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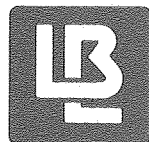
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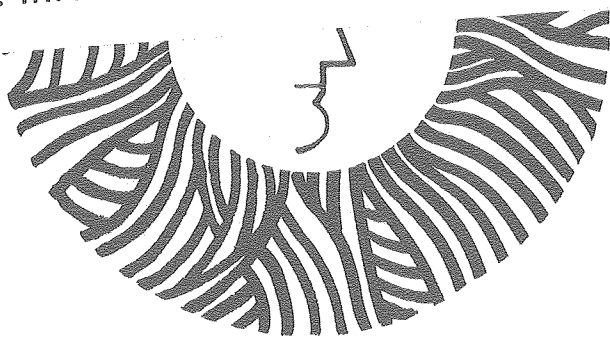
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ECONOMICS OF SUGAR PRODUCTION WITH TRICHODERMA REESEI RUTGERS C-30*

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ABSTRACT

The economics of sugar production from bioconversion of corn stover utilizing cellulase obtained from Trichoderma reesei strain Rutgers C-30 were investigated. The cost of manufacturing sugar is 10.5 cents per pound. This reduced cost is due to cellulase activities ranging from 7-14 IU/ml from batch cultures and from 3.5 to 6 IU/ml from continuous cultures. Hydrolysis can be carried out at substrate concentrations up to 25% and give product streams containing up to 15% sugars. This corresponds to a maximum yield of 86%.

A sensitivity analysis indicates that sugar can be produced at near competitive prices from corn stover costing \$0-50 per ton. By utilizing new fermentation technology, sugar at these prices can be used to produce 95% alcohol at a cost ranging from \$1.80 to \$3.00 per gallon.

I. INTRODUCTION

The development of mutant strains of T. reesei have enhanced the potential for developing commercially viable processes that enzymatically hydrolyze biomass to produce glucose.

Until recently, most of the process development work in this area utilized strain QM-9414 as a source of cellulase for enzymatic hydrolysis. The development of hyperproducing and catabolite repression resistant strain Rut C-30 have led to a re-evaluation of these processes. This report will cover recent hydrolyses utilizing cellulase obtained from Rut C-30 with corn stover as substrate. Updated economic evaluation for a C-30 based process is presented.

II. HYDROLYSIS EXPERIMENTS

Batch hydrolysis experiments were conducted for a period of 24 and 48 hours employing substrate concentrations ranging from 5 to 25% by weight and enzyme activities ranging from 2.6 to 9 IU/ml. Experiments were carried out in 600 ml Erlenmeyer flasks containing 200 ml of substrate-enzyme mixture. The contents were stirred at approximately 150 rpm and were maintained at a constant temperature of 45°C and pH of 5.

Prior to the hydrolysis reaction, corn stover was hammermilled to 2 mm particle size and treated with sulfuric acid to hydrolyze hemicellulose (1). Conditions of the acid treatment are given in Figure 1. Following pretreatment, corn stover was neutralized to a pH of 5, washed and air dried to approximately 10% moisture. The treated substrate contained 57% glucose equivalent by weight.

Cellulase was produced in batch fermentation utilizing T. reesei Rut C-30 following Tangnu, et al (2). Activities of the two enzyme batches used are given in Table 1.

In experiments with substrate concentrations exceeding 10% by weight, a

ACID PRETREATMENT OF CORN STOVER

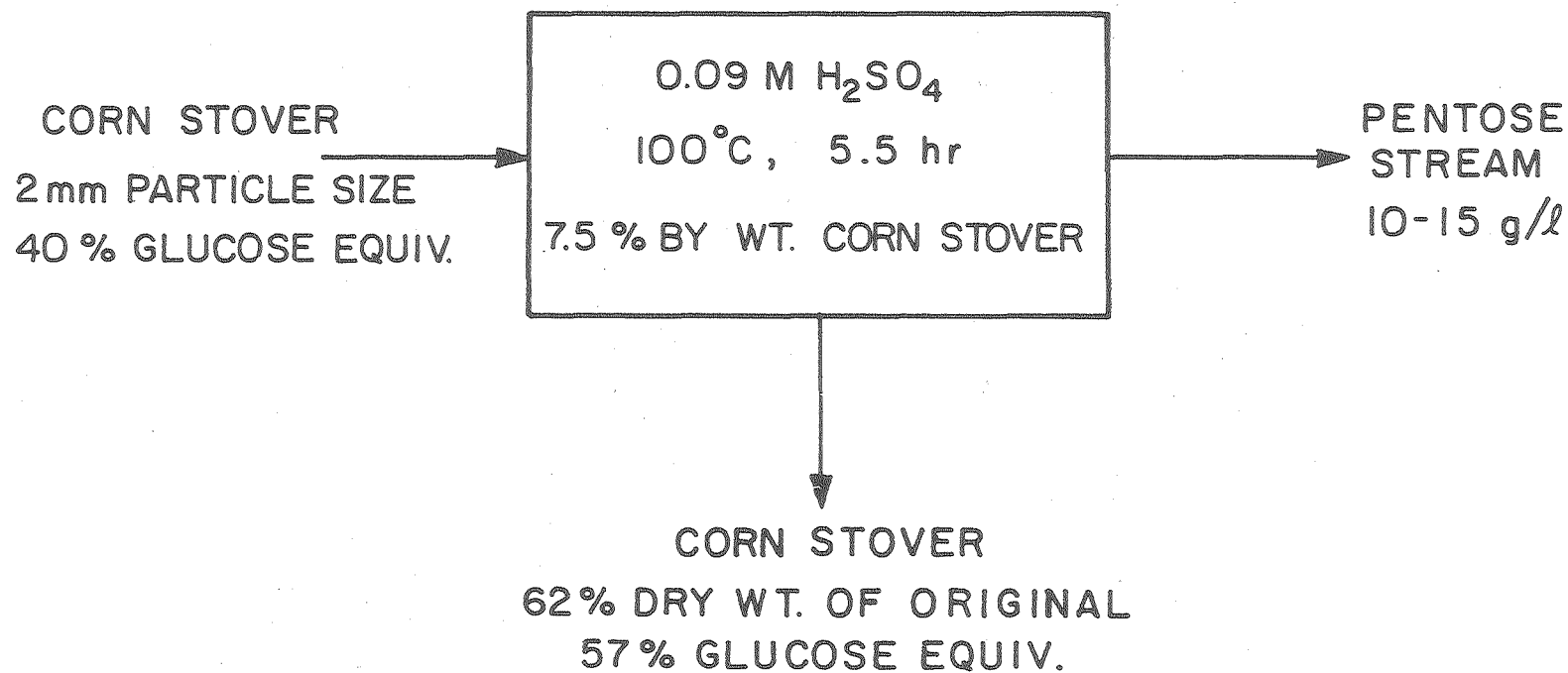


FIGURE 1.

