

Control of Packed Column Fouling in the Continuous Fermentation and Stripping of Ethanol

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By recycling the contents of a 14 L fermentor through a stripping column to continuously remove ethanol and reduce product inhibition, continuous complete conversion of nutrient feed containing 600 g/L glucose was achieved in a small pilot plant. Ethanol was recovered from the carbon dioxide stripping gas in a refrigerated condenser, and the gas was reheated with steam and recycled by a blower. Productivity of ethanol in the fermentor as high as 15.8 g/L/h and condensate production of up to 10 L/day of almost 50% by volume ethanol were maintained for up to 60 days of continuous operation. Weekly washing of the column packing in situ was required to prevent loss of performance caused by attached growth of yeast cells, which restricts the gas flow rate through the stripping column. © 1996 John Wiley & Sons, Inc. Key words: yeast • fuel ethanol • flocculation • glucose conversion

INTRODUCTION

The explosive growth of the U.S. fuel ethanol industry in the last 15 years has been made possible by federal and state tax incentives. Originally introduced as "gasohol," a 10% ethanol blend with gasoline, its value was seen primarily as a gasoline extender, to reduce dependence on imported petroleum while stimulating the U.S. economy, especially in underdeveloped rural areas. The value of ethanol as an antiknock additive to replace the lead formerly added to premium gasoline has also been recognized. With the introduction of reformulated gasolines in areas where winter air pollution has been a problem, fuel ethanol has now taken on its most valuable role as an oxygenated gasoline additive.

With U.S. fuel ethanol sales now well over \$1 billion/year and expected to increase, there is renewed interest in finding improvements in the ethanol production process. Ethanol production today is carried out in fermentors that in their basic design do not differ significantly from those used hundreds of years ago. The only improvement in industrial fermentors has been the recent introduction of continuous cascade fermentors, but these suffer from many of the same disadvantages as simple batch fermentors. The limited ethanol tolerance

of the yeast results in low productivity and large fermentors are required. The high initial investment for fermentors contributes significantly to the cost of producing ethanol.

Many researchers have concentrated their efforts on increasing the productivity of the fermentors. Through the years, many new fermentor designs have been proposed and tested at the laboratory scale. None have so far proven to be cost effective for industrial production, but two on-going efforts are showing promise. At Purdue University in West Lafayette, Indiana, the concept of an immobilized cell reactor-separator (ICRS) was developed.^{1,5} This process involves stripping of ethanol from a packed column of immobilized cells. To recover the ethanol from the gas phase, adsorption into an organic liquid followed by extractive distillation is under development. Solvent extraction coupled with extractive distillation was previously proposed for recovering ethanol from dilute fermentates.⁸ Extractive fermentation has been extensively investigated, and technology transfer is underway at Queen's University in Kingston, Ontario.^{2,3}

Recently, we proposed to improve fermentor productivity by recycling the contents of a continuous fermentor through an ordinary packed column to effectively strip ethanol from the fermentor.⁷ By removing ethanol as fast as it is produced, its concentration is kept below severely toxic levels, and high conversion of a concentrated glucose feed is possible. This, in turn, leads to high cell concentration and productivity in the fermentor. A pilot-scale packed column was assembled with a blower to recycle the carbon dioxide stripping gas through a condenser. The gas was reheated and rehumidified with steam before returning it to the packed column. Initial results from long-term continuous operation with a 1-L fermentor were reported.⁷ Fouling of the packed column with attached growth of yeast cells limited the gas flow through the column, but high conversion of 600 g/L glucose feed was achieved at a stripping rate of approximately 1 L of 50% (v/v) ethanol per day. The objective of the present work was to control the fouling of the packing and to obtain higher

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stripping rates in the same packed column using faster blower speeds and larger fermentor volumes.

