

## Economic Comparison of Calcium Fumarate and Sodium Fumarate Production by *Rhizopus arrhizus*

IRENE C. GANGL,<sup>\*,1</sup> WILLIAM A. WEIGAND,<sup>1,3</sup>  
AND FREDERICK A. KELLER<sup>2</sup>

<sup>1</sup>Department of Chemical Engineering, Illinois Institute  
of Technology, Chicago, IL 60616; <sup>2</sup>Cambridge BioScience  
Corporation, Massachusetts Biotech Research Park, 365 Plantation  
Street, Worcester, MA 01605; and <sup>3</sup>Present address:  
Department of Chemical and Nuclear Engineering, University  
of Maryland, College Park, MD 20742

### ABSTRACT

The economics of using Na<sub>2</sub>CO<sub>3</sub> rather than CaCO<sub>3</sub> as the neutralizing agent in fumaric acid production by *Rhizopus arrhizus* is investigated and compared with the benzene route. Because sodium fumarate, unlike calcium fumarate, is soluble, downstream processing is simplified by requiring less equipment and no heat, and also allows the reuse of cells. In spite of a fumaric acid productivity (g/L/h) 2.4 x lower than the CaCO<sub>3</sub> case, the Na<sub>2</sub>CO<sub>3</sub> alternative with cell reuse has a higher rate of return. However, with the current cost of petroleum, the selling price of fumaric acid from the benzene route is half as much as from an idealized Na<sub>2</sub>CO<sub>3</sub> fermentation.

**Index Entries:** Fumaric acid; economic analysis; fermentation; sodium carbonate; calcium carbonate.

### INTRODUCTION

Currently, industry produces fumaric acid by catalytically isomerizing either purified maleic anhydride or maleic acid. Approximately 83% of maleic anhydride is produced from benzene through catalysis (1). Fumaric acid can be used as a food acidulent, in the manufacture of unsaturated polyester resins, in quick-setting inks, in furniture lacquers, and as sizing resins for paper (1).

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

The microbial production of fumaric acid is confined largely to the order Mucorales, in particular to the genus *Rhizopus* (2). When the cells are grown in a minimal salts medium containing glucose as the carbon source and ammonium sulfate as the nitrogen source, fumaric acid production in *Rhizopus* species is nongrowth associated. As the pH drops in consequence of production, the rate of fumaric acid production slows down and eventually ceases. Therefore, addition of a neutralizing agent is necessary to prevent this self-inhibition. Virtually all previous investigators of fumaric acid production by *Rhizopus* species have used the base, calcium carbonate ( $\text{CaCO}_3$ ), exclusively (2–8). We have found that substituting sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) for  $\text{CaCO}_3$  in the fermentation gives similar yields of fumaric acid from glucose.

Because of its insolubility,  $\text{CaCO}_3$  offers the advantage of all-at-once addition and therefore eliminates the requirement of a control system for base addition. However, because of the low solubility of calcium fumarate (2.11% at 30°C, w/v), the product precipitates out of the broth throughout the fermentation. The presence of insoluble crystals increases power consumption and complicates separation.

Sodium fumarate, unlike calcium fumarate, is soluble (22%, w/v). Because of the low solubility of fumaric acid (0.7%, w/v), one can easily recover fumaric acid by acidifying the salt. Therefore, using  $\text{Na}_2\text{CO}_3$  as the neutralizing agent simplifies downstream processing by requiring less equipment and no heat. Unlike the  $\text{CaCO}_3$  fermentation, the cells of the  $\text{Na}_2\text{CO}_3$  fermentation are not heated and have no residual solids. This allows reuse of the cells, thereby increasing the yield and productivity.

These advantages may be offset by two disadvantages of using  $\text{Na}_2\text{CO}_3$  as the neutralizing agent. Firstly,  $\text{Na}_2\text{CO}_3$  costs about 20% more than purified  $\text{CaCO}_3$ . Secondly, we have not obtained fumaric acid productivities as high as reported in the literature for  $\text{CaCO}_3$  fermentations. However, these differences in productivity may be caused by variations in handling the organism or in mutations of the strain. Lower productivities increase cycle times and therefore increase the size and number of fermentors needed to obtain equal amounts of fumaric acid.

This study will first investigate the comparative economics of several alternate fermentation processes, such as  $\text{CaCO}_3$  vs  $\text{Na}_2\text{CO}_3$  as neutralizing agents, or  $\text{Na}_2\text{CO}_3$  with cell reuse. The best of these alternatives will be compared economically to the chemical route for fumaric acid production. We will note the major factors affecting the selling price of fumaric acid as well as conduct a sensitivity analysis on the return of investment for these two routes. Suggestions for future research will also be presented.

## MATERIALS AND METHODS

### Fermentation

*Rhizopus arrhizus* NRRL 2582 was propagated on the sporulation medium given by Rhodes et al. (2) with D-glucose,  $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$ , and  $\alpha$ -

lactose, being used in place of commercial glucose,  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ , and crude lactose, respectively. The procedure of Rhodes et al. (5) was used to germinate the spores and obtain a pulpy inoculum in two subculturings. The inoculum to the fermentor was 6% (v/v). The fermentation medium was the same as that of Rhodes et al. (5), except that D-glucose was used instead of commercial glucose, the initial concentration of  $(\text{NH}_4)_2\text{SO}_4$  was 0.92 g/L rather than 2 g/L, and  $\text{CaCO}_3$  was omitted. The  $(\text{NH}_4)_2\text{SO}_4$  and methanol (to a final concentration of 1.5%, v/v) were filter-sterilized and added separately to the sterilized medium.

