

Effects of ethanol concentration and stripping temperature on continuous fermentation rate

Abstract The operation of a pilot plant consisting of a 14-l fermentor, 10-cm packed column and condenser for continuous fermentation and stripping of ethanol was stable for more than 100 days. The feed consisted of a non-sterile solution of 560 g/l glucose with 100 g/l corn steep water. Fouling of the packing in the column with attached growth of yeast cells was controlled by in situ washing at intervals of 3–6 days. A computer simulation of the pilot plant was developed and used to analyze the data. The productivity of the continuous fermentor varied from 14 g ethanol to 17 g ethanol $l^{-1} h^{-1}$. The yield was equal to the maximum theoretically possible: 0.51 g ethanol/g glucose consumed. Results are fit to linear models for the effects of ethanol concentration on specific growth rate and cell yield, and for the effect of stripping temperature on specific growth rate.

Introduction

Fuel ethanol production by fermentation of glucose from corn has increased in the United States to almost 5.7×10^9 l (1.5×10^9 US gallons) in 1995, as a result of federal and state incentives (Anon. 1995). Almost 10% of the total gasoline supply is now "gasohol", a 10% ethanol blend with gasoline. Its value was originally seen as a gasoline replacement, to reduce dependence on imported petroleum while stimulating the U.S. economy,

especially in underdeveloped rural areas. The value of ethanol as an anti-knock additive to replace the lead formerly added to premium gasoline has also been recognized. With current regulations on the composition of gasoline in areas where air pollution has been a problem, fuel ethanol has taken on its most valuable role as an oxygenated gasoline additive (Anon. 1996).

The energy of a gallon of ethanol is greater than the total energy of all fossil fuels consumed in its production (Shapouri et al. 1995). Furthermore, because most of these fuels are natural gas and coal, the energy of a gallon of ethanol is seven times the energy of all liquid fuels consumed to produce it (Shapouri et al. 1995). Therefore, dependence on imported petroleum and the foreign trade deficit are reduced, while the security and sustainability of the nation's liquid fuel supply are improved.

Although fermentation has been practiced for centuries and can be considered a mature technology, reduction of process costs through development of improved technology is still possible. Currently available process technology for commercial fuel ethanol production by fermentation incorporates a number of improvements that have reduced the cost of ethanol production. These include the reduction of steam consumption in distillation through improved heat recovery, the reduction of wastewater through the increased reuse of water, the reduction of ethanol losses by recovering ethanol vapors from fermentation CO_2 off gas, the substitution of molecular-sieve or corn-grits drying of ethanol for azeotropic distillation, and the use of continuous-cascade fermentor arrays (Katzen et al. 1994).

The productivity of today's fuel ethanol fermentor will vary, depending on such factors as the amount of yeast added and the final ethanol concentration, but in most plants it is no more than approximately 2 g ethanol $h^{-1} l^{-1}$ fermentor volume, even in continuous-cascade fermentors. The high initial investment for large fermentors contributes at least U.S. \$0.05 to the cost of producing a gallon of fuel ethanol. It has been known

for at least 25 years that the size and cost of fermentors can be greatly reduced by using any of a variety of different highly productive continuous fermentor types. These include immobilized-cell reactors (Dale et al. 1985; Webb et al. 1995), cell-recycle reactors (Dellweg and Luca 1988; Warren et al. 1994), and combined fermentation with ethanol separation (Cysewski and Wilke 1977; Dale et al. 1985; Daugulis et al. 1987; O'Brien and Craig 1996). So far these advanced continuous fermentors have not gained acceptance in the industry.

Recently, we proposed a new variation on the theme of combined fermentation with ethanol separation, continuous fermentation and stripping of ethanol (Taylor et al. 1995). In this process, the fermentor contents are continuously recycled through a packed column. Meanwhile, the CO₂ from the fermentor also goes to the column and accumulates in a gas-recycle loop into which ethanol is stripped from the fermenting beer in the column and condensed in a series of condensers. Results from our pilot plant have shown that, with appropriate measures to limit fouling of the packing in the column by growth of attached yeast cells, stable operation for an extended period can be accomplished routinely (Taylor et al. 1996). Computer simulation and cost analysis of a full-scale plant indicate that, compared with existing technology, a saving of \$0.035/gallon of fuel ethanol (\$0.0092/l) can be realized with this process (manuscript in preparation). The purpose of the research described here was to demonstrate stable long-term ethanol production with non-sterile feed, and to collect data for the effects of ethanol concentration and stripping column temperature on production rate.