MATERIALS AND METHODS

Unless otherwise stated, all materials and methods were the same as previously reported⁷ and shown in Figure 1. The fermentor was a 14-L glass jar Magnaferm (New Brunswick Scientific, New Brunswick, NJ). It was equipped with a mechanical foam breaker as well as automatic antifoam addition. The pH was controlled between 3.6 and 3.7 by automatic addition of ammonium hydroxide solution. Air was supplied to the sparger at approximately 150 mL/min. The fermentor was stirred at 225 RPM and the foam breaker rotated at 750 RPM. The suction side of the column feed pump was connected through the continuous cell settler (if present) to an adjustable dip tube in the fermentor. The fermentor working volume was controlled at one of four different levels: 4.0, 9.0, 11.0, or 13.4 L. The three lower levels were maintained by keeping the column feed pump faster than the recycle pump and adjusting the dip tube appropriately. The highest level was maintained by keeping the recycle pump faster than the column feed pump, causing the fermentor to fill all the way to the head plate. In this case, the recycle rate included some liquid that foamed out through the fermentor exhaust line to the top of the column. The foam breaker and automatic antifoam addition were not used in this case, but antifoam was added manually to control foaming in the column.

Temperature control of the fermentor was provided by heating at lower recycle rates and by cooling at higher recycle rates because the temperature in the column was slightly higher than in the fermentor. The original on-off temperature controller provided with the fer-

mentor was replaced with a more precise proportional controller. The continuous cell settler (if present) consisted of a 250-mL or 2-L separatory funnel. The suction side of the column feed pump (clarified cell suspension) and the fermentor dip tube were connected through a two-hole stopper in the top of the funnel. The suction side of the cell recycle pump (concentrated cell suspension) was connected to the bottom of the funnel. All pump tubes and connecting tubing were 1/4 inch i.d. Marprene (Watson-Marlow, Wilmington, MA).

The column feed heater consisted of a 250 Watt immersion heater inserted into the top of the column through a length of 3/8 inch stainless pipe threaded into the column top plate from below. The top of the immersion heater was sealed to a 3/8 inch stainless tee threaded onto the pipe just above the column top plate. A second 3/8 inch stainless tee attached to the first provided for connections for tubing from the column feed pump and for a thermistor from a proportional temperature controller that cycled the immersion heater on and off to control the column top temperature. The temperature of the stripping gas entering the bottom of the column was controlled by an air-operated control valve on the steam supply line. The valve position was set by a thermostat at the bottom of the column through a mechanical controller. The temperature controls on the column and fermentor provided for temperatures constant to within 1°C when the room temperature changed by 5°C or more.

The column was the same, 10 cm × 1.5 m, as previously described.⁷ The packing in the column was either 1 3/4 inch plastic Tellerettes (Ceilcote Co., Berea, OH) or 1-in. stainless Intalox (Norton, Akron, OH). The condenser was packed with 1-in. stainless Intalox. Condensate from the bottom of the condenser was recycled by a stainless centrifugal pump through a heat exchanger

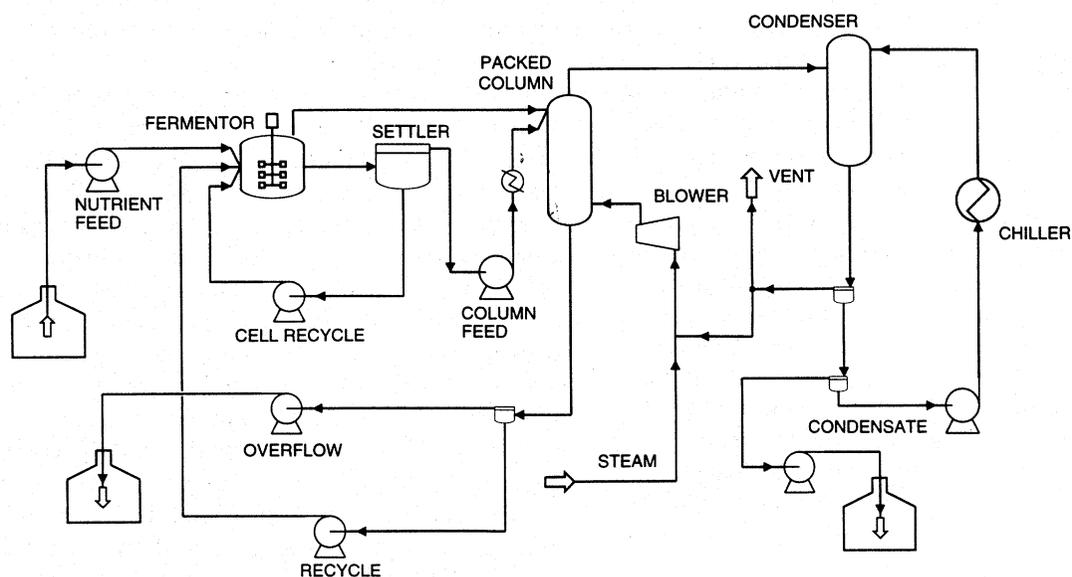


Figure 1. Process flow diagram for continuous fermentation and stripping of ethanol.