All experiments were conducted with a 2-L, computer-controlled fermentor (Queue Systems, ZMS-7000, Parkersburg, WV) at 34°C and 350 rpm, with an airflow of 250 mL/min. Two 5-cm turbine impellers placed at 5.5-cm intervals provided uniform mixing, with the probes and tubes of the top plate acting as the baffles. The air sparger was a coiled and etched Teflon™ tube (3 mm in diameter, W. L. Gore & Associates, Inc., Elkton, MD) that kept the fungus from growing up into the hollow agitator shaft. The microporosity of the Teflon tubing helped form fine air bubbles and therefore produced good aeration. Foaming was prevented by adding 1 mL of a 50% (v/v) solution of Pluronic L-61 (BASF Wyandotte Corp., Parsippany, NJ).

The samples were filtered through Whatman #1 paper (Maidstone, England). These filtrates were analyzed for glucose by a Yellow Springs glucose analyzer (Model 27, Yellow Springs, OH). The concentrations of fumaric acid and other acids in the broth were measured by high-performance liquid chromatography (pump model 510, U6K injector, Waters Associates, Milford, MA) at ambient temperatures using a Bio-Rad Aminex ion exclusion column (HPX-87H, Richmond, CA) and a differential refractometer detector (Model R401, Waters Associates, Milford MA).

## Economics

The economic analysis for fumaric acid production by alternative fermentations is based on the equipment, raw materials, and utilities that differ in the various processes. The return differential of each alternative serves as a relative measure of profitability. The return differential is defined as the ratio of total savings to installed equipment cost, relative to the same ratio of the base case. The base case is the alternative with the lowest total savings.

To compare the fermentation and chemical routes to fumaric acid production and to estimate the selling price, a complete economic analysis is required. We calculated capital investment costs and total product costs based on the method of factors presented by Peters and Timmerhaus (Ch. 5, Tables 26, 27 of [9]). The total capital investment is made up of direct costs, indirect costs, and working capital. The direct cost is estimated as 3.74 x the purchased equipment cost. The indirect costs are equal to 20% of the direct costs plus a contingency of 10% of the fixed capital investment. The fixed capital investment is the sum of the direct and indirect

Table 1  
Comparison of the Effects of Neutralizing Agents  $\text{CaCO}_3$  and  $\text{Na}_2\text{CO}_3$   
on Fumaric Acid Production by *Rhizopus arrhizus*

	Ng et al. (10)	Rhodes et al. (5)	IIT
Neutralizing agent <sup>a</sup>	80 g/L $\text{CaCO}_3$	80 g/L $\text{CaCO}_3$	15% (wt) $\text{Na}_2\text{CO}_3$
pH range	5.5–6.8	–	5.5
Initial glucose, g/L	130	130	102
Duration of fermentation, h	53.3 <sup>b</sup>	72.0	147
Maximum fumaric acid concentration, g/L	107 <sup>b</sup>	88.0	73.3 <sup>c</sup>
Efficiency yield of fumaric acid, % <sup>d</sup>	82	68	72
Fumaric acid productivity, g/L/h <sup>e</sup>	2.00	1.22	0.50 <sup>c</sup>
Fumaric acid selectivity, % (wt) <sup>f</sup>	86	74 <sup>b</sup>	83

<sup>a</sup>  $\text{CaCO}_3$  was charged initially to the fermentor;  $\text{Na}_2\text{CO}_3$  was added on demand.

<sup>b</sup> Estimated.

<sup>c</sup> Corrected for dilution from base addition.

<sup>d</sup> Efficiency yield = (maximum g/L of fumaric acid)/(g/L of glucose consumed).

<sup>e</sup> Productivity = (maximum g/L of fumaric acid)/(h at maximum fumaric acid concentration).

<sup>f</sup> Selectivity = (maximum g/L fumaric acid)/(maximum g/L total acid).

costs. The working capital is taken as 15% of the total capital investment. The total product cost is made up of manufacturing costs plus general expenses. The manufacturing cost is the sum of costs of raw materials, utilities, 1.93 x the operating labor, and 20% of the fixed capital investment. General expenses are taken as the sum of 15% of the operating labor, 16% of the total product cost, and 5% of the total capital investment.

## THE SODIUM CARBONATE FERMENTATION

Table 1 compares the results of fermentations using  $\text{Na}_2\text{CO}_3$  as the neutralizing agent with two  $\text{CaCO}_3$ -neutralized experiments from the literature. The media in these examples are similar, with the exception that Ng et al. (10) use 10  $\mu\text{g/L}$  biotin in place of corn steep liquid in the fermentation medium. Biotin is a cofactor of pyruvate carboxylase, the enzyme that allows the fungus to fix  $\text{CO}_2$  into the citric acid cycle. Corn steep liquor also contains biotin along with many other ingredients. Foster (11) notes that slight changes in culture conditions cause significant metabolic changes in fungi, which "accounts for the difficulties in reproducibility of results between different laboratories employing identical cultures." Therefore, the use of pure biotin may cause the improvement in all the fermentation parameters seen in the Ng et al. fermentation as compared with the Rhodes et al. (5) fermentation. Furthermore, differing storage and handling techniques may also alter the productivity of a strain.

Taking these factors into account, it seems plausible that a productivity of a  $\text{Na}_2\text{CO}_3$  fermentation can equal that of a  $\text{CaCO}_3$  fermentation. Therefore, we will include an idealized case in our alternate investment analysis, where the productivity, yield, and selectivity of the  $\text{Na}_2\text{CO}_3$  fermentation equals that of Ng et al.'s  $\text{CaCO}_3$  fermentation.

Contrary to the results of Rhodes et al. (5), who tested NaOH as a neutralizing agent, we did not observe the "inhibition of fumarate formation... as the concentration of [sodium fumarate] approached 3%." In an experiment designed to test the tolerance of sodium fumarate by the fungus, we added sodium fumarate twice during the fermentation for a total increase of 71.3 g/L sodium fumarate. The cells required 35 h to adjust to this shock. Once adjusted, the cells continued to produce fumaric acid for a total product yield from glucose of 70.7% (wt), and a fumaric acid productivity of 0.67 g/L/h after the adjustment period. The actual final concentration of sodium fumarate in the broth was 9.99% (equivalent to 72.4 g/L fumaric acid). The difference in the observations of Rhodes et al. (5) and our observations probably results from more dissolved  $\text{CO}_2$  being available to the organism in our study. Since  $\text{CO}_2$  fixation is necessary for production of fumaric acid (4,6), the "inhibition" observed by Rhodes et al. may have resulted from low availability of carbonate to the organism for  $\text{CO}_2$  fixation. In this regard, it is worth noting that, besides serving as neutralizing agents,  $\text{Na}_2\text{CO}_3$  and  $\text{CaCO}_3$  are also sources of dissolved  $\text{CO}_2$  needed for fumaric acid production.

