (1.2 g/ L), ethanol (0.5 g/L), acetic acid (1.5 g/L) and butyric acid (1.1 g/ L). XAD4 had the highest separation factor for butanol from the fermentation broth among all the adsorbents they studied (see Table 5). It was found that the selectivity of all the adsorbents decreased when a fermentation broth was used

compared to butanol-water solutions. It was also observed that bonopore had no effect on the fermentability of the medium while XAD4 caused a drastic reduction in medium fermentability. However, normal fermentability was regained when yeast extract was added to the fermenter, which clearly indicated that some of the nutrients were adsorbed more importantly on XAD4 [75].

Yang et al. (1994) used polyvinylpyridine (PVP) resin as a butanol adsorbent in the fermentation broths where the concentration of butanol was varied between 0.43 and 14.65 g/ L. The isotherm for the PVP resin showed a linear relationship with all ABE fermentation products. For butanol adsorption, the equilibrium concentration in the PVP resin was 7.62 times higher than in the fermentation broth whereas it was 6.25 times higher for ABE model solutions. They observed the formation of loose biofilms around the adsorbent particles, concluding that adsorbent particles provided a suitable surface for cell growth and shortened the lag phase leading to the cell growth phase. It was observed that glucose was not adsorbed and the adsorbent had a higher affinity for butyric acid than for butanol. In this integrated process, where inhibition was partly mitigated, the final butanol concentration and the productivity increased by 54 and 130%, respectively, compared to the fermentation without PVP resin [71].

Oudshoorn et al. (2009) studied the performance of ZSM-5 for butanol adsorption from filtered and unfiltered fermentation broths and found that the maximum adsorption capacity was very similar with values of 115 and 100 mg/g, respectively. The maximum adsorption capacity (where the isotherm reaches a plateau) of butanol from an ABE model solution was 111 mg/g (see Table 4). They observed that when the polarity of a component increased, the affinity of the adsorbent for that component decreased, which is a characteristic of hydrophobic adsorbents. Butyric acid behaviour was an exception, since it showed a higher affinity even though it is more polar than butanol. Oudshoorn et al. (2009) explained this behaviour by specific interactions between butyric acid and zeolite [84].

Nielsen and Prather (2009) investigated a wide range of polymeric resins having a high butanol affinity for the separation of butanol from fermentation broths. They found that, in general, resins derived from poly(styrene-co-divinylbenzene) had the highest butanol affinity. For example, Dowex1 Opti-pore L-493 and SD-2 were able to reduce

the aqueous butanol concentration by 85 and 83% with maximum butanol adsorption capacities of 175 and 152 mg/g resin (Table 5). Nielsen and Prather (2009) also examined butanol recovery from the polymeric resin phase under vacuum at 100 °C and condensing the vapour in a cold trap. They suggested that thermal desorption for butanol recovery from resins is an efficient and economic method. In their study, no performance loss was seen for resins after butanol recovery. 83.4% recovery of adsorbed butanol was achieved. In spite of the high adsorption capacity, the selectivity and the cost of these resins are drawbacks for their industrial use as butanol adsorbents [93].

Based on the results of different investigations of butanol separation by adsorption, it can be concluded that this energy efficient technique could be integrated economically to industrial butanol bioproduction processes. In order to design a high performance butanol adsorption process, further investigation is required. For instance, a perfect adsorbent that adsorbs butanol and none of the other components in the fermentation broth needs to be found. Also, an energy-efficient desorption process to recover all the butanol adsorbed in the adsorption process needs to be designed.

Another idea would be to combine adsorption and gas stripping techniques. In this combined process, the stripping gas would pass through the fermenter and the outlet stream containing the solvents would pass through an adsorption column filled with an adsorbent that is selective to butanol. This hybrid process will benefit from the advantages of both of these techniques to produce a product with higher butanol concentrations.

2.5 Perstraction

In a perstraction separation process, a membrane is used with one side contacting a solvent and the other side contacting the solution where the desired products are produced. Perstraction process can therefore be used to remedy the potential problems associated with liquid-liquid extraction such as emulsion formation and toxicity since the fermentation broth and the extractant are not contacted directly. The success of this separation process is in the selection of a membrane highly selective to butanol and of an extractant with a very high partition coefficient. This development of perstraction has been hampered by the high cost of membranes and their tendency to clog and foul

[4,10,15,95]. Some suitable extractants for butanol perstraction are oleyl alcohol, polypropylene glycol, tributyrin, 1-octanol, 1-dodecanol and 2-ethyl-1-hexanol. Jeon and Lee (1987) used oleyl alcohol flowing in a silicone tubing to preferentially recover butanol and found that the substrate utilization increased from 60 to 601 g/L and butanol production increased from 11.5 to 147.4 g/ L when fed-batch fermentation with product removal was used instead of simple batch fermentation [32]. They also investigated the integrated reactor-separator system for continuous operation lasting several weeks. Continuous feeding and withdrawal of extractant were started when the butanol concentration in the extractant reached about 10 g/L. The products contained in the extractant were then recovered by vacuum distillation and the stripped extractant was reused. They found that the average extraction efficiency (the amount of product extracted divided by the total amount produced in fermentation) in 12 subsequent runs was 87% and the average product yield was 0.224 g(product)/g(glucose consumed). They stated that high selective permeability of butanol in silicone membrane and high butanol distribution coefficient in oleyl alcohol were the main reasons for obtaining the high concentration of butanol in the extractant [96].

Shukla et al. (1989) also studied butanol perstraction using hydrophobic hollow fibre tubular fermenter-extractor and 2-ethyl-1-hexanol as the extractant [97]. They stated that butanol distribution coefficient in the extractant was 6.09 and the separation factor [y(1- x)/x(1-y)] was 276.7. But it was mentioned that although gases (hydrogen and carbon dioxide) were removed by the extractant through the tube side of the hollow fibre tubular fermenter-extractor, gas build up was observed repeatedly in the shell side of the fermenter during the perstraction. These researchers recommended increasing the number of fibres or improving the top exit section of the fermenter design to solve this problem. Grobben et al. (1993) studied perstraction for recovery of butanol using a hydrophobic membrane and oleyl alcohol/decane mixture as an extractant [98]. In their perstraction system for butanol separation from fermentation broths, an increase in yield was observed; however, after 50 h of operation, membrane fouling severely affected the separation. To prevent membrane fouling, they used hydrophilic membrane instead of hydrophobic membranes. They also used microfiltration to remove solids and cells. In this integrated process using MFA (methylated fatty acid) as an extractant, they observed

higher yield but lower productivity. They explained that this lower productivity may be due to a slow extraction rate, a detrimental shear effect on the microorganism caused by rotation in the microfiltration membrane or an undesirable effect of polysulphone microfiltration membrane on the cells.

Qureshi and Maddox (2005) investigated butanol perstraction from a fermentation broth using oleyl alcohol as an extractant and silicone tubing as a membrane [70]. They found that there were some problems to be addressed to make perstraction technique more efficient. The first problem was low ABE flux through the membrane. They stated that this problem could be solved using a better membrane having higher flux for butanol such as silicalite-based membranes. In another study, Groot et al. (1990) investigated the butanol separation by perstraction using silicone tubes and different polar and non-polar extractants [95]. They found that the highest selectivity for butanol perstraction from butanol-water solutions can be achieved by non-polar extractants such as hexane, oleyl alcohol and isopropyl myristate. They also compared perstraction results with those obtained in pervaporation. They found that although butanol selectivity in perstraction is high, permeate fluxes in pervaporation are much higher than the fluxes in perstraction, which is believed to be associated to a higher mass transfer resistance in the solvent phase.

2.6 Pervaporation

Pervaporation is a separation process where a binary or multicomponent liquid mixture is separated by partial vaporization through a membrane. For butanol separation via pervaporation, the liquid feed mixture is in direct contact with one side of a hydrophobic membrane whereas the permeate is removed as a vapour on the permeate side, due to the presence of vacuum or a sweep gas. The permeating vapour can then be recovered by condensation in cold traps. During pervaporation, setting the feed temperature provides the required heat of vaporization. The simplified schematic diagram of a pervaporation process is shown in Fig. 5 [5,15,17,99].

Pervaporation possesses strong attributes for butanol separation from fermentation broths since, in addition to low energy requirement, it does not affect microorganisms, and losses of nutrients and substrates are prevented [17,99,100]. Pervaporation is

particularly promising for separating azeotropic mixtures with close boiling points since the separation is dependent on the solubility/sorption and diffusivity of components rather than their volatility [101].

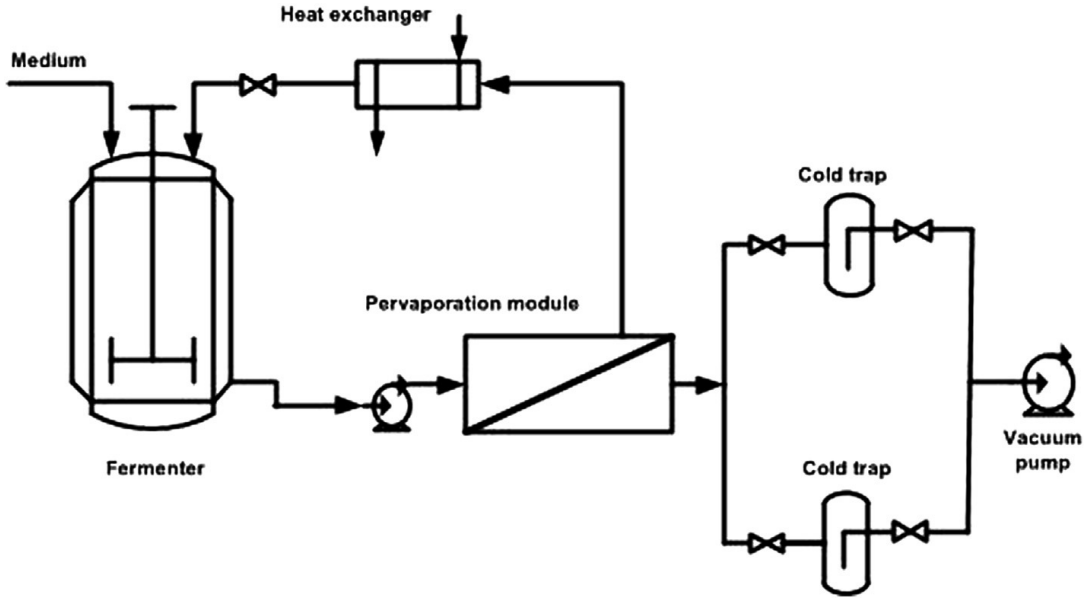


Figure 5 Schematic diagram of butanol separation from fermentation broth by pervaporation.

Other advantages of pervaporation are the high selectivity, low operating temperature, reasonable performance to cost ratio, possibility of modular design and the absence of a separating agent that could cause product contamination [102]. However, since pervaporation is a rate-controlled process, the permeation flux through the membrane is generally low and large membrane surface area is required. The mass transfer in pervaporation is defined by the sorption-diffusion (or solution-diffusion) model and the mechanism of mass transfer includes a three-step process, as shown in Fig. 6: 1) sorption of the permeate from the liquid feed to the upstream side of the membrane, 2) diffusion of the permeate through the membrane, and 3) desorption and evaporation of the permeate at the downstream side of the membrane under low pressure [19,102]. The component solubility/sorption and diffusivity dictate the selectivity and the permeability of the membrane. The solubility/sorption of a component in a membrane is determined by permeate-membrane interaction, and its diffusivity depends on its molecular size, shape

and mass. Pervaporation efficiency may be limited by some factors including low fluxes, membrane swelling and concentration polarization [8,33,100,103,104].

Two parameters, the separation factor and the flux, account for the effectiveness of pervaporation for a given membrane. The separation factor is a measure of the selective removal of the desired component from dilute solution and the flux represents the rate at which the permeate passes through the membrane per unit surface area per time [1,10,24]. The selectivity (S) is given by Equation (1):

$$S = \frac{\frac{y}{1-y}}{\frac{x}{1-x}} \quad (1)$$

where y and x are the mass fraction of the desired component in the permeate and the feed, respectively. The permeate flux (J) is given by Equation (2):

$$J = \frac{W}{A \times t} \quad (2)$$

where J is the permeate flux (g/m^2h) which is calculated as the mass of permeate (W) passing through the membrane per surface area (A) per time (t).

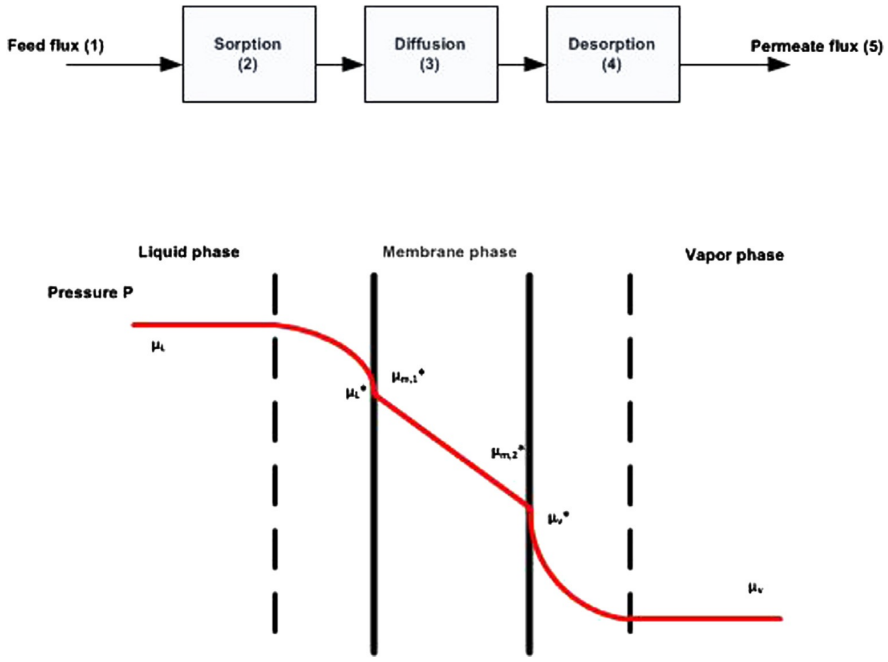


Figure 6 The simplified mechanism of mass transfer in butanol pervaporation.

Table 6 Different results for butanol separation by pervaporation from model solutions by silicone and silicone-silicalite membranes

Membrane	Feed solution	Feed butanol conc. (g/L)	Flux ($g/m^2.h$)	Membrane thickness (µm)	Separation factor	Feed temp. (°C)	Ref.
Silicone tube	Binary	17	8.6[a]	400	45	37	[130]
	Binary	0.94	4.4[a]	400	57	37	
Silicone tube	Binary	12	6.2[a]	1000	64	37	[126]
	Binary	16	10[a]	1000	51	37	
	Binary	18	14.5[a]	1000	45	37	
Silicone tubes	Binary	4.3	22[a]	250	50	37	[132]
	Binary	4	100[a]	180	35	30	
Silicone-silicalite	Binary	8	170[a]	180	28	30	
Silicone tubes	Broth	12.5	8.9[a]	240	-	32	[135]
	Broth	15.5	11.5[a]	240	-	32	
Silicone membrane	Binary	33	69-105	-	25.2-58.2	43	[14]
	Binary	37.6	158-215	-	20.8-68.3	80	
Silicone-silicalite-1 composite membrane (1 :1)	ABE model	10	119	226	70	78	[99]
	ABE model	10	30	306	10	35	
Silicone-silicalite-1 composite membrane (1 :1.5)	ABE model	10	80	306	50	55	
	ABE model	10	100	306	90	78	
	ABE model	10	110	306	120	85	
	ABE model	10	90	50	50	35	
	ABE model	10	100	90	55	35	
	ABE model	10	160	170	70	35	
	ABE model	10	240	306	97	35	
	ABE model	7	105	306	85	78	
	ABE model	30	85	306	130	78	

	ABE model	50	75	306	160	78	
	ABE model	64	60	306	180	78	
	ABE model	9.9	12.5	170	32	35	
	ABE model	9.9	50	170	40	65	
	ABE model	9.9	85	170	44	78	
Silicone	ABE model	10	80	50	30	35	
	ABE model	10	30	90	32	35	
	ABE model	10	10	170	32	35	
	ABE model	10	8	306	30	35	
Thin film silicone	Binary	10	52.8	50	42	30	[33]
membrane	Binary	10	145.3	50	49.6	50	
	Binary	10	350	50	48.6	70	
	Binary	10	8	300	31	30	
	Binary	10	52	300	58	50	
Dense silicalite-silicone	Binary	10	110	300	96	70	
membrane	Binary	10	50	29	44	30	
	Binary	10	137	29	50	50	
	Binary	10	344.4	29	47.8	70	
PERVAP-1070 (silicalite-	Binary	10	62.8	19	85.9	30	
silicone composite)	Binary	10	191	19	111	50	
	Binary	10	607	19	93	70	
Thin-film silicalite-silicone membrane	ABE model	15-20	907	15-16	49	70	

[a] Butanol flux g/m^{2}h

Other parameters that greatly affect the selectivity and the flux of pervaporation are membrane material and thickness, vacuum or sweep gas partial pressure, feed temperature, and feed composition [14,100,102]. To develop membranes with a high permeate flux, efforts have been made to modify the membrane structure from a dense thick film to an asymmetric or composite structure. It has been observed that by reducing the membrane thickness, the flux increased, but the selectivity decreased. The reduction in membrane thickness is limited by the pore size, porosity and surface roughness of the membrane and the support [105].

The comparison of the pervaporation results obtained for butanol by different authors is not trivial because of the myriad of variables that have been considered such as the operating temperature, butanol concentration in the feed, membrane material and thickness, and vacuum pressure. Tables 6 and 7 show the results obtained in butanol pervaporation from model solutions. Table 6 shows that for butanol separation by pervaporation, silicone and silicone-silicalite membranes are the most frequently used membranes. Table 7 presents other hydrophobic pervaporation membranes used for butanol separation: polytetrafluoroethylene (PTFE), poly-propylene (PP), polyurethane

(polyether based) (PUR), polyether block-amide (PEBA), poly(vinylidinedifluoride) (PVDF), polydimethylsiloxane (PDMS), poly(1-(trimethylsilyl)- 1-propyne) (PTMSP) and polyamideimide (PAI) containing cyclodextrin (CD) [16,17,27,33].

Table 7 Some results for butanol separation by pervaporation from model solutions using different kinds of membranes.

Membrane	Feed solution	Butanol Conc. (g/L)	Flux ($g/m^2.h$)	Membrane thickness (µm)	Separation factor	Feed temp. (°C)	Ref.
PDMS	Binary	70	~500	80	30	40	[136]
	Binary	20	~150	80	30	40	
	Binary	10	~100	80	30	40	
T-PDMS	Binary	143	570	145	42	40	[137]
TX-PDMS	Binary	134	530	90	38	40	
PDMS	Binary	3	55	90	32	37	[124]
with dual support:	Binary	10	65	90	30	37	
porous PE sheet and	ABE model	3	54	90	25	37	
perforated alloy metal	ABE model	10	61	90	30	37	
Polydimethylsiloxane	Binary	20	132	65	32	37	[17]
(PDMS)/PE-1/Brass	Binary	20	41	200	52	37	
support composite	Binary	5	80	65	25	37	
	Binary	20	132	65	32	37	
PDMS/ceramic	Binary	10	993	-	16.56	37	[108]
	ABE model	10	1033	-	21.43	37	
PDMS/Ceramic	Binary	10	457.7	10	26.1	40	[128]
composite	Binary	40	730	10	23	40	
Membrane	Binary	10	307	10	28	30	
	Binary	10	822	10	25	60	
Silicalite-filled	Binary	10	90	190-210	36.3	40	[111]
PDMS	Binary	25	130	190-210	-	40	
	Binary	50	230	190-210	-	40	
	Ternary	10	110	190-210	-	40	
	Ternary	25	160	190-210	-	40	
	Ternary	50	250	190-210	-	40	
Silicalite filled	Binary	0.4	125	-	18	25	[138]
PDMS	Binary	0.4	550	-	10	65	
	Binary	0.35		-		25	
	Binary	0.35		-		65	
Silicalite-1/PDMS	Binary	10	17		35	33	
hybrid	Binary	10	120		75	71	
Membrane	Binary	34	27		25	33	
	Binary	34	180		50	71	
	ABE model	10	65		40	33	
	ABE model	10	275		70	71	
Commercial	Binary	6	250	-	14.2	33	[28]
Sulzer Co., Perv.2200	Binary	11	420	-	10	33	
	Binary	20	540	-	7.4	33	

	Binary	50	1640	-	2.4	33	
PDMS with 15% w/w	Binary	10	2	-	12	30	[127]
Silicalite	Binary	100	65	-	40	30	
		10	1	-	12	30	
		100	35	-	20	30	
Styrene Butadiene Rubber (SBR)							
Celfa b	Binary	2	-	10	24	22	[16]
	Binary	2	-	10	35	40	
	Binary	2	-	125	41	22	
P 500-1	Binary	2	-	125	50	40	
PTMSP	Binary	20	2600	16	80	62	[109]
	Binary	20	1100	16	135	37	
PDMS	Binary	20	700	16	84	26	
	Binary	20	250	75	50	62	
	Binary	20	100	75	40	37	
	Binary	20	50	75	30	26	
PTFE	Binary	1.25	170	40	5.6	39	[103]
	Binary	2.5	540	40	2.6	39	
	Binary	3	490	40	2.7	39	
	ABE model	1.25	980	40	9.5	39	
	ABE model	2.5	580	40	12.4	39	
	ABE model	3	530	40	14.8	39	
	Binary	1.25	35	40	5.6	30	
	Binary	1.25	170	40	8.5	40	
	Binary	1.25	805	40	9.9	50	
	Binary	1.25	2100	40	5.2	55	
	ABE model	1.25	19	40	7.2	30	
	ABE model	1.25	980	40	9.5	40	
	ABE model	1.25	1790	40	13.7	50	
PTMSP	Binary	0.3	124	-	51	25	[139]
	Binary	1.5	60	-	55	25	
	Binary	6	436	-	61	70	
	Binary	0.3	762	-	47	70	
	Binary	1.5	1030	-	70	70	
	Binary	6	2097	-	41		
PEBA2533	Binary	50	60.2	100	8.2	23	[131]
	Binary	50	179	30	5.9	23	
	Quaternary	19.1	41	100	13.2	23	
PEBA2533	Binary	4	420	30	11	29	[8]
	Binary	4	300	30	19	40	
	Binary	4	200	30	25	50	
	Binary	4	110	30	31	60	
PEBA	ABE model	75	9.975[a]	-	17	37	[143]
PDMS	ABE model	75	3.911[a]	-	-	37	
Surface modified	MPAW[b]	75	1710	110	5.47	30	[116]
PVDF	MPAW	75	3500	110	4.9	50	
	MPAW	75	5283	110	2.98	70	
	MPAW	25	1000	110	7.2	40	
	MPAW	75	2400	110	5	40	
	MPAW	125	4800	110	3.3	40	
Polyamide-imide	nButanol /t-	200	3.5	80	1.35	-	[101]
(PAI)	Butanol	440	10	80	1.35	-	
		825	12	80	0.8	-	
Polyamide-imide	Binary	~15	846	-	56	75	[103]

(PAI)/polyetherimide (PEI) hollow fiber							
HTPB-Based	Binary	9	14	140	10.5	40	[29]
Polyurethaneurea	Binary	24	17	140	21	40	
Membrane	Binary	27.5	19	140	21	40	
	Ternary	20	17	140	-	40	
	Ternary	45	24	140	-	40	
	Ternary	5	-	140	15	40	
	Ternary	8	-	140	24	40	
Oleyl alcohol membrane supported PP	Binary	4	80	25	180	30	[119]
Liquid	Binary	15	6.4	50	108.4	54	[120]
(trioctylamine(TOA))	Binary	20	8.2	50	126.4	54	
membrane	Binary	25	10	50	141.2	54	

[a]butanol flux ($g/m^2.h$)
[b] MPAW: model pharmaceutical aqueous waste

Membranes made of silicalite have shown some potential for butanol separation because of their high selectivity for butanol. Silicalite, an aluminium free zeolite and molecular sieve, is capable of adsorbing organic solvents such as butanol from liquid solutions. Silicalite selectivity for all alcohols is very high and the selectivity increases as the chain length increases from C1 to C5 [14,19,106]. To improve the performance of silicone membrane, Menchavez and Ha (2013) studied the effect of ultrasound irradiation on butanol pervaporation using silicone tubing as the membrane. They observed that by performing 8 h of pervaporation at 60°C with ultrasound irradiation for ABE model solution (for butanol initial concentration of 10 g/L) butanol concentration and flux increased in the permeate side by 18 g/L (from 203 to 221 g/L) and 13%, respectively [107].

Amongst the polymeric membranes, PDMS membranes are also considered to have a higher potential due to their good process performance (high selectivity), high hydrophobicity, and good thermal, chemical and mechanical stability. In addition, they are easily and economically manufactured [16,17,19,33,108]. Fadeev et al. (2000) investigated PDMS and PTMSP dense films as membranes in butanol pervaporation and found that both selectivity and permeate flux of PTMSP dense film were higher than for PDMS membranes. However, following a very high initial flux, a sharp decline (from 380 to 220 g/m^2h) was observed during the first 20 h of pervaporation and it is likely due

to the membrane fouling [109]. To improve the PDMS membrane performance and achieve a higher permeate flux, ceramic was used as a support of PDMS membrane and the results showed that the flux increased significantly [108,110].

Jonquières and Fane (1997) investigated the possibility of combination of silicalite and PDMS as a membrane to benefit the advantages of both materials as butanol pervaporation membrane. They prepared silicalite filled PDMS and found that the incorporation of silicalite increased the separation factor by 35% [111]. Qureshi et al. (1999) found that incorporating silicalite in silicone membrane can increase both the permeate flux and butanol separation factor [99]. Vankelecom et al. (1995) also investigated the effect of zeolite Y and ZSM-5 embedded in a PDMS membrane and concluded that adding zeolite Y caused an increase in water flux but using ZSM-5 in PDMS membrane reduced the membrane swelling and increased the organic flux [112]. Shao and Kumar (2009) used ZSM-5 filled PDMS membrane and showed that ZSM-5 particles could be uniformly dispersed in PDMS membrane to improve the membrane selectivity for butanol [113]. To improve the performance of silica (SiO_2) filled PDMS membranes, Beltran et al. (2013) investigated butanol pervaporation from model solutions using PDMS membrane filled with surface-functionalized silica nanoparticles [114]. They found that this membrane performed better than PDMS membrane at 60 °C and lower temperatures and outperformed this polymeric membrane even at 70°C. Si-DMPS/PDMS is the PDMS membrane filled with phenyl-functionalized SiO_2 used in that study. In the pervaporation experiment to separate butanol from a model solution containing 1.5 wt% butanol, this membrane had the butanol flux of 48 g/m^2h and separation factor of 51 at 30 °C whereas the butanol flux and separation factor for PDMS membrane were 33 g/m^2h and 46, respectively. They stated that this mixed matrix membrane (MMM) did not have the defects of other nano-sized fumed silica filled polymeric membranes which is due to the hydrophilic (i.e. the presence of silanol groups) property of SiO_2 and consequently its incompatibility with organophilic polymers [114]. Liu et al. (2013) performed similar experiments with PEBA instead of PDMS. They prepared a mixed matrix membrane using zeolitic imidazolate frameworks (ZIF-71) particles as the filler for PEBA polymeric membrane. They also noticed that both separation factor and permeate flux increased compared to the PEBA membrane [115].

In another study, García et al. (2009) used two commercially available membranes for butanol pervaporation: (1) Celfa (composite membrane made of PDMS having an active layer thickness of 10 mm) and (2) P 500-1 (a dense membrane made of a silicone elastomer that consists of dimethyl and methyl vinyl siloxane copolymer having a thickness of 125 mm). They found that Celfa membrane had higher permeation fluxes but lower butanol selectivity [16]. Li et al. (2010) used porous polyethylene (PE) and brass as the supports to prepare the three-layer PDMS/PE-1/brass support composite membrane for butanol pervaporation and found that the total permeate flux and separation factor both increased by placing a layer of hydrophobic polyethylene (PE) between PDMS and the metal support. They reported that this enhancement was due to the high porosity of PE. The use of stiff and porous metallic or ceramic materials is expensive and it is difficult to manufacture modules with large surface area. They indicated that dual support membranes, which should be fabricated using inexpensive materials, require extensive evaluation to determine the optimal thickness of each layer, porosity and surface specifications of the intermediate layer [17].

Srinivasan et al. (2007) used surface modified polyvinylidenedifluoride (PVDF) membrane for butanol separation from model pharmaceutical aqueous waste. They modified the membrane surface by using silicone grease as an ultrathin layer on the membrane surface to improve the pervaporation performance and they obtained higher permeation flux [116].

Böddeker et al. (1990) used polyether block amine (PEBA), polyurethane (PUR) and polydimethylsiloxane (PDMS) membranes for butanol pervaporation. The highest butanol flux was obtained with PEBA membranes but the water flux was also high and therefore its selectivity was low. The PEBA separation factor lies between PDMS and PUR. On the other hand, PUR had the lowest selectivity and flux whereas PDMS had the highest separation factor for butanol [117].

Peters et al. (2006, 2008) attempted to modify the membrane structure from a dense thick film to an asymmetric or composite structure to develop a membrane with a high flux. They used thin-film polyvinyl alcohol (PVA) with intermediate γ-Al_2O_3 layer and α-Al_2O_3 hollow fibre substrate for butanol dehydration. They concluded that ceramic supports exhibit high surface porosity and superior structural stability (no swelling and

compaction) while showing negligible transport resistance. Another advantage of ceramic-supported membranes is the ease of its regeneration at the end of membrane lifetime since the organic layer can be washed by an appropriate solvent or by thermal treatment to recover the expensive ceramic support [33,118].

Liquid membranes such as oleyl alcohol, trioctylamine and supported ionic liquid membranes were also used as pervaporation membranes for butanol separation from model solutions and fermentation broths [114,119-121]. Izák et al. (2008) used tetrapropylammonium tetracyano-burate ionic liquid mixed with PDMS as the liquid membrane to separate butanol from fermentation broth and found that this membrane has a high selectivity and stability throughout the fermentation. They stated that the separation factor increased from 2.2 to 10.9 when they used ionic liquid (IL)-PDMS membrane instead of PDMS membrane alone. Matsumura and Kataoka (1987) used liquid membrane supported by hydrophobic porous polypropylene to separate butanol from model solutions. By performing butanol pervaporation experiments using both silicone membrane and liquid membrane, they found that the selectivity and permeability of the liquid membrane prepared by oleyl alcohol were 2.6 times higher than what was obtained with silicone membrane. However, they stated that if this pervaporation system with the liquid membrane was coupled with a continuous fermentation, the liquid membrane became thinner after directly contacting a large volume of fermentation broth. They postulated that even though the solubility of oleyl alcohol in water is low, it still progressively dissolves. They suggested that saturating the broth with oleyl alcohol would solve the problem [119]. To increase the stability of liquid membranes Thongsukmak and Sirkar (2007) immobilized trioctylamine (TOA) as a liquid membrane in pores of polypropylene hollow fibre substrate having a nanoporous coating. They found that this membrane had a very good stability over many hours of operation and prevented the membrane contamination and loss of liquid membrane to the feed solution [120]. Using gelled ionic liquid membrane was another technique to prevent the liquid loss over the pervaporation process. Plaza et al. (2013) prepared a membrane by the gelation of [bmim][PF6] into the pores of PTFE hollow fibres and found that this gelled liquid membrane has the permeability comparable to the polymeric membrane but more selectivity for butanol [122].

Matsumura et al. (1988) used oleyl alcohol and PVA membranes to investigate its energy saving of pervaporation in butanol purification. For this purpose, they compared the energy required in butanol separation from a 5 g/L aqueous butanol solution, in different separation systems such as conventional distillation and pervaporation coupled with distillation. They found that by using pervaporation as a pre-treatment process of butanol separation, the energy required was one-tenth of that of conventional distillation. According to their report, the energy required for butanol separation in distillation system was 79.5 MJ/kg while the energy requirement for the same process was 7.4 MJ/kg for the combination of pervaporation (oleyl alcohol membrane)/distillation and 6.5 MJ/kg for the combination of two pervaporation techniques using oleyl alcohol and PVA membranes. Butanol final concentration in the product was 99.9 wt% for distillation and pervaporation/distillation processes and 95 wt% for the combination of the two pervaporation methods [123].

2.6.1. Effect of feed composition on flux and separation factor

One of the parameters in pervaporation, having a significant impact on the permeate flux and selectivity, is the initial butanol concentration in the feed. Some researchers stated that an increase in butanol concentration in the feed led to an increase in the permeate flux while the water flux and the butanol selectivity remained nearly constant [8,16,120,124,125]. Other researchers confirmed the increase in the permeate flux but a decrease in selectivity with an increase in butanol concentration [14,126]. According to these reports, the reduction in selectivity may be caused by swelling with higher butanol concentration which loosens the polymer matrix and facilitates the sorption of all solutes [104,116,127]. Li et al. (2010) observed that by increasing butanol concentration in the feed, the water flux increased slightly while butanol flux increased sharply. The separation factor increased when butanol content increased from 5 to 10 g/L and for a further increase in butanol content, the selectivity remained constant in the range of 32-34 up to a butanol concentration of 20 g/L [17]. Surprisingly, Vrana et al. (1993) observed that even a ten-fold increase in butanol concentration in ABE model solution did not have an effect on the permeate flux and selectivity [103]. Tong et al. (2010) studied the effect of the feed composition and its effect on the permeate flux and

selectivity for a ternary solution (butanol-acetone-water). They found that by increasing the butanol concentration in the feed, both acetone and butanol fluxes increased but the increase in butanol flux was much more significant. On the other hand, the water flux was nearly constant. They indicated that butanol selectivity sharply increased with an increase in the butanol concentration in the feed [27].

2.6.2. Effect of temperature on flux and separation factor

Temperature is another parameter to consider in butanol pervaporation for optimizing the pervaporation system. Qureshi and Blaschek (1999) found that by increasing the temperature, the permeate flux increased [14]. Jitesh et al. (2000) observed the same effect on the permeate flux but this increase was accompanied by a decrease in selectivity which was attributed to enhanced thermal motions of polymer segments causing an increase in water permeability [127]. Srinivasan et al. (2007) obtained similar results and found that by increasing the temperature from 30 to 70°C, the total flux increased from 1710 to 5283 g/m^{2}h and the separation factor decreased from 5.47 to 2.98. They stated that this effect may be due to higher polymer chain flexibility and subsequently a larger free volume of the polymer matrix for the diffusion of water [116]. Other researchers found that an increase in temperature caused an increase in both butanol flux and selectivity [16,128,129]. Li et al. (2010) also found that an increase in temperature led to an increase in the total permeate flux. They stated that a temperature increase up to 36°C will increase the selectivity, and a further increase in temperature will cause a slight reduction in selectivity [17]. Fadeev et al. (2000) indicated that the permeate flux increased with temperature and the butanol selectivity increased linearly with temperature for PDMS membranes while for PTMSP membranes, the selectivity reached a maximum of 135 at 37°C [109]. Vrana et al. (1993) indicated that, contrary to the feed composition, the temperature had a pronounced effect on permeate flux and selectivity. They reported that by increasing the temperature, the permeate flux increased 10 and 60 folds for butanol pervaporation from ABE model and butanol-water solutions, respectively [103]. This implies that ABE model solution is less temperature sensitive than butanol-water solution.

2.6.3. Effect of membrane thickness on flux and separation factor

As expected, an increase in thickness led to a decrease in the permeate flux and an increase in selectivity [33,130,131]. Thongsukmak and Sirkar (2007) found that solvent flux increased by a factor of 5 by reducing the trioctylamine (TOA) layer thickness to one-third and one-fifth of the full membrane wall thickness showing that the membrane thickness is a compromise between permeate flux and selectivity [120].

Table 8 Results available in the literature for butanol separation by pervaporation from fermentation broths

Membrane	Butanol Conc. (g/L)	Flux ($g/m^2.h$)	Membrane thickness (μm)	Separation factor	Feed temp. (°C)	Ref.
Silicone	-	34.8	600	18.8	41	[36]
Silicone tube	-	-	250	20	37	[130]
Silicone tubes	17.5	13.5a	1000	46	37	[126]
	16	10a	1000	52	37	
	14.8	8a	1000	57	37	
	14	7a	1000	58	37	
	12	6.2a	1000	64		
Silicone tubes	2	1.5a	240	-	32	[135]
	8.5	6a	240	-	32	
	12.5	8.9a	240	-	32	
	15.5	11.5a	240	-	32	
Silicone	3.3	27	-	7	36	[36]
	4.4	31	-	11	36	
	5	31	-	13	36	
	6	29	-	19	36	
Silicone membrane	10	84	170	44	78	[99]
Silicone membrane	6	13	-	30	35	[13]
Silicalite membrane	7	122	306	70	78	
Silicone-silicalite composite membrane [b]	12	89	306	97	78	[106]
Silicone-silicalite composite membrane [c]	10	119	226	70	78	
Silicone membrane	9.1	84	170	44	78	
Thin film silicalite filled silicone composite membrane	15-16	850-900	13-15	50	70	[33]
PDMS	-	621	201	19.8	37	[142]
PDMS	-	10.4	250	11	30	[140]
PDMS	-	418	201	15.7	35	[141]
PDMS/PE/perforated alloy metal	-	57	-	18-20	37	[124]
PDMS/ceramic	-	741	-	21.4	37	[128]
PDMS/Ceramic composite membrane	11	1050	-	15	37	[110]
PDMS/Ceramic composite membrane	-	338-847	-	5.1-27.1	37	[108]

NA	NA	NA	NA	30	37	[125]
40 membrane fibres made of PP	-	7.1a	400	5		[134]
PP hollow fibre	1	1.5	-	3.2	35	[53]
	4	-	-	3	35	
	6.5	-	-	2.8	35	
Hydroxyterminatedpolybutadiene-baesdpolyethaneurea (HTPB-PU)	11	9.7	140	13.7	40	[29]

[a]butanol flow rate ($g/m^2.h$)
[b]silicalite /silicone ratio: (1.5:1)
[c]silicalite /silicone ratio: (1:1)

2.6.4. Effect of the presence of other components on flux and separation factor

Groot et al. (1991) found that the presence of ethanol did not affect the butanol pervaporation flux but a decrease in selectivity was observed [132]. Jonquières and Fane (1997) compared the permeate flux in butanol pervaporation from binary and ternary (butanol-acetone-water) model solutions and reported that acetone did not have a significant effect on the permeation flux [111]. On the other hand, Li et al. (2011) indicated that the presence of other components in solution had a negative coupling effect on butanol flux. There was a 16 and 28% reduction in overall butanol mass transfer coefficient in ABE model solutions and fermentation broths, respectively [124]. Vrana et al. (1993) also reported that the presence of butyric acid had a negative effect on butanol selectivity and Jitesh et al. (2000) found that the presence of ethanol and acetone in the feed increased the butanol sorption. As it can be seen, no consistent trend is observed on the effect of other components on butanol pervaporation from ABE model solutions and broths [103,127].

Table 8 shows the results of different studies for butanol pervaporation recovery from fermentation broths. Groot et al. (1984) used pervaporation in a continuous fermentation and found that when pervaporation was applied for the separation of butanol from the fermentation broth, a separation factor of 30 was obtained [125]. Table 8 reveals once more that silicone and silicone-silicalite membranes were more frequently used for butanol pervaporation. For silicone tubes (thickness of 1000 mm), the highest separation factor obtained was 64 at 37°C [126]. For silicone-silicalite membranes, a separation factor of 97 was obtained with a thinner membrane (306 mm) at 78 °C [106]. It can be seen that the permeation flux and separation factor were low for butanol pervaporation from fermentation broth using polypropylene (PP) membranes. Matsumura et al. (1992)

used a liquid membrane (oleyl alcohol supported with polypropylene) in butanol separation from fermentation broth which was produced in a continuous fermentation. They found that in comparison to continuous fermentation without butanol removal, using in situ pervaporation recovery doubled the butanol production rate. However they observed that the surface of the membrane after the continuous fermentation was completely fouled with some viscous materials. They used a hollow fibre membrane cartridge with 40 membrane fibres and stated that poor distribution of feed in the hollow fibres was one of the reasons that contributed to the reduction of permeate flux and selectivity. The highest selectivity obtained for that membrane was 5, a very low value [133,134].

3 Concluding remarks

The production of sustainable and renewable liquid biofuels as a replacement for petroleum-based fuels is the subject of unprecedented research that is enthusiastically supported by governments. Biobutanol, with its numerous advantages, is now attracting as much interest as ethanol and biodiesel as a renewable biofuel. However, the production of biobutanol is facing important engineering challenges, the main one being the low final butanol concentration at the end of fermentation due to butanol toxicity for microorganisms used to convert sugars into butanol. This low concentration impacts directly on the efficiency and economics of biobutanol recovery. To partly mitigate this problem, research efforts are mainly directed at improving the ability of the microorganisms to tolerate higher butanol concentration and at using the most efficient separation technique to separate butanol efficiently and economically at the end of the fermentation or in situ recovery during the fermentation.

This paper has mainly examined the aspect of butanol separation and recovery from dilute solutions. Numerous separation techniques have been reviewed such as adsorption, gas stripping, liquid-liquid extraction (LLE), perstraction, reverse osmosis and pervaporation. The LLE method is one of the separation techniques used in butanol removal processes. However, the loss of expensive solvents and their toxicity to microorganisms hinder this technique to emerge as an efficient method in butanol

separation. Perstraction, another separation method, solving some of these problems such as loss of extractant and its toxicity, still has some shortcomings such as low permeate flux due to the high mass transfer resistance in solvent phase.

On the other hand, pervaporation is considered as a potential choice for butanol recovery having high separation factor and permeate flux, simultaneously. Having no effect on microorganism and nutrient and being energy efficient are other specifications that strengthen pervaporation technique as a desired butanol recovery method. This technique has resolved the shortcomings of liquid-liquid extraction and perstraction methods by removing the solvent and its high mass transfer resistance from the process. However, more investigation is needed to find or prepare high performance membranes in addition to optimize the operating conditions such as temperature, permeate side pressure and membrane material and thickness. As discussed, the other promising technique in butanol separation is adsorption. Although many accomplishments have been achieved in finding suitable adsorbents for butanol adsorption from dilute solutions, effective desorption still remains to be developed for this process.

Consequently, based on the available information, it can be stated that more investigation is needed to develop an integrated process using one or a combination of different practical techniques from a technical and economical point of view to separate butanol from fermentation broth in large biobutanol production plants.

4 Nomenclature

A	Membrane surface area (m^2)
J	Permeate flux ($g/m^2.h$)
S	Selectivity (dimensionless)
s	Second (s)
t	Time (h)
x	Mass fraction of component in product/permeate (dimensionless)
y	Mass fraction of component in feed (dimensionless)
μ	Chemical potential

5 List of abbreviations

ABE	Acetone-Butanol-Ethanol
ADD	Adsorption-drying-desorption
ADP	Adenosine diphosphate

CA	*Clostridium acetobutylicum*
CAMD	Computer-aided molecular design
CB	*Clostridium beijerinkii*
CD	Cyclodextrin
IL	Ionic liquid
LLE	Liquid-liquid extraction
MFA	Methylated fatty acid
MMM	Mixed matrix membrane
MOF	Metal organic frameworks
NHOC	Net heat of combustion
MPAW	Model pharmaceutical aqueous waste
PAI	Polyamide-imide
PE	Polyethylene
PEBA	Polyetherblock-amide
PDMS	Polydimethylsiloxane
PP	Polypropylene
PTFE	Polytetrafluoroethylene
PTMSP	Poly(-1-(trimethylsilyl)-1-propyne)
PUR	Polyurethane
PVA	Polyvinyl alcohol
PVDF	Poly(vinylidinedifluoride)
PVP	Polyvinylpyridine
RO	Reverse osmosis
RTIL	Room temperature ionic liquid
ZIF	Zeolitic imidazolate frameworks
ZSM-5	Zeolite Socony Mobil-5

6 References

[1] Ezeji TC, Qureshi N, Blaschek HP. Butanol fermentation research: upstream and downstream manipulations. Chem Rec 2004;4:305-14.

[2] Antoni D, Zverlov VV, Schwarz WH. Biofuels from microbes (mini review). Appl Microbiol Biotechnol 2007;77:23-35.

[3] D-llomonaco C, Fava F, Gonzalez R. The path to next generation biofuels: successes and challenges in the era of synthetic biology. Microb Cell Fact 2010;9:3.

[4] Dürre P. Biobutanol: an attractive biofuel. Biotechnol J 2007;2:1525-34.

[5] Qureshi N, Ezeji TC. Butanol, 'a superior biofuel' production from agricultural residues (renewable biomass): recent progress in technology. Biofuels Bioprod Bioref 2008;2:319-30.

[6] Harvey BG, Meylemans HA. The role of butanol in the development of sustainable fuel technologies. J Chem Technol Biotechnol 2011;86:2-9.

[7] Ezeji TC, Qureshi N, Blaschek HP. Production of acetone, butanol and ethanol by BA101 and in situ recovery by gas stripping. World J Microbiol Biotechnol 2003;19:595-603.

[8] Fouad EA, Feng X. Use of pervaporation to separate butanol from dilutes aqueous solutions: effects of operating conditions and concentration polarization. J Membr Sci 2008;323:428-35.

[9] Shapovalov OI, Ashkinazi LA. Biobutanol: biofuel of second generation. Russ J Appl Chem 2008;81(12):2232-6.

[10] Zheng YN, Li LZ, Xian M, Ma YJ, Yang JM, Xu X, et al. Problems with the microbial production of butanol. J Ind Microbiol Biotechnol 2009;36:1127-38.

[11] Kumar M, Gayen K. Developments in biobutanol production: new insights. Appl Energy 2011;88:1999-2012.

[12] Thirmal C, Dahman Y. Comparisons of existing pre- treatment, saccharification, and fermentation processes for butanol production from agricultural residues. Can J Chem Eng 2012;90:745-61.

[13] Meagher MM, Ghureshi N, Hutkins R. Silicalite membrane and method for the selective recovery and concentration of acetone and butanol from model ABE solutions and fermentation broth. US Patent 5755967, 1998 May 26.

[14] Qureshi N, Blaschek HP. Production of acetone butanol ethanol (ABE) by a hyper-producing mutant strain of *Clostridium beijerinckii* BA101 and recovery by pervaporation. Biotechnol Prog 1999;15:594-602.

[15] Ezeji TC, Qureshi N, Blaschek HP. Bioproduction of butanol from biomass: from genes to bioreactors. Curr Opin Biotechnol 2007;18:220-7.

[16] García V, Pongracz E, Muurinen E, Keiski RL. Recovery of n- butanol from salt containing solutions by pervaporation. Desalination 2009;241:201-11.

[17] Li S, Srivastava R, Parnas RS. Separation of 1-butanol by pervaporation using a novel tri-layer PDMS composite membrane. J Membr Sci 2010;363:287-94.

[18] Richmond C, Han B, Ezeji TC. Stimulatory effects of calcium carbonate on butanol productivity by solventogenic *Clostridium* species. Cont J Microbiol 2011;5(1):18-28.

[19] Ezeji TC, Milne C, Price ND, Blaschek HP. Achievements and perspectives to overcome the poor solvent resistance in acetone and butanol-producing microorganisms. Appl Microbiol Biotechnol 2010;85:1697-712.

[20] Atsumi S, Cann AF, Connor MR, Shen CS, Smith KS, Brynildsen MP, et al. Metabolic engineering of *Escherichia coli* for 1-butanol production. Metab Eng 2008;10:305-11.

[21] Jones DT, Woods DR. Acetone-butanol fermentation revisited. Microbiol Rev 1986;50(4):484-524.

[22] Dürre P. Fermentative butanol production bulk chemical and biofuel. Ann N Y Acad Sci 2008;1125:35-32.

[23] Patakova P, Lipovsky J, Paulova L, Linhova M, Fribert P, Rychtera M, et al. Continuous production of butanol by bacteria of genus *Clostridium*. J Chem Chem Eng 2011;5:121-8.

[24] Ezeji TC, Qureshi N, Blaschek HP. Production of acetone butanol (AB) from liquefied corn starch, a commercial substrate, using *Clostridium beijerinckii* coupled with product recovery by gas stripping. J Ind Microbiol Biotechnol 2007;34:771-7.

[25] Qureshi N, Hughes S, Maddox IS, Cotta MA. Energy-efficient recovery of butanol from model solutions and fermentation broth by adsorption. Bioprocess Biosyst Eng 2005;27:215-22.

[26] El-Zanati E, Abdel-Hakim E, El-Ardi O, Fahmy M. Modeling and simulation of butanol separation from aqueous solutions using pervaporation. J Membr Sci 2006;280:278-83.

[27] Tong C, Bai Y, Wu J, Zhang L, Yang L, Qian J. Pervaporation recovery of acetone-butanol from aqueous solution and fermentation broth using HTPB-based polyurethaneurea membranes. Sep Sci Technol 2010;45:751-61.

[28] Xue C, Zhao J, Liu F, Lu C, Yang ST, Bai FW. Two-stage in situ gas stripping for enhanced butanol fermentation and energy-saving product recovery. Bioresour Technol 2013;135:396-402.

[29] Chen Y, Zhou T, Liu D, Li A, Xu S, Liu Q, et al. Production of butanol from glucose and xylose with immobilized cells of *Clostridium acetobutylicum*. Biotechnol Bioprocess Eng 2013;18:234-41.

[30] He Q, Chen H. Improved efficiency of butanol production by absorbed lignocellulose fermentation. J Biosci Bioeng 2013;115(3):298-302.

