Title
PROCESS DEVELOPMENT STUDIES OF THE ENZYMATIC HYDROLYSIS OF NEWSPRINT

Authors
Wilke, C.R.
Yang, R.D.

Publication Date
1975-04-01

Presented at the Eighth Cellulose Conference on Wood Chemicals--A Future Challenge, Syracuse, New York, May 19-23, 1975.

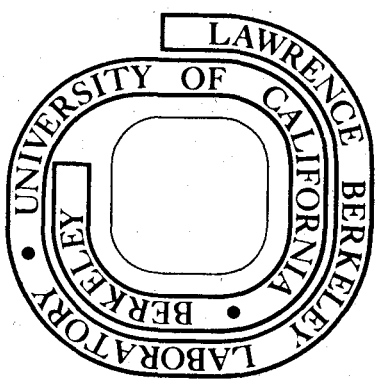
LBL-3283
c. 1

PROCESS DEVELOPMENT STUDIES OF THE ENZYMATIC HYDROLYSIS OF NEWSPRINT

C. R. Wilke and R. D Yang

April 1975

Prepared for the U. S. Energy Research and Development Administration under Contract W-7405-ENG-48



LBL-3283
c. 1

DISCLAIMER

LBL-3283

PROCESS DEVELOPMENT STUDIES OF THE ENZYMATIC HYDROLYSIS OF NEWSPRINT

C.R. Wilke and R.D. Yang
Lawrence Berkeley Laboratory
and
Department of Chemical Engineering
University of California
Berkeley, California 94720

SYNOPSIS

Background laboratory work and a tentative process for enzymatic hydrolysis of waste newsprint are described. Enzyme is produced by T. viride QM 9414 in a two stage system comprising cell growth in soluble sugars and enzyme induction on solid cellulose. Recovery of enzyme from the product sugar solution by adsorption on fresh plant feed and employment of cell recycle around the enzyme induction stages are important features of the process. Design studies indicate that it may be possible to produce sugars at a favorable cost, although additional research is needed to confirm a number of process assumptions

Prepared for presentation at the Eighth Cellulose Conference on Wood Chemicals--A Future Challenge, Jointly sponsored by the Cellulose Research Institute, State University of New York and The Wood Chemistry Committee, Technical Association of the Pulp and Paper Industry, Syracuse, New York, May 19-23, 1975

A tentative processing scheme for hydrolysis of waste newsprint based on laboratory data for ball milled paper has been proposed by Wilke and Mitra. (1) Because of the high cost of ball milling, subsequent studies have been made in this laboratory to assess the possibility of employing a less stringently milled substrate. This work has led to a modified process recently described by Wilke and Yang (2). The present paper will review this latter work and describe a further revised process design and cost analysis based on more recent information.

It should be recognized at the outset, however, that many important features of the proposed processing method have not been fully demonstrated in the laboratory, and that a pilot plant study of the operation will be necessary to establish the performance under realistic practical conditions. Therefore, the processing costs developed herein are primarily intended to provide perspective on important cost factors and do not necessarily represent results which can be obtained in practice. However, such cost studies are valuable as a guide to indicate potentially important areas for future study, and to ascertain that the research has at least some hope of leading to an economically feasible technology.

Laboratory Studies

Newsprint in the form of copies of the Wall Street Journal was chosen as a representative raw material. This paper was found to have a reasonably consistent average composition of 61% α-cellulose, 21% lignin and 16% hemicellulose. No adverse

effects of the printing ink on hydrolysis or fungal growth have been observed.

Cellulase enzyme was prepared in batch fermentation of the fungus Trichoderma viride QM 9414 obtained from the Natick Laboratories (3). Enzyme solutions of varying strength were prepared by redissolving the enzyme protein obtained by acetone precipitation. Enzyme activities were measured and defined in terms C_1, C_x and Filter Paper units according to Mandels and Wilson (4).

Sugars produced by hydrolysis were measured as total reducing sugar by the DNS method (5) and reported as glucose.

1. Hydrolysis Kinetics

A. Effect of Milling

Mitra and Wilke (6), following the earlier work of Ghose and Kostic (7), found that excellent levels of enzymatic hydrolysis could be obtained with newsprint which had been ball milled to -200 mesh. However, this type of milling does not appear practical for large scale processing because of prohibitive equipment and energy costs. To assess the possibilities of alternate milling methods hydrolysis tests in shaken flasks were made by Rubik (8) on a variety of newsprint preparations. Table 1 shows the results for -325 mesh ball milled material and several size distributions obtained with a Wiley mill (a cutting type mill manufactured by the Arthur H. Thomas Company). Suspensions of the newsprint of 5% or 10% concentration were contacted for 48 hours with enzyme solution of varying filter paper activity units

(FPA) at 50°C. Except for the ball milled material the percentage conversion of the cellulose to sugar was found to be independent of the particle size. Even larger particles of the paper, in the form of paper punches, showed the same conversion as equivalent suspensions of the Wiley milled paper. Recent results of Mandels et al. (9) for hydrolysis of a variety of materials support this conclusion. Therefore, it was concluded that a fairly coarse level of milling could be used in large-scale processing by accepting a somewhat lesser degree of conversion than that obtained by ball milling.

The experiments of Rubik also indicated that 5% suspensions gave a much better hydrolysis than 10% suspensions at the same initial enzyme concentration.

B. Hydrolysis with Vigorous Agitation

Because of the obvious low level of mixing obtained in the shaken flask experiments of Rubik, described above, it seemed desirable to determine the hydrolysis rates under more vigorous agitation and with larger volumes of material. Hydrolyses were conducted in 600 ml glass jars immersed in a water bath at 45°C and 50°C, and stirred with a motor driven marine impeller at 200 rpm. Paper suspension was prepared by mixing 15 gm of ground paper with 300 ml crude enzyme filtrate. Figure 1 shows the results of hydrolysis at 45°C and 50°C using 2.5 and 3.5 FPA enzyme solutions. Hydrolysis at 45°C give essentially the same conversion as at 50°C. Conversions at 36% and 52% (based on α-cellulose) were obtained in 48 hours for FPA and 3.5 FPA, respectively.

Comparison of these results with those of Table 1 suggests that the suspensions should be well mixed for maximum conversion.

As illustrated in Figure 2, enzyme is strongly adsorbed at the beginning and then is gradually released into the solution as hydrolysis proceeds. The percentage of original activity remaining in the solution was evaluated for C_1, C_x, and FPA activities by converting the respective activities after hydrolysis to enzyme protein equivalent. The enzyme protein equivalent was determined from calibration curves prepared by dilution of fresh enzyme solution (see Fig. 4 for the case of FPA).

Operation at 45°C gives more potentially recoverable enzyme than at 50°C, presumably because of greater denaturization at the higher temperature. However, the condition of the enzyme on the solids has not been conclusively determined (see Section 3 below).

These results suggest that considerably more enzyme is released on the basis of C_1 and C_x activity than on the basis of FPA. As a design basis for the process proposed below, the amount of enzyme released to the product solution was assumed to be 53% based on the value shown for FPA in Figure 2. Further study of this problem appears desirable.

2. Enzyme Adsorption

From experiments with ball milled paper, Wilke and Mitra (1) proposed in an earlier process design that enzyme release to the sugar solution after hydrolysis can be recovered by

adsorption on the fresh solid feed to the process. Therefore, adsorption measurements were made with the -20 mesh Wiley milled paper to be employed in the present design study. To measure adsorption various quantities of ground newsprint were contacted with enzyme solution of 3.5 FPA and the amount of original activity in the solution was measured after contact of 40 minutes. In washing experiments, solids from the previous experiment were separated by centrifugation and re-suspended in citrate buffer at pH 4.8. The amount of original activity in the solution was measured after 40 minutes contact. Results shown in Figure 3 indicate that C_x and C_1 are adsorbed to about the same extent. Based on Figure 3, for subsequent design of adsorption systems, a distribution coefficient of 0.04 (FP units/ml)/FP units/gm solid) will be assumed (expressed as equivalent protein).

