



UNIVERSITI PUTRA MALAYSIA

***DEVELOPMENT OF AN INTEGRATED FERMENTATION
PERVAPORATION MODEL FOR BIOETHANOL PRODUCTION***

ZENTOU HAMID

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**DEVELOPMENT OF AN INTEGRATED FERMENTATION
PERVAPORATION MODEL FOR BIOETHANOL PRODUCTION**

By

ZENTOU HAMID

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfilment of the Requirements for the Degree of
Doctor of Philosophy**

January 2021

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

**DEVELOPMENT OF AN INTEGRATED FERMENTATION
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January 2021

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The continuous fermentation process where ethanol is selectively removed from the broth is an efficient technique for optimising the bioethanol productivity and limiting the inhibitory effect of both end product and substrate. However, the application of this approach may increase the concentration of minor secondary products to the point where they become toxic to the yeast. Despite that several studies have reported the significant inhibitory effect of byproducts, there is currently no fermentation model that considers the inhibitory effect of these byproducts.

In this study, an integrated model of a fermentation-pervaporation system was developed considering the effect of the interaction between both processes with special attention has been devoted to the inhibitory effect of byproducts. Firstly, a modified Monod model for the alcoholic fermentation process was developed. Then, the optimization and modelling of the pervaporation process for ethanol recovery were conducted. Finally, the integrated model of alcoholic fermentation coupled with a pervaporation system for ethanol recovery was developed and validated.

The findings showed that glycerol, acetic acid and succinic acid were the main byproducts during the fermentation process. It was also noted that the concentration of these byproducts linearly increased with the increase of glucose concentration in the range of 25-250 g/L. A modified Monod model concerning the inhibitory effect of these byproducts was suggested where the specific growth coefficient exponentially decreased with the increase of byproducts concentration in the fermentation broth. The suggested model showed a good agreement with the experimental data and higher accuracy compared to the conventional Monod model.

In optimization of the pervaporation process, the ethanol feed concentration and the permeate pressure positively affected the selectivity, while the feed temperature and the feed flow rate showed a negative effect. The results also revealed that all the four studied factors had a positive effect on the total flux in the selected range. In addition, A solution-diffusion model has been developed and validated using the fermentation broth as a feed solution where it showed high accuracy with R^2 higher than 0.96 for predicting the permeate total flux.

A full model was developed by the integration of the modified Monod model and the solution-diffusion model of the pervaporation process considering the interactions between both processes. The suggested model could accurately predict the biomass concentration, glucose concentration, and ethanol concentration in the fermentation broth simultaneously with predicting the total permeate flux, ethanol flux, and water flux in the collected permeate during a long-term continuous fermentation.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PEMODELAN SISTEM PENAPAIAN ALKOHOL BERSEPADU DENGAN PEMISAHAN PENYEJATAN UNTUK MENAPIS BIOETANOL

Oleh

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Penapaian secara berterusan di mana etanol dikeluarkan secara selektif dari kaldu adalah ideal untuk mengoptimalkan produktiviti bioetanol dan menghadkan kesan perencatan daripada produk akhir dan juga daripada substrat. Namun, proses ini boleh menyebabkan produk sampingan sekunder bertambah sehingga menjadi toksik kepada ragi. Namun begitu, walaupun kesan perencatan dari produk sampingan telah dilaporkan dalam beberapa kajian yang lepas, tiada lagi model penapaian yang melibatkan kesan perencatan daripada produk sampingan.

Dalam kajian ini, model sistem penapaian-penyejatan yang lengkap telah dihasilkan dengan mengambilkira interaksi antara proses penapaian dan penyejatan dengan perhatian istimewa telah diberikan kepada kesan perencatan daripada produk sampingan. Pertama, model baharu Monod yang telah diubahsuai untuk proses penapaian alkohol telah dihasilkan. Kemudian, pengoptimuman dan pemodelan proses penyejatan untuk menapis etanol telah dilakukan. Akhirnya, model lengkap penapaian alkohol yang digabungkan dengan sistem penyejatan untuk menapis ethanol telah dihasilkan dan disahkan.

