0273-2289/00/84–86/0543/$14.25

Coproduction of Ethanol and Glycerol

CHENG S. GONG, JIAU X. DU,
NING J. CAO, AND GEORGE T. TSAO*

*Laboratory of Renewable Resources Engineering,
Purdue University, West Lafayette, IN 47907-1295*

Abstract

Ethanol and glycerol are both metabolic products of yeasts. There are occasions when coproduction of both is considered desirable in industrial operations. In this article, we describe the potential of integrating the two processes. A LORRE Y8 yeast culture isolated from molasses is capable of efficient glycerol production from glucose, and a yeast Culture 1400 is an excellent producer of ethanol. By controlling the process conditions, the ratio of ethanol and glycerol production can be varied.

Index Entries: Glycerol; ethanol; coproduction; yeast fermentation.

Introduction

Normally, glycerol has been considered an undesirable byproduct of ethanol fermentation. In the early 1990s, the price of grain corn was high and more than $3.00 per bushel, many of the producers stopped their ethanol operation because of reduced profit margins. Meanwhile, according to a report by the US Department of Agriculture (USDA), the demand for glycerol has been growing by 30 million lb/yr *(1)*. For many years, glycerol was sold at $0.80 to $0.85/lb. In 1995–1996, because of the increased importation of glycerol into developing countries such as China and India, the price of glycerol went up to as high as $1.20/lb. There were intensified interests in increasing the yield of glycerol in an ethanol operation to obtain better byproduct credits when the profit margin for ethanol was low.

Currently, the price of glycerol has been reduced to $0.55 to $0.60/lb *(2)*, a result of pressure by the currency crisis in Asian countries. The same currency crisis has also reduced the export of corn from the United States. Together with worldwide good farm harvests, the price of grain corn has also been depressed to as low as $1.80 per bushel. Although the price swings are beyond the control of technical persons, developing the necessary

---

*Author to whom all correspondence and reprint requests should be addressed.

processes to coproduce glycerol and ethanol could and should be investigated to prepare for future economical fluctuations.

Glycerol has more than 1000 uses *(3)*. Currently, there are only two methods of industrial production for glycerol: by chemical synthesis; and by isolation as a byproduct from the soap and fatty acid industry, which is dominated by the Asian countries because of the large local supply of palm kernel oil. In this article, we describe a yeast fermentation using cornstarch as the raw material, which has the potential to become the new and third industrial production process for glycerol. Once successfully developed, this will be an important new process of manufacture of a large volume, value-added product from agricultural crops. Glycerol is also the raw material for producing 1,3-propanediol *(4)*, of which there are strong industrial interests for its use in the synthesis of new polyester materials. The fermented beer-containing glycerol can be directly used as a feed stream to a bacterial fermentation batch producing 1,3-propanediol.

An excellent glycerol-producing *Saccharomyces* yeast culture has been isolated from sugar molasses. *Saccharomyces* yeast cells generally are considered safe, and the cells harvested as a byproduct of glycerol fermentation can be marketed as a feed or food additive for better economical returns. This yeast has been temporarily named LORRE Y8. In batch processes, our results show that this yeast can produce 1 lb of glycerol from 2.0 lb of cornstarch leaving no glucose residue. The culture does not require any special expensive nutrient additives. In a batch process, Y8 can consistently provide a productivity of 3.6 g of glycerol/(L of fermenter volume·h) with a high productivity of 4.7 g/(L·h) occasionally observed. Based on experimental results, Y8 can produce a final fermentation broth containing >250 g/L of glycerol.

For increasing the production efficiency of ethanol from glucose, a novel fermenter system called ALSA (airlift with a side arm) has been tested with simultaneous ethanol removal by gas stripping. Recycled CO_2 from ethanol fermentation is compressed and sparged into ALSA to vaporize ethanol from the liquid beer. The CO_2 flow carries ethanol vapor exiting the fermenter. After compression and cooling, liquid ethanol at about 23% is recovered as the condensate and the gaseous CO_2 can be further recycled. An ALSA system is also well suited for glycerol fermentation in which air is sparged into the fermentor to provide dissolved oxygen to support conversion of glucose into glycerol. Here, an airflow replaces the CO_2 flow used in the ethanol batches. Fermentation of ethanol and fermentation of glycerol can be integrated into one overall processing system through the use of a common ALSA reactor.

Two Current Methods of Glycerol Production

Two current methods of glycerol production are chemical synthesis, and hydrolysis of fats and oils. Figure 1 shows a variety of processes for synthesizing glycerol taken directly from the *Kirk-Othmer Encyclopedia of Chemical Technology (3)*. Allyl alcohol is an intermediate in the synthetic

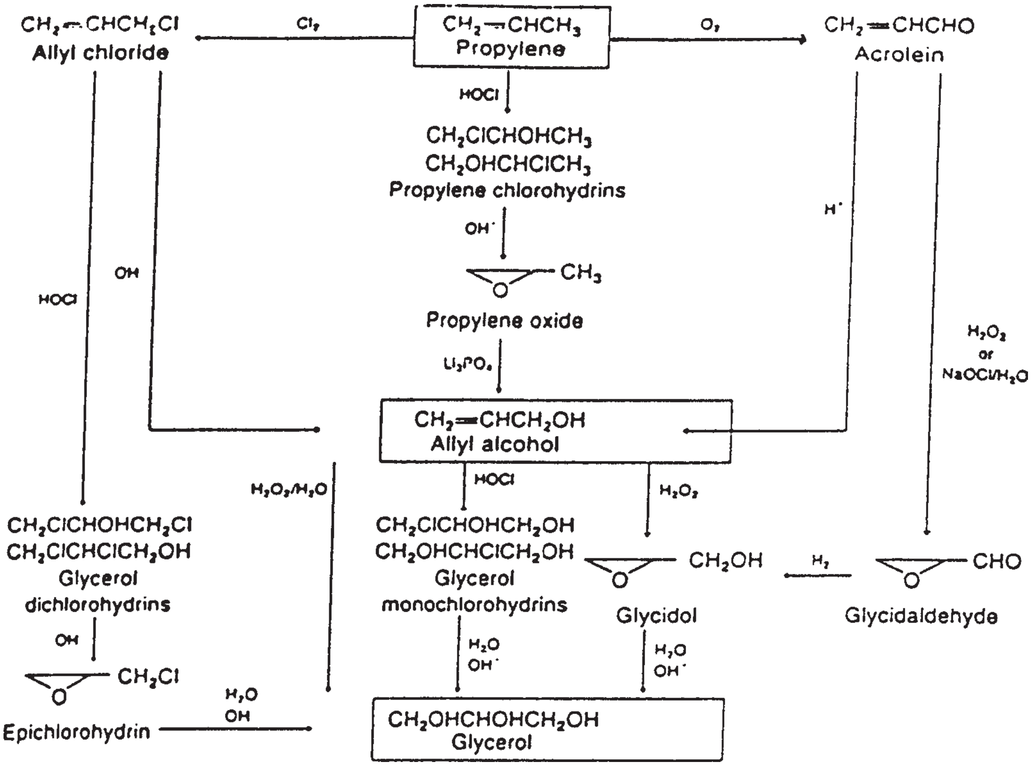

Fig. 1. Chemical synthetic routes for the manufacture of glycerol. (Reproduced from ref. *3*).