[31] Roffler SR, Blanch HW, Wilke CR. In situ extractive fermentation of acetone and butanol. Biotechnol Bioeng 1988;31:135-43.

[32] Jeon YJ, Lee YY. Membrane-assisted extractive butanol fermentation. Ann N Y Acad Sci 1987;506:536-42.

[33] Huang J, Meagher MM. Pervaporative recovery of n-butanol from aqueous solutions and ABE fermentation broth using thin-film silicalite-filled silicone composite membranes. J Membr Sci 2001;192:231-42.

[34] Jang YS, Malaviya A, Lee SY. Acetone-butanol-ethanol production with high

productivity using *Clostridium acetobutylicum* BKM19. Biotechnol Bioeng 2013;110(6):1646-53.

[35] Wu YD, Xue C, Chen LJ, Bai FW. Effect of zinc supplementation on acetone-butanol-ethanol fermentation by *Clostridium acetobutylicum*. J Biotechnol 2013;165:18-21.

[36] Qureshi N, Blaschek HP. Butanol production using *Clostridium beijerinckii* BA101 hyper-butanol producing mutant strain and recovery by pervaporation. Appl Biochem Biotechnol 2000;84-86:225-34.

[37] Ni Y, Xia Z, Wang Y, Sun Z. Continuous butanol fermentation from inexpensive sugar-based feedstocks by *Clostridium saccharobutylicum* DSM 13864. Bioresour Technol 2013;129:680-5.

[38] Ezeji TC, Karcher PM, Qureshi N, Blaschek HP. Improving performance of a gas stripping-based recovery system to remove butanol from *Clostridium beijerinckii* fermentation. Bioprocess Biosyst Eng 2005;27:207-14.

[39] Chen CK, Blaschek HP. Acetate enhances solvent production and prevents degeneration in *Clostridium beijerinckii* BA101. Appl Microbiol Biotechnol 1999;52:170-3.

[40] Park CH, Okos MR, Wankat PC. Acetone-butanol-ethanol (ABE) fermentation and simultaneous separation in a trickle bed reactor. Biotechnol Prog 1991;7:185-94.

[41] Ezeji TC, Qureshi N, Blaschek HP. Acetone butanol ethanol (ABE) production from concentrated substrate: reduction in substrate inhibition by fed-batch technique and product inhibition by gas stripping. Appl Microbiol Biotechnol 2004b;63:653-8.

[42] Qureshi N, Maddox IS. Integration of continuous production and recovery of solvents from whey permeate: use of immobilized cells of *Clostridium acetobutylicum* in a fluidized bed reactor coupled with gas stripping. Bioprocess Eng 1991;6:63-9.

[43] Ezeji TC, Qureshi N, Blaschek HP. Microbial production of a biofuel (acetone-butanol-ethanol) in a continuous bioreactor: impact of bleed and simultaneous product removal. Bioprocess Biosyst Eng 2013;36:109-16.

[44] Mollah AH, Stuckey DC. Feasibility of in situ gas stripping for continuous acetone-butanol fermentation by *Clostridium acetobutylicum*. Enzyme Microb Technol 1993;15:200-7.

[45] Ennis BBM, Marshall CT, Maddox IS, Paterson AHH. Continuous product recovery by in-situ gas stripping/ condensation during solvent production from whey permeate using *Clostridium acetobutylicum*. Biotechnol Lett 1986;8(10):725-30.

[46] Groot WJ, Van der Lans RGJM, Luyben KChAM. Batch and continuous butanol

fermentations with free cells: integration with product recovery by gas-stripping. Appl Microbiol Biotechnol 1989;32:305-8.

[47] Qureshi N, Blaschek HP. Recovery of butanol from fermentation broth by gas stripping. Renew Energy 2001;22:557-64.

[48] Lu C, Zhao J, Yang ST, Wei D. Fed-batch fermentation for n- butanol production from cassava bagasse hydrolysate in a fibrous bed bioreactor with continuous gas stripping. Bioresour Technol 2012;104:380-7.

[49] Setlhaku M, Heitmann S, Go´rak A, Wichmann R. Investigation of gas stripping and pervaporation for improved feasibility of two-stage butanol production process. Bioresour Technol 2013;136:102-8.

[50] Richter H, Qureshi N, Heger S, Dien B, Cotta MA,Angenent LT. Prolonged conversion of n-butyrate to n- butanol with *Clostridium saccharoperbutylacetonicum* in a two- stage continuous culture with in-situ product removal. Biotechnol Bioeng 2012;109:913-21.

[51] Xue C, Zhao J, Lu C, Yang ST, Bai F, Tang IC. High-titer n- butanol production by *Clostridium acetobutylicum* JB200 in fed-batch fermentation with intermittent gas stripping. Biotechnol Bioeng 2012;109:2746-56.

[52] Maddox IS, Qureshi N, Roberts-Thomson IS. Production of acetone-butanol-ethanol from concentrated substrates using *Clostridium acetobutylicum* in an integrated fermentation- product removal process. Process Biochem 1995;30(3):209-15.

[53] Qureshi N, Maddox IS, Friedl A. Application of continuous substrate feeding to the ABE fermentation: relief of product inhibition using extraction, perstraction, stripping, and pervaporation. Biotechnol Prog 1992;8:382-90.

[54] Ennis BBM, Qureshi N, Maddox IS. In-line toxic product removal during solvent production by continuous fermentation using immobilized *Clostridium acetobutylicum*. Enzyme Microb Technol 1987;9:672-5.

[55] Evans PJ, Wang HY. Enhancement of butanol formation by *Clostridium acetobutylicum* in the presence of decanol-oleyl alcohol mixed extractants. Appl Environ Microbiol 1988;54(7):1662-7.

[56] Roffler SR, Blanch HW, Wilke CR. In-situ recovery of butanol during fermentation. Part 1: batch extractive fermentation. Bioprocess Eng 1987;2:1-12.

[57] Cockrem MCM, Meyer RA, Lightfoot EN. Recovery of n- butanol from dilute solution by extraction. Sep Sci Technol 1986;21(10):1059-74.

[58] Eckert G, Schiigerl K. Continuous acetone-butanol production with direct product removal. Appl Microbiol Biotechnol 1987;27:221e8.

[59] Dadgar AM, Foutch GL. Improving the acetone-butanol fermentation process with liquid-liquid extraction. Biotechnol Prog 1988;4(1):36-9.

[60] Adhami L, Griggs B, Himerbrook P, Taconi K. Liquid-liquid extraction of butanol from dilute aqueous solutions using soybean-derived biodiesel. J Am Oil Chem Soc 2009;86:1123-8.

[61] Cascon RH, Choudhari SK, Nisola GM, Vivas EL, Lee DJ, Chung WJ. Partitioning of butanol and other fermentation broth components in phosphonium and ammonium based ionic liquids and their toxicity to solventogenic clostridia. Sep Purif Technol 2011;78:164-74.

[62] Ha SH, Maib NL, Koo YM. Butanol recovery from aqueous solution into ionic liquids by liquid-liquid extraction. Process Biochem 2010;45:1899-903.

[63] Garcia-Chavez LY, Garsia CM, Schuur B, de Haan AB. Biobutanol recovery using nonfluorinated task-specific ionic liquids. Ind Eng Chem Res 2012;51:8293-301.

[64] Dhamole PB, Wang Z, Liu Y, Wang B, Feng H. Extractive fermentation with non-ionic surfactants toenhance butanol production. Biomass Bioenergy 2012;40:112-9.

[65] Kraemer K, Harwardt A, Bronnerberg R, Marquardt W. Separation of butanol from acetone-butanol-ethanol fermentation by a hybrid extractionedistillation process. Comput Chem Eng 2011;35:949-63.

[66] Kraemer K, Harwardt A, Bronnerberg R, Marquardt W. Separation of butanol from acetone-butanol-ethanol fermentation by a hybrid extraction-distillation process. In: 20th European symposium on computer aided process engineering e ESCAPE20 2010.

[67] García A, Lannotti EL, Fischer JL. Butanol fermentation liquor production and separation by reverse osmosis. Biotechnol Bioeng 1986;18:785-91.

[68] Oudshoorn A, Van der Wielen LAM, Straathof AJJ. Assessment of options for selective 1-butanol recovery from aqueous solution. Ind Eng Chem Res 2009;48:7325-36.

[69] Groot WJ, Van der Lans RGJM, Luyben KChAM. Technologies for butanol recovery integrated with fermentations. Process Biochem 1992;27:61-75.

[70] Qureshi N, Maddox IS. Reduction in butanol inhibition by perstraction: utilization of concentrated lactose/whey permeate by *Clostridium acetobutylicum* to enhance butanol fermentation economics. Food Bioprod Process 2005;83(C1):43-52.

[71] Yang X, Tsai GJ, Tsao GT. Enhancement of in situ adsorption on the acetone-butanol fermentation by *Clostridium acetobutylicum*. Sep Technol 1994;4:81-92.

[72] Regdon I, Kiraly Z, Dekany I, Lagaly G. Adsorption of 1- butanol from water on modified silicate surfaces. Colloid Polym Sci 1994;272:1129-35.

[73] Regdon I, Dekany I, Lagaly G. A new way for calculating the adsorption capacity from surface excess isotherms. Colloid Polym Sci 1998;276:511-7.

[74] Takeuchi Y, Iwamotob H, Miyata M, Seiichi Asano S, Haradac M. Adsorption of l-butanol and p-xylene vapour with high silica zeolites and their mixtures. Sep Technol 1995;5:23-34.

[75] Nielsen L, Larsson M, Hoist O, Mattiasson B. Adsorbents for extractive bioconversion applied to the acetone-butanol fermentation. Appl Microbiol Biotechnol 1988;28:335-9.

[76] Abdehagh N, Tezel FH, Thibault J. Adsorbent screening for biobutanol separation by adsorption: kinetics, isotherms and competitive effect of other compounds. Adsorption 2013;19:1263-72.

[77] Saravanan V, Waijers DA, Ziari M, Noordermeer MA. Recovery of 1-butanol from aqueous solutions using zeolite ZSM-5 with a high Si/Al ratio; suitability of a column process for industrial applications. Biochem Eng J 2010;49:33-9.

[78] Groot WJ, Luyben KChAM. In situ product recovery by adsorption in the butanol/isopropanol batch fermentation. Appl Microbiol Biotechnol 1986;25:29-31.

[79] Lin X, Wu J, Fan J, Qian W, Zhou X, Qian C, et al. Adsorption of butanol from aqueous solution onto a new type of macroporous adsorption resin: studies of adsorption isotherms and kinetics simulation. J Chem Technol Biotechnol 2012;87:924-31.

[80] Eom MH, Kim W, Lee J, Cho JH, Seung D, Park S, et al. Modeling of a biobutanol adsorption process for designing an extractive fermentor. Ind Eng Chem Res 2013;52:603-11.

[81] Ying H, Lin X, Chen Y, Chen X, Bai J, Xiaong J, et al. Method for separating butanol. Pub.No.: US 2013/0158303 A1, 2013.

[82] Lin X, Li R, Wen Q, Wu J, Fan J, Jin X, et al. Experimental and modeling studies on the sorption breakthrough behaviors of butanol from aqueous solution in a fixed-bed of KA-I resin. Biotechnol Bioprocess Eng 2013;18:223-33.

[83] Sowerby B, Crittenden BD. Vapour phase separation of alcohol-water mixtures by adsorption onto silicalite. Gas Sep Purif 1988;2:177-83.

[84] Oudshoorn A, van der Wielen LAM, Straathof AJJ. Adsorption equilibria of bio-based butanol solutions using zeolite. Biochem Eng J 2009;48:99-103.

[85] Oudshoorn A, van der Wielen LAM, Straathof AJJ. Desorption of butanol from zeolite material. Biochem Eng J 2012;67:167-72.

[86] Sharma P, Chung WJ. Synthesis of MEL type zeolite with different kinds of morphology for the recovery of 1-butanol from aqueous solution. Desalination

2011;275:172-80.

[87] Águeda, VI, Delgado JA, Uguina MA, Sotelo JL, García Á . Column dynamics of an adsorptionedryingedesorption process for butanol recovery from aqueous solutions with silicalite pellets. Sep Purif Technol 2013;104:307-21.

[88] Faisal A, Zarebska A, Saremi P, Korelskiy D, Ohlin L, Rova U, et al. MFI zeolite as adsorbent for selective recovery of hydrocarbons from ABE fermentation broths. Adsorption 2013. http://dx.doi.org/10.1007/s10450-013-9576-6.

[89] Cousin Saint Remi J, Baron G, Denayer J. Adsorptive separations for the recovery and purification of biobutanol. Adsorption 2012;18:367-73.

[90] Cousin Saint Remi J, Rémy T, Van Hunskerken V, van de Perre S, Duerinck T, Maes M, et al. Biobutanol separation with the metaleorganic framework ZIF-8. ChemSusChem 2011;4:1074-7.

[91] Popescu M, Joly JP, Carre´ J, Danatoiu C. Dynamical adsorption and temperature-programmed desorption of VOCs (toluene, butyl acetate and butanol) on activated carbons. Carbon 2003;41:739-48.

[92] Maddox IS. Use of silicalite for the adsorption of n-butanol from fermentation liquids. Biotechnol Lett 1982;4:759-60.

[93] Nielsen DR, Prather KJ. In situ product recovery of n-butanol using polymeric resins. Biotechnol Bioeng 2009;102:811-21.

[94] Holtzapple MT, Brown RF. Conceptual design for a process to recover volatile solutes from aqueous solutions using silicalite. Sep Technol 1995;4:213-29.

[95] Groot WJ, Soedjak HS, Donck PB, van tier Lans RGJM, Luyben KChAM, Timmer JMK. Butanol recovery from fermentations by liquid-liquid extraction and membrane solvent extraction. Bioprocess Eng 1990;5:203-16.

[96] Jeon YJ, Lee YY. In situ product separation in butanol fermentation by membrane-assisted extraction. Enzyme Microb Technol 1989;11:575-82.

[97] Shukla R, Kang W, Sirkar KK. Acetone-butanol-ethanol (ABE) production in a novel hollow fiber fermentor-extractor. Biotechnol Bioeng 1989;34:1158-66.

[98] Grobben NG, Eggink G, Cuperus FP, Huizing HJ. Production of acetone, butanol and ethanol (ABE) from potato wastes: fermentation with integrated membrane extraction. Appl Microbiol Biotechnol 1993;39:494-8.

[99] Qureshi N, Meagher MM, Hutkins RW. Recovery of butanol from model solutions and fermentation broth using a silicalite/silicone membrane. J Membr Sci 1999;158:115-25.

[100] Qureshi N, Blaschek HP. Butanol recovery from model solution/fermentation broth by pervaporation: evaluation of membrane performance. Biomass Bioenergy 1999;17:175-84.

[101] Wang Y, Chung TS, Wang H, Hong Goh S. Butanol isomer separation using polyamide-imide/CD mixed matrix membranes via pervaporation. Chem Eng Sci 2009;64:5198-209.

[102] Lipnizki F, Hausmanns S, Laufenberg G, Field R, Kunz B. Review: use of pervaporation-bioreactor hybrid processes in biotechnology. Chem Eng Technol 2000;23:569-77.

[103] Vrana DL, Meagher MM, Hutkins RW, Duffield B. Pervaporation of model acetone-butanol-ethanol fermentation product solutions using polytetrafluoroethylene membranes. Sep Sci Technol 1993;28:2167-78.

[104] Wang Y, Goh SH, Chung TS, Na P. Polyamide-imide/ polyetherimide dual-layer hollow fiber membranes for pervaporation dehydration of C1eC4 alcohols. J Membr Sci 2009;326:222-33.

[105] Peters TA, Poeth CHS, Benes NE, Buijs HCWM,Vercauteren FF, Keurentjes JTF. Ceramic-supported thin PVA pervaporation membranes combining high flux and high selectivity; contradicting the flux-selectivity paradigm. J Membr Sci 2006;276:42-50.

[106] Qureshi N, Meagher MM, Huang J, Hutkins RW. Acetone butanol ethanol (ABE) recovery by pervaporation using silicaliteesilicone composite membrane from fed-batch reactor of *Clostridium acetobutylicum*. J Membr Sci 2001;187:93-102.

[107] Menchavez RN, Ha SH. Ultrasound-enhanced recovery of butanol/ABE by pervaporation. Appl Biochem Biotechnol 2013. http://dx.doi.org/10.1007/s12010-013-0196-0.

[108] Wei P, Zhu DW. Acetone-butanol-ethanol (ABE) fermentation using *Clostridium acetobutylicum* XY16 and in situ recovery by PDMS/ceramic composite membrane. Bioprocess Biosyst Eng 2012;35:1057-65.

[109] Fadeev AG, Meagher MM, Kelley SS, Volkov VV. Fouling of poly(-1-(trimethylsilyl)-1-propyne) membranes in pervaporative recovery of butanol from aqueous solutions and ABE fermentation broth. J Membr Sci 2000;173:133-44.

[110] Li G, Wei W, Wu H, Dong X, Jiang M, Jin W. Pervaporation performance of PDMS/ceramic composite membrane in acetone butanol ethanol (ABE) fermentation-PV coupled process. J Membr Sci 2011;373:121-9.

[111] Jonquières A, Fane A. Filled and unfilled composite GFT PDMS membranes for the recovery of butanols from dilute aqueous solutions: influence of alcohol polarity. J Membr Sci 1997;125:245-55.

[112] Vankelecom IFJ, Depre D, De Beukelaer S, Uytterhoeven JB. Influence of zeolites in PDMS membranes: pervaporation of water/alcohol mixtures. J Phys Chem 1997;99:13193-7.

[113] Shao P, Kumar A. Separation of 1-butanol/2,3-butanediol using ZSM-5 zeolite-filled polydimethylsiloxane membranes. J Membr Sci 2009;339:143-50.

[114] Beltran AB, Nisola GM, Choi SS, Kim Y, Chung WJ. Surface- functionalized silica nanoparticles as fillers in polydimethylsiloxane membrane for the pervaporative recovery of 1-butanol from aqueous solution. J Chem Technol Biotechnol 2013. http://dx.doi.org/10.1002/ jctb.4090.

[115] Liu S, Liu G, Zhao X, Jin W. Hydrophobic-ZIF-71 filled PEBA mixed matrix membranes for recovery of biobutanol via pervaporation. J Membr Sci 2013. http://dx.doi.org/10.1016/ j.memsci.2013.06.025.

[116] Srinivasan K, Palanivelu K, Gopalakrishnan AN. Recovery of 1-butanol from a model pharmaceutical aqueous waste by pervaporation. Chem Eng Sci 2007;62:2905-14.

[117] Böddeker KW, Bengtson G, Pingel H. Pervaporation of isomeric butanols. J Membr Sci 1990;54:1-12.

[118] Peters TA, Benes NE, Keurentjes JTF. Hybrid ceramic- supported thin PVA pervaporation membranes: long-term performance and thermal stability in the dehydration of alcohols. J Membr Sci 2008;311:7-11.

[119] Matsumura M, Kataoka H. Separation of dilute aqueous butanol and acetone solutions by pervaporation through liquid membranes. Biotechnol Bioeng 1987;30:887-95.

[120] Thongsukmak A, Sirkar KK. Pervaporation membranes highly selective for solvents present in fermentation broths. J Membr Sci 2007;302:45-58.

[121] Izák P, Schwarz K, Ruth W, Bahl H, Kragl U. Increased productivity of *Clostridium acetobutylicum* fermentation of acetone, butanol, and ethanol by pervaporation through supported ionic liquid membrane. Appl Microbiol Biotechnol 2008;78:597-602.

[122] Plaza A, Merlet G, Hasanoglu A, Isaacs M, Sanchez J, Romero J. Separation of butanol from ABE mixtures by sweep gas pervaporation using a supported gelled ionic liquid membrane: analysis of transport phenomena and selectivity. J Membr Sci 2013;444:201-12.

[123] Matsumura M, Kataoka H, Sueki IM, Yokohama KA. Energy saving effect of pervaporation using oleyl alcohol liquid membrane in butanol purification. Bioprocess Eng 1988;3:93-100.

[124] Li SY, Srivastava R, Parnas RS. Study of in situ 1-Butanol pervaporation from A-B-E fermentation using a PDMS composite membrane: validity of solution-diffusion model for pervaporative A-B-E fermentation. Biotechnol Prog 2011;27(1):111-20.

[125] Groot WJ, Schoutens GH, Van Beelen PN, Van den Oever CE, Kossen NWF. Increase of substrate conversion by pervaporation in the continuous butanol fermentation. Biotechnol Lett 1984;6(12):789-92.

[126] Larrayoz MA, Puigjaner L. Study of butanol extraction through pervaporation in acetobutylic fermentation. Biotechnol Bioeng 1987;30:692-6.

[127] Jitesh DK, Pangarkar1 VG, Niranjan K. Pervaporative stripping of acetone, butanol and ethanol to improve ABE Fermentation. Bioseparation 2000;9:145-54.

[128] Liu G, Hou D, Wei W, Xiangli F, Jin W. Pervaporation separation of butanol-water mixtures using polydimethylsiloxane/ceramic composite membrane. Chin J Chem Eng 2011;19(1):40-4.

[129] Zhou H, Su Y, Chen X, Wan Y. Separation of acetone, butanol and ethanol (ABE) from dilute aqueous solutions by silicalite-1/PDMS hybrid pervaporation membranes. Sep Purif Technol 2011;79:375-84.

[130] Groot WJ, Van den Oever CE, Kossen NWF. Pervaporation for simultaneous product recovery in the butanol/ isopropanol batch fermentation. Biotechnol Lett 1984;6(11):709-14.

[131] Liu F, Liu L, Feng X. Separation of acetone-butanol-ethanol (ABE) from dilute aqueous solutions by pervaporation. Sep Purif Technol 2005;42:273-82.

[132] Groot WJ, den Reyer MCH, Baart de la Faille T, van der Lans RGJM, Luyben KChAM. Integration of pervaporation and continuous butanol fermentation with immobilized cells I: experimental results. Chem Eng J 1991;46:B1-10.

[133] Matsumura M, Takehara S, Kataoka H. Continuous butanol/ isopropanol fermentation in down-flow column reactor coupled with pervaporation using supported liquid membrane. Biotechnol Bioeng 1992;39:148-56.

[134] Friedl A, Qureshi N, Maddox IS. Continuous acetone- butanol-ethanol (ABE) fermentation using immobilized cells of *Clostridium acetobutylicum* in a packed bed reactor and integration with product removal by pervaporation. Biotechnol Bioeng 1991;38:518-27.

[135] Geng Q, Park CH. Pervaporative butanol fermentation by *Clostridium acetobutylicum* B18. Biotechnol Bioeng 1994;43:978-86.

[136] Favre E, Nguyen QT. Extraction of 1-butanol from aqueous solutions by pervaporation. J Chem Technol Biotechnol 1996;65:221-8.

[137] Schnabel S, Roizard SD, Nguyen T, Lochon P, Aptel P. Synthesis of novel block siloxane polymers for the removal of butanols from aqueous feed solutions. Colloids Surf A Physicochem Eng Aspects 1998;138:335-43.

[138] Fouad EA, Feng X. Pervaporative separation of n-butanol from dilute aqueous solutions using silicalite-filled poly(dimethyl siloxane) membranes. J Membr Sci 2009;339:120-5.

[139] Fadeev AG, Selinskaya YaA, Kelley SS, Meagher MM, Litvinova EG, Khotimsky VS, et al. Extraction of butanol from aqueous solutions by pervaporation through poly(1-trimethylsilyl-1-propyne). J Membr Sci 2001;186:205-17.

[140] Groot WJ, Luyben KChAM. Continuous production of butanol from a glucose/xylose mixture with an immobilized cell system coupled to pervaporation. Biotechnol Lett 1987;9(12):867-70.

[141] Hecke WV, Vandezande P, Claes S, Vangeel S, Beckers H, Diels L, et al. Integrated bioprocess for long-term continuous cultivation of *Clostridium acetobutylicum* coupled to pervaporation with PDMS composite membranes. Bioresour Technol 2012;111:368-77.

[142] Hecke WV, Hofmann T, De Wever H. Pervaporative recovery of ABE during continuous cultivation: enhancement of performance. Bioresour Technol 2013;129:421-9.

[143] Yen HW, Lin SF, Yang IK. Use of poly(ether-block-amide) in pervaporation coupling with a fermenter to enhance butanol production in the cultivation of *Clostridium acetobutylicum*. J Biosci Bioeng 2012;113(3):372-7.

SECTION – II: Adsorbent Screening

Chapter III

Adsorption Journal, 19, 1263-1272, (2013)

Adsorbent screening for biobutanol separation by adsorption: kinetics, isotherms and competitive effect of other compounds

N. Abdehagh, F. H. Tezel, J. Thibault

Department of Chemical and Biological Engineering
University of Ottawa
161 Louis-Pasteur, Ottawa, ON, Canada K1N 6N5

Corresponding author: Tel: (613) 652-5800 Ext. 6094; Email: Jules.Thibault@uottawa.ca

Abstract

Butanol, considered as one of the best renewable alternatives for gasoline, has attracted significant attention in recent years. However, biobutanol production via fermentation is plagued by the low final product concentration due to product inhibition. It is possible to enhance productivity by selectively removing biobutanol from the fermentation broth. Adsorption is one of the most promising and energy-efficient techniques for butanol separation and recovery. In the present study, different adsorbents were tested by performing kinetic and equilibrium experiments to find the best adsorbent for butanol separation. Activated carbon (AC) F-400 showed the fastest adsorption rate and the highest adsorption capacity amongst ACs and zeolites tested. AC F-400 also showed the highest affinity toward butanol and to a lesser extent for butyric acid whereas its adsorption capacity for the other main components present in acetone–butanol–ethanol fermentation broths was very low. In addition, the butanol adsorption capacity was not affected by the presence of ethanol, glucose and xylose while the presence of acetone led to a slight decrease in adsorption capacity at low butanol concentrations. On the other hand, the presence of acids (acetic acid and butyric acid) showed a significant effect on

the butanol adsorption capacity over a wide range of butanol concentration and this effect was more pronounced for butyric acid.

1 Introduction

Depletion of oil resources combined with the continuous rising of oil prices, political instability in oil-producing countries and environmental challenges are some of the reasons that have motivated significant interest for producing alternative biofuels from renewable and sustainable resources. Amongst these fermentation-derived fuels, butanol bioproduction is currently attracting attention given the advantages biobutanol has over other biofuels (Ezeji et al. 2004; Antoni et al. 2007; Dellomonaco et al. 2010). Butanol (C_4H_9OH) is a colorless liquid with a very low solubility in water and organic solvents (Thirmal et al. 2012). In recent decades, biobutanol has been considered as one of the most promising biofuels since it has enviable properties in comparison to other biofuels such as bioethanol. Higher energy density, better solubility in existing hydrocarbon fuels, net heat of combustion (NHOC) close to the one for gasoline, low vapor pressure and low corrosiveness are some of the advantages which make butanol easier to handle, transfer and work with (Ezeji et al. 2003; Dürre 2007; Fouad et al. 2008; Qureshi et al. 2005; Shapovalov et al. 2008; Harvey et al. 2011; Thompson et al. 2011). Acetone–butanol–ethanol (ABE) fermentation is the fermentative method used to produce butanol. The microorganisms used in this bioprocess are mostly anaerobic solventogenic clostridia including *Clostridium acetobutylicum* and *C. beijerinkii*. The feedstock used in ABE fermentation could be the low cost substrates such as algal biomass, soy molasses, wheat straw, corn stover, switch-grass and other agricultural wastes. Using these sources as feedstock will reduce the waste build-up that has been one of the serious concerns in recent decades (Ezeji et al. 2004; Zheng et al. 2009; Thirmal et al. 2012). The product of ABE fermentation is typically a mixture of acetone, butanol and ethanol with the ratio of 3:6:1 (by weight) and the butanol concentration in the broth is less than 2 wt% and usually in the order of 1 wt% (Meagher et al. 1998; Qureshi et al. 1999, 2005; Dürre 2007; Ezeji et al. 2007; García et al. 2009; Li and Parnas 2010;

Harvey et al. 2011). Given the very low concentration of butanol and also the presence of other components in the fermentation broth, even at very low concentrations, the major challenge remains to develop a cost-effective technique to separate and recover butanol as the final product. Distillation is the traditional recovery technique used for butanol separation and it is still the technique that is widely used for this purpose. The energy requirement for butanol separation by steam stripping distillation (24.2 MJ/kg) is comparable to the butanol NHOC (36 MJ/kg) (Qureshi et al. 2005; Shapovalov et al. 2008; Harvey et al. 2011; Oudshoorn et al. 2009a). Thus, finding another technique with lower energy demand is very important to make the biobutanol production process economically viable. Other techniques used for butanol separation from fermentation broths include liquid–liquid extraction, gas stripping, perstraction, pervaporation and adsorption. According to different investigations, the energy requirement for adsorption is the lowest compared to other techniques, as shown in Fig. 1 (Qureshi et al. 1999, 2005; El-Zanati et al. 2006; Oudshoorn et al. 2009a; Tong et al. 2010).

An important number of studies have been carried out to separate and recover butanol from model solutions and aqueous mixtures. A wide range of materials such as activated carbon (AC), polymeric resins, polyvinylpyridine, zeolites are commonly used as butanol adsorbents from model solutions and fermentation broths (Maddox 1982; Groot et al. 1986; Nielsen et al. 1988; Sowerby et al. 1988; Yang et al. 1994; Holtzapple and Brown 1995; Nielsen 2009; Oudshoorn et al. 2009b, 2012; Saravanan et al. 2010; Sharma et al. 2011; Thompson et al. 2011; Remi et al. 2011, 2012). According to the results of these studies, zeolites and ACs have the highest adsorption capacity for butanol separation from dilute solutions (Regdon and Dekany 1994; Takeuchi et al. 1995; Regdon and Dekany 1994; Oudshoorn et al. 2009b; Zheng et al. 2009; Saravanan et al. 2010; Sharma et al. 2011). However, only a few studies were performed using different adsorbents to investigate the adsorption rate and the effect of other components present in fermentation broths (Nielsen et al. 1988). The studies carried out in the literature for different adsorbents tested for butanol separation using model solutions were mainly concerned with their adsorption capacity and less attention was devoted to other significant factors influencing the adsorbent selection. In the present study, different types of zeolites and ACs were tested in adsorbent screening experiments to investigate

their performance as butanol adsorbents. The adsorption capacity and the rate of adsorption were measured for different adsorbents. In addition, the effect of the presence of other ABE broth components on butanol adsorption was also investigated.

2 Materials and methods

2.1 Materials

To test the adsorbents, a series of model solutions were prepared to simulate representative concentrations of the main chemicals present in a typical fermentation broth during the production of butanol. n-Butanol (99 % pure, Acros), acetone (95 % pure, Acros), n-butyric acid (99 % pure, Acros) and 99 % pure ethanol and acetic acid were obtained from Fisher Scientific (Fisher Scientific Co., Fair Lawn, NJ). Deionized distilled water was used to prepare all model solutions. The adsorbents tested in this study were ACs F-400 and F-600 (Calgon, Mississauga, ON, CA), zeolites NaY, ZSM-5 (CBV8014) and silicalite (Hisiv3000) (UOP, Des Plaines, IL, USA) and Multi-walled carbon nano tubes (MWCNT), 95 % pure (MK Impex-Cor. Mississauga, ON, CA). Table 1 provides the list of adsorbents used in this study and some of their properties. The adsorbent properties were obtained from information provided by the companies or publications (Feirey et al. 2006; Harlick et al. 2004).

Table 1 Different adsorbents tested in this study for butanol adsorption and some of their properties

Adsorbent	Shape	Surface area (m^2/g)	Mean particle diameter (mm)	Mean particle length (mm)	SiO_2/Al_2O_3	Mesh size	Mean pore diameter (mm)	Micropore volume (cm^3/g)
F-400	Granule	1090	0.55-0.75	-	-	12×40	-	0.43
F-600	Granule	710	1	-	-	12×40	-	0.29
NaY	Rod	-	1.588	2.659	1.8	-	0.8	-
ZSM	Rod	425	1.6	12.11	80	-	0.6	-
Silicalite	Rod	400	1.6	2.86	>1000	-	0.6	0.146
MWCNT	Powder	40-50	$>5\times10^{-5}$	$10\text{-}30\times10^{-3}$	-	-	-	-

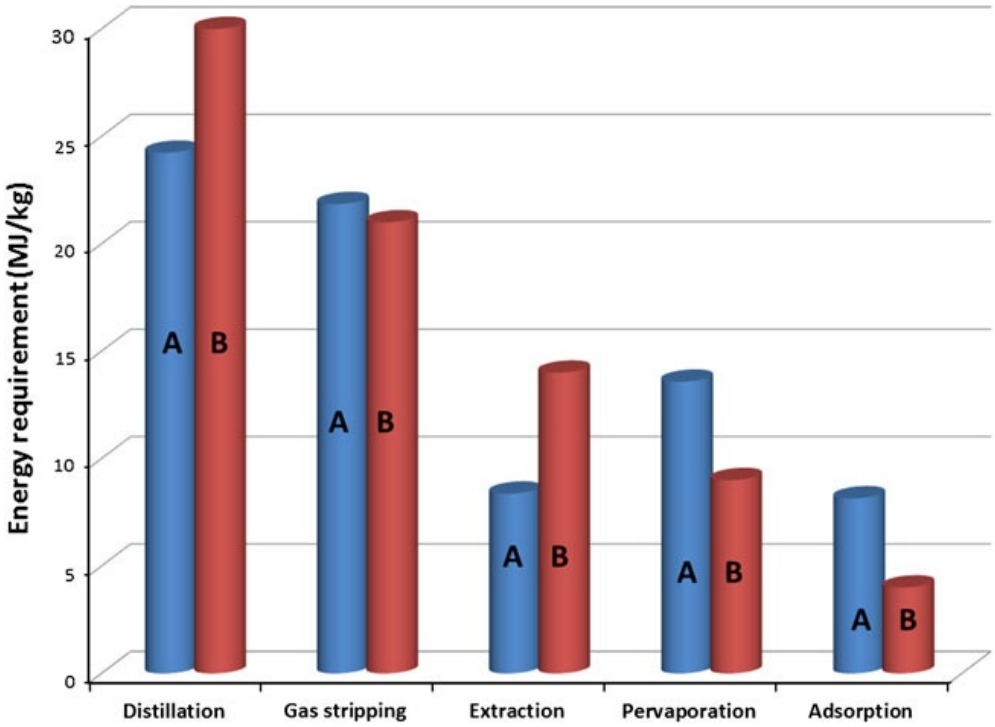


Figure 1 Energy requirements for butanol separation from ABE broth. A for distillation, gas stripping, extraction, and pervaporation are reported in (Qureshi et al. 1999) but the details of the process are not reported. B for distillation: to increase butanol concentration from 1 to 99.99 wt% in the solution; B for gas stripping: to increase the butanol concentration to 50 wt%; B for extraction: to remove 75 % of butanol from the solution using oleyl alcohol as the extractant. B for pervaporation: to remove 25 % of butanol from the solution using silicone rubber as the membrane (Groot et al. 1992); A for adsorption: to remove butanol using silicalite as the adsorbent (Qureshi et al. 1999), and B for adsorption: the estimated energy required for butanol adsorption by Oudshoorn et al. 2009a).

2.2 Methods

2.2.1 Kinetic experiments

In kinetic experiments, the butanol adsorption rate was determined at room temperature for each adsorbent by recording the concentration in the aqueous phase throughout the experiment until thermodynamic equilibium was achieved. Figure 2 shows the schematic diagram of the experimental system used in this investigation for

both kinetic and equilibrium experiments. Aqueous solutions were prepared by adding known amounts of butanol and water in the Erlenmeyer flasks to obtain butanol–water solutions with different initial concentrations. The column was packed with a specific adsorbent. It is important to note that all experiments were conducted in the same column with a specific volume of 30 cm^3. However, based on the differences in the shape and density of the different adsorbents, the amount of materials needed to pack the column was different. The amounts of each adsorbent are provided in the figure legends specifically for each experiment. The butanol solution was pumped through the packed column and recirculated to the feed tank at a high flow rate to ensure a low solution residence time throughout the column to minimize the transient effects of the adsorption column. The comparative determination of the adsorption kinetics was performed in a packed column instead of in a shake flask in order to maintain the physical integrity of the adsorbent particles and alleviate particle attrition. The length and diameter of the packed column were 17.5 and 1.5 cm, respectively. Small samples of approximately 1 mL were periodically removed from the Erlenmeyer flask and analyzed for butanol content. Using a simple mass balance, it was possible to estimate the amount of butanol adsorbed. The rate of change of butanol concentration in the flask was quantified. This allowed the estimation of the rate of adsorption.

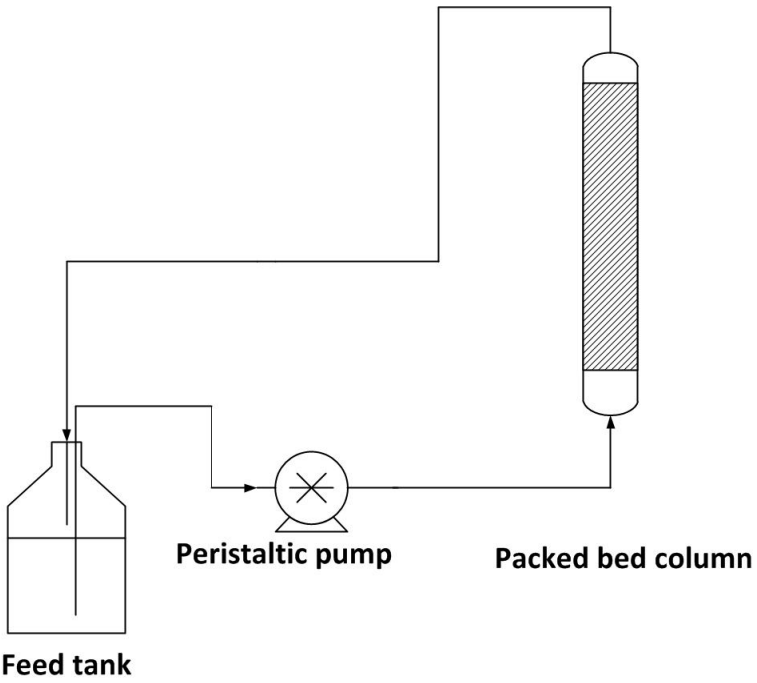


Figure 2 Schematic diagram of adsorption setup used for kinetic and equilibrium studies

2.2.2 Equilibrium experiments

For equilibrium experiments, adsorption runs were carried out with different initial butanol concentrations in model solutions. An aqueous solution containing known amounts of test chemicals was continuously recirculated through the column until equilibrium was reached. After reaching equilibrium for different initial concentrations and determining isotherms of each adsorbent, the performance of all adsorbents in terms of adsorption capacities was compared. To perform butanol adsorption experiments with MWCNT as the adsorbent, batch experiments were used. 250 mL Erlenmeyer was loaded with specific volumes of butanol–water solution with known concentration. A certain amount of adsorbent was added to the flask. The flask was then placed in a shaker at room temperature to ensure mixing was adequate. The samples were filtered using a 0.45 μm filter before measuring the butanol concentrations. For the experiments of binary and ternary solution adsorption isotherms using AC F-400, the amount of adsorbent used varied between 13.5 and 13.8 g.

2.2.3 Kinetic modeling

A first-order kinetic equation (Eq. 1) and a pseudo-second order equation (Eq. 2) adapted from the Lagergren's pseudo-second order kinetic equation, were used to fit the experimental butanol concentration profile in the aqueous solution as a function of time. The time constant for each adsorbent was calculated according to these two kinetic models.

$$C = C_e + (C_0 - C_e)e^{-\frac{t}{\tau}} \quad (1)$$

$$C = C_0 - \frac{a(C_0-C_e)^2 t}{1+a(C_0-C_e)t} \quad (2)$$

The time constant in both equations represents the time in which the butanol concentration in the aqueous solution decreased by 63.2 % of its total change (C_0 - C_e). For the calculation of the time constant, C_e was calculated from the curve fits of these two models to the experimental data. These experiments, done for different adsorbents, allowed comparison of the rate of butanol adsorption and determining the adsorbent with the fastest adsorption kinetics for butanol. The concentration of organic compounds in the solution was determined by high performance liquid chromatography (Waters, CA) using the ICPack column (Waters, Toronto, Canada).

Equations 1 and 2 modeled the variation of the butanol concentration in solution as a function of time. By a simple mass balance and knowing the amount of solids in the adsorption column, it is also possible to calculate the average amount of butanol adsorbed as a function of time. To model the kinetics of adsorption for each adsorbent, Eqs. 3 and 4 were used which are adapted from Eqs. 1 and 2, respectively. Equation 4 is the Lagergren's pseudo-second order equation which highlights the amount of adsorbate adsorbed in the solid, rather than the concentration change in the liquid phase and is usually used for modelling liquid adsorption from aqueous phase.

$$q = q_e(1 - e^{-\frac{t}{\tau}}) \quad (3)$$

$$\frac{t}{q} = \frac{1}{kq_e^2} + \frac{t}{q_e} \quad (4)$$

2.2.4 Equilibrium modeling

An equilibrium isotherm equation was used to characterize the adsorption capacity of a specific adsorbent as a function of equilibrium concentration of the adsorbate in solution at a constant temperature. The isotherm model used in this study to fit the experimental data is the three-parameter Langmuir–Freundlich (Sips) model, given by Eq. 5. This isotherm model was selected since it showed better fits to experimental data compared to Freundlich and Langmuir isotherm models.

$$q_e = q_s \frac{bC_e^{1/n}}{1+bC_e^{1/n}} \quad (5)$$

3 Results and discussion

3.1 Kinetic results

Kinetic experiments were performed at room temperature with different adsorbents using water–butanol binary solutions with different initial concentrations to determine the dynamics of adsorption for each adsorbent. Determination of the time required to reach equilibrium and the initial uptake rate were the main objectives of this set of experiments. Figure 3 shows the results of the kinetic experiments for all adsorbents studied. Results revealed that for an aqueous solution with an initial butanol concentration of approximately 10 g/L, ACs F-400 and F-600 had much faster adsorption rates than the other adsorbents. By fitting the pseudo-second order equation (Eq. 2) to the experimental data and determining the time constant for all adsorbents, it was found that AC F-400 and F-600 had the lowest time constants (8.2 and 11.3 min, respectively), whereas this constant was much higher for other adsorbents specially for NaY which was 109.3 min. A first-order kinetic equation (Eq. 1) was also fitted to experimental data and it was found that the fits for both models were very good. Time constants given by these two equations are fairly similar for AC F-400, F-600 and silicalite. On the other hand, for ZSM-5 and NaY, the difference between calculated time constants for these two different models is significant (see Table 2) and this is due to the difference in the model structure and the number of parameters present in these models. The coefficient of determination

(R^2), calculated for both Lagergren's pseudo-second order and the first-order kinetic equations, revealed that both of these equations can be used with confidence to model the kinetic behavior of the butanol adsorption system using all of the tested adsorbents (Table 2), with first-order kinetics having slightly lower (R^2) values. Figure 3 shows the results of the Lagergren's pseudo-second order equation fits to the experimental data for 10 g/L initial butanol concentration. Similar experiments were performed for other initial concentrations and all results showed the same trend.

The kinetic data for each adsorbent were fitted with Eq. 3 and Lagergren's pseudo second order model (Eq. 4) and Fig. 4 shows the results for the Lagergren's model since it had a slightly better fit. As clearly shown in Fig. 4, the model is a very good fit for the experimental data. Regardless of the amount of butanol adsorbed, the initial uptake rate and the time required to reach equilibrium is fairly similar for the ACs AC F-400 and F-600. In both experiments, the equilibrium state is reached in more or less 150 min whereas the initial uptake rate for NaY and ZSM-5 is significantly slower. For silicalite, the initial uptake rate was higher than the other two zeolites but slower than the ACs uptake rate. Table 3 shows the kinetic parameters obtained by fitting Lagergren's pseudo second order model to the experimental data for each of the adsorbents studied.

3.2 Adsorption equilibrium isotherms for water– butanol binary solutions

Adsorption equilibrium isotherms were used to compare the capacity of different adsorbents for butanol adsorption. As it was observed in kinetic experiments, equilibrium was reached in less than 2.5 h for ACs F-400 and F-600. To err on the safe side, 4-h runs were conducted for the determination of equilibrium data points for these two adsorbents. For the other adsorbents, 7-h experiments were carried out to reach the equilibrium state. Figure 5 shows the adsorption isotherms at room temperature for each of the adsorbents studied. This figure clearly shows that AC F-400 has the highest butanol adsorption capacity in comparison to other adsorbents.

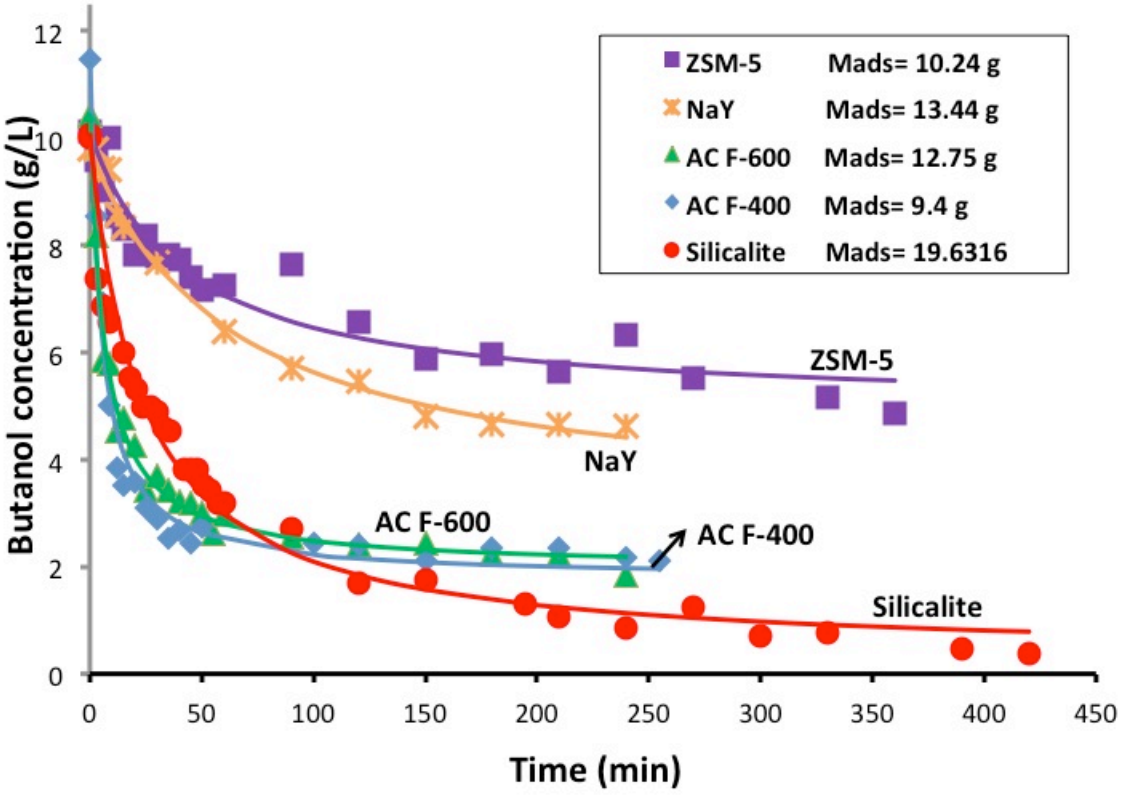


Figure 3 Butanol-water adsorption kinetics at room temperature for 10 g/L initial butanol concentration; *Symbols* represent experimentals data and the *lines* are predictions using the Lagergren's pseudo-second order equation (Eq. 2) (the solution volume was 0.2 L for all experiments)

For example, at a 10 g/L concentration at equilibrium, the butanol adsorption capacity for AC F-400 is 258 mg/g, while it is 149, 139, 107 and 103 mg/g for AC F-600, ZSM-5, NaY, and butanol silicalite, respectively. Interestingly, the adsorption capacities of AC F-400 and AC F-600 are quite different. This difference in adsorption capacities would be due to different raw materials or methods used in preparation processes for these two ACs. MWCNT was also used as butanol adsorbent for a 30 g/L butanol–water solution in a batch experiment and, as shown in Fig. 5, the butanol adsorption capacity obtained was very low (24 mg/g). It should be noted that to reach the equilibrium state, the experiment was run for 96 h and the equilibrium concentration was

obtained at 24 h and did not change for the subsequent 3 days. It was concluded that this specific multi-walled carbon nano tube was not an efficient adsorbent for bibutanol adsorption process. For the three different types of zeolites tested in adsorbent screening experiments, it was observed that ZSM-5 had higher adsorption capacity for butanol in comparison to NaY. This higher adsorption capacity would be due to its higher SiO_2/Al_2O_3 ratio and/or due to different structures of these two zeolites. Increasing SiO_2/Al_2O_3 ratio increases the hydrophobicity of zeolites and since zeolitic hydrophobic adsorbents have higher selectivity for organic compounds over water, it is desired to use a zeolite with higher silica to alumina ratio for butanol adsorption. These hydrophobic zeolites have also some favourable characteristics such as homogeneity, low heat capacity and stability (Oudshoorn et al. 2009b). Accordingly, the experimental results showed that ZSM-5 with the SiO_2/Al_2O_3 ratio of 80 had higher butanol adsorption capacity in comparison to NaY with the SiO_2/ Al_2O_3 ratio of 1.8 over the whole range of butanol concentration. Silicalite which has the same structure as zeolite ZSM-5 with a SiO_2/Al_2O_3 ratio of more than 1,000 was another adsorbent tested in this study. Interestingly, it was observed that although at low butanol concentrations the adsorption capacity of silicalite was higher than that for ZSM-5, at higher butanol concentrations, ZSM-5 showed higher capacity to adsorb butanol (Fig. 5).