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TABLE 1
 ACTIVITY OF CELLULASE FROM BATCH
 FERMENTATION OF RUT C-30

FILTER PAPER (IU/ML)	ACTIVITIES			SOLUBLE PROTEIN
	C ₁	C _X	β -G	(G/L)
9.4	.67	150	8.7	11.9
5.2	.50	158	8.4	8.3

stepwise addition of substrate was employed. After the initial solids loading of 5 to 10%, the reaction was allowed to proceed for one-half hour at which point an increment of corn stover was added. Additional substrate increments enable 25% total substrate concentrations to be achieved in 2.5 hours.

During the course of the reaction samples are withdrawn and analyzed for reducing sugars using the DNS method (3). Selected samples were analyzed for specific sugars using an HPLC and the overall activity of the enzyme remaining in solution was determined using the filter paper assay (3).

Figure 2 presents a series of (DNS) reducing sugar concentration versus time profiles for hydrolyses conducted with cellulase of 5.2 IU/ml activity and substrate concentrations ranging from 5-25% by weight. Total sugar concentrations of up to 9% are possible under these conditions.

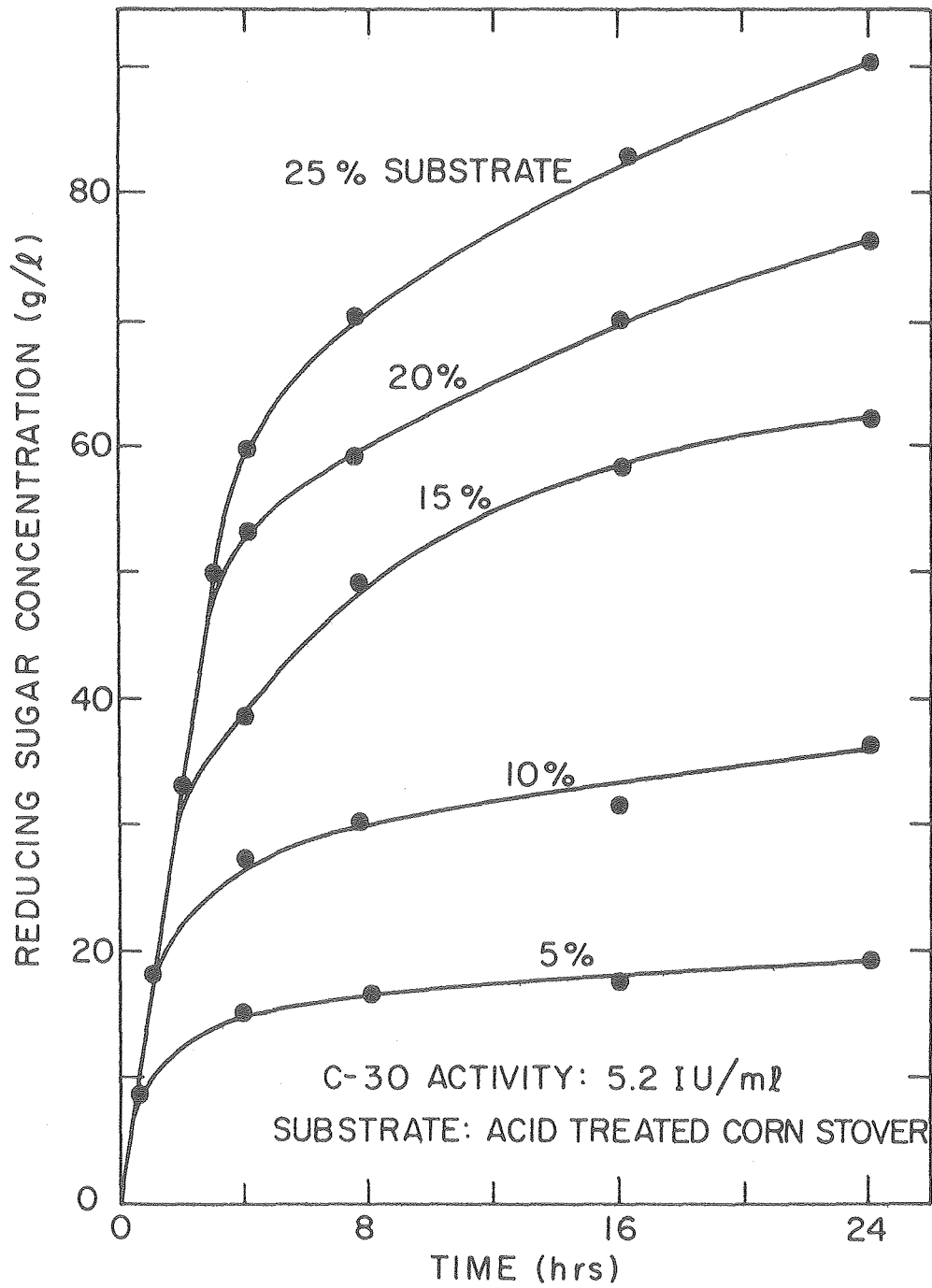
Figure 3 shows a breakdown of the sugar product sugar components corresponding to 25% solid loading case presented above. Glucose comprises approximately 80% of the sugar produced. Xylose concentration levels off to a value of 8 g/l after approximately 8 hours. Cellobiose undergoes a maximum early in the reaction and decreases to a low value after approximately 16 hours.

From the glucose composition of the product streams it is possible to compute glucose yields. Figure 4 shows the yield of glucose for the hydrolyses presented above. Yields range from 60 to 43%, decreasing with increasing solid concentration.

Figure 5 shows the amount of enzyme activity remaining in solution after 24 hours as a percentage of the original activity. The amount remaining is 25 to 80 percent of the original activity, decreasing with increasing substrate concentration.