network. Currently, allyl alcohol is cited in *Chemical Market Reporter (2)* at \$1.00/lb, whereas glycerol is only \$0.50 to \$0.60/lb. For plants already fully depreciated, glycerol production by chemical synthesis might still be in operation. It is hard to justify new production capacity of glycerol by chemical synthesis involving an intermediate valued higher than the final product.

The best-known industrial process involving the hydrolysis of fats and oils is that for manufacturing soap. On hydrolysis, each molecule of a fat or an oil will yield 1 mol of glycerol with a mol wt of 92 and 3 mol of fatty acids with an average mol wt of 266. In other words, for every pound of glycerol from the hydrolysis, there is coproduction of about (266 × 3)/92 = 8.67 lb of fatty acids. Glycerol is recovered from the hydrolysates of fats and oils as a byproduct. First, the dilute hydrolysate is evaporated to remove water. Glycerol is then purified and recovered by vacuum distillation. As a byproduct, it is difficult to increase its production capacity without disturbing the balance between the supply and demand of the main products: soap and fatty acids. If the increased annual demand of about 30 million lb *(1)* of glycerol worldwide is to be met by this hydrolysis route, there must be an increased demand of soap and fatty acids of more than 200 million lb/yr.

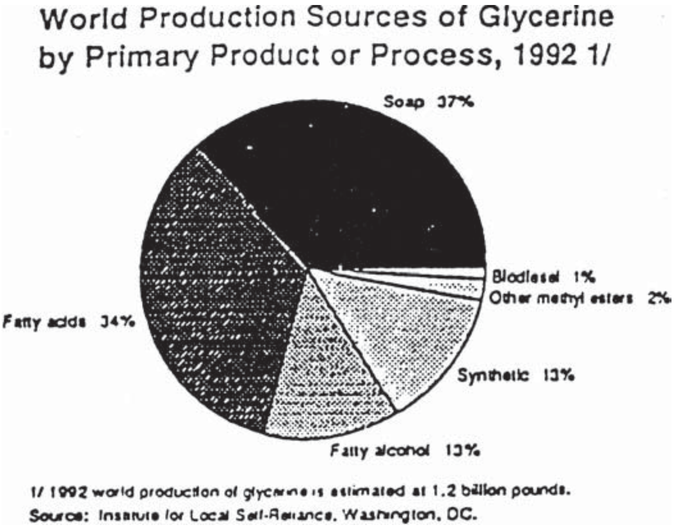

Fig. 2. World production sources of glycerine by primary product or process. In 1992, world production of glycerine was estimated at 1.2 billion lb. *Source*: Institute for Local Self-Reliance, Washington, DC *(1)*.

In view of the limitations of the two current methods of production, a new fermentation method for glycerol should be considered highly desirable for the future development of the bioprocessing industry.

Supply and Demand of Glycerol and Its Economics

The *Kirk-Othmer Encyclopedia of Chemical Technology (3)* states that "glycerol is used in nearly every industry." The largest use is in drugs and oral care products including toothpaste, mouthwash, and oral rinses. Other major users include tobacco, cosmetics, food, urethane polymer, wrapping and packaging material, lubricant, leather, adhesive, and soap and detergent industries. A potentially large volume of use is the conversion of glycerol into 1,3-propanediol, which is the feedstock for synthesis of new polyesters *(4)*.

According to a USDA report *(1)*, the annual production for glycerol worldwide in 1992 was about 1.2 billion lb, as shown in Fig. 2. The annual consumption in the United States was estimated to be 300 million lb, as shown in Fig. 3. Table 1, taken from the same USDA report, provides additional information on various glycerol uses. The report states that "a 2- to 3-percent annual growth is expected through 1997. If glycerol prices fall to 1991 levels, annual demand could grow 5 to 6 percent."

Because of recent economical growth, the living standards in China and India have been improving. The demand of glycerol for the manufacture of cosmetics, toothpaste, and tobacco in those countries has been growing rapidly. Consequently, in 1995–1996, the glycerol price jumped from its usual price of $0.80 to 0.85/lb to $1.10 to 1.20/lb. Because the fermentation

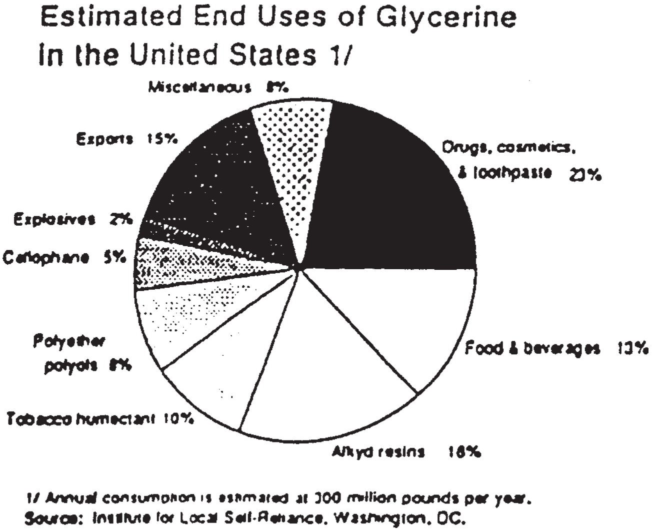

Fig. 3. Estimated end uses of glycerine in the United States. Annual consumption was estimated at 300 million lb/yr. *Source*: Institute for Local Self-Reliance, Washington, DC *(1)*.

glycerol industry does not yet exist and the chemical synthetic glycerol industry cannot grow easily as described above, the supply of glycerol has been met mostly by the industrial processors in Malaysia and Indonesia, where the abundant supply of palm kernel oil provides glycerol through the hydrolysis route. A recent economical crisis in Asia has drastically devalued the currencies in those countries. Consequently, the price of glycerol, traded in US dollars, has dropped to $0.55 to $0.60/lb. In a way, this is a blessing in disguise, because the current low price forces the processors to look for much improved efficiency to cut costs. Once the Asian crisis is over in 2 to 3 yr, as estimated, and the price of glycerol returns to its normal level of $0.85/lb, the fermentation process will likely become the single dominating supply source of glycerol.

