

Fermentation coupled with pervaporation: a kinetic study

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Focus area: Energy Systems, Associate Chair: Bio fuels and Other Clean alternative fuels

Abstract

In recent years the increased demand for energy as well as the depletion of fossil fuels and environmental concerns opened up the search for alternative fuel sources. The production of bioethanol through fermentation is therefore becoming increasingly important due to its application as a renewable liquid fuel. Pervaporation is a membrane separation method that can be combined with fermentation to remove ethanol from the fermentation broth continuously. Combining fermentation with pervaporation can reduce product inhibition by keeping the ethanol concentration in the broth low and simplify downstream processing as the ethanol recovered will be more concentrated. In this study, a mathematical model for ethanol fermentation with *Saccharomyces cerevisiae* coupled with pervaporation was developed. This model incorporates Monod-type kinetics to represent bioethanol fermentation combined with a solution-diffusion model that represents the ethanol flux out of the system. Kinetic parameters of biomass growth, substrate utilization and product formation were estimated using non-linear least squares regression with experimental data obtained in batch fermentation. Kinetic constants of separation of ethanol by pervaporation were estimated using partial fluxes obtained through experimental separation of ethanol and water mixtures.

Keywords: Mathematical modelling; Ethanol; Fermentation; Pervaporation; *Saccharomyces cerevisiae*

1. Introduction

A steady, reliable supply of energy is required for all aspects of development, prosperity, and economic growth in modern society. Fossil fuels are currently under scrutiny because of serious disadvantages regarding the limited supply of fossil fuel resources and the emission of carbon dioxide (and other pollutants) when these fossil fuels are burned (Dresselhaus & Thomas, 2001:333). The search for alternative energy sources has been opened up by an increasing demand for energy worldwide as well as the depletion of fossil fuel sources and environmental concerns (Dresselhaus & Thomas, 2001:332; Sánchez & Cardona, 2008). Alternative energy resources refer to those energy resources that are renewable and therefore sustainable such as biomass, hydro, geothermal, solar, wind, ocean, wave action, and tidal action. The potential of energy from biomass is very promising as it is available worldwide and it has the unique advantage that it offers a solid, liquid, or gaseous fuel that can be stored, transported, and utilized far away from the point of origin (Demirbas, 2008:2107). The solid, liquid, or gaseous fuels obtained from biomass are referred to as biofuels.

Biomass is an alternative energy source from which biofuels such as biogas, biodiesel, and bioethanol can be produced. More environmentally orientated countries are considering biomass fuels to replace fossil fuels as it is generally believed that less carbon dioxide and pollution is emitted when these fuels are burned, compared to fossil fuels (McGowan, 2009:7). When considering the low sulphur content and no net release of carbon dioxide biofuels, such as bioethanol or biodiesel, has a smaller environmental impact compared to fossil fuels (Demirbas, 2008:2107). Liquid biofuels are increasingly considered in Europe as an attractive alternative to fossil fuels to enhance energy security, reduce emissions by transportation, and to contribute to regional development by increasing employment opportunities (Bomb *et al.*, 2007:2256). The Biofuels Industrial Strategy of South Africa propose a 2 percent biofuel use in the transportation sector of South Africa by 2013, amounting to about 400 million litres of biofuel that must be

produced per year (SA, 2007:3). This target will create jobs, thereby reducing unemployment and boosting economic growth (SA, 2007:9).

Ethanol production through biomass fermentation is the major technology available to produce liquid fuel from renewable energy sources (Huber and Dumesic, 2006:122). Bioethanol is produced through fermentation using any sugar or starch rich feedstock and more recently, the use of lignocellulosic feedstocks for bioethanol production has also come under investigation (Bomb *et al.*, 2007:2257). The wide variety of feedstocks that can be used for bioethanol is part of its appeal as an alternative fuel.

The yeast, *Saccharomyces cerevisiae*, is the microorganism usually used for fermentation (Bai *et al.*, 2008:90). *Saccharomyces cerevisiae* is inhibited by high ethanol concentrations, which is a major problem associated with the fermentation process. Inhibition affects the overall productivity of the yeast cells and the ethanol yield of the fermentation process. Currently, product inhibition is overcome by diluting the starting sugar solutions and by the addition of water during fermentation to dilute the ethanol concentration in the fermentation broth. The large amount of water carried through the process amounts to higher equipment cost (due to larger equipment required) and higher separation costs later on in the process to remove the water, as the acceptable water content in bioethanol used as transportation fuel is very low. The amount of water required for dilution is also a concern in water scarce countries such as South Africa. If the ethanol is removed as soon as it is formed, the inhibition effect can be overcome, as the ethanol concentration will be constantly kept low with no additional dilution required.