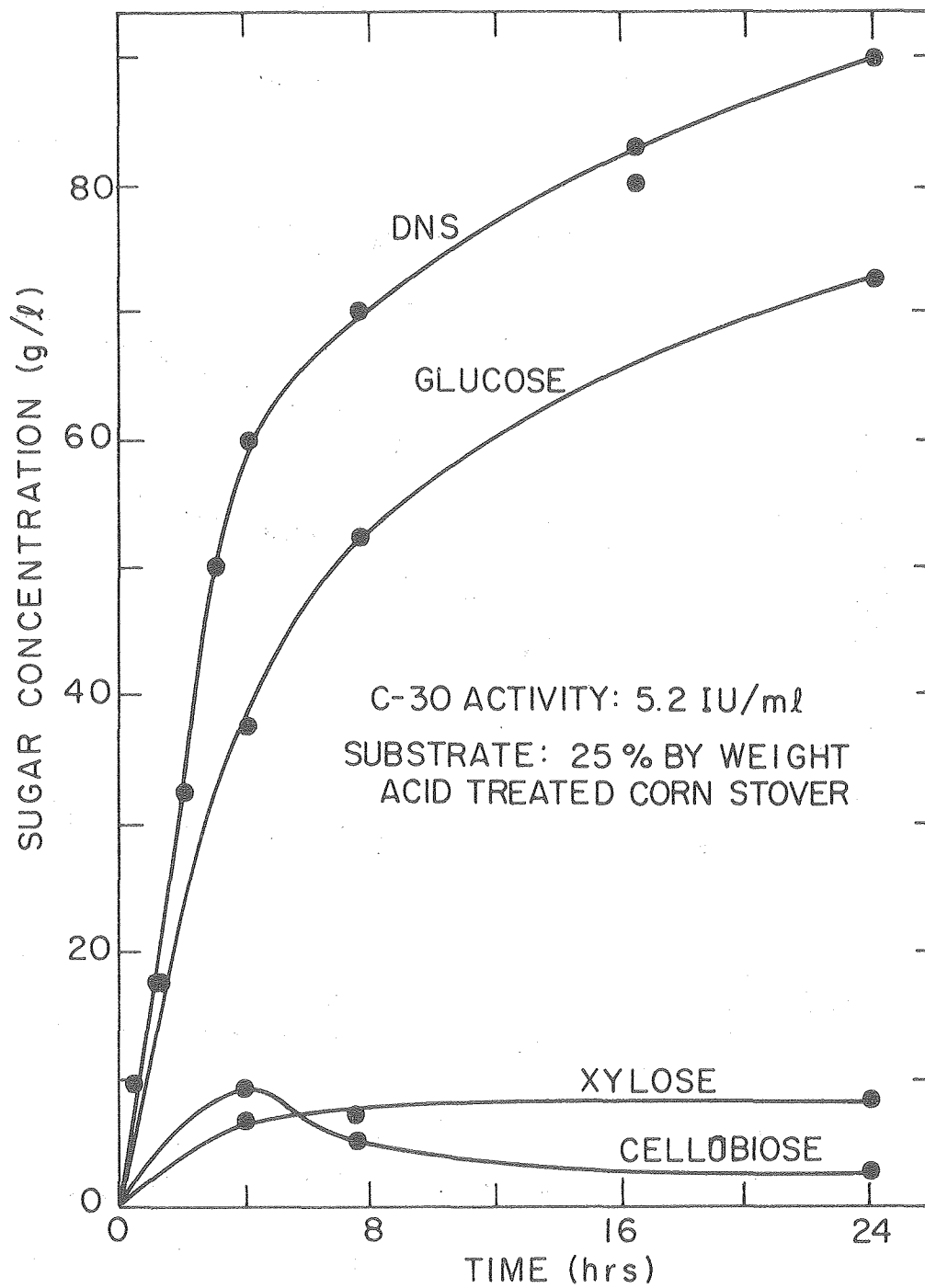
Figure 6 shows a summary of hydrolysis experiments conducted using Rut C-30 cellulase with an activity of 7 IU/ml. The total sugar concentration ranges

FIGURE 2. HYDROLYSIS REDUCING SUGAR CONCENTRATION VERSUS TIME AT VARIOUS SUBSTRATE LOADINGS.



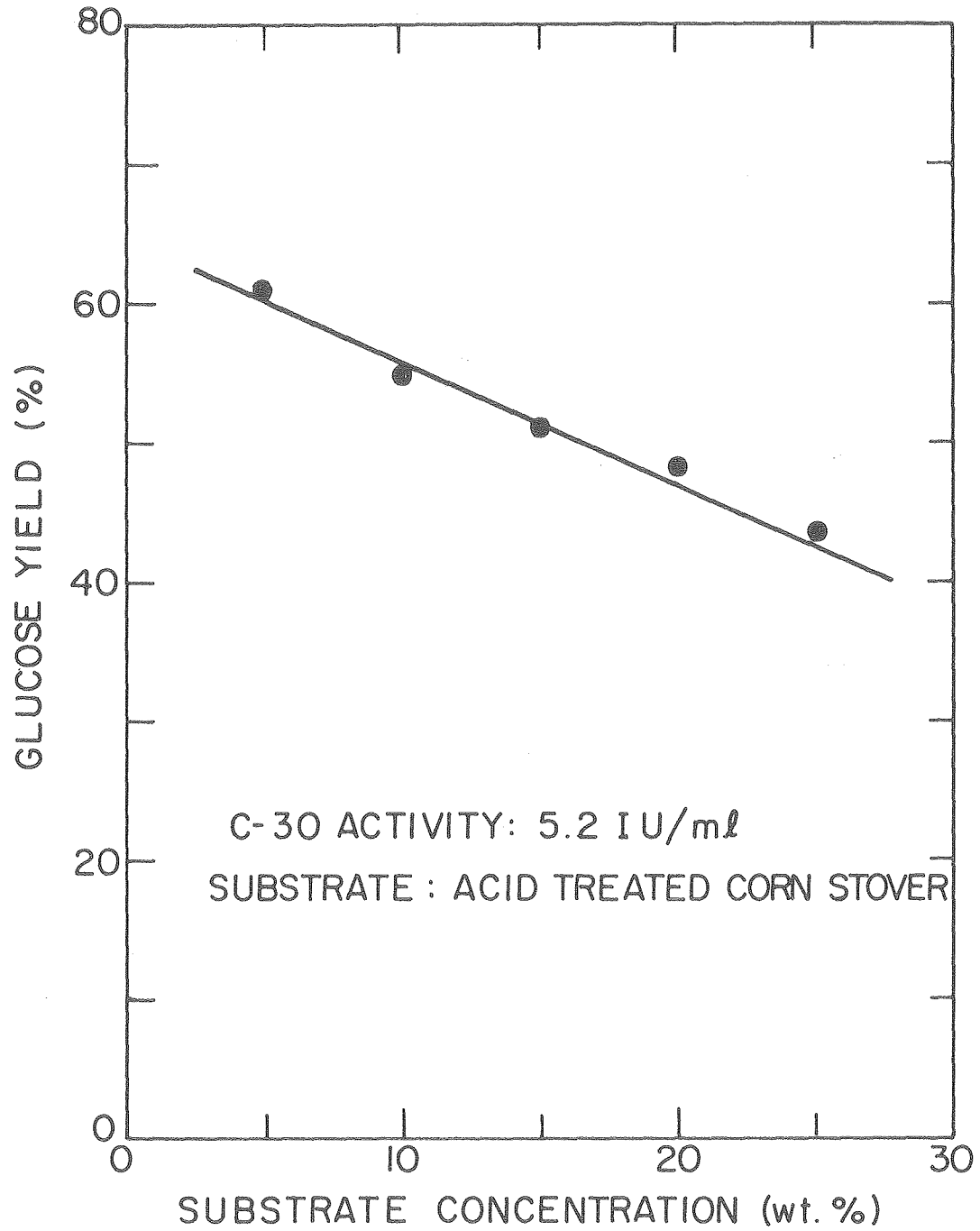
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FIGURE 3. SUGAR CONCENTRATIONS VERSUS TIME.