## PROCESS FLOW SHEETS

The flow sheet for fumaric acid production via benzene is based on information given by Robinson and Mount (1), Kerr (12), and Rosenzweig (13) (see Fig. 1). Benzene is vaporized and mixed with air in a benzene-to-air weight ratio of 1:30. The gas mixture passes through a multiple-tube, fixed-bed reactor containing a vanadium oxide catalyst. The reactor consists of over 900 tubes that are 4 m long and 25 mm wide, made of mild steel. In this tubular reactor, the benzene reacts with oxygen at  $360^\circ\text{C}$  to form maleic anhydride and byproducts (mostly carbon dioxide, carbon monoxide, and water). The reaction converts 94.5% of the benzene at an average maleic anhydride yield of 65% (mol). The reaction requires 0.625 kg catalyst/metric ton of maleic anhydride produced, at a loading of 0.1 kg benzene/kg catalyst. The benzene flow rate is taken as 95 g benzene/L catalyst/h. The salt cooler captures and converts the heat from this highly exothermic reaction to steam. The heat exchangers cooling the effluent gases also produce steam.

A water scrubber converts the maleic anhydride to a 40% (wt) solution of crude maleic acid. To control the heat release, the decolorized maleic acid solution is fed in increments to the isomerizer. The isomerizer

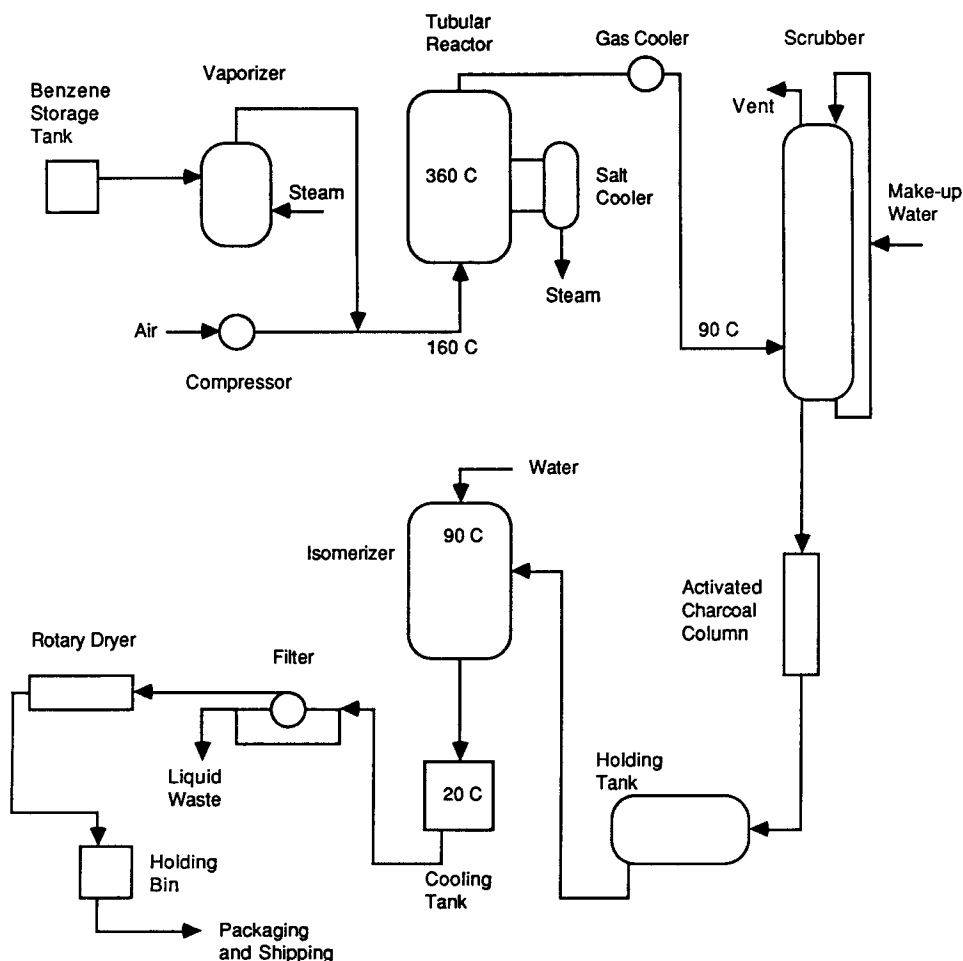


Fig. 1. Flowsheet for fumaric acid production via the benzene route. Equipment: 1 vaporizer (45.6 m<sup>3</sup>), 1 compressor (118 m<sup>3</sup>/min), 1 tubular reactor (144 m<sup>2</sup>), 1 salt cooler (785 m<sup>2</sup>), 2 gas coolers (9.2 m<sup>2</sup>), 1 water scrubber (1.2 m diameter), 1 activated charcoal column (16.8 m<sup>3</sup>), 1 holding tank (1.03 m<sup>3</sup>), 1 isomerizer (1.24 m<sup>3</sup>), 1 cooling tank (1.24 m<sup>3</sup>), 1 rotary drum filter (0.93 m<sup>3</sup>), 1 rotary dryer (25 kg H<sub>2</sub>O/h), 1 holding bin (6.5 m<sup>3</sup>), 5 pumps (30.3 L/min).

operates at 90–100°C with a catalyst made up of NH<sub>4</sub>Br (0.01% [wt]) and (NH<sub>4</sub>)<sub>2</sub>S<sub>2</sub>O<sub>8</sub> (0.03% [wt]) (14). Solubility factors and handling losses result in a 100% (wt) yield of fumaric acid from maleic anhydride (12). The effluent from the isomerizer is cooled to 20°C to precipitate fumaric acid crystals.

