

**Evaluation of Alternate Pretreatment and
Biomass Fractionation Processes for Ethanol Production:
The Xylan Delignification Process (XDP)**

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Abstract

In this subcontract project, corn stalks and switchgrass were treated by Xylan Inc. using their patented Xylan Delignification Process (XDP) which consists of a combined chemical/physical pretreatment regime. The pretreated samples were then frozen and sent to Dr. Dale's lab at Purdue University for enzymatic hydrolysis and fermentation trials. Procedures for analysis of ethanol, lignin, Klason lignin, ash, total solids, hemicellulose, and cellulose were followed as given by NREL. NREL procedures were also used to analyze for enzymatic release of sugars, and for Simultaneous Saccharification and Fermentation (SSF) of the biomass samples- corn stalks and switchgrass.

A QA/QC procedure using unknown samples from NREL was first completed to develop repeatability and confidence in the NREL procedures. Following these analyses, alpha-cellulose, XDP treated corn stalks and switchgrass were tested for digestibility using a cellulose enzyme provided by NREL, and then SSF of the treated biomass was performed. We found that the enzymatic hydrolysis of the XDP treated biomass was quite effective, with an 88% and 78% yield noted on corn stalks and switchgrass respectively within 25 hours. In our SSF trials, with a higher level of biomass added to the media, a 50% conversion efficiency for the XDP corn stalks was determined after a 125 hour fermentation period (conversion efficiency based on cellulose conversion to ethanol). For the XDP switchgrass a 30% yield was noted at 50 hours, after which time ethanol levels dropped. (For the alpha cellulose control, a 60% yield was noted at 125 hours.)

In some experiments beyond the scope of the contracted work, we tested the SSF of corn stalks with a flocculent *S. cerevisiae* strain at 30°C. Conversion efficiency of 25% was noted at 45 hours, after which ethanol levels decreased slightly with time. When a xylose fermenting yeast, *P. stipitus*, was added to the biomass along with our flocc. *S. cerevisiae*, yields improved to 32% at 45 hours. These test indicate that cellulase activity seems to be limiting the conversion, with higher temperature (38°C) fermentations more efficient than low temperature (30°C) tests.

Tests of lignin and hemicellulose solubility of XDP treated straw, corn stalks and switchgrass indicated that perhaps the treatment given during this trial may have been inadequate. We found that only 12 to 15% of the biomass was solubilized as determined by a hot water rinse of the samples. Cellulose content of the rinsed biomass was observed to increase from 39 to 49% , while a larger increase would have been expected. It seems possible that either the temperatures and pressures needed for effective delignification were not reached or perhaps the pretreatment chemicals were not properly added to the biomass. In a second straw sample (straw II) a much higher solubility (32%) was noted along with release of xylose sugars indicating that the pretreatment, using a second extruder, was effective.

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I. Introduction

Biomass is a generic term for plant substances which can be harvested from crop and timberlands and used as a renewable source of organic chemicals. A major goal within the Dept. of Energy and US Dept. of Agriculture is the development of an economic and environmentally responsible bio-fuels program to replace or supplement the use of petroleum as a liquid transportation fuel. Each gallon of ethanol produced in the USA displaces about 0.7 gallons of petroleum on an energy value basis. Our nation is currently importing about 50% of our petroleum needs (Gov. Et. Coal., 1994) with 110 billion gallons of gasoline consumed in 1992 (Biofuels Update, 1994). Non-food crops such as switchgrass and poplars grown on ground not used for food crops could produce 190 to 270 million tons of cellulosic biomass, which could then be turned into 30-50 billion gallons of ethanol (Brower, 1994). The major goal of this project is to help develop cost effective technology for the conversion of cellulose to ethanol, to allow the substitution of a renewable resource, biomass, for a non-renewable resource, oil. There is also a national security issue, in that our nation is dependent on foreign oil for 50% of our oil needs. Renewable resources, within the bounds of our own nation, could replace these imports with ethanol, and provide many jobs in production, collection and processing of the biomass.

Ethanol production in the USA offers a renewable source of liquid fuel produced within the borders of our own nation as well as offering a market for excess grain/biomass crop production capacity of the midwestern states. However, in order for the ethanol fuel industry to be able to expand without governmental subsidies, ethanol production costs must be reduced closer to the level of refined unleaded gasoline (\$0.55-\$0.75 per gallon). We hope that this project will lead to the implementation of cellulose to ethanol processes on full scale plant size using waste cellulose such as waste paper, separated paper/cellulose from municipal solid waste plants, field crops such as switchgrass and corn stalks, and saw dust.

As our nations oil supplies are depleted, and clean air requirements are stiffened, the need for ethanol fuels is becoming a national priority. If, however, current high energy technology (utilizing coal fired boilers) uses 40 to 100,000 BTU's of coal energy to produce 84,000 BTU's of liquid fuel energy per gallon of ethanol, the net effect is to produce a lot of coal fired boiler stack gas emissions to reduce car exhaust emissions. The overall effect on the US environment of this trade-off is open to debate. Current batch fermentation technology for ethanol production from corn requires large scale operations (12-50 million gal/yr of ethanol), a large capital investment (\$2-4.00/ annual gallon), and is energy intensive with an energy usage of 40-100,000 BTU/gal of ethanol produced from corn. About half of this energy is associated with drying and

evaporating the stillage, and half with fermentation and distillation. Presently, ethanol production level in the USA is at about 1,100 million gallons/yr. The total market for ethanol as a 10% blend in gasoline would be 12 billion gallons. As more and more ethanol is produced, it is important to our net energy position that ethanol be produced domestically in an energy efficient fashion. It is also important to develop processing for alternative feed stocks, such as biomass crops, rather than corn. Ethanol fuel production can help utilize excess corn capacity (perhaps 20% of the nations crop) but further ethanol production must use other feedstocks if corn and grain prices are to be kept within 30 to 40% of the current levels.

Biomass substrates for ethanol production include Municipal Solid Waste (MSW) where paper/cellulosics are being picked up and recycled. Goodman and Walters (1991) show that about 66 million tons/yr of paper and paperboard are estimated to be discarded into MSW in the year 2000 along with 19 million tons of yard wastes. If 30% of this stream were converted to ethanol at a conversion rate of .3# ethanol/1 pound biomass, these streams alone could give 2.3 billion gallons/yr of ethanol. Agricultural crop residues such as corn stalks, wheat straw, or cotton stems have been estimated to be about 812 million tons/yr which if 30% utilized could give 22 billion gallons of ethanol. The possibility of biomass crops such as hemp, kenaf, or other highly productive crops grown on marginal lands could expand this biomass availability even more.

Currently corn stover is largely left in the fields, although it is occasionally baled and used as feed extender when cows are fed on corn over the winter. If the biomass is valued at \$20./ton (\$0.01/#) a corn field yielding 100 bushels /acre would yield 2.8 tons of biomass (dry basis, based on a fodder/grain ration of 1/1, Clements, 1991) or a further income to the farmer of \$54/acre over the income of \$220. obtained from the sale of the grain @ \$2.20/bushel (an income increase of 24% per acre for the farmer. Assuming a 90% conversion of the xylose and cellulose fractions (66% of the biomass), 80 gallons of ethanol can be produced per ton of biomass. Thus, an acre of residue should produce about 224 gallons of ethanol with a value of \$264. at a sales price of \$1.18/gal.

Biomass is a mix of three basic components, lignin, cellulose and hemicellulose. In order to break down the hemicellulose and cellulose to sugars, the basic structure of the biomass must be attacked. Once the structure of the biomass is disrupted, the hemicellulose and cellulose can be converted to sugars either with acid or enzymatically. There are several basic method for cellulose breakdown, strong acid, dilute acid, ammonia explosion, steam explosion, and peroxide extrusion. Acid (sulfuric or hydrochloric) can serve both for disruption and hydrolysis of the cellulosic polymers. Strong acid allows complete breakdown of the components in the biomass to sugars , but requires concentrated sulfuric or hydrochloric acid (Goldstein and Easter, 1992; Ladisch

and Swartzkopf, 1991). Dilute acid allows reduced acid concentrations, but requires higher temperatures, and gives furfural as an undesired inhibitory side product. Ammonia explosion is a process being promoted by AFEX (B. Dale et al, 1985; AARC Bulletin, 1994)) which uses a quick pressure reduction after soaking the biomass with liquid ammonia solution. Steam explosion is being developed by Stake Technology which involves extrusion of the biomass at a high temperature and pressure, peroxide extrusion uses a chemical pretreatment along with extrusion to accomplish the same goal of breaking down the internal structure of the biomass fibers. Ammonia, steam and peroxide extrusion/explosion allow enzymatic hydrolysis of the cellulosic polymers.