Table 2 Time constants and coefficients of determination using the first-order and pseudo second order kinetic equations for each adsorbent

Adsorbent	Time constant first-order equation[a], Eq. 1 (min)	R^2	Time constant pseudo-second order equation, Eq. 2 (min)	R^2
AC F-400	7.6	0.989	8.2	0.973
AC F-600	9.6	0.952	11.3	0.986
Silicalite	32.5	0.927	39.2	0.970
ZSM-5	47.5	0.884	71.5	0.920
NaY	59	0.992	109.3	0.990

[a] The time constant represents the time required to achieve a butanol concentration corresponding to 63.2 % of its total change (C_o - C_e)

In Fig. 5, the representation of the isotherms using the Sips adsorption model (Eq. 4) is also plotted. The Sips model was the one that represented the experimental isotherms the best.

It is clear from the adsorbent screening results (kinetics and equilibrium experiments) that AC F-400 is the best adsorbent amongst the different adsorbents tested in this study. This adsorbent showed the highest butanol adsorption capacity and also had the fastest adsorption kinetics.

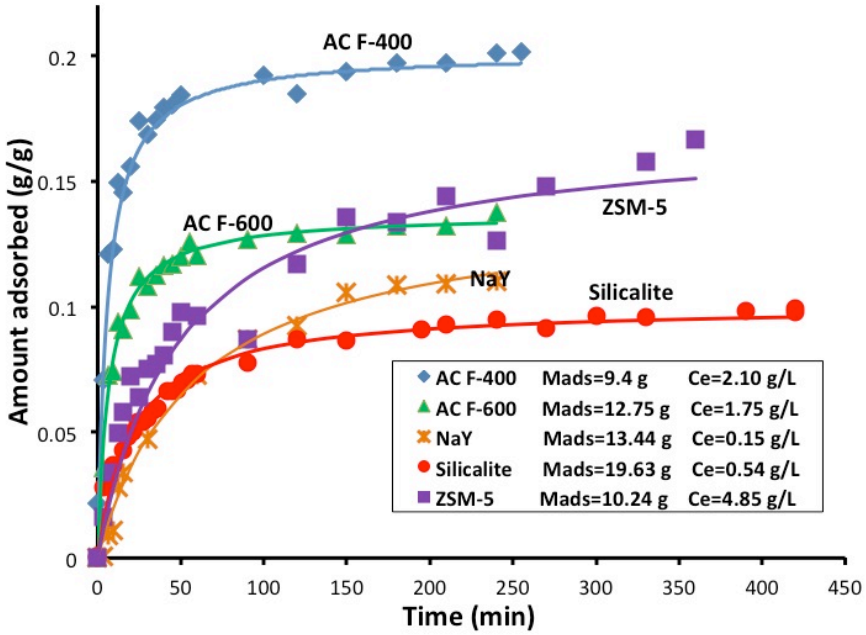


Figure 4 Butanol-Water adsorption kinetics at room temperature with different adsorbents. Symbols represent experimental data and the lines are fitted kinetic model for Lagergren's pseudo-second order equation

3.3 Isotherms of binary solutions of water and other ABE fermentation components

To investigate the adsorbent selectivity toward butanol compared to other components present in the ABE broths, the isotherm experiments with binary solutions of water and each of the components (acetone, ethanol, glucose, xylose, acetic acid and

butyric acid) were performed with AC F-400 and the results are presented in Fig. 6. Results clearly show that AC F-400 had a much higher selectivity toward butanol compared to other components. For example, at a concentration of 10 g/L of each component in the aqueous solutions at equilibrium, the adsorption capacities for butanol, butyric acid, acetone, acetic acid, glucose, xylose and ethanol were 294, 256, 115, 100, 96, 88 and 55 mg/g, respectively. Although the amount of butyric acid adsorbed is much higher than acetone, ethanol, acetic acid and the sugars amounts, it was still less than the amount of butanol that was adsorbed by the AC F-400. Overall, the results of this set of experiments showed that AC F-400 is more selective toward butanol and has a higher affinity for this component over the other compounds in ABE broths. From the results obtained in all adsorbent screening experiments, it can be concluded that it is possible to achieve high performance adsorption of butanol using AC F-400. The results showed that using AC F-400, it is possible to achieve a higher capacity of adsorption in a shorter time in comparison to other adsorbents tested. Thus, AC F-400 was selected in this investigation to be studied further.

Table- 3 Kinetic parameters for Lagergren's pseudo second-order model for 10 g/L initial butanol concentration

Adsorbent	k [(g ads)(g $BuOH^{-1}$)(day^{-1})]	q_e (g BuOH/g adsorbent)	R^2
Activated carbon F-400	1206	0.206	0.97
Activated carbon F-600	1536	0.137	0.99
ZSM-5	173	0.171	0.95
NaY	177	0.140	0.99
Silicalite	686	0.1	0.99

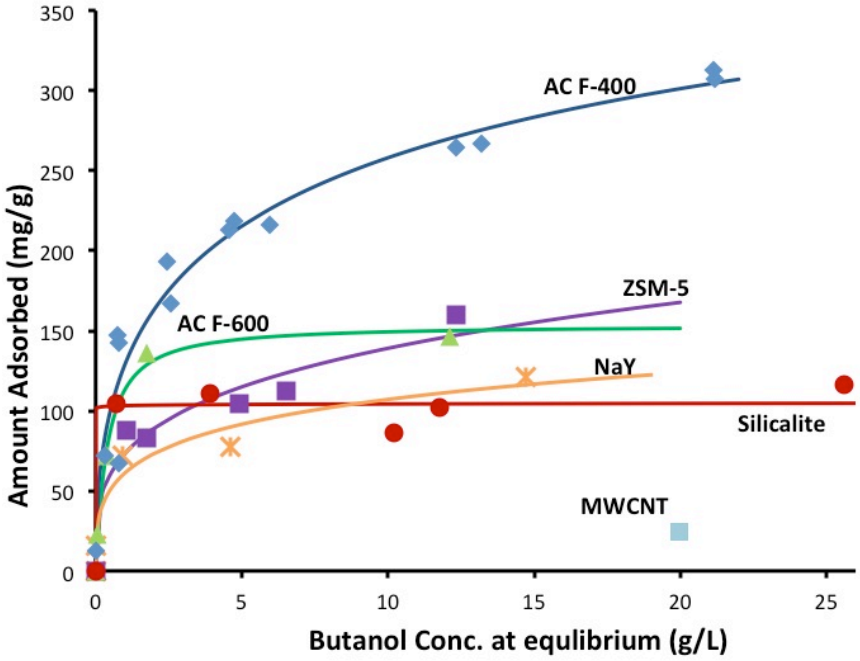


Figure 5 Adsorption isotherms for different adsorbents at room temperature. *Symbols* represent experimental data and the *lines* are fitted Sips adsorption isotherm model

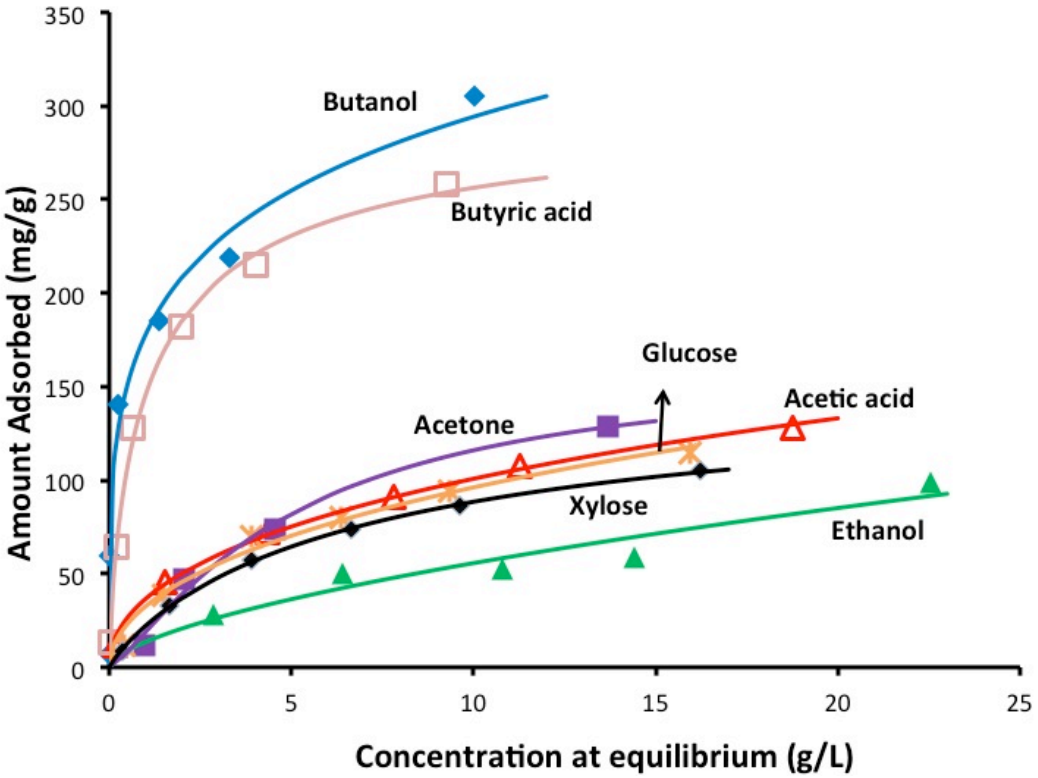


Figure 6 Adsorption isotherm for different components using AC F-400 at room temperature. *Symbols* represent experimental data and the *lines* are fitted Sips adsorption isotherm model

3.4 Isotherms of ternary solutions of water, butanol and other components of ABE broths

To investigate the effect of the presence of other ABE fermentation broth components on butanol adsorption, experiments were performed to study the effect of each component separately with AC F-400 as the adsorbent. Thus, ternary mixtures were prepared, starting with acetone being the next most abundant product in ABE fermentation broths as well as ethanol, glucose, xylose, butyric acid and acetic acid. Equilibrium adsorption experiments were performed with various concentrations of these various ternary solutions. Figure 7a shows butanol isotherms obtained for butanol–water binary solutions, ternary solutions of butanol–water with 5 g/L initial concentration of acetone, and acetone–water binary solutions. Results showed that the presence of acetone does not affect butanol adsorption significantly. As the figure shows, there is a slight

decrease of adsorption capacity at low equilibrium concentrations of butanol. This small decrease in adsorption capacity could be due to competitive adsorption of acetone at lower butanol concentration. Figure 7b shows that the presence of ethanol does not affect butanol adsorption over the whole range of butanol equilibrium concentration. As ethanol adsorption isotherm revealed, the adsorbent affinity for ethanol is very low and the probability of competitive effect of ethanol on butanol adsorption is low. The same effects were observed in the presence of glucose and xylose in Fig. 7c, d. The results showed that even 10 g/L initial concentration of glucose and xylose did not affect the amount of butanol adsorbed for all butanol concentrations. However, the presence of acids (acetic acid and butyric acid) led to a decrease in butanol adsorption capacity and this effect was more significant for the presence of butyric acid as shown in Fig. 7e, f. Figure 7e shows that the presence of 1 g/L initial acetic acid decreased the butanol adsorption capacity by 19 % and this effect became more significant when 5 g/L of acid was added to butanol–water solutions (26.5 % decrease). The effect of the presence of butyric acid was even more pronounced than the effect of acetic acid. As Fig. 7f shows, butanol adsorption capacity decreased significantly in the presence of 1 and 5 g/L butyric acid. The adsorption capacity decreased by 19.5 and 28.8 % in the presence of 1 and 5 g/L butyric acid, respectively. These results nevertheless show that the competitive adsorption appears to favor butanol. To develop an integrated process for butanol adsorption, two strategies can be considered. The first one is the in situ product recovery in which butanol would be removed while the fermentation is in progress. In the second strategy product recovery would be implemented at the end of the fermentation. Favorable characteristics of AC F-400 such as high adsorption rate, capacity and affinity for biobutanol make this adsorbent one of the best candidates for butanol separation from ABE fermentation broths using both strategies.

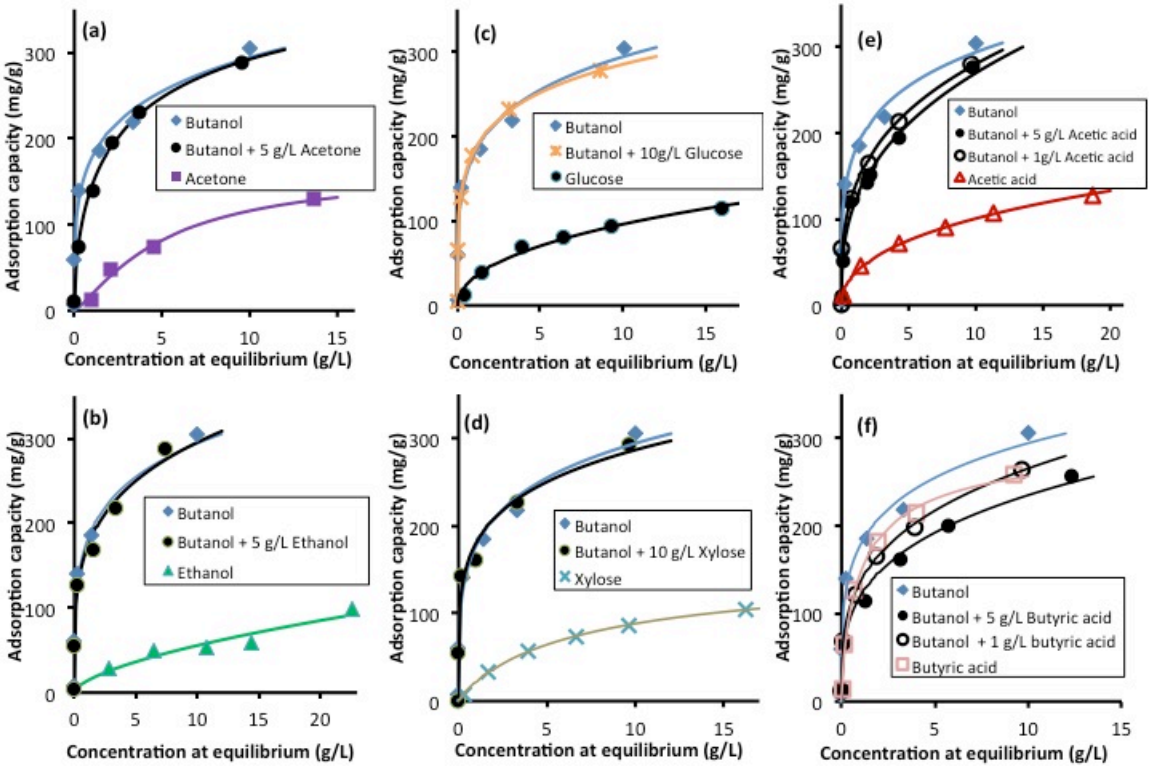


Figure 7 The effect of the presence of other components on butanol adsorption using activated carbon F-400: a acetone; b ethanol; c glucose; d xylose; e acetic acid; f butyric acid. Symbols represent experimental data and the lines are fitted Sips isotherm model

4. Conclusions

Amongst different butanol separation techniques, adsorption can be considered as a promising and energy efficient technique. Adsorption performance mainly depends on the adsorbent used in the process, and adsorption rate and capacity are two important characteristics that should be taken into account in selecting a suitable adsorbent. In this study, by performing the adsorbent screening experiments, these two characteristics were investigated for different adsorbents. Results from the kinetic experiments indicated that ACs F-400 and F-600 had the fastest kinetics for butanol adsorption amongst the tested adsorbents. According to the equilibrium experimental results, AC F-400 had the highest adsorption capacity for butanol. Interestingly, there was a huge difference between AC F-400 adsorption capacity and all other adsorbents capacity for butanol adsorption. To ensure that AC F-400 is suitable for butanol adsorption, its selectivity toward the main

components of ABE broths was investigated and it was confirmed that this adsorbent had the highest affinity for butanol adsorption and to a lesser extent for butyric acid whereas the adsorption capacity for other fermentation broth components was very low. The fast kinetics and the favorable adsorption model indicate the ability of this adsorbent to reach a high adsorption capacity even at low butanol concentrations. Thus, AC F-400 was selected as the adsorbent and further experiments were performed to investigate the effect of the presence of other components in ABE broths. The results showed that the presence of ethanol and sugars (glucose and xylose) did not have any effect on butanol adsorption and the presence of acetone slightly decreased the butanol adsorption capacity at low butanol concentrations. However, the presence of acids affected the adsorbent capacity significantly for butanol and this effect was more pronounced for the presence of butyric acid. These results show that acids compete with butanol to be adsorbed on the adsorbent surface. Future works are planned to couple ABE fermentation with an adsorption unit to selectively remove butanol during the course of fermentation to reduce product inhibition. Also, it is essential to investigate butanol desorption to ensure that butanol can be desorbed efficiently and economically. To gain a deeper understanding of the interactions between different species, breakthrough experiments will be performed in addition to desorption experiments in the future. The complete performance assessment of butanol adsorption using AC F-400 will allow us to design a high performance process to produce a high butanol concentration product from dilute ABE fermentation.

5. Acknowledgments

The authors would like to thank University of Ottawa and Natural Sciences and Engineering Research Council of Canada (NSERC) for their financial contributions for this study.

6. References

Antoni, D., Zverlov, V.V., Schwarz, W.H.: Biofuels from microbes (mini review). Appl. Microbiol. Biotechnol. 77, 23–35 (2007)

Dellomonaco, C., Fava, F., Gonzalez, R.: The path to next generation biofuels: successes and challenges in the era of synthetic biology. Microb. Cell Fact. 9, 3 (2010)

Dürre, P.: Biobutanol: An attractive biofuel. Biotechnol J. 2, 1525–1534 (2007)

El-Zanati, E., Abdel-Hakim, E., El-Ardi, O., Fahmy, M.: Modeling and simulation of butanol separation from aqueous solutions using pervaporation. J. Membr. Sci. 280, 278–283 (2006)

Ezeji, T.C., Qureshi, N., Blaschek, H.P.: Production of acetone, butanol and ethanol by *Clostridium beijerinckii* BA101 and in situ recovery by gas stripping. World J. Microbiol. Biotechnol. 19, 595–603 (2003)

Ezeji, T.C., Qureshi, N., Blaschek, H.P.: Butanol fermentation research: Upstream and downstream manipulations. Chem. Rec. 4, 305–314 (2004)

Ezeji, T.C., Qureshi, N., Blaschek, H.P.: Bioproduction of butanol from biomass: from genes to Bioreactors. Curr. Opin. Biotech- nol. 18, 220–227 (2007)

Feirey, J.L., Speitel, G.E. Jr. Katz, L.E.: Impact of natural organic matter on reduction by granular activated carbon: The role of porosity and electrostatic surface properties. Environ. Sci. Technol. 40, 4268–4273 (2006)

Fouad, E.A., Feng, X.: Use of pervaporation to separate butanol from dilutes aqueous solutions: Effects of operating conditions and concentration polarization. J. Membr. Sci. 323, 428–435 (2008)

García, V., Pongracz, E., Muurinen, E., Keiski, R.L.: Recovery of n-butanol from salt containing solutions by pervaporation. Desalination 241, 201–211 (2009)

Groot, W.J., Luyben, K.Ch.A.M.: In situ product recovery by adsorption in the butanol/isopropanol batch fermentation. Appl. Microbiol. Biotechnol. 25, 29–31 (1986)

Groot, W.J., Van der Lans, R.G.J.M., Luyben, K.Ch.A.M.: Technol-ogies for butanol recovery integrated with fermentations. Process Biochem. 27, 61–75 (1992)

Harlick, P.J.E., Tezel, F.H.: An experimental adsorbent screening study for CO_2 removal from N_2. Microporous Mesoporous Mater. 76, 71–79 (2004)

Harvey, B.G., Meylemans, H.A.: The role of butanol in the development of sustainable fuel technologies. J. Chem. Technol. Biotechnol. 86, 2–9 (2011)

Holtzapple, M.T., Brown, R.F.: Conceptual design for a process to recover volatile solutes from aqueous solutions using silicalite. Sep. Technol. 4, 213–229 (1995)

Li S., Srivastava, R., Parnas, R.S.: Separation of 1-butanol by pervaporation using a novel tri-layer PDMS composite membrane. J. Membr. Sci. 363, 287–294 (2010)

Maddox, I.S.: Use of silicalite for the adsorption of n-butanol from fermentation liquids. Biotechnol. Lett. 4, 759–760 (1982)

Meagher, M.M., Qureshi, N., Hutkins, R.: Silicalite membrane and method for the selective recovery and concentration of acetone and butanol from model ABE solutions and fermentation broth. US patent 5755967, 1998

Nielsen L., Larsson M., Hoist O., Mattiasson B.: Adsorbents for extractive bioconversion applied to the acetone–butanol fermentation. Appl. Microbiol. Biotechnol. 28, 335–339 (1988)

Nielsen D.R., Prather, K.J.: In situ product recovery of n-butanol using polymeric resins. Biotechnol. Bioeng. 102, 811–821 (2009)

Oudshoorn, A., Van der Wielen, L.A.M., Straathof, A.J.J.: Assess- ment of options for selective 1-butanol recovery from aqueous solution. Ind. Eng. Chem. Res. 48, 7325–7336 (2009a)

Oudshoorn, A., Van der Wielen, L.A.M., Straathof, A.J.J.: Adsorption equilibria of bio-based butanol solutions using zeolite. Biochem. Eng. J. 48, 99–103 (2009b)

Oudshoorn, A., Van der Wielen, L.A.M., Straathof, A.J.J.: Desorption ofbutanol from zeolite material. Biochem. Eng. J. 67, 167–172 (2012)

Qureshi, N., Blaschek, H.P.: Production of acetone butanol ethanol (ABE) by a hyper-producing mutant strain of *Clostridium beijerinckii* BA101 and recovery by pervaporation. Biotechnol. Prog. 15, 594-602 (1999)

Qureshi N., Hughes S., Maddox I.S., Cotta M.A.: Energy-efficient recovery of butanol from model solutions and fermentation broth by adsorption. Bioprocess Biosyst. Eng. 27, 215–222 (2005)

Qureshi N., Ezeji, T.C.: Butanol, 'superior biofuel' production from agricultural residues (renewable biomass): Recent progress in technology. Biofuels Bioprod. Bioref. 2, 319–330 (2008)

Regdon I., Kiraly, Z., Dekany I., Lagaly G.: Adsorption of 1-butanol from water on modified silicate surfaces. Colloid Polym. Sci. 272, 1129–1135 (1994)

Regdon I., Dekany I., Lagaly G.: A new way for calculating the adsorption capacity from surface excess isotherms. Colloid Polym. Sci. 276, 511–517 (1994)

Remi, J.C.S., Rémy, T., Van Hunskerken, V., Van de Perre, S., Duerinck, T., Maes, M., De Vos, D., Gobechiya, E., Kirschock, C.E.A., Baron, G.V., Denayer, J.F.M.: Biobutanol separation with the metal-organic framework ZIF-8. ChemSusChem 4, 1074–1077 (2011)

Sharma, P., Chung, W.J.: Synthesis of MEL type zeolite with different kinds of

morphology for the recovery of 1-butanol from aqueous solution. Desalination 275, 172–180 (2011)

Sowerby, B., Crittenden B.D.: Vapour phase separation of alcohol- water mixtures by adsorption onto silicalite. Gas Sep. Purif. 2, 177–183 (1988)

Takeuchi, Y., Iwamotob, H., Miyata, M., Seiichi Asano, S., Haradac, M.: Adsorption of l-butanol and p-xylene vapour with high silica zeolites and their mixtures. Sep. Technol. 5, 23–34 (1995)

Thirmal, C., Dahman, Y.: Comparison of existing pretreatment, saccharification, and fermentation processes for butanol produc- tion from agricultural residues. Can. J. Chem. Eng. 90, 745–761 (2012)

Thompson, A.B., Cope, S.J., Swift, T.D., Notestein, J.M.: Adsorption of n-butanol from dilute aqueous solution with grafted calixa- renes. Langmuir 27, 11990–11998 (2011)

Tong C., Bai Y., Wu J., Zhang L., Yang L., Qian J.: Pervaporation recovery of acetone–butanol from aqueous solution and fermen- tation broth using HTPB-based polyurethaneurea membranes. Sep. Sci. Technol. 45, 751–761 (2010)

Yang X., Tsai GJ., Tsao GT.: Enhancement of in situ adsorption on the acetone-butanol fermentation by *Clostridium acetobutylicum*. Sep. Technol. 4, 81–92 (1994)

Zheng, Y.N., Li, L.Z., Xian, M., Ma, Y.J., Yang, J.M., Xu, X., He, D.Z.: Problems with the microbial production of butanol. J. Ind. Microbiol. Biotechnol. 36, 1127–1138 (2009)

SECTION – III: Analytical method

Chapter IV

American Journal of Analytical Chemistry, 5(16), 1078-1089 (2014)

Improved Acetone-Butanol-Ethanol (ABE) Solution Analysis Using HPLC: Chromatograph Spectrum Deconvolution Using Asymmetric Gaussian Fit

N. Abdehagh[1], M. Bagheri[2], F.H. Tezel[1], J. Thibault[1]

[1]Chemical and Biological Engineering Department, University of Ottawa, Ottawa, Canada
[2]Department of Physics, University of Ottawa, Ottawa, Canada
161 Louis-Pasteur, Ottawa, ON, Canada K1N 6N5

Corresponding author: Tel: (613) 652-5800 Ext. 6094; Email: Jules.Thibault@uottawa.ca

Abstract

Currently, the analysis of acetone-butanol-ethanol (ABE) broths is performed using both High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) for each sample since GC cannot be used in quantifying sugars and HPLC methods are not yet efficient enough to detect all components separately. In this study, a novel method was developed to quantify all main components present in ABE model solutions (acetone, butanol, ethanol, butyric acid, acetic acid, glucose and xylose) using only HPLC. Although the HPLC operating conditions were optimized to obtain the best possible resolution in HPLC chromatograms, it was observed that the peaks for butyric acid, acetone and ethanol overlapped. The same trend was observed for glucose and xylose. Using the asymmetric Gaussian fit, a program was written in MATLAB to detect the overlapped peaks, deconvolute them and calculate the area of each separated peak. The concentrations of each component were then calculated using the areas and the calibration curves for each component. Experimental results show that this method works well for the ABE model solutions and can be used to quantify all components in the solution when there are some overlapped peaks in the HPLC chromatograms.

Keywords: High Performance Liquid Chromatography, Overlapped Peaks, Deconvolution, ABE Broths Analysis, Biobutanol

1 Introduction

Due to environmental challenges and problems associated with fossil fuels such as depletion of resources, high cost and political instability in oil-producing countries, one of industrialists' and scientists' main concerns is to produce fuels from sustainable resources. Amongst the existing options, biofuels have attracted significant attention because their production methods are more environmentally friendly. Biofuels such as bioethanol and biobutanol have shown to be promising alternatives to fossil fuel. Butanol production via fermentation is currently the subject of intense research because it is reputed as one of the best renewable alternative biofuels to replace gasoline. Biobutanol has more favorable characteristics in comparison to other biofuels such as bioethanol. Butanol has a net heat of combustion (NHOC) close to gasoline; it is less hazardous to handle due to its lower vapor pressure and volatility, and it can be blended with gasoline in any proportion and be used in existing car engines without any modifications [1]-[9]. It is however limited by its low final concentration due to product inhibition. Acetone-butanol-ethanol (ABE) fermentation is the most studied bioproduction method to produce butanol. Clostridia species are the most common microorganisms used for this fermentation process [9]-[11].

In ABE fermentation, a number of products are present in the fermentation broth including acetone, butanol, ethanol, acetic acid, butyric acid and sugars (glucose and xylose). To be able to characterize the fermentation process (yield, productivity and concentration of the product and each of the byproducts), precise quantification of all components is required. High performance liquid chromatography (HPLC) and gas chromatography (GC) are used to quantify the components present in ABE fermentation broths. However, the separation and quantification of all the different components in the fermentation broth have not been successfully achieved using only one of these two instruments. Using simultaneously both instruments increases the capital cost and time required to perform an analysis [2]-[6].

Since GC cannot be used to detect sugars in the samples, it is required to use HPLC or other techniques when the solvents and organic compounds are detected by GC. Various GC operating methods and columns (packed and capillary) such as fused silica

(Stabilwax-DA), Innowax 19091N-133 (Agilent Technologies Inc.), capillary column SE-30 (Lanzhou Institute of Chemical Physics) and Porapak Q (80/100) (ALLTECH) have been used to measure the concentration of acetone, butanol, ethanol, butyric acid and acetic acid in the fermentation broths [12-16]. However, in all studies HPLC was required for quantifying sugars. HPLC cannot also be used straightforwardly for measuring the concentration of all components since butyric acid, acetone and ethanol have approximately the same retention time in HPLC chromatograms for most of the HPLC columns used to determine the concentration of alcohols and other components of the fermentation broths. Different HPLC methods (various column temperatures, mobile phase flow rates and different columns) have also been investigated to analyze solvents, organic acids and sugars present in the ABE fermentation broth. Although there are some studies where different HPLC methods were investigated, a precise method with specific methodology to quantify all desired components with a reasonable resolution does not yet exist. Moreover, there is still some controversy among the different results published in the literature. Aminex HPX-87H (300 × 7.8 mm, Bio-Rad) is one of the most common HPLC columns used to quantify the ABE fermentation broth compounds [17-22].

Buday et al. [17] investigated different column temperatures to find the optimum temperature for this column to achieve an adequate separation of ethanol, acetone and butyric acid peaks. They found that at 60°C ethanol and acetone peaks had exactly the same retention time and, by decreasing the temperature to a sub-ambient temperature of 14°C, the peaks were separated but some overlap for acetone and butyric acid was still observed. Wang et al. [18] used the same column at 15°C to detect all ABE fermentation broth components and they have found that this column worked only when it was completely clean and needed to be replaced after a few months when the chromatograms showed low resolutions. Finch et al. [19] also used the Aminex HPX-87H HPLC column at 30°C to quantify the components present in ABE fermentation broths. However, in their paper they have not mentioned acetone as one of the components detected by this column and it seems they have measured the acetone concentration using another method. Cho et al. [20] also used the same HPLC column to investigate the effect of acetic and formic acid on ABE production in ABE fermentation. Although the same microorganism

(*Clostridium acetobutylicum*) was used to produce butanol, they did not measure butyric acid in the fermentation broth as one of the intermediate products.

In a recent study, Kumar et al. [21] developed a methodology to quantify all of the ABE fermentation broth components using the Aminex HPX-87H HPLC column at 65°C. They used a mathematical relationship between the areas and the heights of the peaks to predict the area under the overlapped peaks of acetone and butyric acid assuming the peaks as Gaussian curves. Although they obtained fairly precise results at low concentrations of ABE compounds (up to 5 g/L), the underlying Gaussian curve assumption may not always be valid since the peaks in HPLC chromatograms could be asymmetric Gaussian peaks. Also, since the overlapped peaks are the summation of the two peaks, the height of each separated peak is not the same as the height of the same peak when a significant overlap with other peaks exists. The assumption used in their study would be valid when the widths of the peaks are equal; however, each peak in a HPLC chromatogram has usually a unique width. Therefore, if any of these assumptions are not valid, the correlation used to measure the concentrations of the components with close retention times (RT) having overlapped peaks would lead to incorrect concentrations [21]. Eurokat H Vertex column (300 × 8 mm, 10 µm, KNAUER, Germany) is another HPLC column used for ABE fermentation broth analysis. Setlhaku et al. [23] [24] used this column to quantify the components present in ABE fermentation broths. This column allows performing the HPLC at higher temperatures instead of sub-ambient temperature whereas Aminex HPX-87H column for best performance could only operate at low temperatures. Setlhaku et al. [24] used this column at 80°C and observed that although the RT of ethanol peak was different than the peaks of butyric acid and acetone, there was still a partial overlap between butyric acid and acetone peaks.

In the present study, a novel methodology is developed and used to quantify the main components present in ABE fermentation broths by HPLC using the asymmetric Gaussian fit to deconvolute the overlapped peaks and determine the coefficients of the equations representing separate peaks in order to calculate the area and subsequently the concentration of each component. This proposed method was used to quantify pure component samples as well as multicomponent samples to verify its accuracy and precision.

2 Materials and Methods

2.1 Materials

To prepare samples, n-butanol (99% pure, Acros), acetone (95% pure, Acros), n-butyric acid (99% pure, Acros) and 99% pure ethanol, acetic acid, glucose and xylose, obtained from Fisher Scientific (Fisher Scientific Co., Fair Lawn, NJ, USA), were used. Deionized distilled water was used to prepare all model solutions. The software used to operate the HPLC was Breeze (Waters, Canada) and the mobile phase used was 0.01 N sulfuric acid with a flow rate of 0.8 mL/min. The temperature of the column was kept at 85°C.

2.2 Equipment Specifications

The HPLC used in this study was purchased from Waters, Canada. The detector, pump and autosampler were Refractive Index Detector (Waters 2414), Isocratic HPLC pump (Waters 1515) and Autosampler (Waters 717 plus), respectively. To heat the column to the desired temperature, an external column heater was used. The column used in this study to detect ABE solutions was the Vertex column (300 × 8 mm, KNAUER, Germany) packed with Eurokat H, 10 µm.

The gas chromatograph (GC) used in this study was purchased from chromatographic specialties (SRI Instrument, Brockville, Canada). The GC is equipped with a flame ionization detector (FID). A Stabilwax column, 30 M × 53 MM, 1 µm w/5 M Integra-guard (Restek, purchased from Chromatography Specialties, Brockville, Canada) was used to analyse acetone, ethanol, butanol, acetic acid and butyric acid in model solutions and ABE fermentation broths. Nitrogen and zero air were used as the carrier gas and ignition gas for flame, respectively. The column temperature was 40°C when the samples were injected and this temperature was kept for 2 min and then increased to 200°C with the rate of 20°C/min. The injector and FID detector temperatures were 250°C and 110°C, respectively.

2.3 Fermentation Experiments

The microorganism used in this study to produce butanol was *Clostridium acetobutylicum* ATCC 824 purchased from American Type Culture Collection (ATCC). The bacterium was stored at 4°C in the form of spores in Reinforced Clostridium Medium (RCM) purchased from Sigma-Aldrich Canada. To revive the bacteria, heat shock at 80°C for 10 minutes followed with cooling in ice for one minute was used. The revived bacteria were kept at 37°C in roller incubator for 48 h. The revived bacteria were used to inoculate the fermenter. The fermentation medium contained: yeast extract (5 g/L), KH_2PO_4 and K_2HPO_4 (0.75 g/L), $(NH_4)_2SO_4$ (2 g/L), NaCl (1 g/L), $MgSO_4 \cdot 7H_2O$ (0.2 g/L), $MnSO_4 \cdot H_2O$ and $FeSO_4 \cdot 7H_2O$ (0.01 g/L), L-cysteine HCl (0.5 g/L) and glucose (50 g/L). The inoculated medium of 250 mL was used for each batch fermenter.

2.4 Methods

To quantify the concentrations of each component in ABE solutions, HPLC was used to separate as well as possible all components present in tested samples. The HPLC chromatogram includes different asymmetric Gaussian curves, one for each component. The area under each asymmetric Gaussian curve is related to the concentration of the components in the sample. To properly quantify each component of the fermentation broth, it is necessary to determine the underlying relationship relating the concentration and the area under the asymmetric Gaussian curve. This relationship, which is a linear and referred to as the standard calibration curve, was determined experimentally in the present study. However, due to the characteristics of components leading to similar retention times, some conflicts may occur between the different peaks in the HPLC chromatograms. For the peaks that are conflicting with each other, a computer program, written in MATLAB using an asymmetric Gaussian curve represented by Equation (1), was used to fit the raw data of each peak of the HPLC chromatogram. This equation was fitted to the experimental data either for completely separated peaks or for the over-lapped peaks using an equation comprised of the summation of two or three asymmetric Gaussian curves with specific coefficients that were obtained by minimizing the sum of squares of the errors between the experimental and predicted data. In the program, the overlapped peaks were detected, the parameters of each asymmetric Gaussian curve were

determined and, using the particular coefficients of the asymmetric Gaussian curve associated to each peak, the area underneath the curve was calculated. Then, using the standard calibration curve of each component, the concentration was estimated. The asymmetric Gaussian curve used in the program was:

$$y = \left[\frac{a}{2\times d}\exp\left(\frac{c^2}{2\times d^2}+\frac{(b-t)}{d}\right)\operatorname{erf}\left(\frac{t-b}{\sqrt{2}\times c}-\frac{c}{\sqrt{2}\times d}+1\right)\right] \quad (1)$$

where y is the voltage (mV), t is the time (min) and a, b, c and d are the parameters related to the area, retention time (RT), width and exponential damping terms of the peak. For the overlapped peaks, the summation of two or three asymmetric Gaussian curves (Equation (1)) was used. To calculate the area under each peak, the four model parameters (a, b, c and d) were determined for each peak (eight parameters for two and twelve parameters for three overlapped peaks).

3. Results and Discussion

3.1 HPLC Optimum Operating Conditions

To test the HPLC performance with the Vertex column, the first step was to test each component in a binary aqueous solution to determine the retention time (RT) of all components that will be present in the HPLC chromatogram. Since the retention time of a component depends on the mobile phase flow rate and the column temperature, different values of these two operating variables were tested to find the best operating conditions for the analysis of ABE model solutions. For ABE model solutions, the peaks of butyric acid, acetone and ethanol usually have partial conflicts or are totally overlapped. It is therefore important to find the optimum mobile phase flow rate and column temperature to have minimum conflict between the peaks of these three components. Different mobile phase flow rates (0.3, 0.4, 0.5, 0.8, 0.9 and 1 mL/min) and column temperatures (65℃, 80°C, 85°C, 87°C, 90°C) were tested to find the best operating conditions to perform this analysis. Figure 1 shows the results of these experiments. As it can be observed, at 65°C and 0.5 mL/min, there is major overlapping at elution time between 29 and 31 min (Figure 1(a)). Increasing the temperature to 80°C and decreasing the flow rate to 0.3

mL/min, although the three peaks were detected, the peaks were still overlapping with each other (Figure 1(b)) and similar results were observed at the same temperature for 0.5 and 0.8 mL/min (Figure 1(c) and Figure 1(d)). Therefore, the flow rate was kept at 0.4 mL/min and the temperature was increased to 85°C and still the first two peaks of those three components were not detected separately as two peaks (Figure 1(e)) so the flow rate was increased to 0.8 mL/min at the same temperature and this time it was observed that the overlapped peaks could be detected with a higher resolution (Figure 1(f)). This was the best possible result since by increasing the flow rate to 0.9 or 1 mL/min or the temperature to 90°C, the conflicts between the peaks increased (Figure 1(g) and Figure 1(h)). Thus the optimum operating conditions for HPLC was selected to be at a flow rate of 0.8 mL/min and 85°C as the column temperature.

3.2 Standard Solution Analysis

Standard solutions were prepared at different concentrations (0.5, 1, 5, 10, 15 and 20 g/L) to obtain both the RT of each component as well as to determine the area of the peaks at different known concentrations. The results showed that all components were eluted in the following order in increasing RT: glucose, xylose, acetic acid, butyric acid, acetone, ethanol and butanol. The retention times for all components are shown in Table 1. It was confirmed that the peaks with conflicts are butyric acid, acetone and ethanol having very close retention times. It was also observed that glucose and xylose peaks have also partial overlaps.

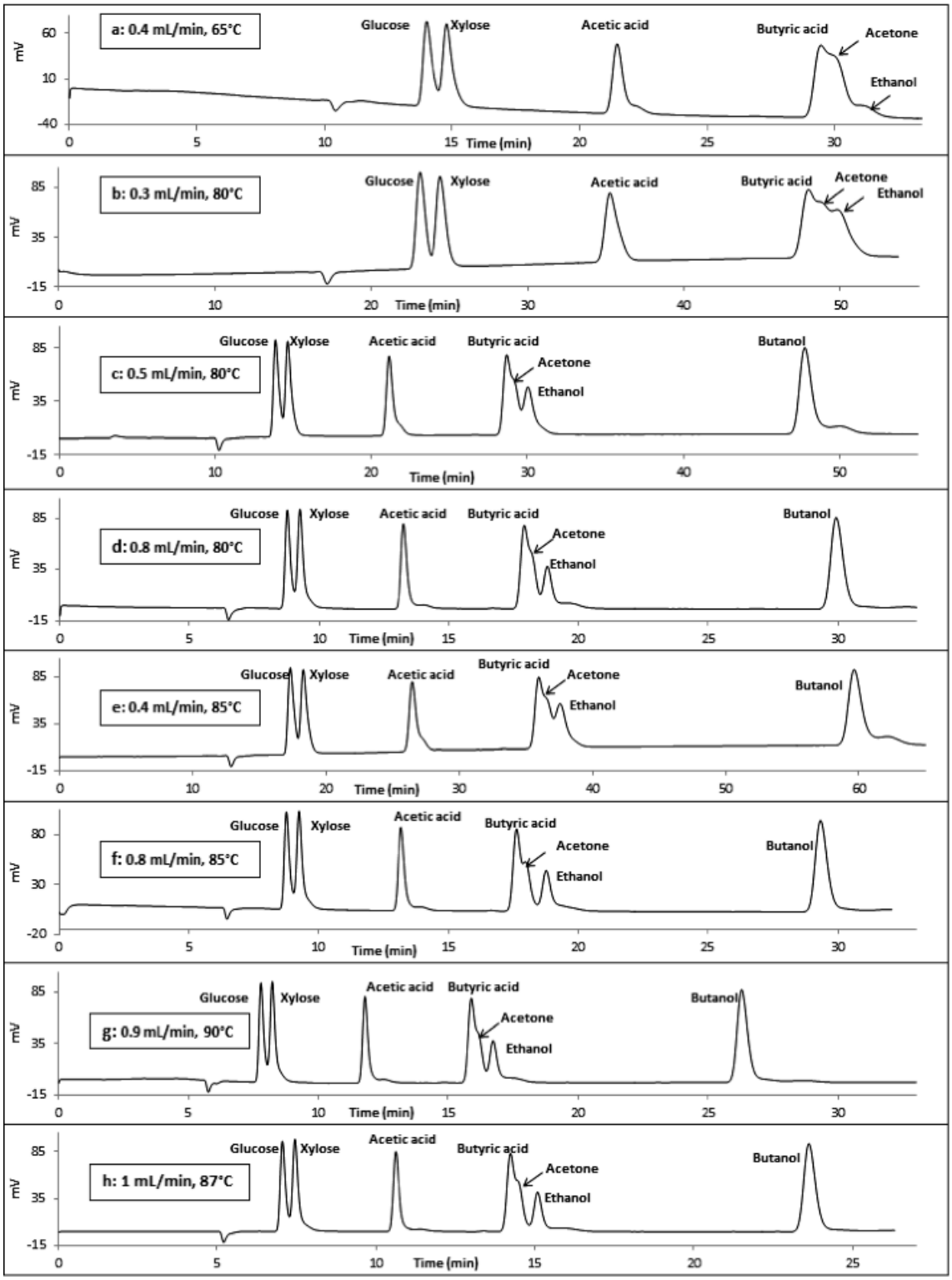


Figure 1 HPLC chromatograms at (a) 0.5 mL/min, 65°C; (b) 0.3 mL/min, 80°C; (c) 0.5 mL/min, 80°C; (d) 0.8 mL/min, 80°C; (e) 0.4 mL/min, 85°C; (f) 0.8 mL/min, 85°C; (g) 0.9 mL/min, 90°C; (h) 1 mL/min, 87°C.

Using these results (peak areas and the corresponding concentrations), standard calibration curves for each component were plotted separately. Figure 2 shows the standard curves estimated using both the results of the HPLC software and the computer program. In each graph, the HPLC raw data (voltage vs time) were analyzed with both the HPLC software (Breeze) and the computer program to calculate the area of the peaks. Using regression with both slope and intercept errors, standard calibration curves were plotted and the equations relating the peak area (mV×s) to the component concentration (g/L) were obtained. Table 1 shows the retention times, the standard calibration equations, and the coefficients of determination of the different compounds obtained by both the HPLC software and the proposed method. Two or three repeats were performed for each experiment and the average value was used to obtain the regression equation (Table 1). Slight differences in the slope of the standard calibration curves obtained by HPLC and the computer program were observed and this might be due to the small bumps after each asymmetric Gaussian peak in HPLC chromatograms that are considered. HPLC software considers these bumps in the area calculation whereas, in the computer program these tailings, were neglected.

3.3 Standard Calibration Curve Validation

The next step was to validate the standard calibration curves using test samples with known concentrations of each compound. Two binary aqueous solutions of 10 g/L of each component were prepared and the HPLC raw data were used to calculate the area of the peaks and the concentrations using both the HPLC software and the MATLAB program. Table 2 shows the results of this comparison. All the experiments were repeated three times and the standard deviation for all the numbers all shown in Table 2. As it can be observed, the concentrations estimated by the computer program are very similar to the ones calculated by the HPLC software and even closer to the concentrations of the test solutions. The standard error of the estimate for using the HPLC and proposed computer program was 0.44 and 0.19, respectively. Thus, the results confirmed that the standard calibration curves, calculated using the MATLAB program, were more accurate.

3.4 Validation of the Method

To validate the proposed method based on the HPLC performance, the limit of detection (LOD), limit of quantification (LOQ), repeatability, intermediate precision and reproducibility were considered [25] [26]. Five different solutions with different concentrations between 0.5 and 20 g/L (0.5 - 30 g/L for butanol) were prepared for each component to determine the LOD and LOQ. 3 and 10 were the signal to noise ratio considered for these parameters. The LODs were 0.29, 0.4, 0.12, 0.27, 0.15 and 0.07 g/L for butanol, acetone, ethanol, butyric acid, acetic acid, glucose and xylose, respectively. The LOQ calculated for butanol, acetone, ethanol, butyric acid, acetic acid, glucose and xylose were 0.96, 1.35, 0.42, 0.95, 0.9, 0.52 and 0.23 g/L, respectively. The ranges of LOD and LOQ showed that HPLC has the adequate sensitivity toward the components present in the ABE fermentation broths.

Table 1 The standard calibration curves and retention times of ABE solution components in HPLC chromatograms. A is the area (μV×s) of the peak for each component.

Component	Retention time (min)	Concentration (g/L)	Coefficient of determination (R^2)	Regression equation using MATLAB program	Coefficient of determination (R^2)
Glucose	8.6 - 8.7	3.1×10^{-6} A − 0.001	0.9999	3.4×10^{-6} A − 0.033	1.0000
Xylose	9.1 - 9.3	3.2×10^{-6} A + 0.010	0.9999	3.5×10^{-6} A−0.018	1.0000
Acetic acid	13.0 - 13.3	6.7×10^{-6} A + 0.224	0.9989	7.4×10^{-6} A + 0.041	0.9998
Butyric acid	17.7 - 17.8	5.5×10^{-6} A + 0.064	0.9998	5.6×10^{-6} A + 0.082	0.9999
Acetone	17.9 - 18.2	9.7×10^{-6} A + 0.162	0.9991	1.0×10^{-5} A + 0.019	0.9998
Ethanol	18.6 - 18.9	1.0×10^{-5} A + 0.259	0.9973	1.1×10^{-5} A − 0.055	1.000
Butanol	29.4 - 29.9	6.2×10^{-6} A − 0.001	0.9993	6.5×10^{-6} A−0.069	0.9999

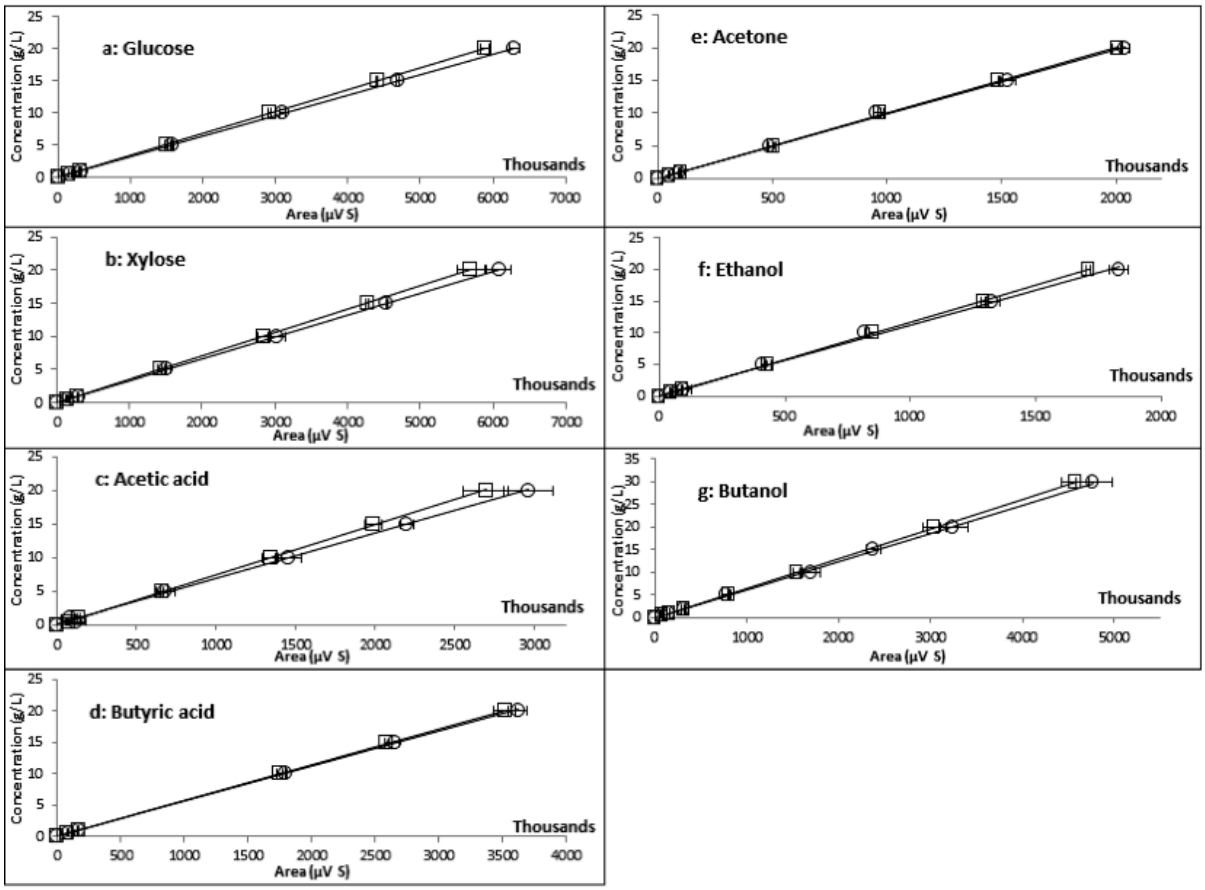


Figure 2 The standard calibration curves for (a) Glucose; (b) Xylose; (c) Acetic acid; (d) Butyric acid; (e) Acetone; (f) Ethanol; (g) Butanol. Standard calibration curves: MATLAB program (□) and HPLC software (O).

