

# Kinetics of continuous fermentation and stripping of ethanol

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A pilot plant consisting of a 30-liter fermenter, and a 10-cm packed column with a blower and condenser to recover ethanol vapors was operated continuously for 185 days. On-line washing of the packing in the column twice weekly with condensed ethanol from the process (approximately 45% v/v) controlled fouling by attached yeast cells. Steady-state glucose consumption rates of up to 800  $\text{g h}^{-1}$ , condensed ethanol production rates of up to 26 l/day, and consistently high ethanol yield of approximately 0.50  $\text{g g}^{-1}$  glucose were observed. Data from the pilot plant showed that the primary inhibitory effect of ethanol on the steady-state fermenter performance was to decrease the cell yield, while the specific glucose consumption rate was almost unaffected by ethanol concentrations up to 65  $\text{g l}^{-1}$ . A new kinetic model is introduced to represent these effects.

## Introduction

In the U. S., the production and use of fuel ethanol reduces consumption of imported petroleum, thus lowering the national trade deficit and conserving non-renewable resources. Also, the ethanol industry increases farm income and creates jobs in rural areas, thus lowering the national budget deficit. Ethanol is a relatively clean burning fuel which can contribute to improving the nation's air quality. Unlike gasoline, the use of ethanol does not produce net greenhouse gas carbon dioxide, or contribute to global warming.

Ethanol is more expensive to produce than gasoline, making federal and state tax incentives necessary. A major bottleneck in the ethanol production process is the fermentation step, where the yeast that convert sugars derived from corn starch to ethanol are unable to tolerate ethanol concentrations higher than approximately 10% w/v. This limitation necessitates the use of large, expensive fermenters and also leads to high costs for separation of large amounts of water from the product and byproduct animal feed. One approach to overcoming this limitation is to remove the ethanol from the fermenter during the fermentation. In this way, higher concentrations of reactants (corn starch) and products (yeast, byproduct) can be maintained throughout the process. Overall costs can be reduced, provided that the additional equipment for ethanol recovery from the fermenter is not too expensive.

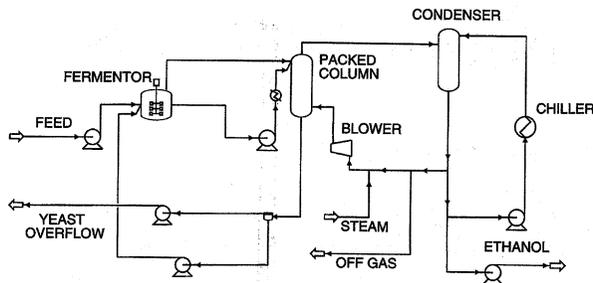
We have recently proposed to use  $\text{CO}_2$ , readily available as a byproduct of the fermentation, to strip the ethanol from

the fermenter contents as it is recycled through a packed stripping column (Taylor *et al.*, 1995). During continuous operation of our pilot plant, we have shown that by periodically washing the column with ethanol to control fouling, stable conversion of a high gravity, non-sterile feed can be maintained indefinitely (Taylor *et al.*, 1996). The productivity of the fermenter is consistently high (approximately 15 g ethanol  $\text{l}^{-1} \text{h}^{-1}$ ), and the yield is consistently nearly theoretical (almost 0.51 g ethanol  $\text{g}^{-1}$  glucose) (Taylor *et al.*, 1997). Cost analysis indicates that continuous fermentation with stripping is less expensive than traditional batch fermentation with distillation (Taylor *et al.*, in preparation).

In our previous paper, we fit data from a 14-l continuous fermenter to a model that included linear terms for the effects of ethanol concentration on specific growth rate and cell yield (Taylor *et al.*, 1997). During cost analysis, we used this model to find the minimum cost operating conditions. The linear model predicted lowest cost at a steady state ethanol concentration of approximately 70  $\text{g l}^{-1}$  in the fermenter. However, the data on which the model was based included steady state concentrations not higher than 60  $\text{g l}^{-1}$ . The work reported here was undertaken to find out whether the linear model could be extrapolated to 70  $\text{g l}^{-1}$ , and, if not, to fit the data to a different kinetic model.