Materials and methods

Unless otherwise stated, all materials and methods were the same as previously reported (Taylor et al. 1996). A flow diagram of the pilot plant equipment for producing ethanol is shown in Fig. 1. The fermentor was a 14-l glass jar Magnaferm (New Brunswick Scientific, New Brunswick, N.J.). Foaming was sensed by a level probe and controlled by automatically pumping in Dow Corning anti-foam emulsion no. 1410 (Dow Corning, Midland, Mich.). The pH was controlled between 3.6 and 3.7 by automatic addition of 2 M ammonium hydroxide. Air was supplied to the sparger at approximately 150 ml/min. The fermentor was stirred at 225 rpm. The temperature in the fermentor was maintained at 34 °C by proportional control of the cooling water. The fermentor working volume was maintained at 12.4 l by an adjustable dip tube connected to the suction side of the stripping-column feed pump. This pump was set slightly faster than the other liquid-recycle pump, which returned the fermenting beer from the bottom of the column to the fermentor at 300–350 ml/min. Peristaltic pumps equipped with 6.4-mm (inner diameter) Marprene tubing (Watson-Marlow, Wilmington, Mass.) were used for liquid recycling. The overflow or excess was removed from the system by a third peristaltic pump connected to drain through a continuous pasteurizer (not shown in Fig. 1). The overflow rate was approximately equal to the feed rate, but varied depending on the temperatures of the gas entering and leaving the stripping column.

The glucose concentration in the fermentor was measured daily with a YSI (Yellow Springs Instrument Co., Yellow Springs, Ohio) glucose analyzer and the feed rate was manually adjusted to maintain a small (less than 5 g/l) but measurable (greater than

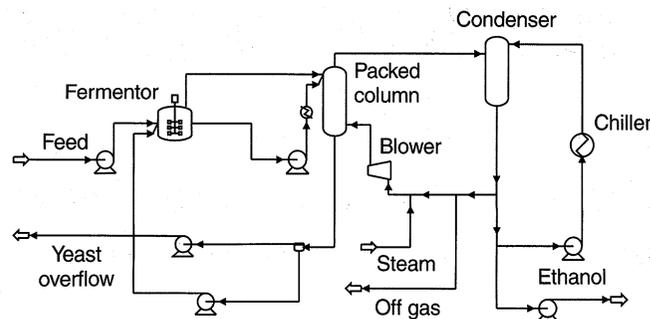


Fig. 1 Simplified flow diagram of pilot-plant equipment

0.2 g/l) glucose concentration. The ethanol concentration was measured by HPLC using an HP 1050 (Hewlett-Packard, San Jose, Calif.) with a fast acid analysis column, 100 × 7.8 mm (BioRad, Hercules, Calif.) and a differential refractometer (Spectra Physics, Riviera, Fla.). The column temperature was 70 °C and the mobile phase was 0.001 M H₂SO₄ at 0.7 ml/min.

The stripping column, 10 cm by 152 cm, was packed with 5-cm plastic Tellerettes (Ceilcote Co., Berea, Ohio). The column-feed heater consisted of a proportional temperature controller (Omega, Stamford, Conn.) and a 250-W immersion heater inserted into the top of the column through a length of 12-mm (inner diameter) stainless-steel pipe threaded into the column top plate from below. Stripping gas was recirculated with a plastic, centrifugal blower having a 38-cm-diameter impeller with 10-cm intake and outlet. The blower speed was constant at 640 rpm. The temperature of the stripping gas entering the bottom of the column was controlled by a pneumatic valve on the steam supply line. The condenser was packed with 2.5-cm stainless-steel Intalox (Norton, Akron, Ohio). Condensate from the bottom of the condenser was recycled by a stainless-steel centrifugal pump through a heat exchanger and back to the top of the condenser. A positive-displacement pump drew the excess condensate off to a product reservoir. The heat exchanger was cooled by methanol at -15 °C to -20 °C recycled through a chiller (FTS Systems, Stone Ridge, N.Y.). The temperature of gas leaving the condenser was -8 °C to -10 °C.

