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# **Extraction of Butanol by Continuous Pervaporation of a Two-Stage Fermentation**

An Undergraduate Honors College Thesis  
in the

Ralph E. Martin Department of Chemical Engineering  
College of Engineering  
University of Arkansas  
Fayetteville, AR

by  
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April 29, 2011

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## INTRODUCTION

Developing alternative fuels is of global importance because of the environmental consequences of fossil fuels, heightened crude-oil prices, and questionable security of the petroleum supply. Interest in liquid biofuel production and use has increased worldwide due to government interest in addressing and minimizing these issues. Currently the biofuel market is dominated by U.S. ethanol production based on cornstarch and Brazilian ethanol production based on sugarcane<sup>1</sup>. Although ethanol has been the primary focus for alternative fuels for the past forty years, butanol is expected to play a major role in the next generation of biofuels. Butanol is less corrosive than ethanol and can be transported through the existing infrastructure, whereas ethanol can only be added to gasoline shortly before use. Butanol is also more energy dense, so fuel consumption is similar to that of pure gasoline, and its usage does not require modifications to car engines<sup>2</sup>. However, butanol production does present challenges.

Butanol can be derived from cellulosic materials, such as grass, leaves, and algae. Such cellulosic materials represent an available and cost-effective sustainable energy source that does not harm the environment or compete with food sources<sup>3</sup>. Although much research has been done on the conversion of lipids from various algae species into biodiesel, the high carbohydrate contents of macroalgae indicate that fermenting the carbohydrates to butanol might be a more cost effective strategy. Butanol is usually produced by the traditional ABE fermentation, where sugars are anaerobically converted by strains of *Clostridium* into acetone, butanol and ethanol<sup>3</sup>. The major problems with butanol fermentation are uneconomical

product recovery, low ABE yield, and product inhibition<sup>4</sup>. Pervaporation is a promising technique that may be preferable to distillation for extraction of butanol from fermentation media because of its lower cost and energy use in small to medium scale production. The lower hydrophilicity of butanol makes it an excellent candidate for this energy efficient separation technique<sup>5</sup>. Although combining continuous pervaporation with a two-stage fermentation process has the potential to overcome the significant problems with butanol fermentation, this technique is presently relatively unexplored.

The key cause of the low butanol concentration in the fermentation broth is associated with the product toxicity/inhibition of the fermenting microorganisms<sup>9</sup>. To improve product specificity and yield it is necessary to ferment and remove the product simultaneously so that a toxic butanol level is never reached<sup>7</sup>. Continuous pervaporation to extract butanol from the reactor is a potential way to overcome this inhibition. Groot et al have shown that the application of continuous pervaporation to a continuous fermentation of glucose to butanol and isopropanol with *Clostridium beijerinckii* cells has the potential to increase the reactor productivity by 65-70%<sup>10</sup>. Geng et al have shown that continuous pervaporation of a continuous fermentation of glucose to butanol with *Clostridium acetobutylicum* cells was able to maintain a butanol concentration below 4.5 g/L<sup>11</sup>.

In 1998, Ramey proposed accomplishing the fermentation in two steps<sup>12</sup>. Through this process the sugars in the fermentation broth are first converted to organic acids such as lactic acid, acetic acid, and butyric acid by a bacterium operating in its acidogenesis phase. The butyric acid from this step is collected and fed to a second fermentation reactor where a

solventogenesis bacterium converts the butyric acid to butanol. There is currently little literature reporting conversion of algal sugars to butanol by the two-step process.

Within this research a fermentation broth is used that has been developed first from the continuous fermentation of algal sugars to organic acids with *Clostridium tyrobutyricum*, followed by the continuous fermentation of the organic acids to butanol using *Clostridium saccharoperbutylacetonium*. The two-stage fermentation has the potential to increase the butanol selectivity from 60% by weight with the traditional ABE process to as high as 100% with the 2-stage process<sup>12</sup>.