Table 2 Results of HPLC and the computer program for 10 g/L aqueous binary solutions of glucose, xylose, acetic acid, butyric acid, acetone, ethanol and butanol.

	HPLC program		Proposed computer program	
Component	Solution 1	Solution 2	Solution 1	Solution 2
Glucose	9.76 ± 0.16	9.94 ± 0.16	9.88 ± 0.12	9.98 ± 0.12
Xylose	9.70 ± 0.30	10.25 ± 0.31	9.79 ± 0.32	10.21 ± 0.33
Acetic acid	9.55 ± 0.51	10.33 ± 0.53	9.71 ± 0.45	10.22 ± 0.47
Butyric acid	10.04 ± 0.33	9.97 ± 0.32	9.89 ± 0.27	10.08 ± 0.28
Acetone	9.63 ± 0.17	9.42 ± 0.39	9.85 ± 0.23	9.67 ± 0.22
Ethanol	9.26 ± 0.64	9.26 ± 0.64	10.10 ± 0.09	9.81 ± 0.09
Butanol	10.26 ± 0.53	10.40±0.54	9.82 ± 0.40	9.93± 0.40

Repeatability, intermediate precision and reproducibility were determined based on three different sets of samples. To determine the repeatability, the samples were analysed in the same day and the time between the samples analysis was short. To determine the intermediate precision of the proposed program, samples were analysed in different days to have a few days gap between each sample. And to determine the reproducibility, the samples were analysed using a different set of standard curves (the standard curves obtained by HPLC software). Both area and the retention time of each component (six samples with same concentration) were considered to evaluate the repeatability, intermediate precision and reproducibility. The results (Table 3) showed that for the retention time the repeatability, intermediate precision and reproducibility were less than 0.99%, 0.93% and 1.4% (coefficient of variation (CV) value), respectively. These values changed to 3.96%, 3.66% and 3.24% for the repeatability, intermediate precision and reproducibility for the peak area, respectively. Therefore, these CV values confirmed that the HPLC operation is pretty accurate and the proposed method using the computer program can be used to analyse the ABE fermentation broth samples.

To be able to use the computer program to separate the overlapped peaks in multi component solutions it was necessary to ensure that the peaks were not correlated. This implies that changing the concentration of one component does not affect the peaks of other components, especially for the peaks that are overlapping. To study the characteristics of the overlapped peaks (butyric acid, acetone and ethanol) in order to track the changes of peak shapes in different cases, a series of experiments were performed using model solutions containing all seven components. In these experiments, the concentrations of two of the three components with overlapped peaks were kept constant and the concentration of the third component was changed to observe the changes of the peaks for the concentration of the other two compounds. The results showed that the peaks are not correlated and changes in each peak are independent of the other overlapped peaks. Results of this analysis are shown in Table 4. Similar experiments were performed to test the computer program for quantifying the glucose and xylose peaks. Results showed that these two peaks are also not correlated and the changes in the concentration of each component do not affect the peaks of other components (Table 5).

Figure 3 also shows the overlapped peaks of HPLC chromatogram for the components (butyric acid-acetone-ethanol and glucose-xylose) that are conflicting. The dotted lines represent the data from HPLC program and the lines with light color is the asymmetric Gaussian curves, fitted to the overlapped peaks and the lines with dark color shows the peaks separated from the overlapped peaks, plotted with the determined coefficients estimated by the computer program (Figure 3).

As the final step, three different ABE model solutions with approximately the same concentrations were prepared and the data of the HPLC chromatogram were used to validate the estimates obtained with the computer program. Table 6 shows the results of the analysis obtained using the computer program to calculate the concentrations of all seven components present in the ABE model solutions.

Solutions with known concentrations of butanol, acetone, ethanol, acetic acid and butyric acid (around 4 g/L of each) were also prepared to compare the analysis of the same sample with GC and the HPLC. The concentrations obtained by the GC were 3.9, 3.98, 3.96, 4.3 and 4.13 g/L for acetone, ethanol, butanol, acetic acid and butyric acid, respectively. The same sample was injected to HPLC and the concentration detected for acetone, ethanol, butanol, acetic acid and butyric acid were 3.98, 4.1, 4.03, 4.5 and 4.16, respectively.

Finally the real fermentation broth was used to test the proposed method and the concentrations obtained for acetone, ethanol, butanol, acetic acid and butyric acid were obtained as 5.37, 0.55, 10.1, 0.6 and 0.2 g/L, respectively. These results were validated with GC analysis and the concentrations of acetone, ethanol, butanol, acetic acid and butyric acid were 5.15, 0.8, 9.6, 0.68 and 0.09, respectively.

Table 3 Repeatability, intermediate precision and reproducibility of the proposed method for all components present in ABE broths in term of coefficient of variation (CV) for retention time and peak area.

Components	Repeatability (n=6)		Intermediate precision (n=6)		Reproducibility (n=6)	
	Retention time (CV%)	Peak area (CV%)	Retention time (CV%)	Peak area (CV%)	Retention time (CV%)	Peak area (CV%)
Glucose	0.55	1.48	0.8	3.14	1.4	2.83
Xylose	0.99	2.84	0.93	3.66	1.28	3.49
Acetic acid	0.73	1.17	0.55	0.87	0.44	0.91
Butyric acid	0.38	1.97	0.29	2.16	0.31	2.45
Acetone	0.26	3.96	0.16	3.62	0.17	3.24
Ethanol	0.22	2.88	0.29	2.07	0.29	2.39
Butanol	0.91	0.55	0.52	0.65	0.77	0.38

Table 4 Concentrations of the solutions used for the validation of Equation (1) using different combinations of concentrations for butyric acid, acetone and ethanol.

	Reference solution	Lower Butyric acid Conc.	Lower Acetone Conc.	Lower Ethanol Conc.
Butyric acid (g/L)	7.47 ± 0.23	3.30 ± 0.14	6.98 ± 0.22	6.79 ± 0.21
Acetone (g/L)	13.83 ± 0.28	13.64 ± 0.28	10.74 ± 0.25	13.45 ± 0.31
Ethanol (g/L)	12.61 ± 0.11	12.87 ± 0.11	12.91 ± 0.11	8.30 ± 0.07

Table 5 Concentrations of the solutions used for the validation of Equation (1) using different combinations of concentrations for glucose and xylose.

	Reference solution	Lower Glucose conc.	Lower Xylose conc.
Glucose (g/L)	4.98 ± 0.08	2.63 ± 0.06	5.29 ± 0.09
Xylose (g/L)	5.14 ± 0.20	5.15 ± 0.20	2.64 ± 0.14

Table 6 Results obtained using the computer program to calculate the concentration of all components in ABE model solutions comprised of glucose: 4 g/L, xylose: 4 g/L, acetic acid: 5 g/L, butyric acid: 5 g/L, acetone: 6 g/L, ethanol: 2 g/L and butanol 12 g/L.

	Concentration calculated by the program (g/L)		
	Solution 1	Solution 2	Solution 3
Glucose	4.20 ± 0.08	4.29 ± 0.08	3.97 ± 0.07
Xylose	4.21 ± 0.18	4.22 ± 0.18	4.14 ± 0.018
Acetic acid	5.13 ± 0.27	5.24 ± 0.28	4.99 ± 0.027
Butyric acid	3.76 ± 0.16	3.83 ± 0.15	4.21 ± 0.16
Acetone	6.5 ± 0.21	6.32 ± 0.21	6.99 ± 0.2
Ethanol	2.00 ± 0.06	2.93 ± 0.06	2.56 ± 0.05
Butanol	11.96 ± 0.47	11.82 ± 0.46	12.17 ± 0.47

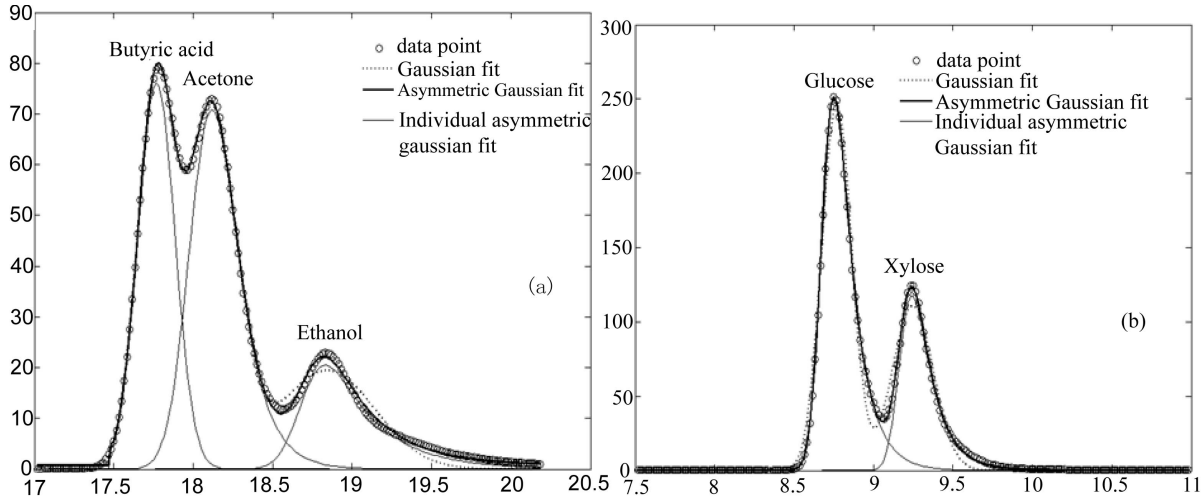


Figure 3 HPLC chromatograms for (a) butyric acid-acetone-ethanol and (b) glucose-xylose. The dotted lines are the HPLC raw data, and the lines are the fitted curves to the experimental data.

Results of this study showed that the computer program is able to deconvolute overlapping component peaks and to provide accurate estimates of the concentrations of the ABE components in a model solution. However, there are still some issues that remain to be investigated to develop a more accurate and precise program. One of the issues is the tailing of the peaks, which is related to the mechanism of component separation in the HPLC column. Each component has a specific adsorption-desorption mechanism based on the HPLC column packing material and this mechanism determines the retention time of that specific component. When two or three components have similar or close retention times, they move together very closely along the column and this affects the shape of their peaks in the final chromatogram. Thus, although it is possible to deconvolute the peaks, each peak has a different shape than the one for pure components due to interactions between different components. This phenomenon will cause errors in the results of peak deconvolution and the precision of the final concentration as measured by the computer program, will certainly be affected. However, in the ranges tested in the present study, the errors were not significant but it is still recommended to perform a more comprehensive investigation to develop an integrated program to obtain more accurate and precise results.

4. Conclusion

A method has been developed to better analyze the overlapping peaks in ABE solutions using HPLC. Due to very similar retention times of butyric acid, acetone and ethanol and also the close retention times of sugars (glucose and xylose) present in ABE fermentation broths, there are several peaks with conflicts in the HPLC chromatograms. This poses a significant challenge to determine the area of each peak and to quantify the corresponding concentrations. In this study, optimum operating conditions for the Vertex column, Eurokat H (300 × 8 mm, KNAUER, Germany) were found for ABE solution analysis (mobile phase flow rate of 0.8 mL/min and the column temperature of 85°C) to ensure that the peaks have minimum conflicts. Using asymmetric Gaussian curves, according to the characteristics of the peaks (being additive and not correlated) and linear regression in plotting the standard calibration curves, a program was written to fit the raw data from HPLC chromatograms to asymmetric Gaussian equations. Achieving an excellent fit, the program was able to determine the coefficients of the equations of each peak separately and to calculate the area of the curve. The standard calibration curves were validated by testing different samples of the components in binary and multi component aqueous solutions with known concentrations. Results show that this method is fairly accurate for ABE solution analysis but still needs some improvements to address some issues such as considering the different shapes of tailings of the peaks in order to make the program even more precise.

5 List of Abbreviations

ABE: Acetone-Butanol-Ethanol;
ATCC: American Type Culture Collection;
CV: Coefficient of Variation;
FID: Flame Ionized Detector;
GC: Gas Chromatograph;
HPLC: High Performance Liquid Chromatography;
LOD: Limit of Detection;
LOQ: Limit of Quantification;
NHOC: Net Heat of Combustion;

RCM: Reinforced Clostridium Medium;
RT: Retention Time.

6 Acknowledgements

The authors would like to thank the University of Ottawa and the Natural Sciences and Engineering Research Council of Canada (NSERC) for their financial contributions for this study.

7 References

[1] Abdehagh, N., Tezel, F.H. and Thibault, J. (2014) Separation Techniques in Butanol Production: Challenges and Developments (Review). Biomass and Bioenergy, 60, 222-246. http://dx.doi.org/10.1016/j.biombioe.2013.10.003

[2] Abdehagh, N., Tezel, F.H. and Thibault, J. (2013) Adsorbent Screening for Biobutanol Separation by Adsorption: Kinetics, Isotherms and Competitive Effect of Other Compounds. Adsorption, 19, 1263-1272. http://dx.doi.org/10.1007/s10450-013-9566-8

[3] Thompson, A.B., Cope, S.J., Swift, T.D. and Notestein, J.M. (2011) Adsorption of n-Butanol from Dilute Aqueous Solution with Grafted Calixarenes. Langmuir, 27, 11990-11998. http://dx.doi.org/10.1021/la202508q

[4] Harvey, B.G. and Meylemans, H.A. (2011) The Role of Butanol in the Development of Sustainable Fuel Technologies. Journal of Chemical Technology & Biotechnology, 86, 2-9. http://dx.doi.org/10.1002/jctb.2540

[5] Dellomonaco, C., Fava, F. and Gonzalez, R. (2010) The Path to Next Generation Biofuels: Successes and Challenges in the Era of Synthetic Biology. Microbial Cell Factories, 9, 3-17. http://dx.doi.org/10.1186/1475-2859-9-3

[6] Dürre, P. (2007) Biobutanol: An Attractive Biofuel. Biotechnology Journal, 2, 1525-1534. http://dx.doi.org/10.1002/biot.200700168

[7] Antoni, D., Zverlov, V.V. and Schwarz, W.H. (2007) Biofuels from Microbes (Mini Review). Applied Microbiology and Biotechnology, 77, 23-35. http://dx.doi.org/10.1007/s00253-007-1163-x

[8] Qureshi, N., Hughes, S., Maddox, I.S. and Cotta, M.A. (2005) Energy-Efficient Recovery of Butanol from Model Solutions and Fermentation Broth by Adsorption. Bioprocess and Biosystems Engineering, 27, 215-222. http://dx.doi.org/10.1007/s00449-

005-0402-8

[9] Ezeji, T.C., Qureshi, N. and Blaschek, H.P. (2004) Butanol Fermentation Research: Upstream and Downstream Manipulations. Chemical Record, 4, 305-314. http://dx.doi.org/10.1002/tcr.20023

[10] Thirmal, C. and Dahman, Y. (2012) Comparison of Existing Pretreatment, Saccharification, and Fermentation Processes for Butanol Production from Agricultural Residues. Canadian Journal of Chemical Engineering, 90, 745-761. http://dx.doi.org/10.1002/cjce.20601

[11] Zheng, Y.N., Li, L.Z., Xian, M., Ma, Y.J., Yang, J.M., Xu, X. and He, D.Z. (2009) Problems with the Microbial Production of Butanol. Journal of Industrial Microbiology and Biotechnology, 36, 1127-1138. http://dx.doi.org/10.1007/s10295-009-0609-9

[12] Wu, Y.D., Xue, C., Chen, L.J. and Bai, F.W. (2013) Effect of Zinc Supplementation on Acetone-Butanol-Ethanol Fermentation by *Clostridium acetobutylicum*. Journal of Biotechnology, 165, 18-21. http://dx.doi.org/10.1016/j.jbiotec.2013.02.009

[13] Lu, C., Zhao, J., Yang, S.T. and Wei, D. (2012) Fed-Batch Fermentation for n-Butanol Production from Cassava Bagasse Hydrolysate in a Fibrous Bed Bioreactor with Continuous Gas Stripping. Bioresource Technology, 104, 380-387. http://dx.doi.org/10.1016/j.biortech.2011.10.089

[14] Sharma, P. and Chung, W.J. (2011) Synthesis of MEL Type Zeolite with Different Kinds of Morphology for the Recovery of 1-Butanol from Aqueous Solution. Desalination, 275, 172-180. http://dx.doi.org/10.1016/j.desal.2011.02.049

[15] Oudshoorn, A., van der Wielen, L.A.M. and Straathof, A.J.J. (2009) Adsorption Equilibria of Bio-Based Butanol Solutions Using Zeolite. Biochemical Engineering Journal, 48, 99-103. http://dx.doi.org/10.1016/j.bej.2009.08.014

[16] Yang, X., Tsai, G.J. and Tsao, G.T. (1994) Enhancement of in Situ Adsorption on the Acetone-Butanol Fermentation by *Clostridium acetobutylicum*. Separations Technology, 4, 81-92. http://dx.doi.org/10.1016/0956-9618(94)80009-X

[17] Buday, Z., Linden, J.C. and Karim, M.N. (1990) Improved Acetone-Butanol-Ethanol Fermentation Analysis Using Subambient HPLC Column Temperature. Enzyme and Microbial Technology, 12, 24-27. http://dx.doi.org/10.1016/0141-0229(90)90175-P

[18] Wang, S., Zhang, Y., Dond, H., Mao, S., Zhu, Y., Wang, R., Luan, G. and Li, Y. (2011) Formic Acid Triggers the "Acid Crash" of Avetone-Butanol-Ethanol Fermentation by *Clostridium acetobutylicum*. Applied and Environmental Microbiology, 77, 1674-1680. http://dx.doi.org/10.1128/AEM.01835-10

[19] Finch, A.S., Mackie, T.D., Sund, C.J. and Sumner, J.J. (2011) Metabolite Analysis of *Clostridium acetobutylicum*: Fermentation in a Microbial Fuel Cell. Bioresource

Technology, 102, 312-315. http://dx.doi.org/10.1016/j.biortech.2010.06.149

[20] Cho, D.H., Shin, S.J. and Kim, Y.H. (2012) Effects of Acetic Acid and Formic Acid on ABE Production by *Clostridium acetobutylicum* and *Clostridium beijerinckii*. Biotechnology and Bioprocess Engineering, 17, 270-275. http://dx.doi.org/10.1007/s12257-011-0498-4

[21] Kumar, M., Sainib, S. and Gayen, K. (2014) Acetone-Butanol-Ethanol Fermentation Analysis Using Only High Performance Liquid Chromatography. Analytical Methods, 6, 774-781. http://dx.doi.org/10.1039/c3ay41717d

[22] Matsumura, M., Takehara, S., and Kataoka, H. (1992) Continuous Butanol/Isopropanol Fermentation in Down-Flow Column Reactor Coupled with Pervaporation Using Supported Liquid Membrane. Biotechnology and Bioengineering, 39, 148-156.

[23] Setlhaku, M., Brunberg, S., Villa, E.A.V. and Wichmann, R. (2012) Improvement in the Bioreactor Specific Productivity by Coupling Continuous Reactor with Repeated Fed-Batch Reactor for Acetone-Butanol-Ethanol Production. Journal of Biotechnology, 161, 147-152. http://dx.doi.org/10.1016/j.jbiotec.2012.04.004

[24] Setlhaku, M., Heitmann, S., Górak, A. and Wichmann, R. (2013) Investigation of Gas Stripping and Pervaporation for Improved Feasibility of Two-Stage Butanol Production Process. Bioresource Technology, 136, 102-108. http://dx.doi.org/10.1016/j.biortech.2013.02.046

[25] Aranda-González, I., Moguel-Ordoñez, Y. and Betancur-Ancona, D. (2014) Rapid HPLC Method for Determination of Rebaudioside D in Leaves of *Stevia rebaudiana* Bertoni Grown in the Southeast of México. American Journal of Anaytical Chemistry, 5, 813-819. http://dx.doi.org/10.4236/ajac.2014.513090

[26] Zhao, L., Liu, L. and Li, L. (2014) Qualitative and Quantitative Analysis of Five Bioactive Flavonoids in Salix bordensis Turcz. by HPLC-DAD and HPLC-ESI-MS. American Journal of Analytical Chemistry, 5, 851-860. http://dx.doi.org/10.4236/ajac.2014.513094

SECTION – IV: Desorption method

Chapter V

Adsorption, 21(3), 185-194 (2015)

Adsorptive separation and recovery of biobutanol from ABE model solutions

N. Abdehagh, P. Gurnani, J. Thibault, F. H. Tezel

Department of Chemical and Biological Engineering
University of Ottawa
161 Louis-Pasteur, Ottawa, ON, Canada K1N 6N5

Corresponding author: Tel: (613) 652-5800 Ext. 6094; Email: Jules.Thibault@uottawa.ca

Abstract

Adsorption is one of the most energy efficient techniques for butanol separation from dilute fermentation broths. To develop an efficient butanol adsorption process, adsorption and desorption are equally important since it is paramount to be able to desorb the adsorbed butanol to produce a high concentration butanol product. Although there are a good number of investigations done to find suitable adsorbents for this process, only few studies considered the desorption process. In this study, activated carbon F-400 was used as the butanol adsorbent since it has a high adsorption rate and capacity and is selective to butanol in the presence of other fermentation broth components. The thermal desorption process was performed in adsorption–desorption cycles both for butanol–water and acetone–butanol–ethanol (ABE) model solutions. The results for 1.5 wt% feed butanol–water binary solutions showed that the butanol adsorption capacity and the recovery of butanol were fairly constant (around 302 mg/g and 84 %, respectively) in subsequent adsorption–desorption cycles confirming that all the amount of adsorbed butanol is desorbed and the adsorption column could preserve its initial adsorption capacity in different cycles. Similar performance was obtained for butanol separation from the ABE model solution containing 1.2, 0.5, 0.2, 0.5, 0.5, 0.4 and 0.4 wt% butanol, acetone,

ethanol, butyric acid, acetic acid, glucose and xylose, respectively. The adsorption capacity and recovery for butanol were 170 mg/g and 80 %, respectively.

Keywords: Bio-butanol, ABE model solution, ABE fermentation, Adsorption, Desorption, Activated carbon, Biofuels

1 Introduction

Biofuels have attracted a lot of attention in recent decades as suitable alternatives for fossil fuels. Although fossil fuels are still the most common fuels being used, there are major concerns about replacing them with eco-friendly fuels produced from sustainable resources (Abdehagh et al. 2013, 2014; Thompson et al. 2011, 2014; Harvey and Meylemans 2011). Amongst the biofuels being studied, biobutanol is considered as one of the most promising since its characteristics are very similar to those of gasoline. Butanol's net heat of combustion (NHOC) of 29.2 MJ/L is close to the NHOC value of 32.5 MJ/L for gasoline (Abdehagh et al. 2013, 2014). In addition, butanol is less volatile, less flammable, less corrosive and thus safer to work with, in comparison to other biofuels. The other main benefit of butanol is its capability to be blended into gasoline in any proportion and can be used as a fuel in existing car engines without requiring modifications (Thompson et al. 2011, 2014; Harvey and Meylemans 2011; Shapovalov and Ashkinazi 2008; Fouad and Feng 2008; Dürre 2007; Qureshi et al. 2005; Qureshi and Blaschek 1999). Also, it can be transferred using existing pipelines and equipment that, as a result, reduces the investment and operating costs for biobutanol production processes significantly (Dellomonaco et al. 2010; Antoni et al. 2007; Ezeji et al. 2003, 2004, 2007). All these reasons have triggered the interest of industrialists and scientists to biobutanol as one of the best alternatives to gasoline.

However, there are some issues in biobutanol production processes. The first issue is related to the butanol fermentation process, called acetone–butanol–ethanol (ABE) fermentation, in which butanol, as the main product of fermentation, is toxic to the microorganism. Approximately 10 g/L (1 wt%) butanol or total ABE concentration of 20 g/ L (20 wt%), the inhibition starts due to the presence of butanol and the fermentation process stops (Thirmal and Dahman 2012; Harvey and Meylemans 2011, Oudshoorn et al.

2009; Zheng et al. 2009; García et al. 2009; Meagher et al. 1998). To partly alleviate this problem, there are a number of investigations being done to (1) genetically modify butanol-producing microorganisms to render them more tolerant to higher concentrations of butanol, and (2) to develop efficient in situ or ex situ separation methods to tackle the low butanol concentration in ABE fermentation broths (Faisal et al. 2014; Ying et al. 2013; Xue et al. 2012, 2013; Lu et al. 2012; Garcia-Chavez et al. 2012; Dhamole et al. 2012; Li et al. 2010; Ha et al. 2010). The present study focuses on the separation of butanol. Distillation, which is the traditional technique used to separate butanol from dilute ABE fermentation broths, is very energy-intensive and not economically viable. Liquid–liquid extraction, gas stripping, pervaporation and adsorption are other techniques that were proposed for the in situ or ex situ separation of biobutanol (Hecke et al. 2012, 2013; Lin et al. 2012, 2013; Yen et al. 2012; Wei et al. 2012; Li et al. 2011).

Adsorption is one of the most energy-efficient techniques to remove butanol from dilute solutions. To develop an efficient integrated adsorption process, it is necessary to use a suitable adsorbent for the specific application. There are some factors that should be considered in adsorbent screening: adsorption capacity, adsorption rate, selectivity for the desired component and ease of desorption. There are numerous studies in the literature about developing an efficient adsorption process for butanol separation using various adsorbents such as Ionic liquids (Rabari and Banerjee 2014), polyvinylpyridine (Yang et al. 1994), polymeric resins (Liu et al. 2014; Nielsen and Prather 2009; Nielsen et al. 1988; Groot and Luyben 1986; Maddox 1982), activated carbon (AC) and zeolites (Thompson et al. 2011; Sharma and Chung 2011; Saravanan et al. 2010; Holtzapple and Brown 1995; Sowerby and Crittenden 1988; Groot and Luyben 1986; Maddox 1982).

According to the results of these studies, zeolites and ACs have the highest adsorption capacity for butanol separation from dilute solutions (Abdehagh et al. 2013, 2014; Oudshoorn et al. 2012; Remi et al. 2011, 2012; Zheng et al. 2009; Takeuchi et al. 1995; Regdon et al. 1994a, 1994b). However, there are only few studies that have considered the effect of the presence of other components on butanol adsorption as well as the desorption process for the adsorbed butanol. In our previous study, the effect of the presence of other compounds in ABE broths was investigated using AC F-400 since this adsorbent showed very high maximum adsorption capacity (around 300 mg/g) and high

adsorption rate for butanol adsorption (Abdehagh et al. 2013). Results showed that this adsorbent is more selective to butanol in comparison to other compounds present in the ABE broths. In the case of butanol recovery, adsorption and desorption are equally important to produce solutions with high concentrations of butanol as the product. In addition, the capability of reusing the adsorbent for numerous separation cycles is equally important. However, desorption methods are not as well considered in the literature as adsorption (Abdehagh et al. 2014; Faisal et al. 2014; Rabari and Banerjee 2014; Sharma and Chung 2011; Remi et al. 2011; Groot and Lluyben 1986). Saravanan et al. (2010) have reported a desorption recovery of 80 % with the hypothesis that the rest of the butanol was strongly adsorbed and could not be desorbed using argon as the purge gas at a temperature of 150 °C and at higher temperature (more than 250°C), butanol was desorbed as butene. Remi et al. (2012) have reported that the effluent of the desorption column had a butanol concentration of 43.3 % when the column was saturated with ethanol and butanol. The latter result did not change when they increased the temperature from 90 to 150°C. In the present study, the butanol desorption process from butanol–water and ABE model solutions was investigated for numerous adsorption–desorption cycles to assess the performance of the process in long-term applications.

2 Materials and methods

2.1 Materials

To prepare butanol–water and ABE model solutions, n-butanol (99 % pure, Acros), acetone (95 % pure, Acros), n-butyric acid (99 % pure, Acros) and 99 % pure ethanol, acetic acid, glucose and xylose (Fisher Scientific Co., Fair Lawn, NJ) were used. Deionized distilled water was used to prepare all model solutions. The adsorbent used for this study was F-400 activated carbon (AC F-400) purchased from Calgon Corporation, Mississauga, ON, Canada, which showed the best potential, among tested adsorbents, for butanol adsorption in our previous studies (Abdehagh et al. 2013). Table 1 shows some of the properties of this adsorbent.

Table 1 Some of the properties of activated carbon F-400 used as butanol adsorbent in this study.

Activated carbon F-400	
Shape	Granule
Surface area	1090 (m^2/g)
Mean particle diameter	0.55-0.75 (mm)
Mesh size	12 x 40
Micropore volume	0.43 (cm^3/g)

2.2 Methods

2.2.1 Adsortion breakthrough experiments

Adsorption experiments were performed in a packed bed having an inner diameter of 0.02 m and a length of 0.195 m. For breakthrough experiments, the feed solution was pumped to the column using a peristaltic pump and samples of the effluent were taken at specific time intervals until the adsorbent bed reached complete saturation. The concentrations of butanol, ethanol, acetone, acetic acid, butyric acid and sugars in the solutions and samples were determined by high performance liquid chromatography (HPLC) (Waters, Canada). Figure 1a shows the schematic diagram of the experimental system used in this investigation for breakthrough adsorption experiments.

2.2.2 Thermal desorption experiments

When an adsorption experiment was complete and the column reached complete saturation, the column was carefully drained to remove the liquid present in the column. It was then placed in the desorption setup (Fig. 1b) within an oven, where a purge stripping gas (CO_2) was circulated through the column until the adsorbed components were completely desorbed and the adsorbent was regenerated. The gas stream exiting the column during desorption was passed through a cold trap to condense the majority of the desorbed products.

The adsorption capacities were determined using the breakthrough curves by integrating the area above the breakthrough curve which is proportional to the total amount adsorbed in the column as expressed by Eq. 1.

$$q_i = \frac{t_i \times m_{flow,i}}{m_{adsorbent}} \quad (1)$$

where q_i, $m_{flow,i}$ and $m_{adsorbent}$ represent the adsorption capacity for component i (g/g adsorbent), the mass flow rate for component i (g/s), and the mass of adsorbent (g) in the column, respectively.

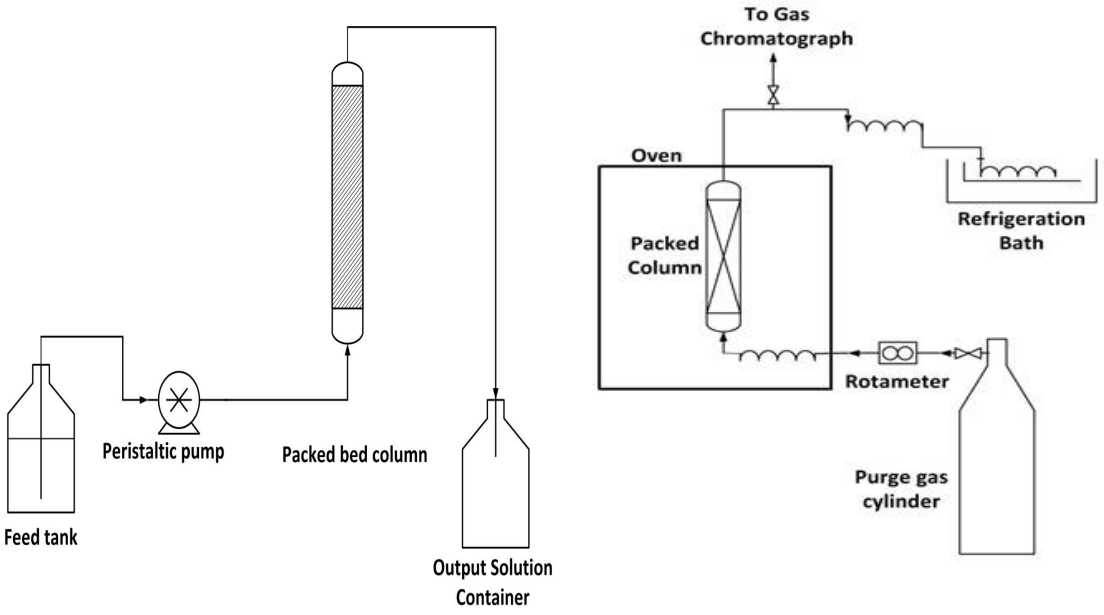
The term t_i corresponds to the integration of the area above the normalized breakthrough curve (Eq. 2).

$$t_i = \int_0^{\infty} (1 - \frac{c_i}{c_{i0}}) dt \quad (2)$$

where c_i and c_{i0} represent the outlet and inlet concentrations for component i (g/L), respectively.

2.2.3 Analytical method

The concentrations of butanol, ethanol, acetone, acetic acid, butyric acid, glucose and xylose were determined using HPLC. The HPLC used in this study was purchased from Waters, Canada. The detector, pump and auto-sampler were Refractive Index Detector (Waters 2414), Isocratic HPLC pump (Waters 1515) and Autosampler (Waters 717 plus), respectively. To heat the column to the desired temperature, an external column heater was used. The column used to detect the ABE solutions in this study was the Vertex column (300 x 8 mm, KNAUER, Germany) packed with Eurokat H, 10 µm. The software used to operate the HPLC was Breeze purchased from Waters, Canada. The mobile phase used in HPLC analysis was 0.01 N sulfuric acid with a flow rate of 0.8 mL/min and the temperature of the column was kept at 85°C (Abdehagh et al. 2015). The experiments in this study were repeated once. More repetitions were considered in some minor experiments based on the results.



(a) (b)

Figure 1 a Schematic diagram of the adsorption experimental setup used for breakthrough adsorption experiments. b Schematic diagram of the desorption experimental setup used in this study

3 Results and discussion

3.1 Desorption process operating conditions

To select the operating conditions for the butanol desorption process, butanol–water model solutions with 1.5 wt% of butanol were used as the feed for the adsorption runs. After the column reached complete saturation, desorption runs were performed under different conditions. To find the optimum operating conditions for desorption experiments, different purge gas temperatures and flow rates were tested to achieve the highest butanol recovery. For an accurate operating condition optimization, it is desired to minimize the time of desorption, maximize butanol recovery, minimize the stripping gas temperature, minimize the amount of CO_2 used and to maximize the cold trap temperature. Obviously, for this multicriteria optimal problem it is not possible to find a unique solution to satisfy all these objectives simultaneously and a judicious compromise must be struck between all these objectives. However, it would be sufficient for our study

to reach the highest butanol recovery. To find the best operating conditions based on the butanol recovery, a set of experiments was tested at different purge gas flow rates and temperatures to measure their impact on the achieved butanol recovery. The selected condenser temperature was determined by performing steady-state UniSim simulations to find the temperature at which most butanol would condense while trying to have the highest possible temperature.

The temperature of the purge gas was the first parameter to be tested, since it determines the most appropriate compromise between a high temperature for faster desorption and a low temperature for minimum heating requirements for the purge gas. The purge gas flow rate and the cold trap temperature were other parameters that were tested for the butanol desorption process. To find the suitable values of parameters, a series of experiments were performed and the results were compared. Five different oven temperatures were tested: 100, 125, 150, 175 and 200°C. It was found that at temperatures higher than 175 °C the recovery was constant at its maximum value (Fig. 2a). Different purge gas flow rates (20, 40, 60, 80, 100 mL/min) were also tested and it was observed that the highest recovery was obtained at a flow rate of 40 mL/min as shown in Fig. 2b. The corresponding purge gas superficial velocities were 0.001, 0.002, 0.003, 0.004 and 0.0053 m/s for the column size that was used.

To find the cold trap temperature, a simple simulation was performed using Honeywell UNISIM. The cold trap temperature was varied in the range from -40 to 20°C to investigate its impact on the butanol recovery for an effluent purge gas stream containing a butanol:water mass ratio of 0.25:1 at atmospheric pressure. The fluid package used for this model was non-random two liquid model (NRTL) and was validated using vapour–liquid equilibrium data for butanol–water binary system (Khoury 2005). Figure 3 shows the predicted butanol recovery and mass fraction at different cold trap temperatures. It can be observed that the difference between the percentage recovery obtained at -40 and 2°C is not significant with values of 100 and 98%, respectively. It was also observed that the butanol mass fraction in the condensed phase obtained at -40, 2 and 20°C were 0.25, 0.248 and 0.242, respectively. Even though there were some assumptions considered in Honeywell UNISIM simulations such as equilibrium conditions between the flowing gas phase and the condensed phase, 2°C was selected as

the experimental cold trap temperature since the difference between the recoveries at -40 and 2°C was negligible (Rohani et al. 2014). This is justified considering the high energy requirement for low cold trap temperatures. Assuming thermodynamic equilibrium conditions implies that the rate of mass transfer from the flowing desorption gas and the cold trap walls is very high.

To find the optimum operating conditions for butanol desorption from ABE model solutions the same conditions (purge CO_2 gas flow rate of 40 mL/min (0.002 m/s) at 175°C) were used for 6 h since based on the previous results, the desorption is almost complete within a few hours. For the purge gas temperature and flow rate in the next step of desorption, flow rates of 40, 60 and 80 mL/min at 185 and 200°C were tested and it was observed that the highest butanol recovery was achieved at 60 mL/min (0.003 m/s) at 185°C. The results showed that using these conditions would lead to a complete desorption of all components and the adsorption capacity of the adsorbent particles would be the same as its initial capacity afterwards.

3.2 Adsorption–desorption for butanol–water solutions

The desorption process were then used to perform a series of adsorption–desorption experiments using butanol–water binary solutions. The concentration of butanol in the solution for the adsorption experiments was 15 g/L (1.5 wt%) which is typically the maximum butanol concentration that could be encountered in the ABE fermentation broths. After completion of the breakthrough experiment, the free liquid was allowed to drip off the column and then the saturated packed column was placed in the desorption setup and desorption was carried out for 24 h with a CO_2 purge gas passing through the column placed in an oven maintained at 175°C. During the desorption experiments, samples of the condensed liquid phase were taken at different time intervals and analyzed by HPLC. It was observed that after 15 h, desorption appears to be complete as no more condensate was collected in the cold trap. However, the purge gas continued circulating within the column to ensure that there is no more adsorbed component in the packed column. Using the breakthrough curve, the adsorption capacity was calculated using Eqs. 1 and 2 and the amount of butanol adsorbed was determined.

For the adsorption breakthrough curve shown in Fig. 4, the estimated butanol amount adsorbed was 12.24 g and the adsorption capacity was 312 mg/g.

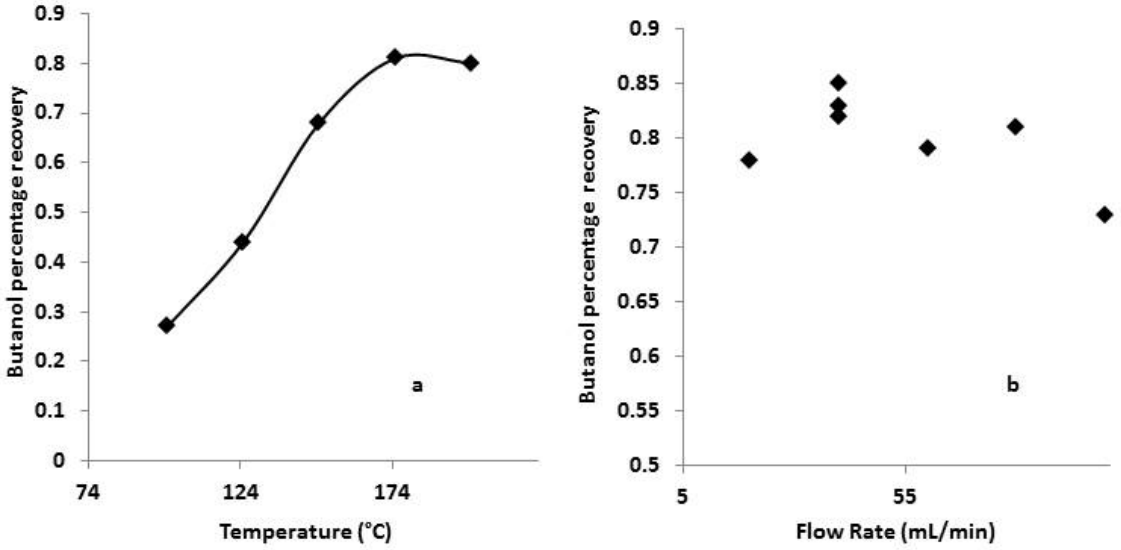


Figure 2 Butanol desorption recovery results from butanol–water solutions for cold trap temperature of 2°C for: a different purge gas temperatures at purge gas flow rate of 40 mL/min and b different purge gas flow rates at purge gas temperature of 175°C

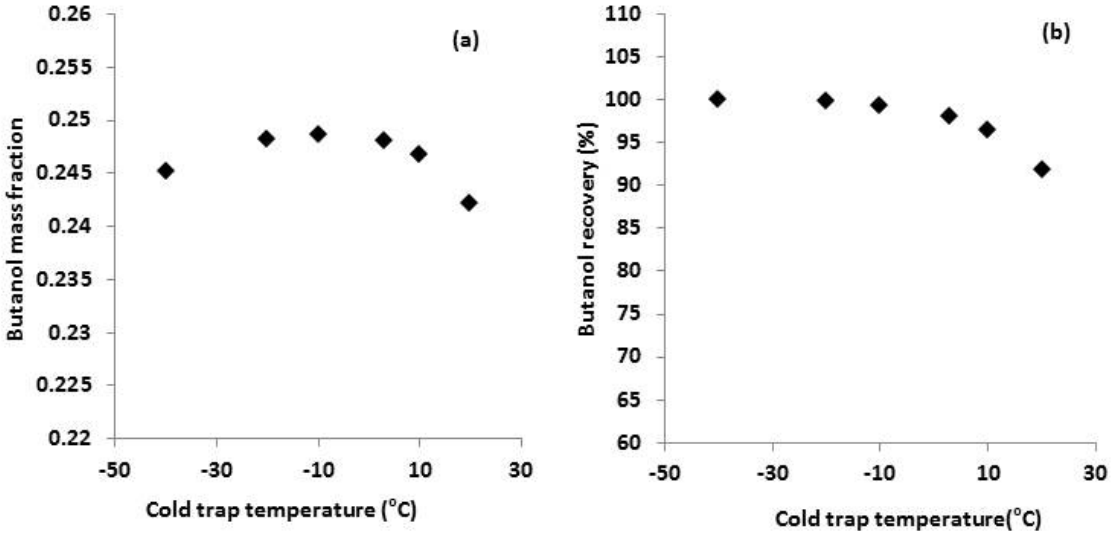


Figure 3 Butanol mass fraction (a) and recovery (b) in the butanol desorption process from an effluent purge gas stream containing a butanol:water mass ratio of 0.25:1, using Honeywell UNISIM simulation

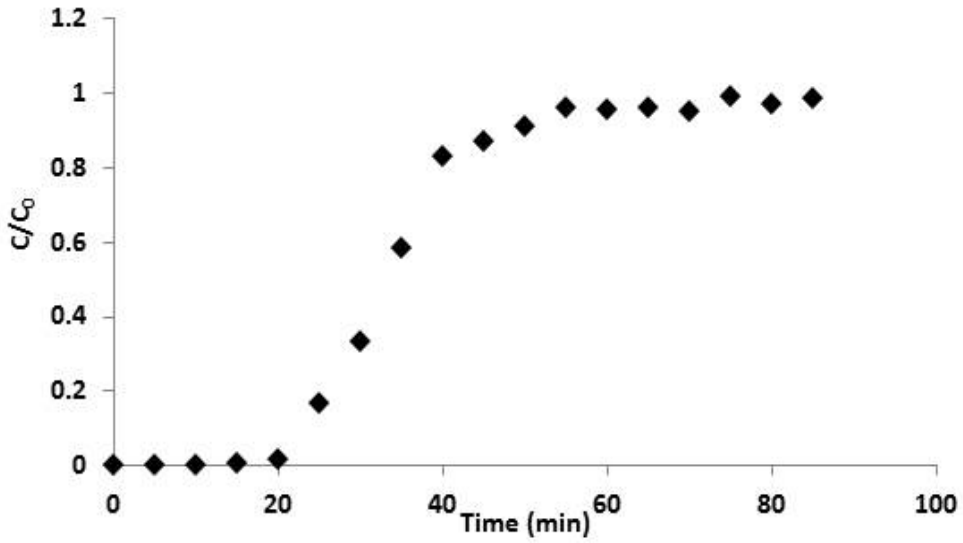


Figure 4 Adsorption breakthrough curve for butanol– water solution at a flow rate of 24.4 mL/min at room temperature for activated carbon F-400

Following the completion of the adsorption breakthrough curve, desorption experiment was carried out at 175°C with the purge gas flow rate of 40 mL/min (0.002 m/s) for 24 h. Results showed that 10.05 g of butanol was recovered. For this experiment, the ratio of the amount of recovered butanol to adsorbed butanol was 0.82 (g/g). Since the time given to one desorption experiment was definitely enough to displace all the compounds adsorbed onto the adsorbent particles, it was assumed that the unrecovered fraction of butanol is due to the cold trap conditions. The purge gas at the outlet of the column contains butanol and water in vapor phase which upon contact with the cold trap, the majority of butanol and water should ideally condense. However, this assumes that equilibrium between the purge gas stream and the walls of the cold trap prevails. The butanol recovery of 82 % is undoubtedly due to the loss of butanol and water being carried by the purge gas exiting the cold trap after the condensation. Águeda et al. (2013) used a cold trap temperature of -10°C, 12°C lower than that in this investigation, for desorption of butanol–water binary solution from silicalite pellets, for a butanol recovery of 95 %. Thus, although it is believed that the whole amount of adsorbed butanol in the column is desorbed, the condensed amount of butanol in the cold trap is less than the amount initially adsorbed onto the adsorbent surface in the column during adsorption. To

investigate this phenomenon, subsequent cycles of adsorption–desorption experiments were performed.

Results obtained for the second adsorption–desorption cycle showed that the adsorption capacity was 294 mg/g and the recovery of butanol was 85.6 %. Since a slight decrease was observed in the adsorption capacity for the second cycle, a third cycle was carried out. The resulting adsorption capacity and the butanol recovery were 308 mg/g and 89.1 %, respectively. These results confirmed that the total amount of butanol adsorbed in each adsorption experiment was desorbed during the subsequent desorption cycle and the percentage recovery is relatively constant from cycle to cycle and the adsorption capacity is identical to the original adsorbent. Thus, these results reaffirm that the loss of butanol (between 10 and 18 wt%) is due to the incomplete condensation during the desorption process. In addition, the packed adsorption column preserved its initial adsorption capacity for several adsorption–desorption cycles.

To test the adsorbent lifetime and its capability to be used for numerous adsorption–desorption cycles, further adsorption–desorption cycles were carried out. A total of six adsorption–desorption experiments were carried out using the same adsorbent particles in the column. Table 2 presents the results for the six adsorption–desorption experiments. It can be observed that the adsorption capacity and percentage recovery are roughly identical for all cycles confirming that the AC F-400 is an excellent adsorbent for butanol separation since it has all the desired characteristics of a suitable adsorbent. AC F-400 has a very high adsorption capacity (293–312 mg/g) and adsorption rate. In addition, it is selective to butanol in the presence of other components (Abdehagh et al. 2013) and the desorption process can be completed using a thermal desorption method.

To improve the desorption process in increasing the recovery of the butanol, it would be possible to set a lower cold trap temperature as used by Águeda et al. (2013) but at the expense of higher cooling cost. It is believed that a better solution would be to reheat and recirculate the purge gas stream exiting the cold trap to the packed column such that the amount of butanol, which did not condense in the cold trap, would not be discarded and the majority of the butanol would eventually condense in the cold trap. This would significantly increase the total percentage recovery. The current desorption system did not allow to recycle the exiting CO_2 stream.

Table 2 Results of the six adsorption-desorption cycles for butanol separation from butanol-water binary solutions using adsorption.

Cycle	Initial butanol conc. (g/L)	Adsorption capacity (mg/g)	Butanol adsorbed (g)	Butanol desorbed (g)	Butanol conc. in desorbed phase (g/L)	Recovery (%)
1	15.14	312	12.24	10.05	302.25	82.1
2	15.38	294	11.56	9.9	279.26	85.6
3	15.14	308	12.11	10.8	293.47	89.1
4	15.2	303	11.9	9.8	272.98	82.3
5	14.97	293	11.5	9.44	259.34	82.0
6	14.98	306	12.00	9.7	260.58	81.0

3.3 Adsorption–desorption for ABE model solutions

After testing numerous adsorption–desorption cycles for butanol–water binary solutions, the next step was to perform the adsorption–desorption processes for the ABE model solutions containing all of the main chemical components present in an actual ABE fermentation broth. The ABE model solutions contained butanol, acetone, ethanol, acetic acid, butyric acid, glucose and xylose at concentrations of 1.2, 0.6, 0.2, 0.5, 0.5 0.4 and 0.4 wt%, respectively, as the feed into the column to carry out the adsorption runs.