Hasil kajian telah menunjukkan bahawa gliserol, asid asetik dan asid sukina adalah produk sampingan utama semasa proses penapaian. Terdapat juga pemerhatian bahawa kepekatan produk sampingan ini turut meningkat secara linear mengikut peningkatan kepekatan glukosa dalam julat 25-250 g/L. Model baharu Monod yang telah diubahsuai dengan mengambilkira kesan perencatan produk sampingan adalah disarankan, di mana pekali pertumbuhan spesifik menurun secara eksponen dengan peningkatan kepekatan produk sampingan dalam penapaian kaldu. Model baharu ini telah menunjukkan persamaan yang baik dengan data eksperimen dan ketepatan yang lebih tinggi berbanding dengan model Monod konvensional.

Kajian pengoptimuman mengenai kesan keadaan operasi dalam proses penyejatan menunjukkan bahawa kepekatan suapan ethanol dan tekanan telapan mempunyai kesan positif yang ketara pada pemilihan. Sebaliknya, kesan negatif suhu suapan dan kadar aliran suapan pada pemilihan telah direkodkan. Selain itu, keempat-empat faktor telah menunjukkan kesan positif pada jumlah fluks dalam julat yang dipilih. Di samping itu, model larutan-resapan telah dihasilkan dan disahkan menggunakan penapaian kaldu dan menunjukkan ketepatan tinggi dengan R^2 lebih tinggi daripada 0.96 untuk meramalkan jumlah fluks telapan.

Model lengkap telah dihasilkan dengan persepaduan model Monod yang telah diubah suai dan model larutan-resapan untuk proses penyejatan dengan mempertimbangkan interaksi antara kedua-dua proses tersebut. Model yang dicadangkan mempunyai kemampuan untuk meramal kepekatan biomas, kepekatan glukosa, dan kepekatan ethanol dalam penapaian kaldu secara serentak dengan meramalkan jumlah fluks telapan, fluks ethanol, dan fluks air dalam telapan yang terkumpul semasa penapaian secara berterusan dalam jangka panjang.

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LIST OF ABBREVIATIONS

AC	Activated Carbon
ADP	Adenosine Tri-Phosphate
ANOVA	Analysis of Variance
ATP	Adenosine Tri-Phosphate
CCD	Central Composite Design
CV	Coefficient of Variation
CoA	Coenzyme A
CS	Chitosan
DF	Degree of Freedom
GSEF	Gas Stripping Ethanol Fermentation
HEC	Hydroxy Ethyl Cellulose
HPLC	High Performance Liquid Chromatography
MAPE	Mean Absolute Percentage Error
MAVS	Membrane Assisted Vapor Stripping
MBRs	Membrane Bio-Reactors
MMMs	Mixed Matrix Membranes
NADH	Nicotinamide Adenine Dinucleotide + Hydron
NAD	Nicotinamide Adenine Dinucleotide
NREL	National Renewable Energy Laboratory
NRTL	Non-Random-Two-Liquid
OD	Optical Density
PAA	Poly Acrylic Acid
PAN	Poly Acrylo-Nitrile

PDMS	Poly Di-Methyl Siloxane
PI	Poly Imide
POMS	Poly Octyl Methyl Siloxane
PRESS	Predicted Error Sum of Squares
PTFE	Poly Tetra Fluoro Ethylene
PTMSP	Poly [1-(Tri-Methyl-Silyl)-1-Propyne
PV	Pervaporation
PVA	Poly (Vinyl Alcohol)
PVDF	Poly (Vinylidene Fluoride)
PVI	Poly (2-Vinyl-Imidazoline)
RSM	Response Surface Methodology
SDS	Sodium Dodecyl Sulfate
SSF	Simultaneous Saccharification and Fermentation
UNIQUAC	Universal Quasichemical
USA	United States American
VHG	Very High Gravity
YEP	Yeast Extract Peptone
YNP	Yeast Nitrogen Base
YPD	Yeast Peptone Dextrose
3D	Three Dimensions

CHAPTER 1

INTRODUCTION

1.1 Background

In the past decades, the industrial revolution has remarkably increased the demand for energy that is derived from conventional fossil fuel resources such as coal, oil and natural gas. Growing concerns over the consequences of climate change may severely limit future access to fossil fuels. A forced choice between energy and environment could precipitate a major economic crisis, an environmental crisis, or both. Averting such a crisis will be difficult because fossil energy resources are an essential part of the world's energy supply and climate change is mainly driven by the build-up of carbon dioxide in the atmosphere (Hester et al., 2010). Nevertheless, fossil fuels will be substituted partially by new energy sources that can fulfil the energy needs of humanity and overcome the environmental challenges resulting from the extensive use of fossil fuels. In this regard, biofuels provide an excellent alternative to traditional fossil fuel-derived energy sources, as they can be produced from abundant supplies of renewable biomass (Davis et al., 2000).