## BACKGROUND

Pervaporation is a separation process in which a liquid containing two or more miscible components is fed to one side of a non-porous polymeric membrane or molecularly porous inorganic membrane while a vacuum is applied to the other side. According to the solution-diffusion model, the components in the liquid stream sorb into the membrane, diffuse through the membrane, and evaporate into the vapor phase<sup>6</sup>. The resulting permeate is then condensed. The success of the separation is determined by the selectivity of the membrane and the flux of the desired component. Mathematically, selectivity is defined as:

$$\alpha = \frac{y/(1-y)}{x/(1-x)} \quad [1]$$

where x and y are weight fractions of butanol in the feed and permeate, respectively<sup>7</sup>. The flux of butanol through the membrane is given by:

$$J_B = \frac{P_M}{\delta_M} (x_B \gamma_B P_B^S - y_B P_P) \quad [2]$$

where  $P_m$  is the permeability of the membrane,  $\delta_m$  is the membrane thickness,  $x_B$  is the mole fraction of butanol in the feed,  $\gamma_B$  is the activity coefficient for butanol,  $P_B^s$  is the vapor pressure of butanol,  $y_B$  is the mole fraction of butanol in the permeate, and  $P_p$  is the permeate pressure<sup>8</sup>. Higher butanol yield from the fermentation broth is expected to increase the selectivity and the flux of the butanol.

In order to determine whether the solution-diffusion model is appropriate to use, a theoretical flux through the membrane was predicted and compared to experimental data. Data for the two experiments can be found below in Table 1. Data from Experiment 1 was used to determine the membrane permeability. This constant was used within the solution-diffusion model to predict that Experiment 2 would yield a total flux of 27.8 g/m<sup>2</sup>h. The theoretical flux matched the experimentally determined flux of 37.3 g/m<sup>2</sup>h within 25.5%. The model is assumed to predict experimental values reasonably well.

**Table 1. Experimental data**

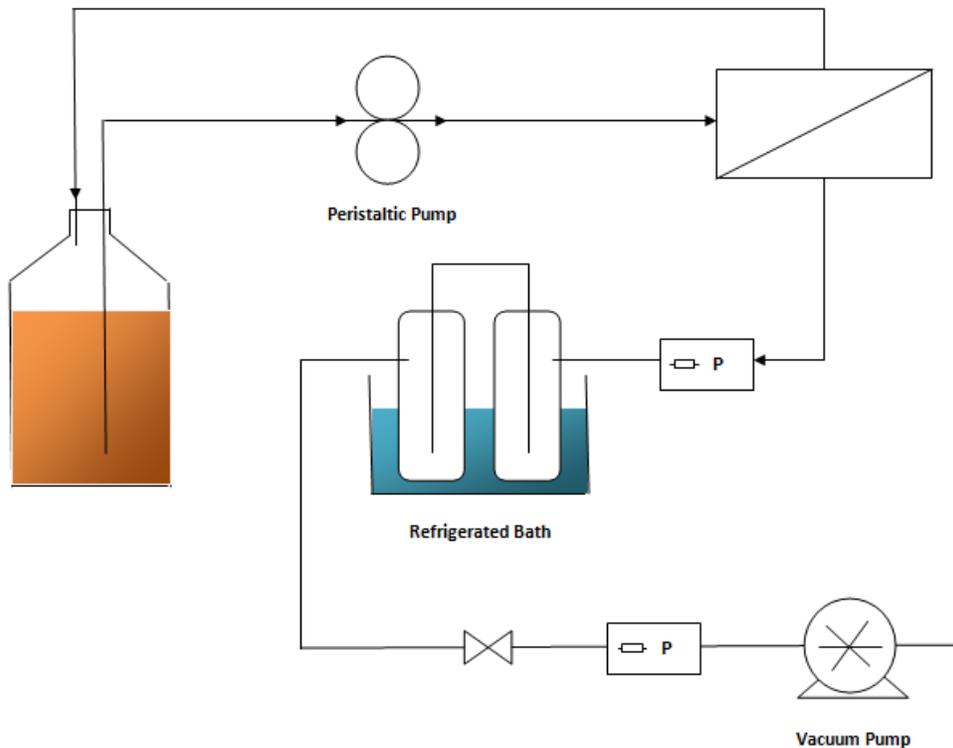
Experiment	Butanol in Feed (g/L)	Butanol in Permeate (g/L)	Temperature (°C)	Time (hrs)	Selectivity	Total Flux (g/hr-m <sup>2</sup> )
1	0.098	1.965	40	22	20.3	40.6
2	0.385	1.082	33	12.5	5.81	37.3