ALSA Fermentor System

The use of a novel reactor called ALSA will allow an increased fermentation rate. An ALSA fermenter can be easily designed to operate in a batch, fed-batch, or continuous mode. Figure 4 is a photograph of such a unit used in ethanol-yeast fermentation. The construction is very simple: one or two side arms attached to an air-lift unit. In the main body, there is a high level of turbulence. A side arm, away from the vertical motions, provides a relatively calm zone where yeast cells can settle. In Fig. 4, there is a clear liquid layer above the cell-heavy broth. The clear liquid will exit and leave cells behind to build up a high cell population. The length and the location of the side arm can be adjusted to create sedimentation of cells to achieve good cell retention. Not shown in Fig. 4 is another side arm that can also be

Table 1
Industrial Uses of Glycerol[a]

Product category	Uses
Food and beverage	Humectant, solvent, sweetener, and preservative
Pharmaceuticals	Solvent, moistener, humectant, and bodying agent in tinctures, elixirs, ointments, and syrups; plasticizer for medicine capsules; suppositories, ear infection remedies, anesthetics, cough remedies, lozenges, gargles, and carrier for antibiotics and antiseptics
Cosmetics and toiletries	Humectant, vehicle, and emollient in toothpaste, skin creams and lotions, shaving preparations, deodorants, and makeup
Tobacco	To keep tobacco moist and soft to prevent breaking and crumbling during processing; to ensure freshness in packaged cigarettes and other tobacco products
Surface coatings	In the manufacture of alkyd resins, which are an important component of surface coatings
Paper and printing	Plasticizer, humectant, and lubricant in the manufacture of paper; used with other ingredients in specialty treatments such as grease-proofing; alkyd resins also an important constituent of many priming inks
Lubricants	Because of its nontoxic character, used in lubricants for food and other machinery in which product purity is essential
Textiles	Widely used conditioning agent in lubricating, sizing, and softening yarn and fabric; lubricates many kinds of fibers in spinning, twist setting, knitting, and weaving operations
Rubber and plastics	Lubricant and plasticizer for plastic
Urethane polymers	Fundamental chemical component of polyethers for urethane foams
Electrical and electronics	In the manufacture of electrolytes for electrolytic condensers, which are used in radios and neon lights, and in processes for electrodeposition and treatment of metals
Nitration	To make nitroglycerine, which is the usual explosive in dynamite and a cardiovascular agent

[a] Source: *Glycerine: An Overview*, Soap and Detergent Association, Glycerine and Oleochemical Division, New York, NY, undated.

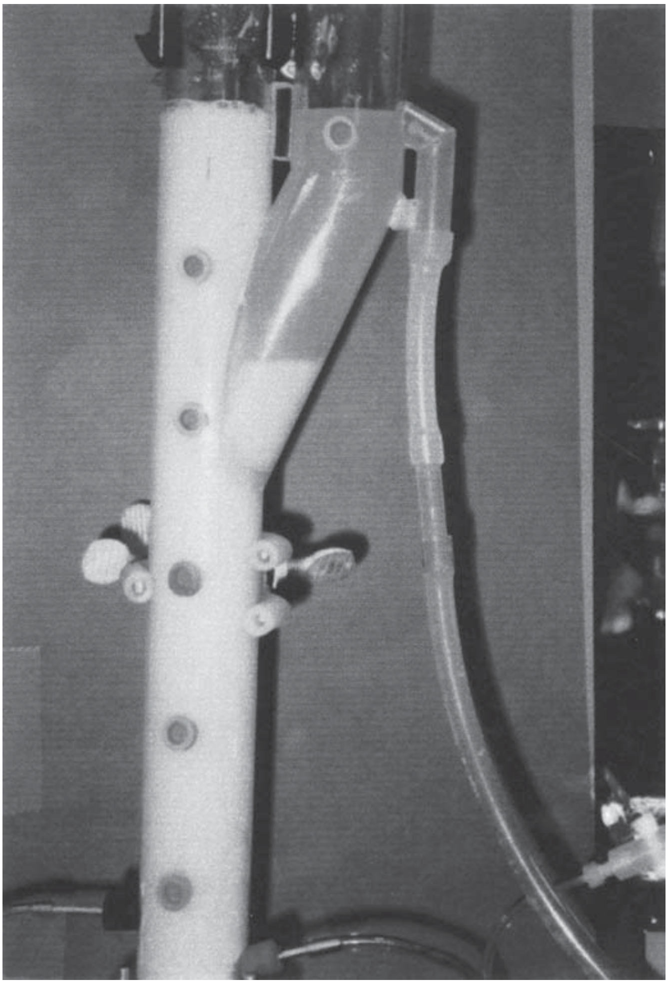

Fig. 4. Photograph of an ALSA (clear liquid in the side arm to exit).

attached to achieve another useful function. In this case, the opening of the side arm is attached to the main air lift at a higher position so that the liquid together with the foam layer on top can be circulated into the side arm, as shown in Fig. 5. When the fluid is flowing downward in the annular space (see the arrows in Fig. 5), its velocity is relatively fast. According to the Bernoulli theory *(5)*, the pressure should be low in the annular space in the main body. This creates a draft and enhances the downward motion of the fluid from the side arm to be back-mixed into the main body. This simple setup helps circulation, and, more important, it acts effectively as a foam breaker, reducing or eliminating the need of adding an antifoaming agent. Antifoaming agents are surface active, and they usually coat the gas-liquid interface and perhaps even the microbial cell surface, hindering material transport. Without the surface-active antifoaming agents, interracial mass transfer between the liquid and the gas bubbles is usually enhanced.