Biofuels are produced directly or indirectly from natural feedstocks, which include vegetables, raw materials, and animal waste. There are two main industrial sectors in biofuel production, namely bioethanol and biodiesel (Correa et al., 2017). Bioethanol can be produced by the fermentation of sugars, whereas biodiesel is derived from vegetable or animal fat through the process of transesterification. Brazil and the USA are the two largest biofuel producers in the world (Alonso-Pippo et al., 2013).

The biofuel industry is facing several challenges to substitute totally and limit the use of fossil fuels and meet the market demand. The availability of an efficient separation and purification technique is one of these major challenges, as it typically represents at least 40% (up to 80%) of bioethanol production cost (Le et al., 2011). Moreover, the product (ethanol) inhibition is another limiting factor for the efficient production of ethanol (Garhyan et al., 2004). It was previously reported that yeast cells do not grow in ethanol concentration above 11 wt.% where the ethanol-producing capability of the cells is totally blocked at a concentration of 10 wt.% (Luong, 1985).

Fermentation and purification are the major steps during bioethanol production. Ethanol and total water are the major components of the broth after fermentation. Separation is necessary to purify the ethanol from the fermentation broth (Tian et al., 2013). Distillation has been used as the main purification method for ethanol recovery for many years (Lei et al., 2003). Distillation has a lot of advantages that place it as the preferred choice for industrial application due to: high alcohol recovery (99+ %), sufficient energy efficiency at moderate feed concentrations, and easy

simulation process with different available software programs. On the other hand, distillation has some negative aspects such as the high energy consumption and costs, the high operating temperatures which cause the deactivation of proteins and enzymes, and the need for additional separation to reach product dryness specifications (Vane, 2008).

Therefore, developing new separation techniques become a hot issue to improve biofuels production efficiency, and reduce energy consumption (Nigiz et al., 2013). Bioethanol recovery techniques from fermentation broth were classified by Serra et al. (Serra et al., 1987) into conventional or modified conventional systems (distillation system) and nonconventional systems (non-distillation systems).

The non-conventional systems are recently proposed as alternatives for ethanol recovery with energy saving and low investments such as pervaporation, vacuum stripping, gas stripping, solvent extraction, adsorption and various hybrid processes were mostly developed during the 70's when there was the interest to produce chemicals using less fossil fuel (Offeman et al., 2005).

In the last decade, the application of membrane technology for biological separation processes have flourished throughout the world because it overcomes several constraints associated with conventional techniques. Membrane systems have several advantages over conventional separation processes such as distillation, adsorption, and extraction (Schmidt et al., 1997). Pervaporation technology is currently developed to be integrated with the fermentation process for bioethanol recovery during the continuous alcoholic fermentation process. Fermentation systems operated in continuous mode offer several advantages compared to batch processes, generally resulting in enhanced volumetric productivity and, consequently, smaller bioreactor volumes and lower investment and operational costs (Ivanova et al., 2011). In this context, several studies have been conducted to develop new designs of bioreactors and new membrane modules to optimize bioethanol production; parallelly, researchers turned their focus towards using computing methods to optimize the bioethanol production process. Mathematical model-based simulations of actual bioreactor runs suggest how process variables such as substrate and product concentrations change and how nutrient feeding should be "tuned" with respect to time, pattern, concentration, and composition to elicit the desired response. Insights gained from modelling can guide us in the adjustment of a process, reducing the number of characterization rounds required. Furthermore, comparing actual experimental results with model predictions helps improve the models themselves.

