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Economic Potential of Recombinant Aerobes for Producing Specialty Chemicals

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ABSTRACT

Productivity of aerobic fermentations is often limited by the rate at which cells can aerobically catabolize the carbon source. Oxygen limitations frequently result in generation of growth-limiting metabolites. All approaches thus far have been concentrated on improving oxygen mass transfer rates by mechanical means. In contrast, a recent discovery has shown that insertion of hemoglobin into *E. coli* by genetic engineering accelerates cell growth and increases final cell density.

Stimulated by this innovation, this study appraised the techno-economic potential for improved fermentations based on recombinant aerobes that grow faster, to considerably greater cell densities, and with higher product concentrations than conventional cells. Possible reductions in fermentation cost for four specialty products based on this genetic approach were determined. It was concluded that, so far as fermentation economics are concerned, research in this area should focus primarily on means for reducing process investment.

Index Entries: Recombinant aerobes; fermentation; economics; aeration; specialty chemicals.

INTRODUCTION

The transfer of oxygen to aerobic cells has been a central focus of biochemical engineering since the advent of asceptic submerged fermentation in the mid-1940s. A central problem in the aerobic growth of any cell

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culture has been to maintain dissolved oxygen concentrations above growth-limiting levels, especially in high cell density fermentations. Productivity is often limited by the rate at which cells can aerobically catabolize the carbon source. Oxygen limitations frequently result in generation of growth-limiting metabolites (*1,2*).

The transfer process basically involves two sequential steps: (1) the transfer of oxygen from the gas phase into the liquid phase at a sufficiently high rate so as to maintain the dissolved oxygen concentration at an optimal level for (2) assimilation by the organism at its maximum biological limit.

All previous strategies for improving oxygen transfer have concentrated on improving the delivery of oxygen from air or oxygen gas to the surface of the cell by manipulating various environmental parameters of the fermenter medium.

In contrast, an alternative genetic strategy was recently developed for improving the cellular utilizationof oxygen and, possibly, also altering aerobic metabolism control under a less than optimum oxygen environment in the liquid phase (*3–5*). This approach involves isolating the gene for a novel hemoglobin molecule, which is expressed by the aerobic bacterium *Vitreoscilla* in poorly-oxygenated environments, and expressing the gene in *Escherichia coli*. The recombinant cells contain heme as well as active hemoglobin and grow faster and to considerably greater cell densities than comparable plasmid-containing cells that do not express hemoglobin.

The genetic approach is in a very early research stage, with little known about the specific potential for process improvement. Consequently, it was the objective of this study to evaluate various aerobic fermentation systems of commercial importance, in order to identify process shortcomings of economic significance that might be overcome by applying the genetic approach or, indeed, improved physical aeration approaches and, as a result, to direct this research along economically relevant pathways. The processes chosen for the analysis were those for producing bovine growth hormone (BGH), a functional cloned protein product, glutamic acid, and citric acid. Each of these is illustrative of a broad range of pharmaceuticals or chemical specialties produced over a wide range of plant capacities.

MECHANICAL AERATION

As noted by Hubbard (*6*), maintaining the dissolved oxygen content at an optimal value in the fermenter broth is usually the limiting factor in the design of an aeration system for an aerobic fermentation. This usually translates into providing sufficient energy in the form of agitator shaft horsepower and/or compressed air to a sparger so as to provide small air bubbles having a high surface/volume ratio and consequently having a high rate of diffusion between the gas and liquid phase.

Since it was not the intention of this study to evaluate the effects of mechanical aeration strategies on economics, power requirements for the economic models were set at a level of 3.4 HP/m^3. From a brief review of the literature, this appeared adequate to ensure sufficient oxygen transfer within the broth to provide for the needs of the organism (*7–12*).