The nutrient feed to the fermentor consisted of approximately 560 g/l glucose and 100 g/l corn steep water (Grain Processing, Muscatine, Iowa). Corn steep water contains approximately 50% water, so the feed contained approximately 50 g/l corn steep water solids. Corn steep water was diluted with one-third part by weight deionized water, sterilized for 20 min at 121 °C and clarified with a continuous laboratory centrifuge (Sharples, Warminster, Pa.). The non-sterile supernatant was mixed with tap water and Cerelose (dextrose, dry hydrate, approximately 90% glucose and 10% water; Corn Products, Franklin Park, Ill.) in a clean but not sterile 380-l stainless-steel tank. The tank was agitated and heated with a steam sparger to 72 °C before final adjustment of the glucose concentration with tap water.

Between runs the fermentor and all connecting tubing was cleaned. At the start of each new run, the fermentor was filled with 12 l tap water and sterilized in an autoclave. The yeast *Saccharomyces cerevisiae*, ATCC 4126 (American Type Culture Collection, Rockville, Md.), was maintained on agar slants. A 1-l portion of sterile YM broth (Difco, Detroit, Mich.) in a 2-l flask was inoculated with the resuspended cells from one slant. After shaking at 25–30 °C overnight, the contents of the flask were used to inoculate the fermentor. Immediately after inoculation, the stripping column recycle pumps were started, and the nutrient feed pump was started, slowly at first, then gradually faster over the first several days to maintain complete conversion of glucose as the cell mass in the fermentor accumulated. The chiller temperature was also gradually decreased from 0 °C at first to prevent freezing of the condensate in the heat exchanger.

Two methods were used to clean the packing in the column. Between runs, the packing was removed from the column, placed in

a sink and sprayed with a hose. The cleaned packing was sterilized in an autoclave before being returned to the column. The column and gas-recycle loop were then sterilized by recycling 70% ethanol through the column overnight with the heater and blower on. The in situ washing system consisted of a high-volume (approximately 10 l/min) centrifugal pump connected into a loop of 1-cm (inner diameter) tubing from the bottom of the column to the top. Once every 3–6 days, the loop was filled with condensed ethanol from the process (approximately 45% ethanol by volume). The blower, chiller, column feed heater and fermentor recycle pumps were turned off, and ethanol was recycled through the column for approximately 8 min. Yeast cells flushed from the column were drained and the procedure repeated once. The entire washing procedure took approximately 25 min, during which time continuous feeding to the fermentor, without stripping, temporarily caused a slightly increased ethanol concentration.

Simulation and modeling

The averages of ethanol, glucose and cell concentrations as well as flow rates and temperatures for 12 selected time periods of from 2 to 12 days, when the pilot plant was judged to be operating at steady state, were used as inputs to a computer simulation developed with Aspen Plus, release 9.2 with Bioprocess Simulator (Aspen Technology, Cambridge, Mass.). The number of measured variables was more than required to specify a solution of the simulation uniquely. Therefore the measured variables were reconciled with the simulation by adjusting them to their most likely values using the data-fitting feature of Aspen Plus. These most likely values of input variables and the corresponding simulation results are reported here as measured (Table 1) and calculated (Table 2) variables. In no case did the most likely value of an input variable differ from the actual average of measured values by more than the estimated standard error: 2 °C for temperatures and 5% for flow rates and concentrations.