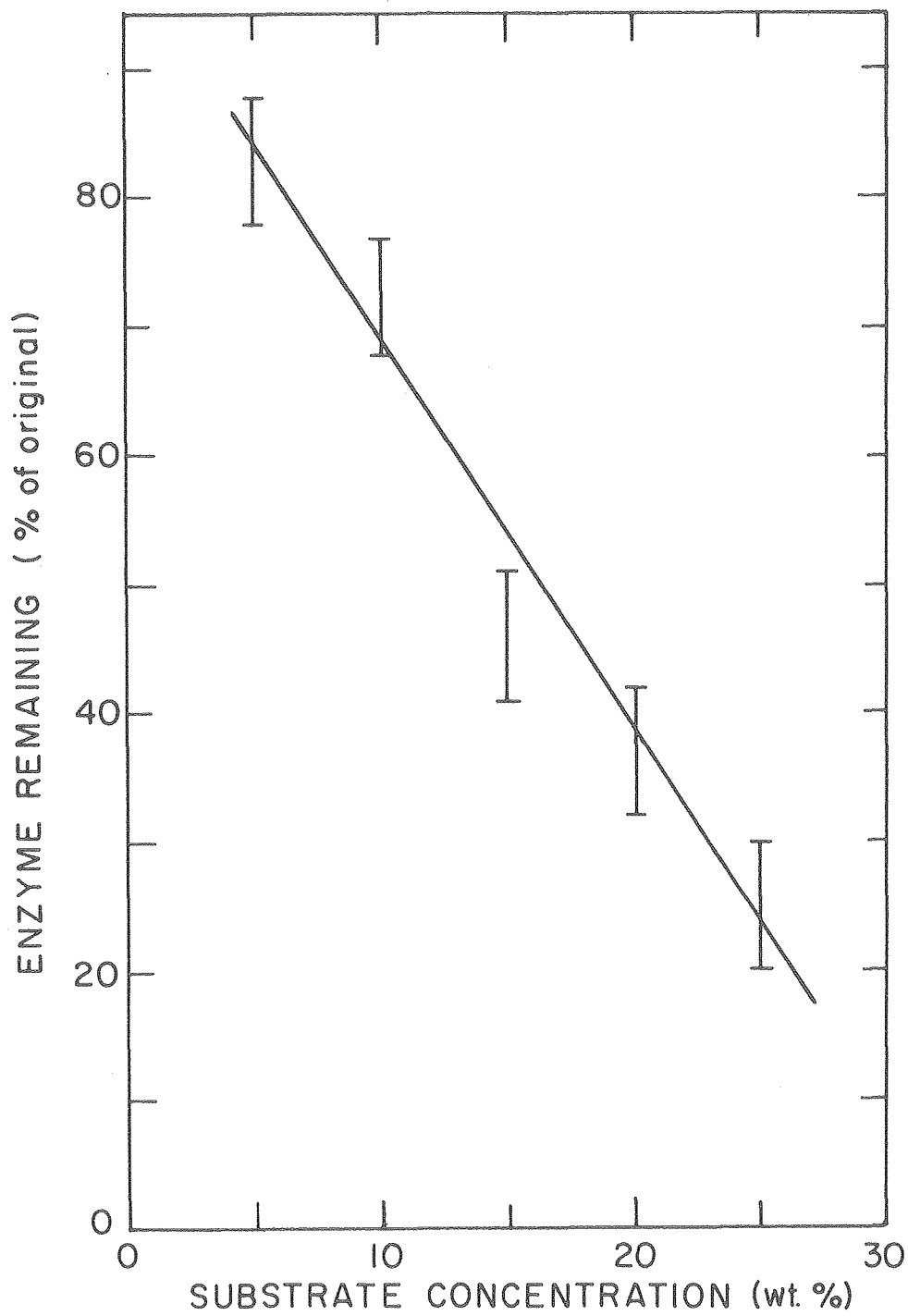
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FIGURE 4. GLUCOSE YIELD AT 24 HOURS VERSUS SUBSTRATE CONCENTRATION.



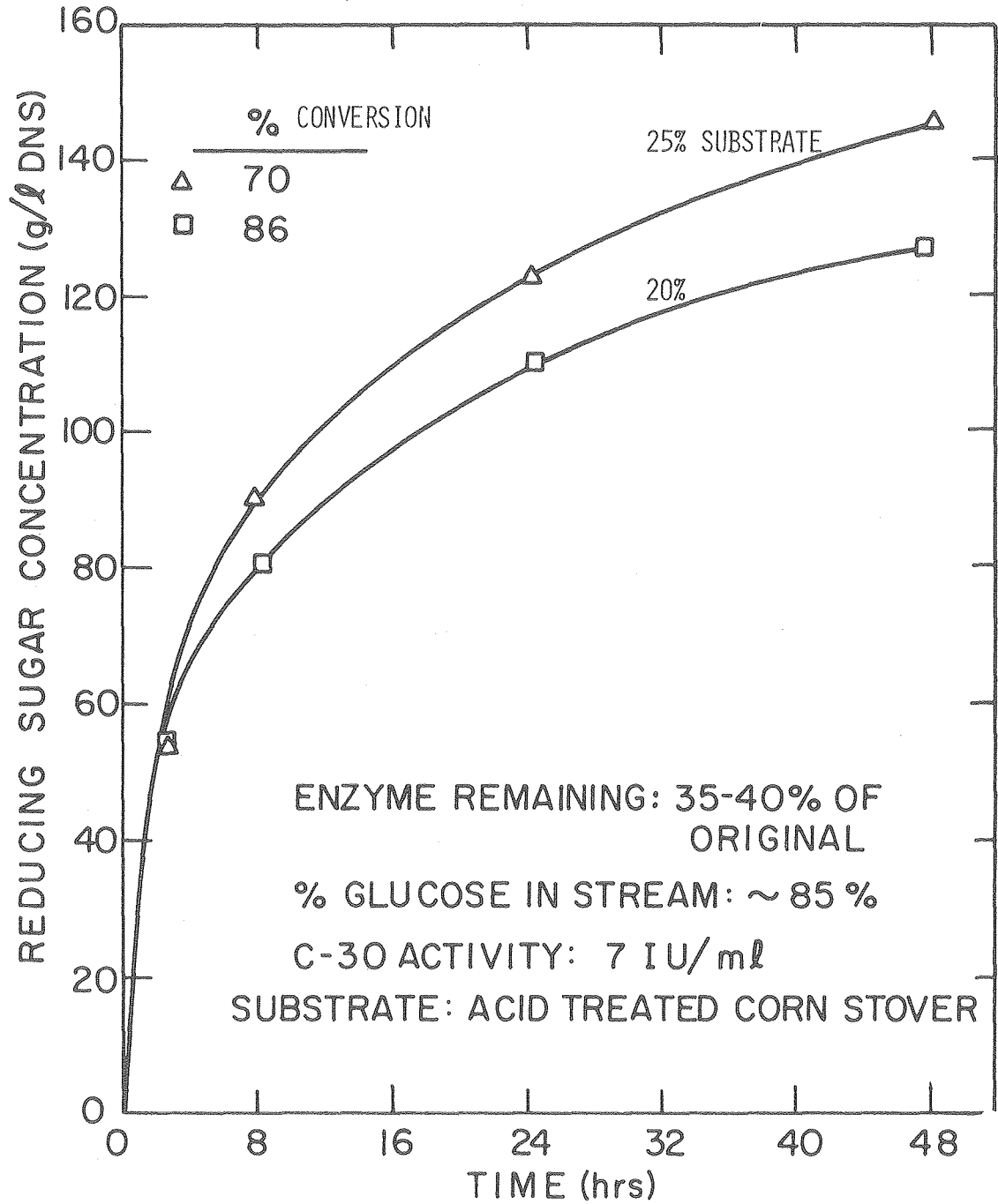
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FIGURE 5. ENZYME ACTIVITY AT 24 HOURS VERSUS SUBSTRATE CONCENTRATION.