THE BIOCATALYST APPROACH

The genetic approach is based on cloning into the production strain of interest a functional gene encoding the hemoglobin of the bacterium *Vitreoscilla*. The strategy has been demonstrated to date in *E. coli*, in which functional *Vitreoscilla* hemoglobin has been expressed after inserting the hemoglobin gene on a multicopy plasmid (*3*). *E. coli* cells containing hemogloblin respire more rapidly than plasmid-containing controls, especially under microaerobic conditions. Similarly, *E. coli* containing hemoglobin grow substantially more rapidly and attain higher cell densities under oxygen-limited conditions than control strains that do not synthesize hemoglobin (*4,5*). Preliminary data also suggest that the presence of hemoglobin reduces acetate production under microaerobic conditions.

Implementation of this strategy in other industrial aerobic organisms is now in progress. *Vitreoscilla* hemoglobin has been successfully expressed in one type of eucaryotic cell, suggesting that there are no fundamental barriers to applying this strategy to many different types of aerobic bioprocesses.

EXPECTED IMPACT OF RECOMBINANT AEROBES

The final concentration achieved in an aerobic bioprocess is often directly correlated to the maximum achievable biomass. Maximum biomass concentration is in turn often limited by oxygen supply to the system. Because it has been demonstrated that cloned hemoglobin enhances respiration and growth under oxygen-limiting conditions and enables growth to higher cell densities in laboratory situations, it is expected that this technology will enable fermentation operations to attain higher cell densities in batch and fed-batch processes and to enhance cell recycling in continuous processes. A second benefit of extending the duration of culture activity will be to increase the length of the batch operating cycle at the time when volumetric productivity is greatest.

There is insufficient data at present to estimate accurately how much cell density can be increased using this technology. In shake flask and fed-batch fermentations, cell density increases of between 50% and almost 100% have been observed as a result of the presence of cloned hemoglobin. However, these cultures were not growth in an optimized fashion to reach maximum attainable cell densities in the control.

Exploratory experiments in the laboratory have also indicated that increased specific growth rates can be provided by the presence of cloned *Vitreoscilla* hemoglobin. The possibility of acetate formation being reduced in fed-batch fermentations would also enable rapid feeding of the culture and yield accordingly higher growth rates. In a continuous process this behavior would permit a higher dilution rate.

One objective of further research on recombinant aerobes is to define the magnitude of potential improvements to the fermentation systems. This economic analysis was carried out with the aim of guiding this research.

PRODUCTS SELECTED FOR STUDY

Processes for producing BGH, a cloned protein, glutamic acid, and citric acid were selected as the evaluation "vehicles" because they represented a broad range in plant production scale for specialty chemicals and also allow a comparison between endogenous protein products and exogenous chemical products.

The BGH process, and the conclusions drawn thereon in this study, might also serve as a crude model for the production of a great number of biologically active drugs, such as interferon, human insulin, interleukin II, and the like, with due regard for the effects of production scale on cost. The cloned protein product category is actually an extension of the BGH model to a production scale of 1 million pounds annually to represent other protein products in this production volume category.

The production of glutamic acid in its preferred form as the flavor enhancer, monosodium glutamate monohydrate, outstrips all other amino acids in world volume (860 million pounds) and value ($750 million) (*13*). Citric acid is a specialty chemical, bordering on a commodity chemical in production volume (235 million pounds valued at $191 million) (*14*).

BASE CASE CONDITIONS FOR PROCESS MODELS

Process operating data for the models were obtained from the literature (*11,15–23*). The base case conditions are summarized in Table 1.

A batch mode was assumed in all base cases, although the economic potential for continuous operation coupled with cell recycling was evaluated for the exogenous chemicals. For the proteins, a common stage was used for concurrent cell growth and product expression. The models for the chemicals assumed that cell growth and product formation could occur in either common or sequential stages.