Figure 5 shows the adsorption breakthrough curve obtained for the ABE model solution experiment performed with AC F-400. This figure shows that glucose and xylose breakthrough curves are nearly identical. Sugars, acetic acid, ethanol and acetone were adsorbed initially in the column and were later desorbed and displaced by butyric acid and butanol. This is the reason for the column exit concentrations of sugars, acetic acid, ethanol and acetone to exceed their concentrations in the feed upon desorption. For the AC F-400, butanol and butyric acid are preferentially adsorbed such that they will be adsorbed in the unsaturated part of the column and decrease to very low concentrations outside of the butanol/butyric acid adsorption zone in the column and, of course, at the exit of the column. Because their concentrations are very low toward the exit in the column in fluid phase, the adsorption sites there can be occupied by the other chemical species until the adsorption zone of butyric acid and butanol reaches these adsorption sites and the other less selective molecules are displaced from the adsorbed phase.

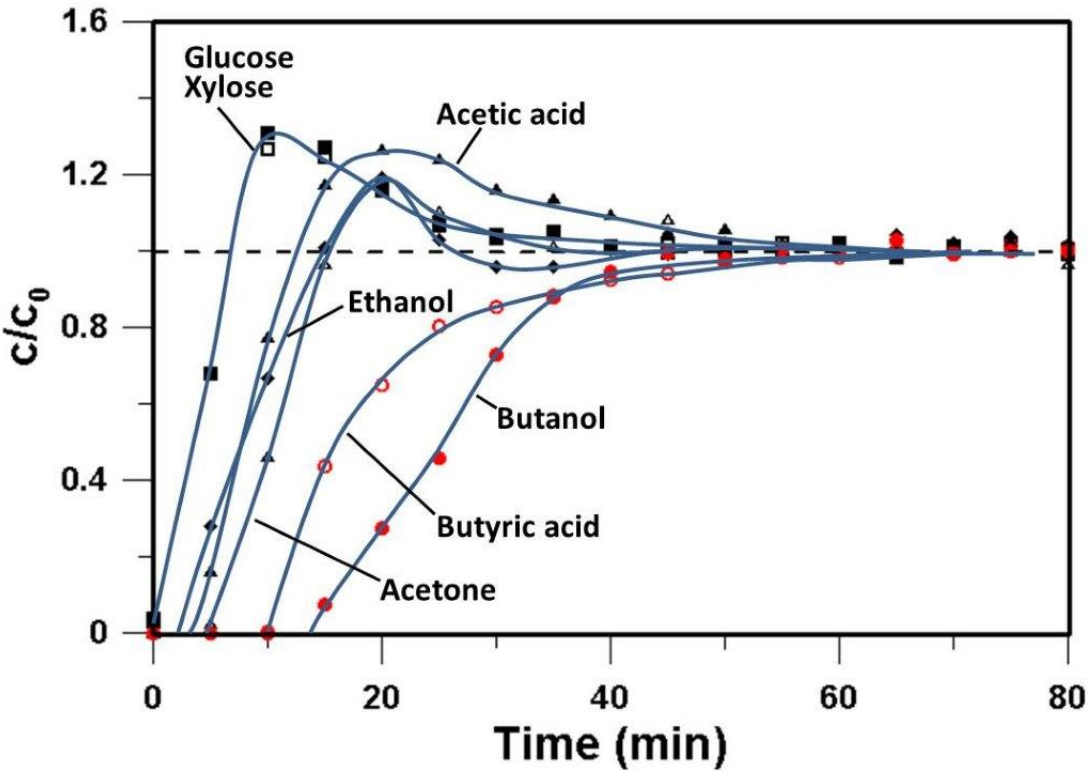


Figure 5 Adsorption breakthrough curve for the ABE model solution at a flow rate of 24.4 mL/min at room temperature for activated carbon F-400

For the breakthrough curves of Fig. 5, the final adsorption capacity for butanol, acetone, ethanol, acetic acid, butyric acid, glucose and xylose upon saturation of the column were 193.3, 25, 7, 4.5, 64.5, 0 and 0 mg/g, respectively. It was observed that in the presence of other compounds, the adsorption capacity of butanol decreased from around 300–170 mg/g and this decrease is mainly due to the presence of butyric and acetic acid according to our previous published results (Abdehagh et al. 2013). Based on the effluent concentration profiles of Fig. 5, it appears that glucose and xylose are first excluded from the adsorption sites by the other species that have greater adsorptive affinity. It seems that other components such acetone, ethanol and acetic acid have displaced the sugars and, in turn, these components were excluded from the adsorption sites by the stronger affinity of butyric acid and butanol. These results will be confirmed by modelling of the breakthrough experiments, which is currently being conducted. After the completion of the adsorption of all the components in the fluid phase (i.e. after the

complete saturation of the column), all the column outlet compositions return to the feed compositions for all the components in the system.

It is important to note that the final amount of sugar being adsorbed is estimated to be zero. This observation is important because if sugars were to occupy adsorption sites, they would not be desorbed with the hot purge gas and would accumulate in the column. The fact that the adsorption capacity is not altered from cycle to cycle confirms these results.

Table 3 Results of butanol adsorptive butanol separation from ABE model solutions for the 7 adsorption-desorption cycles.

Cycle	Initial butanol conc. (g/L)	Adsorption capacity (mg/g)	Amount adsorbed (g)	Amount desorbed (g)	Butanol conc. in desorbed phase (g/L)	Recovery (%)
1	12.04	193.3	7.53	5.4	153.8	72
2	12	167.3	6.52	5.2	142.9	80
3	11.9	165	6.42	5	143.14	78
4	11.9	145.4	5.67	4.6	128.87	81
5	11.95	169.2	6.6	5.71	165.5	86.5
6	12	172.6	6.73	5.2	146.68	78
7	12	162.9	6.31	5.2	140.92	82.5

Table 4 Adsorption-desorption results for acetone, ethanol, acetic acid, butyric acid and sugars in 7 adsorption-desorption cycles for adsorptive butanol separation from ABE model solutions.

Cycle	Initial conc. (g/L)	Adsorption capacity (mg/g)	Recovery (%)	Cycle	Initial conc. (g/L)	Adsorption capacity (mg/g)	Recovery (%)
Acetone				**Ethanol**			
1	5.23	25.0	62	**1**	2.8	7.0	20
2	5.31	11.7	100	**2**	2.7	9.6	100
3	5.25	24.3	15	**3**	2.93	13.7	20
4	5.5	18.6	42	**4**	2.83	9.0	85
5	5.8	27.8	44	**5**	2.96	14.5	100
6	5.53	24.5	40	**6**	2.99	14.3	80
7	5.69	0.4	100	**7**	3.13	7.2	95
Acetic acid				**Butyric acid**			
1	4.92	4.5	100	**1**	5.16	64.5	74
2	4.99	9.7	80	**2**	5.28	49.5	96
3	5.06	7.2	75	**3**	5.28	49.4	96
4	5.17	3.9	100	**4**	5.44	44.7	100
5	5.2	5.1	100	**5**	5.11	57.8	67
6	5.01	8.9	66	**6**	5.1	51.7	90
7	5.17	8.7	100	**7**	5.26	58.9	100

To ensure that the adsorption system can preserve its performance in subsequent cycles, the adsorption–desorption experiments were also performed for a total of seven cycles using the same adsorbent particles for the ABE model solution. Table 3 shows the results for butanol desorption from the ABE model solutions for these seven cycles. These results showed that although the butanol adsorption capacity slightly decreased in the second cycle, it was fairly constant for the next six cycles and the recovery obtained varied between 72 and 86.5 % in all seven adsorption–desorption experiments that were performed. For acetone, ethanol, acetic acid and butyric acid, the adsorption capacity and recovery were also constant in all seven adsorption–desorption cycles (Table 4). Traces of sugars were observed in the desorbed phase, which might be due to the liquid retained in the column after draining.

Table 3 shows that the adsorption process for butanol separation from a dilute ABE model solution was able to increase the concentration of butanol from 1.2 wt% (12 g/L) to approximately 15 wt% (150 g/L).

To improve the recovery of butanol, the same method applied for desorption of butanol–water binary solution could be implemented for the ABE model solution. Recirculation of the purge gas from the cold trap to the packed bed column would prevent the loss of butanol as well as other solvents in the gas phase. However, it was concluded that during the desorption process, all of the adsorbed components were desorbed completely and the adsorbent particles preserved their adsorbent characteristics during the entire adsorption process, that is for the seven adsorption–desorption cycle experiments.

4 Conclusions

Although biobutanol has attracted a lot of attention as a biofuel to replace fossil fuels, there are significant issues in its bioproduction process such as the separation methods used to remove and recover butanol from dilute ABE fermentation broths. Adsorption is one of the most efficient methods to separate butanol from dilute solutions. There are numerous investigations done to find the best adsorbent for this application. However, only few studies have investigated the desorption and recovery of butanol following the adsorption process. AC F-400 is one of the best adsorbents for this

application since it has high adsorption capacity and high adsorption rate as well as being selective to butanol in comparison to other components present in the ABE fermentation broths.

In this study, butanol adsorption and desorption from butanol–water and ABE model solutions were investigated and it was found that a thermal desorption method is an efficient technique for the butanol separation and recovery process. In different subsequent adsorption–desorption cycles, the adsorption capacity of AC F-400 for butanol was between 293 and 312 mg/g and the butanol recovery varied between 81 and 89.1 % for butanol separation from butanol–water binary solutions. For butanol separation from the ABE model solutions the average butanol adsorption capacity and recovery were 170 mg/g and 80 %, respectively. It was observed that the adsorption capacity and recovery were fairly constant both for the butanol–water and the ABE model solutions. Thus, it was concluded that the whole amount of adsorbed butanol and other compounds were desorbed completely in each desorption experiment. The results confirmed that AC F-400 is one of the best adsorbents for butanol separation processes since in addition to the high adsorption capacity and adsorption rate, adsorbed butanol could be desorbed and recovered as a high butanol concentration product, using a thermal desorption process.

5 Acknowledgements

The authors would like to thank the University of Ottawa and Natural Sciences and Engineering Research Council (NSERC) of Canada for their financial contributions for this study.

6 References

Abdehagh, N., Tezel, F.H., Thibault, J.: Adsorbent screening for biobutanol separation by adsorption: kinetics, isotherms and competitive effect of other compounds. Adsorption 19, 1263–1272 (2013)

Abdehagh, N., Tezel, F.H., Thibault, J.: Separation techniques in butanol production: challenges and developments (Review). Biomass Bioenergy 60, 222–246 (2014)

Águeda, V.I., Delgado, J.A., Uguina, M.A., Sotelo, J.L., García, A.: Column dynamics of

an adsorption–drying–desorption process for butanol recovery from aqueous solutions with silicalite pellets. Sep. Pur. Technol. 104, 307–321 (2013)

Antoni, D., Zverlov, V.V., Schwarz, W.H.: Biofuels from microbes (mini review). Appl. Microbiol. Biotechnol. 77, 23–35 (2007)

Dellomonaco, C., Fava, F., Gonzalez, R.: The path to next generation biofuels: successes and challenges in the era of synthetic biology. Microb. Cell Fact. 9, 3–17 (2010)

Dhamole, P.B., Wang, Z., Liu, Y., Wang, B., Feng, H.: Extractive fermentation with non-ionic surfactants to enhance butanol production. Biomass Bioenergy 40, 112–119 (2012)

Dürre, P.: Biobutanol: an attractive biofuel. Biotechnol. J. 2, 1525–1534 (2007)

Ezeji, T.C., Qureshi, N., Blaschek, H.P.: Production of acetone, butanol and ethanol by *Clostridium beijerinckii* BA101 and in situ recovery by gas stripping. World J. Microbiol. Biotechnol. 19, 595–603 (2003)

Ezeji, T.C., Qureshi, N., Blaschek, H.P.: Butanol fermentation research: upstream and downstream manipulations. Chem. Rec. 4, 305–314 (2004)

Ezeji, T.C., Qureshi, N., Blaschek, H.P.: Bioproduction of butanol from biomass: from genes to Bioreactors. Curr. Opin. Biotechnol. 18, 220–227 (2007)

Faisal, A., Zarebska, A., Saremi, P., Korelskiy, D., Ohlin, L., Rova, U.: MFI zeolite as adsorbent for selective recovery of hydrocarbons from ABE fermentation broths. Adsorption 20, 465–470 (2014)

Fouad, E.A., Feng, X.: Use of pervaporation to separate butanol from dilutes aqueous solutions: effects of operating conditions and concentration polarization. J. Membr. Sci. 323, 428–435 (2008)

García, V., Pongracz, E., Muurinen, E., Keiski, R.L.: Recovery of n-butanol from salt containing solutions by pervaporation. Desalination 241, 201–211 (2009)

Garcia-Chavez, L.Y., Garsia, C.M., Schuur, B., de Haan, A.B.: Biobutanol recovery using non-fluorinated task-specific ionic liquids. Ind. Eng. Chem. Res. 51, 8293–8301 (2012)

Groot, W.J., Luyben, KChAM: In situ product recovery by adsorption in the butanol/isopropanol batch fermentation. Appl. Microbiol. Biotechnol. 25, 29–31 (1986)

Ha, S.H., Maib, N.L., Koo, Y.M.: Butanol recovery from aqueous solution into ionic liquids by liquid–liquid extraction. Process Biochem. 45, 1899–1903 (2010)

Harvey, B.G., Meylemans, H.A.: The role of butanol in the development of sustainable fuel technologies. J. Chem. Technol. Biotechnol. 86, 2–9 (2011)

Hecke, W.V., Vandezande, P., Claes, S., Vangeel, S., Beckers, H., Diels, L.: Integrated bioprocess for long-term continuous cultivation of *Clostridium acetobutylicum* coupled to pervaporation with PDMS composite membranes. Bioresour. Technol. 111, 368–377 (2012)

Hecke, W.V., Hofmann, T., De Wever, H.: Pervaporative recovery of ABE during continuous cultivation: enhancement of performance. Bioresour. Technol. 129, 421–429 (2013)

Holtzapple, M.T., Brown, R.F.: Conceptual design for a process to recover volatile solutes from aqueous solutions using silicalite. Sep. Technol. 4, 213–229 (1995)

Khoury, F.M.: Multistage Separation Processes, 3rd edn. CRC Press, Boca Raton (2005)

Li, G., Wei, W., Wu, H., Dong, X., Jiang, M., Jin, W.: Pervaporation performance of PDMS/ceramic composite membrane in acetone butanol ethanol (ABE) fermentation-PV coupled process. J. Membr. Sci. 373, 121–129 (2011)

Li, S., Srivastava, R., Parnas, R.S.: Separation of 1-butanol by pervaporation using a novel tri-layer PDMS composite membrane. J. Membr. Sci. 363, 287–294 (2010)

Lin, X., Li, R., Wen, Q., Wu, J., Fan, J., Jin, X.: Experimental and modeling studies on the sorption breakthrough behaviors of butanol from aqueous solution in a fixed-bed of KA-I resin. Biotechnol. Bioprocess Eng. 18, 223–233 (2013)

Lin, X., Wu, J., Fan, J., Qian, W., Zhou, X., Qian, C.: Adsorption of butanol from aqueous solution onto a new type of macroporous adsorption resin: studies of adsorption isotherms and kinetics simulation. J. Chem. Technol. Biotechnol. 87, 924–931 (2012)

Lu, C., Zhao, J., Yang, S.T., Wei, D.: Fed-batch fermentation for n-butanol production from cassava bagasse hydrolysate in a fibrous bed bioreactor with continuous gas stripping. Bioresour. Technol. 104, 380–387 (2012)

Liu, D., Chen, Y., Ding, F.Y., Zhao, T., Wu, J.L., Guo, T., Ren, H.F. i, B.B., Niu, H.Q., Cao, Z., Lin, X.Q., Xie, J.J., He, X.J., Han Jie Ying, H.J.: Biobutanol production in a *Clostridium acetobutylicum* biofilm reactor integrated with simultaneous product recovery by adsorption. Biotechnol. Biofuels 7, 5–17 (2014)

Maddox, I.S.: Use of silicalite for the adsorption of n-butanol from fermentation liquids. Biotechnol. Lett. 4, 759–760 (1982)

Meagher, M.M., Qureshi, N., Hutkins, R.: Silicalite membrane and method for the selective recovery and concentration of acetone and butanol from model ABE solutions and fermentation broth. US patent 5,755,967 (1998)

Nielsen, D.R., Prather, K.J.: In situ product recovery of n-butanol using polymeric resins. Biotechnol. Bioeng. 102, 811–821 (2009)

Nielsen, L., Larsson, M., Hoist, O., Mattiasson, B.: Adsorbents for extractive bioconversion applied to the acetone-butanol fermentation. Appl. Microbiol. Biotechnol. 28, 335–339 (1988)

Oudshoorn, A., Van der Wielen, L.A.M., Straathof, A.J.J.: Assessment of options for selective 1-butanol recovery from aqueous solution. Ind. Eng. Chem. Res. 48, 7325–7336 (2009)

Oudshoorn, A., Van der Wielen, L.A.M., Straathof, A.J.J.: Adsorption equilibria of bio-based21 Recovery of 1-butanol from aqueous solutions using zeolite ZSM-5 with a high Si/Al ratio; suitability of a column process for industrial applications. Biochem. Eng. J. 49, 33–39 (2012)

Qureshi, N., Hughes, S., Maddox, I.S., Cotta, M.A.: Energy-efficient recovery of butanol from model solutions and fermentation broth by adsorption. Bioprocess Biosyst. Eng. 27, 215–222 (2005)

Qureshi, N., Blaschek, H.P.: Production of acetone butanol ethanol (ABE) by a hyper-producing mutant strain of *Clostridium beijerinckii* BA101 and recovery by pervaporation. Biotechnol. Prog. 15, 594–602 (1999)

Rabari, D., Banerjee, T.: Experimental and Theoretical Studies on the Effectiveness of Phosphonium-Based Ionic Liquids for Butanol Removal at T = 298.15 K and p = 1 atm. Ind. Eng. Chem. Res. (2014). doi:10.1021/ie500833h

Regdon, I., Kiraly, Z., Dekany, I., Lagaly, G.: Adsorption of 1-butanol from water on modified silicate surfaces. Colloid Polym. Sci. 272, 1129–1135 (1994a)

Regdon, I., Dekany, I., Lagaly, G.: A new way for calculating the adsorption capacity from surface excess isotherms. Colloid Polym. Sci. 276, 511–517 (1994b)

Remi, J.C.S., Baron, G.V., Denayer, J.F.M.: Adsorptive separation for the recovery and purification of biobutanol. Adsorption 18, 367–373 (2012)

Remi, J.C.S., Rémy, T., Van Hunskerken, V., Van de Perre, S., Duerinck, T., Maes, M., De Vos, D., Gobechiya, E., Kirschock, C.E.A., Baron, G.V., Denayer, J.F.M.: Biobutanol separation with the metal-organic framework ZIF-8. ChemSusChem 4, 1074–1077 (2011)

Rohani, A.S., Mehrani, P., Thibault, J.: Comparison of in situ recovery methods of gas stripping, pervaporation, and vacuum separation by multi-objective optimization for producing biobutanol via fermentation process. Can. J. Chem. Eng. In print (2014)

Saravanan, V., Waijers, D.A., Ziari, M., Noordermeer, M.A.: Recovery of 1-butanol from aqueous solutions using zeolite ZSM-5 with a high Si/Al ratio; suitability of a column process for industrial applications. Biochem. Eng. J. 49, 33–39 (2010)

Shapovalov, O.I., Ashkinazi, L.A.: Biobutanol: biofuel of second generation. Russ. J. Appl. Chem. 81(12), 2232–2236 (2008)

Sharma, P., Chung, W.J.: Synthesis of MEL type zeolite with different kinds of morphology for the recovery of 1-butanol from aqueous solution. Desalination 275, 172–180 (2011)

Sowerby, B., Crittenden, B.D.: Vapour phase separation of alcohol water mixtures by adsorption onto silicalite. Gas Sep. Purif. 2, 177–183 (1988)

Takeuchi, Y., Iwamotob, H., Miyata, M., Seiichi Asano, S., Haradac, M.: Adsorption of l-butanol and p-xylene vapour with high silica zeolites and their mixtures. Sep. Technol. 5, 23–34 (1995)

Thirmal, C., Dahman, Y.: Comparison of existing pretreatment, saccharification, and fermentation processes for butanol production from agricultural residues. Can. J. Chem. Eng. 90, 745–761 (2012)

Thompson, A.B., Scholes, B.C., Notestein, J.M.: Recovery of dilute aqueous acetone, butanol, and ethanol with immobilized calixarenecavities. Appl. Mater. Interfaces 6, 289–297 (2014)

Thompson, A.B., Cope, S.J., Swift, T.D., Notestein, J.M.: Adsorption of n-butanol from dilute aqueous solution with grafted cal- ixarenes. Langmuir 27, 11990–11998 (2011)

Wei, P., Zhu, D.W.: Acetone-butanol-ethanol (ABE) fermentation using *Clostridium acetobutylicum* XY16 and in situ recovery by PDMS/ceramic composite membrane. Bioprocess Biosyst. Eng. 35, 1057–1065 (2012)

Xue, C., Zhao, J., Liu, F., Lu, C., Yang, S.T., Bai, F.W.: Two-stage in situ stripping for enhanced butanol fermentation and energy- saving product recovery. Bioresour. Technol. 135, 396–402 (2013)

Xue, C., Zhao, J., Lu, C., Yang, S.T., Bai, F.W., Tang, I.C.: High-titer n-butanol production by *Clostridium acetobutylicum* JB200 in fed-batch fermentation with intermittent gas stripping. Biotechnol. Bioeng. 109, 2746–2756 (2012)

Yang, X., Tsai, G.J., Tsao, G.T.: Enhancement of in situ adsorption on the acetone-butanol fermentation by *Clostridium acetobutylicum*. Sep. Technol. 4, 81–92 (1994)

Yen, H.W., Lin, S.F., Yang, I.K.: Use of poly(ether-block-amide) in pervaporation coupling with a fermenter to enhance butanol production in the cultivation of *Clostridium acetobutylicum*. J. Biosci. Bioeng. 113(3), 372–377 (2012)

Ying, H., Lin, X., Chen, Y., Chen, X., Bai, J., Xiaong, J.: Method for separating butanol. Pub. No.: US 2013/0158303 (2013)

Zheng, Y.N., Li, L.Z., Xian, M., Ma, Y.J., Yang, J.M., Xu, X., He, D.Z.: Problems with

the microbial production of butanol. J. Ind. Microbiol. Biotechnol. 36, 1127–1138 (2009)

SECTION – V: Adsorption-gas stripping method

Chapter VI

Submitted to Journal of Chemical Technology and Biotechnology

Biobutanol Separation from ABE Model Solutions and Fermentation Broths Using a Combined Adsorption-Gas Stripping Process

Niloofar Abdehagh, Bo Dai, Jules Thibault, F. Handan Tezel

Department of Chemical and Biological Engineering
University of Ottawa
Ottawa, Ontario, Canada K1N 6N5

Corresponding author:
Email: handan.tezel@uottawa.ca Tel.: 1 (613) 562-5800 Ext. 6107

Abstract

BACKGROUND: Butanol is considered as a promising sustainable biofuel to partly replace petroleum-based fuels. However, to become an economically viable biofuel, some challenges need to be overcome in the biobutanol production process such as the low final product concentration caused by product toxicity to the microorganism. Few separation techniques have been proposed to extract biobutanol in situ or ex situ from dilute fermentation broths. In this investigation, the combination of gas stripping and adsorption has been studied experimentally as a process to effectively separate butanol from dilute model solutions and fermentation broths using the advantages of both separation techniques.

RESULTS: Results showed that the butanol adsorption capacity of activated carbon F-400 was 261 mg/g, for a stripped gas stream from butanol-water binary solution with an

initial liquid phase composition of 15 g/L butanol, which ended up having a vapour phase composition of 5.8 mg/L after gas stripping. This capacity is relatively high compared to the values reported in the literature. Butanol adsorption capacities for a stripped gas stream in equilibrium with ABE model solutions (5.1 mg/L) and fermentation broths (2.3 mg/L) for this adsorbent (211.6 and 219.8 mg/g, respectively), were also higher than the capacities reported in the literature.

CONCLUSION: Combined gas stripping and adsorption method could be considered as an effective technique for biobutanol separation processes.

Keywords: biobutanol, adsorption, gas stripping, ABE fermentation, biofuel, separation

1 Introduction

In recent years, biofuel production processes have attracted a lot of attention from the scientific community and industry. There are several reasons motivating biofuel production to replace petroleum-based fuels: increased oil price, higher demand for energy, depleting fossil fuel resources, environmental challenges and political instability in oil-producing countries. The need for producing sustainable fuel from renewable resources has been a serious concern recently. Second generation biofuels such as bioethanol and biobutanol produced from the non-food feedstocks are considered as promising alternatives to partly replace petroleum-based fuels.[1-3] Biobutanol, one of the most promising biofuels, possesses characteristics that are close to those of gasoline making it a viable alternative for partly replacing petroleum-based fuels. These biobutanol characteristics include similar net heat of combustion (NHOC) with gasoline,

low flammability, better blending properties with gasoline and its ability to be used in unmodified car engines.[4-9]

Acetone-butanol-ethanol (ABE) fermentation is known as the biological process of biobutanol production using agricultural non-food by-products as feedstock. The most common bacteria used in these processes are the *Clostridia* species such as *Clostridium acetobutylicum* and *Clostridium beijerinkii*. However, some challenges need to be addressed to make the ABE fermentation a viable process to produce biobutanol as the main final product. Low product concentration in the fermentation broth (1 to 1.2 wt%) due to the toxicity of butanol for the microorganisms is one of the main issues in ABE fermentation. As a result, it is essential to develop an efficient separation technique to separate butanol from the dilute fermentation broth.[10-26]

Conventional distillation, liquid-liquid extraction (LLE)[23], pervaporation[27], adsorption[5] and gas stripping[28] are the most common separation methods considered for butanol separation. Distillation cannot be considered as an efficient method due to its high-energy requirement. LLE has some serious drawbacks such as finding a non-toxic selective extractant and the need for second separation method to separate the extractant from butanol. Gas stripping could be considered as one of the cost effective methods but the results achieved by this method have not been completely satisfactory.[29-35] Adsorption and pervaporation are the two potential methods when considering both the energy and the process efficiency. The critical issue in developing an integrated separation process for butanol using adsorption or pervaporation would be to find or prepare the most efficient adsorbent or membrane, which is selective to butanol in comparison to other components.

In an adsorption process, finding an effective adsorbent with high adsorption capacity, high adsorption rate, and high selectivity for the targeted compound are very important factors. The ease of desorption is another important factor to be considered to develop an efficient process. In our previous study, activated carbon F-400 was selected as one of the best commercially available solid adsorbent for butanol removal due to its high adsorption capacity, fast kinetics, high selectivity for biobutanol, and its low price.[5] The desorption process has also been investigated in another study conducted by our group showing that, using thermal desorption process, a complete butanol desorption could be achieved following the adsorption process.[36]

In this study, adsorption and gas stripping, two potential separation methods are combined to develop a new process to separate butanol from ABE model solutions and fermentation broths. The process efficiency has been investigated using the combination of gas stripping to remove the solvents from liquid solutions using CO_2 as the stripping gas and adsorption to separate butanol from the vapor phase leaving the gas stripping system.

2 Materials and methods

2.1 Materials

To prepare all model solutions, n-butanol (99 % pure, Acros), acetone (95 % pure, Acros), n-butyric acid (99 % pure, Acros) and 99 % pure ethanol, acetic acid, glucose and xylose were all purchased from Fisher Scientific (Fair Lawn, NJ, USA). Deionized distilled water was used to prepare all model solutions. F-400 activated carbon (AC F-400) purchased from Calgon Corporation (Mississauga, ON, Canada) with the mean

particle size of 0.55-0.75 mm and the surface area of 1090 cm^2/g was used as the butanol adsorbent. All chemicals used in the fermentation medium were purchased from Sigma Aldrich (Oakville, ON, Canada).

2.2 Experimental setup

The adsorption-gas stripping experimental setup used in this study (Fig. 1) consisted of two parts: a gas stripping system with a feed reservoir placed in a water bath at a controlled temperature and an adsorption column (height and diameter of 4 and 1.5 cm, respectively) packed with AC F-400 (around 3.4 g with bulk density of 640 kg/m^3) placed in an oven at a controlled temperature. For each experiment, the feed reservoir was filled with a certain volume of model solution or fermentation broth and CO_2 was sparged in the solution with a specific flow rate controlled by a mass flow controller (MKS, Ottawa, Ontario, Canada). To minimize the change in the concentrations of all compounds in the solution and in the associated vapour phase composition during the breakthrough experiments, a relatively large volume of solution was used. The CO_2 gas stream leaving the gas stripping system contains mostly water and the ABE solvents (butanol, acetone and ethanol), stripped from the aqueous solution. The gas stream was then passed through an adsorption column maintained at a constant temperature. In the adsorption column, each component of the gas stream was adsorbed progressively onto the surface of the adsorbent particles based on their specific multi-component adsorption isotherm. The equilibrium adsorption capacities were determined using the breakthrough curves by integrating the area defined by the difference between the feed concentration and the breakthrough curve of each individual chemical species as a function of time. This method was described in our previous study.[36] The outlet stream of the adsorption

column was analysed using gas chromatography (GC) system to determine the gas stream composition. Using a three-way valve, it was also possible to analyze the composition of the gas stream after the gas stripping unit prior to entering the adsorption system.

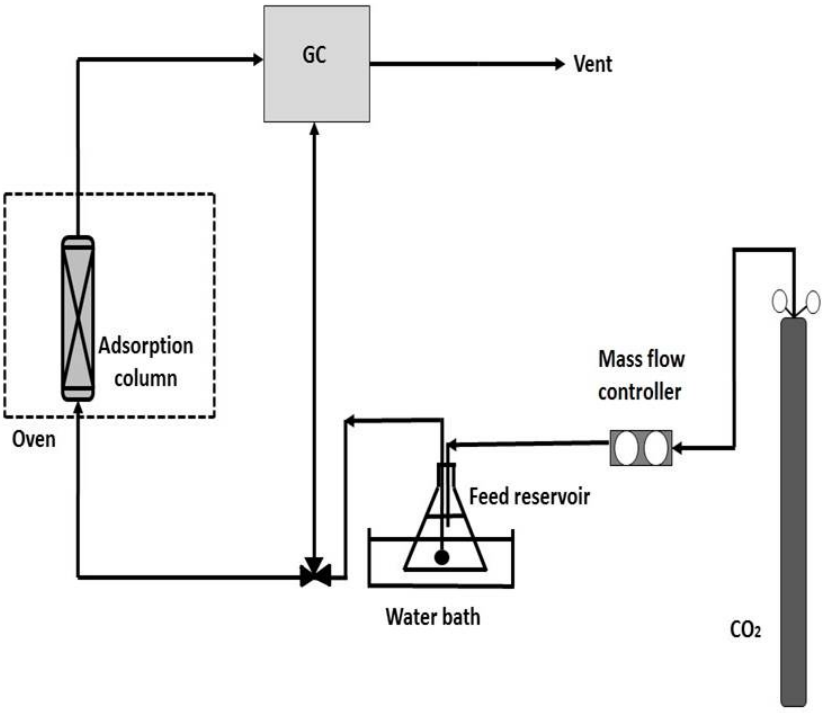


Figure 1. Schematic diagram of the gas stripping-adsorption setup used for butanol separation from model solutions and fermentation broths.

2.3 Culture, medium preparation and fermentation

Clostridium acetobutylicum (ATCC 824) was purchased from the American Type Culture Collection (ATCC) to be used as the butanol producing microorganism. The stock culture of this strain was stored in a 15% glycerol stock solution at -80°C freezer. To prepare the seed culture for the batch fermentation experiments, 2 mL of the glycerol

stock culture was inoculated to 25 mL of 3.8% of reinforced clostridia medium (RCM, Sigma Aldrich, Oakville, ON, Canada) in anaerobic autoclaved hungate tubes. The tubes were incubated at 37°C in a shaker for 48 h until cells were highly active. For the fermentation experiments, each hungate tube was used as the seed culture in 500 mL of the ABE fermentation medium in an anaerobic autoclaved 1-L bottle. The composition of a 1-L medium solution used in this study is shown in Table 1. Solutions I to V were separately prepared and then mixed after autoclaving under anaerobic conditions before inoculation. Anaerobic condition inside the bottles was achieved by purging ultra-pure nitrogen inside the bottles for 5 to 15 min. Resazurin (5 mg/L) was used as an oxygen indicator (Sigma Aldrich, Oakville, ON, Canada). All the culture transfers were done through sterilized syringes and hypodermic needles in a glove bag to prevent oxygen contamination.

Table 1. Composition of a 1-L medium solution used for *C. acetobutylicum* in ABE fermentation.

*Solution I	Glucose: Varies	320 mL D.I.Water
*Solution II	Yeast Extract: 5g	320 mL D.I. Water
*Solution III	KH_2PO_4: 0.75 g K_2HPO_4: 0.75 g $(NH_4)_2SO_4$: 2 g NaCl: 1 g	320 mL D.I. Water
**Solution IV	$MgSO_4.7H_2O$: 0.2 g $MnSO_4.H_2O$: 0.01 g $FeSO_4.7H_2O$: 0.01 g	20 mL D.I. Water
**Solution V	L-cysteine.HCl: 0.5 g	20 mL D.I. Water

*Sterilize in autoclave for 20 minutes at 121°C.
**Sterilize using 0.2 µm syringe filter (Millipore, Etobicoke, ON, Canada).

2.4 Analytical methods

To analyze the composition of liquid sample solutions, a high pressure liquid chromatograph (HPLC, Waters, Mississauga, ON, Canada) was used. It is equipped with a Refractive Index Detector (Waters 2414), together with an Isocratic HPLC pump (Waters 1515) and Autosampler (Waters 717 plus). An external column heater was used to heat the column to the desired temperature. The column used to detect the ABE compounds in the HPLC was a Vertex column (300 X 8 mm, KNAUER, Germany) packed with Eurokat H, 10 µm. The software used to operate the HPLC was Breeze purchased from Waters (Mississauga, ON, Canada). Mobile phase used in HPLC analysis was 0.01 N sulfuric acid with a flow rate of 0.8 mL/min. The temperature of the column was kept at 85°C.[37]

The composition of the gas stream was analyzed using a gas chromatograph (GC, SRI 8610C, Chromatographic Specialties Inc. CSI, Brockville, ON, Canada) equipped with a flame ionization detector and a 30-m fused silica column (Stabilwax, 30M X 53MM, 1µm W/5M Integra-Guard column, EAC, Chromatographic Specialties Inc. CSI, Brockville, ON, Canada). The carrier gas was nitrogen and the column temperature was held at 40°C for 2 min, then raised to 200°C at a rate of 20°C/min, and held at 200°C for 4 min. The injector and detector temperatures were set at 110°C and 250°C, respectively. The adsorption capacities of the components were calculated using the concentrations measured by the HPLC and GC and mass balance equations.[36]

3 Results and Discussion

3.1 Operating conditions for gas stripping-adsorption system

The stripping of butanol from binary butanol-water solutions was tested at different system temperatures (both for gas stripping and adsorption): 37, 50 and 60°C at a constant stripping CO_2 flow rate of 600 mL/min for butanol-water binary solution. The initial concentration of the butanol solution in the feed reservoir was 15 g/L. Results showed that at 37°C, the highest butanol adsorption capacity was obtained (224.6 mg/g) whereas, for 50 and 60°C, the adsorption capacities were 216.6 and 64.5 mg/g, respectively. The corresponding equilibrium concentrations in the vapour phase for 37, 50 and 60°C were 5.8, 7.7 and 9 mg/L, respectively (Fig. 2). The vapour phase compositions increase as temperature is increased as expected. If the system temperatures were the same for these different vapour compositions, the adsorption capacity would have increased with increasing vapour composition in the feed to the adsorption column. But since adsorption is an exothermic process, the capacity increases as temperature is decreased. In this case, the increase in adsorption capacity is compensating for the decrease in vapour composition at the lowest temperature of 37°C. The resulting adsorption capacity recorded for each temperature is therefore a compromise between a higher vapour phase concentration and lower adsorption capacity as the temperature is increased.

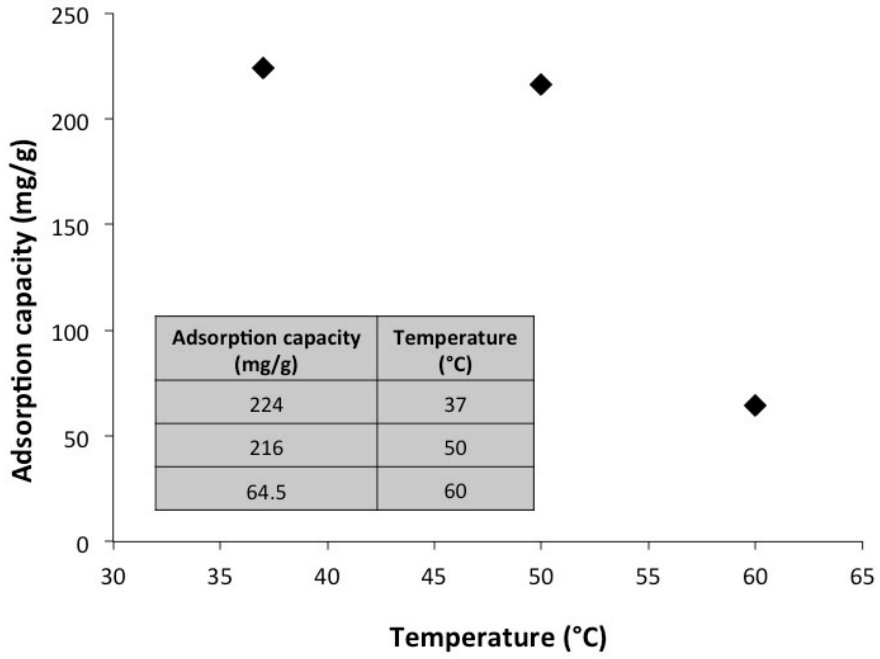


Figure 2. The effect of the temperature of adsorption and gas stripping system on butanol adsorption capacity of AC F-400 in the vapour phase, for 600 mL/min CO_2 flow-rate and 15 g/L butanol concentration in the liquid in the feed reservoir. Values for data points are given in the inserted table.

The other important parameter tested was the CO_2 gas flow rate. Three different flow rates, 600, 900 and 1000 mL /min, were tested to strip butanol from binary aqueous solutions with 15 g/L butanol concentration in the feed reservoir which gave the corresponding butanol vapour phase concentrations of 5.8, 5.8 and 5.6 mg/L, respectively. The temperature of the system both at the feed reservoir and the column was kept constant at 37°C which is the typical temperature at which ABE fermentation is performed. As Figure 3 shows, adsorption capacity was fairly similar for all three different flow rates showing that the gas flow rate reached an equilibrium state prior to

exiting the stripping column for these different flow rates. It means that at these stripping gas flow rates, the bubbles leaving the sparger raised to the surface of the liquid with adequate turbulence, thus the residence time was sufficient for the gas bubbles to achieve equilibrium saturation with the liquid medium. The adsorption capacity for butanol was between 224 and 261 mg/g for the three different gas flow rates tested. It is interesting to note that the butanol adsorption capacity obtained with the same adsorbent (AC F-400) for a liquid stream with the same binary aqueous solution in the feed reservoir (15 g/L butanol in liquid phase) at the same temperature was 215 mg/g.[5]

Based on the results of these experiments on the effect of the system temperature and CO_2 gas flow rate, the temperature of 37°C (giving the maximum adsorption capacity) and flow rate of 900 mL/min were selected as the operating conditions for further gas stripping-adsorption experiments. The 900 mL/min flow rate was selected since it resulted in the highest butanol adsorption capacity. In addition, a higher gas flow rate has the advantage of shortening the time required to perform the breakthrough experiment.

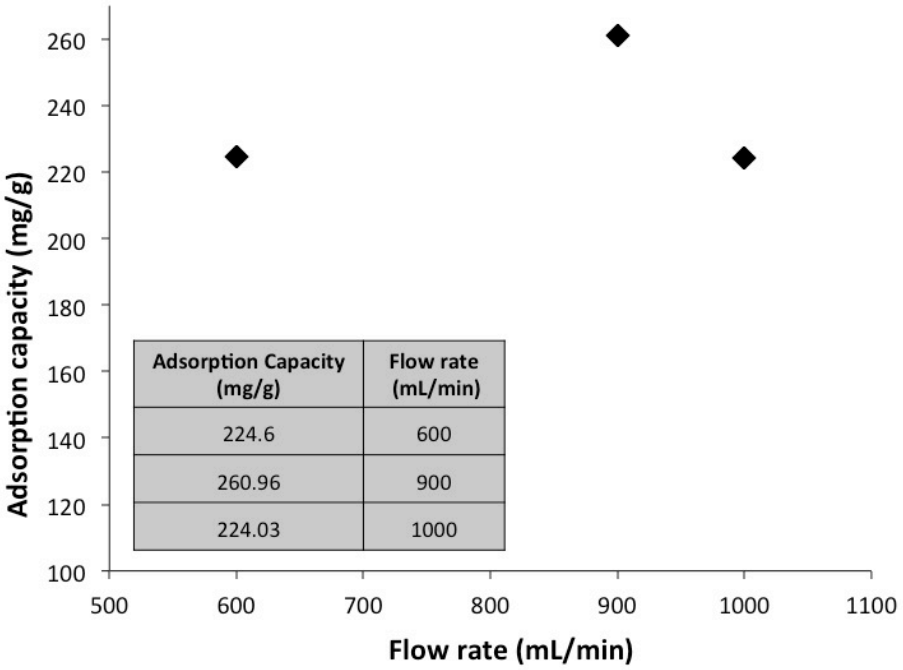


Figure 3. The effect of CO_2 flow rate on butanol adsorption capacity at 37°C with 15 g/L butanol concentration in the liquid in the feed reservoir. Values for data points are given in the inserted table.

3.2 Adsorption capacity of butanol-water binary solutions with different initial butanol concentrations in the feed reservoir

In the next set of experiments, butanol-water binary solutions with different initial butanol concentrations in the liquid phase in the feed reservoir (5, 10, 15, 20 and 25 g/L) were tested at 37°C (both gas stripping and adsorption units) and CO_2 gas flow rate of 900 mL/min. Results of this set of experiments are presented in Figure 4. The corresponding vapour phase concentration of butanol for 5, 10, 15, 20 and 25 g/L liquid phase concentrations in the feed reservoir were 2.6, 4.9, 5.8, 7.1 and 8 mg/L, respectively. The results show that the butanol adsorption capacity is fairly similar for all

concentrations and it is between 220 and 260 mg/g. Cao et al. (2015) conducted a similar investigation using helium as the carrier gas that was purged through a butanol-water binary solution prior to entering an adsorption column packed with activated carbon Sabre Series CR2050C-75. Their column was very small column, containing only 50 mg of adsorbent and a very low flow rate of helium (10 mL/min) at 50°C was used. The adsorption capacity obtained with their system was 260 mg/g which is interestingly the same value achieved in the present study.[4]

In Figure 4, butanol adsorption isotherm using adsorbent AC F-400 for the liquid phase adsorption at 25°C is also shown together with the results of butanol adsorption isotherm for vapor phase adsorption experiments at 37°C performed using different initial butanol concentrations in binary solutions in the liquid feed reservoir in this study.[5] It can be observed that in both systems, although the scales of the equilibrium concentrations are totally different, the maximum adsorption capacity appeared as a plateau of nearly constant adsorption capacity (Fig. 4).

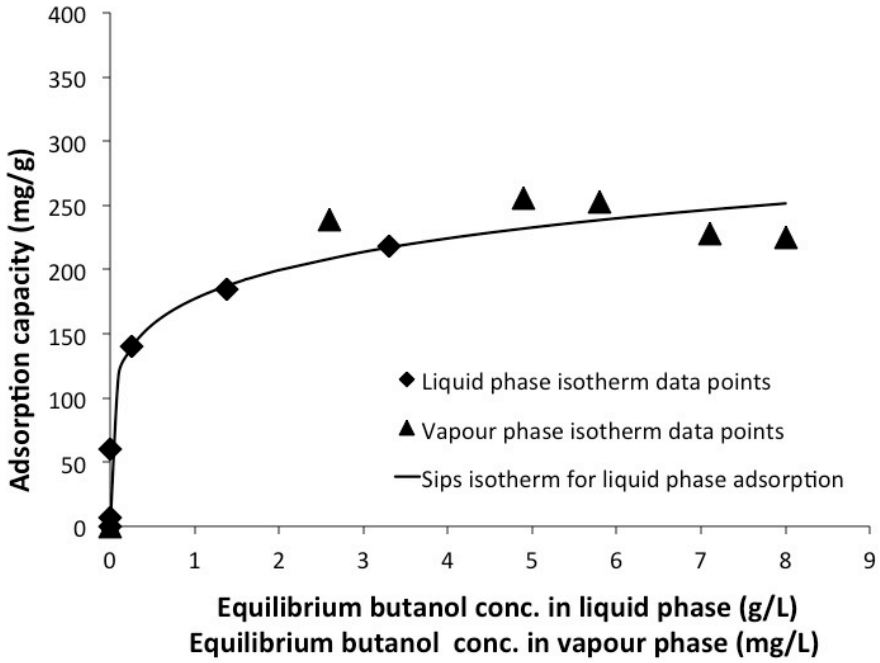


Figure 4. Isotherms of butanol adsorption in the liquid and vapour phase adsorption system. ♦: Butanol adsorption isotherm in the liquid phase (—) at 25°C (the experimental data taken from our previous study)[5]; ▲: Butanol adsorption isotherm in the vapour phase at 37°C.

3.3 Results for ABE model solution experiments

One of the very important factors affecting the butanol removal for all separation methods is the presence of other compounds in the ABE fermentation broths, such as ethanol, acetone, acetic acid, butyric acid and sugars. AC F-400 has been tested in our previous study in adsorption experiments using ABE model solutions containing all these main components and it was observed that this adsorbent was highly selective towards butanol even in the presence of other compounds.[5] In the present study, the effect of the presence of other components on the butanol adsorption was also examined using ABE

model solutions prepared with the following composition: butanol 15 g/L, acetone 7.5 g/L, ethanol 2.5 g/L, butyric acid 4 g/L and acetic acid both 4 g/L. This concentration in the liquid solution is in the vicinity of the composition typically observed in an ABE fermentation broth. The corresponding vapour phase composition after the gas stripping unit was 5.1, 8.1 and 0.40 mg/L for butanol, acetone and ethanol, respectively. It was observed that butyric and acetic acids in the vapour phase were not present, showing that these two components were not stripped by the purging gas. This is a very important bservation since it shows that butyric and acetic acids will remain in the fermentation broth and, as a result, will be converted to produce more butanol and acetone, respectively. Figure 5 shows the results for two separate breakthrough experiments performed with ABE model solutions in the feed reservoir at 37^{o}C with the adsorption column operating at 37^{o}C and with a CO_2 gas flow rate of 900 mL/min. In this figure, for the y-axis, the vapour phase compositions exiting the adsorption column were normalized with respect to the feed vapour phase composition C_0. The adsorption capacities for butanol in the two breakthrough experiments (denoted as a and b in Figure 5) were estimated to be 203 and 212 mg/g, respectively. The adsorption capacities for acetone were estimated from the response curves to be 40.6 and 45.6 mg/g, respectively. Based on the breakthrough curves, the equilibrium adsorption capacity for ethanol in both the breakthrough experiments was negligible. In addition, since butyric acid and acetic acid were not stripped in detectable proportion into the vapour phase, these two organic acids did not appear in the breakthrough curves of Figures 5 and 6. The plots of Figure 5 clearly show the competitive adsorption that occurs where ethanol and butanol displaced acetone partly and, ethanol was completely displaced from the adsorption sites by butanol,

causing the exit compositions of acetone and ethanol increasing above their feed compositions in the vapour phase. The relative selectivity of butanol-acetone pair was calculated by dividing the ratio of the adsorbed mass of butanol over acetone to the ratio of these two components in the vapour stream ($\alpha_{Bu-Ac} = (M_{Bu}/M_{Ac})/(x_{Bu}/x_{Ac})$). The relative selectivity for butanol-acetone pair for the ABE model solution experiments in this study was 7.6. These results are in accordance with the results obtained for ABE liquid adsorption performed in our previous study, confirming that AC F-400 is a suitable and effective adsorbent for butanol removal from multicomponent solutions.[5] Wu et al. (2015) investigated the ternary (acetone-butanol-ethanol) solution gas stripping and similarly observed that butanol was the strongest adsorbate with the highest adsorption capacity. The breakthrough curve in their study also showed that both acetone and ethanol were initially adsorbed in the column but then displaced by butanol molecules as the main adsorbate of the system.[2] It was also found that combining the gas stripping and adsorption methods has the advantage of eliminating the negative effect of the presence of acetic and butyric acids since these two compounds have a low relative volatility and are stripped negligibly from the liquid solutions. As stated in our previous study with respect to the liquid adsorption, the presence of organic acids had a negative impact on butanol adsorption capacity and this effect was significant when butyric acid was present.[5] In this study, this effect was completely removed by using a gas stripping system before the adsorption process. To desorb the adsorbed butanol in the adsorption column, thermal desorption method could be used as described in our previous study.[36]

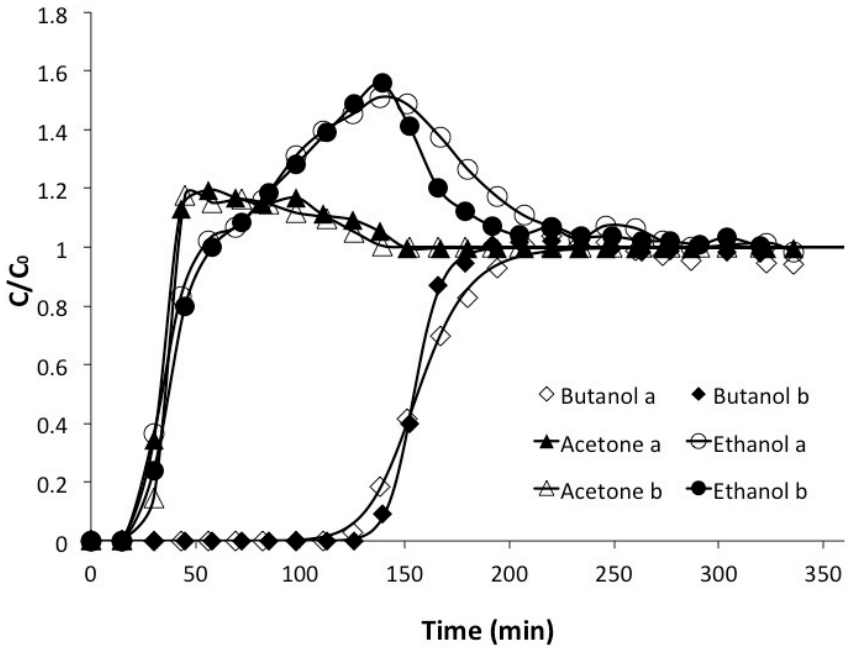


Figure 5. Breakthrough adsorption experiments for ABE model solutions using an adsorption-gas stripping process at 37°C and a CO_2 gas flow rate of 900 mL/min. for AC F-400.