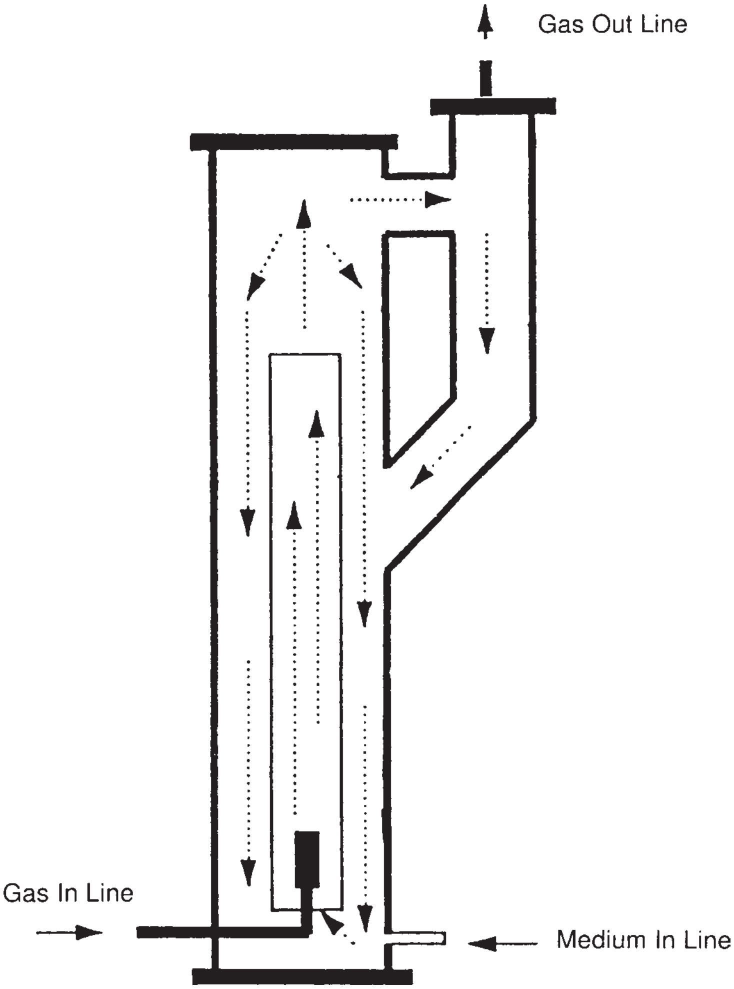

Fig. 5. Diagram of an ALSA showing liquid circulation pattern.

Side arms are inexpensive to install but they can serve rather useful purposes in fermenter designs. A well-designed ALSA has one side arm for cell sedimentation and liquid exit and another side arm for foam breaking. ALSA is much less expensive than an agitated-sparged fermenter in the initial investment and in the consumption of electricity because no mechanical agitator is used.

If the exit liquid from an ALSA system still contains residual glucose, a multiple-stage continuous system can be installed to, complete the sugar consumption. For glycerol production, it is important to leave no glucose in the final fermented broth. We presently have plans to carry out a two-stage fermentation in a multiple ALSA system with the first stage being the glycerol producer and the second stage for the conversion of glycerol in beer into 1,3-propanediol.

Materials and Methods

Saccharomyces LORRE Y8 was used for glycerol production and the yeast culture no. 1400 was used in the ethanol fermentation. The 1400 yeast culture is a fusion product of *S. diastaticus* and *S. uvarum (6)*. Both the Y8 and 1400 cultures are normally kept on slants until enlargement for experimental work. To prepare inoculum for glycerol fermentation, slant cultures were transferred into seed medium consisting of YMP medium enriched with 2 g/L of KH_2PO_4 and 20 g/L of glucose. For ethanol by the culture 1400, YEP medium enriched with 20 g/L of glucose is used for preparing inoculum. For fermentation runs, a variety of media were used and are subsequently described for the given individual experiments. The seed cultures are usually incubated at 30°C on shakers for 24 h before use.

An experimental ALSA fermenter, together with the gas flow and other accessories shown in Fig. 6, was used in our experimental studies. It had a working liquid capacity of 350 mL and consisted of a column having a diameter of 3.8 cm and a height of 40 cm with an inner draft tube of 2.5 cm diameter and 22 cm height. A side arm of a diameter of 3.8 cm and a height of 16.5 cm was attached to the column. This side arm was used mainly as a downdraft to break foam as described previously, similar to the one in Fig. 5. An air line was also connected to the gas inlet. In some experiments, a mixture of air and CO_2 was used as the gas inlet. A 20-L ALSA was also constructed for use in this study. The diameter and height of its outer column were 10.2 and 198 cm, respectively. The diameter and height of its inner tube were 10.2 and 150 cm, respectively. The diameter and height of the side arm were 10.3 and 47.5 cm, respectively. The top of the side arm was at the same level of the top of the main column. In addition to the ALSA, 2-L agitated and sparged glass jar fermenters (New Brunswick) of typical designs were used in many experimental runs.

Samples taken from the experimental runs were analyzed mostly with high-performance liquid chromatography (Hitachi with a Bio-Rad HPX-87H ion exclusion column) for ethanol, glycerol, glucose, and other minor byproducts. In some cases, glucose was also analyzed with a YSI 2700 Select Biochemistry Analyzer (YSI, Yellow Springs, OH).

In the experiments in which CO_2 was used to strip off ethanol from the fermenting broth, the gas flow rate varied from 0.5 to 1.0 vvm. CO_2 from a gas cylinder was first heated through a tube submerged in a water bath at 50°C.

Results and Discussion

Production of Glycerol by Saccharomyces *Yeast*

The culture *Saccharomyces* LORRE Y8 can withstand very high osmotic pressure. From a series of preliminary experiments, we decided that an initial glucose concentration of about 30% was desirable. In a fed-batch operation with two discrete glucose additions, shown in Fig. 7, the rate of