Currently pervaporation is a method that can be effectively combined with fermentation to remove ethanol from the fermentation broth continuously. Pervaporation is a membrane process in which a phase change takes place over the membrane. The liquid mixture comes into contact with one side of the membrane and the permeated product (known as the permeate) is removed as a low pressure vapour on the other side. The driving force for mass transport over the membrane is the chemical potential gradient created by applying a vacuum pump on the permeate side to lower the partial pressure of the feed liquid and thus lowering the chemical potential of the permeate stream on the downstream side (Feng & Huang, 1998:1048). Pervaporation is an attractive separation method as it is operated at low feed pressures and temperatures and no additional chemicals are necessary for separation. There is also no significant economy of scale meaning that pervaporation can be used in small and large processing plants (Feng & Huang, 1998:1049). Pervaporation combined with fermentation can keep the ethanol concentration in the fermentation broth low enough so that product inhibition will not take place resulting in higher productivity. The bioethanol achieved through this combined process will contain less water, reducing separation costs to achieve the high grade of bioethanol required for fuel grade ethanol.

Most of the research in the field of fermentation coupled with pervaporation, focus on the different membranes that can be used, the effect that components in fermentation broth have on membranes and the effect that pervaporation has on fermentation. There is however a definite lack of research on kinetics of the membrane-reactor system where fermentation and pervaporation is combined. By investigating the kinetics of fermentation and the mass transfer of ethanol over a membrane using pervaporation, a mathematical model describing the process of fermentation combined with pervaporation can be constructed. This model can be used to describe and better understand the process and to predict performance under different process conditions as well as for process design, process optimization, and process control, and in doing so reduce research and development time and cost (Dunn *et al.*, 1992:10).

1.1. Objectives

Bioethanol is already produced commercially but to combine this process with pervaporation, membrane-reactor kinetics is required. Therefore, the main objective of this study is to develop a model that represents the membrane-reactor kinetics when fermentation is coupled with pervaporation.

The following sub objectives are necessary to achieve the main objective mentioned above.

- Investigate traditional batch fermentation
 - Evaluate the influence of different conditions, such as sugar concentration and yeast concentration on the fermentation performance
 - Investigate fermentation kinetic models in literature and develop a simple model to describe traditional batch fermentation
- Investigate separation of ethanol and water by pervaporation
 - Examine the influence of different feed compositions on the separation performance of pervaporation
 - Explore pervaporation separation models in literature and develop a simple model to describe the separation process

2. Mathematic modelling

2.1. Fermentation kinetics

Reaction kinetics deals with how fast a reaction proceeds (also known as the reaction rate) and the effects of different process conditions (such as pressure, temperature, composition, and catalysts) on the reaction rate. Usually models proposed to describe fermentation kinetics use a system of nonlinear differential equations to describe cell growth, product formation, and substrate uptake respectively, as shown by Eqs. (1)-(3). Two main products formed during fermentation namely ethanol and glycerol and this model has been adapted to incorporate both products.

$$\frac{dX}{dt} = \mu X \quad (1)$$

$$\frac{dP_i}{dt} = v_i X \quad (2)$$

$$\frac{dS}{dt} = - \left(\frac{1}{\sum Y_{P_i/S}} \frac{d\sum P_i}{dt} \right) \quad (3)$$

The most well known unstructured nonsegregated kinetic model is the Monod model for cell growth, which was introduced to the world in 1949 by a Frenchman named Jacques Monod (Monod, 1949). The Monod model is used as a basis for most recently developed models and as a result, Monod-type models dominate the field. The Monod equation, shown in Eq. (4) and Eq. (5), is used to describe the relation between specific growth rate (μ) and the specific product production rate (v) and the substrate concentration respectively.

$$\mu = \mu_{\max} \frac{S}{K_{sx} + S} \quad (4)$$

$$v_i = v_{\max,i} \frac{S}{K_{sp,i} + S} \quad (5)$$

2.2. Model equations for separation by pervaporation

Fick's law, Eq. (6), is used to model the pervaporation flux.