It is hoped that the base case conditions represent the state of the art. Realistically, however, it is recognized that up-to-date proprietary process information is seldom available from producers. Accordingly, the

Table 1
Aerobic Fermentation Performance Batch Fermenter Cases

	Bovine growth hormone	Cloned protein	Glutamic acid	Citric acid
Production level, million PPY	0.02	1.0	25.0	50.0
Fermenter mode	Batch	Batch	Batch	Batch
Growth/production stages	Common	Common	Sequential	Sequential
Product concentration, g/L	7	7	90	90
Cell density, g/L	42	42	18	8
Specific productivity, g/g cells · h	0.007	0.007	0.18	0.24
Fermentation yield, % of glucose converted	5	5	54	89
Recovery yield, % of product in broth	60	60	98	98
Batch cycle, h				
Growth	24	24	6	24
Production			29	46
Turnaround	3.5	8	12	12
Volumetric productivity, g/L ·h	0.25	0.22	2.2	1.6
Production fermenters, gallons	3×1000	3×60,000	3×100,000	6×130,000
Fermentation power, HP/1000 L	3.4	3.4	3.4	3.4
Aeration ratio, 02 fed/transferred	3:1	3:1	3:1	3:1
Oxygen transferred, m*M*/L · h	14	14	55	15

cost estimates may err on the high side, although the conclusions to be drawn are still believed to be valid.

In addition to the large difference in production scale between the protein and chemical products, a number of other inherent process differences exist: The intracellular proteins have only low product concentrations, very low yields relative to substrate converted, very low yields across the recovery and refining operations, and are not amendable to the potential benefits of cell recycling as are the extracellular chemical products.

BASE CASE ECONOMICS

Base case economics for the four processes were determined using technoeconomic models developed by Bio En-Gene-Er Associates, Inc. The time frame was that of a plant built at a Midwest site on the Mississippi River with a midpoint of construction in 1984, a start-up in 1986, and operating in 1988 at full production following two start-up learning years. Stainless steel fermenters were used in each case.

The analysis includes only the process steps common to all the processes, namely: raw materials receiving, medium preparation and sterilization, fermentation, beer/cell filtration, and final product storage. *Recovery and refining operations were not included in the analysis since these vary from process to process.*

A summary of product economics for the base cases is shown in Table 2. The given cost factors were based on actual operating experience for a plant like that described in the site scenario. Considering that recovery costs are not included, selling prices yielding a 30–40% pretax return on investment appear reasonable compared with market prices as of February 1989, of $0.86/lb for monosodium glutamate and $0.835/lb for anhydrous citric acid (*24*). BGH has more of the attributes of an expensive pharmaceutical—which it is—than those of a chemical (*25*).

EFFECT OF PRODUCTION SCALE

The relative importance of the major cost elements is indicated in Table 2 and Fig. 1. The production scale of BGH is so low that economics are overwhelmed by the cost of labor—a cost element for which improvements in aeration hold little promise. Nevertheless, improvements in aeration leading to reductions in investment could result in large absolute reductions in capital charges, even though the relative effect is overshadowed by high labor charges.

Among the cases studied, the contribution of labor to total cost decreases as production scale increases. In contrast, the importance of investment increases.

For the higher-volume processes, capital-related costs (including earnings required for an acceptable return on investment) are the most important of the cost elements. Fermenter investment for the protein products is high relative to the chemical products for two reasons: (1) product concentration is low, and volumetric productivity is accordingly low and (2) for BGH, the small 1000-gallon fermenters cost $500/installed gross gallon compared with $20/gross gallon for fermenters in the 150,000-gallon size. The relative position of capital-related charges for fermenter investment increases faster with increases in scale than other investment elements, because at high production levels, fermenters are added in multiples of large fermenter units, and investment is less scale sensitive than investment for other equipment pieces (Table 3). Clearly, research on aeration should be concentrated on ways to reduce investment.

The cost of raw materials ranks a distant second to capital charges. High substrate demand and raw material cost are related to

1. Poor product stoichiometry, i.e., a low chemical yield for the metabolic pathway followed by the organism;
2. High cell growth relative to protein product formation; and
3. Poor recovery of protein products across the recovery and refining steps.

It does not appear that improvements in aeration effectiveness can help this situation. However, this point needs to be explored more fully.

The effect of scale on the production of cloned proteins is shown in Fig. 2. A 10-fold increase in production rate results in a 10-fold increase in fermenter investment, but only a sevenfold increase in total plant investment. Cost-plus-return is cut in half. In Fig. 2 as well as other figures in this report, the state-of-the-art base case value is shown as a black dot.