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FIGURE 6. REDUCING SUGAR CONCENTRATION VERSUS TIME.



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from 12-15%, the conversions range from 70-86% and enzyme activity remaining after hydrolysis is 35-40% of original.

III. PROCESS DESIGN

To enable economic evaluation, the information obtained from the hydrolysis work was applied to a processing scheme designed to manufacture 214 tons of glucose per day from corn stover. A block flow diagram of this process, previously developed for evaluation of the performance of QM-9414 (4), is shown in Figure 7.

Incoming corn stover is milled and treated with sulfuric acid as previously described. Liquors from the acid treatment reactors are partly recycled, the remainder being sent to a process which will utilize xylose. Solids leaving the acid treatment section are contacted with the hydrolysis product stream to adsorb some of the enzyme remaining in solution. After passing through the enzyme recovery section, the solids enter the hydrolysis reactors along with fresh enzyme being continuously produced.

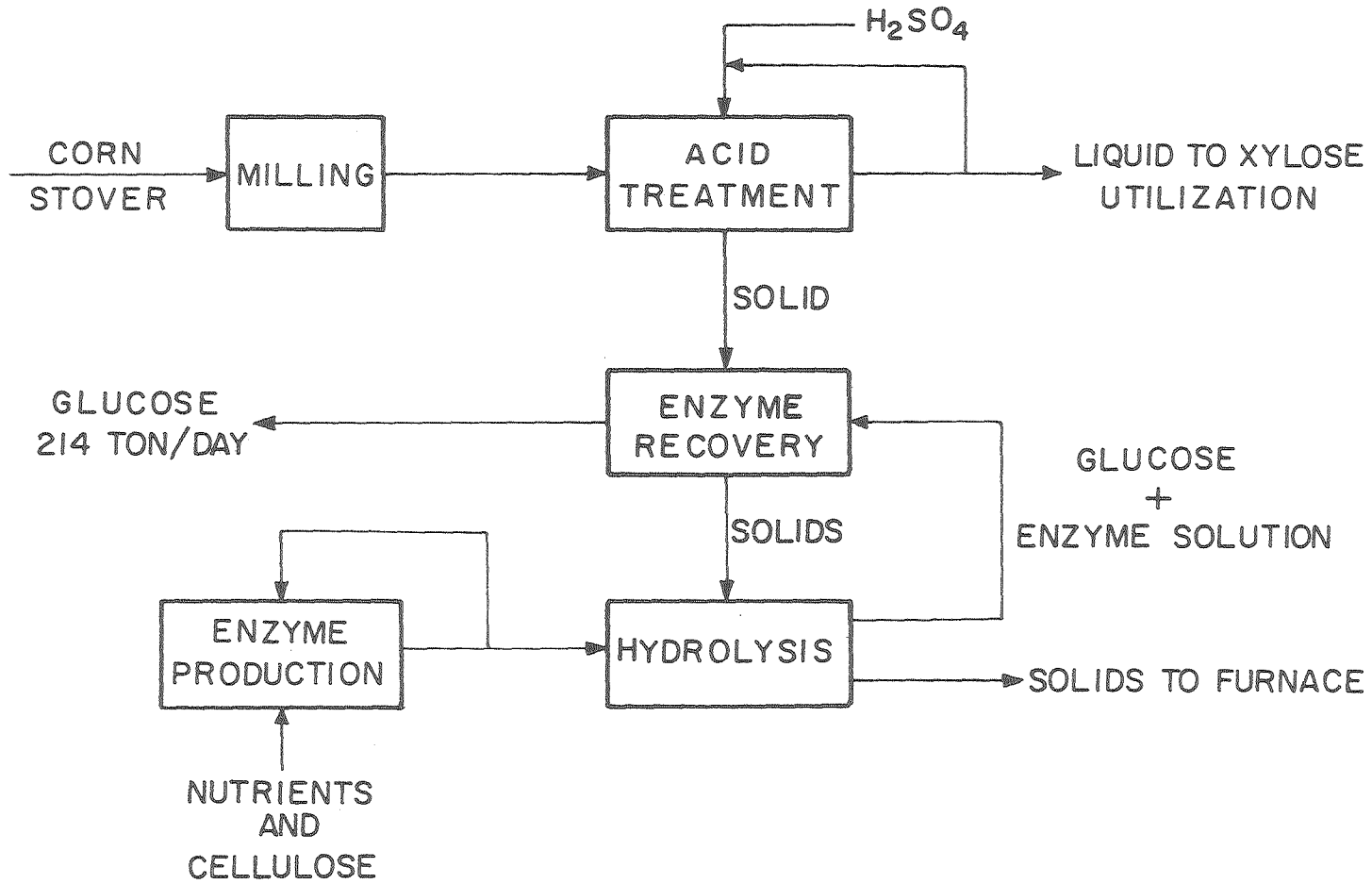
A detailed description of the process has been given by Yang, et al. (4). Enzyme production is based on the work of Tangnu, et al. (5)

IV. ECONOMIC EVALUATION

Using the process described above, a preliminary cost estimate was made for the required fixed capital and cost of sugar production. The evaluation was performed on all hydrolysis experiments presented.

The cost estimation procedure recommended by Peters and Timmerhaus was used. Fixed capital costs were estimated as a multiple of purchased cost. A multiplier

ENZYMATIC HYDROLYSIS PROCESS : BLOCK FLOW DIAGRAM



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FIGURE 7

of 4.1 corresponding to a solid-fluid handling facility was used. Capital costs were updated from previous reports (6) and obtained from Peters and Timmerhaus or Happel and Jordan (7,8). A Marshall and Swift Index of 635 corresponding to the first quarter of 1980 was used. The manufacturing cost is comprised of capital and labor related factors, utilities and raw materials. Capital and labor factors, and utility based rates are given in Tables 2-4. Taxes have been omitted on the assumption that the plant is tax exempt.