## Materials and methods

Unless otherwise indicated, all methods and materials were the same as previously reported (Taylor *et al.*, 1995; 1996; 1997). *Saccharomyces cerevisiae*, ATCC 4126, and the methods of cell culturing and inoculation were the same. As



**Figure 1** Schematic flow-diagram of pilot plant equipment.

previously described and shown in Figure 1, the stripping system consisted basically of two interlocking recycle loops, a liquid recycle loop between the fermentor and the stripping column, and a gas recycle loop between the stripping column and the condenser. Two different fermenters were used, the 14-l glass jar fermenter with working volume 12.4 l as previously described, and a 60-l stainless steel jacketed fermenter with 31 l working volume (including recycle holdup). The fermenter was equipped with automatic antifoam addition and pH control, however neither was required during continuous operation as previously explained (Taylor *et al.*, 1997). The fermenter temperature was maintained at 34°C by proportional control of cooling water addition to the jacket recirculation loop. Air was supplied through a sparger at 300 ml/min with agitation at 160 rpm. The fermenter holdup volume (30 l) was maintained by a dip tube connected to the suction side of the stripping column feed pump, which pumped slightly faster than the recycle return pump from the bottom of the stripping column to the fermenter. The recycle rate, determined by the recycle return pump speed, was set to one of two values, either 325 ml/min or 590 ml/min. The recycle holdup volume (1 l) was maintained by a yeast overflow pump which removed the excess from the bottom of the stripping column to drain through a continuous pasteurizer.

The stripping column, packing and temperature controls were the same as previously described (Taylor *et al.*, 1997). Two times per week during continuous operation, the blower and recycle pumps were turned off and, using a centrifugal wash pump, approximately 6–8 l of 45 vol% ethanol were recirculated through the packed column at approximately 8–10 l/min for approximately 10 min to wash attached yeast cells from the packing. The condenser and blower were the same as previously except that the blower speed was increased from 640 rpm to 1350 rpm during the last two steady state data sets. The feed composition and preparation were the same (Taylor *et al.*, 1997). The startup of the 30-l fermenter was the same

as the 14-l fermenter except that non-sterile tap water initially filled the fermenter, and the inoculum was two liters instead of one. The sampling and measurement techniques were the same, and, as before, the feed rate was manually adjusted to maintain a small but measurable glucose concentration in the fermenter.

As before, selected sets of steady state operating data were averaged, then the most likely values of non-measured result variables such as the stripping gas flow rate, and of some measured input variables were determined from regression of the data using a computerized simulation of the process (Aspen Technology, Cambridge, MA). Data reported here include these most likely values, which in no case differed by more than 5% from the actual averages of measured data. The set of regressed variables differed slightly from the previous analysis. In particular, the number of theoretical stages in the stripping column (previously fixed at one) was introduced as a regressed variable. Also in contrast to the previous analysis, the total holdup volume including recycle holdup was used as the fermenter working volume.

Previous results showed that the cell yield,  $Y_{X/S}$ , (g dry cells  $g^{-1}$  glucose) decreases with increasing ethanol (1997). In using the standard equation for fermentation kinetics:

$$\frac{dX}{dt} = \mu X$$

where  $X$  is cell mass and  $\mu$  is the specific growth rate, it could not be determined to what extent the effect of ethanol concentration on the specific growth rate was actually due to its effect on cell yield. For this reason, we now report kinetic data as the rate of glucose consumption, defined by:

$$-\frac{dS}{dt} = q_s X \quad (2)$$

where  $S$  is substrate (glucose) and  $q_s$  is the specific glucose consumption rate ( $g g^{-1}$  dry cells  $h^{-1}$ ). The specific growth rate and specific ethanol production rate,  $q_p$ , are obtained from the specific glucose consumption rate, cell yield, and ethanol yield,  $Y_{P/S}$ , according to:

$$\mu = Y_{X/S} q_s \quad (3)$$

$$q_p = Y_{P/S} q_s \quad (4)$$

A new measured variable was also introduced, the concentration factor,  $C$ , defined as the glucose concentration in the feed multiplied by the ratio of feed flowrate to overflow flowrate. This variable is an indicator of the relative concentration of nonvolatile feed components and yeast byproducts. Such components may be inhibitory when

accumulated to sufficiently high concentration (Maiorella, *et al.*, 1983; 1984). Although the feed glucose concentration was constant at approximately 560 g l<sup>-1</sup>, variation of the concentration factor resulted from different amounts of net evaporation of water and ethanol in the stripping column at different stripping temperatures.

## Results

The data in Tables 1 and 2 present results from two separate runs, one with the 14-l fermenter and one with the 30-l fermenter. The 14-l results in data sets 1, 2, and 3 are from the continuation of the second of two 14-l runs for which results were previously reported (Taylor *et al.*, 1997). This run continued for a total of 153 days without interruption or contamination. The remaining data in

Tables 1 and 2 are from one uninterrupted run with the 30-l fermenter lasting for a total of 185 days of continuous operation without contamination.

When the 30-l fermenter was started up, the only change from the previous run was the increased fermenter volume. Based on extrapolation of the linear model for fermentation rate that had been derived from previous data from the 14-l fermenter (Taylor *et al.*, 1997), it was expected that ethanol concentrations over 70 g l<sup>-1</sup> would be achieved in the 30-l fermenter. Instead, although the ethanol concentration increased slightly, the cell yield decreased from approximately 0.04 to 0.05 in the 14-l fermenter to only 0.02 to 0.03 in the 30-l fermenter (data sets 6–10 in Tables 1 and 2). As a result, the overall glucose conversion

**Table 1** Continuous fermenter/stripper measured variables

Data Set No.	Gas into column bottom (°C)	Gas from column top (°C)	Nutrient Feed		Fermenter			Overflow		Condensate	
			Feed Rate (ml/min)	Glucose (g l <sup>-1</sup> )	Glucose (g l <sup>-1</sup> )	Ethanol (g l <sup>-1</sup> )	Yeast Dry Wt. (g l <sup>-1</sup> )	Overflow (ml/min)	Ethanol (g l <sup>-1</sup> )	Condensate (ml/min)	Ethanol (g l <sup>-1</sup> )
1	41.4	37.2	9.67	573	0.64	43.6	33.8	8.35	35.5	8.43	309
2	36.9	36.6	10.82	573	0.69	45.9	36.0	7.64	37.5	8.64	324
3	36.1	36.9	11.28	555	0.91	49.1	34.7	7.72	40.2	8.70	340
4	41.9	37.9	22.37	569	1.23	53.8	30.1	19.00	36.9	18.34	304
5	39.2	36.2	24.17	565	1.24	54.6	30.2	19.24	37.6	17.60	325
6	42.1	36.6	15.25	551	0.84	60.3	20.6	13.08	49.6	9.69	365
7	37.9	33.6	14.41	546	28.60	62.5	12.7	11.98	57.4	8.11	385
8	40.7	35.1	14.63	577	3.35	64.3	17.7	12.54	58.7	9.34	367
9	40.3	33.4	14.68	556	32.10	64.4	12.3	13.44	59.4	8.46	361
10	42.0	37.9	16.02	549	2.44	64.4	21.2	13.17	53.6	10.04	364

Note: Fermenter total working volume (including recycle holdup) was 13.2 l in data sets 1 to 3 and 31 l in data sets 4 to 10.