EFFECT OF PRODUCT CONCENTRATION

Product concentration is the most important of the process variables affecting investment and, hence, cost. Its reciprocal, liters of medium per gram of product, is an indicator of the volume and, hence, investment required for the *entire* plant at a desired level of production. Preferably, concentration should be increased at constant cell density and fermentation time. Provided product inhibition is not a factor, this can be accomplished, at least theoretically, by increasing specific productivity, g product/g cells · h, or, in the case of exogeneous products, by recycling cells to build density without adversely affecting yield. If product inhibition is a factor, a genetic change would have to be made to the organism to reduce sensitivity to product.

The effects of increasing product concentration are shown in Fig. 3 for a cloned protein and in Fig. 4 for glutamic acid. The data for BGH and

Table 2
Aerobic Fermentation Comparisons Batch Fermenter Basecases

		Bovine growth hormone	Cloned protein	Glutamic acid	Citric acid
Production level, Million PPY		0.020	1.0	25.0	50.0
Investment, $million (1984)					
Direct Permanent Investment (DPI)		$1.97	$8.8	$15.0	$32.7
Allocated Power, Service, & Gen'l (APS&G)		$0.21	$1.3	$2.6	$5.5
Working Capital		$0.69	$2.2	$2.8	$4.5
Total Investment (TI), $Million		$2.87	$12.3	$20.4	$42.7
, $/lb		$143.49	$12.29	$0.82	$0.85
Cost, $/lb (1988)					
Raw Materials					
Sugar Syrup	@$0.081/lb glucose	$2.56	$2.56	$0.129	$0.076
Nutrients	@Current prices	$0.47	$0.47	$0.016	$0.004
Total Raw Materials		$3.03	$3.03	$0.145	$0.080
Utilities					
Electricity	@$0.040/KWH	$0.32	$0.36	$0.027	$0.044
Steam	@$2.20/1000 lb	$0.02	$0.02	$0.001	$0.001
Cooling Water	@$0.040/1000 gal	$0.00	$0.01	$0.002	$0.002
Process Water	@$0.500/1000 gal	$0.01	$0.01	$0.001	$0.001
Biodegradation	@$0.040/lb dry solids	$0.11	$0.11	$0.001	$0.001
Total Utilities		$0.46	$0.51	$0.032	$0.049
Labor-Related					
Dir. Op. Wages & Benefits (DOW&B)	@$26.40/man-hour	$51.68	$1.55	$0.069	$0.042
Dir. Salaries & Benefits	@18% DOW&B	$9.30	$0.28	$0.012	$0.007

Op. Supplies & Services	@6% DOW&B	$3.10	$0.09	$0.004	$0.002
Gen. Plt. O'head on Operations	@23% DOW&B	$14.02	$0.42	$0.019	$0.011
Control Laboratory	@$19.22/man-hour	$30.26	$0.81	$0.016	0.008
Tech. Assistance to Mfg.	@$22.06/man-hour	$0.00	$0.09	$0.002	$0.001
Total Labor-Related		$108.36	$3.24	$0.122	$0.071
Capital-Related					
Maint. Wages & Benefits (MW&B)	@1.7% DPI	$1.68	$0.15	$0.010	$0.011
Maint. Salaries & Benefits	@25% MW&B	$0.42	$0.04	$0.003	$0.003
Maint. Materials & Services	@40% MW&B	$0.67	$0.06	$0.004	$0.004
Maint. Overhead	@4% MW&B	$0.07	$0.01	$0.001	$0.001
Gen. Plt. O'head on Maintenance	@23% MW&B	$0.48	$0.04	$0.003	$0.003
Taxes & Insurance	@0.3% DPI	$0.30	$0.03	$0.002	$0.002
Depreciation on DPI	@8% DPI	$7.91	$0.70	$0.047	$0.052
Depreciation on APS&G	@6% APS&G	$0.62	$0.08	$0.006	$0.007
Total Capital-Related		$12.14	$1.11	$0.076	$0.083
Cost of Manufacture		$123.99	$7.89	$0.374	$0.282
Selling Expense	@6% Sales	$13.04	$0.39	$0.021	$0.019
Distribution	Varies	$2.00	$0.01	$0.010	$0.010
Research & Development	@6% Sales	$13.04	$0.59	$0.033	$0.029
Administrative Expense	@2% Sales	$4.34	$0.26	$0.014	$0.012
Incentive Compensation	@6% Earnings	$3.45	$0.22	0.014	$0.015
Cost of Sales		$159.86	$9.36	$0.466	$0.367
Pretax Earnings	@30%-40% TI	$57.45	$3.69	$0.241	$0.256
Cost-Plus-Return Selling Price		$217.31	$13.05	$0.707[a]	$0.623