3. Enzyme Desorption from Hydrolyzed Solids

The hydrolyzed solids described in Section 1 were centrifuged from the original sugar solution and resuspended in buffer solution to determine the amount of enzyme released by desorption. After centrifugation no activity could be detected in the supernatant suggesting that the washing method for enzyme recovery proposed by Wilke and Mitra would not be effective in this case. This result is in sharp contrast to results obtained by Mitra (6) for the distribution of fresh enzyme between buffer solution and paper which had been hydrolyzed to 82% conversion. Further work is in progress to see if some desorption technique can be developed or if

the adsorbed enzyme is irreversible denatured. Meanwhile, it will be assumed that no enzyme recovery from the hydrolyzed solids is possible and that the quantity of enzyme retained on the solid will be consistent with the results described in Section 2 above.

4. Enzyme Activity in Relation to Soluble Protein Concentration

A calibration curve for FPA versus enzyme protein was established as shown in Figure 4. Enzyme protein was measured by modified Biuret method after precipitation with acetone. Enzyme solution of FPA 2.75, corresponding to 0.85 mg/ml protein, was used as the starting material. More concentrated solutions were prepared by precipitating the enzyme at room temperature with acetone (3:1 v/v) and re-dissolving the precipitate in appropriate volumes of citrate buffer of pH 4.8. More than 90% recovery of enzyme activity can be obtained by acetone precipitation, as shown in the graph.

A Tentative Hydrolysis Process

The foregoing laboratory results are employed as a basis for revision of the original Wilke-Mitra process (1). In addition a major modification in the enzyme production operation involving use of cell recycle is introduced. This latter change is justified as follows.

Mitra and Wilke (6)(10)specified a two-stage continuous fermentation operation for cellulase production. In the first stage T. viride is grown rapidly on glucose to produce

a relatively dense cell suspension. In the second stage cellulosic material is added in the absence of externally supplied glucose to induce the enzyme. It was also shown that the enzyme productivity was directly proportional to the cell density in the induction stage as illustrated in Figute 5. On the basis of this latter observation use of cell recycle around the induction stages is proposed to produce a higher cell density than would be possible otherwise. It is also assumed that the recycle cells will become fully adapted metabolically to enzyme induction following their synthesis on glucose in the growth stage.

The foregoing data and assumptions are incorporated in the processing scheme shown in Figure 6. Flow quantities are given on the diagram for a particular design specification which will be referred to as the "base case." Principal items of equipment corresponding to the flow sheet quantities are described in Table 2. For simplicity the facilities for milling, heat exchange, induction solids sterilization and residual solids combustion have been omitted in the flow diagram, although they were designed and are included in the processing cost analyses.

The primary plant feed consists of 885 tons per day of

newsprint containing 6% moisture. By means of moderate shredding and hammermilling the feed is reduced to approximately -20 mesh. The size reduction is not critical so long as the material will form aqueous suspensions which can be pumped, agitated and filtered. An additional 59 tons per day of feed material is diverted to the first enzyme induction fermentor after sterilization with steam. The product sugar stream from the hydrolyzer is contacted countercurrently with feed solids for enzyme recovery. Each mixer filter stage consists of a mixing tank to provide 30 minutes contact time and a horizontal belt vacuum filter to separate the solids from the liquid. A total enzyme recovey of 95% is predicted by theory based on the adsorption studies described in Section 2.

Hydrolysis is conducted over 40 hours at 45°C at a solid/liquid ratio of 1/20 w/w based on imputs to the hydrolyser. The latter consists of 5 agitated cylindrical concrete digestors of the type used for solid waste treatment in sanitary engineering. Cellulose conversion of 50% is assumed, based on the data of Fig. 2, at an overall enzyme strength equivalent to 3.5 FPA in the hydrolyser. Provision is made for recycle of a portion of the product sugar solution (plus enzyme) back to the hydrolysis vessel. A sugar concentration of 3.4% is obtained for the case shown. A range of sugar levels is possible depending on the mode of operation and amount of sugar recycle employed.

Make-up enzyme is produced in a two-stage fermentation system as discussed above. Cell growth is obtained in the

first stage at a dilution rate of 0.2 hr^{-1} employing a medium containing 1% product sugars plus minerals and Proflo oil as given in Table 2. The induction system is operated at an overall dilution rate of 0.02 hr^{-1} using a 1.5% suspension of sterilized feed solids as inducer. Both stages employ agitated stainless steel vessels operated at 30°C with aeration rates of 0.15 and 0.015 v.v.m. in growth and induction stages, respectively. The growth stage feed is sterilized in a heat exchange system (not shown). The induction section effluent is passed through a centrifuge from which a portion of the underflow is fed back to the first induction stage. From three to five induction stages in series are employed depending on the fraction of cells which is recycled. The flow quantities in Figure 7 correspond to a cell concentrate recycle fraction of 0.11. Recycle fraction is defined as the ratio of the flow of cell concentrate from the centrifuge to the flow from the growth stage. The actual fraction of cells recycled is 0.59. For the case shown the use of recycle increases the cell density in the induction system from 0.44% to 1.0% dry weight, and increase of 125% over the cell concentration from the growth stage. The resultant increase in enzyme production is sufficient to provide an enzyme concentration of 3.5 FPA in the hydrolyser without use of acetone precipitation. A portion of the centrifuge underflow is filtered and the cells discarded to maintain adequate cell viability. The centrifuge overflow will contain a small concentration of cells (0.029% in the case shown). Removal of these cells prior to

hydrolysis is assumed unnecessary because T. viride will not grow at the hydrolysis temperature. However, further study of other possible problems of microbial contamination in the hydrolysis system is needed.

Spent solids from the hydrolyser following filtration are fed to a furnace and steam-power plant to provide process steam and electricity for the process. A substantial excess of energy is available in the spent solids, sufficient to operate an alcohol fermentation plant, for example, and to produce some additional by-product power. No credit is assumed for this excess energy in the processing cost analysis described below, pending a more detailed study of the combustion operation.

Base Case Cost Estimation

For the process described above a preliminary cost estimate was made for the required capital investment and cost per pound of sugars produced in aqueous solution.

The general cost estimation procedure was that recommended by Peters (11), and by Holland (12).

The fixed capital cost is estimated as a multiple of purchased cost of the principal items of equipment. In the present case a multiplier of 3.1 was used, except in the case of the concrete digestors for which the multiple was reduced to 1.68 because the unit cost already included engineering construction and contractor's fees. Estimated costs of the principal equipment items are listed in Table 1. The total manufacturing cost is broken down into investment related costs, labor related costs, utilities costs and raw material

costs. Multipliers and unit costs for the component cost items are listed in Tables 3-6. A base labor rate of $4.00 per hour is assumed. Taxes are omitted on the assumption that the installation would be part of a municipal waste processing complex. No charge or credit has been assigned to the newsprint. Costs of process steam and power were estimated assuming that they could be generated on the plant site using spent solids as fuel. Capital costs for steam-power facilities are not included in the fixed capital costs on the assumption that the specified unit costs for steam and electricity include both investment and labor charges. An on-stream efficiency of 90% is assumed, corresponding to 330 days operation per calendar year.

The resulting fixed capital cost, total manufacturing costs and costs per unit of product are listed in Table 7 for each of the major processing sections: (1) hydrolysis, (2) pretreatment, (3) enzyme recovery, and (4) enzyme make-up.

For this base case a fixed capital cost of $24,448,000 and a sugar cost of 6.56¢ per pound is obtained. Enzyme make-up is the major cost factor, comprising over 60% of the total. Alternative design specifications will now be considered.