$$J_i = -D_i \frac{dc_i}{dz} \quad (6)$$

This model (solution-diffusion model) was chosen due to its simplicity and popularity, according to Chen *et al.* (2010). The models based on the solution-diffusion model vary from each other in the way that the diffusion coefficient varies with concentration. For this study the Greenlaw's linear dependence of diffusivity on concentration, Eq. (7) was considered.

$$J_i = \frac{D_i^0}{L} \left(\frac{(c_i)^2}{2} + \beta_{ij} c_i (1 - c_i) \right) \quad (7)$$

3. Research methodology

3.1. Batch fermentation

Saccharomyces cerevisiae obtained from Anchor Yeast in South Africa was used to ferment glucose obtained from ACE. Solutions containing from 5 to 35 wt% glucose was fermented with a 10 g/L starting yeast concentration. The optimum glucose concentration was used to determine the effect of starting yeast concentration which was varied from 1 g/L to 10 g/L. Inoculated flasks were incubated without shaking at 30°C for 72 hours with samples taken at regular intervals. The pH was kept constant at 4 by adding H₂SO₄ to decrease or NaOH to increase the pH. The samples were analysed using a HPLC fitted with a Shodex sugar column. Cell growth was determined by measuring the optical density at 600nm using a spectrophotometer.

3.2. Pervaporation of water-ethanol mixtures

The standard pervaporation set-up used in this study is well described in literature (see for example Schmidt *et al.*, 1997; Mohammadi *et al.*, 2005; Weyd *et al.*, 2008 and Wu *et al.*, 2005). The feed composition was varied to contain between 5 and 15wt% ethanol and between 0 and 15wt% glucose. The feed was heated in an 8 litre stainless steel container and kept at constant temperature of 30°C by a temperature control unit. Every 60 to 120 minutes, permeate was collected in a glass cold trap cooled by liquid nitrogen. A commercially available membrane, PERVAP®4060, purchased from Sulzer Chemtech in Germany, with an active membrane area of $2.54 \times 10^{-2} \text{m}^2$ was used. The total amount of permeate collected (m_{tot}) was measured to determine the total membrane flux (J_{tot}) based on Eq. (8). The ethanol content of the sample was determined using a refractometer to calculate the partial flux of each component.

$$J_{\text{tot}} = \frac{m_{\text{tot}}}{At} \quad (8)$$

where A is the total active membrane area (m^2) and t stands for the collection time (h). The partial fluxes are calculated by multiplying the total flux with the mass fractions of the component in the permeate

3.3. Fermentation coupled with pervaporation

The same setup used for the pervaporation experiments were used for the combined experiments. Fermentation was done directly in the feed vessel. After a set amount of time, the pervaporation unit was started to remove ethanol continuously from the fermentation broth.

4. Results

4.1. Batch fermentation

4.1.1. Effect of feed composition

The effect of the varying starting glucose concentration on the final yield (after 72 hours) is shown in Figure 1. The final ethanol yield is high at 5 wt%, 10 wt%, 15 wt%, and 20 wt% glucose with a maximum ethanol yield of $0.431 \pm 0.015 \text{ g.g}^{-1}$ achieved when 15 wt% glucose was used. When the starting glucose concentration is increased above 20 wt% the ethanol yield declines sharply due to substrate inhibition. At high osmotic pressure conditions, such as high substrate concentration, the yeast cells do not grow or reproduce normally (Munene *et al.*, 2002:311) and a decrease in cell growth will be observed resulting in lower product yield (D'Amore *et al.*, 1988:111).