In this project the peroxide extrusion process as developed by Xylan Inc was utilized as a pretreatment technology. The Xylan-Delignification-Process (XDP) utilizes extrusion technology in conjunction with alkaline hydrogen peroxide. The method continuously treats lignocellulosic biomass by reacting the biomass with a reaction medium containing an aqueous solution of alkali agent (pH 11.5) which softens the lignin and allows water to enter the biomass. The cellulosic biomass is then fed into a pressurized extruder reactor. Exiting the reactor barrel is a liquid/solid mixture stream containing lignin and hemicellulose sugars, and cellulose fibers, suitable for paper, cattle feed, or enzymatic hydrolysis to glucose. The wet/fibrous product from the extrusion process consists of a lignin/soluble hemicellulose stream and a fibrous cellulose. Squeezing and washing the cellulose gives two streams, the lignin/soluble hemicellulose, and the solid fibrous cellulose. The liquid lignin/soluble hemicellulose stream is easily converted to xylose enzymatically, after which it can be converted to ethanol using various fermentation strategies. The cellulosic fibers can be broken down to glucose via an enzymatic treatment. Simultaneous saccharification and fermentation (SSF) of cellulose and hemicellulose from the XDP process on hardwood were demonstrated (Tyson, 1993).

II. QA/QC Analysis of NREL Samples

A glucose unknown, an ethanol unknown, and a biomass sample of unknown origin was provided to our lab for analysis. The following procedures were given by NREL to be used for determinations of biomass compositions and fermentation broth characterization.

Ethanol- Following Ethanol QC Sample Requirements (John Brigham)... 0.1 ml. sample is diluted with 0.9 ml of solution containing isopropyl alcohol (internal standard).

Total solids of biomass- Following Procedure 001 (Tina Ehrman)...hold sample (1-5 g) at 105 C for between 3 to 24 hours - until constant weight is attained.

Klason Lignin- Following Procedure 003 (Kate Magill)...the sample is digested in 72% sulfuric acid, with the lignin remaining undigested and determined by gravimetric analysis. Three samples were run to establish the repeatability of the analysis.

Acid soluble lignin- Following Procedure 004 (Kate Magill)...acid soluble lignin is determined by spectrophotometric absorbance at 205 nm on the filtrate from procedure 003. Again, three samples were run on the samples before and after the XDP treatment.

Ash- Following Procedure 005 (Tina Ehrman)...the sample is ashed in a furnace at 575 C for 3-5 hours. There may be a slight increase in ash due to the chemicals added in the XDP processing.

Cellulase Activities- Following Procedure 006 (B. Adney and J. Baker)...The FPU (enzyme quantity able to breakdown 4% of a filter paper solution) of the cellulase esymes provided were determined as per this procedure. Three replicates were run.

Saccharification of XDP samples- Following Procedure 009 (R. Torget)...The ability of the enzymes to saccharify the treated vs. untreated biomass were shown. Hemicellulase and cellulase activities on the four samples (treated/untreated corn stover/switchgrass) were tested with two to three replicates of the enzymatic digestion carried out. An alpha-cellulose sample were run simultaneously as a baseline control sample.

SSF of XDP samples- Following Procedure 008 (G. Philippidis, T. Smith, and S. Schmidt)...The XDP treated biomass stream was fermented with three fermentations carried out simultaneously. Finally, an alpha-cellulose sample was fermented as a baseline control sample.

b. Description of standards and blanks.

Glucose, ethanol, xylose standards were prepared using pure chemicals purchased from reputable chemical supply companies (Sigma, Aldrich, etc). These standards were held in tightly capped vials in a refrigerator.

c. Samples w/ standards

At least one standard sample containing known ethanol /xylose /glucose levels were run when samples are being analyzed. These standards were included at a frequency of equal to or greater than one per every 8th HPLC/GC injection. Initial standard curves were run for each analysis using four concentrations in the range of interest.

d. Quality analysis (QA)

In general, three samples were run to allow the sample repeatability to be determined. If variability was larger than 2-3%, one or two more samples were run, with the reasons for the variability hopefully determined and eliminated or reduced.

e. QA statistics.

Variability in the samples can be determined using standard linear regression for standards, with a goodness of fit determined by a r squared correlation coefficient, or by a standard deviation when multiple samples are run. We ran two to three replicates of various analyses, and we report the results with an "average deviation" , where the average deviation is defined as:

$$\text{av. deviation} = \Sigma\{\text{abs}(x - \bar{x})\} / N$$

where \bar{x} is the average value measured for the samples and N is the number of samples (generally three). QA/QC data as determined at Purdue's laboratory are given in Appendix 1, and Table 1 gives the amount of biomass (wet basis) added to the various saccharification and fermentation trials.

Table 1.

Procedure	Figure	Substrate	g Biomass (wet wt)
008-Hydr	1	XDP corn stalks	2.6g
008-Hydr	2	XDP switchgrass	2.7g
008-Hydr	3	alpha cellulose	0.5g
008-Hydr	4	fresh corn stalk	2.0g
009-Hydr	5	XDP corn stalk	0.7g
009-Hydr	6	XDP switchgrass	0.6g
009-Hydr	7	alpha cellulose	0.1g
008-SSF	9	XDP corn stalk	7.0g
008-SSF	10	XDP switchgrass	4.5g
008-SSF	11	alpha cellulose	2.0g
008-SSF	13	XDP corn stalk	5.0g
008-SSF	14	XDP corn stalk	5.0g

III. Xylan Delignification Process.

Background

The Xylan-Delignification-Process (XDP) utilizes extrusion technology in conjunction with alkaline hydrogen peroxide. Carr and Doane (1984) reported on the use of a similar process for straw pretreatment. The Xylan method continuously treats lignocellulosic biomass by reacting the biomass with a reaction medium containing an aqueous solution of alkali agent (pH 11.5) which softens the lignin and allows water to enter the biomass. The cellulosic biomass is then fed into a pressurized extruder/ reactor in an oxygen atmosphere at a temperature between 115-150^o C. and pressures up to 400 psi. These high temperatures and pressures allow minimization of chemicals as compared to other technologies for cellulose pretreatment (low acid-high temp, or high acid-low temp). The mechanical extrusion system mixes, grinds, sterilizes, and disrupts the cellulosic biomass cell walls. Exiting the reactor barrel is a liquid/solid mixture stream containing lignin and hemicellulose sugars, and cellulose fibers, suitable for paper, cattle feed, or enzymatic hydrolysis to glucose. Exit temperatures from the extruder are in the range of 115 to 125^oC .

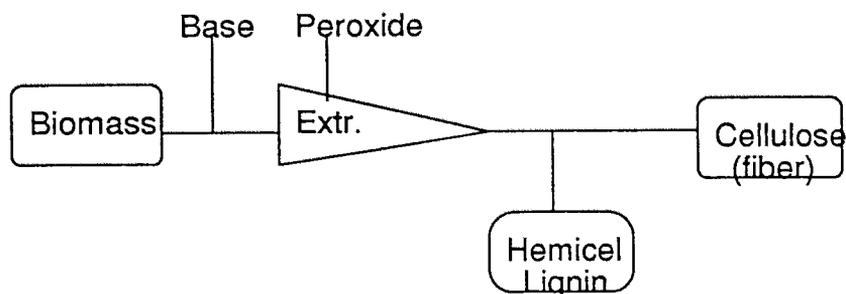


Figure 1. Xylan Delignification Process

Tyson (1993) suggests that temperatures of 138^oC and a pressure of 340 psi seemed best during tests with hardwoods. Hydrogen peroxide is added to the barrel of the extruder to help catalyze the breakdown of the fibrous biomass structure. The wet /fibrous product from the extrusion process consists of a lignin /soluble hemicellulose stream and a fibrous cellulose. Squeezing and washing the cellulose gives two streams, the lignin/soluble hemicellulose, and the solid fibrous cellulose.

The liquid lignin/soluble hemicellulose stream is easily converted to xylose enzymatically, after which it can be converted to ethanol using various fermentation strategies. In previous lab tests, when treating this stream (from a pre-treated wood chip process) with hemicellulase, the conversion of the

hemicellulose to xylose was noted, with 20 g/l xylose being determined after hydrolysis along with 2.2 g/l of glucose (Tyson, 1993). The cellulosic fibers stream can then be broken down to glucose via a similar enzymatic treatment. The cellulosic fiber stream, when added to water and hydrolyzed with cellulase gave a 10.7 g/l ethanol product during a Simultaneous Saccharification Fermentation (SSF) with a 31.9% yield of ethanol from dry matter yield (Tyson, 1993). Co-products of the fermentation included lactic acid and acetic acid at 3.3 and 1.6 g/l final concentrations respectively with 4.2 g/l glucose also remaining unfermented (Tyson, 1993).

Methods

The process began with the addition of sodium hydroxide, at approximately 3# of 50% NaOH solution per 100 # of biomass, 2 oz of magnesium salts, and a few drops of Versonex 80, a metal chelation product of Dow Chemical Co are also added. The base and chemicals were mixed with the shredded biomass in a 15HP Grainger stainless steel mixer for a period of fifteen minutes. The biomass was then taken to a "cram feeder" attachment to a SX 20 Wenger extruder. The barrel of the extruder was heated to 200 °C using low pressure steam. Hydrogen peroxide, at about a 9% solution strength, was injected near the entrance to the extruder barrel at a rate of about 1 # per 100# of biomass. A pressure of about 300 to 400 psi develops within the extruder barrel. After about a 15 second residence time, the biomass 'exploded' from the die at the end of the extruder barrel. A 3/8" die aperture was used when processing the straw, corn stalks, and switchgrass used in this project.