Many aspects complicate the modelling of the bioprocess since the fermentation process has both non-linear and dynamic properties, and the metabolic processes of the microorganisms are very complicated and cannot be modelled precisely. The most important properties of a biological mathematical model were defined in the Edwards and Wilke' that postulates: (a) it is capable to represent all the culture phases; (b) it is flexible enough to approximate different data types without the

insertion of significant distortions; (c) it must be continuously derivable; (d) it must be easy to operate, once the parameters evaluated; (e) each model parameter is to have a physic significance and must be easy to evaluate (Bellgardt, 2000).

Although the continuous operation is known to be advantageous over the batch process in term of reducing operational costs, it has not yet enjoyed the same measure of acceptance in the industry as that of the batch operation. Some of the major hindrances to the industrial applications of continuous fermentation are susceptibility to contaminations and complex operational problems like a nonlinear process that is difficult to control (O'Brien et al., 2000). Kinetic modelling may be regarded as an important tool in developing an efficient ethanol fermentation process, since models help in process control, reducing process costs, and optimization of the performance of biotechnological processes.

1.2 Problem Statement

Besides the complexity of fermentation modelling itself, coupling the alcoholic fermentation with a separation system such as pervaporation separation for ethanol recovery implies another challenge to developing an 'integrated model' of the fermentation-pervaporation system. The current models do not ideally represent the integrated system since it is standard models describing the fermentation process and pervaporation process as separate units. Thus, modified models should be developed specifically for the modelling of a fermentation-pervaporation integrated system by taking into account the effects of the interaction between both processes.

In this regard, several issues have been highlighted in the present study to be addressed. First of all, it is known that ethanol is selectively removed from the fermentation broth during continuous fermentation using a separation process such as pervaporation which eliminates the ethanol inhibitory effect. On the other hand, this process can concentrate minor secondary products to the point where they become toxic to the yeast. The inhibitory effect of byproducts on the fermentation process has been confirmed in previous studies (Maiorella et al., 1983). However, most available fermentation models highlighted the inhibitory effect of ethanol (Brown et al., 1981; Ghose et al., 1979; Luong, 1985; Palmqvist et al., 2000; Q. Zhang et al., 2015), the inhibitory effect of substrate (Ghose et al., 1979; Mota et al., 1984; Starzak et al., 1994; Q. Zhang et al., 2015), and inhibition effect of cell density on the growth called " self-inhibition "(Contois, 1959; Mazzoleni et al., 2015), whereas of inhibition effect of byproducts did not receive the same attention. Thus, a vital part of the present study was devoted to developing a new modified Monod model which takes into account the inhibitory effect of byproducts.

Indeed, defining the optimal operating conditions of a process plays an important role to enhance its performance and productivity. While the optimal conditions of the fermentation process using *S. cerevisiae* yeast have been well-known for several decades ago, there no agreement about the optimal conditions of the pervaporation process due to the differences in the properties of the used membrane, the selected

range of factors, and the characteristics of the components of the experimental setup. Therefore, using Response Surface Methodology (RSM) approach for the optimization of ethanol pervaporation process was used due to the reliability RSM approach compared to the one-factor-at-a-time approach which does not consider the interactions between variables during the optimization process.

In addition, the majority of studies investigating the modelling of pervaporation for ethanol recovery have used the ethanol/water mixture as a feed whereas the fermentation broth normally contains other metabolites which may influence the pervaporation separation performance (Hietaharju et al., 2019; Kanse et al., 2017; Qiu et al., 2019). Using ethanol/water standard solution is understood as it allows better control of the operating conditions, however, the fermentation broth should be used during the validation of the model for more reliability.

The integration biological fermentation model and pervaporation separation model is a very challenging step. The notion of 'integrated model' provides a platform to infuse the researches from two different fields biological process modelling and separation modelling for bioethanol production and recovery in a harmonized way and hence provokes interconnected investigations from both fields. The previous studies have focused more on the interaction between both process in term of productivity of performance, for example, the effect of product removal by a pervaporation on ethanol fermentation (Miyazawa et al., 1998), or the effect of fermentation broth components on the pervaporation process (García et al., 2009) whereas a lack in studies discussing an integrated model for the integrated fermentation-pervaporation system was noted. The few reported modelling studies have considered the conventional modelling approach of continuous fermentation at a fixed dilution rate ignoring the fact that output flux is variable due to the change of the ethanol concentration in fermentation broth and its effect on the pervaporation process performance. These issues and points will be fully covered in the present study.