Figure 8 presents the cost of manufacturing glucose at various substrate concentrations for the 5.2 IU/ml enzyme activity case presented earlier. The cost of sugar ranges from 12.2 to 11.4 cents per pound with the lowest cost corresponding to a substrate concentration of 10 to 15%.

Figure 9 shows a breakdown of manufacturing costs by processing step. Enzyme production is the predominant cost comprised approximately 50% of the manufacturing cost. Initial savings in required enzyme volume stemming from increased solids loading per volume are offset by decreased enzyme recovery experienced at higher substrate loading. Acid treatment cost rise significantly as solids are increased due to loss in yields. Savings in hydrolysis cost due to volume reduction are countered by increased agitation requirements.

Figure 10 presents a breakdown of manufacturing cost by manufacturing component. Capital related factors make up over 50% of the manufacturing cost. Raw materials comprise 25% of the total.

Fixed capital investment for the cases presented range from 37 to 40 million dollars. The production costs presented were based on a plant receiving corn stover free of charge. Figure 11 is a sensitivity analysis of the cost of producing sugar at various corn stover costs. When the cost of corn stover is increased from \$0 to \$50 per ton the cost of sugar is increased from 11 to 24 cents per pound. The minimum production costs shifts to lower substrate

TABLE 2
CAPITAL RELATED COST FACTORS
(COST PER ANNUM = FACTOR X FIXED CAPITAL)

ITEM	COST FACTOR
DEPRECIATION	0.1
INTEREST	0.06
MAINTENANCE	0.06
INSURANCE	0.01
PLANT SUPPLIES	0.01
TAXES	0.0
TOTAL	0.24

TABLE 3
LABOR RELATED COST FACTORS

(COST PER ANNUM = FACTOR X OPERATING LABOR)

ITEM	COST FACTOR
OPERATING LABOR	1.0
FRINGE BENEFITS	0.22
SUPERVISOR, CLERICAL	0.18
OPERATING SUPPLIES	0.10
LABORATORY	0.15
TOTAL	<u>1.65</u>

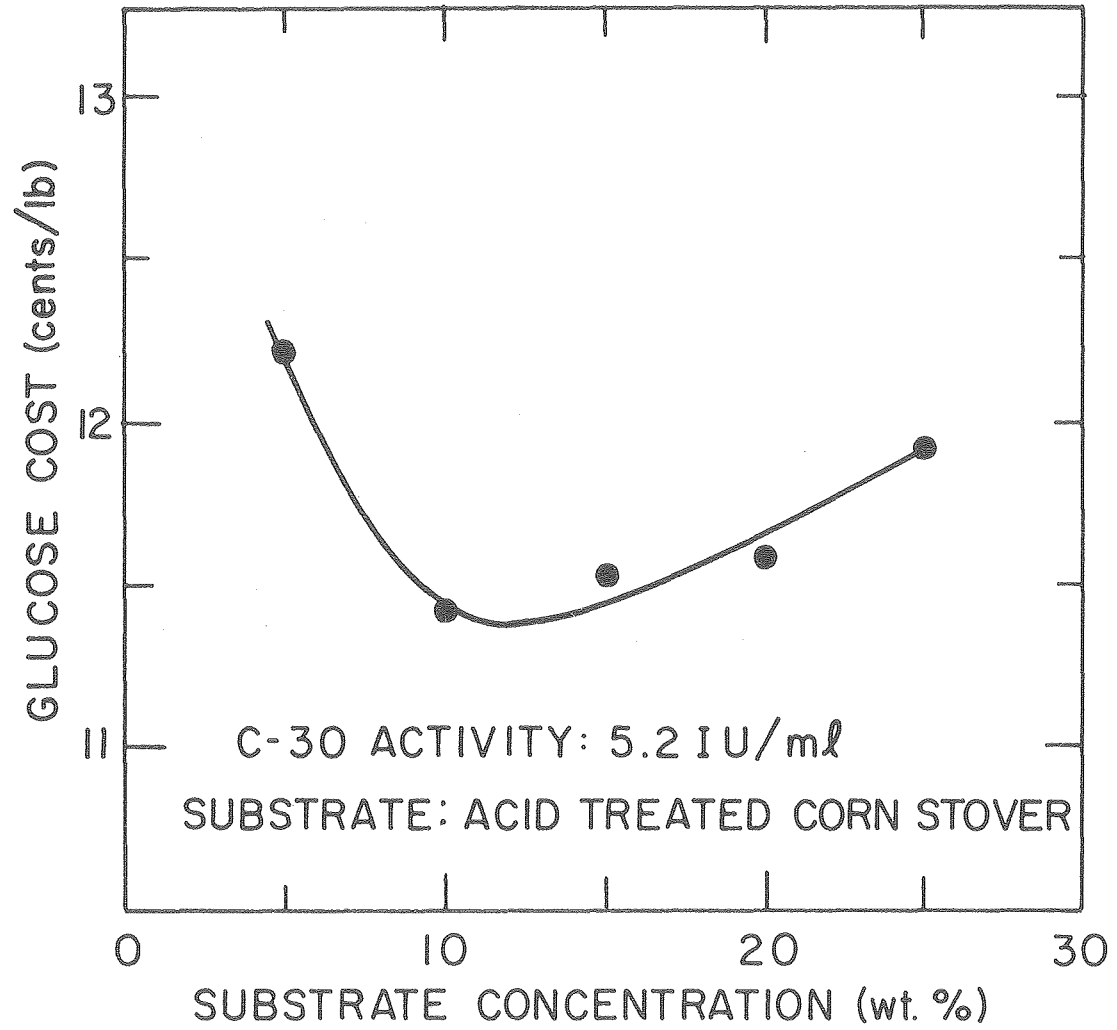
BASE LABOR RATE: \$22,000/MAN YR.