## MATERIALS & METHODS

The two-stage fermentation is designed to split the process into two separate fermentations to increase butanol yield. In the first step, sugar is converted to butyric acid via continuous fermentations of peptone yeast glucose (PYG) media with *Clostridium tyrobutyricum*. For this process a pH of 6-7 is maintained. The butyric acid can be recovered through electrodeionization (EDI). In the second step the butyric acid is converted to butanol

with *Clostridium saccharoperbutylacetonium*. Tryptone yeast glucose (TYG) media are used and the pH is adjusted to about 4.8 with 10N sodium hydroxide.

Experiments were conducted to extract butanol from the second step fermentation. A diagram of the lab set-up is shown in Figure 1. The feed liquid flows over the membrane, and the retentate, the portion of the feed which does not diffuse through the membrane, is recycled back to the feed source to ensure maximum butanol extraction. The permeate diffuses through the membrane due to a chemical potential difference created by the vacuum pump and is immediately collected in a vacuum trap immersed in a refrigeration bath. The permeate and feed broth from before and after the experiment are sampled and analyzed using high-performance liquid chromatography (HPLC) to determine their composition. The first set of experiments were conducted to remove butanol from a fermentation broth, and to determine the butanol flux and selectivity of the membrane. The average separation factor for the polydimethylsiloxane (PDMS) membrane, supplied by Membrane Technology and Research, Inc, is  $20.3 \text{ [mol butanol in permeate/mol water in permeate]/[mol butanol in feed/mol water in feed]}$  for a feed at 40°C and 1 g/L butanol. This separation factor is comparable to that obtained by other researchers under similar conditions<sup>13, 14</sup>. The average flux of butanol is  $8 \times 10^{-2} \text{ g/m}^2\text{-h}$ . The feed was maintained at 40°C to simulate the conditions for pervaporation directly connected to the reactor.



**Figure 1. Experimental Set Up**

To achieve a higher conversion of butyric acid to butanol within the reactor the pervaporation apparatus was connected directly to the reactor. Butanol was to be continuously removed in the permeate while the retentate was recycled back to the reactor. This keeps the level of butanol low enough that it prevents product inhibition. Complications were encountered when attempting to conduct the in-situ pervaporation experiments. Numerous attempts were made to make the pervaporation apparatus leak-tight. However, five consecutive experiments were run where the fermentation broth went directly into the retentate rather than only the butanol being vaporized across the membrane. It was determined that the membrane being used was no longer effective. However, reactor kinetic data that does not include simultaneous butanol extraction has been collected. Using a classic

Monod approach it is possible to model this data and then further develop the model to predict the levels of butanol expected within the reactor when simultaneous product removal is achieved.

## RESULTS & DISCUSSION

A mathematical model has been developed to describe the conversion of butyric acid to butanol via continuous fermentation with *Clostridium saccharoperbutylacetonium* coupled with in-situ pervaporation to extract butanol. To begin, the classic Monod equation was used as it is known for representing the specific cell growth rate as a function of a limiting substrate concentration. The Monod equation is expressed as follows:

Substrate  $\longrightarrow$  (S) Cells (C) + Product (R)

$$r_C = k \frac{C_S C_C}{C_S + C_M} \quad [3]$$

where  $r_C$  is the cell growth rate,  $k$  is the reaction rate constant,  $C_S$  is the limiting substrate concentration, and  $C_M$  is the Monod constant<sup>15</sup>. The Monod equation fails to account for product inhibition which is essential for modeling the kinetics of *Clostridium saccharoperbutylacetonium* due to its sensitivity to its products. Han and Levenspiel proposed the following generalized form of the Monod equation to account for product inhibition<sup>15</sup>:

$$r_C = k \left(1 - \frac{C_R}{C_R^*}\right)^n \frac{C_S C_C}{C_S + C_M \left(1 - \frac{C_R}{C_R^*}\right)^m} \quad [4]$$