[a] $0.60 as monosodium glutamate monohydrate.

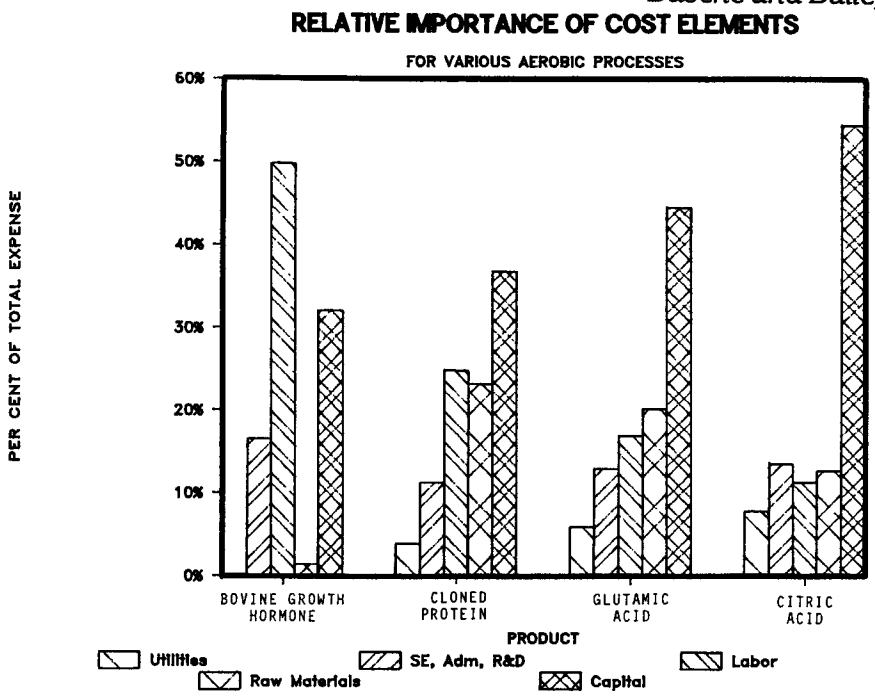

Fig. 1. Relative importance of major cost elements for various aero processes.

Table 3
Distribution of Investment Among Process Elements

Process	Bovine growth hormone	Cloned protein	Glutamic acid	Citric acid
Production, Million PPY	0.020	1	25	50
Investment, $/annual lb				
Electric	$0.18	$0.21	$0.02	$0.03
Other allocations	$9.08	$0.98	$0.06	$0.07
Aeration	$1.24	$1.24	$0.10	$0.04
Prep, sep and storage	$17.15	$3.58	$0.21	$0.17
Fermenters	$70.55	$3.10	$0.23	$0.38
Total plant	$98.20	$9.11	$0.62	$0.69
Investment, % of total plant				
Electric	0.2%	2.3%	3.2%	4.3%
Other allocations	9.2%	10.8%	9.7%	10.1%
Aeration	1.3%	13.6%	16.1%	5.8%
Prep and storage	17.5%	39.3%	33.9%	24.6%
Fermenters	71.8%	34.0%	37.1%	55.1%
Total	100.0%	100.0%	100.0%	100.0%