The simulation corrected for small losses of ethanol with the steam condensate (not shown in Fig. 1) and carbon dioxide off gas, which were not measured. The overall ethanol productivity could not be calculated because the volume for the fermentation taking place in the packed column and recycle lines (approximately 500 ml) was not accurately measurable. To calculate the ethanol productivity in the fermentor, where the volume was constant at 12.4 l, the glucose conversion in the fermentor was determined separately from the overall glucose conversion and multiplied by the overall ethanol yield. The stripping-gas flow rate was calculated by the simulator using the Wilson equation (Chao and Greenkorn 1975) to approximate the non-ideal vapor/liquid equilibrium for ethanol and water. The simulator calculated the specific growth rate, μ , in the fermentor, defined by:

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

where X is cell mass. The results obtained from the simulation were fit by least-squares regression to the empirical model:

Table 1 Continuous fermentor/stripper measured variables

Data set no.	Gas temperature (°C)		Nutrient feed		Fermentor		Overflow		Condensate		
	Gas into column bottom	Gas from column top	Feed rate (ml/min)	Glucose (g/l)	Glucose (g/l)	Ethanol (g/l)	Yeast dry wt. (g/l)	Overflow (ml/min)	Ethanol (g/l)	Condensate (ml/min)	Ethanol (g/l)
1	38.4	38.3	10.81	561	1.01	46.5	32.2	8.44	39.1	8.85	299
2	38.4	36.8	11.48	535	0.62	49.7	32.0	9.72	42.1	8.56	316
3	39.6	36.8	11.36	570	1.50	51.4	28.8	9.85	43.5	8.92	325
4	38.2	37.1	11.54	578	0.76	51.6	30.9	9.02	43.7	8.83	325
5	39.4	36.4	11.28	589	1.63	52.6	28.9	9.75	44.6	8.82	330
6	38.6	35.3	10.70	567	0.41	52.7	28.8	9.52	44.6	8.23	329
7	39.2	35.4	10.77	569	0.83	55.0	27.6	9.66	47.3	7.82	340
8	38.2	35.3	11.09	548	1.47	55.7	25.2	9.73	48.5	7.96	343
9	38.1	35.4	10.56	580	1.72	56.0	25.6	9.06	48.8	7.98	344
10	39.9	35.4	12.23	606	2.99	56.7	27.8	10.99	48.4	8.63	342
11	38.9	35.9	11.16	559	1.22	58.2	28.0	9.75	50.0	8.15	352
12	39.4	35.1	11.45	569	3.06	60.1	26.0	10.39	52.0	7.92	361

Table 2 Continuous fermentor/stripper calculated variables

Data set no.	Stripping gas flow (kg/h)	Overall glucose conversion (g/h)	μ , specific growth rate in fermentor (h^{-1})	$Y_{X/S}$ cell yield (g/g)	$Y_{P/S}$ ethanol yield (g/g)	Ethanol productivity in fermentor ($\text{g l}^{-1} \text{h}^{-1}$)
1	11.49	364	.0401	0.0458	0.498	14.1
2	11.72	369	.0468	0.0518	0.514	14.9
3	11.97	389	.0457	0.0446	0.559	16.5
4	11.67	400	.0443	0.0438	0.497	15.5
5	12.01	399	.0455	0.0431	0.550	16.8
6	12.00	364	.0461	0.0459	0.522	15.1
7	11.04	367	.0460	0.0442	0.516	14.8
8	11.30	364	.0453	0.0409	0.535	14.9
9	11.21	367	.0420	0.0384	0.529	14.8
10	12.28	444	.0501	0.0412	0.477	16.1
11	10.87	374	.0450	0.0435	0.544	15.7
12	10.92	390	.0476	0.0421	0.526	15.4

$$\mu = \mu_{\max} \frac{S}{0.28 + S} \left(1 - \frac{P}{P_{\max}}\right) \left(1 - \frac{T - 35}{T_{\max} - 35}\right), \quad (2)$$