3.4 Results for real ABE fermentation broth experiment

In the next experiment, the gas stripping-adsorption separation process was investigated using a real fermentation broth with the same experimental system. 500 mL of ABE fermentation broth was produced using the *C. acetobutylicum* ATCC 824 fermentation. The fermentation broth was centrifuged and filtered to separate the cells. The cell-free ABE fermentation broth was used as the liquid feed in the reservoir for the gas stripping and adsorption processes, both carried out at 37°C, with a CO_2 gas flow rate of 900 mL/min. The composition of the filtered cell-free fermentation broth was analyzed. The concentrations of butanol, acetone, ethanol, butyric acid and acetic acid were 9.68, 6.72, 2.5, 0.88 and 1.12 g/L, respectively. The vapor phase composition after the gas

stripping unit was 2.3, 5.3 and 0.1 mg/L for butanol, acetone and ethanol, respectively. Again the presence of acetic and butyric acids was not detected in the vapour phase. The result of this breakthrough adsorption experiment is shown in Figure 6. As it was the case in Figure 5, for the y-axis of Figure 6, the vapour phase compositions exiting the adsorption column were normalized with respect to the feed vapour phase composition C_0. The estimated adsorption capacities for butanol, acetone and ethanol were 220, 56 and 0 mg/g, respectively. The relative selectivity of butanol-acetone for this experiment was 9.5 which is slightly higher than the relative selectivity for ABE model solution experiments. The small increase in the butanol equilibrium adsorption capacity for the fermentation broth solution compared to the ABE model solution experiments could be due to the significantly lower butyric acid and acetic acid concentrations present in the liquid solutions in the feed reservoir which could change the butanol vapour-liquid equilibrium. The pH was measured for the both ABE model solution and fermentation broth experiments and it was observed that pH was constant. Since the solution is always saturated in CO_2, the circulating CO_2 stream did not affect the pH of the solution. In both ABE model solution and fermentation broth experiments, it was observed that acetone is first adsorbed and then competitively partly desorbed with the front of ethanol moving through the column. Subsequently, the same phenomenon occurred for ethanol where it was completely desorbed upon the progression of the butanol adsorption front within the adsorption column. In addition to the results presented in the figures, the equilibrium adsorption capacity for these two sets of experiments is shown in Table 2. Once again, these results clearly show that AC F-400 is much more selective for butanol which is in accordance with our results published previously.[36]

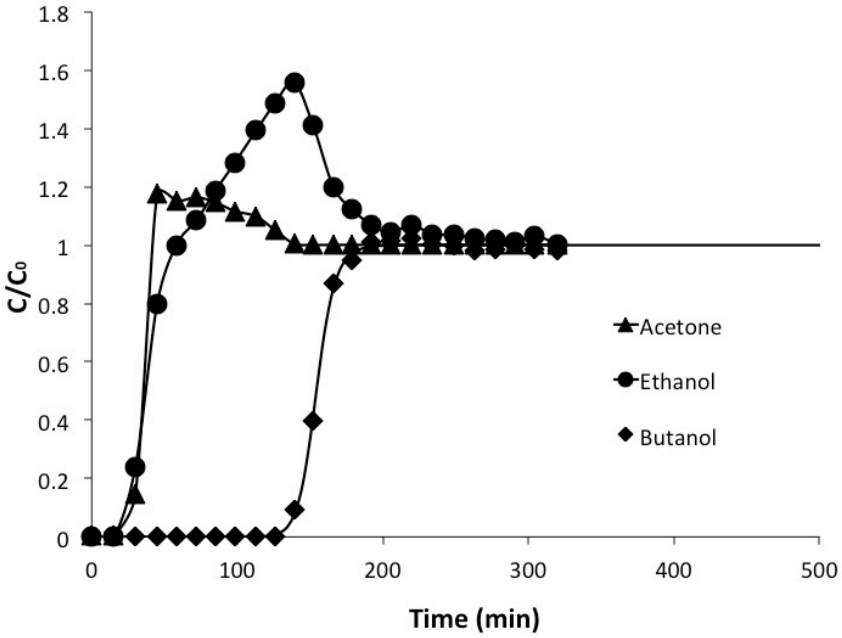


Figure 6. Breakthrough adsorption experiment for ABE fermentation broth using adsorption-gas stripping process at 37°C and with a CO_2 gas flow rate of 900 mL/min for AC F-400.

Table 2. Liquid and vapour phase composition of the ABE model solution and fermentation broth experiment with the adsorption capacity for ABE components.

Compound	Liquid phase conc. (g/L)	Vapour phase conc. (mg/L)	Equilibrium adsorption capacity (mg/g)
ABE model solution			
Butanol	15	5.1	203[a] and 212[b]
Acetone	7.5	8.1	40.6[a] and 45.6[b]
Ethanol	2.5	0.4	0
Butyric acid	4	0	0
Acetic acid	4	0	0
Cell-free fermentation broth			
Butanol	9.68	2.3	220
Acetone	6.62	5.3	56
Ethanol	2.5	0.1	0
Butyric acid	0.88	0	0
Acetic acid	1.12	0	0

[a] the concentration in ABE model solution a and [b] the concentration in ABE model solution b

4 Conclusions

Butanol separation from binary solutions, ABE model solutions and fermentation broths were investigated using the combination of gas stripping and adsorption processes. CO_2 was used as the carrier gas for the gas stripping process since it is produced in the ABE fermentation process and can be used as the stripping gas to make the process more economically viable. The adsorbent used in the adsorption process was AC F-400 that is highly selective to butanol and has a high adsorption capacity for this component. The adsorption capacity for a binary solution with 5.8 mg/L butanol in the vapour phase was 261 mg/g. The adsorption experiment was performed both for an ABE model solution and ABE fermentation broth at the same operating conditions: 37^{o}C and 900 mL/min CO_2 flow rate. For ABE model solutions, containing 5.1 mg/L butanol in the vapour phase, and for ABE fermentation broths, with 2.3 mg/L butanol in the vapour phase, the adsorption capacities of butanol were 212 and 220 mg/g, respectively. These results showed that this combined method of separation is an effective technique for biobutanol separation since the adsorption capacity of biobutanol is relatively higher than the values reported in the literature. Also the competing effect of the presence of sugars and organic acids on the adsorption capacity are eliminated since these components are not stripped to the gas phase and therefore do not have any negative effect on biobutanol adsorption in the packed column. It is strongly believed that this method could be considered as one of the most efficient biobutanol separation techniques benefiting from advantages of both gas stripping and adsorption processes.

5 List of Abbreviations

ABE	Acetone-butanol-ethanol
AC	Activated carbon
ATCC	American type culture collection
DI	Deionized
GC	Gas chromatography
HPLC	High performance liquid chromatography
LLE	Liquid-liquid extraction
NHOC	Net heat of combustion
RCM	Reinforced clostridium medium

6 Nomenclature

$\propto_{Bu-Ac}$	Relative selectivity of butanol in comparison to acetone
M_{Bu}	Adsorbed mass of butanol
M_{Ac}	Adsorbed mass of acetone
x_{Bu}	Weight fraction of butanol in the vapour stream
x_{Ac}	Weight fraction of acetone in the vapour stream

7 Acknowledgements

The authors would like to acknowledge the Natural Science and Engineering Research Council (NSERC) of Canada and Ontario Graduate Scholarship (OGS) for their financial support.

8 References

1. Farzaneh A, Zhou M, Potapova E, Bacsik Z, Ohlin L, Holmgren A, Hedlund J and Grahn M, Adsorption of Water and Butanol in Silicalite‑1 Film Studied with in Situ Attenuated Total Reflectance−Fourier Transform Infrared Spectroscopy. *Langmuir* 31(17): 4887–4894 (2015).

2. Wu J, Zhuang W and Ying H, Acetone–Butanol–Ethanol Competitive Sorption Simulation from Single, Binary, and Ternary Systems in a Fixed-Bed of KA-I Resin. *Biotechnolgy progress* 31(1) : 124-134 (2015).

3. Abdehagh N, Tezel FH and Thibault J, Separation techniques in butanol production: challenges and developments (Review). *Biomass and Bioenergy* 60 : 222–246 (2014a).

4. Cao Y, Wang K, Wang X, Gu Z, Gibbons W and Vu H, Butanol vapor adsorption behavior on active carbons and zeolite crystal. *Applied Surface Science* 349 : 1–7 (2015).

5. Abdehagh N, Tezel H and Thibault J, Adsorbent screening for biobutanol separation by adsorption: kinetics, isotherms and competitive effect of other compounds. *Adsorption* 19 : 1263–1272 (2013).

6. Venugopal T and Ramesh A, Experimental studies on the effect of injection timing in a SI engine using dual injection of n-butanol and gasoline in the intake port. *Fuel* 115 : 295–305 (2014).

7. Merola SS, Valentino G, Tornatore C and Marchitto L, In-cylinder spectroscopic measurements of knocking combustion in a SI engine fuelled with butanol–gasoline blend, *Energy* 62: 150–161 (2013).

8. Thompson AB, Scholes BC and Notestein JM, Recovery of dilute aqueous acetone, butanol, and ethanol with immobilized calixarene cavities. *Appl. Mater. Interfaces* 6 : 289–297 (2014).

9. Thompson AB, Cope SJ, Swift TD and Notestein JM, Adsorption of n-butanol from dilute aqueous solution with grafted calixarenes. *Langmuir* 27: 11990–11998 (2011).

10. Trinh LTP, Lee YJ, Bae HJ and Lee HJ, Pervaporative separation of butanol using a composite PDMS/PEI hollow fiber membrane. *J. Ind. Eng. Chem.* 20: 2814–2818 (2014).

11. Faisal A, Zarebska A, Saremi P, Korelskiy D, Ohlin L and Rova U, MFI zeolite as adsorbent for selective recovery of hydrocarbons from ABE fermentation broths. *Adsorption* 20: 465–470 (2014).

12. Ying H, Lin X, Chen Y, Chen X, Bai J and Xiaong J, Method for separating butanol. US patent 20130158303 (2013).

13. Xue C, Zhao J, Liu F, Lu C, Yang ST and Bai FW, Two-stage in situ stripping for enhanced butanol fermentation and energy saving product recovery. *Bioresour. Technol.* 135: 396–402 (2013).

14. Xue C, Zhao J, Lu C, Yang ST, Bai FW and Tang IC, High-titer n-butanol production by *Clostridium acetobutylicum* JB200 in fed-batch fermentation with intermittent gas stripping. *Biotechnol. Bioeng.* 109 : 2746–2756 (2012).

15. Garcia-Chavez LY, Garsia CM, Schuur B and de Haan AB, Biobutanol recovery using non-fluorinated task-specific ionic liquids. *Ind. Eng. Chem. Res.* 51 : 8293–8301 (2012).

16. Dhamole PB, Wang Z, Liu Y, Wang B and Feng H, Extractive fermentation with non-ionic surfactants to enhance butanol production. *Biomass and Bioenergy* 40 : 112–119 (2012).

17. Thirmal C and Dahman Y, Comparison of existing pretreatment, saccharification, and fermentation processes for butanol production from agricultural residues. *Can. J. Chem. Eng.* 90 : 745–761 (2012).

18. Oudshoorn A, Van der Wielen LAM and Straathof AJJ, Adsorption equilibria of bio-based Recovery of 1-butanol from aqueous solutions using zeolite ZSM-5 with a high

Si/Al ratio; suitability of a column process for industrial applications. *Biochem. Eng. J.* 49 : 33–39 (2012).

19. Lu C, Zhao J, Yang ST and Wei D, Fed-batch fermentation for n-butanol production from cassava bagasse hydrolysate in a fibrous bed bioreactor with continuous gas stripping. *Bioresour. Technol*. 104 : 380-837 (2012).

20. Shen CR, Lan EI, Dekishima Y, Baez A, Cho KM and Liao JC, 2011. Driving forces enable high-titer anaerobic 1-butanol synthesis in *Escherichia coli*. *Appl. Environ. Microbiol.* 77 : 2905–2915 (2011).

21. Harvey BG and Meylemans HA, 2011. The role of butanol in the development of sustainable fuel technologies. *J. Chem. Technol. Biotechnol.* 86 : 2–9 (2011).

22. Li S, Srivastava R and Parnas RS, Separation of 1-butanol by pervaporation using a novel tri-layer PDMS composite membrane. *J. Membr. Sci.* 363 : 287–294 (2010).

23. Ha SH, Maib NL and Koo YM, Butanol recovery from aqueous solution into ionic liquids by liquid–liquid extraction. *Process Biochem.* 45 : 1899–1903 (2010).

24. Zheng YN, Li LZ, Xian M, Ma YJ, Yang JM, Xu X and He DZ, Problems with the microbial production of butanol. *J. Ind. Microbiol. Biotechnol.* 36 : 1127–1138 (2009).

25. García V, Pongracz E, Muurinen E and Keiski RL, Recovery of n-butanol from salt containing solutions by pervaporation. *Desalination* 241 : 201–211 (2009).

26. Dürre P, New insights and novel developments in clostridial acetone/butanol/isopropanol fermentation. *Appl. Microbiol. Biotechnol.* 49 : 639–648 (1998).

27. Borisov IL, Malakhov AO, Khotimsky VS, Litvinova EG, Finkelshtein ES, Ushakov NV and Volkov VV, Novel PTMSP-based membranes containing elastomeric fillers:

enhanced 1-butanol/water pervaporation selectivity and permeability. *J. Membr. Sci.* 466 : 322–330 (2014).

28. Vrije TD, Budde M, van der Wal H, Claassen PA and Lopez-Contreras AM, In situ removal of isopropanol, butanol and ethanol from fermentation broth by gas stripping, *Bioresour. Technol.* 137 : 153–159 (2013).

29. Hecke WV, Hofmann T and De Wever H, Pervaporative recovery of ABE during continuous cultivation: enhancement of performance. *Bioresour. Technol.* 129 : 421–429 (2013).

30. Hecke WV, Vandezande P, Claes S, Vangeel S, Beckers H and Diels L, Integrated bioprocess for long-term continuous cultivation of *Clostridium acetobutylicum* coupled to pervaporation with PDMS composite membranes. *Bioresour. Technol.* 111 : 368–377 (2012).

31. Lin X, Li R, Wen Q, Wu J, Fan J and Jin X, Experimental and modeling studies on the sorption breakthrough behaviors of butanol from aqueous solution in a fixed-bed of KA-I resin. Biotechnol. *Bioprocess Eng.* 18 : 223–233 (2013).

32. Lin X, Wu J, Fan J, Qian W, Zhou X and Qian C, Adsorption of butanol from aqueous solution onto a new type of macroporous adsorption resin: studies of adsorption isotherms and kinetics simulation. *J. Chem. Technol. Biotechnol.* 87 : 924–931 (2012).

33. Yen HW, Lin SF and Yang IK, Use of poly(ether-block-amide) in pervaporation coupling with a fermenter to enhance butanol production in the cultivation of *Clostridium acetobutylicum*. *J. Biosci. Bioeng.* 113(3) : 372–377 (2012).

34. Wei P and Zhu DW, Acetone-butanol-ethanol (ABE) fermentation using *Clostridium acetobutylicum* XY16 and in situ recovery by PDMS/ceramic composite membrane. *Bioprocess Biosyst. Eng.* 35 : 1057–1065 (2012).

35. Li G, Wei W, Wu H, Dong X, Jiang M and Jin W, Pervaporation performance of PDMS/ceramic composite membrane in acetone butanol ethanol (ABE) fermentation-PV coupled process. *J. Membr. Sci.* 373 : 121–129 (2011).

36. Abdehagh N, Gurnani P, Tezel FH and Thibault J, Adsorptive separation and recovery of biobutanol from ABE model solutions. *Adsorption* 21(3) : 185-194 (2015).

37. Abdehagh N, Bagheri M, Tezel FH and Thibault J, Improved Acetone-Butanol-Ethanol (ABE) Solution Analysis Using HPLC: Chromatograph Spectrum Deconvolution Using Asymmetric Gaussian Fit. *American Journal of Analytical Chemistry* 5 : 1078-1089 (2014b).

SECTION – VI: Adsorption process modeling

Chapter VII

Accepted for publication in Adsorption journal

Multicomponent Adsorption Modeling: Isotherms for ABE Model Solutions Using Activated Carbon F-400

N. Abdehagh, F. H. Tezel, J. Thibault

Department of Chemical and Biological Engineering
University of Ottawa
161 Louis-Pasteur, Ottawa, ON, Canada K1N 6N5

Corresponding author: Tel: (613) 652-5800 Ext. 6094; Email: Jules.Thibault@uottawa.ca

Abstract

Biobutanol has attracted significant interest in recent decades and is seriously considered as a potential biofuel to partly replace gasoline. However, some production challenges must be addressed to make butanol economically viable such as the low product concentration and product toxicity inhibiting the microorganism. To alleviate these limitations, several in situ or ex situ separation techniques have been investigated in view of their integration to the biobutanol production process to enhance its economic viability. One of these techniques is adsorption which is one of the most energy-efficient techniques used for biobutanol separation. Considering the number of chemical species present in the ABE fermentation broth, it is essential to develop multicomponent adsorption isotherms for all components as a first step to design a high performance adsorption process. Few multicomponent isotherm models have been proposed such as multicomponent Langmuir and Freundlich. In this study, these two models as well as artificial neural networks (ANN) were used to model the isotherms of each component in an ABE fermentation broth as a function of the equilibrium concentrations of all components for activated carbon F-400. Results showed that the multicomponent Langmuir model was not accurate due to the many simplifying assumptions. The multicomponent Freundlich and feedforward neural network isotherm models were able

to predict the behavior of multicomponent systems very well. Indeed, the predictive model of the experimental data had a coefficient of determination (R^2) of 0.97 and 0.99, for multicomponent Freundlich and feedforward neural network isotherm models, respectively.

Keywords: *Biobutanol, Adsorption, Isotherm model, Artificial neural network, Langmuir adsorption isotherm, Freundlich adsorption isotherm*

1 Introduction

The rising cost of crude oil, depletion of natural resources and environmental challenges have motivated researchers and industrialists to produce eco-friendly fuels from sustainable resources. Biofuels have been regarded as the best alternatives to petroleum-based fuels due to the significant development of biotechnology (Abdehagh et al. 2014). Biobutanol is considered as one of the most promising biofuels due to advantages over other biofuels such as ethanol and methanol. In biobutanol production processes, the product separation is one of the major challenges since biobutanol concentration in acetone-butanol-ethanol (ABE) fermentation broths is very low (typically less than 1.2% wt) due to its toxic inhibition for butanol-producing microorganism. The other challenge is the presence of other components in the fermentation broth such as acetone and ethanol (by-products), acetic acid and butyric acid (intermediates) and potentially unconsumed sugars. Therefore, developing an integrated separation technique for butanol removal is one of the promising resolutions for this issue (Ezeji et al. 2003, 2004,2007; Antoni et al. 2007; Dellomonaco et al. 2010; Dürre2007; Fouad et al. 2008; Qureshi et al. 1999, 2005; Shapovalov et al. 2008; Harvey et al. 2011; Thompson et al. 2011; Abdehagh et al. 2013, 2014).

Adsorption is one of the most energy-efficient separation techniques for butanol separation from dilute solutions. In an adsorption process, one or more components are separated from a fluid stream using an adsorption column packed with adsorbent particles. Using the best adsorbent for a specific application (high adsorption rate and capacity, high selectivity for the desired component and low cost) allows good separation of the desired component from all other components present in the adsorption feed stream (Maddox 1982; Groot et al. 1986; Nielsen et al. 1988 and 2009; Sowerby et al.1988;

Yang et al. 1994; Holtzapple and Brown 1995; Oudshoorn et al. 2009b, 2012; Saravanan et al. 2010; Sharma et al. 2011; Thompson et al. 2011;Remi et al. 2011, 2012).

To develop an integrated adsorption process for butanol separation, it is first necessary to investigate the behavior of all components present in the fluid stream (Abdehagh et al. 2013). A measurement of the adsorption capacity over a wide range of component composition at a fixed temperature is called an adsorption isotherm that can be used to predict the process behaviour precisely. There are several adsorption isotherm models developed over the years using particular equations based on different assumptions. Langmuir, Freundlich, Sips, and Toth isotherm models are some of these classic models (Do, 1998). Since these models were developed based on the data from traditional systems, they are not capable of representing the equilibrium data of non-traditional systems adequately and display a significant lack of fit for them. Modeling the adsorption isotherms would be even more complicated when there is more than one component present in the fluid stream competing for adsorption sites. Multicomponent Langmuir model is one of the multicomponent adsorption isotherm models that usually have difficulty in predicting the adsorption behavior of different components in a multicomponent system due to its limiting assumptions (Morse et al. 2011). To model the adsorption behavior of each component in a multicomponent system, it is paramount to develop a unifying equation able to represent the behavior of varying adsorption processes as well as the interactions between the different components.

The scope of this paper was to examine multicomponent Langmuir, Freundlich and artificial neural networks as a general class of nonlinear models to represent the multicomponent adsorption isotherms for the different components present in ABE fermentation broths. Feedforward artificial neural networks have been successfully used in modelling isotherms of different adsorption processes such as wastewater treatment, ethanol separation, nitrogen separation from air and isopropanol separation to simulate the dynamics of adsorption in adsorption columns (Bulsari and Palosaafi 1993; Yang et al. 1993; Lewandowski et al. 1998; Carsky and Do 1999; Basu et al. 2002).

Butanol separation from dilute solutions has been investigated in a vast number of studies but only a few describe the competitive adsorption of components other than butanol present in the ABE fermentation broths (Oudshoorn et al. 2009b; Zheng et

al.2009; Saravanan et al. 2010; Sharma et al. 2011, Abdehagh et al. 2013; Thompson et al. 2011; Remi et al. 2011, 2012). To the best of our knowledge, there has not been any study proposing multicomponent adsorption isotherm models for all the components present in ABE fermentation broths. Thus, the aim of the present study has been to model the simultaneous adsorption behavior of the main components present in ABE fermentation broths (butanol, acetone, ethanol, butyric acid, acetic acid, glucose and xylose) on activated carbon from aqueous model solutions. Some of the single component adsorption data from our previous study (Abdehagh et al. 2013) have been used in the present paper to augment the databank and improve the multicomponent adsorption isotherm models. The multicomponent Langmuir and Freundlich isotherm models and an artificial neural network model (ANN) were used to describe the adsorption equilibrium for each component in multicomponent adsorption systems.

2 Materials and methods:

2.1 Materials

n-Butanol (99.5 % pure, Acros), acetone, n-butyric acid, ethanol (99 % pure, Acros) and acetic acid (99.7 % pure) were purchased from Fisher Scientific Co. (Whitby, ON, Canada). Glucose and xylose were also purchased from Fisher Scientific Co. (Fair Lawn, NJ, USA). Deionized distilled water was used to prepare all model solutions. F-400 activated carbon (AC F-400) purchased from Calgon Corporation (Mississauga, ON, Canada) was used as the adsorbent. In our previous study, it was found that this adsorbent was the best in terms of adsorption capacity and kinetics for the separation of butanol (Abdehagh et al. 2013). Some of the properties of this adsorbent are shown in Table 1.

Table 1. Some of the properties of the adsorbent used in this study (activated carbon F-400)

Activated carbon F-400	
Shape	Granule
Surface area	1090 (m^2/g)
Mean particle diameter	0.55-0.75 (mm)
Mesh size	12 x 40
Micropore volume	0.43 (cm^3/g)

2.2 Experimental methods

To determine the adsorption isotherm equilibrium data, adsorption experiments were performed at room temperature in a packed bed having an inner diameter of 0.02 m and a length of 0.195 m. The adsorption experiments were carried out using different initial concentrations of the different ABE fermentation components (butanol, ethanol, acetone, butyric acid, acetic acid, glucose and xylose) to determine adsorption capacities at different solution compositions for each component in the system. The experimental procedure and the method used to calculate the adsorption capacity were described in previous studies (Abdehagh et al. 2013, 2015). The concentrations of the different components in the feed solutions and final equilibrium solutions were determined by high pressure liquid chromatography (HPLC) (Waters, Mississauga, ON, Canada). To be able to validate the model for different compositions and ratios of the components, adsorption experiments were performed for each particular component by changing its initial concentration while keeping the initial concentrations of the other components constant. Isotherm experiments were carried out for two different levels of concentrations. The two levels of concentrations for each component are shown in Table 2. The levels of concentrations for each component were determined based on approximate concentrations that are typically observed in ABE fermentation broths. Since the ratio of butanol:acetone:ethanol in the typical ABE fermentation broth is roughly 6:3:1 (by weight) and the objective of the present study was to model the multicomponent adsorption system for this specific application, approximately the same ratio of solvents was considered for the model solutions in the adsorption experiments. For other components present in the solution, the low and high levels of the components were chosen based on the literature results for the possible minimum and maximum concentrations in typical ABE fermentation broths. To make the isotherm models even more general over a wider range of concentration, some of the data from single component adsorption isotherms from our previous study (Abdehagh et al. 2013) were incorporated to the multicomponent adsorption databank. To independently validate the models used in this study, few additional experiments with new and different compositions of all components were performed. The results of the validation

experiments were not used in obtaining the modeling but were only used to validate the different models.

Table 2. The two levels of initial concentrations for each component in ABE model solutions used in multicomponent adsorption experiments.

Components	Low level (LL) Concentration (g/L)	High level (HL) Concentration (g/L)
Butanol	10.85 ± 0.09	23.19 ± 0.14
Acetone	5.21 ± 0.17	12.03 ± 0.89
Ethanol	1.85 ± 0.05	3.6 ± 0.2
Butyric acid	4.72 ± 0.09	10.29 ± 0.22
Acetic acid	5.01 ± 0.06	10.17 ± 0.09
Glucose	6.26 ± 0.09	12.5 ± 0.17
Xylose	3.2 ± 0.05	6.34 ± 0.07

2.2.1 Multicomponent Langmuir adsorption isotherms

The Langmuir isotherm model is one of the most common adsorption isotherm models used in modeling the adsorption behavior in different systems (Langmuir, 1918). A modified version of this model can be used to model multicomponent systems. Equations (1) and (2) show the Langmuir isotherm equations for the single and each component i in the multicomponent adsorption systems, respectively.

$$q = \frac{q_s b C}{1 + bC} \quad (1)$$

$$q_i = \frac{q_{si} b_i C_i}{1 + \sum_{j=1}^{N} b_j C_j} \quad (2)$$

where q is the amount adsorbed in equilibrium with the liquid solution (g of adsorbate/g of adsorbent), q_s is the saturation adsorption capacity (g of adsorbate/g of adsorbent), all b variables are constant model parameters (L/g adsorbate) and C is the adsorbate concentration (g/L) of each component in the liquid solution. N is the total number of components in the system, and subscripts i and j are indices representing each component in the system.

The assumptions considered in the Langmuir isotherm model include: (1) localized adsorption of monolayer surface coverage, i.e. each adsorbed molecule occupies one adsorption site, (2) homogenous surface (no polarity on the surface of the adsorbent), (3) equivalent adsorption sites and (4) no interaction between the adsorbate molecules adsorbed on adjacent sites. The Langmuir isotherm model was investigated in this study to examine its applicability to predict the multicomponent adsorption isotherms for all components of a model solution mimicking the ABE fermentation broth. The multicomponent Langmuir models will also be compared to Freundlich and ANN models for predicting the equilibrium adsorption capacity of each component.

2.2.2 Multicomponent Freundlich adsorption isotherms

Freundlich isotherm is an entirely empirical model without a theoretical basis that is often used to model adsorption for heterogeneous adsorbent surfaces (Freundlich 1906). This isotherm model can be used for both single and multicomponent systems as defined in Equations (3) and (4), respectively.

$$q = KC^{1/n} \quad (3)$$

$$q_i = K_i C_i \left(\sum_{j=1}^{N} a_{ij} C_j \right)^{\frac{1}{n_i} - 1} \quad (4)$$

where q is the equilibrium adsorption capacity (g of adsorbate/g of adsorbent). The matrix of model parameters a_{ij} are defined as competitive adsorption coefficients for the components in the system with the diagonal elements $a_{11} = a_{22} = \cdots = a_{NN} = 1$. K and n are the isotherm constants determined from the single component systems. C is the adsorbate concentration (g/L) of each component at equilibrium. N is the total number of components in the system, and subscripts i and j are the indices representing each component in the system. This isotherm model was also investigated and compared to multicomponent Langmuir isotherm and ANN models to find the best model to describe the simultaneous adsorption behavior of all components present in the ABE fermentation broths.

2.2.3 Artificial neural network model

Artificial neural networks (ANN) have been used successfully to model a myriad of processes. ANNs mimic the biological neural systems using multiple interconnected non-linear processing elements to mathematically represent complex and nonlinear mathematical problems. The computational ability of ANNs is based on their plasticity and learning skills to encapsulate the possible relationship that exists between a series of inputs and outputs of a given system.

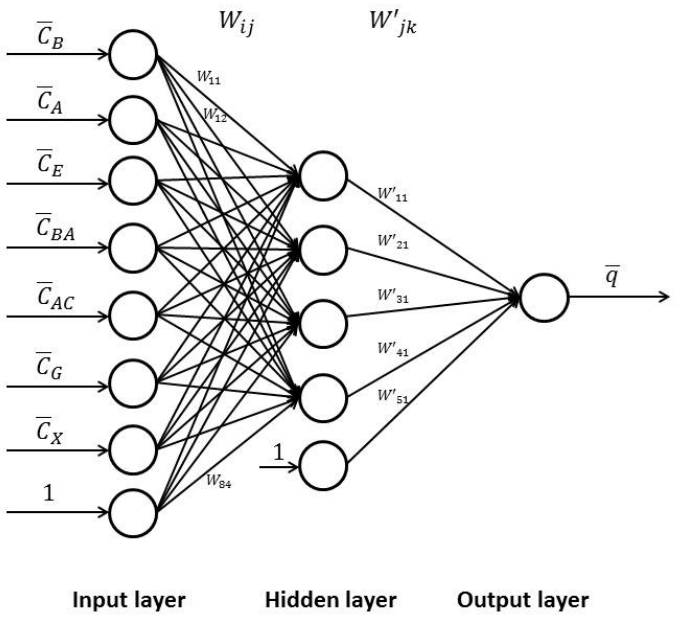


Figure 1. Feedforward neural network to model adsorption isotherms for each of the 7 ABE model solution components at room temperature.

Amongst different neural networks, feedforward neural networks (FFNN) are the most common neural network structures used in engineering applications. FFNNs have been used successfully to model the adsorption of phenol, methylene blue and heavy metal mixtures (Basu et al., 2002; Bulsari et al. 1993; Carsky and Do, 1999). The structure of a FFNN usually consists of three layers: an input layer to fan out the model inputs, a hidden layer containing a judicious number of processing neurons and an output layer providing the predicted output(s) of the model. Figure 1 shows the three-layer

FFNN used in this study. The input layer is comprised of eight neurons, one for each species (B: butanol, A: acetone, E: ethanol, BA: butyric acid, AA: acetic acid, G: glucose, X: xylose) and one bias neuron with a unit output which, when associated to a weight, serves as a constant term to shift each functional neuron of the next layer. The scaled inputs of the input layer are transferred without further transformation to all functional neurons of the hidden layer. Each functional neuron of the hidden layer performs two simple tasks: a weighted summation of all inputs to the hidden layer and the processing of this weighted sum through a non-linear transfer function such as a sigmoid or a hyperbolic tangent. The hidden layer also contains a bias neuron. The outputs of each neuron of the hidden layer become the inputs to each neuron of the output layer. All neurons of the output layer perform the same tasks as the neurons in the hidden layer to produce the final outputs of the FFNN. To find an appropriate model, it is recommended to normalize all process inputs and outputs to remove the effect of units and magnitudes of the different variables used in the models. In this study, all the input and output variables were normalized and scaled between 0 and 1 and the sigmoid function was used as the transfer function for the hidden and output layers. The calculations were performed for different neural networks using different numbers of neurons in the hidden layer to find the best fit of the experimental data sets while trying to minimize the number of hidden neurons to avoid over training. Figure 1 shows the feedforward neural network with the eight input neurons, five hidden neurons and one output neurons. In this investigation, one such neural network was used for each of the seven species of the fermentation broth. Equations (5) and (6) give the mathematical equation and the model parameters (weights) used in the neural network for each of these species.

$$\bar{q} = \frac{1}{1 + e^{-\sum_{j=1}^{4}\left(\frac{W'_{j1}}{1 + e^{-\left(W_{1j}\bar{C}_B + W_{2j}\bar{C}_A + W_{3j}\bar{C}_E + W_{4j}\bar{C}_{BA} + W_{5j}\bar{C}_{AC} + W_{6j}\bar{C}_G + W_{7j}\bar{C}_X + W_{8j}\right)}}\right) + W'_{51}}} \quad (5)$$

$$\bar{q} = \frac{q - q_{min}}{q_{max} - q_{min}} \;,\; \bar{C} = \frac{C - C_{min}}{C_{max} - C_{min}} \quad (6)$$

As it is shown in Equation (5) (for 4 functional neurons plus the bias in the hidden layer), the fitting information (model parameters) of the FFNN model is encapsulated into the weights (W_{ij} and W'_{jk}). Equation (6) was used to normalize the adsorption

capacity q and the equilibrium concentration of the components C for each component prior to using these variables in the ANN modeling. In an ANN, through the learning process, the weight matrices are formed to produce the best fit by minimizing the sum of squares of the errors between the predicted and experimental outputs. The quasi-Newton optimization algorithm was used in this study to fit the experimental adsorption data (Morse et al. 2011). For butanol, butyric acid and acetic acid isotherms, five neurons were used in the second layer of the network whereas for acetone, ethanol, glucose and xylose isotherms, four hidden neurons were used.

3 Results and discussion

The adsorption isotherms of butanol, acetone, ethanol, butyric acid, acetic acid, glucose and xylose were modeled using the multicomponent isotherm models (Langmuir and Freundlich) and neural networks. The results of each model will be discussed in turn.

Langmuir Isotherm -, The adsorption isotherms of each of the seven components in binary aqueous solutions were first modeled using the single component Langmuir isotherm equation (Eq. 1). Then, the multicomponent Langmuir isotherm model (Eq. 2) was used to represent the adsorption isotherms in the multi-component systems. In the adsorption isotherm modeling using multicomponent Langmuir model, to obtain the best fit, different b values were used for single and multicomponent systems as shown in Table 3. This allows obtaining a model with a better fit for the multicomponent system that is potentially capable of representing isotherms of each particular component in the mixture, regardless of the concentrations of other components present in the solution. For an ideal system, the value of q_s (that can be determined from the single component isotherms and is different for each component) should be constant and independent of the presence of the other components in the mixture such that the resulting adsorbent equilibrium capacity for each component would only be influenced by the equilibrium concentration terms of other components found in the denominator of the multicomponent isotherm. This would result in a universal multicomponent Langmuir model for each component. However, since there are interactions between different components in a competitive adsorption process, nonlinear interactions exist between

different adsorption equilibrium capacities of each component. Therefore, the parameter q_s for each component must be modified to represent the change in saturation adsorption capacity due to the presence of the other components in the system. This is evidenced in ABE solution breakthrough experiments where some more strongly adsorbed components desorbed other less strongly adsorbed components in the mixture (Abdehagh et al. 2015; Jiao et al. 2015; Wu et al. 2014). If the concentration level of each component changes, a new value of the parameter q_s must be determined separately for that specific solution composition.

Figure 2 shows the results for multicomponent Langmuir isotherm models for the three sets of experimental data obtained for each component, i.e. data for pure components in a water solution and for multicomponent aqueous solutions at the two different levels of compositions. The corresponding constants for each multicomponent isotherm model are shown in Table 3. As the experimental results showed, different levels of ABE components present in the model solution have different effect on the adsorption capacity for each component. Since different component compositions could be considered in the ABE solutions, it is essential to have a unified multicomponent adsorption isotherm model, which is able to predict the adsorption behaviour of the ABE components in any possible solution composition. It was observed that multicomponent Langmuir isotherm model could be used to model (for each specific data set) the isotherm of butanol. However, even though the experimental data for other components are relatively well represented, the behaviour of the resulting equations for these components would definitely not be used due to the unreasonable trends given by the isotherm models. One reason for this weak representation of the model could be the assumption considered in the model that might not be applied in the ABE model solution adsorption process. The coefficients obtained for the model are given in Table 3. It can be seen that although the Langmuir equation forced the q_s to reach a plateau, for most of the components the isotherm trends show an increasing q value with increasing equilibrium concentrations and the q_s of the multicomponent isotherms are larger than the single component isotherms which is not the case in reality. Indeed, due to the presence of other components, the value of q_s should obviously decrease compared to the single component adsorption capacity.

Table 3. Parameters for the multicomponent Langmuir adsorption isotherm model for all components in the ABE fermentation for predicting the adsorption capacity of single components and multiple components in the mixture with different concentration levels of other components.

	Butanol	Acetone	Ethanol	Butyric acid	Acetic acid	Glucose	Xylose
b pure component	2.83	0.121	0.0485	1.356	0.216	0.231	0.174
b multicomponent	1.335	0.225	0.216	0.407	0.001	0.115	0.158
q_s*	272.76	207.579	173.143	264.828	153.435	141.407	139.901
q_s**	287.18	506.605	739.58	851.33	62.498	434.254	334.067
q_s***	252.84	753.93	861.502	381.38	111.615	524.16	427.534

* Value for pure components;

** Value for lower level of other components (see Table 2) in the ABE fermentation;

*** Value for higher level of other components (see Table 2) in the ABE fermentation.

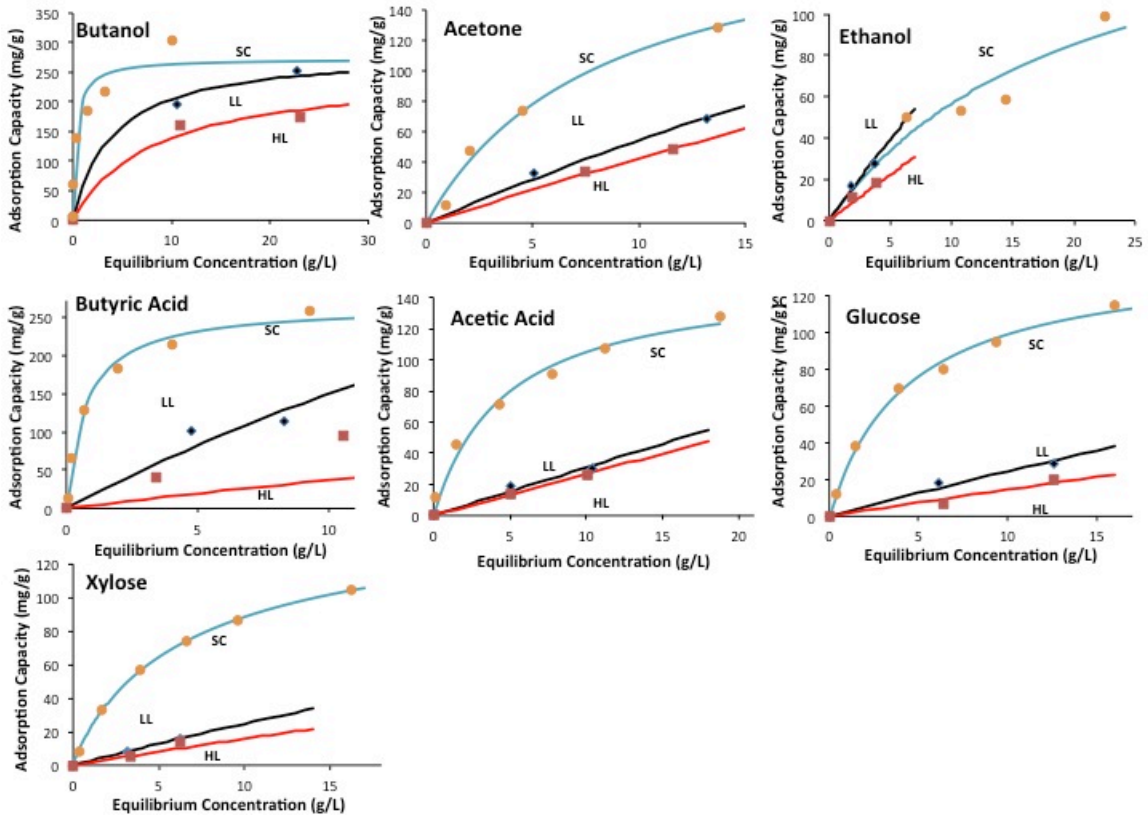


Figure 2. Plots of experimental (symbols) and predicted (curves) multicomponent Langmuir isotherm fits for butanol, butyric acid, ethanol, acetone, acetic acid, glucose and xylose for single component (SC), low level concentrations of other components

(LL), and high level concentrations of other components (HL) in the model ABE fermentation at room temperature for activated carbon F-400.

It is important to note that even for the components with relatively good fits, the model can only be used for the specific levels (the high and low levels specified in this study) of other components since the coefficient q_s for each component in each level are specifically determined and would not be used for other component compositions. As can be seen from Table 3, if the composition of the solution changes, the value of q_s will also change accordingly. It is necessary to re-evaluate this parameter using a new set of experimental data for the specific compositions of the other components in the mixture. To obtain a universal model, it would be required to derive an equation for each component for parameter q_s as a function of the concentrations of other components in the model solution. In its present form, due to the lack of generality, this model is not very useful for predicting the equilibrium adsorption capacities of each component of the ABE fermentation broth where the concentration of each species will vary continuously. The poor ability of multicomponent Langmuir isotherm for fitting multicomponent competitive adsorption has been reported in previous studies. For example, even for a binary system, Wu and Lin (2009) showed that the competitive Langmuir model represented data accurately at the low concentration range. When they used the isotherm model at higher concentrations, the relative error became larger and increased with increasing concentration. They postulated that the single Langmuir isotherm model cannot consider the impact of competition of different adsorbates on the adsorbed species which depends on the interaction energy between molecules as well as with the adsorbent. In addition to the lack of accuracy, it has been shown that the use of the same parameters in single and multicomponent isotherms violates the Gibbs-Duhem relationship unless the relative adsorption capacities of all components are identical (Ruthven 1984; Lim et al. 1995).

Table 4. Parameters of the Freundlich adsorption isotherm model for all components individually (K and n from Equation (3)) and as mixtures (a_{ij}'s from Equation (4)) with different compositions in the multicomponent mixture.

	Butanol	Acetone	Ethanol	Butyric acid	Acetic acid	Glucose	Xylose
K	188.870	26.340	13.271	130.930	37.406	33.139	27.010
n	6.627	1.619	1.612	3.050	2.352	2.171	1.995
a_{i1}	1	1.579	0.039	0.424	1.737	5.904	3.965
a_{i2}	0.449	1	0.101	1.672	3.114	3.478	2.140
a_{i3}	0.707	2.356	1	0	0.650	2.246	1.086
a_{i4}	0.000014	0	0.060	1	0	3.118	2.381
a_{i5}	0.013	2.249	0.165	0	1	3.109	2.349
a_{i6}	0.0005	3.042	0	0.362	1.557	1	2.693
a_{i7}	0.380	1.836	0.487	0	1.338	2.343	1

1: butanol, 2: acetone, 3: ethanol, 4: butyric acid, 5: acetic acid, 6: glucose, 7: xylose

Freundlich isotherm - The Freundlich isotherm model is another common isotherm model that can be used for single component (Eq. 3) and multicomponent (Eq. 4) adsorption systems. To predict the adsorption behavior of the ABE fermentation broth components (butanol, acetone, ethanol, butyric acid, acetic acid, glucose and xylose), multicomponent Freundlich isotherm model was also used in this study (Eq. 4). For each component, the model parameters K, n and a_{ij} were determined via the minimization of the sum of squares of the differences between the experimental and predicted values for the mixture equilibrium adsorption capacity. Parameters K, n and a_{ij} for multicomponent adsorption isotherm for each component are given in Table 4. The coefficients shown in this table reveal how the adsorption of a specific component is influenced by the presence of the other components in the solution. For example, it could be observed that glucose and xylose adsorption are affected by the presence of all the other components specially butanol, whereas in the case of butanol, the presence of most other components on butanol adsorption is rather low (Table 4). This is again an indication that biobutanol is the main component affecting the adsorption behaviour of other components in the adsorption column using AC F-400 as the adsorbent and it adsorbed preferentially to other components. The effect of the presence of other components and the competitive effects of components present in a typical ABE broth have been discussed previously (Abdehagh et al. 2013, 2015). Results for multicomponent Freundlich isotherm models are shown in Figure 3 for the three sets of experimental data obtained for each component.

Results showed that the model fits the data obtained from all experiments relatively well and could be considered as one of the applicable models to predict the adsorption behavior of all the components present in ABE fermentation broths. The coefficient of determination (R^2) for multicomponent adsorption Freundlich isotherm model of all components was 0.97 (Figure 4). To validate the multicomponent Freundlich model, two additional experiments were performed with different concentrations and the results of these validation experiments were used via the multicomponent Freundlich model to predict the equilibrium adsorption capacity of each component. Results of these validation data are plotted on the parity plot of Figure 4 which clearly shows that the model is able to predict the equilibrium adsorbed concentrations from solutions that are significantly different from concentrations of the original three series of experiments used to fit the multicomponent isotherm models. The black circle symbols in the parity plot of Figure 4 show the relationship between the predicted and the experimental results for the validation experiments. The multicomponent Freundlich isotherm is a more general isotherm model that is capable of representing the mutual competitive effect of all components of the ABE solutions with a very good precision. Its better fitting ability compared to multicomponent Langmuir isotherm comes mainly from the higher number of parameters. The multicomponent Langmuir isotherm model has 7 parameters that are common to all components and one parameter that is specific for a given component and given concentration level. On the other hand, the multicomponent Freundlich isotherm model has 9 parameters for each component in the mixture.

Artificial Neural Network - Finally, an ANN was used to model the isotherms for all seven components in the ABE model solution. Because an ANN requires a large data set to obtain a good model, the set of experimental data used by the multicomponent Langmuir and Freundlich isotherm models was augmented by all the equilibrium data that have been obtained over the last four years for binary, ternary and multicomponent mixtures for the activated carbon F-400. ANNs, as depicted in Figure 1, were used to model the equilibrium adsorption capacity of each component in ABE model solutions. The same ANN structure was used for each component with the only difference being that five neurons (including the bias) were used in the hidden layer for the ANN isotherms of butanol, butyric acid and acetic acid whereas, for the other four components,

the number of neurons in the hidden layers was four. The number of functional neurons was selected based on a good coefficient of determination (R^2) obtained by testing different numbers of neurons for each component while keeping the number to a minimum to overcome overfitting. Table 5 lists the resulting weights for the neural network obtained for each component (see Eqs. (5) and (6)).