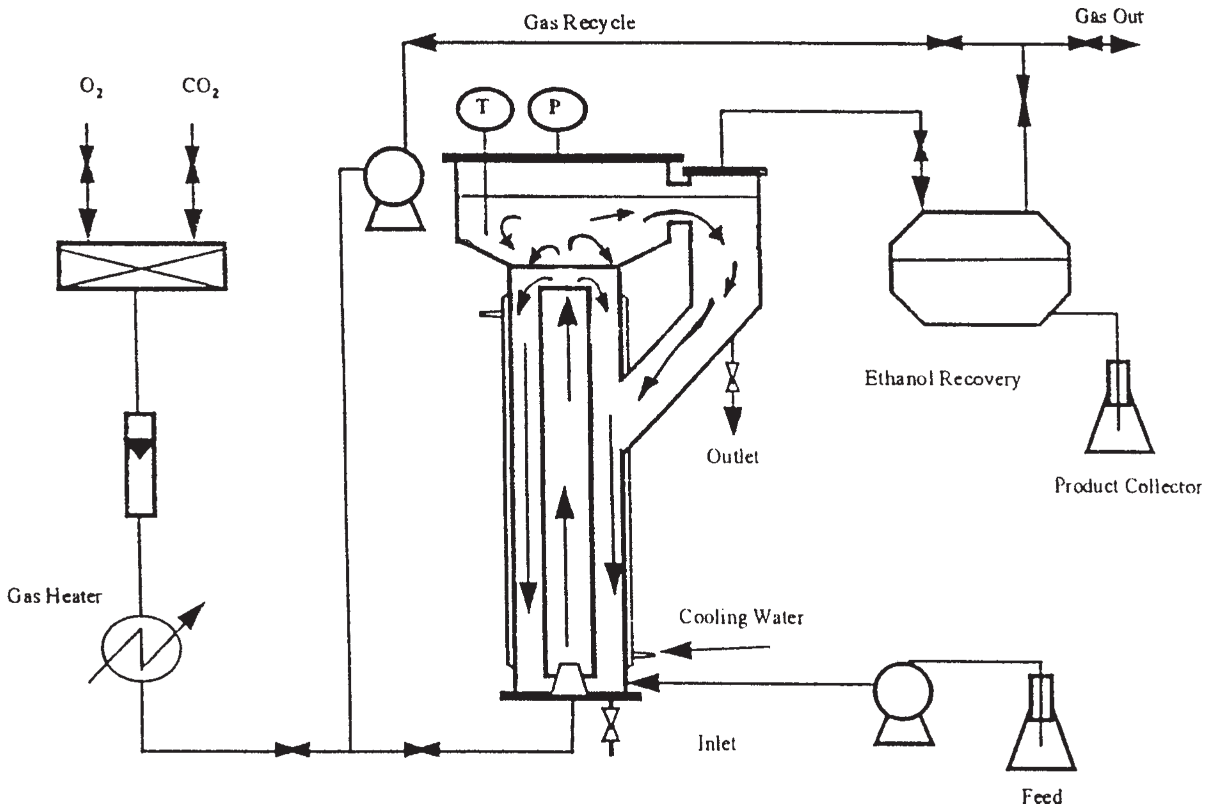

Fig. 6. ALSA fermentation system with gas stripping for ethanol recovery.

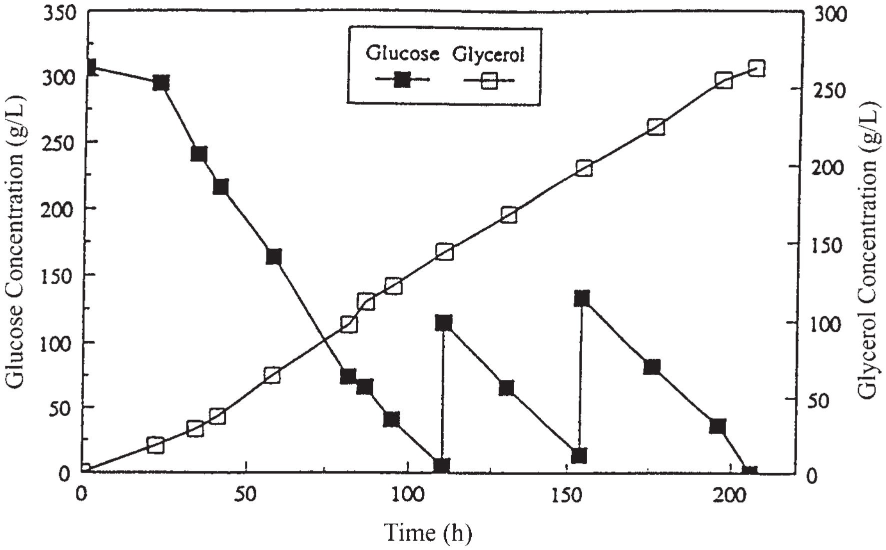

Fig. 7. Glycerol fermentation in stirred tank.

glycerol production by Y8 can be maintained at a steady rate showing little product feedback inhibition at a glycerol concentration of >250 g/L. This is not surprising, because syntheses of glycerol and other polyols by living microbial cells are often considered a protective mechanism against external osmotic pressures *(7)*. One of the early reported methods of glycerol production involves the addition of large amounts of salt into the fermenting broth as a way to induce cells to synthesize glycerol. The fact that LORRE Y8 showed little sufferance from product feedback inhibition even at a high glycerol concentration of >250 g/L is very desirable because it suggests that an even higher final glycerol concentration in the fermented broth might still be possible. Figure 8 presents a simple flow diagram for glycerol production showing that the final recovery and purification of glycerol from the fermented broth is by vacuum distillation. Glycerol has a high boiling point under atmospheric pressure. All the water from the filtered broth must be evaporated before vacuum can be applied to recover glycerol. The higher the glycerol concentration in the fermented broth, the less water will be there in the final broth to evaporate. The results in Fig. 7 give an average productivity of glycerol of about (260 g/L)/(210 h) = 1.24 g/(L·h). In this experiment, the liquid medium contained 1 g/L of yeast extract and 2 g/L of KH_2PO_4. The level of seed culture was 10% of the initial fermenter content.