Effects of Alternative Process Design Bases

1. Cell Recycle

Because the use of cell recycle over the induction stages is a major factor in achieving a low enzyme cost, several additional design cases were evaluated at other recycle

fractions, including one case with no recycle. In the latter case it is necessary to concentrate the enzyme solution from the growth stage to 3.5 FPA by precipitating the protein with acetone and redissolving it in the hydrolyser liquid. The design and costs for the acetone system were estimated in the manner described previously by Wilke and Mitra (1) and will not be described in detail here since this method of operation is not attractive economically.

The top line of Figure 7 depicts approximately the variation of sugar cost with the fraction of cells recycled. A probable most favorable case is that for a recycle fraction of 0.76, which is about the maximum which seems feasible. As shown by the dotted line, the cell density increases from 4.4 gm dry weight per liter to 17 gm per liter over the recycle range. Detailed investment and operating costs for this case are given in Table 8. In comparison with the base design case the fixed capital cost is reduced to approximately $19,000,000 and the sugar cost becomes 4.2¢ per lb.

2. Enzyme Recovery

In the design cases considered thus far, it has been assumed that 53% of the enzyme in the hydrolysers is discharged in the hydrolysis solution and is recoverable in the adsorption section. Further study of this problem seems desirable in view of the difference in release of C_1 and C_x activities shown in Figure 2 compared to that of filter paper activity, and since the experimental data are rather limited in scope. Therefore, additional cost estimates were prepared for enzyme recoveries of 65% and 20%, on the assumption that the recovery ultimately

obtainable in practice will fall within this range. The effects of these assumptions on the sugar production cost are illustrated by the corresponding lines on Figure 7. It is apparent that efforts to improve the degree of enzyme recovery could be well worth-while.

3. Percentage Cellulose Conversion

The dotted line of Figure 7 shows the substantial cost improvement which would be possible if somehow the level of cellulose hydrolysis could be increased from 50% to 80% without increase of the processing costs. Table 9 shows the pretreatment (chemical or milling) cost which would be allowable to achieve various levels of conversion and corresponding sugar costs. For example, it would be feasible to pay an additional $16 per ton for pretreatment to increase the conversion level to 80% and match the cost for the design case with 50% conversion and $2.70 per ton for milling. This type of consideration could be particularly important if a charge must be made for the raw material.

4. Effects of Taxes, Interest, and Raw Material Cost

Table 10 shows the incremental cost per pound of sugar at a cell recycle of 0.76 resulting from variations in the costs of taxes, interest, and raw materials. The base case assumptions of zero taxes and 6% interest corresponding to a municipal operation do not appear particularly serious, since increasing these variables to values typical of the present private economy will not increase the product costs

very much. A more serious factor is the potential cost of the raw material. A zero raw material cost may be reasonable for a municipal waste disposal operation in which the material has to be collected and disposed of whether or not a hydrolysis plant is used. Because of the variability of waste paper costs in the open market (presently quoted at $4.00 per ton in the Wall Street Journal) no attempt will be made in this paper to evaluate the raw material question in further detail.

Conclusions

The foregoing analyses suggest that it may be economically feasible to produce sugars from waste newsprint by enzymatic hydrolysis, and that a similar conclusion may be extended to various other cellulosic materials pending more specific studies. However, it should be recognized that many uncertainties exist in the proposed processing methods and cost estimates. Research and development work, including pilot plant studies, should be continued to improve the technology and provide a more firm basis for design of large scale processing plants.

More effective utilization of other components of cellulosic materials including lignin and hemicellulose should also be considered for their potential economic benefit.

Acknowledgment

The assistance of Thomas F. Anderson in laboratory studies and specification of the vacuum filtration equipment is gratefully acknowledged. This work is part of a general program on utilization of cellulose as a chemical and energy resource conducted under the joint auspices of the Energy Research and Development Administration (ERDA) and the National Science Foundation.

References

1. Wilke, C. R., and G. Mitra, "Process Development Studies on the Enzymatic Hydrolysis of Cellulose," Paper presented at the National Science Foundation Special Seminar, "Cellulose as a Chemical and Energy Resource," Berkeley, California, June 25-29, 1974. (to be published in Bioengineering-Biotechnology)
2. Wilke, C. R. and R. D. Yang, SITRA Symposium on Enzymatic Hydrolysis of Cellulose, Hämeenlinna, Finland, March 1975
3. Mandels, M., J. Weber, and R. Parizek, Applied Microbiol., 21, 152 (1971).
4. Mandels, M., and J. Weber, Advan. Chem. Ser. 95, 391 (1969).
5. Summer, J. B., and G. F. Somers, "Laboratory Experiments in Biological Chemistry," Academic Press, N.Y. (1949).
6. Mitra, G., and C. R. Wilke, "Enzymatic Utilization of Waste Cellulosics," Lawrence Berkeley Lab. Report 2334 (Revised) April, 1975.
7. Ghose, T. K., and J. A. Kostick, "Saccharification of Cellulose in Semi- and Continuously Agitated Systems," Advan. Chem. Ser. 95, 415 (1969).
8. Rubik, Beverely, private communication, Lawrence Berkeley Lab., October 1974
9. Mandels, M., L. Hontz, and J. Nystrom, Biotechnol. Bioeng., 16, 1471 (1974)
10. Mitra, G., and C. R. Wilke, Biotechnol. Bioeng., 17, 1 (1975).

11. Peters, J. S., and K. D. Timmerhaus, "Plant Design and Economics for Chemical Engineers," McGraw-Hill Book Co., New York, N.Y. (1968).

12. Holland, F. A., F. A. Watson, and J. K.Wilkinson, "Introduction to Process Economics," John Wiley & Sons, Ltd., London (1974).

13. "M and S. Equipment Cost Index--Process Industries Averages," Chem. Eng., 82, No. 3, 120 (1975).

SUBSTRATE	FPA	CONVERSION
10% WILEY (-20)	4.06	24.8%
10% BALL (-325)	4.06	72.9%
5% WILEY (-20)	4.06	41.1%
5% BALL (-325)	4.06	86.1%
10% WILEY (-20)	2.70	18.0%
10% WILEY (-100)	2.70	17.0%
10% WILEY (-325)	2.70	17.2%

Table 1. Newsprint Hydrolysis v.s., Milling-Shaken Flasks at 50°C, 48 hr.

Table 2 -- Major Items of Equipment -- Base Case

Item	Unit Specification	No. of units	Cost/unit, $[1]
Enzyme Production		Total	$ 4,371,000
Cell growth fermentor (B_1)	Vol. 5.56×10^4 gals. agitated, stainless steel construction	10	75,000
Induction fermentor (B_2)	Vol. 5.2×10^5 gals. agitated, stainless steel construction	10	287,000
Agitator motor coupled with B_1	Variable speed drive unit rating 60 HP	10	5,000
Agitator motor coupled with B_2	Unit rating 300 HP	10	11,000
Air compressor coupled with B_1 and B_2	Centrifuger typ, 30 psig 17,000 CFM, 1650 HP	1	300,000
Heat exchanger for media sterilization	Shell and tube type, multiple exchange units for a total of 10,900 ft^2 of surface	1	53,870
Induction solid sterilizer	9' x 160' modified rotary kiln or dryer	1	31,200
Seed tank	Vol. 1500 gals. vessel, agitated and motor	1	14,000

Table 2 (continued)

Item	Unit Specification	No. of units	Cost/unit $
Raw material mixing tank	Vol. 23,200 gals., agitated carbon steel construction, 60 HP motor	1	35,000
Centrifuge for cell recycle	De laval centrifuge 70 M^3/hr throughput	2	41,150
Mycelium filter	Pressure filter, effective area 175 ft^2	1	7,270
Hammermill for induction solids	Welded steel construction 3600 RPM motor, 3000 lb/hr	2	30,400
Screw conveyor for inducation solids		1	3,500
Heat exchange tubing for temp. control in fermentors	A total of 400 ft^3 area, stainless steel	1	3,500
Pumps and drivers		12	2,500
Hydrolysis		Total	$ 3,214,400
Hydrolyzer (H)	Concrete digestor, agitated place below ground level. Vol. 1 x 10^6 gals.	7	395,000
Agitator motor coupled with H	Rating 500 HP	7	14,600