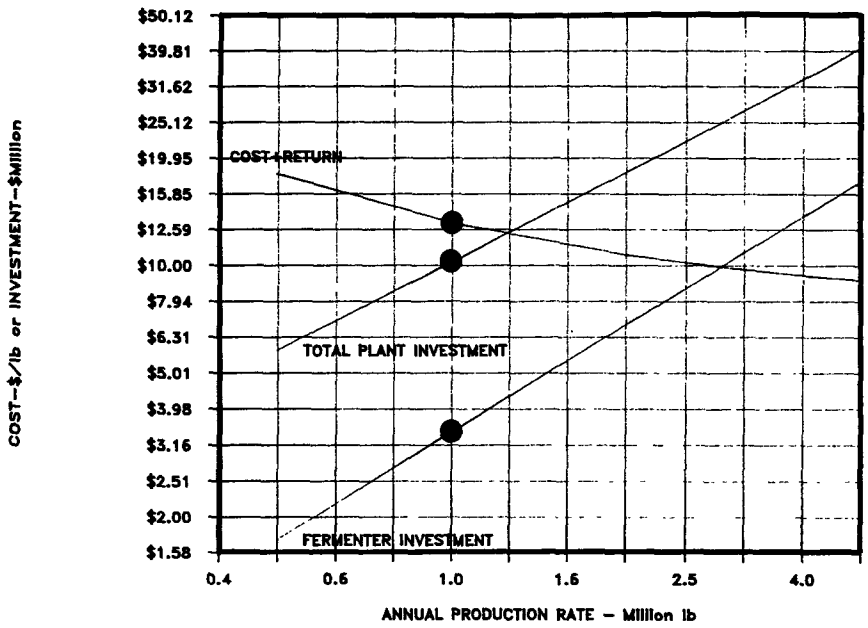

Fig. 2. Cloned protein fermentation: effect of production scale and total plant investment on cost-plus-return selling price.

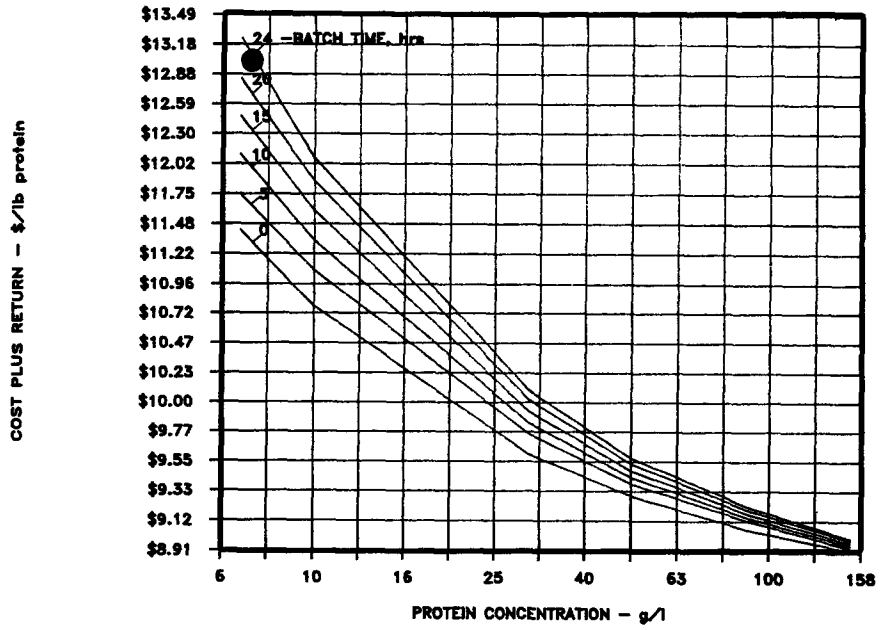

Fig. 3. Cloned protein fermentation: effect of protein concentration batch time on cost-plus-return selling price.