Figure 9 shows another set of batch curves of glycerol fermentation. In this case, a solution of oligosaccharides derived from starch was used as the substrate This substrate is known in the corn-processing industry as the low dextrose equivalent (DE) liquefied starch. It is prepared by treating starch slurries with α-amylase usually through a continuous heat exchanger to gelatinize and also partially hydrolyze the starch to reach a DE value of from 12 to 20%. Glucoamylase (sample provided by Grain Processing,

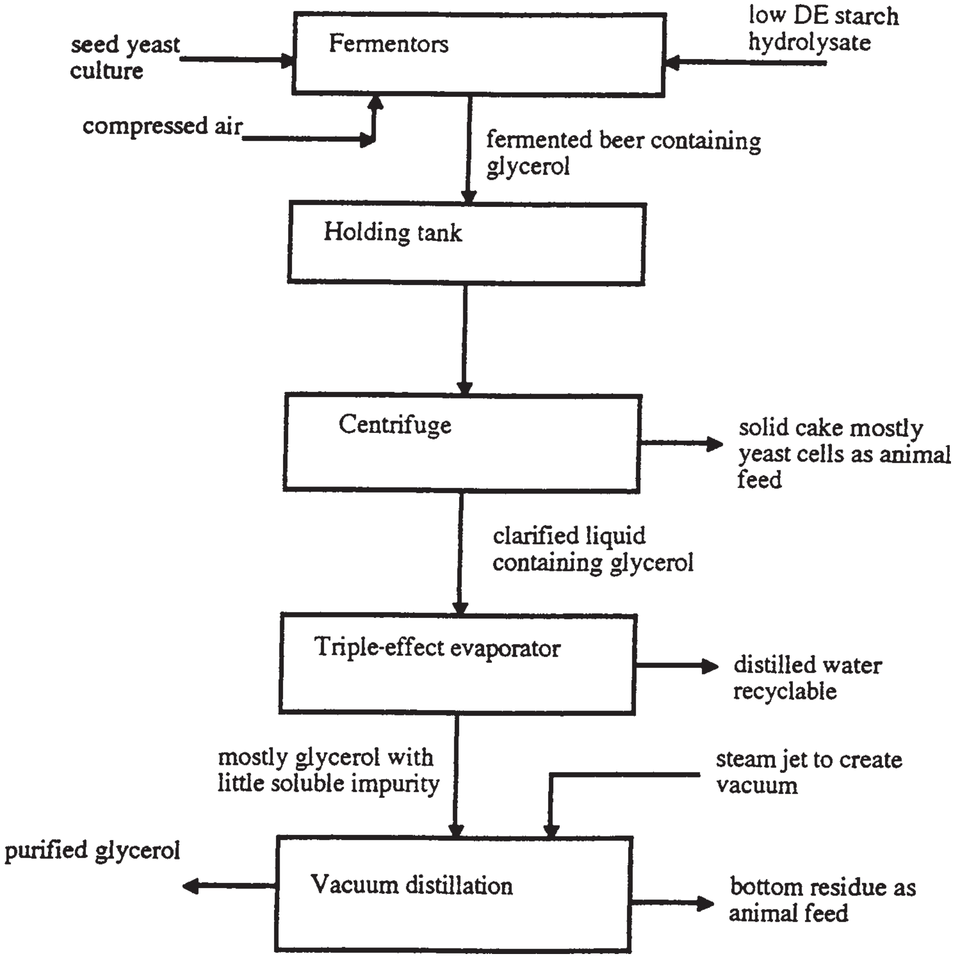

Fig. 8. Simple flow diagram for glycerol production by yeast fermentation.

Muscatine, IA) was added to the solution to carry out the simultaneous saccharification and fermentation process. The final glycerol concentration from this run was 144 g/L in 110 h, with an overall average productivity of 1.3 g/(L·h). In this case, 4 mL/L of corn steep liquor (with 50% solids; from A. E. Staley Manufacturing, Lafayette, IN) and 2 g/L of KH_2PO_4 were added to enrich the liquid medium.

Figure 10 shows another set of batch curves. In this case, a fivefold preprepared inoculant was used to start the batch. The fermentation was completed in 37 h, yielding a final glycerol concentration of 137 g/L, with an overall productivity of 3.7 g/(L·h). In this batch, there was a short lag phase of about 5 h during which more cell growth and less glycerol accumulation occurred. During a period of about 21 h (from h 16 to the final h 37), the productivity was maintained at about 4.43 g/(L·h). A higher cell population density apparently gives higher productivity.

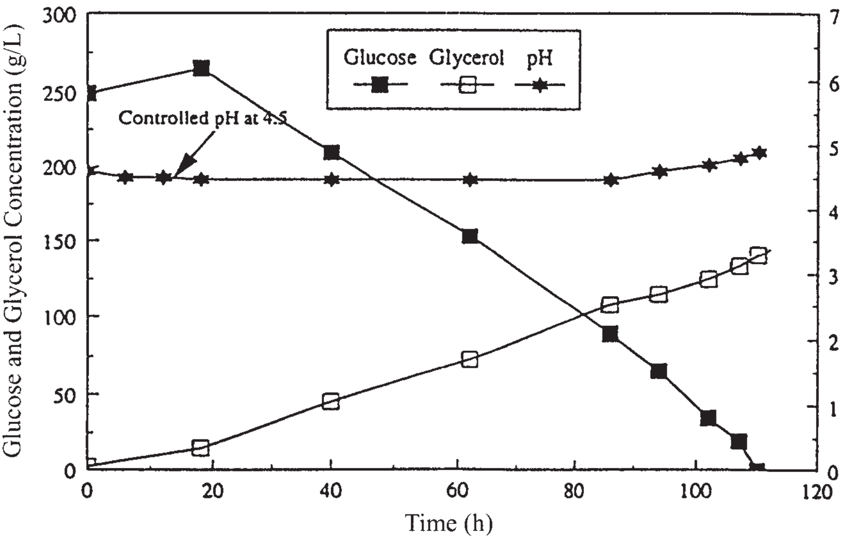

Fig. 9. Glycerol fermentation in stirred tank (working volume: 1.5 L).

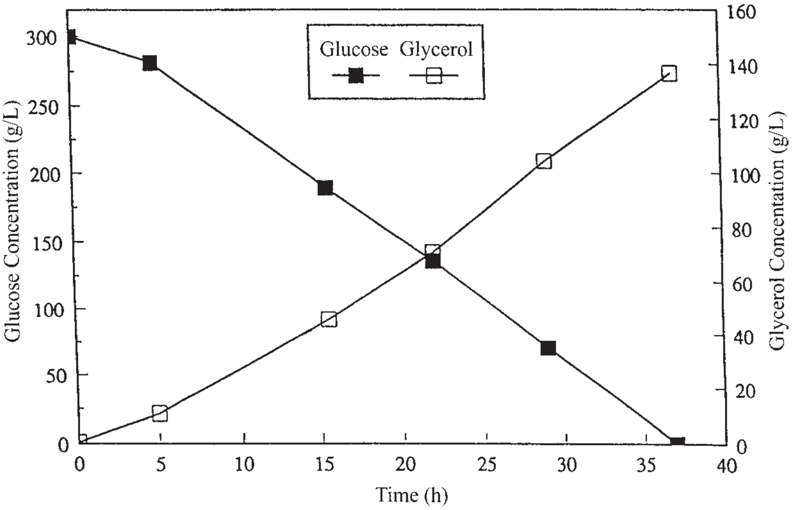

Fig. 10. Glycerol fermentation in stirred tank (effect of fivefold inoculant).