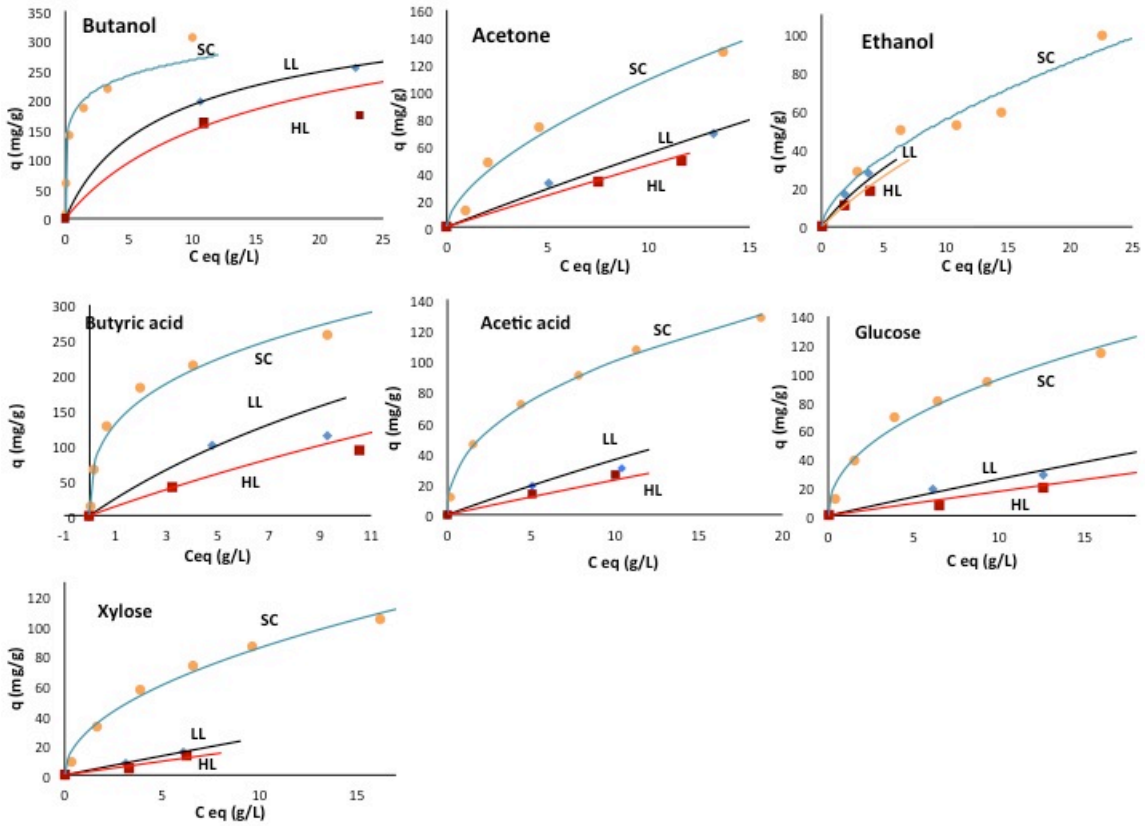


Figure 3. Plots of experimental (symbols) and predicted (curves) multicomponent Freundlich isotherm fits for butanol, butyric acid, ethanol, acetone, acetic acid, glucose and xylose for single component (SC), low level concentrations of other components (LL), and high level concentrations of other components (HL) in the model ABE fermentation solution at room temperature for activated carbon F-400.

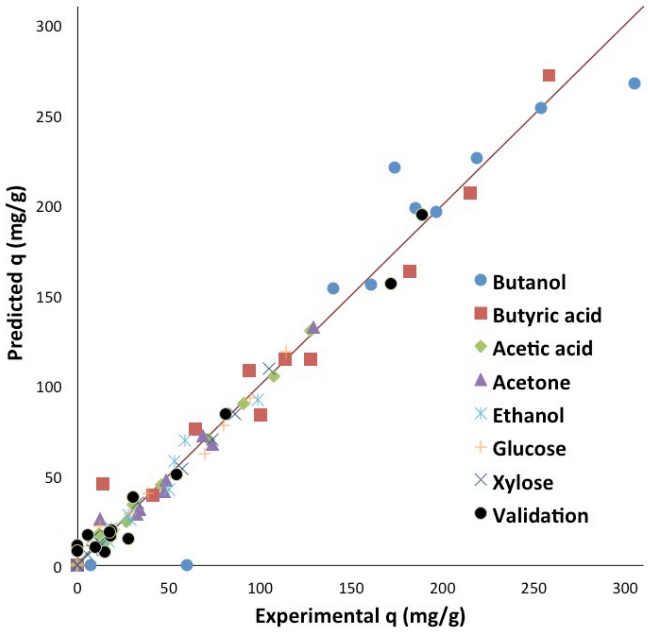


Figure 4. Comparison of experimental and predicted adsorption capacities for butanol, butyric acid, acetone, ethanol, acetic acid, glucose and xylose isotherms modeled using multicomponent Freundlich isotherm models for the model ABE fermentation at room temperature for activated carbon F-400.

In the ANN of Figure 1, the input variables for butanol, acetone, ethanol and acetic acid are the normalized concentrations of each component (Eq. 6). For butyric acid, glucose and xylose, the input variable for the butanol input neuron was the equilibrium butanol adsorption capacity ($\overline{q_B}$) instead of the normalized butanol concentration $\overline{C_B}$ due to its very low equilibrium concentration in solution for a relatively high adsorption capacity. This very low concentration is due to the very steep increase of the butanol adsorption isotherm curve at very low butanol concentrations (see Figs. 2 and 5) where relatively large adsorption is observed at nearly undetectable concentration. In the case of the ethanol isotherm, the input to account for the concentration of butyric acid was also its equilibrium adsorption capacity. The experimental data and the predicted isotherms obtained with the neural networks for the model solutions comprised of the individual components and their mixtures are shown in Figure 5.

Table 5. Weights obtained in the FFNN modeling for butanol, butyric acid, acetone, ethanol, acetic acid, glucose and xylose.

	Butanol	Acetone	Ethanol	Butyric acid	Acetic acid	Glucose	Xylose
W_{11}	-400.53	-12.939	24.202	0.39012	135.31	3.7469	0.47074
W_{12}	-11.446	63.578	10892	-0.38116	-64.794	45.763	3.8742
W_{13}	-0.63557	10.202	-20.67	3.9351	26.997	-3.8593	30.478
W_{14}	-0.89185	-	-	3.8186	3.2937	-	-
W_{21}	18.606	-25.187	58.512	12.329	13.403	-3.1575	7.0327
W_{22}	-28.635	-4.0269	12276	-2.9077	12.498	34.741	11.252
W_{23}	-5.5558	-3.7103	-37.763	17.9	-6.5579	3.1582	11.996
W_{24}	5.8433	-	-	11.644	4.1125	-	-
W_{31}	-1.644	-38.548	-1.5847	39.925	14.353	2.8051	1.5911
W_{32}	6.5091	-11.122	-55081	-0.85523	-8.2293	-165.08	-48.362
W_{33}	-6.5068	21.952	-13.582	-8.5594	0.37748	-2.9113	1.4271
W_{34}	5.5927	-	-	-8.5449	2.7972	-	-
W_{41}	-11.701	16.322	-8.3408	-72.16	13.849	10.353	-11.702
W_{42}	15.86	2.0576	-13527	-3.0635	-6.4094	181.1	13.148
W_{43}	6.6409	-10.527	7.3717	0.50165	9.9501	-10.47	6.1011
W_{44}	-4.487	-	-	0.45178	-28.626	-	-
W_{51}	-1.0054	-8.4129	-92.259	-33.081	-1.093	-23.849	-4.5943
W_{52}	2.7556	45.663	6065.7	-0.9739	-5.5717	85.21	28.88
W_{53}	3.7239	-14.417	40.49	10.697	-1.2019	24.458	8.7751
W_{54}	-2.7749	-	-	6.0056	-26.457	-	-
W_{61}	-1.4735	18.412	37.105	-51.609	18.508	-1.1876	6.39
W_{62}	-0.11043	0.91939	-24768	50.713	62.628	-49.549	-8.2228
W_{63}	1.4701	-1.038	-2.8704	-23.473	-28.624	1.1491	8.2287
W_{64}	-0.98176	-	-	-14.52	10.014	-	-
W_{71}	-0.95537	30.952	-6.4013	74.9	12.884	15.416	-17.442
W_{72}	-0.52944	-178.53	16346	-77.596	-3.2457	117.32	-4.6422
W_{73}	-0.19713	16.326	5.1211	15.71	4.4337	-16.043	-0.83184
W_{74}	0.24237	-	-	14.633	-0.67092	-	-
W_{81}	-9.5789	-10.874	2.952	-7.0956	8.0826	-4.0712	-8.3939
W_{82}	-1.4185	6.2653	1923.3	-3.6921	-0.36155	-0.13998	0.84344
W_{83}	2.705	0.82977	-2.0552	3.098	1.3865	4.0233	7.7704
W_{84}	-2.0379	-	-	3.7895	-8.7341	-	-
W'_{11}	-40827	-180700	-18.151	-3413	-3348.2	-2666.9	-10101
W'_{21}	-5.4343	-2.6302	-1010.8	-59.601	-1.9942	-4.7247	-2.0429
W'_{31}	-21.636	-4.6721	-18.133	2138.2	-2.2896	-2332.3	-2620.3
W'_{41}	-17.592	4.6838	16.46	-4270.7	-12346	2335	2619.9
W'_{51}	22.751	-	-	2131.6	3349.1	-	-

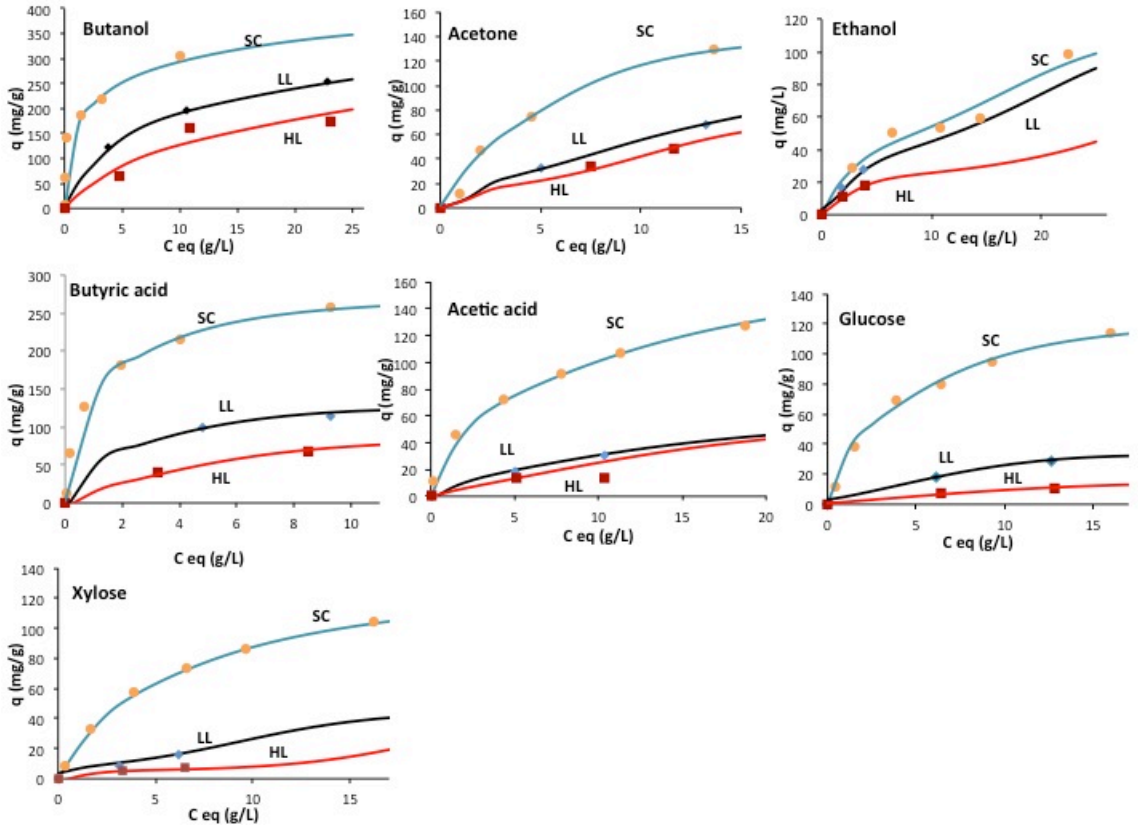


Figure 5. Experimental and predicted adsorption isotherms for butanol, butyric acid, ethanol, acetone, acetic acid, glucose and xylose for single component (SC), low level concentrations of other components (LL), and high level concentrations of other components (HL) modeled with FFNN in the model ABE fermentation at room temperature for activated carbon F-400.

Predicted results using neural networks to model multicomponent isotherms for all main components of the ABE fermentation broths are presented in Figure 5. This figure clearly shows that all isotherms represented the experimental data points very well and the predicted isotherms are of sufficient precision to be used with confidence for adsorption process simulation and design. The coefficient of determination (R^2) of neural network isotherm models of all components was 0.99. Neural networks use a series of transfer function curves (sigmoid transfer functions in this investigation) as piece-wise assemblage to construct the trends of different isotherms that best represent the experimental equilibrium concentrations. Because of steep changes in the adsorption isotherms for some components such as butanol and butyric acid, the predicted curves

experience at times rapid slope changes. These rapid changes will not affect the predictions of this model as it is able to follow quite well the overall behaviour of the isotherms.

For some curves, the predictions were made over a larger range to assess the predictive behaviour of the neural isotherms beyond the experimental range of concentration at a given level. Since the trend of the extrapolated at one level is guided by the experimental isotherms at other concentration levels, the extrapolated data appear to follow an expected behaviour. Nevertheless, it is not recommended to extrapolate beyond experimental data and additional experiments would need to be performed to cover the desired range of concentrations.

To independently validate the predictive ability of the neural network models, the results of the validation experiments were used. Figure 6 presents the parity plots for each component in the ABE model solution for the complete data bank to compare the experimental data and calculated values using the neural network isotherms. The large square symbols in this figure show the results of the validation experiments. The results of these experiments showed that for most of the components, the model predictions for the validation experimental points are accurate (Figure 6). These parity plots clearly show that the neural network isotherm models can be used with confidence to predict the equilibrium adsorption capacities of all components in the ABE model solutions.

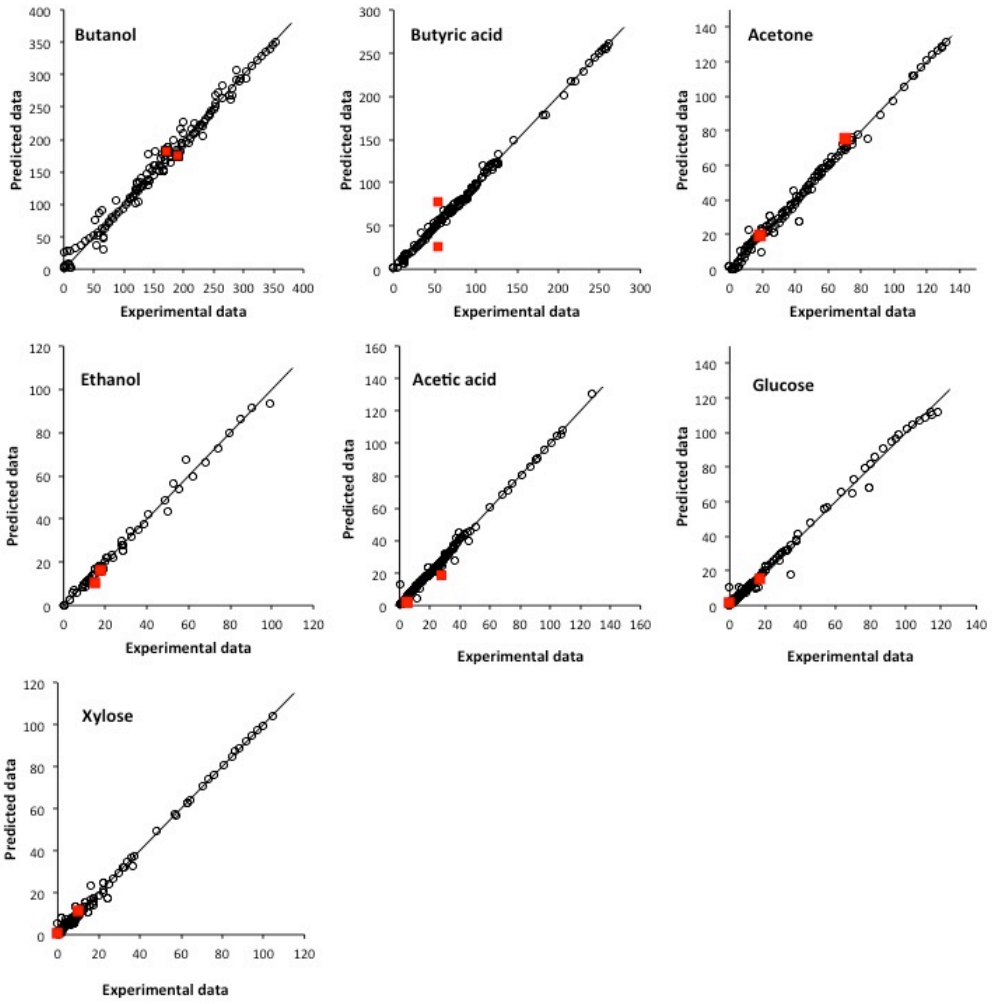


Figure 6. Comparison of experimental and calculated adsorption capacities for butanol, butyric acid, acetone, ethanol, acetic acid, glucose and xylose isotherms modeled using neural networks. o: Experimental learning data points; ■: Experimental validation data points.

The results of Freundlich and artificial neural network isotherms derived in this investigation showed that these two models could be used with reasonable confidence to predict the adsorption capacities for all components in the ABE model solutions provided that the concentrations of each species are not very different from the typical ABE concentration ranges. It would be possible to make the model even more general by performing experiments over a wider range of concentrations for all components. However, the purpose of this paper was to model the multicomponent isotherms for

concentrations typically encountered in ABE fermentation. On the other hand, the multicomponent Langmuir isotherms are valid only for the component concentrations for which they were derived for, because of the strong competitive adsorption occurring amongst all components. However, it is doubtful that these models can be used directly in the modelling of a packed bed adsorption process since in that model, the isotherm models are typically used to inversely predict the equilibrium concentrations of all components in the fluid phase by using the equilibrium concentrations in the adsorbed phase. This prediction will have to be performed for all components at the same time. This issue, called the "inverse adsorption problem", is the main obstacle in the modeling of an adsorption process in a packed bed for a multicomponent system. However, it is possible to rearrange the mass balance equation for the packed beds to include the adsorption driving force defined in terms of the adsorbed phase compositions, (q* - q), rather than in terms of the liquid phase concentrations (C - C*). This rearrangement has the net advantage of being able to use the direct isotherm model, as defined in this investigation, to calculate the equilibrium value q* of each component given the concentrations of all components in the liquid phase, without having to deal with "inverse adsorption problem".

4 Conclusions

Butanol adsorption is one of the most efficient separation techniques which can be used in biobutanol production processes for its production as a promising biofuel. One main challenge in butanol adsorption is the presence of other components in the fermentation broth and their effect on adsorbent selectivity for butanol. Since there are other components such as acetone, ethanol, acetic acid, butyric acid and sugars in the ABE fermentation broths, it is important to determine the behavior of each component and their effect in the adsorption process. In this study, predicting the isotherms for a multicomponent system (containing all components at different levels of concentrations) using multicomponent Langmuir and Freundlich adsorption isotherm models were studied as well as the Feedforward neural networks (FFNNs). Amongst these three models, the multicomponent Freundlich isotherm model showed reasonable prediction

results for all the seven components present in ABE fermentation broths and could be considered as a promising method to model the multicomponent adsorption system. Feedforward neural networks (FFNNs) were also used as an efficient approach to model the multicomponent adsorption isotherms of all the components in the presence of each other. Results showed that FFNNs were able to represent a wide range of adsorption isotherm data. The power of multicomponent Freundlich and neural network models lies in the universality of their application. The ability of a unifying model capable of representing data for different combinations of different components' adsorption isotherms is an achievement that traditional adsorption models such as multicomponent Langmuir model cannot attain individually. Adsorption data over a wider range of concentration of all components would allow making the isotherm models even more general.

5 Nomenclature

a_{ij}	Competition coefficient in multicomponent Freundlich isotherm model
b	Constant in multicomponent Langmuir isotherm model (L/g adsorbate)
C	Adsorbate concentration at equilibrium (g/L)
$\bar{C}$	Normalized adsorbate concentration at equilibrium (g/L)
C^*	Adsorbate concentration in equilibrium with adsorbed phase concentration q (g/L)
n	Constant in multicomponent Freundlich isotherm model
K	Constant in multicomponent Freundlich isotherm model (L/g adsorbent)
q	Adsorption capacity (g adsorbate/ g adsorbent)
q^*	Adsorption capacity in equilibrium with bulk liquid concentration C (g adsorbate/ g adsorbent)
q_s	Saturation adsorption capacity (g adsorbate/ g adsorbent)
$\bar{q}$	Normalized adsorption capacity (g adsorbate/ g adsorbent)
W	Weights in FFNN model

6 Abbreviations

ABE	Acetone-butanol-ethanol
ANN	Artificial neural network
FFNN	Feed forward neural network
HL	High level
LL	Low level
SC	Single component

7 Acknowledgements

The authors would like to acknowledge the Natural Science and Engineering Research Council (NSERC) of Canada and Ontario Graduate Scholarship (OGS) for their financial support.

8 References

Abdehagh N, Tezel FH, Thibault J. Adsorbent screening for biobutanol separation by adsorption: kinetics, isotherms and competitive effect of other compounds. Adsorption, 19, 1263-1272 (2013).

Abdehagh, N., Tezel, F.H., Thibault, J.: Separation techniques in butanol production: Challenges and developments (Review). Biomass and Bioenergy, 60, 222-246 (2014).

Abdehagh, N., Gurnani, P., Tezel, F.H., Thibault, J. Adsorptive separation and recovery of biobutanol from ABE model solutions. Adsorption, 21, 185-194 (2015).

Antoni, D., Zverlov, V.V., Schwarz, W.H.: Biofuels from microbes (mini review). Appl. Microbiol. Biotechnol. 77, 23–35 (2007).

Basu, S., Henshaw, P.F., Biswas, N., Kwan, H.K.: Prediction of gas phase adsorption isotherms using neural nets. Can. J. Chem. Eng.80, 1‑7 (2002)

Bulsari, A.B., Palosaafi, A.: Application of neural networks for system identification of an adsorption column. Neural Comput. Appl. 1, 160‑165 (1993)

Carsky, M., Do, D.D.: Neural network modeling of adsorption of binary vapour mixtures. Adsorption 5, 183‑192 (1999)

Dellomonaco, C., Fava, F., Gonzalez, R.: The path to next generation biofuels: successes and challenges in the era of synthetic biology. Microb. Cell Fact. 9, 3 (2010).

Do, D.D., Adsorption Analysis: Equilibria and Kinetics, Imperial College Press, Jan 1, 1998.

Dürre, P.: Biobutanol: An attractive biofuel. Biotechnol. J. 2, 1525–1534 (2007).

Ezeji, T.C., Qureshi, N., Blaschek, H.P.: Production of acetone, butanol and ethanol by *Clostridium beijerinckii* BA101 and in situ recovery by gas stripping. World J. Microbiol. Biotechnol. 19, 595–603 (2003).

Ezeji, T.C., Qureshi, N., Blaschek, H.P.: Butanol fermentation research: Upstream and downstream manipulations. Chem. Rec. 4, 305–314 (2004).

Fouad, E.A., Feng, X.: Use of pervaporation to separate butanol from dilutes aqueous solutions: Effects of operating conditions and concentration polarization. J. Membr. Sci. 323, 428–435 (2008).

Freundlich, H.M.F. Über die Adsorption in Lösungen. Z Phys Chem., 57(A), 385-470 (1906).

Groot, W.J., Luyben, K.Ch.A.M.: In situ product recovery by adsorption in the butanol/isopropanol batch fermentation. Appl. Microbiol. Biotechnol. 25, 29–31 (1986).

Harvey, B.G., Meylemans, H.A.: The role of butanol in the development of sustainable fuel technologies. J. Chem. Technol. Biotechnol. 86, 2-9 (2011).

Holtzapple, M.T., Brown, R.F.: Conceptual design for a process to recover volatile solutes from aqueous solutions using silicalite. Sep. Technol. 4, 213–229 (1995).

Jiao, P, Wu, J., Zhou, J., Yang, P., Zhuang, W., Chen, Y., Zhu, C., Guo, T., Ying, H. Mathematical modeling of the competitive sorption dynamics of acetone–butanol–ethanol on KA-I resin in a fixed-bed column. Adsorption, 21, 165-176 (2015).

Langmuir, I. The Adsorption of gases on plane surface of glass, mica and platinum. The Research Laboratory of the General Electric Company: 1361-1402 (1918).

Lewandowski, J., Lemcoff, N.O., Palosaari, S.: Use of neural networks in the simulation and optimization of pressure swing adsorption processes. Chem. Eng. Technol. 21(7), 593‑597 (1998).

Lim, B.G., Ching, C.B., Tan, R.B.H. Determination of competitive adsorption isotherms of enantiomers on dual-side adsorbent. Separation Technology, 5, 213-228 (1995).

Maddox, I.S.: Use of silicalite for the adsorption of n-butanol from fermentation liquids. Biotechnol. Lett. 4, 759-760 (1982).

Morse G., Jones R., Thibault J., Tezel F.H., Neural network modelling of adsorption isotherms. Adsorption 17, 303‑309 (2011).

Nielsen L., Larsson M., Hoist O., Mattiasson B.: Adsorbents for extractive bioconversion applied to the acetone-butanol fermentation. Appl. Microbiol. Biotechnol. 28, 335–339 (1988).

Nielsen D.R., Prather, K.J.: In situ product recovery of n-butanol using polymeric resins. Biotechnol. Bioeng. 102, 811–821 (2009).

Oudshoorn, A., Van der Wielen, L.A.M., Straathof, A.J.J.: Adsorption equilibria of bio-based butanol solutions using zeolite. Biochem. Eng. J. 48, 99–103 (2009)

Oudshoorn, A., Van derWielen, L.A.M., Straathof, A.J.J.: Desorption of butanol from zeolite material. Biochem. Eng. J. 67, 167–172 (2012)

Qureshi, N., Blaschek, H.P.: Production of acetone butanol ethanol (ABE) by a hyper-producing mutant strain of *Clostridium beijerinckii* BA101 and recovery by pervaporation. Biotechnol. Prog. 15, 594-602 (1999).

Qureshi N., Hughes S., Maddox I.S., Cotta M.A.: Energy-efficient recovery of butanol from model solutions and fermentation broth by adsorption. Bioprocess Biosyst. Eng. 27, 215–222 (2005)

Remi, J.C.S., Remy, T., Van Hunskerken, V., Van de Perre, S., Duerinck, T., Maes, M., De Vos, D., Gobechiya, E., Kirschock, C.E.A., Baron, G.V., Denayer, J.F.M.: Biobutanol separation with the metal-organic framework ZIF-8. ChemSusChem 4, 1074–1077 (2011).

Remi, J.C.S., Baron, G.V., Denayer, J.F.M.: Adsorptive separation for the recovery and purification of biobutanol. Adsorption 18, 367–373 (2012).

Ruthven, D.M. Principles of adsorption and adsorption processes. New York: John Wiley & Sons (1984).

Saravanan, V., Waijers D.A., Ziari M., Noordermeer M.A.: Recovery of 1-butanol from aqueous solutions using zeolite ZSM-5 with a high Si/Al ratio; suitability of a column process for industrial applications. Biochem. Eng. J. 49, 33–39 (2010).

Shapovalov, O.I., Ashkinazi, L.A.: Biobutanol: Biofuel of second generation. Russ. J. Appl. Chem. 81(12), 2232–2236 (2008).

Sharma, P., Chung, W.J.: Synthesis of MEL type zeolite with different kinds of morphology for the recovery of 1-butanol from aqueous solution. Desalination 275, 172–180 (2011).

Sowerby, B., Crittenden B.D.: Vapour phase separation of alcohol water mixtures by adsorption onto silicalite. Gas Sep. Pur. 2, 177–183 (1988).

Thompson, A.B., Cope, S.J., Swift, T.D., Notestein, J.M.: Adsorption of n-butanol from dilute aqueous solution with grafted calixarenes. Langmuir 27, 11990–11998 (2011).

Wu, X.-H., Lin, B.-C. Model Modification of Binary Competitive Isotherm. J. Liquid Chromatography & Related Technologies, 32: 2465–2483 (2009).

Wu, J., Zhuang, W., Ying, H. Acetone-Butanol-Ethanol competitive sorption simulation from single, binary, and ternary systems in a fixed-bed of KA-I resin. BioTechnology Progress, 31(1) 124-134 (2014).

Yang, M., Hubble, J., Fang, M., Locke, A.D., Rathbone, R.R.: A neural network for breakthrough prediction in packed bed adsorption. Biotech. Tech. 7(2), 155‑158 (1993)

Yang X., Tsai GJ., Tsao GT.: Enhancement of in situ adsorption on the acetone-butanol fermentation by *Clostridium acetobutylicum*. Sep. Technol. 4, 81-92 (1994).

Zheng, Y.N., Li, L.Z., Xian, M., Ma, Y.J., Yang, J.M., Xu, X., He, D.Z.: Problems with the microbial production of butanol. J. Ind. Microbiol. Biotechnol. 36, 1127–1138 (2009).

Chapter VIII

Adsorption packed bed simulation for butanol-water and ABE model solutions using multicomponent Freundlich isotherm

Niloofar Abdehagh, Mehran Bagheri, F. Handan Tezel, Jules Thibault

Department of Chemical and Biological Engineering
University of Ottawa
Ottawa, Ontario, Canada K1N 6N5

Corresponding author: Tel: (613) 652-5800 Ext. 6094; Email: Jules.Thibault@uottawa.ca

Abstract

Adsorption method has been considered as one of the most energy-efficient techniques for biobutanol separation from acetone-butanol-ethanol (ABE) fermentation broths. Activated carbon F-400 has been identified as one of the best adsorbents for biobutanol separation due to its high adsorption rate and capacity as well as its high selectivity toward butanol. To better comprehend and to design an efficient separation method in biobutanol production process, it is essential to be able to simulate the behaviour of the compounds present in the ABE broth during the adsorption process. In this study, a model comprised of a set of partial differential equations describing the components' mass balance was developed and solved by finite differences to predict the adsorption behaviour of all components present in an ABE fermentation broth. To account for the multicomponent competitive adsorption of all ABE components, the competitive multicomponent Freundlich isotherm model was used. The dynamic model along with the multicomponent isotherms was validated using experimental breakthrough curves for binary and ABE model solutions. Results showed that the packed column adsorption

model was able to predict relatively well the dynamic behaviour of each component in breakthrough adsorption experiments.

Keywords: ABE fermentation, biobutanol, adsorption modeling, multicomponent isotherm model, multicomponent Freundlich isotherm model

1 Introduction

Biobutanol has attracted significant attention as a potential alternative fuel for gasoline. Due to a myriad of reasons such as environmental concerns, depletion of fossil fuel resources and generally increasing cost of crude oil, the scientific community and industry are currently focusing more heavily on the development of biofuel production processes. Biobutanol is one of the most promising biofuels to replace gasoline due to its advantages over other biofuels: lower volatility, decreased flammability, better solubility in existing hydrocarbon fuels and higher energy density. In addition, the net heat of combustion (NHOC) of butanol is close to the one for gasoline. The possibility of using butanol in the existing car engines without any modifications has contributed to enhance the wave of interest toward biobutanol production. Typical microorganisms responsible for the production of butanol are able to use all sugars from lignocellulosic biomass without genetic modification. The feedstock used in biobutanol production, as a second generation biofuel, can therefore be agricultural and forestry residues and, as a result, avoids the "food versus fuel" controversy as it is often an issue in the first generation biofuels production such as first generation bioethanol (Abdehagh et al. 2013 and 2014a; Harvey et al. 2011; Thompson et al. 2011; Ezeji et al. 2004; Harvey et al. 2011; Dellomonaco et al. 2010; Antoni et al. 2007; Dürre 2007; Qureshi et al. 2005).

Clostridium species are the most common bacteria used in biobutanol production which are classified as gram-positive, strictly anaerobic and spore forming bacteria. In acetone-butanol-ethanol (ABE) production using *Clostridium* species (mainly *C. acetobutylicum* and *C. beijerinkii*) butanol is produced as one of the main products in a two-phase fermentation process. Acetone and ethanol are the other products in ABE fermentation and the products ratio in the broth is typically 3:6:1 wt% for

acetone:butanol:ethanol. In the acidogenic phase of the fermentation, in the exponential growth phase of the microorganism, acetate and butyrate are produced. In the second phase of fermentation, called solventogenic phase, the intermediate organic acids are re-assimilated and converted into acetone and butanol, respectively, in the late exponential and stationary phases of bacterial growth (Thirmal et al. 2012; Zheng et al. 2009; Ezeji et al. 2004). However, due to toxicity of butanol to the cell culture, the product concentration in the fermentation broth is limited to a maximum concentration of 20 g/L (2 wt%) and usually is in the order of 1 wt% of butanol. Thus, one way to achieve higher productivity in the ABE fermentation is to remove butanol from the fermentation broth to prevent its concentration to reach the toxicity threshold for the cell culture (Faisal et al. 2014; Harvey et al. 2011; Li and et al. 2010; García et al. 2009; Dürre 2007; Ezeji et al. 2007; Qureshi et al. 2005 and 1999).

There are several methods that can be used to recover butanol (in situ or at the end of fermentation) from dilute fermentation broth such as distillation, gas stripping, liquid-liquid extraction, pervaporation and adsorption (Farzaneh et al. 2015; Cao et al. 2015; Borisov et al. 2014; Thompson et al. 2014; Faisal et al. 2014; Xue et al. 2013; Abdehagh et al. 2013; Ying et al. 2013; Lin et al. 2012 and 2013; Hecke et al. 2012 and 2013; Lu et al. 2012; Xue et al. 2012; Garcia-Chavez et al. 2012; Dhamole et al. 2012; Yen et al. 2012; Wei et al. 2012; Li et al. 2011; Ha et al. 2010; Dürre 2007). Distillation is one of the separation techniques traditionally used for butanol separation but due to its high energy requirement (higher than the energy density of butanol) this method is not economically viable (Dürre 2007; Ezeji et al. 2004). The energy requirements and the performance of each method have been discussed in our previous study (Abdehagh et al. 2013). Amongst the other techniques, adsorption is considered as one of the most energy efficient techniques to remove butanol from dilute fermentation broths. In this technique, butanol is adsorbed onto the surface of a suitable adsorbent and subsequently desorbed by increasing the temperature and/or using displacers to produce a concentrated butanol solution (Abdehagh et al. 2014a; Faisal et al. 2013; Harvey et al. 2011; Oudshoorn et al. 2009a; Shapovalov et al. 2008; Qureshi et al. 2005).

There are some key factors that should be considered in adsorptive separation of butanol such as finding the best adsorbent with high adsorption capacity and adsorption

rate as well as being highly selective for the desired component. The feasibility of desorption and the cost of the adsorbent are other factors to consider in butanol adsorption processes. The adsorption capacity is one of the most important factors for the selection of an adsorbent since a high adsorption capacity leads to a smaller amount of adsorbent needed for the adsorption process. The adsorption rate also directly affects the contact time required between the adsorbent and the adsorbate; with fast kinetics, the time needed for the adsorption process decreases and less adsorbent is also required (Abdehagh et al. 2014a; Abdehagh et al. 2013; Yang et al. 1994). Even though a number of investigations have been done using adsorption technique to separate butanol from dilute solutions and a variety of adsorbents such as activated carbons, zeolites and polymeric resins, have been evaluated (Cao et al. 2015; Abdehagh et al. 2014a; Remi et al. 2011, 2012; Thompson et al. 2011; Oudshoorn et al. 2009b, 2012; Sharma et al. 2011; Saravanan et al. 2010; Nielsen 2009; Holtzapple and Brown 1995; Yang et al. 1994; Nielsen et al. 1988; Groot et al. 1986; Maddox 1982), in most of these studies, the focus was mainly placed on the adsorption capacity and only in a few of them have considered the adsorption rate and the impact of other components present in fermentation broths (Abdehagh et al. 2013; Nielsen et al. 1988). In addition, in butanol separation process by adsorption, adsorption and desorption are equally important to obtain a high butanol concentration solution (Abdehagh et al. 2015 and 2014a; Saravanan et al. 2010).

To better comprehend the dynamic behaviour of multicomponent ABE adsorption in a packed bed and to design an optimal packed bed adsorber for an industrial application, it is important to develop a model that will predict the fate of all ABE components. Only a few studies on the modeling of the adsorption process for ABE model solutions or fermentation broths have been undertaken (Wu et al. 2015). Wu et al. (2015) performed an interesting study where the adsorption process of butanol, acetone and ethanol in a ternary solution was simulated. Although their model was able to predict accurately their experimental data, the presence of sugars (glucose and/or xylose) and organic acids (butyric and acetic acids), observed in ABE fermentation broths, was not considered.

In our previous study, an adsorbent screening was performed where different kinds of activated carbons and zeolites were evaluated for butanol recovery. Activated

carbon F-400 was selected as the best adsorbent due to its high adsorption capacity, adsorption rate and selectivity toward butanol (Abdehagh et al. 2013). In this study, breakthrough experiments for binary butanol solutions and ABE model solutions were carried out to examine the dynamic behaviour of each component during the adsorption process. A dynamic adsorption model has been developed to simulate the breakthrough behaviour under different operating conditions in an adsorption packed bed for butanol separation from binary butanol-water and ABE model solutions. The model was validated with data of experimental breakthrough concentration profiles.

2 Materials and methods

2.1 Materials

Butanol-water and ABE model solutions were prepared to simulate representative concentrations of the main chemicals present in a typical fermentation broth during the production of butanol. To prepare the solutions, n-butanol (99 % pure, Acros), acetone (95 % pure, Acros), n-butyric acid (99 % pure, Acros) and 99 % pure ethanol, acetic acid, glucose and xylose were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Deionized distilled water was used to prepare all model solutions. The adsorbent used for this study was F-400 activated carbon (AC F-400) purchased from Calgon Corporation (Mississauga, ON, Canada). Some of the AC F-400 properties are given in Table 1.

Table 1 Some of the properties of activated carbon F-400 used as butanol adsorbent.

Activated carbon F-400	
Shape	Granule
Surface area (m^2/g)	1090
Mean particle diameter (mm)	0.55-0.75
Mesh size	12 x 40
Micropore volume (cm^3/g)	0.43

2.2 Methods

Breakthrough experiments using an adsorption packed bed were performed over a range of experimental conditions with varying length-to-diameter ratio, inlet flow rate and feed concentration. Experiments were carried out in two different columns. One glass column having the diameter (D) of 0.015 m and the height (L) of 0.175 m and one steel column with a diameter of 0.02 m and a height of 0.195 m were used. In the breakthrough

experiments, the feed solution was pumped to the column using a peristaltic pump and samples of the effluent were taken at specific intervals until the adsorbent bed reached complete saturation. The concentration of butanol, ethanol, acetone, acetic acid, butyric acid, glucose and xylose in the feed solutions and output samples were determined using High Pressure Liquid Chromatography (HPLC) and Gas Chromatography (GC). The adsorption capacities for each compound were then determined using the breakthrough curves by integrating the area above the curve. This method was described in our previous study (Abdehagh et al. 2015). Figure 1 shows the schematic diagram of the experimental system used in this investigation for breakthrough experiments.

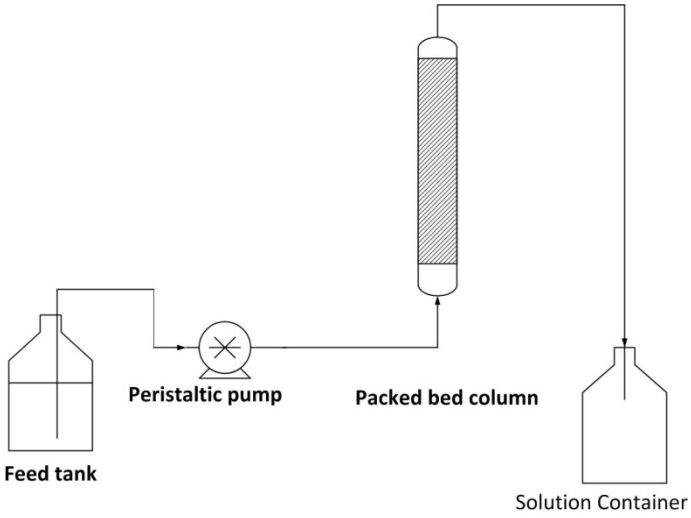


Figure 1 Schematic diagram of the adsorption setup used for breakthrough experiments.

2.3 Analytical method

The HPLC used in this study was purchased from Waters (Mississauga, ON, Canada). The detector, pump and autosampler were Refractive Index Detector (Waters 2414), Isocratic HPLC pump (Waters 1515) and Autosampler (Waters 717 plus), respectively. To heat the column to the desired temperature an external column heater was used. The column used to determine the concentration of each component of ABE solutions in this study was the Vertex column (300 X 8 mm, KNAUER, Germany) packed with Eurokat H, 10 µm. The software used to operate the HPLC was Breeze purchased from Waters (Mississauga, ON, Canada). Mobile phase used in HPLC analysis

was 0.01 N sulfuric acid with a flow rate of 0.8 mL/min. The temperature of the column was kept at 85°C (Abdehagh et al. 2014b).

The gas chromatograph (GC, SRI 8610C, Chromatographic Specialties Inc. CSI, Brockville, ON, Canada) used in this study was equipped with a flame ionization detector and a 30-m fused silica column (Stabilwax, 30M X 53MM, 1µm W/5M Integra-Guard column, EAC, Chromatographic Specialties Inc. CSI, Brockville, ON, Canada). Nitrogen was used as the carrier gas and the column temperature was held at 40°C for 2 min, raised to 200°C at a rate of 20°C/min, and held at 200°C for 4 min. The injector and detector were set at 110°C and 250°C. The adsorption capacity of the components was calculated using the concentrations measured by the HPLC and GC and mass balance equations (Abdehagh et al. 2015).

3 Modeling

3.1 Modeling multicomponent isotherms

Freundlich isotherm is an entirely empirical model without a theoretical basis that is often used to model adsorption for heterogeneous adsorbent surfaces (Freundlich, 1906). This isotherm model can be used for both single and multicomponent systems as defined in Equations (1) and (2), respectively.

$$q = KC^{1/n} \tag{1}$$

$$q_i = K_i C_i \left(\sum_{j=1}^{N} a_{ij} C_j \right)^{\frac{1}{n_i}-1} \tag{2}$$

where q is the equilibrium adsorption capacity (g of adsorbate/g of adsorbent). a_{ij} parameters are defined as competition coefficients with $a_{11} = a_{22} = \cdots = 1$. K and n are the isotherm constants under isothermal conditions. C is the concentration (g/L) of each component. The total number of components in the system is defined by N. i and j are the indices representing each component in the system. To fit and validate the isotherm

models for the multicomponent system with different combinations and ratios of the components, a set of adsorption experiments were performed for each particular component at different initial concentrations while keeping the initial concentrations of the other components constant. Isotherm experiments were carried out for two different levels of concentrations. The high level concentration of butanol, acetone, ethanol, butyric acid, acetic acid, glucose and xylose were 23, 12, 4, 10, 10, 12 and 6, g/L, respectively. The low level concentrations were 10, 5, 2, 5, 5, 6 and 3 g/L, respectively, for butanol, acetone, ethanol, butyric acid, acetic acid, glucose and xylose. These two levels of concentrations for the components were determined based on approximate concentrations of a typical ABE fermentation broth. The coefficients and constants for all the components determined by the multicomponent Freundlich isotherm model are shown in Table 2.

Table 2 Freundlich adsorption isotherm model coefficients for all components for multicomponent systems.

	Butanol	Acetone	Ethanol	Butyric acid	Acetic acid	Glucose	Xylose
K	188.870	26.340	13.271	130.930	37.406	33.139	27.010
n	6.627	1.619	1.612	3.050	2.352	2.171	1.995
a_{i1}	1	1.579	0.039	0.424	1.737	5.904	3.965
a_{i2}	0.449	1	0.101	1.672	3.114	3.478	2.140
a_{i3}	0.707	2.356	1	0	0.650	2.246	1.086
a_{i4}	0.000014	0	0.060	1	0	3.118	2.381
a_{i5}	0.013	2.249	0.165	0	1	3.109	2.349
a_{i6}	0.0005	3.042	0	0.362	1.557	1	2.693
a_{i7}	0.380	1.836	0.487	0	1.338	2.343	1

1: butanol, 2: acetone, 3: ethanol, 4: butyric acid, 5: acetic acid, 6: glucose, 7: xylose

3.2 Adsorption breakthrough modeling

To understand the dynamics of packed bed adsorbers and to design large-scale adsorption systems, the development of a mathematical model validated with experimental data is highly desirable. A sufficiently accurate model will allow predicting reasonably the adsorption of each component given a set of known operating conditions

(feed flow rate, composition, adsorption column characteristics, adsorbent properties and multicomponent isotherms). Modeling of breakthrough experiments was carried out using mass balance equations for each component in the liquid bulk and on the surface of the adsorbent.

Mass balance of the components in the liquid bulk: Assuming isothermal conditions and no radial dispersion, the rate of change in the concentration of each component throughout the adsorption column is given in the Equation 3. The terms in Equation 3, from left to right, represent the change of the concentration as a function of time of each component in the liquid bulk phase, the axial dispersion along the column, the mass transferred from the liquid bulk to the adsorbent expressed as a rate of change of the amount adsorbed, and the amount of a specific component flowing by convection, respectively.

$$\frac{\partial C_L}{\partial t} = D_z \frac{\partial^2 C_L}{\partial z^2} - \frac{(1-\varepsilon)}{\varepsilon} \rho_s \frac{\partial q}{\partial t} - u_L \frac{\partial C_L}{\partial z} \quad (3)$$

The mass transfer rate of the components at the surface of the adsorbent particles is defined in Eq. 4.

$$\frac{\delta q}{\delta t} = k_{eff}(q_e - q) \quad (4)$$

where k_{eff} is the effective mass transfer coefficient (s^{-1}) and q and q_e are the adsorption capacity at time t and the adsorption capacity (g of adsorbate/g of adsorbent) that would be in equilibrium with the concentration of one specific species in the liquid bulk defined by its multicomponent Freundlich isotherm model (Freundlich 1906).

Initial and boundary conditions: Initially, none of the ABE components are present in the column such that the concentrations in liquid and solid are zero (Equation 5):

$$At\ t = 0:\ C_L = 0,\ \ q = 0,\ \ \text{for all z} \quad (5)$$

Two boundary conditions are required to solve Equations 3 and 4 for each component. These boundary conditions show that at the entrance and the exit of the adsorption column, the diffusive flux is equal to zero (Equations 6 and 7).

$$\frac{\partial C_L}{\partial z} = 0 \qquad z = 0, t \geq 0 \qquad (6)$$

$$\frac{\partial C_L}{\partial z} = 0 \qquad z = L, t \geq 0 \qquad (7)$$

The sets of partial differential equations were solved using finite difference method. The time step and grid size were chosen to be size and grid independent.

4 Results and discussion

4.1 Breakthrough experiments for butanol-water binary solutions

Having obtained the adsorption isotherm models governing the packed bed adsorption operation and an estimate of the effective diffusion coefficient, the system of partial differential equation was first solved to validate the model for butanol-water (15 g/L) binary solutions following a series of breakthrough experiments that were performed for three inlet flow rates: 9, 17 and 25 mL/min in an adsorption column with the height and diameter of 17.5 and 1.5 cm, respectively. Results of the validation experiments are shown in Figure 2.

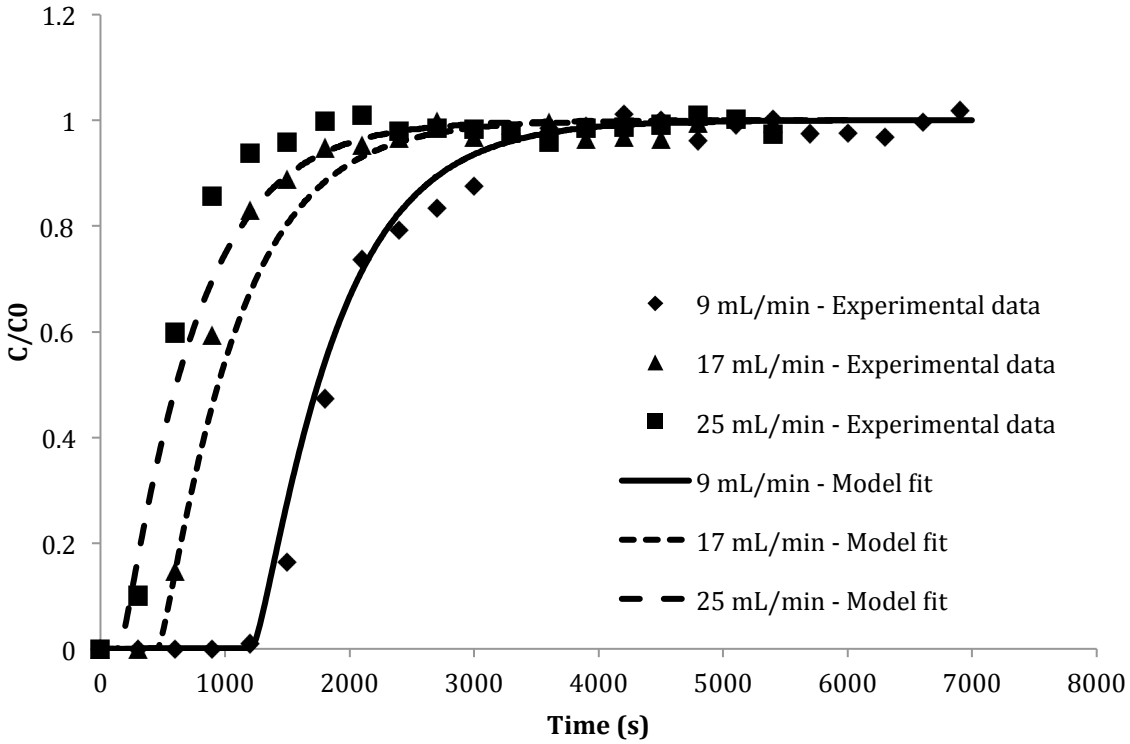


Figure 2 Butanol adsorption on activated carbon F-400 in a packed bed breakthrough experiment for butanol-water binary solutions. Breakthrough data are plotted along with the model predictions for various flow rates. The k_{eff} and D_z of butanol in the model were 0.002 s^{-1} and 7.78 × 10^{-7} m^2/s (Wu et al. 2015).