where  $C_R$  is the concentration of the inhibiting product,  $C_R^*$  is the critical concentration of the inhibiting product above which the reaction stops, and  $n$  and  $m$  are constants. For the purpose

of developing this model it is assumed that butanol is the single limiting product. The constants can be evaluated by inverting the equation and plotting  $C_R/r_C$  versus  $1/C_S$ . This yields the following equation:

$$\frac{C_C}{r_C} = \frac{C_M \left(1 - \frac{C_R}{C_R^*}\right)^m}{k \left(1 - \frac{C_R}{C_R^*}\right)^n} \frac{1}{C_S} + \frac{1}{k \left(1 - \frac{C_R}{C_R^*}\right)^n} \quad [5]$$

The constants  $m$  and  $n$  are determined based on the pattern of inhibition for the specific type of fermentation. In the ABE fermentation the butanol product of the reaction acts as a noncompetitive inhibitor which yields constants of  $n > 0$  and  $m = 0$ . Thus, Equation 4 reduces to:

$$r_C = k \left(1 - \frac{C_R}{C_R^*}\right)^n \frac{C_S C_C}{C_S + C_M} \quad [6]$$

which can be inverted and rearranged to yield:

$$\frac{C_C}{r_C} = \frac{1}{k \left(1 - \frac{C_R}{C_R^*}\right)^n} \frac{1}{C_S} + \frac{1}{k \left(1 - \frac{C_R}{C_R^*}\right)^n} \quad [7]$$

$$= \frac{1}{k_{obs}} \frac{1}{C_S} + \frac{1}{k_{obs}} \quad [8]$$

where:

$$k_{obs} = k \left(1 - \frac{C_R}{C_R^*}\right)^n \quad [9]$$

and taking the logarithm of both sides:

$$\ln(k_{obs}) = n \ln \left(1 - \frac{C_R}{C_R^*}\right) + \ln(k) \quad [10]$$

In order to evaluate the constants  $k$  and  $n$ ,  $\ln(k_{obs})$  is plotted versus  $\ln \left(1 - \frac{C_R}{C_R^*}\right)$ . The constant  $k$

is taken to be the y-intercept while  $n$  is the slope of the line. The work from Han and Levenspiel

is reproduced in Figure 2 with data from reference 16 to ensure that this method can be reproduced. The values for constants  $k$  and  $n$  were found to be equivalent to those reported.