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 -28 to -30°C recycled through a chiller. The temperature of gas leaving the condenser was maintained at approximately -10°C .

The nutrient feed to the fermentor consisted of 600 g/L glucose and 120 g/L corn steepwater. Addition of Ca and Mg salts and ethylenediaminetetraacetic acid (EDTA) as previously described⁷ was found to be unnecessary and, in fact, caused precipitation of solids which clogged the feed pump lines. Glucose (dextrose, dry hydrate, approximately 10% water, Corn Products Corp., Franklin Park, IL) was dissolved in tap water and sterilized for 45 min. at 121°C . Corn steepwater (Grain Processing, Muscatine, IA) was sterilized and clarified with a continuous laboratory centrifuge (Sharples, Warminster, PA) before adding to the glucose solution to make nutrient feed solution. Removable parts of the centrifuge were sterilized in an autoclave, and the rest of the centrifuge was sterilized with 70% ethanol.

Between runs the fermentor and all connecting tubing were cleaned and sterilized. At the start of each new run, the fermentor was filled to the desired continuous level with tap water and autoclaved. The yeast ATCC 4126 (American Type Culture Collection, Rockville, MD) was maintained on agar slants kept in the refrigerator for up to 2 years. One liter of YM Broth (Difco, Detroit, MI) in a 2 L shake flask was inoculated with the resuspended contents of one slant. After shaking at 25 – 30°C overnight, the contents were used to inoculate the fermentor. At the time of inoculation, the nutrient feed pump was started slowly, then gradually increased over a period of several days to maintain complete conversion of glucose while the cell mass in the fermentor built up. During the first run, to increase the fermentor volume from 1.2 to 4 L, 3 L of sterile water in the 14 L fermentor was inoculated by aseptic transfer of 1.2 L from the 2 L fermentor previously described.⁷

Two methods were employed 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 returning it 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. For the last two runs, a wash pump was installed to clean the column packing in situ. The system consisted of a high-volume (several gallons per minute) centrifugal pump connected into a loop of 3/8 inch I.D. tubing from the bottom of the column to the top. The loop was initially sterilized with 70% ethanol; then once a week during the last two runs, the blower and fermentor recycle pumps were turned off, and approximately 12 L of sterile water with antifoam was recycled through the packed column for approximately 30 min.

Measurement of flow rates and sampling and measurement of glucose, ethanol, and cell concentrations were the same as previously described,⁷ except that for higher cell concentrations the samples were diluted 1 to 100 or 1 to 200 instead of 1 to 20 before measurement of optical absorbance. The glucose concentration of each batch of nutrient feed was checked after diluting 1 to 200. The data were always within 5% of 600 g/L. As before, ethanol concentration in the fermentor was measured both on-line and by sampling, but only the data from samples are reported here.

The averages of data from selected time periods of from 2 to 8 days when the system was judged to be operating at steady state were used as inputs to a computer simulation developed with Aspen Plus, release 8.5-6 with Bioprocess Simulator (Aspen Technology, Cambridge, MA). For some simulation results, such as the overall glucose conversion and cell yield, the results were no different than those obtainable by simple calculation on the feed and product (overflow) glucose and cell concentrations. To obtain the ethanol yield, the simulation corrected for small losses of ethanol in the steam condensate and carbon dioxide vent which were not measured. The ethanol productivity was calculated by subtracting the glucose conversion in the column from the overall glucose conversion, multiplying by the ethanol yield, and dividing by the sum of the fermentor and settler volumes. The ethanol productivity was thus an average productivity for the fermentor and settler, ignoring the ethanol produced in the column. The theoretical maximum specific growth rate, μ_{max} , was calculated by the simulator only for the fermentor assuming adherence to the empirical formula:

$$\mu = \mu_{\text{max}} e^{-0.033p} \frac{S}{0.28 + S}$$

where p is product (ethanol) concentration and S is substrate (glucose) concentration. This formula was previously obtained by fitting data from a simple continuous fermentor.⁷ These data also gave a value for the theoretical maximum specific growth rate, $\mu_{\text{max}} = 0.51 \text{ h}^{-1}$. The above formula was valid only for ethanol values up to 60 g/L. At higher values, the actual growth rate was less than predicted.⁷ The use of this empirical formula was only for comparison to the previous work and does not imply its validity, which could not be verified from these limited data. In the simulation, the stripping gas flow rate was determined from input temperatures, ethanol concentrations, and flow rates using Wilson coefficients to model the vapor-liquid equilibrium data for ethanol and water.