Lab Analysis

The XDP treated samples of straw, corn stalks and switchgrass were received at Purdue from Mr. Tyson in early and mid 1994. The samples were tested for basic composition by Dr. Lei following the suggested NREL procedures.

	Corn Stalk	Switchgrass	Straw
1. Total Solids	38.0%	56.8%	87.9%
av. dev.	+/- 0.47	0.85	0.3
2. Ash	15.0%	11.4%	10.7%
3. K. Lignin	18.8%	21.0%	23.4%
4. A. S. Lignin	2.0%	1.7%	0.3%
5. Acid Hydrol.			
a) Glucan	39.2%	31.4%	39.5%
av. dev.	+/-0.34	1.1	1.0
b) Xylan	24.9%	20.5%	19.1%
av.dev.	+/-0.3	2.6	0.3

Biomass samples were sealed in zip-lock bags and frozen to prevent deterioration.

IV. Enzymatic Hydrolysis of XDP Samples

The XDP treated corn stalks and switchgrass were treated with cellulase enzyme following procedures NREL 008 (with nutrients) and NREL 009 (without nutrients). The enzyme activity was listed by the manufacturer as being 95.3 FPU/ml on July 5, 1994. We ran our tests in late 1994 and early 1995. The enzymatic activity of the cellulase was determined using NREL procedure 006 as shown below.

Cellulase activity....Lot # 17-92262-09 from Env. Biotech. Inc.

Test #1 67.8 FPU/ml

Test #2 69.1 FPU/ml

Test #3 66.9 FPU/ml

avg. 67.9 FPU/ml av. dev +/- 0.76 FPU/ml

The hydrolysis of the biomass with the cellulase (Procedure 009) was measured by taking 0.1 g of cellulose in the biomass sample (by calculation based on dry weight and cellulose composition) added to 9.9 ml water. 90 microliters of cellulase enzyme is then added to the solution (60 FPU/g cellulose), and the sample is then allowed to digest at 50 C. Glucose liberation rate is then measured. Procedure 008 is performed on 0.3-.5 g cellulose in 125 ml flasks w/ fermentation nutrients (Yeast extract/Peptone) and digests at 38°C with 200 to 300 microliters of cellulase to get about 25 FPU/g.,

The results of Procedure 008 (w/ nutrients) enzyme hydrolysis of XDP treated corn stalks are shown in Figure 1-A and 1-B. The yield or conversion efficiency (as defined as grams cellobiose and glucose released per gram of cellulose) can be seen to be about 85% within 7 hours. During hours 7 through 24, cellobiose concentration drops with a concomitant increase in glucose concentration. Final glucose concentrations of 4.8 g/l glucose, 3.2 g/l cellobiose, and 2.6 g/l xylose were noted. The variability between the three replicates is given as a range about the average. Figure 1-B shows the experimental data range for cellobiose and glucose (to prevent overlapping of ranges if included on Figure 1-A).

Similar data for XDP treated switchgrass is shown in Figure 2-A. Conversion efficiencies of 78% were noted at 24 hours, with conversion being somewhat slower than was seen with the XDP treated corn stalks, although a 65% conversion yield was noted at 7 hours. Cellobiose levels were not observed to drop as seen in Figure 2B, although glucose levels continued to rise slowly from hours 7 to 24 of the treatment. As a control, alpha-cellulose similarly treated showed a 78% conversion to cellobiose and glucose in a 24 hour period as seen in Figure 3-A and B.

The effectiveness of the XDP treatment as compared to untreated corn stalks was determined finally as a test of the XDP efficacy. 'Fresh' corn stalks were gathered from an Indiana field about 2 months after harvest (late October '94), manually cut into short 2 cm lengths, milled in Waring blender, and sieved using

a 40 mesh sieve. This untreated corn stalk sample was then subjected to the same enzymatic evaluation as the pretreated samples. As shown in Figure 4-A and B, conversion was considerably slower, with only a 50% yield noted in 7 hours, and a final yield of 72% noted at 24 hours.

It was determined that the yeast nutrients had a beneficial effect or possibly some cellulosic enzymatic properties when the hydrolysis experiments were repeated (Procedure 009 without nutrients) on the XDP corn stalks, the XDP switchgrass, and the alpha cellulose. As seen in Figure 5A-B, XDP treated corn stalks showed a much slower release of sugars, with a 65% yield determined at 95 hours, and a final yield of 75% determined at 170 hours. Switchgrass showed similar drop in performance without the fermentation nutrients as shown in Figure 6. A 55% yield was determined at 50 hours, with a final yield of 72% noted at 170 hours. Alpha cellulose was found to be much slower to convert as shown in Figure 7, with a 98% conversion recorded at 170 hours as compared to 78% in 24 hours when nutrients were present (Figure 3). A comparison of the various biomass samples hydrolyzed with (Figure 8-A) and without (8-B) nutrients are presented. As per Figure 8-A (with fermentation nutrients), the XDP treated corn stalks hydrolyzed more quickly than the other samples, with a final yield of 82% reached in 7 hours. Alpha cellulose hydrolyzed at a slower but more constant rate reaching the same yield noted as with XDP switch grass (78%) at 24 hours. Figure 8-B shows a comparison for hydrolysis w/o fermentation nutrients. We noted more comparable hydrolysis rates between the samples, with XDP corn stalks and switchgrass showing similar glucose liberation rates until 60 hours, after which, the biomass samples slowed, reaching a final yield of 75% in 170 hours as compared to the alpha cellulose which slowly but steadily hydrolysed to reach near complete conversion at 170 hours.

V. SSF of XDP treated Biomass

The Simultaneous Saccharification and Fermentation of the biomass samples was next tested following NREL procedure 008 (Phillipidis, Smith and Schmidt, 1993) with the D5A *S. cerevisiae*, and then repeated using some of our labs yeast cultures. Following the NREL procedure, approximately 30 g/L of cellulose (based on the cellulose content of the biomass analysis) was simultaneously saccharified and fermented with an enzyme dosage of 25 FPU/ml in the fermentation mixture (actual biomass dosages are given in Table 1). The SSF of XDP treated corn stalks is shown in Figure 9. We measured a 46% yield in 48 hours with an ethanol concentration of 12 g/liter, after which time there was little change in ethanol level over the next 75 hours. SSF of the XDP treated switchgrass is shown in Figure 10 with a 33% yield noted at 48 hours, after which time ethanol levels were observed to drop with time. An ethanol concentration of close to 6 g/l was reached at 48 hours. Several tests of SSF of alpha cellulose (Figure 11A and 11B) were run as a control with 14 to 16.5 g/l

ethanol reached at 100 hours. These tests gave reproducible yield of 62-65% at 100 hours. Conversations with D. Hsu and D. Spindler of NREL suggested that these yields were somewhat lower than usually attained. Dr. Hsu discussed procedures with Dr. Dale and Mr. Zhao of Purdue, and we repeated these tests again in August of '95. We changed from using a magnetic stir bar at 150 RPM in the fermenter to using an orbital shaker at 150 RPM. With this minor change, we noted yields of 81% as shown in Figure 11C. In this experiment, the initial concentration of ethanol was 1.45 g/l and the highest concentration of ethanol was 18.44 g/l at 120 hrs. The initial broth pH was 5.09, and the final pH was 4.24 which is slightly lower than the first two trials. (The alpha cellulose curve in Figure 12 are from the first trials, Figure 11A and 11B)

Yields from the various biomass samples are compared in Figure 12, corn stalks and alpha cellulose fermentation rates were comparable over the first 50 hours, after which alpha cellulose continued to convert, while the corn stalk fermentation was stagnant. Switchgrass performance was significantly lower than corn stalks.

SSF of the XDP treated cornstalks was run using a flocculent *S. cerevisiae* maintained in our labs, NRRL 11878. This yeast is not as temperature tolerant as the D5A strain, so the fermentation was run at 30°C. As seen in Figure 13 performance was much worse at the lower temperature, with only a 25% yield noted at 48 hours. A residual glucose concentration of 2 g/l was noted with no measurable xylose observed. Co-culturing of glucose (11878) and xylose fermenting (*P. stipitus 11545*) yeast strains was next tested as shown in Figure 14 (similar to a study by Grooten et al, 1991). Yield improved slightly to 33% in about 48 hours, with lower levels of glucose determined (0.5 g/l) at 70 hours. Again, no xylose was noted in our chromatograms.

A comparison of the SSF experiments on XDP corn stalks is shown in Figure 15. The higher temperature SSF gave the best yields. The use of even higher temperature tolerant yeast, perhaps *K marxianus* strains might be explored in the future (Dale et al, 1990). In results not shown here, it was found that contamination of the SSF broth led to poor fermentation performance. We found it important to filter sterilize the cellulase enzyme to prevent any contamination of the SSF experiments.