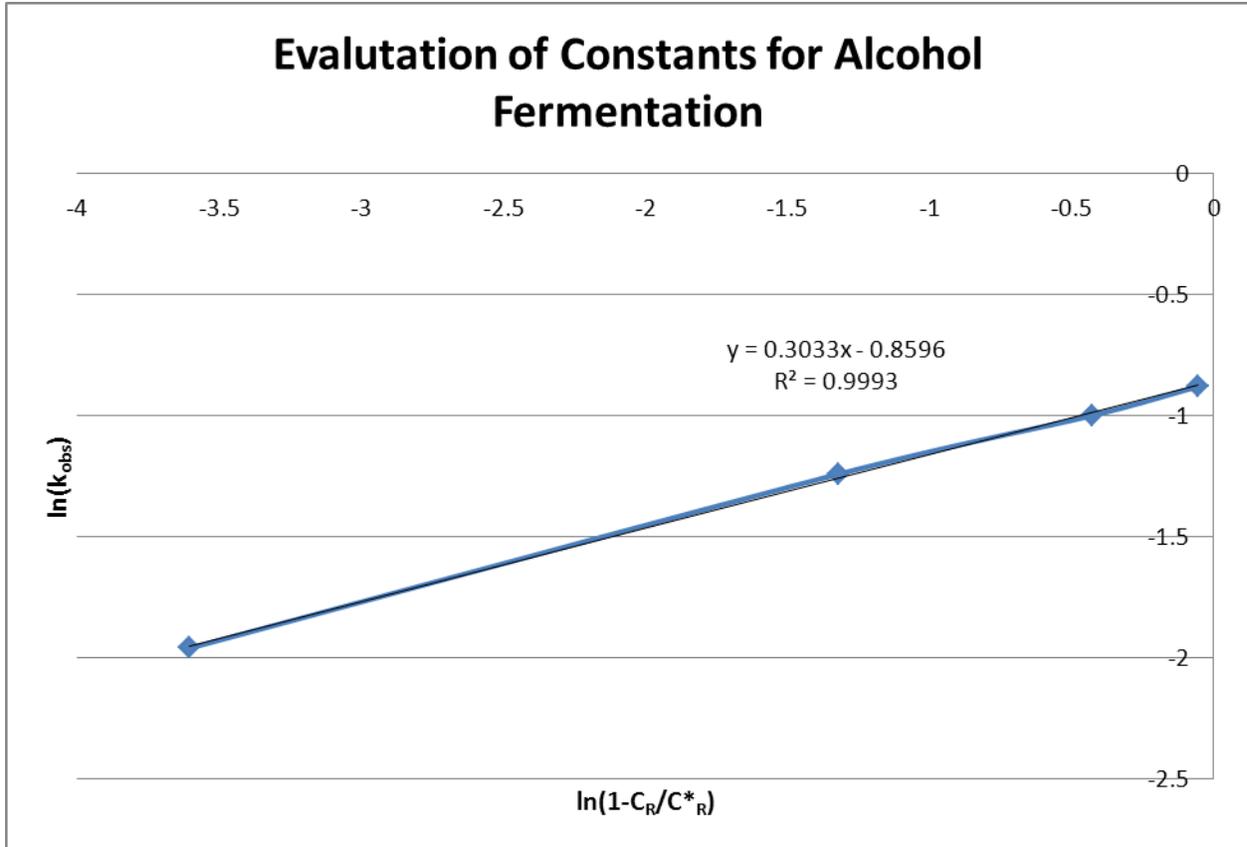


Figure 2. Evaluation of Constants  $k$  and  $n$

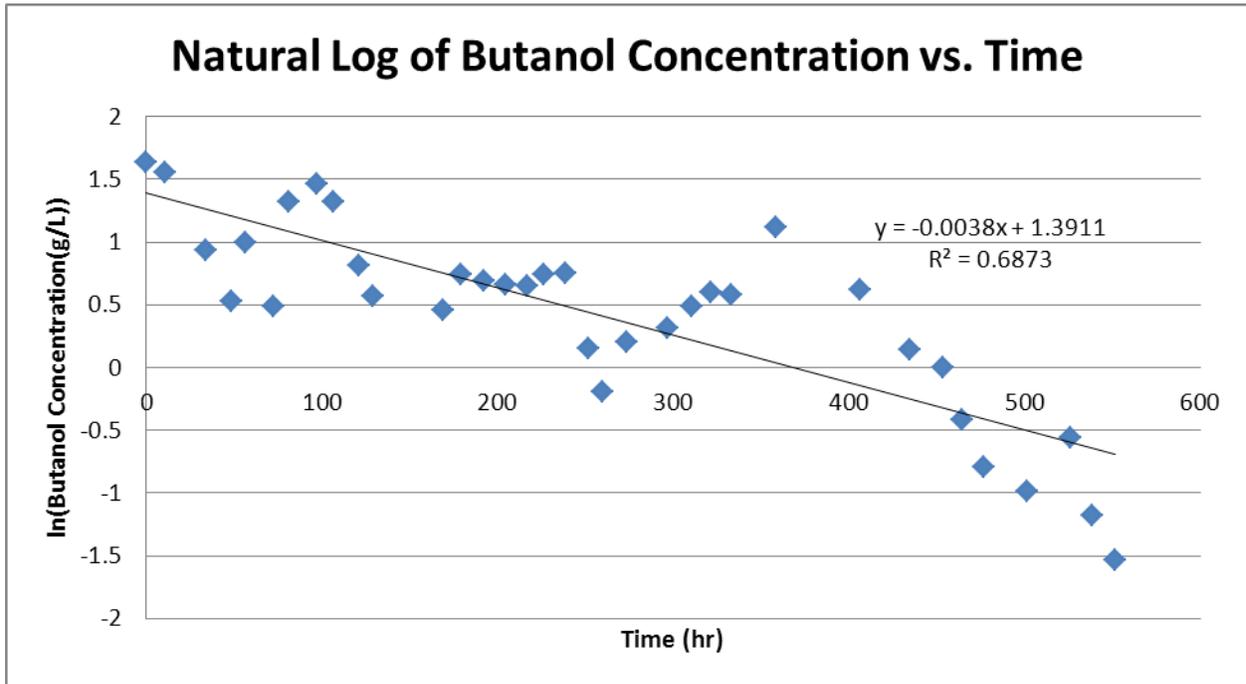
There was not sufficient experimental data obtained in this research to evaluate  $k_{obs}$  and plot it for various inhibitor concentrations. In order to evaluate  $k$  for the butanol fermentation a different approach was taken, assuming that the fermentation followed the first order rate law:

$$r = k[C_4H_9OH] \quad [11]$$

This yields the integrated rate law:

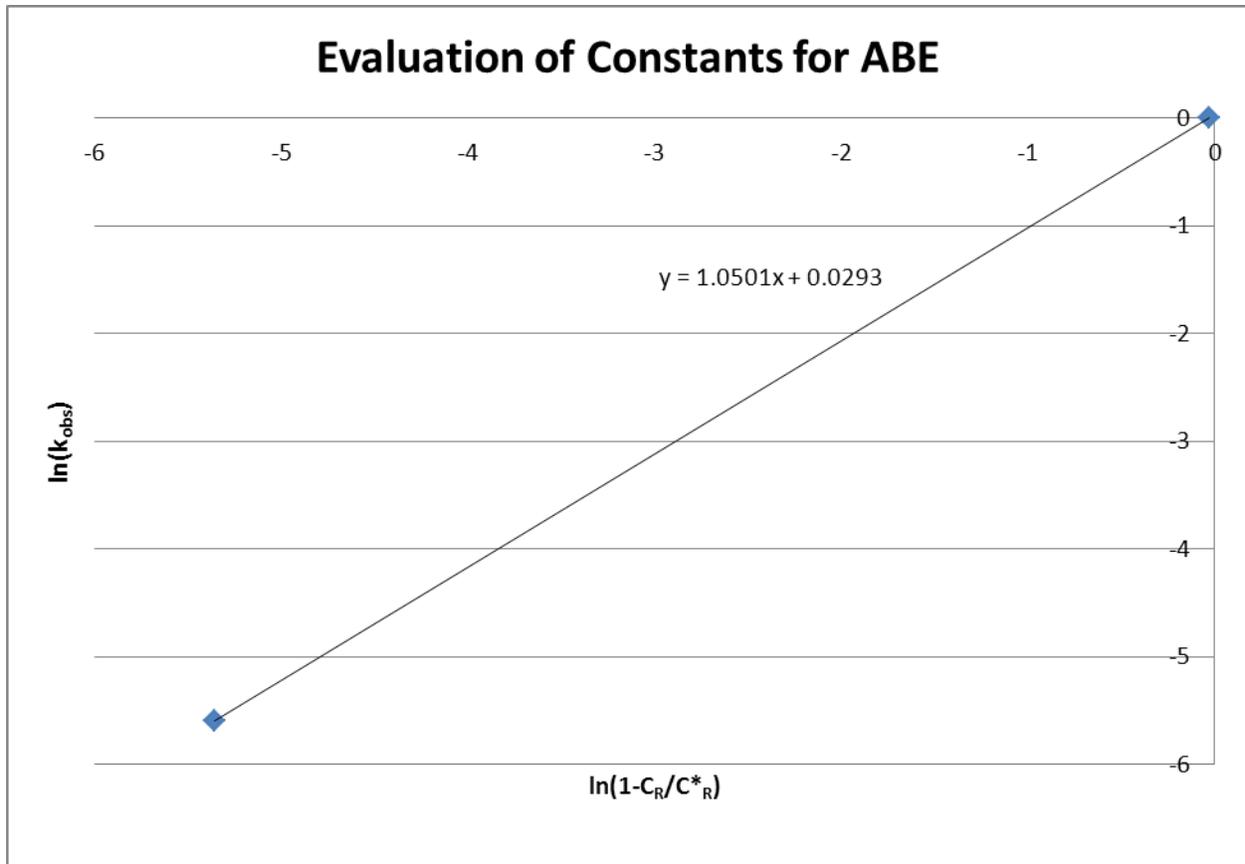
$$\ln[C_4H_9OH] = -kt + \ln[C_4H_9OH]_0 \quad [12]$$

Thus, plotting the natural log of the butanol concentration versus time allows the value of  $k$  to be obtained from the negative slope of the line. Equation 12 is plotted below in Figure 3 for a batch fermentation to produce butanol from glucose and  $k$  is estimated to be  $0.0038 \text{ hr}^{-1}$ .