Figure 2 gives the process flow sheet for fumaric acid production from fermentation. Glucose, 80 g/L CaCO<sub>3</sub>, and the mineral salts are sterilized together in the fermentor. The (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> solution is sterilized separately and then fed to the cooled fermentor. The fermentor operates at 34°C with 0.5 vvm airflow. We will base the fermentation parameters for the CaCO<sub>3</sub> fermentation on the Rhodes et al. (5) protocol, because these

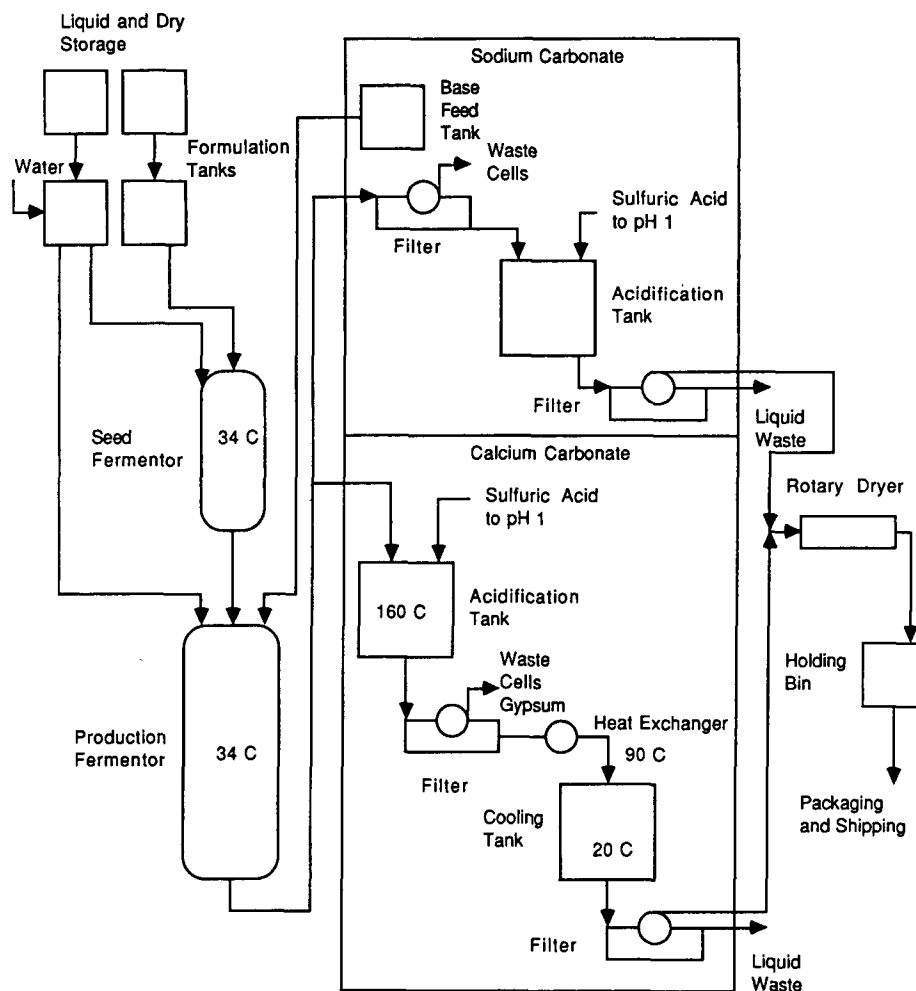


Fig. 2. Flowsheet for fumaric acid production via fermentation. Equipment in common: 2 formulation tanks ( $0.5 \text{ m}^3$ ,  $11 \text{ m}^3$ ), 1 seed fermentor ( $11 \text{ m}^3$ ), 2 production fermentors ( $100 \text{ m}^3$ ), 2 rotary drum filters ( $0.93 \text{ m}^3$ ), 1 rotary dryer ( $25 \text{ kg H}_2\text{O/h}$ ), 1 holding bin ( $6.5 \text{ m}^3$ ), 2 compressors ( $60 \text{ m}^3/\text{min}$ ,  $0.015 \text{ m}^3/\text{min}$ ). For  $\text{Na}_2\text{CO}_3$ : 1 stirred tank ( $100 \text{ m}^3$ ), 2 base-feed tanks ( $36.4 \text{ m}^3$ ), 4 pumps ( $775 \text{ L/min}$ ). For  $\text{CaCO}_3$ : 1 jacketed reactor ( $105 \text{ m}^3$ ), 1 heat exchanger ( $141 \text{ m}^2$ ), 1 cooling tank ( $105 \text{ m}^3$ ), 5 pumps ( $775 \text{ L/min}$ ).

values are intermediate among those reported in the literature. Therefore, the parameters are taken as 74% (wt) yield of fumaric acid from glucose, a fumaric acid productivity of  $1.2 \text{ g/L/h}$ , and a selectivity of 75% (wt) of fumaric acid/total acids produced. The fermentation parameters for the  $\text{Na}_2\text{CO}_3$  process are taken as 74% (wt) yield of fumaric acid from glucose, a productivity corrected for dilution of  $0.5 \text{ g/L/h}$ , and a selectivity of 83% (wt) fumaric acid over total acids. We will assume the organism has adapted to growth on corn syrup (containing 60.9% (wt) sugar).