VI. Solubilization of Lignin and hemicellulose

The XDP treatment of biomass is supposed to solubilize the lignin and hemicellulose fractions of the biomass. The effectiveness of the XDP treatment was evaluated by a few tests in which the biomass was rinsed with hot water to release the soluble hemicellulose and lignin. The cellulose, hemicellulose, lignin fractions of the rinsed biomass were compared using the standard methods before and after the rinsing. If the XDP were totally effective, little lignin or hemicellulose would remain after the rinsing, with the fiber being largely cellulose. Acid hydrolysis of the rinsed remaining cellulose fiber should release mainly glucose. Solubilization tests of four substrates are shown in Table 2. As

per this table, only 12.2-15.6% of the solids obtained during '94 were soluble, but the second straw sample received in early 1995, which had been run through a different extruder and a physical post-treatment, showed good solubility (Straw II).

Table 2. Solubilization of Biomass by the XDP

	<i>Corn Stalks</i>	<i>Switch Grass</i>	<i>Wheat Straw</i>	<i>Straw II</i>
Dry Solid/50 g	20.5	28.3	44.1	44.9
Soluble Solids	2.52	4.4	6.6	14.5
% Soluble Solids	12.20%	15.60%	14.90%	32.30%

Compositions before and after hot rinsing and additional treatments of the biomass are shown in Table 3 using XDP treated cornstalks. Treatment 1 consisted of adding peroxide (3% d.b., 4 hours at 20 °C), followed by a hot rinse. Treatment 2 was a hot base/peroxide treatment (3% d.b. peroxide, pH adjusted to 12, 70°C for 1 hr). As seen in the table, substantial amounts of lignin and hemicellulose (L&H) remained in the biomass, although the fraction of lignin and hemicellulose dropped from 45.8% of the total solids to 37.2% of the total biomass solids after the initial hot rinse. Treatment 1, a second peroxide treatment had little effect, dropping the percentage of L&H to 36.6% of the solids, while treatment 2 had no beneficial effect at all with a 38.8% L&H determined after the hot rinse. These results make us suspect that the correct temperatures or chemical addition rates were not quite reached during the pretreatment process for the first set of samples.

Table 3. Composition of Extracted XDP Corn Stalk

		<i>Hot Rinsed</i>	Treatment1 <i>H202</i>	Treatment 2 <i>Hot Base</i>
Total Solids	40.40%	37.97%	34.09%	34.09%
ash	15.01%	15.02%	14.05%	14.34%
Glucans	39.25%	47.65%	49.23%	46.65%
Xylans	24.87%	19.31%	21.50%	22.57%
K-Lignin	18.80%	17.64%	14.29%	15.04%
A-Lignin	2.04%	0.28%	0.85%	1.20%
Sum Xy/Lig	45.71%	37.23%	36.64%	38.81%

The enzymatic hydrolysis of the second sample of straw (Straw II) was tested. The product was separated into two streams as per Figure 1, a solids fraction remaining after water extraction (3.5 g biomass into 50 ml water at 95C for 1 hour) and the liquid stream containing the solubles. The liquid stream was treated with cellulase (Env. Biot) at about 20-30 FPU/g solids. We noted (Figure 16) an initial xylose concentration of 5.3 g/l increasing to a final concentration of 6.8 g/l in 3 hours. Similarly, the solid stream was hydrolyzed as shown in Figure

17. We measured a 68% yield of the cellulose to sugars (yield defined based on compositions shown in Table 1) in 12 hours with a fairly large amount of xylose also released (an initial concentration of 2 g/l increasing to 8.0 g/l over 12 hours) indicating that the recovery of hemicellulose/xylose in the liquid extraction was not total. A mass balance on the xylose indicates 48% of the hemicellulose was extracted in the hot water extraction, perhaps more could have been extracted in repeated washings. Therefore it seems that delignification and hemicellulose release was achieved in the second straw sample (Straw II).

We feel that the XDP for biomass pretreatment could be coupled with a two stage fermentation process to produce ethanol from biomass in an efficient and economical fashion. The solubilized liquid stream could be fermented using an efficient xylose to ethanol yeast, while the cellulose could be fermented using either a thermotolerant glucose to ethanol yeast/bacteria in an SSF type reactor, or pre-hydrolysed and fermented. Co-fermentation of xylose and glucose is difficult as noted by Grooten et al. (1990,1991), although apparently some progress is being made on the development of a genetically altered yeast which can sequentially ferment both xylose and glucose (Ho et al, 1994). To achieve a high concentration of xylose, a counter current washing of the biomass could be used. In a preliminary simulation of this process, we found that when a solution was used 4 times to extract solubles from four straw II samples, acetic acid built up in an almost linear fashion from 1.2 g/l in the first rinse to 7.6 g/l in the fourth rinse as shown in Figure 18. A study by Wilson et al. (1989) on the fermentability of stream extracted hemicellulose suggests that the acetic acid is quite inhibitory to *P. stipitus*, with no fermentation noted at 5 g/l acetic acid and little at 2 g/l. The removal of the acetic acid was required to achieve a fast and efficient conversion of the hemicellulose hydrolysis.

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Figure 1-A, Biomass Hydrolysis

(XDP-treated Corn Stalk with Nutrition; PH Initial=5.05, Final=5.25)

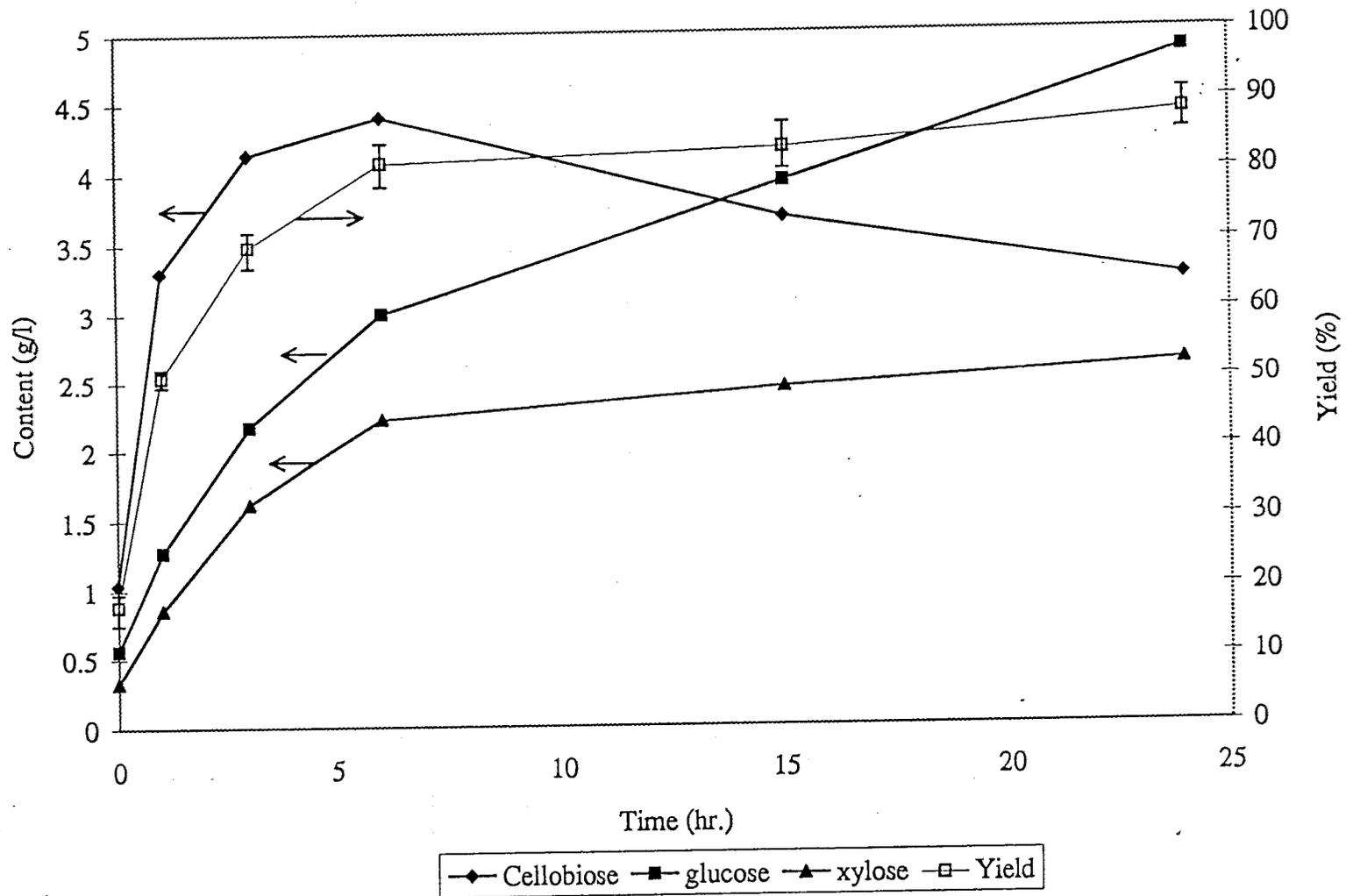


Figure 1-B. Biomass Hydrolysis
(XDP-treated Corn Stalk with Nutrition)