Results of these experiments showed as expected that, as the flow rate decreased, the breakthrough of butanol at the column effluent was delayed. Experimentally, the shape of the breakthrough concentration profiles for the three flow rates are very similar with a sharp increase when the adsorption column is nearing saturation. The simulated concentration profiles were also able to predict the breakthrough of butanol fairly well and the model simulation qualitatively matches the trend of the experimental data.

4.2 Breakthrough adsorption for ABE model solutions

The packed bed adsorption experiment was also performed with ABE model solutions that were mimicking a typical composition of the real ABE fermentation broth. The feed solution contained 12.0, 5.5, 2.0, 5.1, 5.2, 4.3 and 4.25 g/L of butanol, acetone, ethanol, butyric acid, acetic acid, glucose and xylose, respectively. Figure 3a to 3d

present the normalized concentration profiles for a breakthrough experiment performed at a flow rate of 25.6 mL/min at room temperature in an adsorption column with the height and diameter of 0.0195 and 0.002 m, respectively. The D_z for butanol was 7.78×10^{-7} m^2/s and for the other components the axial dispersion coefficient was considered as 7.8×10^{-7} m^2/s (Wu et al. 2015).

Figure 3a shows the breakthrough concentration profiles at the exit of the column for all components present in the feed solution. It was observed that glucose and xylose breakthrough normalized concentration profiles were roughly identical. These two components are desorbed by other components during the adsorption process. Then acetic acid and ethanol are desorbed by acetone, butyric acid and butanol. Finally, acetone is desorbed by butyric acid and butanol in the adsorption column. These two components are not desorbed in the adsorption column due to higher selectivity of AC F-400 toward butanol and butyric acid. However, the dsorption capacity of butyric acid and butanol is nevertheless reduced by the presence of the other ABE components. This competitive adsorption phenomenon is observed in the breakthrough concentration profiles exceeded their feed concentrations. These results clearly show that AC F-400 has a higher selectivity and affinity for butanol and butyric acid than for the other ABE components. As a result, butanol and butyric acid were initially adsorbed in the earlier section of the packed column. The significantly reduced concentration of butanol and butyric acid in the subsequent section of the column allowed the other components with less favourable adsorption affinity to occupy adsorption sites until the adsorption zone of butyric acid and butanol eventually reaches these adsorption sites. The components with lower selectivity were then partly or totally displaced from the adsorbed phase. The final adsorption capacity for butanol, acetone, ethanol, butyric acid, acetic acid, glucose and xylose upon saturation of the column were estimated to be 193.3, 25, 7, 64.5, 4.5, 0 and 0 mg/g, respectively (Abdehagh et al. 2013). The mass balance model developed in this study was validated using these experimental breakthrough concentration profiles and, as it is shown in Figures 3a to 3d, the model was able to fit the adsorption breakthrough experimental data adequately for most of the components present in the solution.

Figure 3b shows the breakthrough of butanol, acetone and ethanol of the same experiment separately. It can be observed that the model can predict the behaviour of

butanol and ethanol perfectly. For the acetone breakthrough concentration profile, the model was not able to fit the time of occurrence and the magnitude of the overshoot in the concentration profile adequately but was nevertheless able to predict the displacement of acetone by butanol. This lack of fit could be due to the inaccurate multicomponent adsorption isotherm model coefficients for acetone. Since it is relatively complex to find a multicomponent adsorption isotherm model to account for the interactions between seven compounds, it is not surprising to have one compound that will not represent as accurately as desired the competitive adsorption that prevails in this complex system. To have a better fit in the concentration profiles for the ABE compounds in a breakthrough curve, additional data of varied compositions are required to derive better multicomponent isotherms. The identification of better isotherm models is currently being investigated using different methods and models to find the most accurate isotherm structure for this specific multicomponent system.

Figure 3c shows the concentration profiles for the experimental and predicted breakthrough curves for butyric acid and acetic acid adsorption of the same experiment. Similarly, the experimental and simulated glucose and xylose concentration profiles are shown in Figure 3d. In all case, it was observed that the model was able to simulate adequately the dynamic adsorption behaviour of all these compounds in the adsorption process. Results also showed that butanol and butyric acid were the two components that were retained the strongest in the adsorption column using AC F-400 as the adsorbent. Based on the results of Figure 3, the selectivity for all ABE components, ranked from highest to lowest, are in the following order: butanol, butyric acid, acetone, acetic acid, ethanol, glucose and xylose. These results confirm once more that activated carbon F-400 has good adsorption selectivity toward butanol over other ABE components.

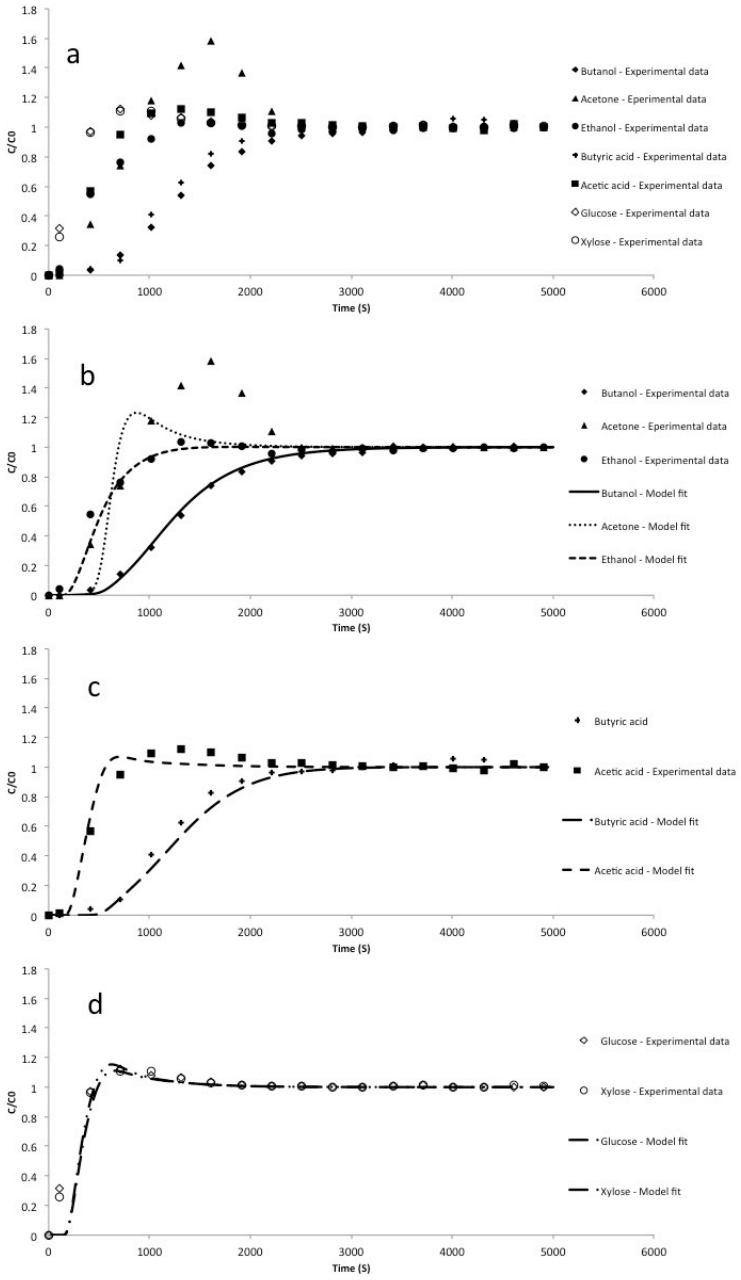


Figure 3 Breakthrough normalized concentration profile curves for ABE model solution adsorption at room temperature with the flow rate of 25.6 mL/min: a) the breakthrough for all components, b) butanol, acetone and ethanol breakthrough curves, c) Butyric acid and acetic acid breakthrough curves, and d) glucose and xylose breakthrough curves. The k_{eff} for butanol, acetone, ethanol, butyric acid, acetic acid, glucose and xylose in the model was 0.003, 0.05, 0.01, 0.006, 0.009, 0.01 and 0.012 s^{-1}, respectively.

The improvement of the model relies on the accurate representation of the competitive multicomponent system. In this study, the interaction and selectivity of all seven compounds potentially present in ABE fermentation needs to be encapsulated in a multicomponent isotherm model. This is currently being performed and will need additional equilibrium data of various concentrations to improve the simulation model. Other model structures are also investigated to find better adsorption isotherm models such as multicomponent Langmuir or neural network. The breakthrough experiment modeling would gain in representativity by considering the intrapellet diffusion within the pores of the adsorbent particles in which the mass transfer resistance in the particles often controls the adsorption rate. This option is also currently being studied.

5 Conclusions

Developing an effective separation method for biobutanol separation and recovery has been one of the major concerns in biobutanol production processes and adsorption is considered as one of the most efficient techniques in biobutanol separation from ABE fermentation broths due to its reasonable energy efficiency. Activated carbon F-400 has been known as one of the best adsorbents due to its high adsorption rate and capacity in addition to high selectivity toward butanol. However, there are some aspects in biobutanol production process that have not been studied comprehensively. The adsorption breakthrough modeling is one of these aspects since there are only a few studies focusing on modeling the butanol adsorption process from binary or ternary solutions and not even a multicomponent system containing acids and sugars as the present compounds in the real ABE fermentation broths. In this study, the focus was on developing a model to simulate the butanol adsorption process from ABE model solutions using a set of differential equations for mass balance in the packed column incorporated with the multicomponent Freundlich isotherm equation to simulate the breakthrough curves of adsorption process for ABE model solutions containing all the main compounds present in the real fermentation broths. The computational and theoretical model developed in this study could adequately characterize the process of the butanol adsorption in a packed bed. The relevant breakthrough curves modeled in this study provide valuable information for the designing the adsorption unit in the process.

As the future work the model will be modified using more accurate adsorption isotherm models for multicomponent system. In addition, developing the model to simulate desorption process will be considered as the future work in addition to justify the model for the adsorption-gas stripping system as well.

6 Abbreviations

ABE	*Acetone-butanol-ethanol*
ATCC	*American type culture collection*
D.I	*Deionized*
GC	*Gas chromatography*
HPLC	*High pressure liquid chromatography*
NHOC	*Net heat of combustion*
RCM	*Reinforced Clostridia medium*

7 Notations

ε	Void fraction of the column, (m^3 void/m^3 bed)
ρ_s	Solid density, kg m^{-3}
a_{ij}	Competition coefficient in multicomponent Freundlich isotherm model
C_L	Adsorbate concentration in the liquid bulk (g/L)
D_z	Axial dispersion coefficient (m^2/s)
K	Constant in multicomponent Freundlich isotherm model (L/g adsorbent)
k_{eff}	Effective mass transfer coefficient (s^{-1})
n	Constant in multicomponent Freundlich isotherm model
q	Adsorption capacity (g adsorbate/ g adsorbent)
q_e	Amount of component adsorbed at equilibrium (g adsorbate/ g adsorbent)
t	Time (S)
U_L	Superficial velocity of the fluid, m s^{-1}
z	Distance in the axial direction (m)

8 References

Abdehagh, N. Gurnani, P., Tezel, F.H., Thibault, J., Adsorptive separation and recovery of biobutanol from ABE model solutions. Adsorption 21(3), 185-194 (2015).

Abdehagh, N., Bagheri, M., Tezel, F.H., Thibault J., Improved Acetone-Butanol-Ethanol (ABE) Solution Analysis Using HPLC: Chromatograph Spectrum Deconvolution Using Asymmetric Gaussian Fit. American Journal of Analytical Chemistry 5, 1078-1089 (2014b).

Abdehagh, N., Tezel, F.H., Thibault, J.: Separation techniques in butanol production: Challenges and developments (Review). Biomass and Bioenergy 60, 222-246 (2014a).

Abdehagh, N, Tezel, F.H, Thibault, J., Adsorbent screening for biobutanol separation by adsorption: kinetics, isotherms and competitive effect of other compounds. Adsorption 19:1263-1272 (2013).

Antoni, D., Zverlov, V.V., Schwarz, W.H., Biofuels from microbes (mini review). Appl. Microbiol. Biotechnol. 77, 23–35 (2007).

Borisov, I.L., Malakhov, A.O., Khotimsky, V.S., Litvinova, E.G., Finkelshtein, E.S., Ushakov, N.V., Volkov, V.V., Novel PTMSP-based membranes containing elastomeric fillers: enhanced 1-butanol/water pervaporation selectivity and permeability. J. Membr. Sci. 466, 322–330 (2014).

Cao, Y., Wang, K., Wang, X., Gu, Z., Gibbons, W., Vu H., Butanol vapour adsorption behaviour on active carbons and zeolite crystal. Applied Surface Science 349, 1–7 (2015).

Dellomonaco, C., Fava, F., Gonzalez, R., The path to next generation biofuels: successes and challenges in the era of synthetic biology. Microb. Cell Fact. 9, 3 (2010).

Dhamole, PB, Wang, Z, Liu, Y, Wang, B, Feng, H., Extractive fermentation with non-ionic surfactants to enhance butanol production. Biomass Bioenergy 40, 112-119 (2012).

Dürre, P., Biobutanol: An attractive biofuel. Biotechnol. J. 2, 1525–1534 (2007).

Ezeji, T.C., Qureshi, N., Blaschek, H.P., Butanol fermentation research: Upstream and downstream manipulations. Chem. Rec. 4, 305–314 (2004).

Ezeji, T.C., Qureshi, N., Blaschek, H.P., Bioproduction of butanol from biomass: from genes to Bioreactors. Curr. Opin. Biotechnol. 18, 220–227 (2007).

Faisal, A., Zarebska, A., Saremi, P., Korelskiy, D., Ohlin, L., Rova, U.: MFI zeolite as adsorbent for selective recovery of hydrocarbons from ABE fermentation broths. Adsorption 20, 465–470 (2014).

Farzaneh, A., Zhou, M., Potapova, E., Bacsik, Z., Ohlin, L., Holmgren, A., Hedlund, J., Grahn, M.: Adsorption of Water and Butanol in Silicalite-1 Film Studied with in Situ Attenuated Total Reflectance−Fourier Transform Infrared Spectroscopy. Langmuir 31 (17), 4887–4894 (2015).

Freundlich, H.M.F. Über die Adsorption in Lösungen. Z Phys Chem., 57(A), 385-470 (1906).

Garcia-Chavez, L.Y., Garsia, C.M., Schuur, B., de Haan, A.B., Biobutanol recovery using nonfluorinated task-specific ionic liquids. Ind Eng Chem Res, 51, 8293-8301 (2012).

García, V., Pongracz, E., Muurinen, E., Keiski, R.L., Recovery of n-butanol from salt containing solutions by pervaporation. Desalination 241, 201–211 (2009).

Groot, W.J., Luyben, K.Ch.A.M., In situ product recovery by adsorption in the butanol/isopropanol batch fermentation. Appl. Microbiol. Biotechnol. 25, 29–31 (1986).

Ha, S.H., Maib, N.L., Koo, Y.M., Butanol recovery from aqueous solution into ionic liquids by liquid-liquid extraction. Process Biochem. 45:1899-1903 (2010)

Harvey, B.G., Meylemans, H.A., The role of butanol in the development of sustainable fuel technologies. J. Chem. Technol. Biotechnol. 86, 2–9 (2011).

Hecke, W.V., Vandezande, P., Claes, S., Vangeel, S., Beckers, H., Diels, L., Integrated bioprocess for long-term continuous cultivation of *Clostridium acetobutylicum* coupled to pervaporation with PDMS composite membranes. Bioresour .Technol. 111, 368-377 (2012).

Hecke, W.V., Hofmann, T., De Wever, H., Pervaporative recovery of ABE during continuous cultivation: enhancement of performance. Bioresour. Technol. 129, 421-429 (2013).

Holtzapple, M.T., Brown, R.F., Conceptual design for a process to recover volatile solutes from aqueous solutions using silicalite. Sep. Technol. 4, 213–229 (1995).

Li, S., Srivastava, R., Parnas, R.S., Separation of 1-butanol by pervaporation using a novel tri-layer PDMS composite membrane. J. Membr. Sci. 363, 287–294 (2010).

Li, G., Wei, W., Wu, H., Dong, X., Jiang, M., Jin, W., Pervaporation performance of PDMS/ceramic composite membrane in acetone butanol ethanol (ABE) fermentation-PV coupled process. J. Membr. Sci. 373, 121-129 (2011).

Lin, X., Wu, J., Fan, J., Qian, W., Zhou, X., Qian, C., Adsorption of butanol from aqueous solution onto a new type of macroporous adsorption resin: studies of adsorption isotherms and kinetics simulation. J. Chem. Technol. Biotechnol. 87, 924-931 (2012).

Lin, X., Li, R., Wen, Q., Wu, J., Fan, J., Jin, X., Experimental and modeling studies on the sorption breakthrough behaviors of butanol from aqueous solution in a fixed-bed of KA-I resin. Biotechnol. Bioprocess. Eng. 18, 223-233 (2013).

Lu, C., Zhao, J., Yang, S.T., Wei, D., Fed-batch fermentation for n-butanol production from cassava bagasse hydrolysate in a fibrous bed bioreactor with continuous gas stripping. Bioresour. Technol. 104, 380-387 (2012).

Maddox, I.S., Use of silicalite for the adsorption of n-butanol from fermentation liquids. Biotechnol. Lett. 4, 759–760 (1982).

Nielsen, L., Larsson, M., Hoist, O., Mattiasson, B., Adsorbents for extractive bioconversion applied to the acetone–butanol fermentation. Appl. Microbiol. Biotechnol. 28, 335–339 (1988).

Nielsen, D.R., Prather, K.J., In situ product recovery of n-butanol using polymeric resins. Biotechnol. Bioeng. 102, 811–821 (2009).

Oudshoorn, A., Van der Wielen, L.A.M., Straathof, A.J.J., Assessment of options for selective 1-butanol recovery from aqueous solution. Ind. Eng. Chem. Res. 48, 7325–7336 (2009a).

Oudshoorn, A., Van der Wielen, L.A.M., Straathof, A.J.J., Adsorption equilibria of bio-based butanol solutions using zeolite. Biochem. Eng. J. 48, 99–103 (2009b).

Oudshoorn, A., Van derWielen, L.A.M., Straathof, A.J.J., Desorption of butanol from zeolite material. Biochem. Eng. J. 67, 167–172 (2012).

Qureshi, N., Blaschek, H.P.: Production of acetone butanol ethanol (ABE) by a hyper-producing mutant strain of *Clostridium beijerinckii* BA101 and recovery by pervaporation. Biotechnol. Prog. 15, 594-602 (1999).

Qureshi, N., Hughes, S., Maddox, I.S., Cotta, M.A., Energy-efficient recovery of butanol from model solutions and fermentation broth by adsorption. Bioprocess Biosyst. Eng. 27, 215–222 (2005).

Remi, J.C.S., Rémy, T., Van Hunskerken, V., Van de Perre, S., Duerinck, T., Maes, M., De Vos, D., Gobechiya, E., Kirschock, C.E.A., Baron, G.V., Denayer, J.F.M., Biobutanol separation with the metal-organic framework ZIF-8. ChemSusChem 4, 1074–1077 (2011).

Remi, J.C.S., Baron, G.V., Denayer, J.F.M., Adsorptive separation for the recovery and purification of biobutanol. Adsorption 18, 367–373 (2012).

Saravanan, V., Waijers D.A., Ziari M., Noordermeer M.A., Recovery of 1-butanol from aqueous solutions using zeolite ZSM-5 with a high Si/Al ratio; suitability of a column process for industrial applications. Biochem. Eng. J. 49, 33–39 (2010).

Shapovalov, O.I., Ashkinazi, L.A., Biobutanol: Biofuel of second generation. Russ. J. Appl. Chem. 81(12), 2232–2236 (2008).

Sharma, P., Chung, W.J.: Synthesis of MEL type zeolite with different kinds of morphology for the recovery of 1-butanol from aqueous solution. Desalination 275, 172–180 (2011).

Thirmal, C., Dahman, Y., Comparison of existing pretreatment, saccharification, and fermentation processes for butanol production from agricultural residues. Can. J. Chem. Eng. 90, 745–761 (2012).

Thompson, A.B., Scholes, B.C., Notestein, J.M., Recovery of dilute aqueous acetone, butanol, and ethanol with immobilized calixarene cavities. Appl. Mater. Interfaces 6, 289–297 (2014).

Thompson, A.B., Cope, S.J., Swift, T.D., Notestein, J.M., Adsorption of n-butanol from dilute aqueous solution with grafted calixarenes. Langmuir 27, 11990–11998 (2011).

Wei, P, Zhu, D.W., Acetone-butanol-ethanol (ABE) fermentation using *Clostridium acetobutylicum* XY16 and in situ recovery by PDMS/ceramic composite membrane. Bioprocess. Biosyst. Eng. 35, 1057-1065 (2012).

Welty, J.R., Wicks, C.E., Wilson, R.E., and Rorrer, G., Fundamentals of Momentum, Heat and Mass Transfer, John Wiley and Sons, New York, (2000).

Wu, J., Zhuang, W., Ying, H., Acetone–Butanol–Ethanol Competitive Sorption Simulation from Single, Binary, and Ternary Systems in a Fixed-Bed of KA-I Resin. Biotechnolgy Progress 31(1), 124-134 (2015).

Xue, C., Zhao, J., Lu, C., Yang, S.T., Bai, F.W., Tang, I.C., High-titer n-butanol production by *Clostridium acetobutylicum* JB200 in fed-batch fermentation with intermittent gas stripping. Biotechnol. Bioeng. 109, 2746-2756 (2012).

Xue, C., Zhao, J., Liu, F., Lu, C., Yang, S.T., Bai, F.W., Two-stage in situ gas stripping for enhanced butanol fermentation and energy-saving product recovery. Bioresour. Technol. 135, 396-402 (2013).

Yang, X., Tsai, G.J., Tsao, G.T., Enhancement of in situ adsorption on the acetone-butanol fermentation by *Clostridium acetobutylicum*. Sep. Technol. 4, 81-92 (1994).

Yen, H.W., Lin, S.F., Yang, I.K., Use of poly(ether-block-amide) in pervaporation coupling with a fermenter to enhance butanol production in the cultivation of *Clostridium acetobutylicum*. J. Biosci. Bioeng. 113(3), 372-7 (2012).

Ying, H., Lin, X., Chen, Y., Chen, X., Bai, J., Xiaong, J., Method for separating butanol. Pub.No.: US 2013/0158303 A1, 2013.

Zheng, Y.N., Li, L.Z., Xian, M., Ma, Y.J., Yang, J.M., Xu, X., He, D.Z.: Problems with the microbial production of butanol. J. Ind. Microbiol. Biotechnol. 36, 1127–1138 (2009).

SECTION – VII: Conclusions and recommendations

Chapter IX

Conclusions and Recommendations

This thesis has examined the adsorption and also adsorption-gas stripping methods to improve butanol bioproduction processes to produce butanol economically as an alternative to other biofuels and petroleum-based fuels. The application targeted by this research project is the integration of a butanol separation and recovery method to a butanol fermentation system to remove and recover butanol from the dilute solutions and fermentation broths. The main accomplishments of this thesis and recommendations for future work are provided below.

1 Main accomplishments and contributions

A brief summary of the major thesis accomplishments and an assessment of how the thesis objectives were met are presented below. The introduction of the objectives can be found in Section I (Chapter I and Chapter II): Introduction.

1) In adsorbent screening experiments, some of the potential commercially available adsorbents were tested for their ability to selectively adsorb butanol from model solutions containing different combinations of the ABE components present in ABE fermentation broths. Adsorption performance was characterized based on the equilibrium adsorption capacity and the kinetics of the adsorption. Activated carbon F-400 was selected among the tested adsorbents due to its strong performance showing the fastest adsorption rate and adsorption capacity in addition to high selectivity toward butanol in comparison to other ABE components.
2) The competitive adsorption was comprehensively investigated to evaluate each ABE fermentation components' effect on butanol adsorption separately to achieve a better understanding of the adsorption process. The results showed that the butanol adsorption capacity was not affected by the presence of ethanol, glucose and xylose while the presence of acetone led to a slight

decrease in adsorption capacity at low butanol concentrations. On the other hand, the presence of acids (acetic acid and butyric acid) showed a significant negative effect on the butanol adsorption capacity over a wide range of butanol concentration and this effect was more pronounced for butyric acid.

3) ABE fermentation components' detection using only HPLC was investigated. A computer program was developed to use the HPLC chromatograms with overlapped peaks to detect all the components (acetone, butanol, ethanol, butyric acid, acetic acid, glucose and xylose) using a deconvolution method to calculate the area of each overlapped peak to estimate the concentration of each component for a multicomponent HPLC chromatogram.
4) Thermal desorption method was studied to investigate the ability of the adsorbent to be used in different cycles of adsorption-desorption as well as the desorption process performance as a valid method for completion of the adsorption process for ABE fermentation and butanol bioproduction. The results showed that AC F-400 retained its initial adsorption capacity after 6 adsorption-desorption cycles with the butanol recovery of 84 and 80% with butanol adsorption capacity of 302 and 171 mg/g, respectively, for butanol-water (1.5 wt%) binary and ABE model solutions.
5) Single and multicomponent isotherms for different components in the ABE model solutions were determined by testing different adsorption isotherm models. Sips model, single and multicomponent Langmuir and Freundlich models, as well as the neural network models were used to simulate the behaviour of each component in the ABE fermentation in binary and multicomponent adsorption systems. The Sips isotherm model was found to adequately fit the experimental data for binary adsorption systems. Multicomponent Freundlich and neural network models were able to effectively represent the behaviour of the different components in the ABE fermentation broth for multicomponent adsorption behaviour with AC F-400.
6) A method was designed for the combination of adsorption and gas stripping processes and it was compared to the liquid phase adsorption. An adsorption process was combined with a gas stripping system benefiting from the

stripping gas CO_2 to remove the solvents from the liquid solution into the vapour phase and performing the adsorption in the vapour phase. The butanol vapour adsorption capacity achieved for this system was 261 mg/g for a binary butanol-water solution of 15 g/L (vapour concentration of 5.8 mg/L). For ABE model solutions (containing 5.1 mg/L butanol in vapour phase) and for ABE fermentation broths (with 2.3 mg/L butanol in the vapour phase) the adsorption capacity of butanol in the vapour phase was 211.6 and 9.8 mg/g. The adsorption capacities achieved in this study were higher than what has been published in the literature.

7) A mechanistic model was developed to represent the adsorption process to predict the butanol adsorption in the packed bed. Isotherm models obtained in previous sets of experiments were incorporated into the model. The model consisted of a series of differential equations and used to simulate the performance of the packed bed adsorption column where multicomponent components were fed. The model adequately represented the experimental data for the simulation of the behaviour of butanol and other ABE fermentation components in the adsorption process.

2 Recommendations for Future work

Optimizing the desorption process was not addressed in detail in this particular study. The results for thermal desorption experiments in this project showed that the AC F-400 could be used in subsequent adsorption-desorption cycles. However, the process was not effectively optimized to maximize the desorption rate and performance. Therefore it is still essential to investigate the process more in detail to develop the integrated butanol adsorption process in the packed bed using AC F-400 as the adsorbent.

Developing an in-situ recovery system consisting of fermentation and separation system to reduce the butanol inhibition in the fermentation process to increase the butanol productivity is one of the other future studies that could be performed to improve butanol bioproduction processes. The in-situ separation system could be either adsorption or the combined adsorption-gas stripping process.

In addition, more work is needed to adjust the model regarding modeling the multicomponent adsorption isotherms and also the packed bed adsorption process. Different scenarios can be assumed for a packed bed adsorption process such as considering the diffusion in the adsorbent particles or the instantaneous adsorption ($k_{ads} \approx \infty$) onto the surface of the adsorbent particles in the packed bed that could be considered in the modeling. In addition, the modeling of the integrated fermentation process combined with the in-situ separation and recovery system can be considered as one of the future studies to predict the continuous butanol production, separation and recovery processes.

Although these schemes were only tested at the lab scale to examine the technical feasibility, a setup that would be industrially relevant for large-scale operations would need to be tested to better study butanol separation and recovery in an industrial context. This study did not aim to determine the ideal desorption process that would be employed industrially. More work is needed on this front and in particular in the design of:

- Suitable downstream processes such as distillation and purification unit operations after the separation and recovery by adsorption-desorption system.
- The whole plant from the fermentation step to the final step that is producing high concentration of butanol as the final product.

Section VIII: Appendix

Chapter A

C.V. information

1. Paper in Refereed Journals

1. N. Abdehagh, F.H. Tezel, J. Thibault, "A REVIEW: Biobutanol Production and Separation: Challenges and Developments", Biomass and Bioenergy Journal, 60, 222- 246, (2014)
2. N. Abdehagh, F.H. Tezel, J. Thibault, "Adsorbent Screening for Biobutanol Separation by Adsorption: Kinetics, Isotherms and Competitive Effect of Other Compounds", Adsorption Journal, 19, 1263-1272, (2013)
3. N. Abdehagh, M. Bagheri, F.H. Tezel, J. Thibault, "Improved Acetone-Butanol-Ethanol (ABE) Solution Analysis Using HPLC: Chromatograph Spectrum Deconvolution Using Asymmetric Gaussian Fit", American Journal of Analytical Chemistry, 5(16), 1078-1089 (2014).
4. N. Abdehagh, P. Gurnani, F.H. Tezel, J. Thibault, "Adsorptive Separation and Recovery of Biobutanol from ABE Model Solution", Adsorption, 21(3), 185-194 (2015).
5. N. Abdehagh, B. Dai, J. Thibault, F.H. Tezel, "Biobutanol separation from ABE model solutions and fermentation broths using a combined adsorption-gas stripping process", Submitted to Chemical Engineering Science journal
6. N. Abdehagh, F.H. Tezel, J. Thibault, "Multicomponent Adsorption Isotherm Modeling for ABE Model Solutions", Submitted to Adsorption journal
7. N. Abdehagh, F.H. Tezel, J. Thibault, "Simulation and validation of butanol separation from ABE model solutions and fermentation broth in breakthrough experiments for an adsorption packed bed", in preparation to be submitted shortly.

2. Papers in Conference Proceedings (Abstracts only)

8. N. Abdehagh, F.H. Tezel, J. Thibault, "Biobutanol separation by adsorption: Ad- sorbent screening, kinetics and equilibrium", Oral presentation in the 14th CSChE Ontario-Quebec Biotechnology Meeting, University of Ottawa, Ottawa. ON, May 30- 31, (2012)
9. N. Abdehagh, F. H. Tezel, J. Thibault, "Improving Biobutanol Production by Adsorption: Equilibrium and Kinetic Studies for Adsorbent Screening", Oral presentation in the 62nd Canadian Chemical Engineering Conference (CSChE), Vancouver, BC, October 14-17 (2012)
10. N. Abdehagh, F. H. Tezel, J. Thibault, "Improvements in Biobutanol Separation by Adsorption: Adsorbent Screening, Kinetics and Equilibrium", Oral presentation in the 2012 Annual Meeting taking place in Pittsburgh, PA, USA, October 28–November 2 (2012)
11. N. Abdehagh, A. Sharif, F. H. Tezel, J. Thibault, "In situ removal of biobutanol from fermentation broth", Oral presentation in the XXVI Interamerican Congress of Chemical Engineering, Montevideo, Uruguay, November 12-14, (2012)
12. N. Abdehagh, F.H. Tezel, J. Thibault, "Adsorption Separation of Biobutanol: Kinetic and Equilibrium studies and Effect of Impurities", Oral presentation in 11th International Conference on Fundamentals of Adsorption FOA11, Baltimore, Mariland, May 19-24 (2013)
13. N. Abdehagh, A. Sharif, F.H. Azimi, H. Tezel, P. Mehrani, J. Thibault, "Biobutanol: A Viable Fuel", (Opening Plenary presentation), International Conference and Exhibition on Clean Energy, Ottawa, Canada, Sep 9-11, (2013)
14. H. Azimi, N. Abdehagh, P. Gurnani, A. Sharif Rohani, F.H. Tezel, Mehrani P., Thibault J., "Butanol Recovery via Pervaporation Separation from ABE Fermentation", 15th CSChE Quebec-Ontario Biotechnology Meeting, Quebec, Canada, 30-31 May (2013)

15. N. Abdehagh, F.H. Tezel, J. Thibault, “Adsorptive Separation of Biobutanol: Adsorbent Screening, Effect of Impurities and Breakthrough Experiments”, 15th CSChE Quebec-Ontario Biotechnology Meeting, Quebec, Canada, 30-31 May (2013)
16. A. Sharif Rohani, N. Abdehagh, P. Mehrani, F.H. Tezel, J. Thibault, “In-situ Removal of Biobutanol During Fermentation Process”, 10th World Congress on Industrial Biotechnology, Montreal, Canada, June 16-19 (2013)
17. N. Abdehagh, F. H. Tezel, J. Thibault, “Biobutanol Separation By Adsorption: Adsorbent Screening and Breakthrough Experiments”, AIChE 2013 Annual Meeting, San Francisco, CA, USA, Nov 3-8 (2013).
18. N. Abdehagh, F. H. Tezel, J. Thibault, “Adsorptive biobutanol separation from ABE model solutions”, 11th Annual world Congress on Industrial Biotechnology, Philadelphia, PA, USA, May 12-16 (2014).
19. N. Abdehagh, F. H. Tezel, J. Thibault, “Adsorption of Biobutanol from ABE Fer- mentation Broths ”, 10th International Conference on Diffusion in Solids and Liquids, Paris, France, 23-27 June (2014)
20. N. Abdehagh, F. H. Tezel, J. Thibault, “Biobutanol Separation and Recovery by Adsorption”, 12th Annual World Congress on Industrial Biotechnology July 19-22, Montreal, CA (2015)

Chapter B

Chromatograph Spectrum Deconvolution Code

```
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Decounvoluter Code                                              %
%Written by                                                       %
% Mehran Bagheri, UOttawa, Jan14                                  %
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%======================%
% Input & output                %
%======================%

inputdata = importdata('peaks-data/7. Multicomponent Isotherm/16/initial.arw','\t');


G_m  = 4.902; G_0  = 0.162;            % slope and intercept ...
X_m  = 4.762; X_0  = 0.086;            % ... obtained from Standard curve
AA_m = 2.252; AA_0 = 0.092;            %  area = slope * concentration + itcp
A_m  = 1.666; A_0  = 0.317;            % AA = Acetic acid, A = Acetone
BA_m = 2.976; BA_0 = 0.244;            % BA = Butyric acid, E = Ethanol
E_m  = 2.751; E_0  = 0.083;            % G = Glucose, X = Xylose
B_m  = 2.564; B_0  = 0.177;            % B = Butanol
area_fac = [G_m X_m AA_m A_m BA_m E_m B_m];% sort based on retention time
area_ini = [G_0 X_0 AA_0 A_0 BA_0 E_0 B_0];

% specify the peaks x-range
region_1 = [7.5, 11];              % x-range of the peak1, peak2
region_2 = [12.5, 15];             % x-range of the peak3
region_3 = [17, 20.2];             % x-range of peak4, peak5, peak6;
region_4 = [28, 33];               % x-range ofh.5e peak7

% plot initial range for peaks area
fig_all_peaks = figure( 'Position', [100 300 1000 400]);   % figure position & size
plot(inputdata(:,1),inputdata(:,2));                        % plot initial peak
hold on;
plot([region_1(1,1) region_1(1,1)] ,[-5 5], '-r', 'LineWidth', 2);
plot([region_1(1,2) region_1(1,2)] ,[-5 5], '-r', 'LineWidth', 2);
plot([region_1(1,1) region_1(1,2)] ,[-5 -5], '--r', 'LineWidth', 2);
plot([region_2(1,1) region_2(1,1)] ,[-5 5], '-r', 'LineWidth', 2);
plot([region_2(1,2) region_2(1,2)] ,[-5 5], '-r', 'LineWidth', 2);
plot([region_2(1,1) region_2(1,2)] ,[-5 -5], '--r', 'LineWidth', 2);
```

```
plot([region_3(1,1) region_3(1,1)] ,[-5 5], '-r', 'LineWidth', 2);
plot([region_3(1,2) region_3(1,2)] ,[-5 5], '-r', 'LineWidth', 2);
plot([region_3(1,1) region_3(1,2)] ,[-5 -5], '--r', 'LineWidth', 2);
plot([region_4(1,1) region_4(1,1)] ,[-5 5], '-r', 'LineWidth', 2);
plot([region_4(1,2) region_4(1,2)] ,[-5 5], '-r', 'LineWidth', 2);
plot([region_4(1,1) region_4(1,2)] ,[-5 -5], '--r', 'LineWidth', 2);
% count number of data-point in different peaks region
count_12 = 0; count_3 = 0;
count_456 = 0; count_7 = 0;
for i = 1:size(inputdata,1)
   if inputdata(i,1) > region_1(1,1) && inputdata(i,1) < region_1(1,2)
      count_12 = count_12+1;
   elseif inputdata(i,1) > region_2(1,1) && inputdata(i,1) < region_2(1,2)
      count_3 = count_3+1;
   elseif inputdata(i,1) > region_3(1,1) && inputdata(i,1) < region_3(1,2)
      count_456 = count_456+1;
   elseif inputdata(i,1) > region_4(1,1) && inputdata(i,1) < region_4(1,2)
      count_7 = count_7+1;
   end;
end;


% seperate different peaks data
peak12 = zeros(count_12,2); peak3 = zeros(count_3,2);
peak456 = zeros(count_456,2); peak7 = zeros(count_7,2);
count_12 = 0; count_3 = 0;
count_456 = 0; count_7 = 0;
for i = 1:size(inputdata,1)
   if inputdata(i,1) > region_1(1,1) && inputdata(i,1) < region_1(1,2)
      count_12 = count_12+1;
      for j = 1:2
         peak12(count_12,j) = inputdata(i,j);
      end
    elseif inputdata(i,1) > region_2(1,1) && inputdata(i,1) < region_2(1,2)
      count_3 = count_3+1;
      for j = 1:2
         peak3(count_3,j) = inputdata(i,j);
      end
   elseif inputdata(i,1) > region_3(1,1) && inputdata(i,1) < region_3(1,2)
      count_456 = count_456+1;
      for j = 1:2
         peak456(count_456,j) = inputdata(i,j);
      end
    elseif inputdata(i,1) > region_4(1,1) && inputdata(i,1) < region_4(1,2)
      count_7 = count_7+1;
      for j = 1:2
```

```
            peak7(count_7,j) = inputdata(i,j);
        end
    end;
end;

% correction for non-zero baseline
min_peak12 = min(peak12(:,2));
min_peak3 = min(peak3(:,2));
min_peak456 = min(peak456(:,2));
min_peak7 = min(peak7(:,2));
peak12(:,2) = peak12(:,2) - min_peak12;
peak3(:,2) = peak3(:,2) - min_peak3;
peak456(:,2) = peak456(:,2) - min_peak456;
peak7(:,2) = peak7(:,2) - min_peak7;

%=======================%
% fit asy_guassian fun              %
% to data points                    %
%=======================%

% asymetric gaussian fit function :
% f = a0/2/d0 * exp(c0^2/2/d0^2 + (b0-x)/d0).*(erf((x-b0)/(sqrt(2)*c0) - c0/sqrt(2)/d0)
+1)

%-----------------------------------------%
% First region: region_1                  %
% including two peaks (1) & (2)       %
%-----------------------------------------%

fit_gauss_peak12 = fit(peak12(:,1),peak12(:,2),'gauss2');   % two gaussian functions
fig_peak12 = figure('Position', [100 100 500 500]);             % figure position & size
plot(fit_gauss_peak12, peak12(:,1) , peak12(:,2));               % plot gaussian fit
hold on;
par_guess = coeffvalues(fit_gauss_peak12);                          % guess initial value for
a1=par_guess(1,1);b1=par_guess(1,2);c1=par_guess(1,3    % asymetric-Guassian
a2=par_guess(1,4);b2=par_guess(1,5);c2=par_guess(1,6);  %from gaussian fit
d1 = 1.4*c1; d2 = 1.4*c2; s=0;
fit_init_par_peak12 = [a1 b1 c1 d1 a2 b2 c2 d2 s];           % asy Gasussian parameters

% find parameter for the best fit and plot the results
options= statset('MaxIter',1000);
find_fit_par = nlinfit(peak12(:,1),peak12(:,2), @fit_fun_2peaks, fit_init_par_peak12,
options);
plot( peak12(:,1), fit_fun_2peaks(find_fit_par,peak12(:,1)),'g-','LineWidth', 2);

% plot peaks seperately &
```

```
%  find area undeneath of each one &
%   calculate the concentration
fit_par_peak1 = find_fit_par(1:4);

fit_par_peak2 = find_fit_par(5:8);
final_peak1 = asym_gauss(fit_par_peak1,peak12(:,1));
final_peak2 = asym_gauss(fit_par_peak2,peak12(:,1));
plot( peak12(:,1), final_peak1,'b-','LineWidth', 0.5);
plot( peak12(:,1), final_peak2,'b-','LineWidth', 0.5);
area_peak1 = trapz(peak12(:,1),final_peak1);
area_peak2 = trapz(peak12(:,1),final_peak2);

%--------------------------------------%
% Second region: region_2          %
% including one peaks (3)          %
%--------------------------------------%

fit_gauss_peak3 = fit(peak3(:,1),peak3(:,2),'gauss1');     % one Gaussian function fit
fig_peak3 = figure('Position', [100 100 500 500]);         % figure position & size
plot(fit_gauss_peak3, peak3(:,1) , peak3(:,2));              % plot Gaussian fit
hold on;
par_guess = coeffvalues(fit_gauss_peak3);           % guess initial value for assymetric-
a1=par_guess(1,1);b1=par_guess(1,2);c1=par_guess(1,3)%  Guas from Gaussian fit
d1 = 1.4*c1;s=0;
fit_init_par_peak3 = [a1 b1 c1 d1 s];                   % assymetric Gasussian parameters

% find parameter for the best fit and plot the result
options= statset('MaxIter',1000);
find_fit_par = nlinfit(peak3(:,1),peak3(:,2), @fit_fun_1peak, fit_init_par_peak3, options);
peak3(:,2) = peak3(:,2) - find_fit_par(1,5);
plot( peak3(:,1), fit_fun_1peak(find_fit_par,peak3(:,1)),'g-','LineWidth', 2);

% plot peaks seperately &
%  find area undeneath of each one &
%   calculate the concentration
fit_par_peak3 = find_fit_par(1:4);
final_peak3 = asym_gauss(fit_par_peak3,peak3(:,1));
plot( peak3(:,1), final_peak3,'b-','LineWidth', 0.5);
area_peak3 = trapz(peak3(:,1),final_peak3);

%-----------------------------------------------%
% First region: region_3                        %
% including three peaks (1) & (2) & (3)%
%-----------------------------------------------%

fit_gauss_peak456 = fit(peak456(:,1),peak456(:,2),'gauss3');  % three Gaus. Func. fit
```

```
fig_peak456 = figure('Position', [100 100 500 500]);              % figure position & size
plot(fit_gauss_peak456, peak456(:,1) , peak456(:,2));              % plot Gaussian fit

hold on;
par_guess = coeffvalues(fit_gauss_peak456);       % guess initial value for assymetric-
a1=par_guess(1,1);b1=par_guess(1,2);c1=par_guess(1,3) %  Guas from Gaus fit
a2=par_guess(1,4);b2=par_guess(1,5);c2=par_guess(1,6);
a3=par_guess(1,7);b3=par_guess(1,8);c3=par_guess(1,9);
d1 = 1.4*c1; d2 = 1.4*c2; d3 = 1.4*c3; s=0;
fit_init_par_peak456 = [a1 b1 c1 d1 a2 b2 c2 d2 a3 b3 c3 d3 s];
% assymetric Gasussian parameters

% find parameter for the best fit and plot the result
options= statset('MaxIter',1000);
find_fit_par = nlinfit(peak456(:,1),peak456(:,2),
@fit_fun_3peaks, fit_init_par_peak456, options);
plot( peak456(:,1), fit_fun_3peaks(find_fit_par,peak456(:,1)),'g-','LineWidth', 2);

% plot peaks seperately &
%  find area undeneath of each one &
%   calculate the concentration
fit_par_peak4 = find_fit_par(1:4);
fit_par_peak5 = find_fit_par(5:8);
fit_par_peak6 = find_fit_par(9:12);
final_peak4 = asym_gauss(fit_par_peak4,peak456(:,1));
final_peak5 = asym_gauss(fit_par_peak5,peak456(:,1));
final_peak6 = asym_gauss(fit_par_peak6,peak456(:,1));
plot( peak456(:,1), final_peak4,'b-','LineWidth', 0.5);
plot( peak456(:,1), final_peak5,'b-','LineWidth', 0.5);
plot( peak456(:,1), final_peak6,'b-','LineWidth', 0.5);
area_peak4 = trapz(peak456(:,1),final_peak4);
area_peak5 = trapz(peak456(:,1),final_peak5);
area_peak6 = trapz(peak456(:,1),final_peak6);

%---------------------------------------%
% First region: region_4               %
% including one peaks (7)          %
%---------------------------------------%

fit_gauss_peak7 = fit(peak7(:,1),peak7(:,2),'gauss1');     % one Gaussian function fit
fig_peak7 = figure('Position', [100 100 500 500]);         % figure position & size
plot(fit_gauss_peak7, peak7(:,1) , peak7(:,2));              % plot Gaussian fit
hold on;
par_guess = coeffvalues(fit_gauss_peak7);                % guess initial point from gaussian
fit
a1=par_guess(1,1);b1=par_guess(1,2);c1=par_guess(1,3);
```

```
d1 = 1.4*c1; s=0;
fit_init_par_peak7 = [a1 b1 c1 d1 s];                    % assymetric Gasussian parameters
% find parameter for the best fit and plot the result
options= statset('MaxIter',1000);
find_fit_par = nlinfit(peak7(:,1),peak7(:,2),
@fit_fun_1peak, fit_init_par_peak7, options);
plot( peak7(:,1), fit_fun_1peak(find_fit_par,peak7(:,1)),'g-','LineWidth', 2);

% plot peaks seperately &
%  find area undeneath of each one &
%   calculate the concentration
fit_par_peak7 = find_fit_par(1:4);
final_peak7 = asym_gauss(fit_par_peak7,peak7(:,1));
plot( peak7(:,1), final_peak7,'b-','LineWidth', 0.5);
area_peak7 = trapz(peak7(:,1),final_peak7);

%======================%
% sort area based on                %
% their retetion time               %
% & calculate the                     %
%   concentration                     %
%======================%

% list of peaks area and their retion time
area_val = [area_peak1 area_peak2 area_peak3 area_peak4 area_peak5 area_peak6
area_peak7]
retention_t      =      [fit_par_peak1(1,2)      fit_par_peak2(1,2)      fit_par_peak3(1,2)
fit_par_peak4(1,2) fit_par_peak5(1,2) fit_par_peak6(1,2) fit_par_peak7(1,2)]

% sort peaks area based on their retion time
for i = 1:6
   for j = 1:6
      if retention_t(1,j) > retention_t(1,j+1)
      temp_t = retention_t(1,j);
      temp_a = area_val(1,j);
      retention_t(1,j) = retention_t(1,j+1);
      area_val(1,j) = area_val(1,j+1);
      retention_t(1,j) = temp_t;
      area_val(1,j) = temp_a ;
      end
   end
end

%calculate concetration
concentration = zeros(1,7);
for i = 1:7
```

```
    concentration(1,i) = (area_val(1,i) - area_ini(1,i) )/area_fac(1,i);
end

%print results
area_val
retention_t
concentration

%======================%
% functions                    %
%======================%

% asymetric Gaussian function
function f = asym_gauss(init_par,x)
    a0 = init_par(1); %height of peak
    b0 = init_par(2); %centre of peak
    c0 = init_par(3); %width of peak
    d0 = init_par(4); %damping term
    f =   a0/2/d0 * exp(c0^2/2/d0^2 + (b0-x)/d0).*(erf((x-b0)/(sqrt(2)*c0) - c0/sqrt(2)/d0)
+1)
end

% fit function for single peak
function f = fit_fun_1peak(init_par,x)
    a1 = init_par(1);
    b1 = init_par(2);
    c1 = init_par(3);
    d1 = init_par(4);
    s = init_par(5);
    f1 = asym_gauss([a1 b1 c1 d1],x)
    f = s + f1;
end

% fit function for double peaks
function f = fit_fun_2peaks(init_par,x)
    a1 = init_par(1);
    b1 = init_par(2);
    c1 = init_par(3);
    d1 = init_par(4);
    a2 = init_par(5);
    b2 = init_par(6);
    c2 = init_par(7);
    d2 = init_par(8);
    s =  init_par(9);
    f1 = asym_gauss([a1 b1 c1 d1],x)
    f2 = asym_gauss([a2 b2 c2 d2],x);
```

```
    f = s + f1 + f2;
end

% fit function for triple peaks
function f = fit_fun_3peaks(init_par,x)
    a1 = init_par(1);
    b1 = init_par(2);
    c1 = init_par(3);
    d1 = init_par(4);
    a2 = init_par(5);
    b2 = init_par(6);
    c2 = init_par(7);
    d2 = init_par(8);
    a3 = init_par(9);
    b3 = init_par(10);
    c3 = init_par(11);
    d3 = init_par(12);
    s = init_par(13);
    f1 = asym_gauss([a1 b1 c1 d1],x);
    f2 = asym_gauss([a2 b2 c2 d2],x);
    f3 = asym_gauss([a3 b3 c3 d3],x);
    f = s + f1 + f2 + f3;
end
```