Table 2 (continued)

Item	Unit Specification	No. of units	Cost/unit, $[1]
Solid filter	Vaccum drum filter plus accessory	1	227,200
Pumps and drivers	1400 GPM	4	2,500
Storage tank	1×10^6 gals., carbon steel	1	100,000
Heat exchange tubing	450 ft^2 area	1	2,500
Enzyme Recovery		Total	$ 775,140
Mixer	Agitated, carbon steel mixing vessel, vol. 7.4×10^4 gals.	3	21,700
Agitator coupled with above	Variable speed drive unit rating 50 HP	3	4,480
Filters for Adsorption	Horizontal belt filter plus accessory, 300 ft^2 effective area	3	227,200
Pumps and drivers	1400 GPM	6	2,500
Pretreatment		Total	$ 893,500
Hammermill	Welded steel construction 1800 RPM motor, air system with cyclone, capacity 2500-4000 lb/hr.	20	43,800
Screw conveyor	400 ft^3/hr, 10' x 30' 1.69 HP	5	3,500

[1]Costs are estimated for the 4th quarter 1974, Marshall Stevens Index = 431 (~~412~~ 413)

ITEM	COST FACTOR
DEPRECIATION	0.10
INTEREST	0.06
MAINTENANCE	0.06
INSURANCE	0.01
PLANT SUPPLIES	0.009
TAXES	0.00

Table 3. Investment related cost factors
(Annual Cost = Factor x Fixed Capital)

ITEM	COST FACTOR
DIRECT LABOR COST	1.00
SUPERVISION	0.15
PAYROLL OVERHEAD	0.15
LABORATORY	0.15
PLANT OVERHEAD	0.50

Table 4. Labor Related Cost Factors
(Cost = Factor x Labor Cost)

	UNIT	UNIT COST	UNITS/HR.
POWER	KWH	0.75¢	8338
STEAM	1000 LB.	32.5¢	36
WATER	1000 GAL.	40.0¢	83

Table 5. Utilities Costs--Base Case

COMPONENT	GM/LIT.	$/TON	TONS/DAY
AMMONIUM SULFATE	1.4	90	11.68
POTASIUM PHOSPHATE	2.0	120	16.70
CALCIUM CHLORIDE	0.3	33	2.50
MAGNESIUM SULFATE	0.3	110	2.50
UREA	0.3	160	2.50
PROTEIN NUTRIENT	0.5	300	4.17

Table 6. Medium Raw Materials--Base Case

	HYDROLYSIS	PRETREATMENT	ENZYME RECOVERY	ENZYME MAKE-UP	TOTAL
FIXED CAPITAL COST, $	6,020,350	2,734,110	2,319,300	13,375,000	24,448,760
Annual investment related costs, $	1,438,900	653,450	554,310	3,196,600	5,843,260
Annual labor related costs, $	122,990	61,495	122,990	122,990	430,465
Annual Utilities costs, $	189,090	79,860	39,270	287,430	605,650
Annual raw materials costs, $	-	-	-	1,656,800	1,656,800
ANNUAL MANUFACTURING COSTS, $	1,750, 980	794,800	716,580	5,263,820	8,526,180
DAILY MANUFACTURING COST, $	5,306	2,408	2,171	15,950	25,837
SUGARS COST, ¢/lb.	1.35	0.61	0.554	4.07	6.56

Table 7 Process Cost Analysis--Base Case (Cell recycle fraction = 0.59)

	HYDROLYSIS	PRETREATMENT	ENZYME RECOVERY	ENZYME MAKE-UP	TOTAL
FIXED CAPITAL COST, $	6,020,350	2,734,110	2,319,300	7,918,000	18,991,760
Annual investment related costs, $	1,438,900	653,450	554,310	1,892,390	4,539,050
Annual labor related costs costs, $	122,990	61,495	122,990	122,990	430,465
Annual Utilities costs, $	189,090	79,860	39,270	170,158	478,378
Annual raw materials costs, $	-	-	-	90,826	980,826
ANNUAL MANUFACTURING COST, $	1,751,000	794,800	716,500	3,166,360	6,428,180
DAILY MANUFACTURING COST, $	5,306	2,408	2,171	9,595	19,479
SUGARS COST, ¢/lb.	1.14	0.52	0.47	2.07	4.2

Table 8. Process Cost Analysis (Cell Recycle Fraction = 0.76)

CELL RECYCLE	% CONVERSION	¢/LB. SUGAR COST	$/TON ALLOWABLE PRETREATMENT
0.76	50	4.2	2.7
0.76	70	3.5	8.0
0.76	80	2.4	2.7
0.76	80	3.6	13.0
0.76	80	4.2	19.0

Table 9. Allowable pretreatment cost to obtain higher conversion

VARIABLE	INCREASE OF VARIABLE	¢/LB INCREASE OF SUGAR COST
COST OF PAPER	$20/TON	3.85
TAXES	0 TO 3%	0.36
INTEREST	6% TO 12%	0.75

Table 10. Effect of variables on product cost (Cell recycle fraction = 0.76)