**Figure 3. Natural Log of Butanol Concentration vs. Time**

Additional information is needed in order to plot Equation 10 and solve for the constant  $n$ . The critical concentration of butanol above which the reaction cannot proceed,  $C_R^*$ , is reported in literature to be  $13 \text{ g/L}^{17}$ . This concentration, the known y-intercept, and an experimentally observed reaction rate of  $0.0047 \text{ hr}^{-1}$  for a product concentration of  $0.358 \text{ g/L}$  are enough to generate a plot for various product concentrations and evaluate the remaining constant  $n$  for the system. Figure 4 shows this plot for a range of butanol product concentrations. From the equation of the line it is deduced that  $n = 1.05$ .



**Figure 4. Evaluation of Constant  $n$  for ABE with Butanol Product Inhibition**

As a result, the following equation represents the observed reaction rate constant for product inhibition associated with butanol concentration in the ABE fermentation:

$$k_{obs} = 0.0037 \text{ hr}^{-1} \left(1 - \frac{C_R}{13 \text{ g/L}}\right)^{1.05} \quad [13]$$

In 2000, Mutschlechner *et al* experimentally determined the reaction rate constant to be  $0.022 \text{ h}^{-1}$  for a butanol concentration of  $7.8 \text{ g/L}^{18}$ . Using the model obtained in this research, a reaction rate constant of  $0.0014 \text{ h}^{-1}$  would be predicted for the same product concentration.

This yields a percent error of 93.6%, indicating that the model was not accurate in determining  $k_{obs}$ . In 2004, Ramey *et al* found the reaction rate constant to be  $0.1 \text{ h}^{-1}$  for a butanol concentration of  $3.5 \text{ g/L}^{19}$ . The model again predicts a much lower constant,  $0.0027 \text{ h}^{-1}$ , with a

97.3% error. It appears that the model is unsuccessful at predicting  $k_{obs}$ , but the model was generated from a product concentration of 0.358 g/L, so this does not give an accurate representation of whether or not the model will work for lower levels of butanol that are closer to 0.358 g/L. The model would also be more accurate if  $k$  were obtained from plotting the natural log of observed reaction rate constants and finding the y-intercept as the procedure for determining the constants described, rather than using  $k$  to create a line. Hence, more reactor kinetic data for various product concentrations could provide more accurate constants for Equation 13, leading to more accurate predictions for  $k_{obs}$ .

Extending the mathematical model to describe the conversion of butyric acid to butanol via continuous fermentation coupled with in-situ butanol extraction requires an additional term to account for pervaporation. Since the solution-diffusion model was shown to model the butanol flux, Equation 2 incorporated into the current rate equation to account for the rate of butanol leaving the fermentation via pervaporation:

$$r_C = k \left( 1 - \frac{C_R}{C_R^*} \right)^n \frac{C_S C_C}{C_S + C_M} - \left[ \frac{P_M}{\delta_M} (x_B y_B P_B^S - y_B P_P) \right] \left[ \frac{S}{V} \right] \quad [14]$$

where  $S$  is the surface area of the membrane and  $V$  is the volume of the liquid fed to the pervaporation unit.

## CONCLUSIONS

A new technique using pervaporation as an in situ product removal method for a two-step fermentation was developed to continuously remove butanol from an ABE fermentation and keep its concentration below the level of toxicity. Experiments for simultaneous product

extraction were unsuccessful but a model was developed to characterize the product inhibition due to butanol in the ABE fermentation. This model was not found to agree with literature values but further research would determine whether the model could be appropriate over a specific range of concentrations. The model was extended to include a pervaporation term to predict the butanol concentration resulting from in-line pervaporation.

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