TABLE 4
BASE UTILITY RATE

	UNIT	UNIT COST
POWER	Kw-Hr	3¢
STEAM	1000 #	32.5¢*
WATER	1000 GAL	12.8¢

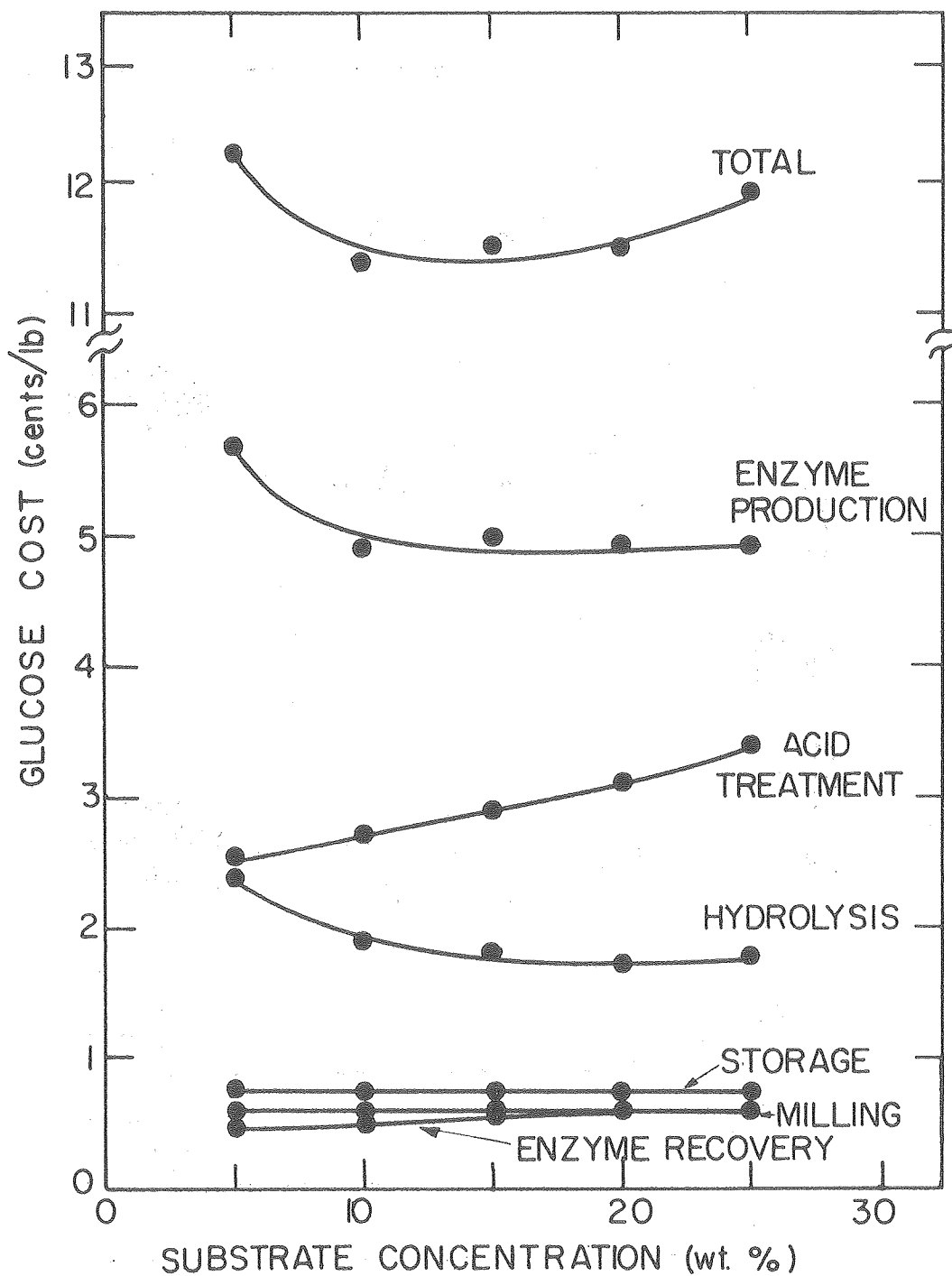
*SELF GENERATED FROM RESIDUAL SOLIDS

FIGURE 8. SUGAR PRODUCTION COST VS. SUBSTRATE CONCENTRATION



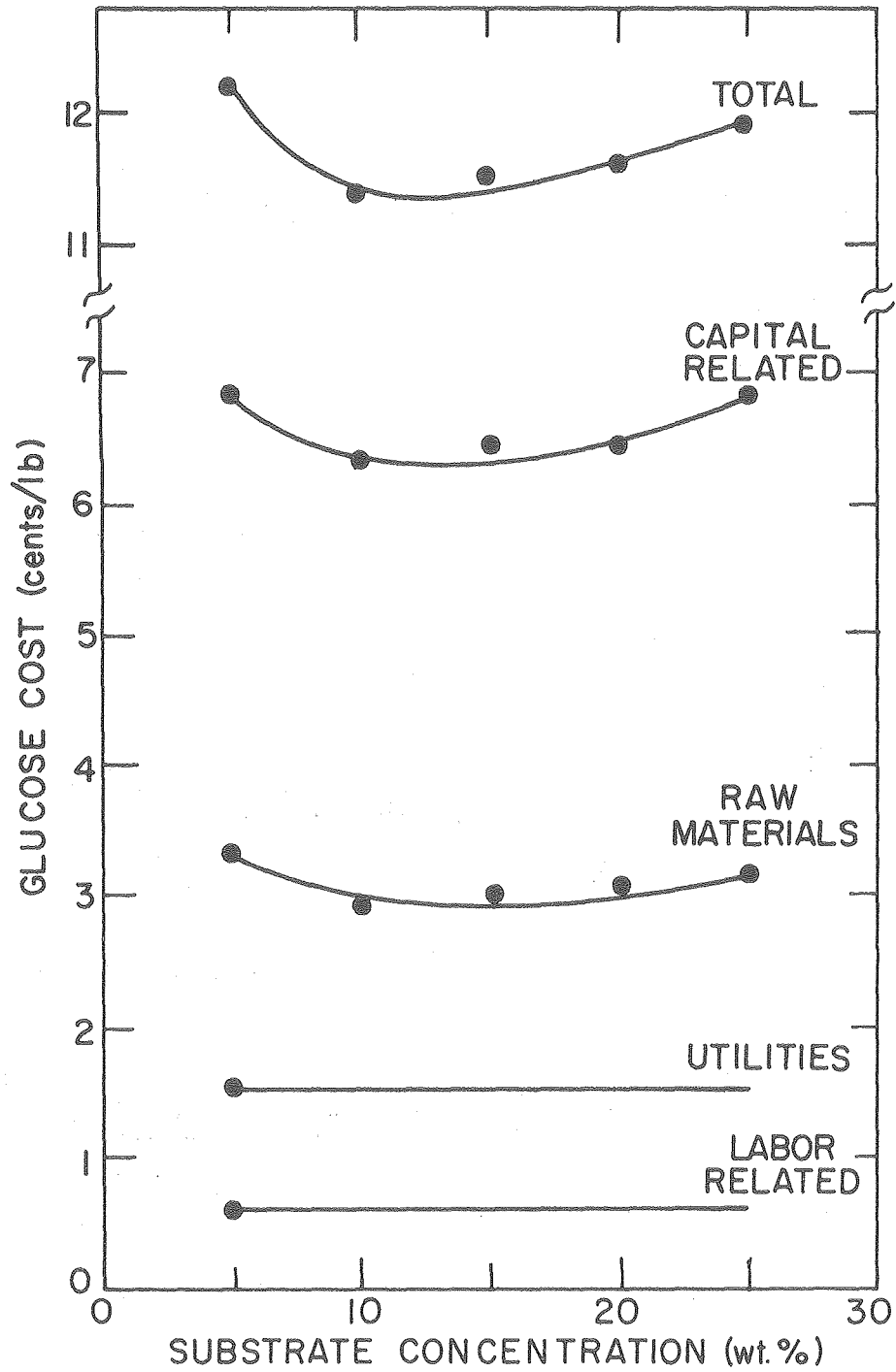
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FIGURE 9. PRODUCTION COST BY OPERATION VS. SUBSTRATE CONCENTRATION.