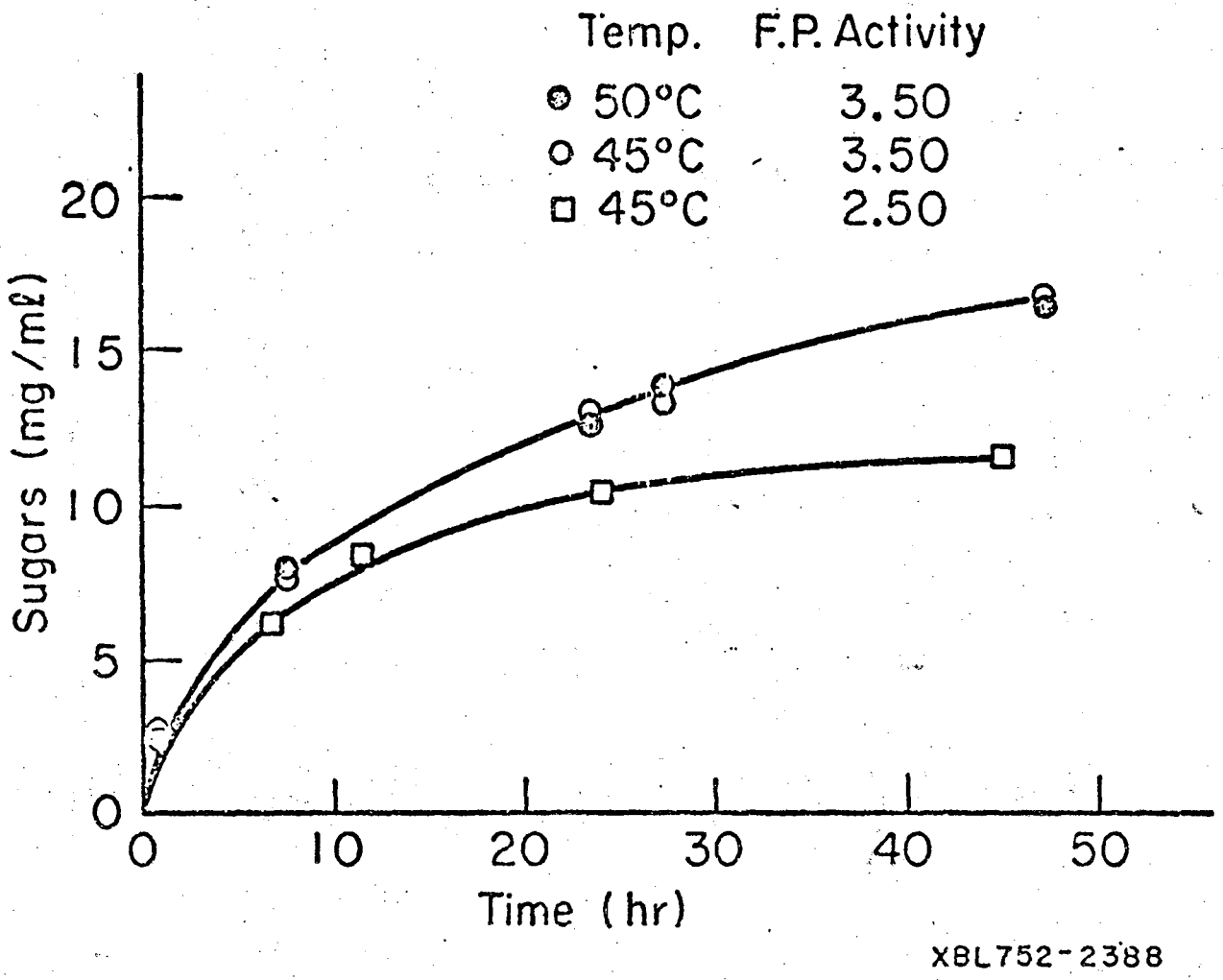


Figure 1. Hydrolysis of 5% Suspensions of -20 mesh Newsprint

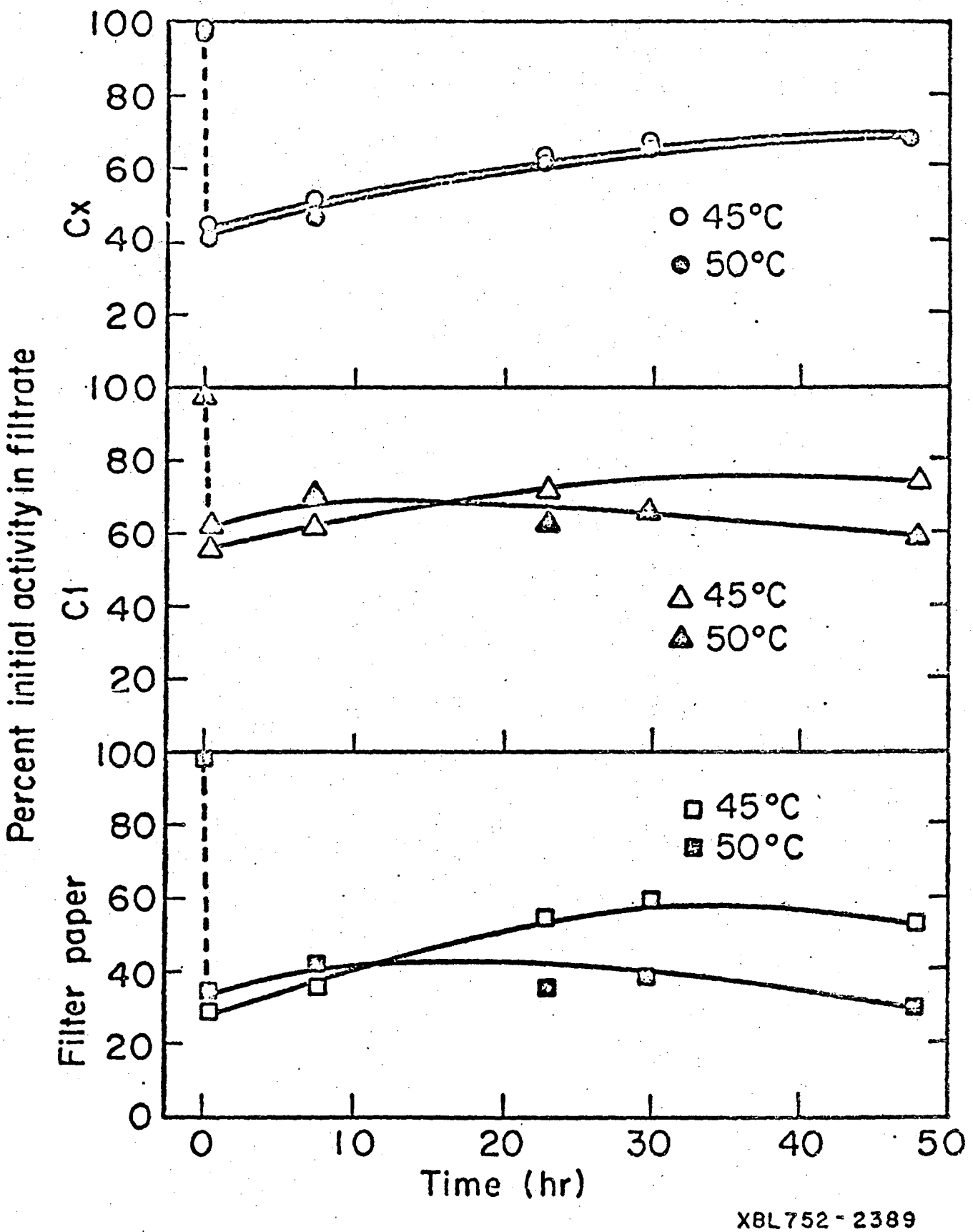


Figure 2. Release of Adsorbed Enzyme during Hydrolysis for Initial FPA of 3.5 and 5% solids.