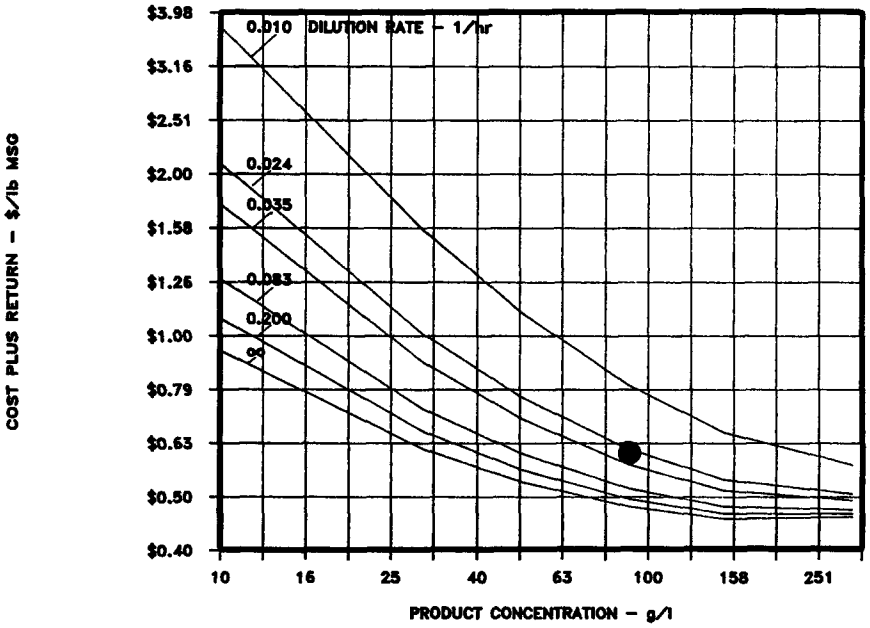

Fig. 4. Glutamic acid fermentation: effect of product concentration dilution rate on cost-plus-return selling price.

citric acid are similar. In all cases, product cost drops rapidly at low product concentration in the 10–40 g/L range, but begins to level off at concentrations above 100 g/L, showing the diminishing effect of the reciprocal of concentration at high concentrations.

EFFECT OF FERMENTATION TIME

Batch time or, for a continuous process, its reciprocal, dilution rate, is another important variable affecting investment. However, unlike product concentration, which affects all investments, changes in time affect only the investment in fermenters. Nevertheless, since fermenter investment is usually a major part of total investment (*see* Table 3), time is accordingly important to cost.

From the above discussion of the effects of product concentration, it appears that strategies for reducing time should not do so at the expense of concentration; for example, developing a thermophilic organism having a lower product inhibition threshold. The interrelation of time and concentration is shown in Figs. 3 and 4. The effect of time is strongest at low product concentrations, but becomes increasingly insignificant at high concentrations as capital charges, and in particular, fermenter in-

Table 4
Bovine Growth Hormone Effect of Productivity
on Fermentation Performance for 6.9 g/L Product Concentration

	For batch operation—3.5-h turnaround					
Specific productivity, g/g · h	Batch time, h	Volume productivity, g/L · h	Oxygen transfer, mM/L · h	Cost + return, $/lb	Ferm. invest., $MM	FI/TPI
0.0023	72.0	0.09	4.6	$317	$4.3	83%
0.0027	60.0	0.11	5.5	$292	$3.6	81%
0.0034	48.0	0.13	6.8	$267	$2.9	80%
0.0045	36.0	0.18	9.1	$242	$2.2	77%
0.0068	24.0	0.25	13.7	$217	$1.6	72%
0.0136	12.0	0.45	27.3	$192	$0.9	62%
0.0271	6.0	0.73	54.6	$180	$0.5	52%

vestment, becomes less significant elements of total cost. Note also that even at a zero batch time for the cloned-protein case, cost is still quite high.

EFFECT OF CONTINUOUS OPERATION

The very real advantage of continuous-mode operation in producing extracellular products is also illustrated in Fig. 4. In this plot, dilution rate is expressed as the reciprocal of time for continuous operation or as the reciprocal of batch time (bt) plus turnaround time (tt), i.e., $1/(bt+tt)$, for batch operation. For the large fermenters used in these models, a turnaround time of 12 h was assumed in batch operation for emptying, cleaning, sterilizing, refilling, and inoculating the fermenter for the next batch. None of this would be required for continuous operation over extended campaign periods. The maximum apparent dilution rate for a large batch fermenter at zero batch time would be 0.083 h^{-1}, i.e. 1/12 h. The cost curve for that rate represents the lowest cost for the batch model. However, the cost for continuous fermentation could be reduced even more, to near the costs for "infinite" dilution rate. At or near that point, the effect of fermenter investment on cost is not significant.