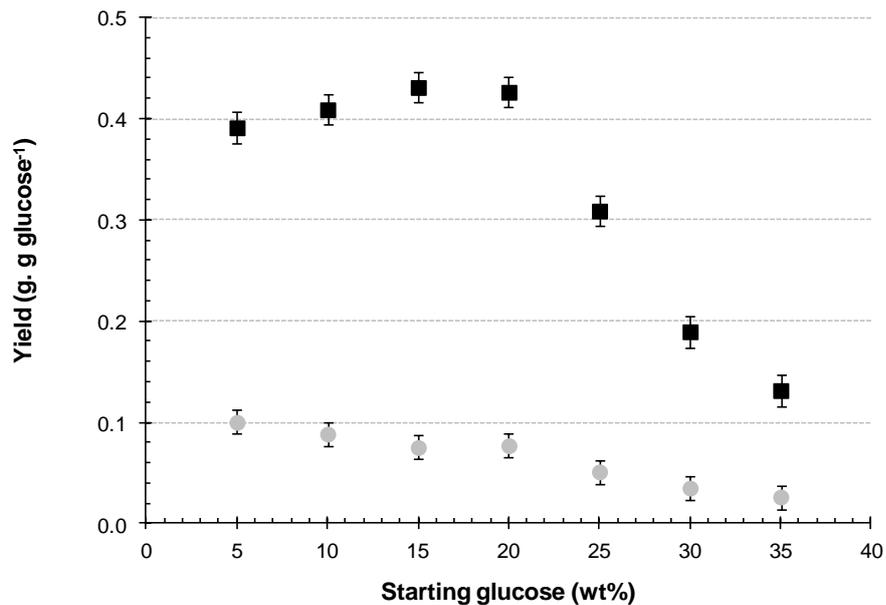


Figure 1. Effect of feed composition on final ethanol and glycerol yield (■ ethanol, ● glycerol)

4.1.2. Effect of yeast concentration

The effect of the starting yeast concentration on the product yield after 72 hours is shown in Figure 2. The starting yeast concentration has a significant effect on the ethanol yield over time and ethanol forms with higher yeast concentrations. This is expected as a higher yeast concentration means that more yeast cells are available to convert glucose to ethanol, thereby increasing the rate of ethanol formation. The highest ethanol yield ($0.432 \pm 0.001 \text{ g.g}^{-1}$) after 72 hours of fermentation was achieved using 10 g.L^{-1} starting yeast concentration.

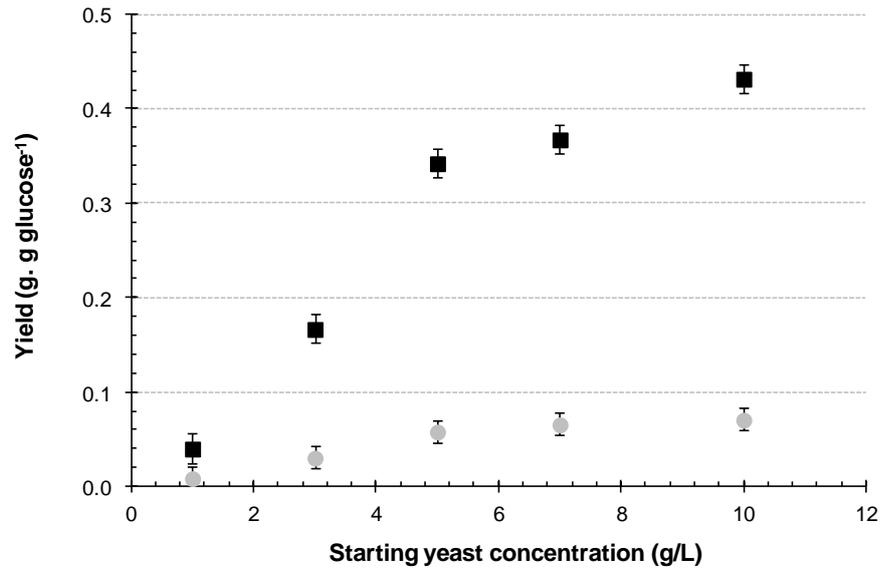


Figure 2. Effect of starting yeast concentration on final ethanol and glycerol yield (■ ethanol, ● glycerol)

4.2. Fermentation modelling

4.2.1. Kinetic parameters of fermentation

The kinetic parameters of the fermentation model (shown in Table 1) were determined through non-linear least squares regression using the Nelder-Mead simplex optimisation method by applying experimental fermentation data to minimize the deviation between the predicted values and the experimental data. The fourth order Runge-Kutta method was combined with the simplex method to solve the set of differential equations simultaneously (Eqs. 1-5). The standard error of the parameters was determined with the Bootstrap method.

The initial yeast concentration had a significant effect on the model parameters μ_{\max} and $v_{\max,i}$. Therefore, it was necessary to account for the effect of initial yeast concentration on these parameters as shown by the empirical equations in Table 1, similar in form to those used by Krishnan *et al.* (1999:377). These empirical equations were determined by finding the u_{\max} and $v_{\max,i}$ parameters at different initial yeast concentrations and determining an equation that incorporates the effect of cell concentration on these parameters.