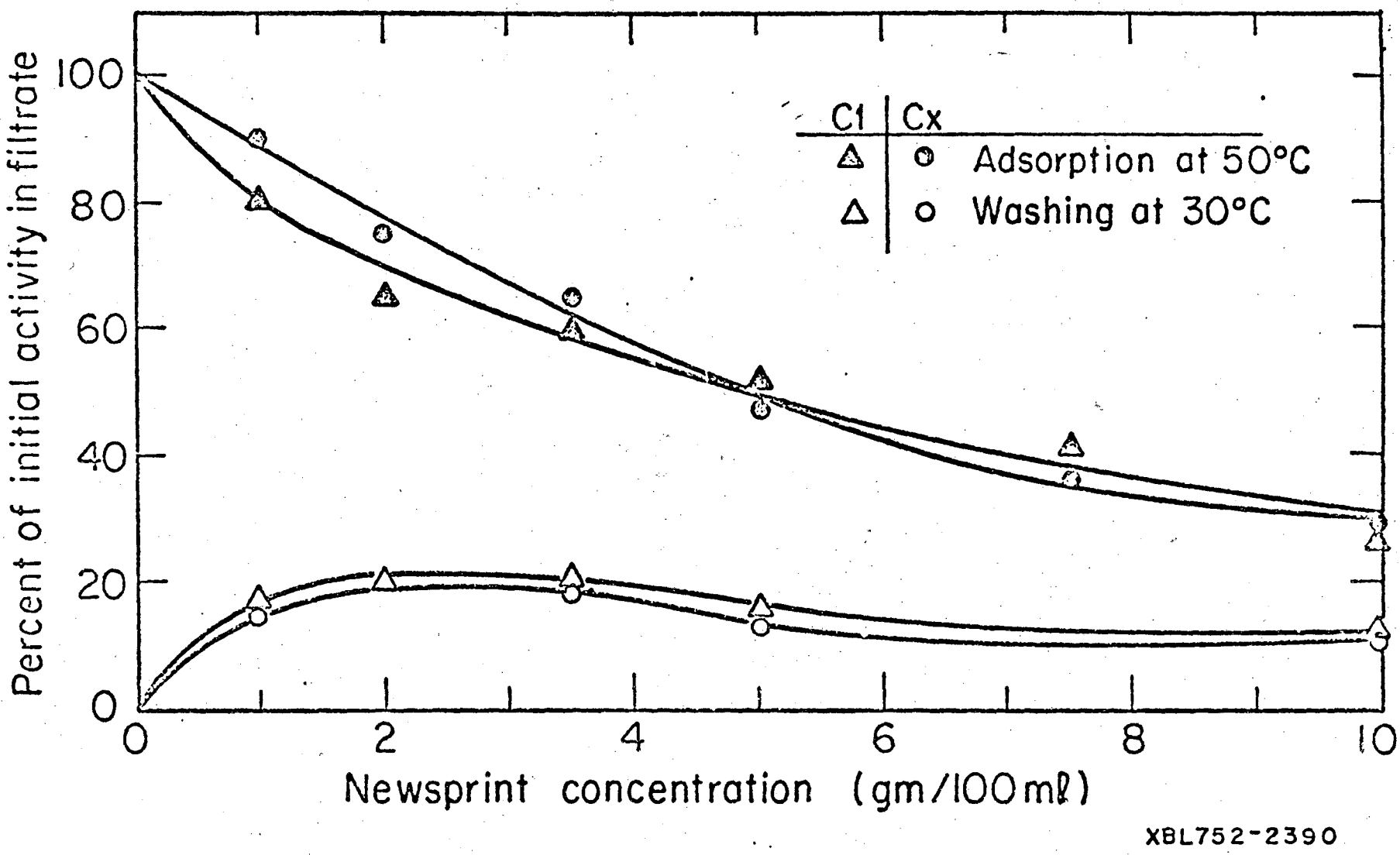


Figure 3. Enzyme Adsorption Equilibria on Newsprint at initial FPA of 3.5

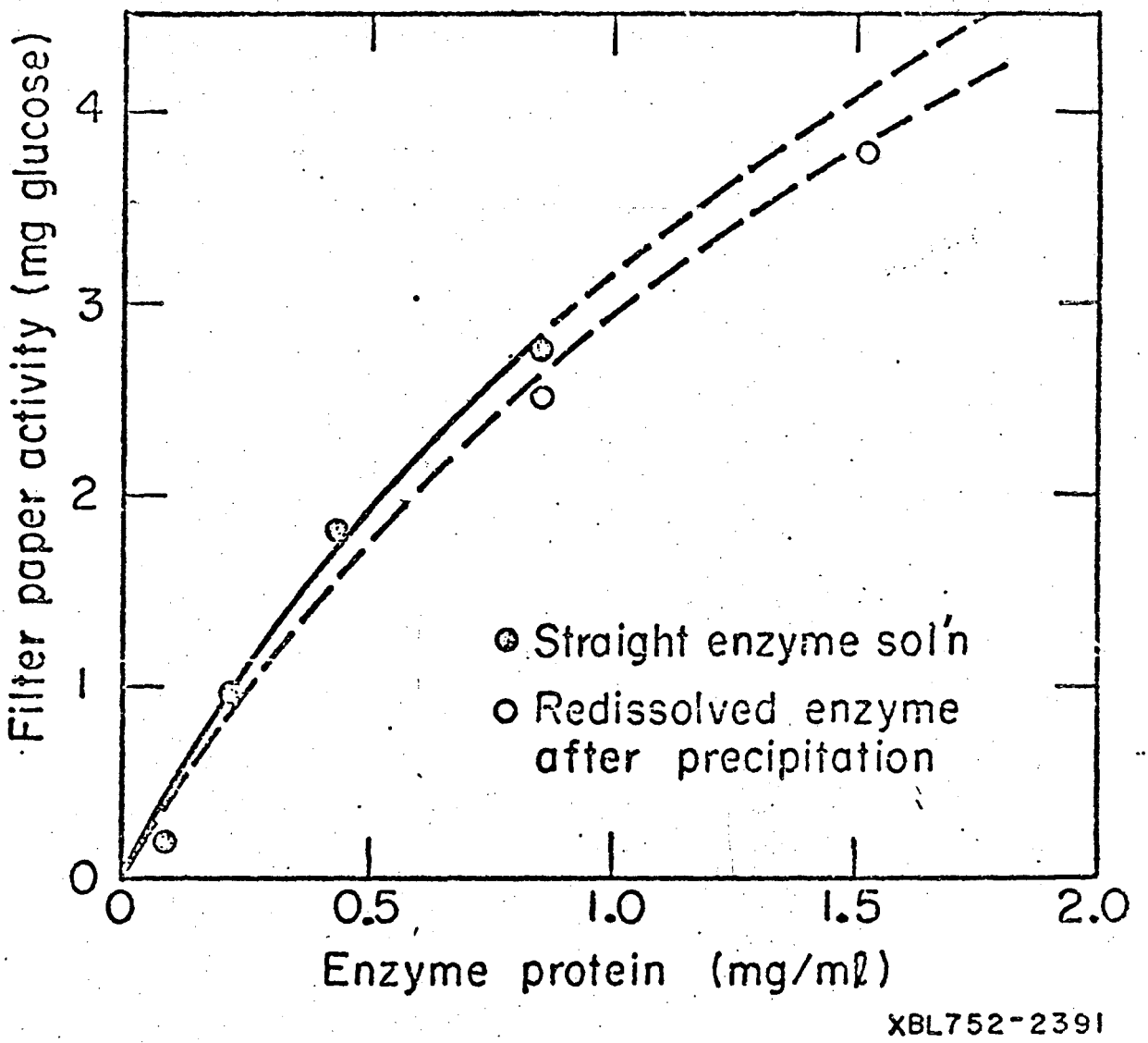


Figure 4. Filter Paper Activity vs. Enzyme Protein Concentration.

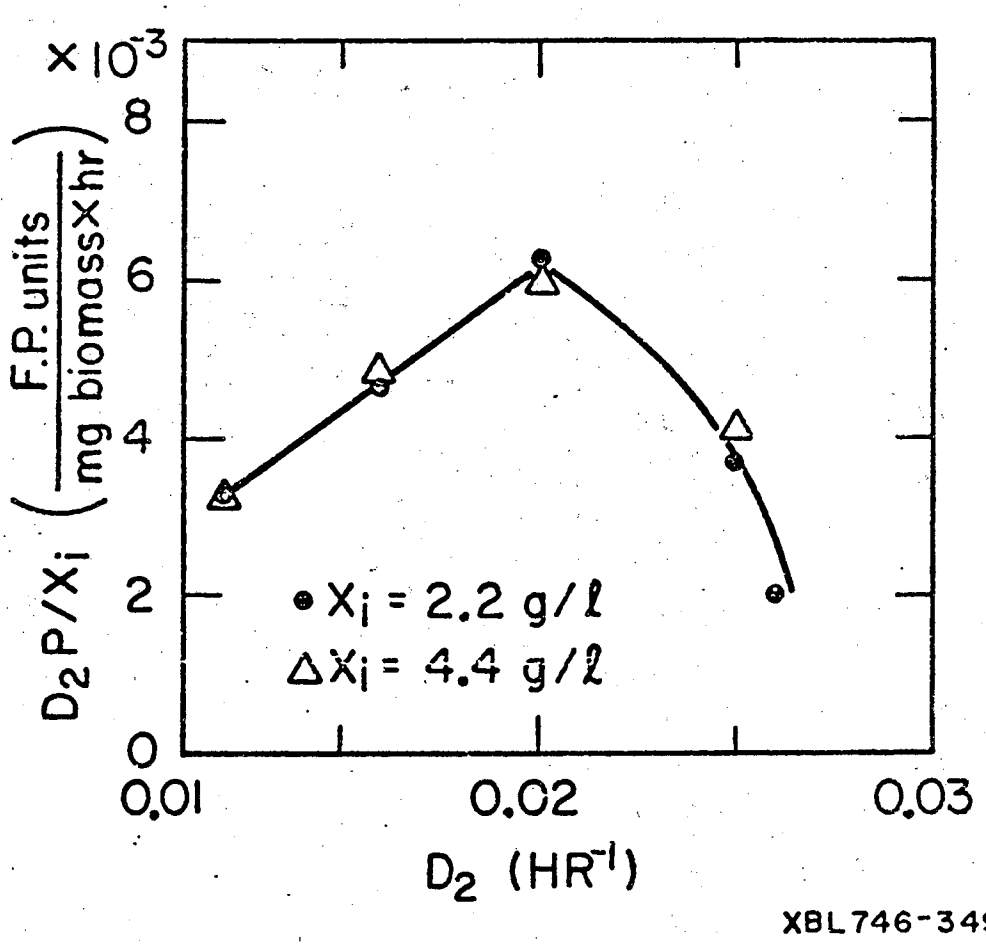


Figure 5. Specific enzyme productivity per unit inlet biomass to the cellulose stage as a function of dilution rates. X_1 → inlet biomass to the cellulose stage, P → enzyme activity, D_2 → dilution rate. (After Mitra and Wilke)[10].