**Table 2** Continuous fermenter/stripper calculated variables

Data Set No.	Stripping Gas Flow (kg h <sup>-1</sup> )	Stripping Column Number of Theoretical Stages	Overall Glucose Conversion (g h <sup>-1</sup> )	q <sub>s</sub> , Specific Glucose Consumption (h <sup>-1</sup> )	Y <sub>X/S</sub> , Cell Yield (g g <sup>-1</sup> )	Y <sub>P/S</sub> , Ethanol Yield (g g <sup>-1</sup> )	Ethanol Productivity in Fermenter (g l <sup>-1</sup> h <sup>-1</sup> )
1	11.7	1.8	333	0.746	0.051	0.53	13.4
2	12.2	2.0	372	0.784	0.045	0.50	14.2
3	11.7	2.0	375	0.821	0.041	0.53	15.1
4	25.7	1.8	763	0.818	0.045	0.50	12.3
5	26.1	1.7	819	0.874	0.043	0.48	12.6
6	12.4	1.0	505	0.789	0.032	0.50	8.2
7	12.1	0.8	453	1.153	0.020	0.51	7.5
8	13.1	0.4	505	0.918	0.027	0.50	8.1
9	13.3	0.3	465	1.223	0.020	0.50	7.5
10	12.0	0.7	527	0.799	0.032	0.50	8.5

see Note, Table 1

was only slightly higher and the ethanol productivity was much lower than in the 14-l fermenter.

At first, it was not clear whether the low productivity in the 30-l fermenter may have been caused by some difference in the conditions in the two different fermenters. The pH and temperature were the same. Neither increasing the agitation rate to 270 rpm nor increasing the sparger air flowrate to 900 ml/min had any effect (data not shown). Insulating the recycle lines which were longer than for the 14-l fermenter improved the 30-l fermenter performance slightly (data from before insulation are not included here), but decreasing the recycle rate from 590 ml/min to 325 ml/min had no effect. It was soon realized that we were observing a large departure from linearity in the effect of ethanol concentrations above 60 g l<sup>-1</sup> on the cell yield.

To further test whether there was any effective difference (other than size) between the 30-l and 14-l fermenters, the blower speed was increased, approximately doubling the stripping gas flow rate (data sets 4 and 5 in Tables 1 and 2) and returning the ethanol concentration to less than 60 g l<sup>-1</sup>. The cell yield and fermenter productivity were restored to values similar to those observed in the 14-l fermenter, confirming that conditions in the two fermenters were essentially the same. These results show that a non-linnol concentration on cell yield is required. At the higher blower speed, the glucose consumption rate increased to approximately 800 g h<sup>-1</sup>, corresponding to a feed rate of approximately 24 ml/min of 560 g glucose l<sup>-1</sup>. The ethanol production rate from the condenser increased to approximately 18 ml/min (26 l/day) of over 300 g ethanol l<sup>-1</sup>. The ethanol yield, Y<sub>P/S</sub>, (g ethanol g<sup>-1</sup> glucose), including smaller amounts of ethanol in the yeast overflow and exhaust gas, remained constant at approximately 0.50 (Table 2). This value is high compared with typical values reported for yeast fermentation, but not impossible. Although 0.51 g ethanol g<sup>-1</sup> glucose is the maximum theoretically possible, the nitrogen source, consisting of crude protein in corn steepwater, and amounting to approximately 4% of the glucose, also provided some organic carbon for cells and/or ethanol.

Results from the last three runs (two 14-l and one 30-l) were combined into the complete data set shown in Table 3. After fitting the data by least squares regression to several different models, the following expressions produced the best fit:

$$q_s = 1.612 \left( \frac{S}{S + 0.1347} \right) \left( 1 - \frac{T - 34}{12.33} \right) \left( 1 - \frac{P \cdot C}{162000} \right) \quad (5)$$

$$Y_{X/S} = 0.1238 \left( 1 - \frac{P}{66.46} \right)^{0.3108} \left( 1 - \frac{C}{1632} \right) \quad (6)$$

Where T is the stripping temperature (°C), P is product (ethanol) concentration (g l<sup>-1</sup>) and the other symbols are as previously defined. The general mathematical form of the non-linear factor expressing the effect of ethanol concentration (P) on cell yield (Y<sub>X/S</sub>) was introduced by Levenspiel to represent ethanol inhibition of the specific growth rate (Levenspiel, 1980). These mathematical expressions are pragmatic or empirical, not mechanistic. However, expression of results in mathematical form is essential to computerized process modeling and optimization. These equations should not be used to extrapolate beyond the range of actual measured values as given in Table 3.