EFFECT OF SPECIFIC CELL PRODUCTIVITY

In an endogenous batch process, the only way to reduce fermentation time and, as a result, fermenter investment, at the desired maximum product concentration attainable would be to increase the specific productivity of the cell. The effect of increasing specific productivity of intracellular systems is shown in Table 4 for BGH. As specific productivity

Table 5
Citric Acid Manufacture Effect of Cell Recycling
on Fermentation Performance for 90 g/L Product Concentration

	For batch operation—12-h turnaround					
Cell density, g/L	Batch time, h	Volume productivity, g/L · h	Oxygen transfer, m*M*/L · h	Cost+ return, $/lb	Ferm. invest., $MM	FI/TPI
8.2	46.0	1.55	15	$0.62	$21.3	56%
15.0	25.2	2.42	28	$0.55	$15.9	50%
30.0	12.6	3.66	56	$0.50	$12.6	45%
50.0	7.5	4.60	93	$0.49	$11.3	42%
80.0	4.7	5.38	149	$0.48	$10.6	41%
110.0	3.4	5.83	205	$0.48	$10.3	40%
150.0	2.5	6.20	279	$0.48	$10.0	39%
	For continuous operation					
Cell density, g/L	Dilution rate, 1/h	Volume productivity, g/L · h	Oxygen transfer, m*M*/L · h	Cost+ return, $/lb*	Ferm. invest., $MM	FI/TPI
8.2	0.02	1.96	15	$0.58	$18.2	53%
15.0	0.04	3.58	28	$0.50	$12.8	45%
30.0	0.08	7.16	56	$0.46	$9.5	39%
50.0	0.13	11.93	93	$0.44	$8.2	36%
80.0	0.21	19.08	149	$0.43	$7.5	34%
110.0	0.29	26.24	205	$0.42	$7.1	33%
150.0	0.40	35.78	279	$0.42	$6.9	32%

is increased, volumetric productivity increases, and batch time can be reduced accordingly. Fermenter investment (FI) is reduced as a result, and becomes an increasingly smaller part of total plant investment (TPI). Because of the flywheel effect of other cost elements, however, product cost does not drop in proportion to productivity.

EFFECT OF CELL RECYCLING

Although exogenous product systems would also benefit from increases in specific productivity, the same effect could be obtained much easier by recycling cells to higher cell densities at constant specific productivity. This effect is shown in Table 5 for both batch and continuous production of citric acid. Both the glutamic acid and the citric acid fermentations reach high product concentrations. As noted earlier, this diminishes the relative effect of reductions in fermentation time. Recycling cells would have a greater effect on reducing costs for systems that are severely

product inhibited. Nevertheless, product costs for the acid fermentations could be reduced 15–28% by recycling cells to high (150 g/L) levels.

As a result of the adverse effect of broth viscosity on aeration (*26–30*), such high levels may not be attainable unless recombinant aerobes can be developed to tolerate such microaeration environments.

CONCLUSIONS AND RECOMMENDATIONS

The transfer of the hemoglobin gene into aerobic organisms of commercial interest appears to have merit from both a product expression and an economic point of view. For systems such as those analyzed in this study, it is recommended that research on improved aerobes for product expression be directed toward developing properties that can lead primarily to a reduction in plant investment, with emphasis on reducing fermenter investment. Even for functional protein products to be produced at small scale, the cost of investment could be effectively reduced by advances in aeration. Although the relative savings might be masked by high labor charges, high absolute savings could result. Reducing investment might be accomplished by

1. Increasing product concentration;
2. Reducing fermenter time and volume (*a*) For extracellular products, by recycling cells to a higher density at constant specific productivity, or (*b*) For either extracellular or intracellular products, by increasing the specific productivity (g product/g cells · h) of the cells;
3. Converting to continuous operation.

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