Table 1. Kinetic parameters of fermentation model

Parameter	Substrate limiting model
u_{\max}	$0.015 \times X_0^{0.551} - 0.012$
$v_{\max, \text{ethanol}}$	$9.17 \times 10^{-7} \times X_0^{5.034} + 0.093$
$v_{\max, \text{glycerol}}$	$8.29 \times 10^{-7} \times X_0^{4.590} + 0.018$
K_{sx}	21.461 ± 0.005
$K_{sp, \text{ethanol}}$	0.145 ± 0.015
$K_{sp, \text{glycerol}}$	1.413 ± 0.006

4.2.2. Evaluation of the fermentation kinetic model

Typical experimental values of batch fermentations of glucose with *Saccharomyces cerevisiae* as well as the values calculated using on Eqs. (1)-(5) with Table 1 are shown in Figure 3.

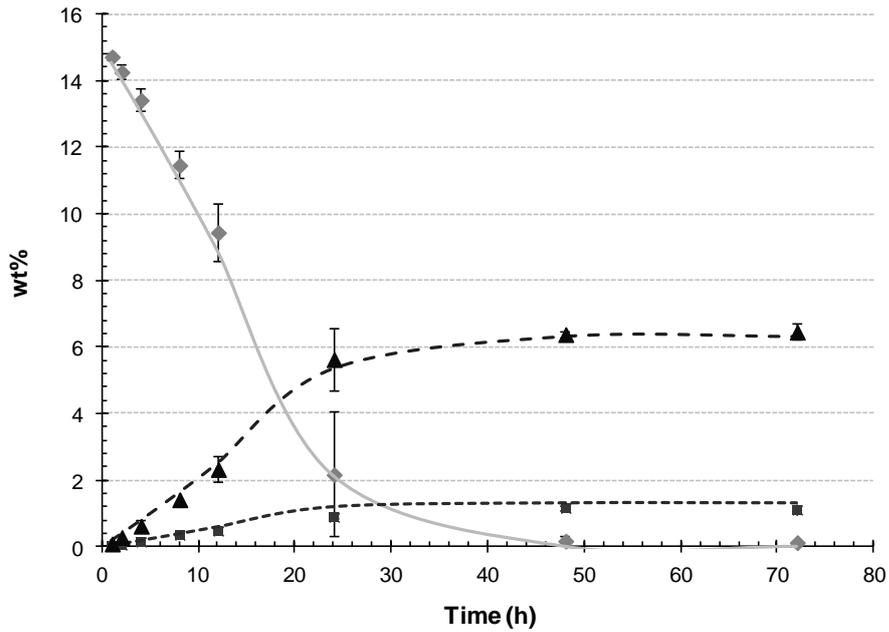


Figure 3. Comparison of experimental fermentation with the values predicted by the model using 15wt% starting glucose and 10g/L starting yeast (◆ Experimental glucose, ▲ Experimental ethanol, ■ Experimental glycerol, — glucose model, --- ethanol model, glycerol model)

The fermentation model showed very accurate results compared to the experimental data when no substrate inhibition took place (glucose < 20 wt%). The accuracy of the model (Table 2) was determined through the R^2 value (van der Grypt, 2003:179), as shown by Eq. 9.

$$R^2 = \frac{\text{Sum of squares differences}}{\text{Sum of squares}} = 1 - \frac{\sum (x - y)^2}{\sum (x^2 - y^2)} \quad (9)$$

with x the experimental value and y the theoretical model value

Table 2. Accuracy of substrate limiting fermentation model

Starting glucose (wt%)	Starting yeast (g/L)	Ethanol R^2	Glycerol R^2	Glucose R^2
5	10	0.994	0.943	0.993
10	10	0.998	0.997	0.999
15	10	0.999	0.986	1.000
20	10	0.995	0.958	0.996
25	10	0.909	0.802	0.967
30	10	0.692	0.613	0.929
35	10	0.533	0.497	0.911
15	1	0.984	0.987	1.000
15	3	0.997	0.998	1.000
15	5	0.996	0.997	1.000
15	7	0.995	0.994	0.999

4.3. Membrane performance of a pure ethanol, water and glucose mixture

The effect of feed composition on the performance of the PERVAP®4060 membrane was investigated by varying the weight percentage ethanol and weight percentage glucose in the feed. The temperature of all the pervaporation experiments was kept constant at 30°C.