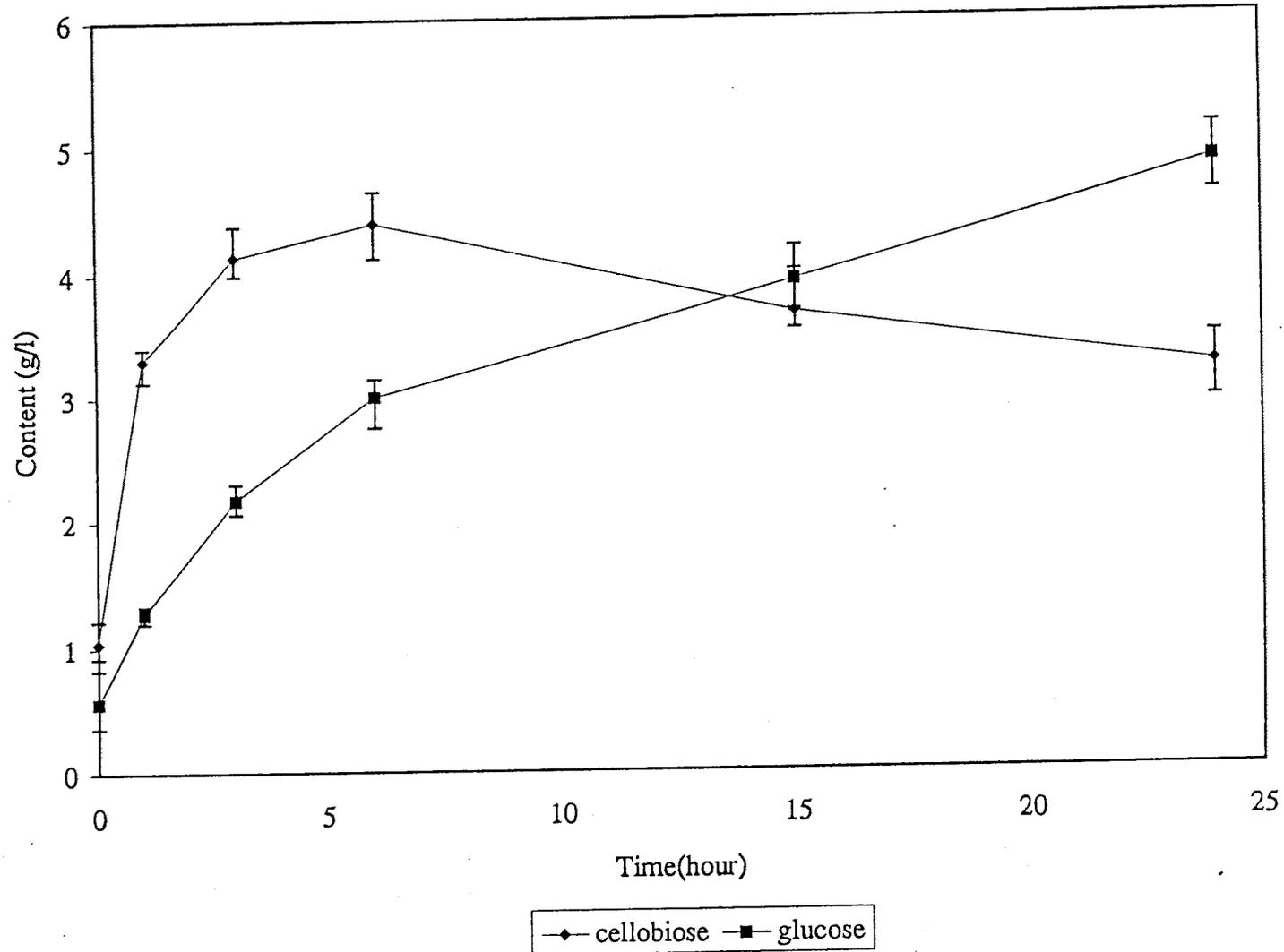


Figure 2-A. Biomass Hydrolysis

(XDP-treated Switch Grass with Nutrition; PH Initial=5.02, Final=5.30)

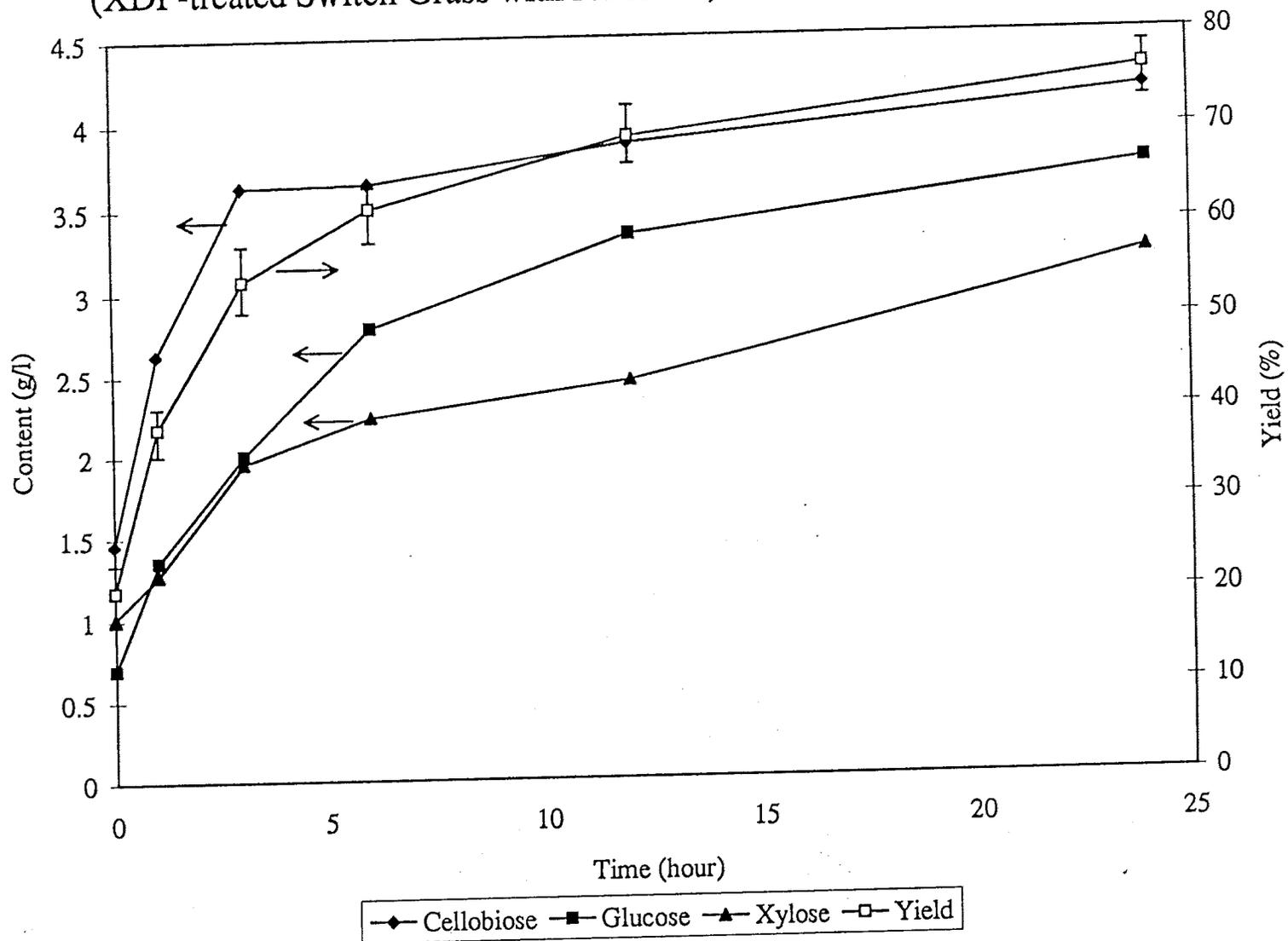


Figure 2-B. Biomass Hydrolysis
(XDP-treated Switch Grass with Nutrition)