Agreement of the model with the data is presented in Figures 2 to 4. In Figure 2, the data are corrected by dividing the specific glucose consumption by the expressions in the model for the effects of stripping temperature (T), ethanol (P), and concentration factor (C) to show the effect of glucose concentration alone. Similarly, in Figure 3, the data are corrected for glucose (S), ethanol (P), and concentration factor (C) to show the effect of stripping temperature alone. A third plot to show the effect of ethanol (P) and concentration factor (C) on specific glucose consumption is not provided, because the effect is small. The fit of the model to the specific glucose consumption data is only slightly worse when ethanol and the concentration factor are excluded from the model. Study of the effect of the concentration of non-volatile inhibitory feed components and byproducts was not a major objective of this work, so the range of data for the concentration factor is small (603-818 g l<sup>-1</sup>). Also, the specific glucose consumption is relatively unaffected by ethanol concentration within the experimental range (43 to 65 g l<sup>-1</sup>).

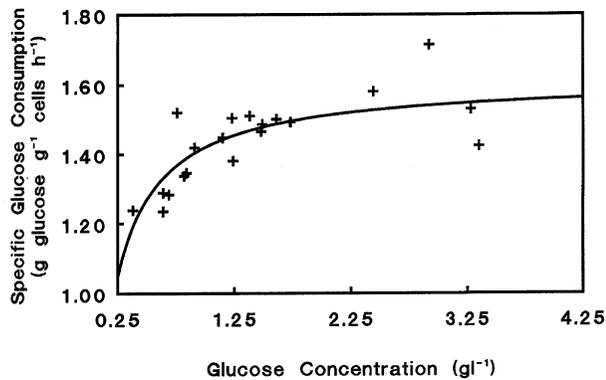
In Figure 4, the data are corrected by dividing the cell yield by the expression in the model for the effect of the concentration factor (C), to show the effect of ethanol alone. Again, graphical display of the effect of the concentration factor is omitted because the range of data and thus the effect within that range are small. The data and model clearly show that the inhibitory effect of ethanol on the specific growth rate is primarily due to decreased cell yield.

## Discussion

The kinetics of ethanol fermentation have been well studied over the years (Aiba and Shoda, 1969; Bazua and Wilke, 1977; Luong, 1985). There is not general agreement in the literature on the effect of ethanol concentration. Results may differ depending on whether data were

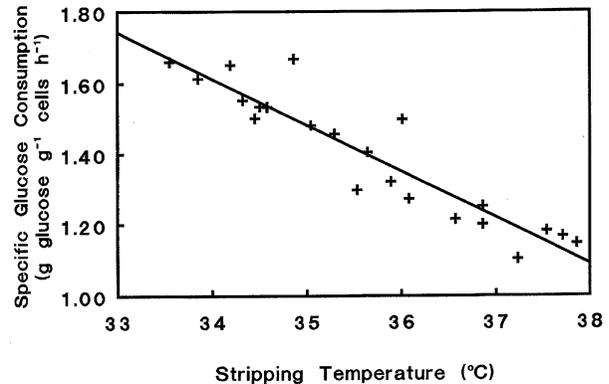
**Table 3** Complete data set for kinetic model.