In the  $\text{CaCO}_3$  process, the harvested broth, containing a slurry of calcium fumarate, cells, and trace amounts of  $\text{CaCO}_3$ , is heated to  $160^\circ\text{C}$  in a jacketed reactor and is acidified to pH 1.0 by  $\text{H}_2\text{SO}_4$ . At this temperature, both calcium fumarate and fumaric acid are soluble, but the calcium sulfate precipitates out of solution. Fumaric acid crystals are recovered by cooling the filtrate. In the  $\text{Na}_2\text{CO}_3$  process, the pH is kept at 5.5 by a 40% (wt) solution of  $\text{Na}_2\text{CO}_3 \cdot 1\text{H}_2\text{O}$ . The fumaric acid crystals are recovered by acidification.

## RESULTS AND DISCUSSION

### Alternative Investment Analysis

We will consider the following alternative fermentations: (1) neutralized with  $\text{CaCO}_3$ , (2) neutralized with  $\text{Na}_2\text{CO}_3$ , (3) neutralized with  $\text{Na}_2\text{CO}_3$ , reusing the cells 5 x, and (4) idealized  $\text{Na}_2\text{CO}_3$  case, reusing the cells 5x. Because the only solid present in the  $\text{Na}_2\text{CO}_3$  fermentation is the cell mass, the broth can be drained while retaining the cells in the fermentor. Waksman (7) has proposed such a repeated fed-batch mode of operation to produce fumaric acid commercially. He claims that the cells can undergo several sugar replacements with minimal loss in vigor. Because the cells are already grown, this mode of operation has the advantage of reducing both the cycle time and the raw material costs (by increasing the fumaric acid yield from glucose). We have assumed that cell reuse reduces the cycle time by 20 h (equivalent to the length of the growth phase). We estimated the average yield of repeated fed-batch operation as 83.1% (wt) from glucose. The idealized  $\text{Na}_2\text{CO}_3$  case with cell reuse assumes that improved media and handling techniques allow *R. arrhizus* to produce fumaric acid at the same productivity and yield as obtained by Ng et al. (10). Reusing the cells 5 x increases the yield to 91.6% (wt) and the productivity to 2.7 g/L/h. Handling losses of fumaric acid were not taken into account in the alternative analysis. We also assumed that the waste-treatment costs of the  $\text{CaCO}_3$  and  $\text{Na}_2\text{CO}_3$  processes are on the same order of magnitude.

Table 2 summarizes the alternative investment analysis. The  $\text{Na}_2\text{CO}_3$  case has the highest raw material costs, and therefore it is the basis of comparison for the other cases. At a return differential of 49.5%, the idealized  $\text{Na}_2\text{CO}_3$  case is by far the best alternative. This case achieves such a high return differential as a result of a high savings in raw material costs because of high yield, and a relatively low installed-equipment cost because of high productivity. In spite of a productivity 2.4 x lower than the  $\text{CaCO}_3$  case, reusing the cells allows the real  $\text{Na}_2\text{CO}_3$  case to surpass the  $\text{CaCO}_3$  case.

Figure 3 examines the effect of steam and neutralizing-agent costs on the return differential. High costs for steam favor the  $\text{Na}_2\text{CO}_3$  case, because



Table 2  
Alternate Investment Analysis for Fumaric Acid Production by Fermentation

	Alternates			
	CaCO <sub>3</sub>	Na <sub>2</sub> CO <sub>3</sub>	Na <sub>2</sub> CO <sub>3</sub> with cell reuse	Idealized Na <sub>2</sub> CO <sub>3</sub> with cell reuse
Basis: $2.30 \times 10^6$ kg/yr of Fumaric Acid				
Production fermentors, m <sup>3</sup>	170	225	210	100
Final operating volume, m <sup>3</sup>	136	180	165	84
Number of fermentors	2	4	4	2
Cycle time, h	95.2	202	185	53.5
Fumaric acid production, 10 <sup>3</sup> kg/batch	13.1	13.8	12.6	7.32
Batches/yr	176	167	182	314
Analysis				
I = installed equipment cost (1000 \$)	1720	2460	2310	687
R = raw material cost (1000 \$/yr)	2480	2650	2500	2310
O = operating cost (1000 \$/yr)	90.3	0	0	0
F = fixed charges (0.1 × L, 1000 \$)	172	246	231	68.7
S <sub>r</sub> = savings in R (1000 \$/yr)	170	0	150	340
S <sub>t</sub> = total Savings (S <sub>r</sub> - O - F, 1000 \$/yr)	-92.3	-246	-81.0	271
Annual return differential (%)	4.63	basis	6.49	49.5
= (S <sub>t</sub> /I) - (S <sub>t</sub> /I) <sub>basis</sub>				

steam is not required to recover the product. The cost of the neutralizing agent also affects the return differential significantly.

Unlike the CaCO<sub>3</sub> case, the mycelium produced in the Na<sub>2</sub>CO<sub>3</sub> process is free of clinging solids. Therefore, as in the commercial citric acid process using *Aspergillus niger*, the waste mycelia may be sold as an animal feed or as a soil conditioner (15,16). The additional revenue from the mycelia, which was not considered here, would further increase the return differential of the Na<sub>2</sub>CO<sub>3</sub> case relative to the CaCO<sub>3</sub> case.

### Comparison of the Chemical and Fermentation Routes

Next we will compare the economics of the benzene route for fumaric acid synthesis with the best fermentation alternative—the idealized Na<sub>2</sub>CO<sub>3</sub> fermentation with cell reuse. In the following analysis, we will assume that the cost of waste treatment is on the same order of magnitude between the two routes. Figure 4 reveals that the benzene route for fumaric acid production has lower costs than the fermentation route (purchased equipment, raw materials, direct labor, and utilities). The resulting lower