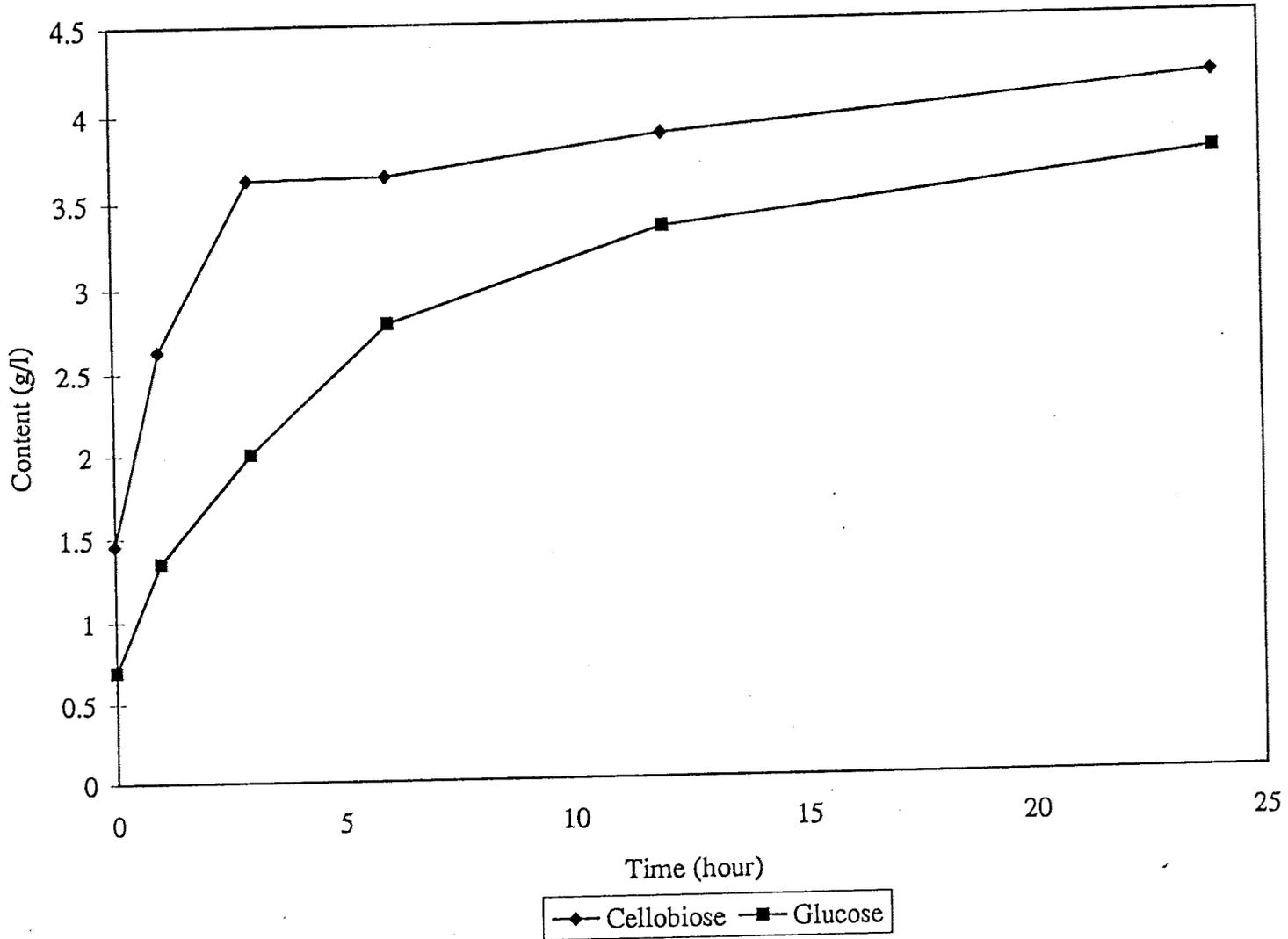


Figure 3-A. α -Cellulose Hydrolysis

(With Nutrition; PH Initial=5.00, Final=5.06)

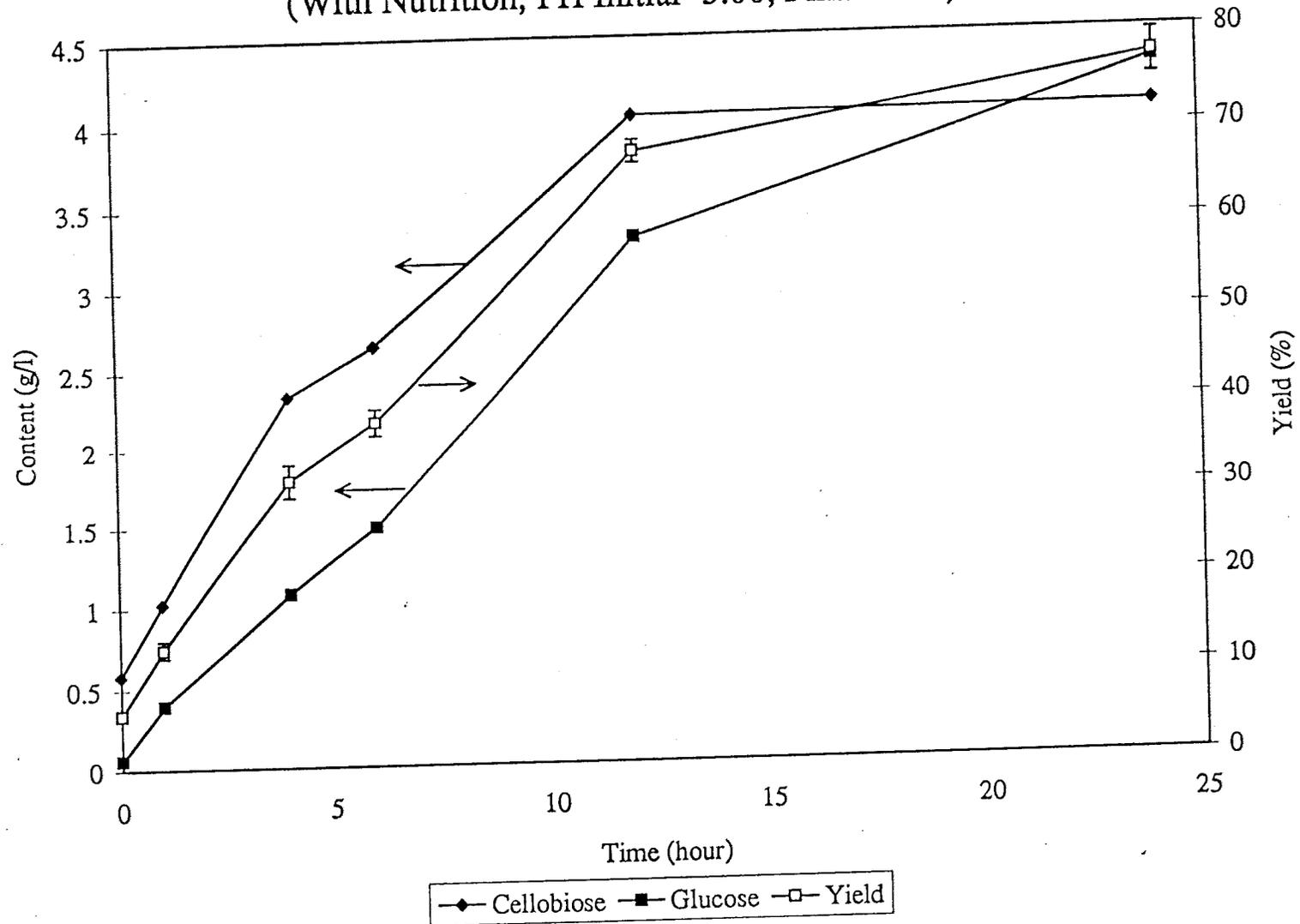


Figure 3-B. α -Cellulose Hydrolysis
(with Nutrition)