$q_S$ , Specific Glucose Consumption ( $h^{-1}$ )	$Y_{x/s}$ Cell Yield ( $gg^{-1}$ )	S, Glucose Concentration in Fermenter ( $gl^{-1}$ )	P, Ethanol Concentration in Fermenter ( $gl^{-1}$ )	T, Stripping (Top) Temperature ( $^{\circ}C$ )	C, Concentration Factor ( $gl^{-1}$ )
0.746	0.0511	0.64	43.6	37.23	677
0.784	0.0447	0.69	45.9	36.57	809
0.847	0.0451	1.38	47.0	37.54	739
0.821	0.0410	0.91	49.0	36.86	818
0.876	0.0501	0.64	49.7	35.89	645
1.019	0.0437	1.49	51.3	35.64	663
0.980	0.0463	0.76	51.6	36.01	724
0.966	0.0446	0.38	52.5	34.19	643
1.044	0.0421	1.61	52.5	35.29	691
0.818	0.0454	1.23	53.8	37.71	672
0.874	0.0431	1.24	54.6	36.08	711
1.010	0.0434	0.82	55.3	34.45	637
1.097	0.0400	1.48	56.3	34.50	635
1.084	0.0377	1.73	56.5	34.58	685
1.213	0.0407	2.92	57.4	34.86	678
1.021	0.0432	1.15	58.1	35.04	643
0.789	0.0323	0.84	60.3	36.86	639
1.139	0.0412	3.28	61.0	34.32	629
1.153	0.0202	28.60	62.5	33.55	787
0.918	0.0265	3.35	64.3	35.53	668
1.223	0.0196	32.10	64.3	33.85	603
0.799	0.0322	2.44	64.4	37.86	667



**Figure 2** Effect of Glucose Concentration on Specific Glucose Consumption Rate.

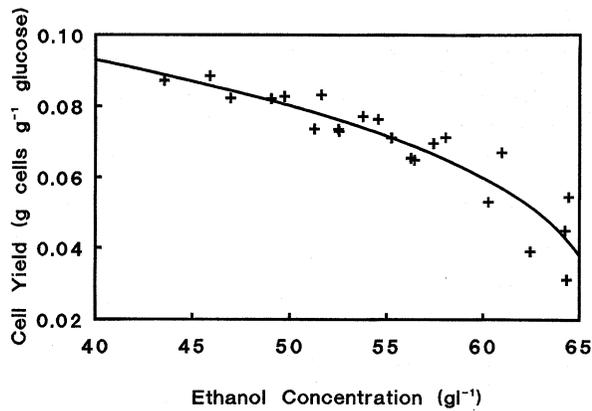
obtained from batch fermentations or by steady state operation of continuous fermenters. Whether ethanol was added to or removed from the fermenter, or controlled simply by adjusting the feed glucose concentration may also affect results. Many different mathematical models have been proposed. Usually the cell growth rate, and sometimes the ethanol production rate are the quantities of interest. Models that describe the glucose consumption rate are not found. Most often, the cell yield and ethanol yield are assumed to be constant. Sometimes maintenance or cell death is invoked to account for varying cell and/or



**Figure 3** Effect of Stripping Temperature on Specific Glucose Consumption Rate.

product yields. Cell death rates have been measured (Dale *et al.*, 1990), but in most experimental situations, including the present one, the separate influences of cell growth and cell death cannot be distinguished.

The data presented here clearly show that in continuous, steady-state fermentation, the primary inhibitory effect of ethanol is on the cell yield, which falls off sharply above 60 g ethanol  $l^{-1}$ , while the glucose consumption rate is almost unaffected. The loss of cells places an upper limit on continuous fermentation at a steady-state ethanol concen-



**Figure 4** Effect of Ethanol Concentration on Cell Yield.

tration of approximately 65  $\text{gl}^{-1}$ . The non-linear mathematical model presented here displays this effect, fitting the data well. Although derived from data obtained by stripping of ethanol, this model may be applicable to continuous fermentation in general. Application of this model to the design of a corn-to-ethanol plant incorporat-

ing continuous fermentation with integrated ethanol recovery may lower the cost of fuel ethanol production. Adoption of this technology by the fuel ethanol industry may be beneficial, not only to the industry, but to farmers, transportation fuel consumers and the general public.

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