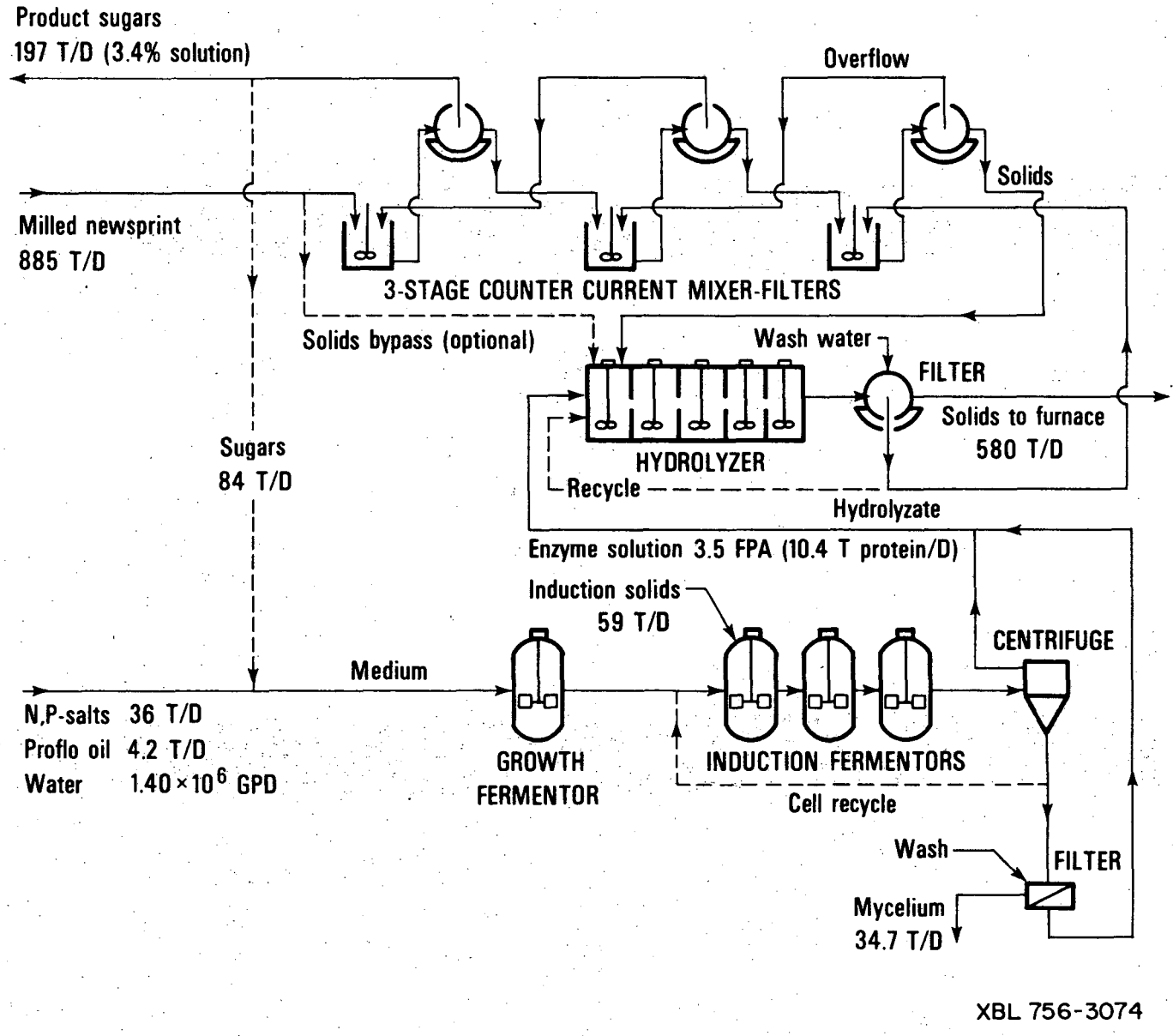


Figure 6. Flow Diagram for Modified Hydrolysis Process

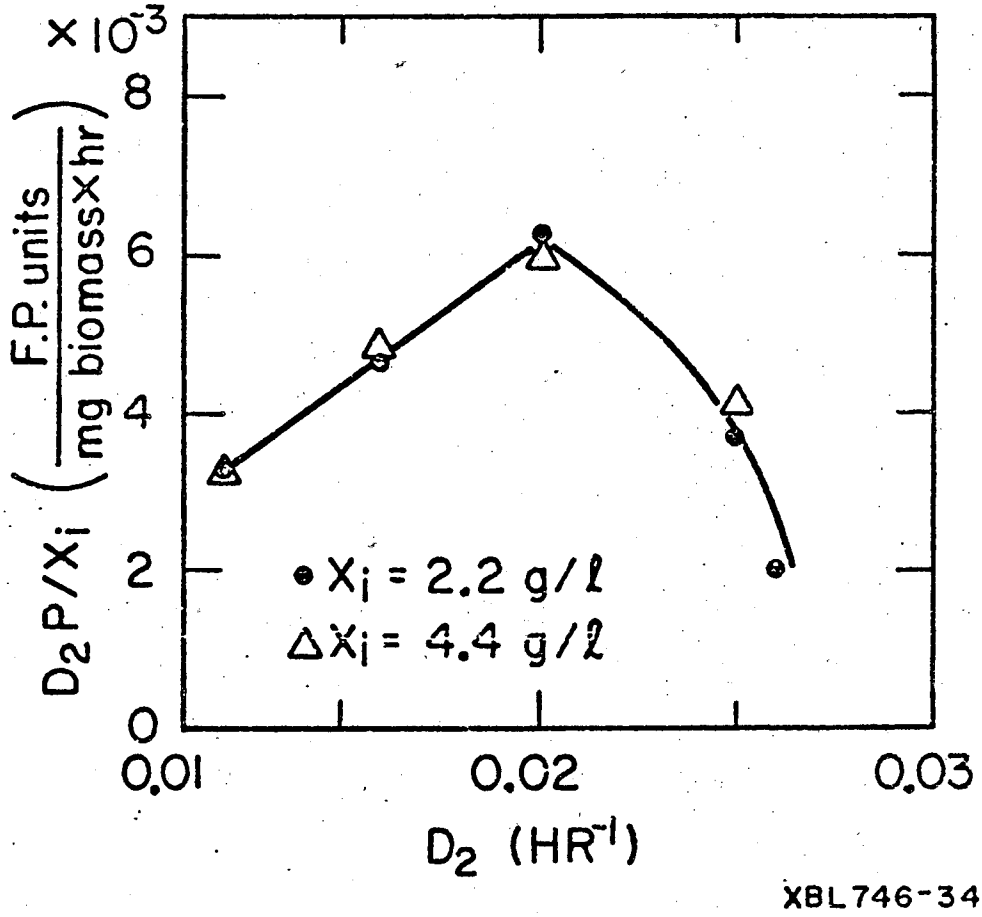


Figure 5. Specific enzyme productivity per unit inlet biomass to the cellulose stage as a function of dilution rates. X_1 → inlet biomass to the cellulose stage, P → enzyme activity, D_2 → dilution rate. (After Mitra and Wilke)[10].