RESULTS AND DISCUSSION

Fouling

Averaged data from selected time periods when the system was judged to be operating at steady state are

Table I. Continuous fermentor/stripper operating variables.

Run	Packing	Days from inoculation	Fermentor volume (L)	Settler volume (L)	Nutrient feed (mL/min)	Recycle (mL/min)	Settled cell recycle (mL/min)	Fermentor temperature (°C)	Gas into column bottom (°C)	Gas from column top (°C)	Blower speed (RPM)
1	Plastic	108-112	1.2	0.25	1.51	37	34	33.2	30.6	33.5	100
		136-137	4.0	0.25	1.35	43	36	34.2	35.7	33.0	100
		147-148	4.0	0.25	1.73	45	35	30.0	39.0	33.8	100
		203-206	9.0	2.00	2.78	86	59	29.2	44.9	36.1	290
2	Plastic	35-39	13.4	2.00	7.94	425	123	29.1	40.9	32.6	290
		84-88	13.4	2.00	3.19	433	125	31.6	40.2	34.5	290
3	Stainless	15-19	13.4	0.00	8.02	441	0	30.5	40.7	33.2	440
4	Stainless	21-28	11.0	0.00	5.27	250	0	29.8	40.1	32.7	440
5	Plastic	31-35	11.0	0.00	9.54	218	0	33.3	38.1	34.5	440

shown in Tables I-III for five consecutive runs numbered 1-5. Run 1 is the continuation of the first continuous fermentor/stripper run, earlier data for which were previously reported.⁷ This run lasted a total of 215 days. At 108 days, the earliest point included in the data reported here, the column was already fully fouled. Any further attachment of yeast cells to the packing or growth of cells already attached to the packing was balanced by sloughing of clumps of attached cells from the packing. This fully fouled condition was accompanied by a transition of the entire culture to a flocculating state.

Floc particles observed in samples were not simply clumps of cells sloughed from the packing, but were true floc particles resulting from flocculation of individual suspended cells. These floc particles could be broken up by rapid swirling, but re-formed quickly on standing. Furthermore, the flocculating characteristic was genetically transmitted to a limited number of subsequent generations of cells. When a flask containing 200 mL of sterile YM medium was inoculated with 1 mL from the continuous fermentor/stripper and grown overnight, the result was a flocculating culture. However, when 1 mL from this flask was transferred to fresh YM medium and grown overnight, the flocculating characteristic was lost.

Most yeast are nonflocculating during exponential growth but flocculate to varying degrees in the stationary phase. In the brewing industry, flocculation during the stationary phase is generally desirable because it aids in the separation of yeast from the beer. Flocculation is a very complex process that may be induced or inhibited by various soluble factors in the beer.⁶ Genes that confer flocculating ability have been identified and are different in top- and bottom-fermenting strains.⁴ Highly flocculent strains that flocculate during the exponential phase have been isolated. Flocculation in ATCC 4126 has not been described, and flocculation was never observed in YM medium even several days into the stationary phase, except when inoculated from the fouled fermentor/stripper as described above.

To check whether flocculation was induced by some soluble factor, YM broth was made using centrifuged overflow from the fermentor/stripper instead of water. After filter sterilization and inoculation from a slant, ATCC 4126 grew normally without flocculation even after several transfers into this medium. Therefore, flocculation in the continuous system was not caused by a soluble factor but probably by some physical characteristic of the fermentor/stripper environment, possibly simply physical contact with the packing in the column.

Table II. Continuous fermentor/stripper observed response variables.