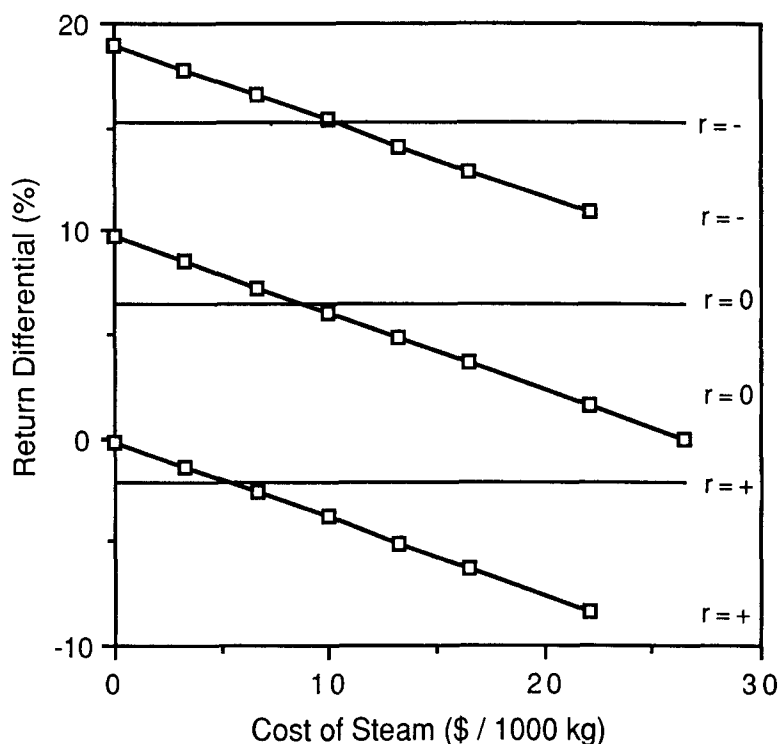


Fig. 3. The effects of steam and neutralizing agent costs on the return differential for the fermentation routes.  $\square$ — $\square$   $\text{CaCO}_3$ ; — real  $\text{Na}_2\text{CO}_3$  with cell reuse;  $r=0$ , current raw material costs;  $r=-$ , 20% lower cost of neutralizing agent;  $r=+$ , 20% higher cost of neutralizing agent.

fixed capital investment and total product costs make the benzene route economically superior to the fermentation route.

The selling price of fumaric acid for the two processes is based on a 20% return on investment over 10 years. Table 3 gives the sensitivity analysis of the return on investment, illustrating the effects on the base case(A) of a fixed capital investment overrun(C) and a reduction in the selling price(B). The selling price of fumaric acid from the fermentation route is twice as high as from the benzene route. For both routes, the selling price is the greatest factor influencing the return on investment. In both cases, a 20% higher fixed capital investment would not greatly affect the return on investment.

Figure 5 shows the effect of plant design capacity on selling price, assuming an unsaturated market. The selling price is relatively insensitive to plant capacity for fumaric acid production of over  $20 \times 10^6$  kg/yr. At lower capacities, the selling price rises steeply with decreasing fumaric acid production capacity. In 1978, fumaric acid plant capacities in the US ranged from  $2.3$ – $13.6 \times 10^6$  kg/yr (1).

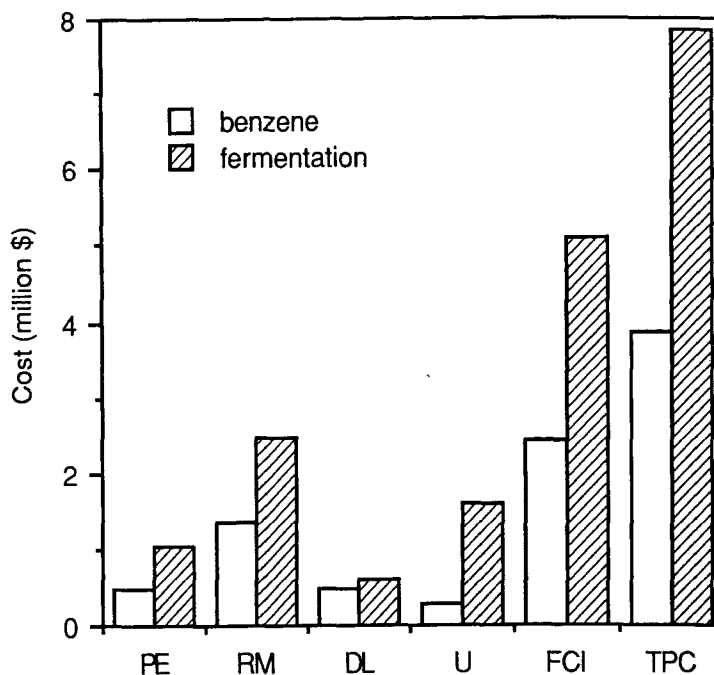


Fig. 4. Economic comparison of the benzene and fermentation routes. PE=purchased equipment; RM=raw materials; DL=direct labor; U=utilities; FCI=fixed capital investment; TPC=total product cost. PE from (9), except rotary dryer (17), fermentors (18); Marshall and Swift Indexes were used to adjust costs. RM from (19), except estimated activated charcoal from (20), and packaging from (21). DL from (9). U for benzene route estimated from (12), surplus steam taken as a revenue; power requirements for fermentors taken as 0.7 hp/100 gal (seed), 1.27 hp/100 gal (22); power requirements for compressed air (9), for dryers (17).

The selling price for both processes is relatively insensitive to the cost of steam, process water, and tower water over a wide range. Figure 6 reveals that the selling price of fumaric acid produced via fermentation is affected by electricity costs to a much greater extent than is the price of fumaric acid produced via the benzene route. The electricity requirements of the fermentation route are almost 6 x higher than those of the benzene route. The production fermentors and the compressed air supply draw the most electricity in the fermentation route.