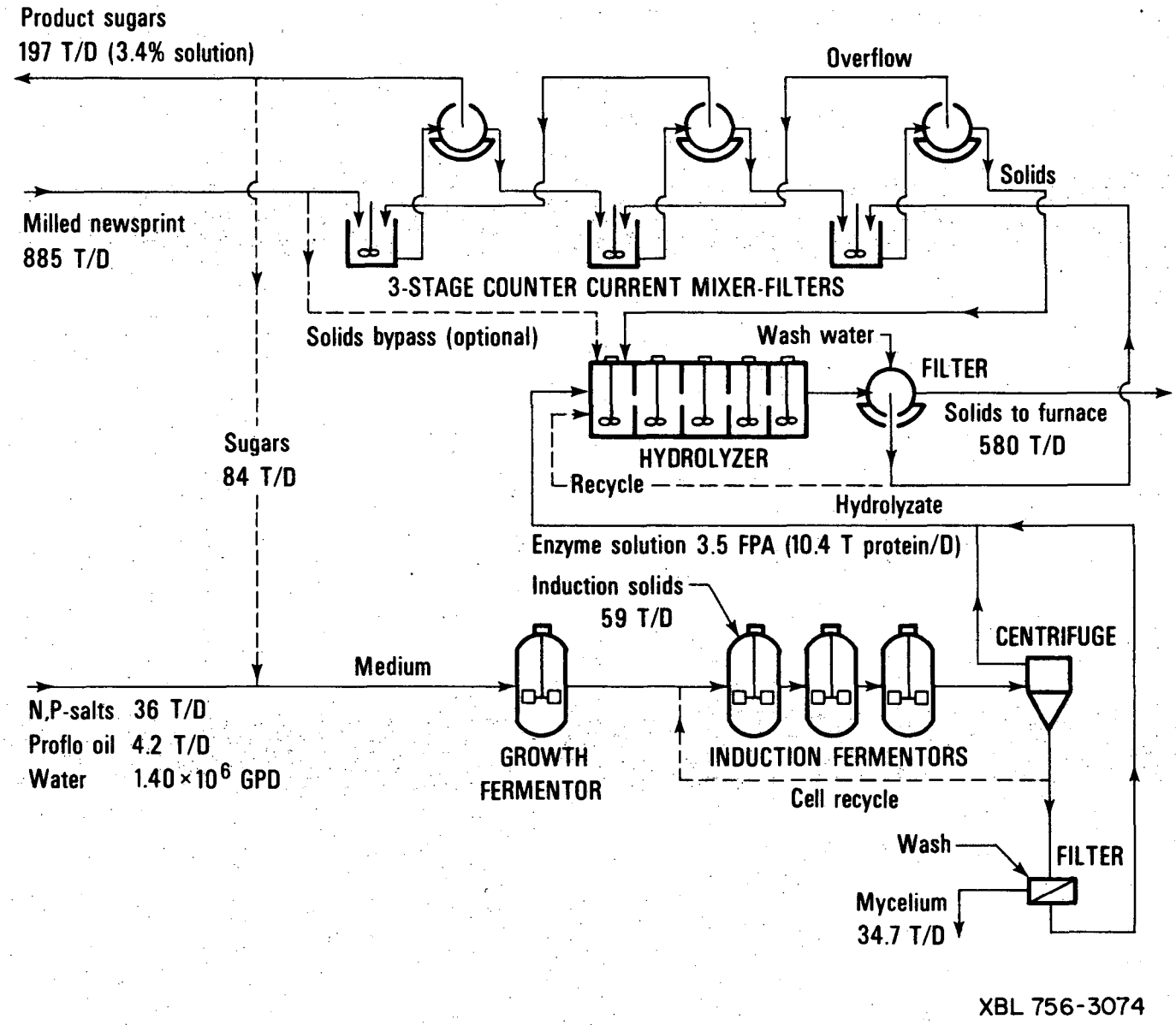


Figure 6. Flow Diagram for Modified Hydrolysis Process

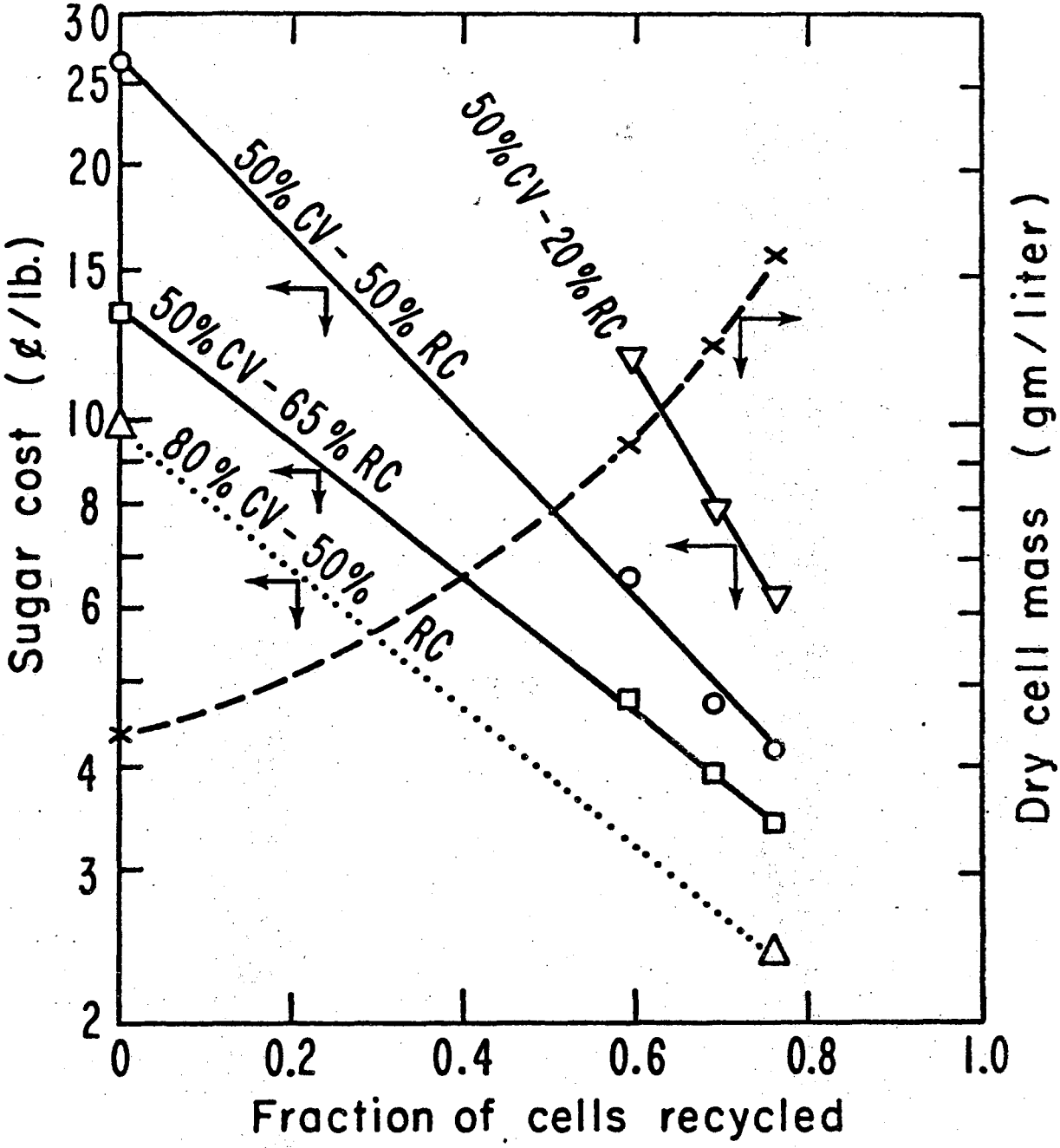


Figure 7. Sugar cost as a Function of the Fraction of Cells Recycled over the Induction Stages.

XBL755-2968

TECHNICAL INFORMATION DIVISION
LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720