$$0 < P < P_{\max}, \quad 35 < T < T_{\max}$$

where S is the substrate (glucose) concentration (g/l), P is the product (ethanol) concentration (g/l), and T is the temperature ($^{\circ}\text{C}$) of the gas leaving the top of the stripping column. The glucose term is that of the commonly used Monod model (Luong 1985). The Monod constant, 0.28 g/l, was previously obtained by fitting data from a simple continuous fermentor (Taylor et al. 1995). The ethanol term is a linear model originally used by Hinshelwood (Luong 1985). The temperature term is linear, and similar to the ethanol term except that it contains a threshold temperature, the lowest temperature at which heating in the column begins to affect the specific growth rate in the fermentor. This was assumed to equal the maximum or optimum growth temperature for ATCC 4126, 35 $^{\circ}\text{C}$ (Cysewski and Wilke 1977). The maximum specific growth rate, μ_{\max} , as well as the maximum ethanol concentration, P_{\max} , and maximum stripping temperature, T_{\max} , are the constants to be determined by least-squares treatment of the data.

The ethanol production rate is linked to the specific growth rate through the cell and ethanol yield factors, $Y_{X/S}$ and $Y_{P/S}$, defined as the mass (g) dry cells and ethanol, respectively, produced per gram of glucose consumed. The cell yield results obtained from the simulation were fit by linear regression to the empirical model:

$$Y_{X/S} = mP + b \quad (3)$$

where m and b are the slope and intercept of the best straight line.

Results

Fouling and contamination

Figures 2 and 3 show the stability over time of the two runs from which the data in Tables 1 and 2 were ob-

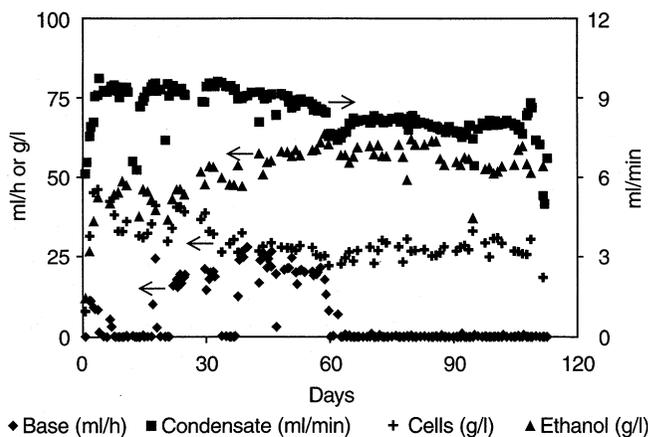


Fig. 2 Pilot-plant data for ethanol and cell concentrations in the fermentor, and for flow rates of condensate (approximately 45% ethanol by volume) from the condenser, and base (2 M ammonium hydroxide) to the fermentor. First run, column washing at 4- to 6-day intervals

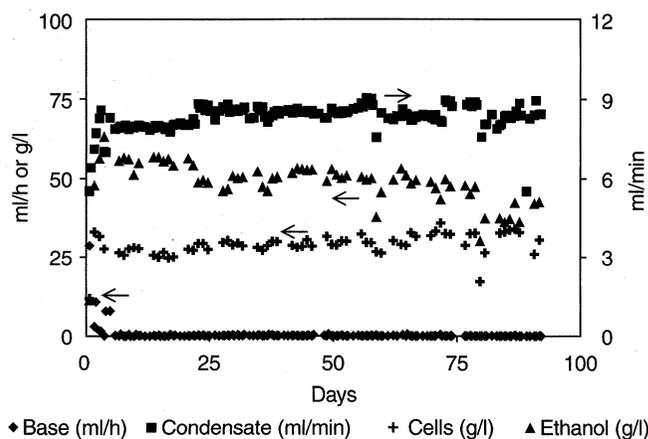


Fig. 3 Pilot-plant data for ethanol and cell concentrations in the fermentor, and for flow rates of condensate (approximately 45% ethanol by volume) from the condenser, and base (2 M ammonium hydroxide) to the fermentor. Second run, column washing at 3- to 4-day intervals, blower throttle closed