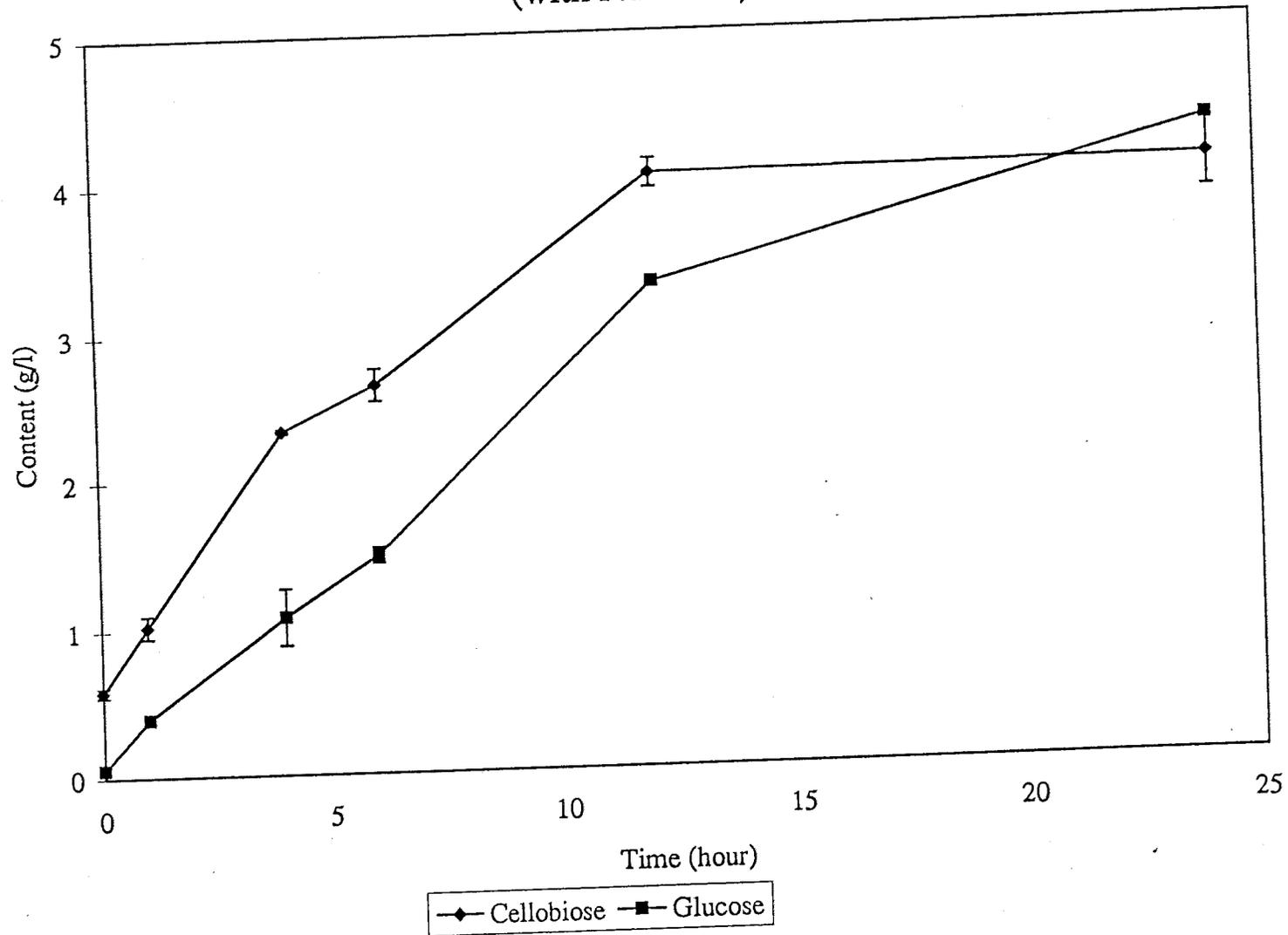


Figure 4-A. Biomass Hydrolysis

(Non-treated Corn Stalk with Nutrition; PH Initial=5.00, Final=5.12)

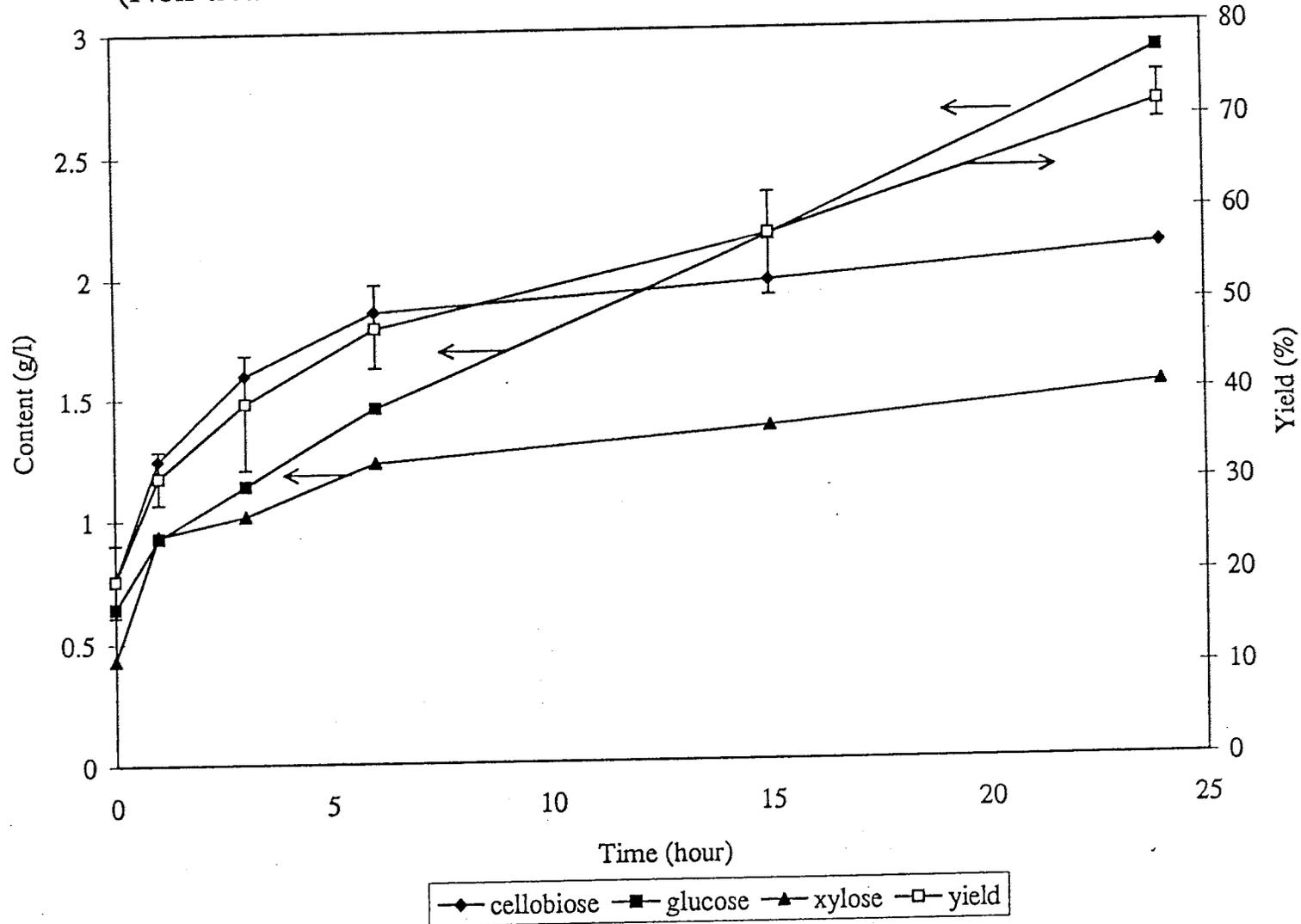


Figure 4-B. Biomass Hydrolysis
(Non-treated Corn Stalk with Nutrition)

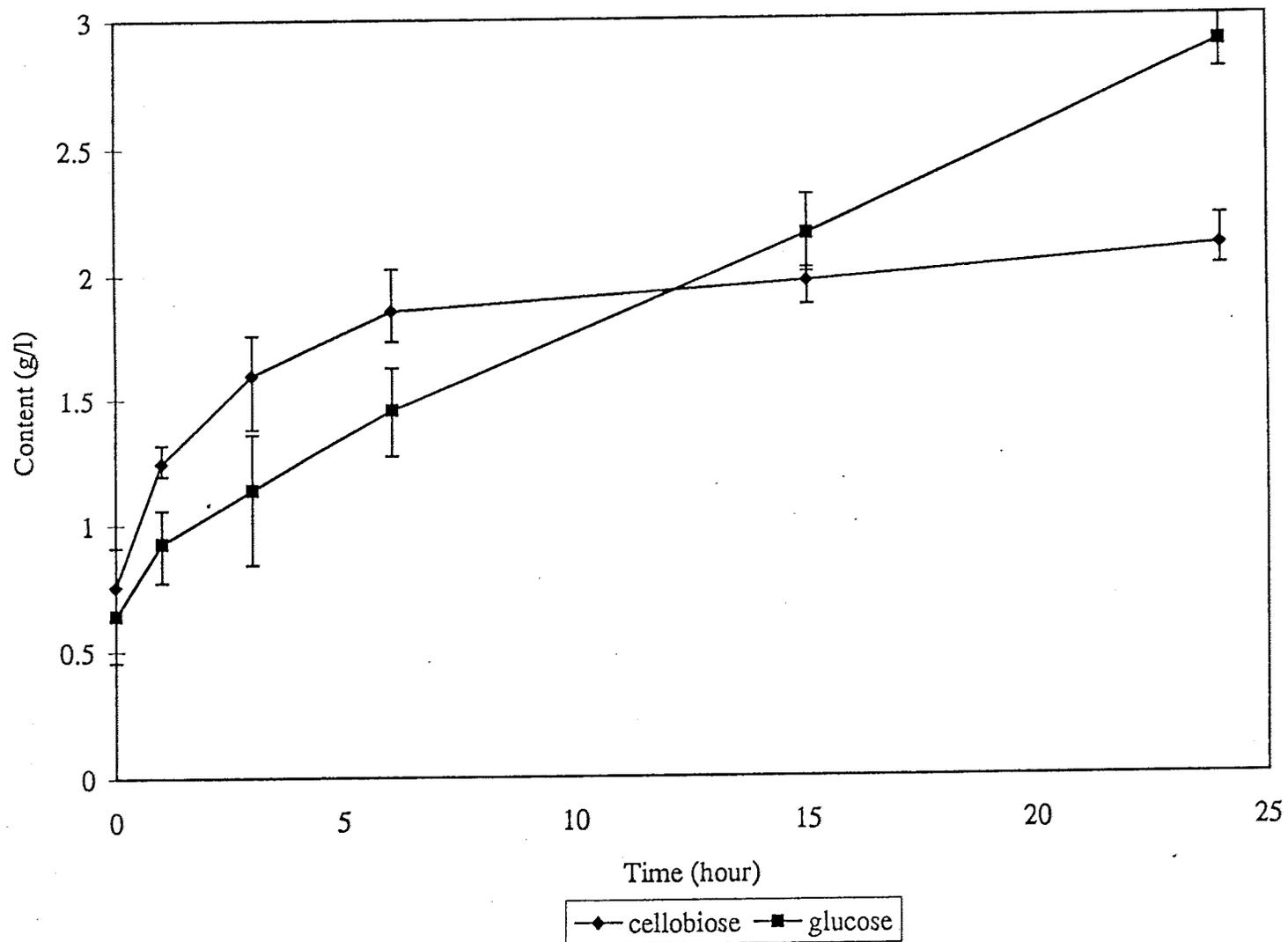


Figure 5-A. Biomass Hydrolysis
(XDP-treated Corn Stalk without Nutrition)