The fermentation route will become profitable when the selling price of fumaric acid is high because of a high benzene cost. Using benzene cost data over 1970–1975 given by Ferber (23) and crude oil cost data over the same period given by Dale (24), we found that the price of benzene is linearly related to the price of crude oil. Extrapolating these data, we find the rough approximation that the cost of crude oil must reach \$61/bbl for

Table 3  
Sensitivity Analysis for Fumaric Acid Production<sup>a</sup>

	Benzene route			Fermentation route		
	A Base	B 20% reduced selling price	C 20% higher FCI	A Base	B 20% reduced selling price	C 20% higher FCI
Selling price (\$/kg)	2.34	1.87	2.34	4.79	3.83	4.79
TI = total income (1000 \$/yr)	5050	4040	5050	10300	8270	10300
TPC = total product cost (1000 \$/yr)	3860	3860	4010	7850	7850	8110
GI = gross income (TI - TPC, 1000 \$/yr)	1190	179	1040	2500	423	2140
SU = start-up cost (0.1 × FCI, 1000 \$)	244	244	293	509	509	611
NE <sub>1</sub> = net earnings, yr 1 (0.5GI - SU, 1000 \$)	353	-154	227	741	-298	459
NE <sub>10</sub> = net earnings, yr 2-10 (0.5GI, 1000 \$)	597	89.6	520	1250	211	1070
WC = working capital (1000 \$)	431	431	517	898	898	1080
FCI = fixed capital investment (1000 \$)	2440	2440	2930	5090	5090	6110
ROI = return of investment <sup>b</sup>	20.0	2.27	14.2	20.0	2.68	14.0

<sup>a</sup>Method of calculation from (9).

<sup>b</sup> $ROI = (1/10) \times (NE_1 + 9NE_{10}) / (WC + FCI)$ .

the benzene and fermentation routes to give the same return on investment. This value is approximately 3.4 × higher than prices for crude oil in December of 1988.

*Aspergillus niger* produces citric acid at low pH without a neutralizing agent. Acid production stops in *Rhizopus* species as the pH drops. One goal of future research may be to adapt or mutate *Rhizopus* species to tolerate and produce acids at low pH. At low pH, fumaric acid crystals will precipitate out of solution throughout the fermentation. Because of density differences, the crystals can be separated from the cells in a fermentor with a conical bottom. This eliminates one rotary filter and the acidification tank from the process. The low-pH fermentation would lower raw material costs, because Na<sub>2</sub>CO<sub>3</sub> and H<sub>2</sub>SO<sub>4</sub> would no longer be required. The low-pH fermentation would also lower utility costs, because no steam would be required in the fermentor for sterilization. These factors would bring the selling price down to \$3.59/kg. This is still 1.5 × higher than the selling price of fumaric acid from the benzene route. For the two routes to give the same return on investment at this lower selling price,

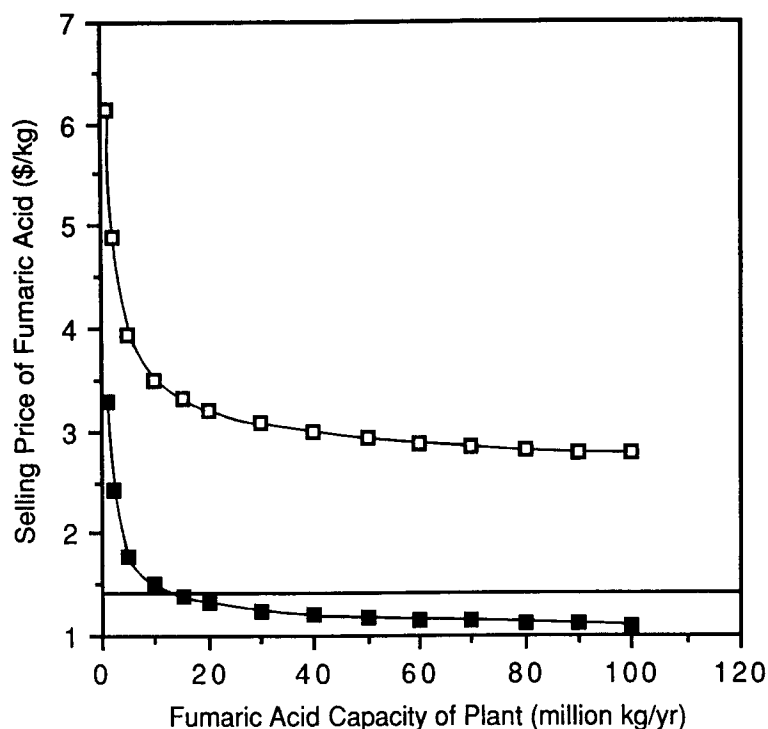


Fig. 5. Comparison of the benzene and fermentation routes: selling price of fumaric acid vs plant capacity.  $\square$ — $\square$  idealized  $\text{Na}_2\text{CO}_3$  fermentation;  $\blacksquare$ — $\blacksquare$  benzene route; — fumaric acid price in Dec., 1988 (19).

the price of crude oil must reach \$34/bbl (or about 1.8 x higher than current prices). The cost of corn syrup may be cut in half if lower-quality syrup is used. In such a case, the selling price of fumaric acid would be cut to \$3.31/kg, which would require a crude oil price of \$28/bbl for the fermentation route to become economically attractive.

## CONCLUSION

Substituting  $\text{Na}_2\text{CO}_3$  for  $\text{CaCO}_3$  as the neutralizing agent for fumaric acid production by *Rhizopus arrhizus* requires less equipment and no steam for product recovery; it also allows the reuse of the cells. At equal fumaric acid yields from glucose, these advantages give the  $\text{Na}_2\text{CO}_3$  fermentation with cell reuse a higher rate of return than the  $\text{CaCO}_3$  fermentation. Compared with an idealized, high-yielding, high-productivity  $\text{Na}_2\text{CO}_3$  fermentation, the benzene route produces fumaric acid with lower equipment, raw material, labor, and utility costs. Relative to the fermentation route, these advantages of the benzene route cut the selling price of fumaric acid in half. The fermentation route could compete with the chemical route if