1.3 Objectives

The major objective of this study is to develop an integrated model of a fermentation-pervaporation system. This will include the following secondary objectives and tasks:

1. To develop a modified Monod model for bioethanol fermentation process with the incorporation of byproducts inhibitory effects.
2. To model pervaporation process based on solution- diffusion mechanism.
3. To develop an integrated model based on a continuous combined fermentation-pervaporation system for bioethanol production.

1.4 Scope of Study

The presented work is focused on the modelling of a fermentation-pervaporation system by taking the effect of the interactions on both fermentation and pervaporation processes. Firstly, a preliminary investigation was conducted to evaluate the amount of byproducts formation during the alcoholic fermentation and study the effect of initial substrate concentration on the formation of these byproducts. Based on this investigation, an experimental design was set to determine the effect of these byproducts during the alcoholic fermentation to end up with a new Modified Monod model for the alcoholic fermentation with taking the byproducts inhibitory effect into account.

Secondly, RSM approach was used to optimize the pervaporation process and study the effect of temperature, feed concentration, flow rate and vacuum pressure which may be dependent on each other and it was needed to consider their interactions in this study. Besides the RSM model, a solution-diffusion model will be developed to predict the total flux and separation factor during the pervaporation process, and the developed model will be validated against experimental data using the fermentation broth for more reliability and to assess the ability to integrate the model with the fermentation process.

Finally, an integrated mathematical model to describe the fermentation-pervaporation system was built based on the developed models. The obtained model was validated against experimental data and then compared to previous models reported in the literature to assess its performance.

In the present study, the proposed models were developed assuming that the fouling of the membrane is negligible during the pervaporation process. Although that this assumption is valid for the present study due to the use of glucose solution as a feed of the fermentation process, the fouling problem was reported as the most factor reducing the permeation flux during the pervaporation process (Kamelian et al., 2020; Sun et al., 2020; Zhao et al., 2021; Zhu et al., 2020). Therefore, the application of the suggested integrated model in this study is only limited in the cases where the fouling factors are not significant. Thus, it is suggested to take the fouling phenomena into account in the modelling of the integrated fermentation-pervaporation system especially in the presence of foulants particles in the feed. Moreover, the solution-diffusion model in the present work does not consider the variation of feed concentration along with the module. Therefore, this model is suitable only for flat sheets or small membrane modules as the feed concentration varies along the x-direction for the large modules.

1.5 Outline of Thesis

The presented thesis is divided into five chapters. Chapter one covers the introduction, problem statement, objectives, scope and thesis structure. Chapter two presents the theory and literature review closely related to this work including principles, mechanism, and modelling of both fermentation and pervaporation process and the integration of these process for bioethanol production. Chapter three includes the different materials and methods used in the presented study and describe the experimental design of different stages of the project. Chapter four presented the obtained results and discussed the findings. Conclusions have summarized in chapter five which involves some recommendations for future works as well.



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This work has been conducted by Zentou Hamid. He is an Algerian student who was born on 4th March 1988 in Khemis Miliana, Algeria. In 2010, he received his bachelor degree in Industrial Chemistry at University of Khemis Miliana. He completed his postgraduate studies at the same university to receive a master degree in Processes Engineering in 2013. In the period between 2010 and 2014, Zentou has been working as a chemical engineer in Rassila Sucre Sarl in a sugar factory. He moved to Malaysia to complete his doctoral studies in Chemical Engineering where he enrolled at Universiti Putra Malaysia in February 2015 under the supervision of Prof. Zurina Zainal Abidin. His research focused on the biofuels field highlighting the optimization of the production process, separation techniques, modelling and simulation.

LIST OF PUBLICATIONS

- Zentou, H., Abidin, Z. Z., Yunus, R., Biak, D. R. A., & Issa, M. A. A New Model of Alcoholic Fermentation Under Byproducts Inhibitory Effect. *Omega ACS*.2021(<https://pubs.acs.org/doi/abs/10.1021/acsomega.0c04025>)
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