Run	Column condition	Fermentor			Overflow				Condensate	
		Glucose (g/L)	Ethanol (g/L)	Yeast dry wt. (g/L)	Overflow (mL/min)	Glucose (g/L)	Ethanol (g/L)	Yeast dry wt. (g/L)	Condensate (mL/min)	Ethanol (g/L)
1	fouled	95.9	47.3	22.0	1.07	82.1	46.9	8.2	0.75	338
	fouled	71.1	51.5	20.2	1.20	63.9	49.9	5.2	0.64	343
	fouled	98.8	61.3	18.3	1.57	89.8	59.0	6.2	0.62	372
	fouled	70.6	59.0	24.3	2.68	68.1	58.2	5.9	0.93	416
2	clean	2.8	76.9	18.1	8.12	1.8	73.7	17.3	4.51	367
	fouled	18.5	72.0	10.7	3.12	17.0	70.9	7.3	1.45	370
3	clean	22.0	45.3	24.9	8.44	20.9	42.6	24.1	5.29	300
4	clean	0.3	42.1	32.7	5.65	0.1	36.9	32.2	4.99	271
5	clean	2.2	64.7	30.4	8.19	1.9	55.5	25.3	6.83	361

Table III. Continuous fermentor/stripper calculated response variables and simulation results.

Run	Stripping gas flow (kg/h)	Overall glucose conversion (g/h)	Overall glucose conversion (%)	Glucose conversion in column (g/h)	Fermentor μ_{\max} (h^{-1})	Cell yield, $Y_{X/S}$ (g/g)	Ethanol yield, $Y_{P/S}$ (g/g)	Ethanol productivity (g/L/h)
1	1.3	49	90.4	34	0.16	0.011	0.49	5.8
	1.3	44	90.5	21	0.07	0.008	0.49	2.7
	1.1	53	86.5	26	0.16	0.011	0.48	3.1
	1.3	89	89.1	14	0.25	0.011	0.45	3.1
2	7.9	276	99.7	10	0.49	0.031	0.51	8.8
	2.6	108	97.1	18	0.26	0.013	0.49	3.0
3	9.4	258	96.1	30	0.20	0.046	0.48	8.1
4	8.5	190	100.0	4	0.18	0.057	0.50	8.4
5	9.5	347	99.7	3	0.42	0.036	0.51	15.8

The possibility of contamination of the system with flocculent strains of wild yeast cannot be ruled out. However, the fact that the flocculent yeast reverted to non-flocculent after two transfers to YM broth makes contamination an unlikely explanation.

Whether fouling is caused by flocculation or flocculation is caused by fouling, the effects on the packed column and continuous fermentor/stripper are the same. Fouling limits the stripping gas flow rate at constant blower speed, thus limiting the stripping rate, and in turn the fermentation rate. As shown in Tables I–III, when the fermentor volume was increased from 1.2 to 4 L, there was little change in the overall glucose conversion, and the ethanol productivity decreased from 5.8 to 2.7 g/L/h. Similarly, increasing the settler volume from 0.25 to 2 L, increasing the fermentor volume from 4 to 9 L, and increasing the blower speed from 100 to 290 RPM had little effect on the overall glucose conversion. A dramatically increased rate of glucose consumption was observed when the second run was started with clean packing. These results show that, during the first run, the performance of the system was severely limited by fouling of the packed column with attached growth of yeast cells, which restricted the gas flow rate.

Although the blower speed was not increased between the first and second runs, the stripping gas flow increased from 1.3 to 7.9 kg/h because of unrestricted flow through the cleaned packing. The gas flow is a simulation result. A more direct indicator of the gas flow is the condensate flow. If the gas temperatures in and out of the condenser and the ethanol concentration remain constant, then the condensate flow is directly proportional to the gas flow. The condensate flow or stripping rate increased to 4.5 mL/min (6.5 L/day) of 367 g/L (almost 50% by volume) ethanol when the packing was cleaned before the start of the second run. Also, the overall glucose conversion increased from 89 to 276 g/h and from 89.1 to 99.7% partly because the fermentor volume increased from 9 to 13.4 L. Because the settler was not effective on nonflocculating cells, the

cell concentration in the fermentor decreased from 24 to 18 g/L even though the cell yield increased from 0.01 to 0.03 g/g. However, the productivity increased from 3.1 to 8.8 g/L/h.