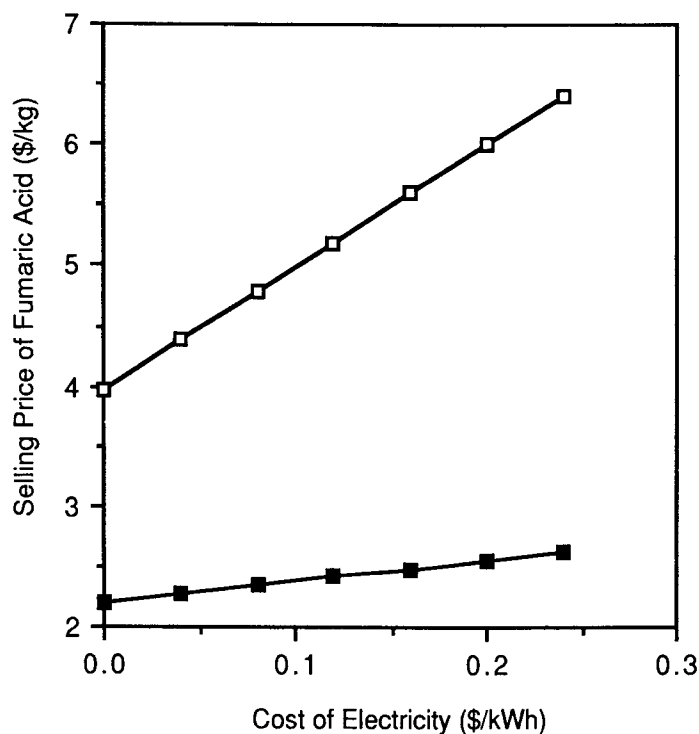


Fig. 6. Comparison of the benzene and fermentation routes: selling price of fumaric acid vs the cost of electricity. □——□ idealized Na<sub>2</sub>CO<sub>3</sub> fermentation; ■——■ benzene route.  $2.16 \times 10^6$  kg/yr fumaric acid.

the price of crude oil were to reach \$61/bbl. Development of a *Rhizopus* mutant that does not require a neutralizing agent will improve the fermentation economics. If an inexpensive source of corn syrup were available, such a low-pH fermentation would compete with the benzene route if the price of crude oil were to reach \$28/bbl.

## ACKNOWLEDGMENT

We would like to express our gratitude to Corn Produces, Inc. (Summit-Argo, IL), who supported this work in addition to allowing F. K. a 2-yr leave of absence to teach at IIT and granting I. G. a fellowship.

## REFERENCES

1. Robinson, W. D. and Mount, R. A. (1981), *Kirk-Othmer Encyclopedia of Chemical Technology*, vol. 14, Grayson, M. and Eckroth D., eds., Wiley, New York, p. 770.

2. Rhodes, R. H., Moyer, A. J., Smith, M. L., and Kelly, S. E. (1959), *Appl. Microbiol.* **7**, 74.
3. Goldberg, I. and Stieglitz, B. (1985), *Biotech. Bioeng.* **27**, 1067.
4. Kenealy, W., Zaady, E., du Prez, J. C., Stieglitz, B., and Goldberg, I. (1986), *Appl. Environ. Microbiol.* **52**, 128.
5. Rhodes, R. H., Lagoda, A. A., Misenheimer, T. J., Smith, M. L., Anderson, R. F., and Jackson, R. W. (1962), *Appl. Microbiol.* **10**, 9.
6. Romano, A. H., Bright, M. M., and Scott, W. E. (1967), *J. Bacteriol.*, **93**, 600.
7. Waksman, S. A. (1943), US Patent 2,326,986.
8. Yasin, M., Kausar, T., and Akbar, M. D. (1975), *Pak. J. Biochem.* **8**, 41.
9. Peters, M. S. and Timmerhaus, K. D. (1980), *Plant Design and Economics for Chemical Engineering*, 3rd ed., McGraw-Hill, New York.
10. Ng, T. K. Hesser, R. J., Stieglitz, B., Griffiths, B. S., and Ling, L. B. (1986), *Biotech. Bioeng. Symp. No. 17*, p. 355.
11. Foster, J. W. (1949), *Bact. Rev.* **11**, 167.
12. Kerr, R. O. (1975), *Benzene and its Industrial Uses*, Hancock, E. G., ed., Wiley, New York, p. 524.
13. Rosenzweig, M. D. (1972), *Chem. Eng.* **79**, 64.
14. Scientific Design, (1966), US Patent 3,262,972.
15. Bouchard, E. F. and Merritt, E. G. (1981), *Kirk-Othmer Encyclopedia of Chemical Technology*, vol. 6, Grayson, M. and Eckroth D., eds., Wiley, New York, p. 150.
16. Faust, U. (1987), *Fundamentals of Biotechnology*, Weinheim, FRG, Ch. 15, p. 601.
17. Perry, R. H. and Chilton, C. H. (1973), *Chemical Engineering Handbook*, 5th ed., McGraw-Hill, New York.
18. Bailey, J. E. and Ollis, D. F. (1986), *Biochemical Engineering Fundamentals*, 2nd ed., McGraw-Hill, New York, Ch. 12, p. 802.
19. *Chem. Marketing Reporter*, **234**, 34 (1988).
20. Sussman S. (1981) *Kirk-Othmer Encyclopedia of Chemical Technology*, volume 24, Grayson, M. and Eckroth D., eds., Wiley, New York, p. 383.
21. Bartholomew, W. H. and Reisman, H. B. (1979), *Microbial Technology*, volume II, 2nd ed., Peppler, H. J. and Perlman, D., eds., Academic, New York, Ch. 18, p. 463.
22. Busche, R. M. (1986), *Biotech. Bioeng. Symp. No. 17*, 565.
23. Ferber, K. H. (1981), *Kirk-Othmer Encyclopedia of Chemical Technology*, vol. 3, Grayson, M. and Eckroth D., eds., Wiley, New York, p. 744.
24. Dale, G. H. (1981), *Kirk-Othmer Encyclopedia of Chemical Technology*, vol. 12, Grayson, M. and Eckroth D., eds., Wiley, New York, p. 870.