The total flux increases with an increase in the mass fraction ethanol in the feed. This occurrence has been reported by other researchers as well such as Zhou *et al.* (2011), Ikegami *et al.* (2002) and Kaewkannetra *et al.* (2011).

There was no glucose ever detected in the permeate and it is considered an impermeable component. In comparison to the pure ethanol and water mixture, the addition of glucose led to a slight decrease in the total flux. The reason for this decrease in total flux was mainly due to a reduction in water flux, which decreased due to addition of glucose. On the other hand, changes in ethanol flux were found to be within the experimental error. This is due to the preferential interaction of the hydroxyl groups in the glucose with water and a lower bonding capacity of water molecules to ethanol thereby decreasing the transport of water through the membrane because of a decrease in water vapour pressure (Chovau *et al.*, 2011:1671).

4.4. Pervaporation modelling

4.4.1. Kinetic parameters of ethanol removal by pervaporation

The parameters of ethanol and water removal (Table 3) through pervaporation were determined using the Nelder-Mead simplex optimisation method and the standard error of the parameters was determined with the Bootstrap method.

Table 3. Parameters for partial flux models

Model	D_i^0 (m ² /s)	B_{ij}
Ethanol	$9.55 \times 10^{-9} \pm 1.87 \times 10^{-10}$	$0.39 \pm 1.18 \times 10^{-5}$
Water without glucose	$6.52 \times 10^{-10} \pm 7.59 \times 10^{-12}$	$0.75 \pm 9.46 \times 10^{-7}$
Water with glucose	$3.79 \times 10^{-10} \pm 5.93 \times 10^{-11}$	0.5 ± 0.025

4.4.2. Evaluation of the pervaporation model

The model presented in Eq. (7) with the parameters presented in Table 3 was used to predict theoretical partial flux values for the pervaporation setup used in this study. The theoretical partial flux values were predicted very accurately (R^2 above 0.998), shown in Figure 4.

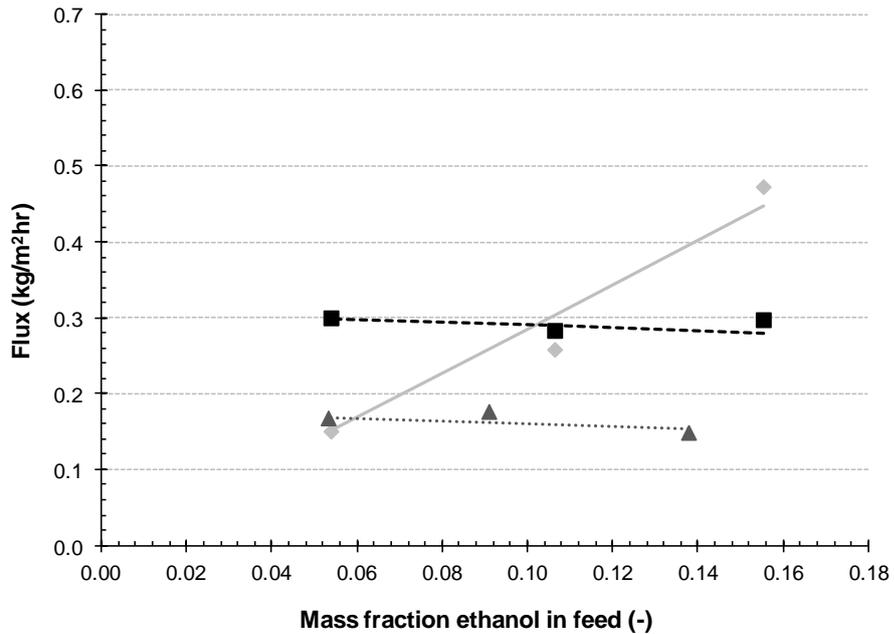


Figure 4. Comparison of the experimental partial flux with the Greenlaw model (♦ experimental ethanol flux, ■ experimental water flux without glucose, ▲ experimental water flux with glucose, — ethanol model, --- water model without glucose, ··· water model with glucose)

4.5. Fermentation coupled with pervaporation modelling

The fermentation model was combined with the pervaporation models to propose a new model to represent the membrane-reactor system when fermentation is coupled with pervaporation. In Figure 5, the model is compared to experimental data; pervaporation was started after 24 hours.