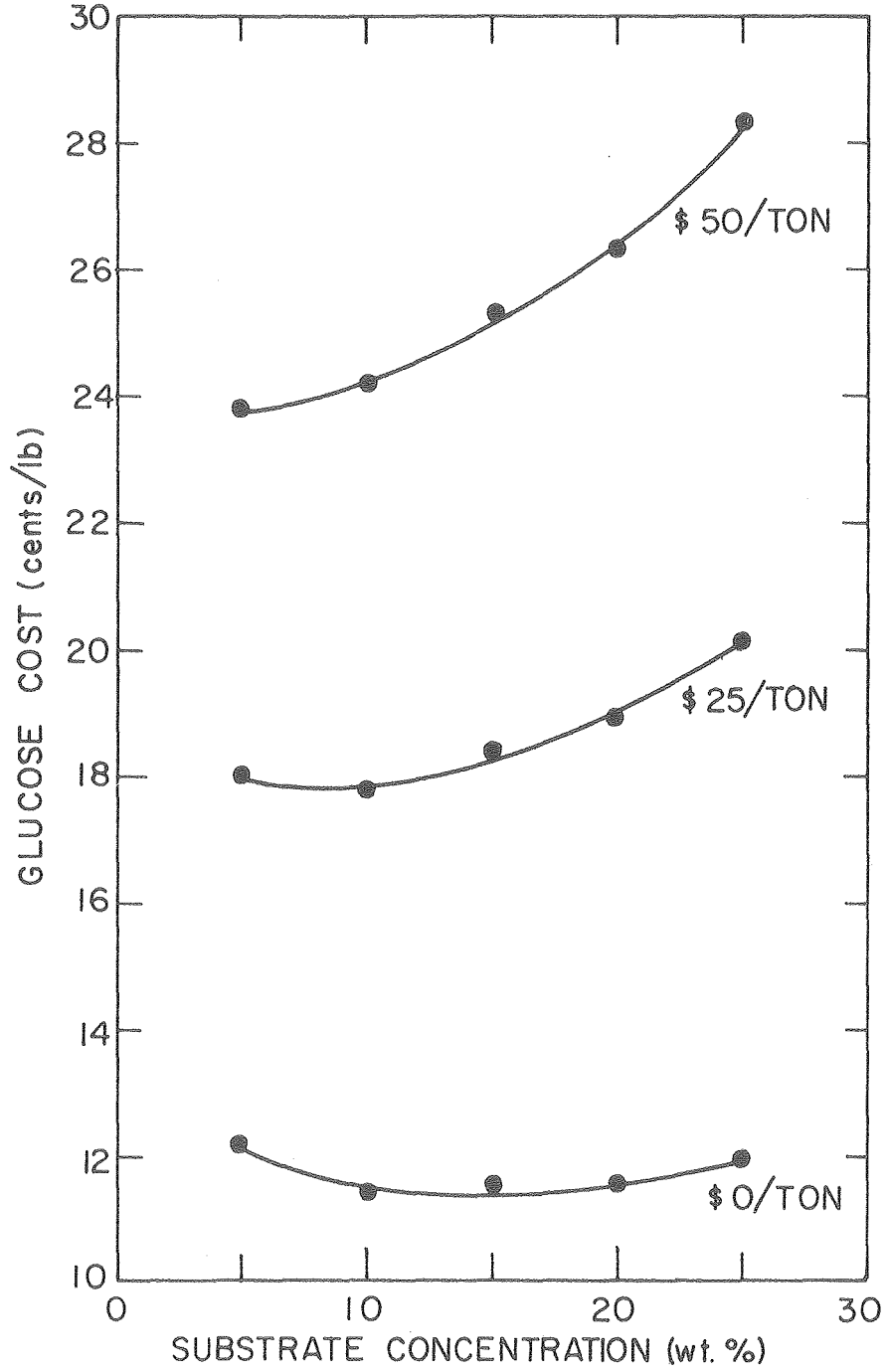
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FIGURE 10. PRODUCTION COST BY MANUFACTURING COMPONENT VS. SUBSTRATE CONCENTRATION.



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FIGURE. 11. SUGAR PRODUCTION COST VS SUBSTRATE CONCENTRATION AT VARIOUS CORN STOVER COSTS.



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concentration plants as the price of corn stover is increased. This is due to higher yields found under these conditions.

A similar evaluation was conducted for a process utilizing 20% substrate loading and using 7 IU/ml activity enzyme obtained from batch production of 14 IU/ml enzyme. No work has reported activities in the range from continuous production. The cost of manufacturing sugar for this case is 10.5 cents per pound and constitutes the lowest production cost calculated. For this case, benefits obtained from the 86% yield obtainable are offset by increased capital costs due to batch production of enzyme. Fixed capital for this case is approximately 38 million dollars.

Table 5 shows a comparison between the lowest manufacturing cost obtained using cellulase from C-30 and that obtained using QM-9414. The QM-9414 production cost were updated from the work of Yang, et al. (4). The lowest manufacturing cost for a process using QM-9414 is 14.9 cents per pound obtained from a plant utilizing a 5% corn stover stream at a conversion of 40% to produce a 2% sugar stream. Processing with C-30 results in a cost savings of 30-40% over that obtained with QM-9414.

If current technology (9) is employed to manufacture 95% alcohol from the sugars produced, the price of ethanol per gallon ranges from \$1.81 to \$2.92 depending on the cost of corn stover.

V. CONCLUSION

Cost reductions obtained by using C-30 to produce glucose from corn stover can produce sugar at near market prices. However, the manufacture of ethanol for agricultural residues does not appear economical at this point. A sensitivity analysis showed the price of sugar to be heavily dependent on the assumed price of corn stover. Therefore, even with significant process improvements the cost of producing ethanol from biomass is likely to be controlled by the market value of the biomass.

TABLE 5
 COST OF SUGAR PRODUCTION CELLULASE QM-9414 vs C-30
 AT VARIOUS CORN STOVER COST

CORN STOVER COST:		\$0/TON	\$25/TON	\$50/TON
SUGAR COST	QM9414	14.9	22.9	30.9
(¢/LB)	C-30	10.5	14.6	18.7
% DIFFERENCE		30%	36%	40%

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