tained. During the first run (Fig. 2), the interval between consecutive column washings varied from 4 days to 6 days. During the second run (Fig. 3), the washing interval was every 3–4 days. The data suggest that increased frequency of washing was most effective. During the first run, the gradual increase in ethanol concentration from approximately 50 g/l to 60 g/l from 30–60 days correlated with the visual observation (by removing insulation from the glass column) of a gradual accumulation of attached yeast cells growing on the packing. Also, the dip in the curve for the condensate flow rate at 60 days correlated with a decrease in the stripping-gas flow rate from approximately 12 kg/h to 11 kg/h. Fouling of the column by attached yeast cells probably caused a slight increase in the pressure drop through the packing, which resulted in slightly less gas flow, and therefore slightly less condensate production. This, in turn, caused a slight increase in the ethanol concentration, which reduced the cell concentration from approximately 40 g/l to 30 g/l. Beyond 60 days, the data, along with visual observation, indicated that the fouling reached a steady state such that further attachment or growth of attached yeast cells was balanced by removal during washing. There was even some indication that the stripping-gas flow rate returned to approximately 12 kg/h at the end of the run. When the packing was removed for cleaning, a few small deposits of a filamentous fungal growth were observed. These were too small to have affected column performance, amounting to no more than approximately 100 ml in total volume. However, unlike the attached yeast cells, which could be removed by in situ washing, continued filamentous fungal growth may have eventually affected performance.

By contrast, during the second run (Fig. 3), when washing was more frequent, there was no evidence that fouling by attached yeast cells affected the performance of the column. Attached growth was visually observed, becoming heavy in several patches and individual pieces of packing. But, after the first 2–3 weeks, the fouled areas did not enlarge and most of the packing remained almost free of yeast cells. Moreover, filamentous fungal growth was not observed on the packing, even at the end of the run. The initial stripping gas flow rate was slightly less than in the first run because a throttle valve on the blower was closed for this run, simulating the effect of fouling on the gas flow rate. Thus the initial steady-state ethanol and cell concentrations were similar to values observed after 60 days in the first run. The higher initial ethanol concentration and lower initial cell concentration during this run may have helped to control fouling. In any case, the gas flow remained constant within experimental error throughout this run, most likely because of the increased frequency of washing.

Occasionally, contamination of the feed reservoir with fungi growing on the liquid surface, and with osmotolerant yeast was observed. This only happened when a batch of feed, which was sized to last approximately 2 weeks, was almost gone. It was necessary to

install a degasser on the feed line to remove carbon dioxide gas bubbles, produced by the contaminating yeast, which interfered with pumping at a constant feed rate. However, growth of the contaminants was very slow, and any decrease in the glucose concentration of the feed was within experimental error. Feed contaminants were not observed in the fermentor or column.

pH effect

As shown in Figs. 2 and 3, when the ethanol concentration in the fermentor was 50–55 g/l or less, addition of up to 25 ml/h 2 M ammonium hydroxide was required to maintain the pH at 3.6–3.7. Once a threshold value of approximately 60 g/l ethanol was reached, no further base was required, and the pH held at 3.6–3.7 without any need for pH control, even though the ethanol concentration subsequently fell to lower values later in the second run.

Fermentation rate

As shown in Table 2, the overall glucose conversion rate varied from 364 g/h to 444 g/h. The glucose concentration in the overflow (data not shown) varied from 0.1 g/l to 1.7 g/l, and the overall glucose conversion was always greater than 99%. The glucose conversion in the fermentor was always greater than 95% of the overall glucose conversion, and the ethanol productivity in the fermentor varied from 14.1 g l⁻¹ h⁻¹ to 16.8 g l⁻¹ h⁻¹. The ethanol yield was, within experimental error, constant and equal to the theoretical maximum, 0.51 g/g. The average of calculated values is 0.522 g/g, with a standard deviation of 0.024 g/g. Data for the specific growth rate, and for the column top temperature and the fermentor ethanol and glucose concentrations were fit to the empirical model described in Simulation and modeling. The best fit was obtained with a maximum specific growth rate of 0.233 h⁻¹, a maximum ethanol concentration of 76.0 g/l and a maximum stripping temperature of 43.04 °C. The complete model for specific growth rate is thus:

$$\mu = 0.233 \left(\frac{S}{0.28 + S} \right) \left(1 - \frac{P}{76.0} \right) \left(1 - \frac{T - 35}{8.04} \right), \quad (4)$$

$$0 < P < 76.0, \quad 35 < T < 43.04$$

Although mathematically valid within the limits specified above for P and T , and for any positive value of S , the model should not be used for extrapolation beyond the range of data. The range for ethanol is 46.5–60.1 g/l, and the range for temperature is 35.1–38.3 °C. To display graphically the agreement of the data with the model, the data for specific growth rate are corrected for glucose and temperature and plotted against ethanol in Figure 4. The line shown as “Model” in Fig. 4 is a plot of the above model with the glucose and temperature

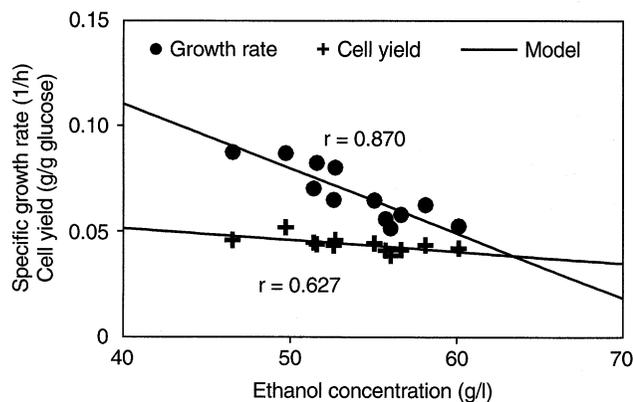


Fig. 4 Agreement of data with model. Specific growth rate (corrected for glucose and stripping temperature) and cell yield versus ethanol concentration

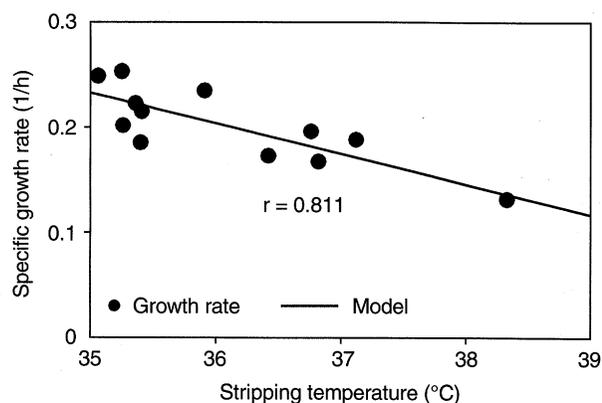


Fig. 5 Agreement of data with model. Specific growth rate (corrected for glucose and ethanol concentration) versus stripping temperature

terms set to 1. Similarly, in Fig. 5 are shown the specific growth rate data corrected for glucose and ethanol and plotted against temperature. The "Model" line in Fig. 5 is for the above model with the glucose and ethanol terms set to 1.

Data for the cell yield and ethanol concentration were fit to the linear empirical model described in Simulation and modeling. Best fit was obtained with m equal to -0.000546 and b equal to 0.0732 . The complete model for cell yield is thus:

$$Y_{X/S} = -0.000546(P) + 0.0732 \quad (5)$$

The agreement of the data with the model is shown in Fig. 4. Extrapolation significantly beyond the range of data is not recommended.

Discussion

Stable operation of a continuous fermentor with a packed column for stripping ethanol can be maintained

for at least 100 days. Washing the packed column with condensed ethanol from the process (approximately 45% by volume) at intervals of 3–4 days is more effective than at 4- to 6-day intervals, and minimizes the effects of fouling on the performance of the column. The productivity of the continuous fermentor described here is approximately ten times as much as that of a typical batch fermentor. The ethanol yield, 0.51 g ethanol/g glucose consumed, is also slightly higher than for a batch fermentor. The cell yield decreases with increasing ethanol concentration. The effects on growth rate and cell yield of ethanol concentration and stripping temperature are described by linear mathematical models within the range of data. Application of these models to the design and optimization of a full-scale plant, incorporating continuous fermentation and stripping, may reduce the cost of fuel ethanol production.

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