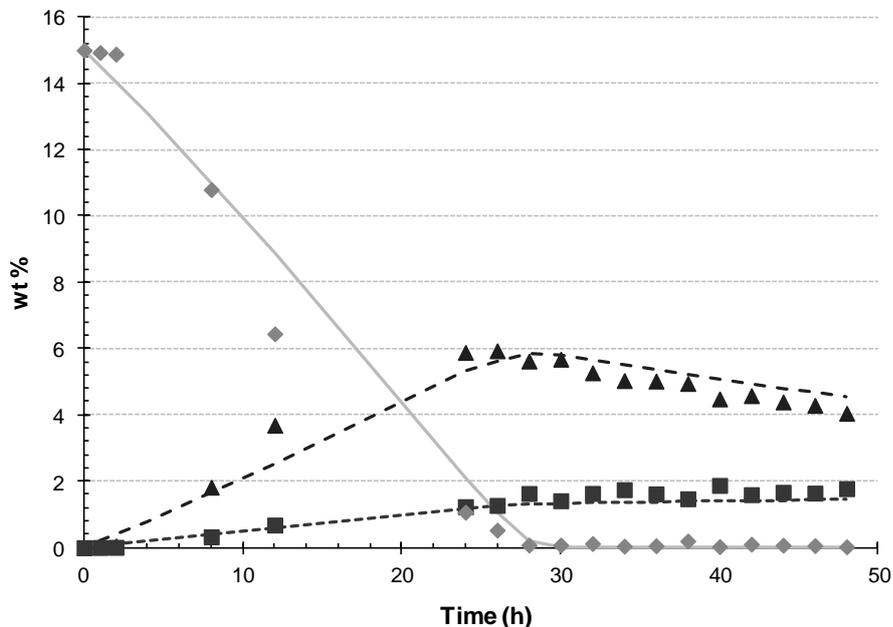


Figure 5. Comparison of the membrane-reactor system model with experimental data (♦ Experimental glucose yield, ▲ Experimental ethanol yield, ■ Experimental glycerol yield, — glucose yield model, --- ethanol yield model, ··· glycerol yield model)

The accuracy of the fit of the membrane-reactor system model, given by the R^2 value, is 0.995 for the ethanol data, 0.984 for the glycerol data and 0.993 for the glucose data.

5. Conclusions and recommendations

The main objective of this study was to investigate the membrane-reactor kinetics when fermentation is coupled with pervaporation. A model that predicted the membrane-reactor kinetics, the membrane-reactor system model, was proposed and it was shown that this model could accurately predict the ethanol, glycerol, and glucose contents of the feed as well as the ethanol and water flux out of the system. This model can now be used to optimise the membrane reactor system by predicting the optimal process conditions including the fermentation time after which pervaporation should be started.

5.1. Fermentation

The effect of different operating conditions on fermentation was investigated in the first part of this study. The starting glucose and starting yeast content of the fermentation broth had a remarkable effect on the final ethanol yield and it was found that the optimum ethanol yield was achieved using 15 wt% glucose and 10 g/L yeast. Glucose concentrations higher than 20 wt% resulted in low yields due to inhibition of the yeast.

5.2. Pervaporation

The effect of the weight percentage ethanol in the feed as well as the influence of glucose on the flux and selectivity of the membrane was investigated. The water flux was not influenced by the ethanol concentration in the feed but an increase in ethanol in the feed resulted in an increase in ethanol flux. Glucose did not have an effect on the ethanol flux but it significantly lowered the water flux.

5.3. Fermentation modelling

A simplified model based on the Monod model was developed by using the fermentation data obtained in the first part of this study. The model only incorporated substrate limitation and showed very accurate results for low (<20 wt%) initial sugar concentrations. At high sugar concentrations, where substrate inhibition had an effect, this model did not accurately predict the fermentation process.

5.4. Pervaporation modelling

A model based on the solution-diffusion model was developed by using the pervaporation data obtained with the PERVAP®4060 membrane. Fick's first law with the Greenlaw model for diffusion coefficients was used to describe the transport of the permeating species through the membrane. Very accurate partial flux predictions were obtained using this model.

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