Production of Ethanol by Culture 1400

The results in Fig. 11 are from a typical batch fermentation by *Saccharomyces* culture 1400 at 32°C in an ALSA with 162 g/L of initial glucose in a liquid medium also containing 7 g/L of Bactopeptone (Difco, Detroit, MI) and 6 g/L of yeast extract (Difco). The product ethanol existed in both the liquid broth and the condensate collected from the stripping CO_2 gas stream (*see* Fig. 6 for the equipment setup). In Fig. 11, the solid product line shows the ethanol in the fermenting broth, whereas the dotted line shows the sum of ethanol in the broth and also in the condensate. In this case, the final yeast

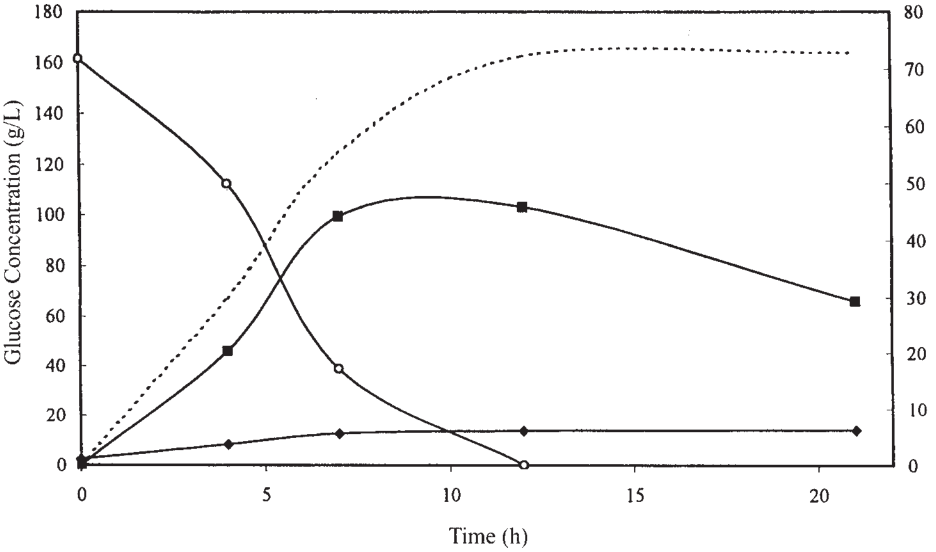

Fig. 11. Ethanol fermentation by yeast 1400 in ALSA with CO_2 stripping. (—○—), Glucose; (—◆—), cell density; (—■—), ethanol in broth; (– – – –), total ethanol.

cell mass concentration was almost 14 g/L, which is higher than usual. The cell growth was probably enhanced owing to ethanol removal by CO_2 stripping, resulting in less feedback inhibition on both ethanol synthesis and cell growth. In this run, all glucose was exhausted in <12 h. Afterward, the gas stripping was continued for several more hours for the purpose of recovering more ethanol. The overall calculated ethanol yield was 86% based on glucose consumed.

In another experiment, cells were prepared and harvested from shaker cultures, centrifuged, and resuspended in an ALSA to a relative high cell density of 16.1 g/L in an initial medium containing 3 g/L of yeast extract, 3 g/L of malt extract (Difco), 5 g/L of Bactopeptone, and 135.7 g/L of glucose. The batch was run at 35°C with CO_2 stripping. All glucose was exhausted in 7.5 h, giving a yield of 92%. In Fig. 12, the dotted line represents the total ethanol produced, whereas the solid line represents ethanol concentration in the liquid broth. The batch was terminated immediately after the glucose exhaustion. The motivation of this run was to ferment with a high cell density, similar in concept to the "rapid fermentation" tested by Nagodawithana and Steinkraus *(8)*, but under the condition of relatively low ethanol inhibition by gas stripping.

The high cell density process was further tested in a fed-batch operation with a 500 g/L sterilized glucose solution added in three discrete pulses into the fermentation broth at h 5, 9, and 13 after the start. The results in Fig. 13 show a total production of 129 g/L (fermenter volume) of ethanol in 24 h (the dotted line), while the ethanol concentration in the broth was kept below 50 g/L throughout the fed-batch to reduce inhibition. The ethanol yield based on glucose consumed was 90%. The fermenter productivity

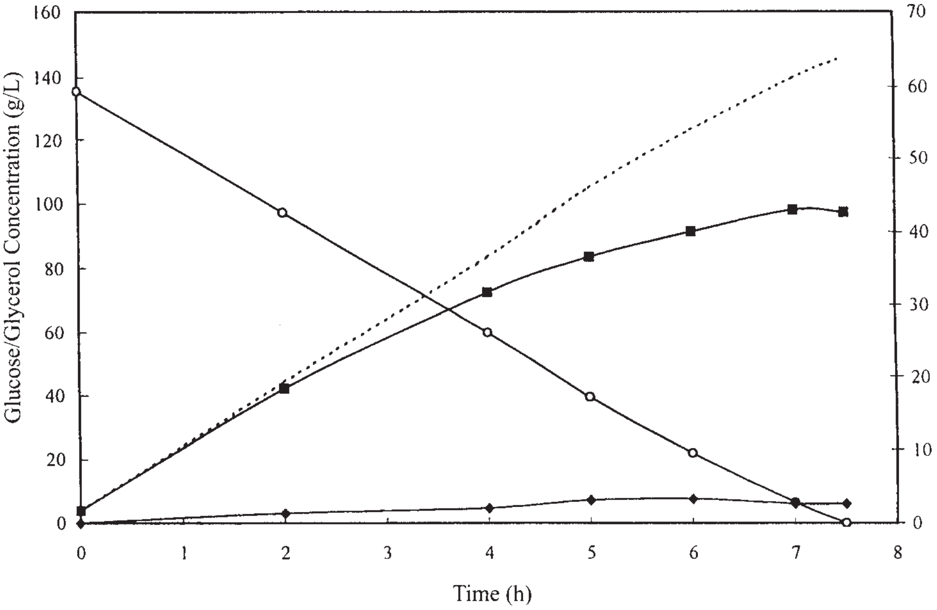

Fig. 12. High cell density fermentation in ALSA with CO_2 stripping. (—○—), Glucose; (—◆—), glycerol; (—■—), ethanol in broth; (– – – –), total ethanol.