The general operating strategy for all the runs was to manually adjust the nutrient feed rate to match the observed fermentation rate, so that a high percent conversion and low but measurable glucose concentration in the overflow would be achieved. When the performance of the system dramatically improved at the start of the second run, the feed rate was slowly and incrementally increased, and it was not until more than 30 days into the run that a steady state with measurable glucose concentration in the overflow was achieved. Because this glucose concentration was a required input to the simulator, the data could not be formally analyzed and reported before the 35–39-day time period, as shown in Table I. On day 47 of the second run the power was out for 10 h. When the system was restarted, the samples were observed to be flocculating. No flocculation had been observed in previous samples of this run.

Fouling of the column had been observed from the first week visually through the glass walls by temporarily removing the column insulation. The packing became unevenly but progressively more fouled by thickening layers of attached yeast cells, but the performance of the system was unaffected until after flocculation was observed. Then there was a gradual and steady decrease in the performance. Averaged data for the 84–88-day time period during this decline are shown in Tables I–III. The stripping rate eventually decreased to the fully fouled value of the first run and leveled off. After 106 days the system was shut down and cleaned.

For the third run, new stainless steel Intalox packing was installed in the column. Because the settler effectively concentrated only flocculating cells, and because it was desired to somehow prevent the fouling and flocculation, the settler and cell recycle pump were removed from the system following the second run. Also, the blower speed was increased slightly, but this only compensated for the increased pressure drop of the smaller

packing, so that the clean performance was approximately the same as the second run. It was hoped that the stainless steel would be more hydrophilic than the plastic so that the yeast cells would not adhere to it as readily. However, this did not turn out to be the case. Again, fouling was observed to gradually accumulate, but without affecting the stripping rate until flocculation was observed. This time there was no power outage, but there was a close proximity between the time that flocculation was first observed and the time that the performance started to decrease. In run 3 this happened on approximately day 31. This was actually sooner than in run 2, probably because of the size of the packing. The smaller stainless packing had less void space to fill with attached cells, so the space was filled sooner. Only the clean packing performance is shown in Tables I–III. Run 3 was continued for a total of 39 days.

For run 4 the in situ column washing system was installed. This technique proved to be quite effective. Although the weekly washing did not restore the packing to absolute cleanliness, it did remove the worst fouling and prevent the onset of flocculation and concomitant loss of performance, or at least delayed it beyond the 31 days of run 3. Compared to run 3, the overall glucose conversion was lower because the fermentor volume was reduced from 13.4 to 11 L, but the cell yield (0.057 g/g) and fermentor cell concentration (32.7 g/L) were higher because the ethanol concentration was kept low by the increased ratio of stripping rate to fermentor volume. Cell yield was previously reported to be an inverse function of ethanol concentration.⁷ This run was operating at a steady rate when it was shut down after 37 days to re-position the washing nozzle at the top of the column. During washing, wash water was splashing over to the condenser where it diluted the condensate temporarily. This was a problem because it raised the freezing point of the condensate above the temperature in the chiller, freezing the condensate in the heat exchanger and interrupting the operation of the system.

For run 5, the plastic packing was reinstalled. The stripping rate was almost 10 L/day of almost 50% by volume ethanol. This was the highest yet observed because the combination of higher blower speed and larger packing produced a higher gas flow than with the smaller packing. As shown in Figure 2, the cell mass in the fermentor built up to almost 50 g/L within the first 100 h of run 5 and held constant while the nutrient feed rate was gradually increased and the glucose in the overflow remained below the detection limit. Between 400 and 900 h the ethanol concentration continued to increase, causing the cell yield and concentration to decrease. When the glucose concentration finally increased to a measurable value at 900 h, it continued to increase and the feed rate was decreased to keep it low. The constant condensate flow shown in Figure 2 indicates there was no effect of fouling on the stripping gas flow rate. At the time of writing, run 5 had continued

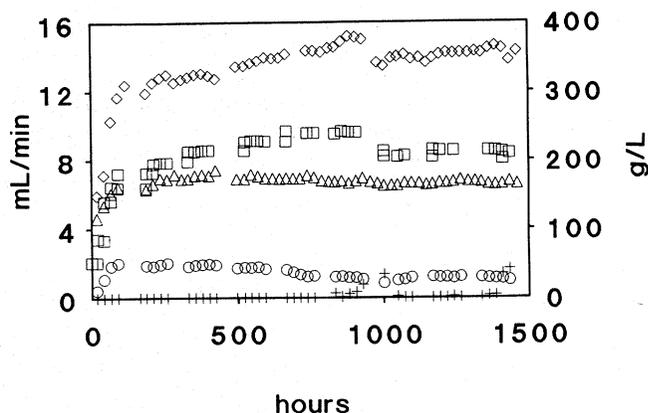


Figure 2. Data for run 5: (□) mL/min nutrient feed; (△) mL/min condensate; (◇) g/L ethanol in condensate; (○) g/L cell dry weight in fermentor; (+) g/L glucose in fermentor.

to operate for 60 days, well beyond day 47, when the plastic packing performance had begun to decline in run 2. The ethanol productivity had continued at approximately 15 g/L/h. At the time of writing there had been no flocculation in the samples and no decrease in the stripping rate.

During run 5, the pressure rise across the blower, which is equal to the combined pressure drop through the column and condenser, was approximately only 1 in. of water. It will be possible to increase the blower speed as much as 10-fold. It therefore seems likely that much higher stripping rates can be achieved and sustained in this column. The results presented here have shown that during future scale-up it is important to maintain the ratio of stripping gas flow rate to fermentor volume above some minimum value in order to achieve high productivity in the fermentor. While the optimum ratio will be determined by overall cost analysis, these data indicate the need for approximately 1 kg/h of stripping gas per liter of fermentor volume, or approximately 1 CFM per gallon.

Temperature and pH Effects

Although the effect of fouling was the primary focus of this work, several other inconclusive observations can be made. The equivalent of the dilution rate for a simple continuous fermentor is determined for continuous fermentation and stripping by the ratio of the overflow rate, not feed rate, to fermentor volume. The overflow rate is generally somewhat less than the feed rate because the ethanol is stripped out. The precise overflow rate is controlled by the gas temperatures in and out of the column. Because the gas is saturated with water vapor at both points, a higher inlet gas temperature results in a net condensation of water in the column and a higher overflow and dilution rate, while a higher outlet gas temperature results in a net evaporation of

water in the column and a lower overflow and dilution rate. This effect can be seen in comparing runs 4 and 5 in Tables I–III. The gas inlet temperature decreased from 40 to 38°C while the gas outlet temperature increased from 33 to 35°C. As a result the overflow rate was slightly higher than the feed rate for run 4 but significantly less than the feed rate for run 5. This effect may have contributed to the high productivity of run 5 by concentrating the cells in the fermentor. However, a low overflow rate could also cause the accumulation of toxic feed components or yeast byproducts, inhibiting the growth rate and productivity.

As shown in Table I, the fermentor temperature was approximately 30°C during most of the runs, but at the beginning of run 1 and for run 5, the fermentor temperature was 3–4 degrees higher. Although there were too many other variables to make a definite conclusion, the data seem to indicate that there was improved performance at the lower temperature during run 1 but the higher temperature was better later. Because the main difference was that the culture was flocculating during run 1 but nonflocculating during runs 4 and 5, one may tentatively conclude that the flocculating culture has a lower optimum growth temperature.

The quantity of base addition required to maintain the pH above 3.6 was not measured. However, it was observed that during each run, after the first few weeks of continuous operation, little or no base addition was required, the pH holding on its own at approximately 3.6–3.8. During run 5 this transition occurred at approximately 900 h. As previously discussed,⁷ cell death and autolysis can reduce or eliminate the requirement for base addition, and lower cell yields at higher ethanol concentrations are probably due to cell death and autolysis. Therefore, it appears that once the ethanol concentration builds up to a sufficiently high level, further addition of base to maintain pH may be unnecessary.

CONCLUSIONS

Fouling of a packed column with attached growth of yeast cells affects the performance of a continuous fermentation and stripping process by restricting the gas flow through the column. Severe fouling was accompanied by the appearance of a flocculating characteristic in ATCC 4126. Performance of a fully fouled column was restored after removing and cleaning the packing. With in situ washing, a stripping rate of almost 10 L/day of almost 50% by volume ethanol was maintained for 60 days, sustaining ethanol productivity of 15 g/L/h in the fermentor. It is likely that significantly higher rates and productivities can be similarly maintained if only the blower speed and feed rate are increased.

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