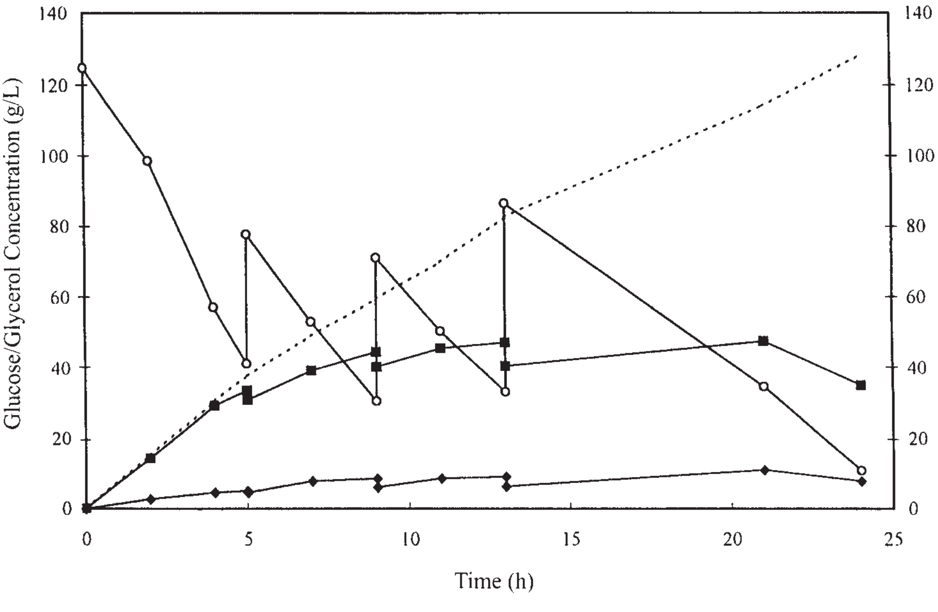

Fig. 13. Fed-batch ethanol fermentation in ALSA with CO_2 stripping. (—○—), Glucose; (—◆—), glycerol; (—■—), ethanol in broth; (– – – –), total ethanol.

was calculated to be 5.3 g/(L·h). One encouraging observation is that the total ethanol formation (the dotted line in Fig. 13) was maintained more or less linearly up, indicating no serious ethanol feedback inhibition to slow down ethanol synthesis. Control runs without gas stripping were done with different initial glucose concentrations in typical batch operations without further addition of glucose after the start. The results indicate that the ethanol concentrations were unable to reach beyond 105 g/L in about 40 h, without CO_2 stripping. The culture was able to tolerate ethanol concentration in the batch up to about 90 g/L reasonably well, but not higher than that. With CO_2 stripping, the net effect was that the relatively high rate of ethanol synthesis was maintained until the glucose was exhausted, as shown in Fig. 13.

Preliminary Cost Analysis for Glycerol Production

The current industry knows the economy of ethanol production far better than what we can do. We therefore will restrict our cost analysis to only the new glycerol production method. The recovery of glycerol from vacuum distillation is essentially 100% with very little loss. The following list gives the important but conservatively assumed process parameters:

1. Raw material requirements: 2.2 lb of glucose equivalent/lb of glycerol product
2. Aeration rate: 0.1 L of air/(L of liquid broth·min) (0.1 vvm)
3. Initial substrate concentration: 300 g/L
4. Yield: 0.48 lb of glycerol/lb of glucose equivalent
5. Final glycerol concentration: 144 g/L
6. Final glucose concentration: none
7. Fermentation duration: 36 h (with a productivity of 4.0 g/(L·h)
8. Byproduct: yeast in the final fermented broth at 12 g of dry cell mass/L.

According to trade information, crude glucose equivalent in the form of starch hydrolysate can be obtained at $0.07/lb. Based on this information, one can calculate the following cost per pound of glycerol:

1. Substrate cost: 2.2 × 0.07 = $0.154
2. Utilities
   a. Electricity: 0.6 kW-h
   b. Steam for sterilization, evaporation, and vacuum distillation: 11.3 lb
   c. Cooling water: 192.2 lb

The total raw material and utility cost is about $0.22/lb of glycerol. Assuming the total raw material and utility cost to be 70% of the total production cost, the total production cost can be estimated as $0.31/lb. Currently, glycerol is priced *(2)* at $0.50/lb. A new glycerol plant producing 40 million lb/yr of glycerol can generate a return of about 40,000,000 × (0.50 – 0.314) = $7,480,000/yr. In the case of fermentation of glycerol, there are no other volatile byproducts. Therefore, the final distilled glycerol product is of high quality, which is unlike the glycerol recovered from hydrolysis of fats and oils. Some fatty acids in the hydrolysates in their free-acid form can be carried over into the final glycerol product by vacuum distillation. High-quality polymer-feedstock grade glycerol can get $0.60 to 0.65/lb *(2)*.

Conclusion

We described a potentially useful method of coproduction of glycerol and ethanol. When the price of corn grains is high and the profit margin of ethanol producers is reduced, coproduction of glycerol and ethanol can be considered as an alternative to ethanol production alone for better process economy.

References

1. US Department of Agriculture. (1993), Report IUS-2.
2. *Chemical Market Reporter* (2000), 257(6).
3. (1991), *Kirk-Othmer Encyclopedia of Chemical Technology*, 4th ed., vol. 12, "Glycerol" John Wiley & Sons, New York, pp. 681–694.
4. Cameron, D. C., Altaras, N. E., Hoffman, M. L., and Shaw, A. J. (1998), *Biotechnol. Prog.* **14,** 116–125.
5. Brown, G. G. (1950), *Unit Operations*, John Wiley & Sons, New York.
6. D'Amore, T., Celotto, G., Russell, I., and Stewart, G. G. (1989), *Enzyme Microb. Technol.* **11,** 411–416.
7. Underkofler, L. A. (1954), *Industrial Fermentations*, vol. 1, Underkofler and Hickey, eds., Chemical Publishing, New York.
8. Nagodawithawa, T. W. and Steinhause, K. H. (1976), *Appl. Environ. Microbiol.* **31,** 158–162.