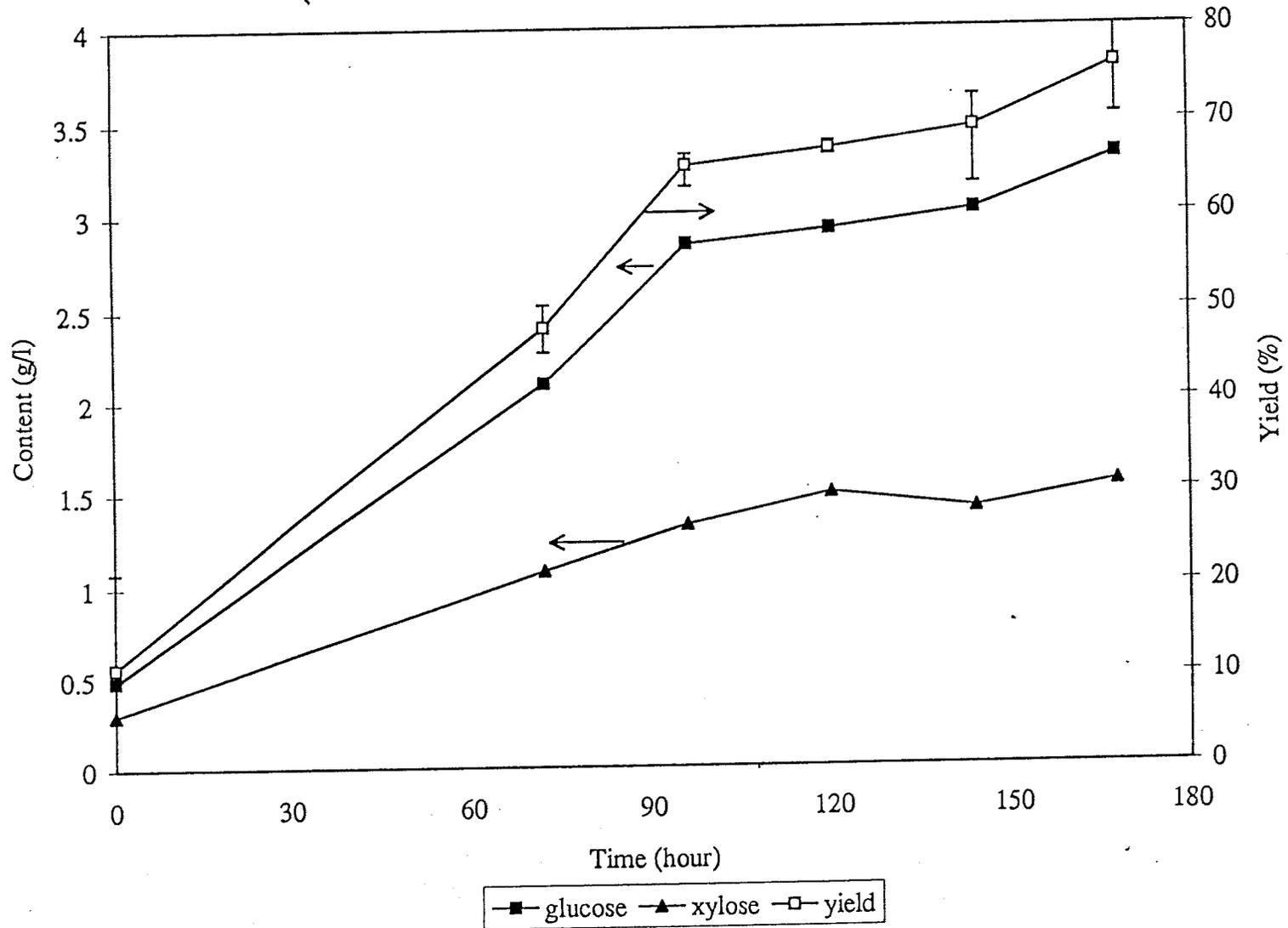


Figure 5-B. Biomass Hydrolysis
(XDP-treated Corn Stalk without Nutrition)

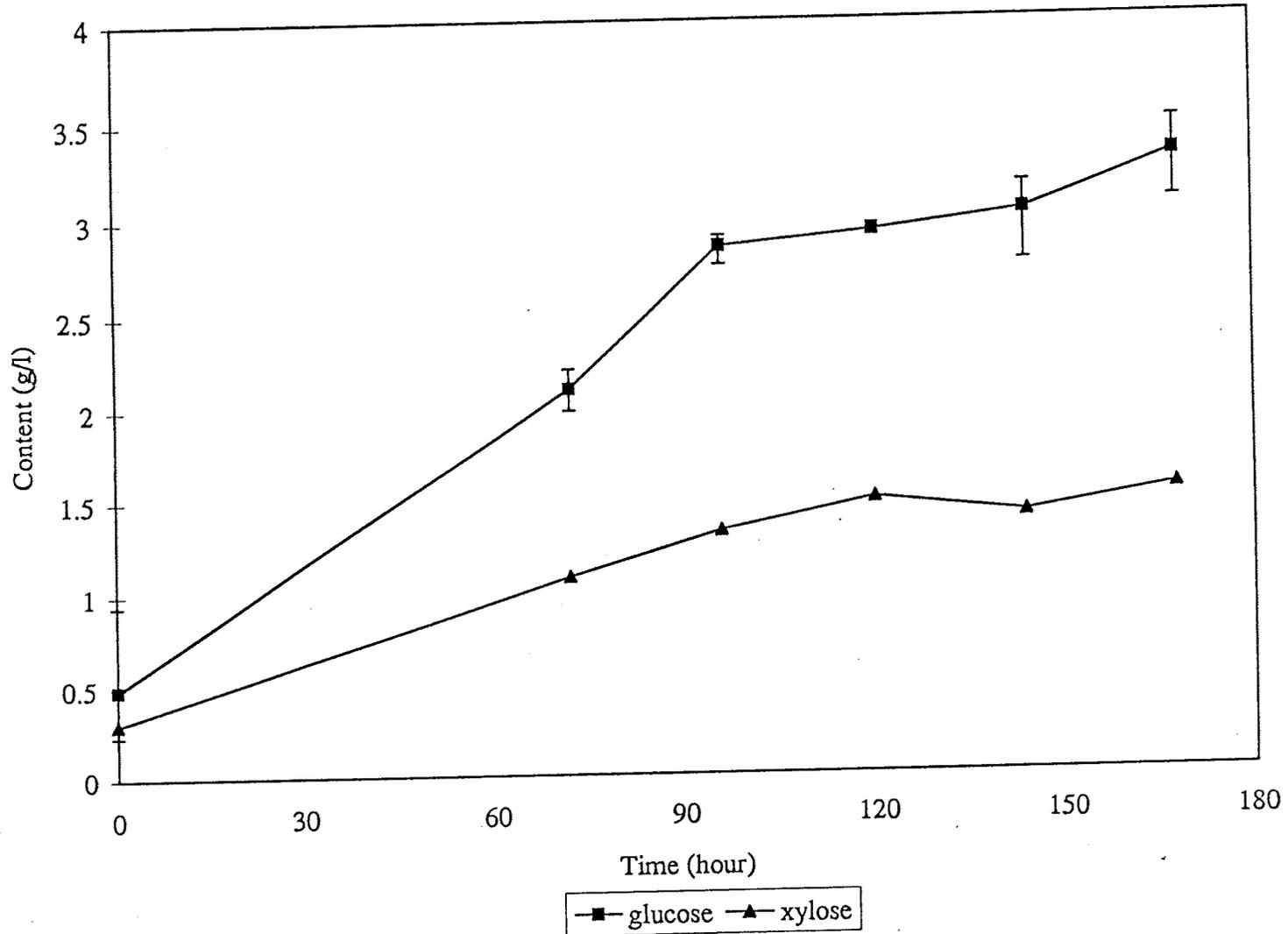


Figure 6. Biomass Hydrolysis
(XDP-treated Switch Grass without Nutrition)

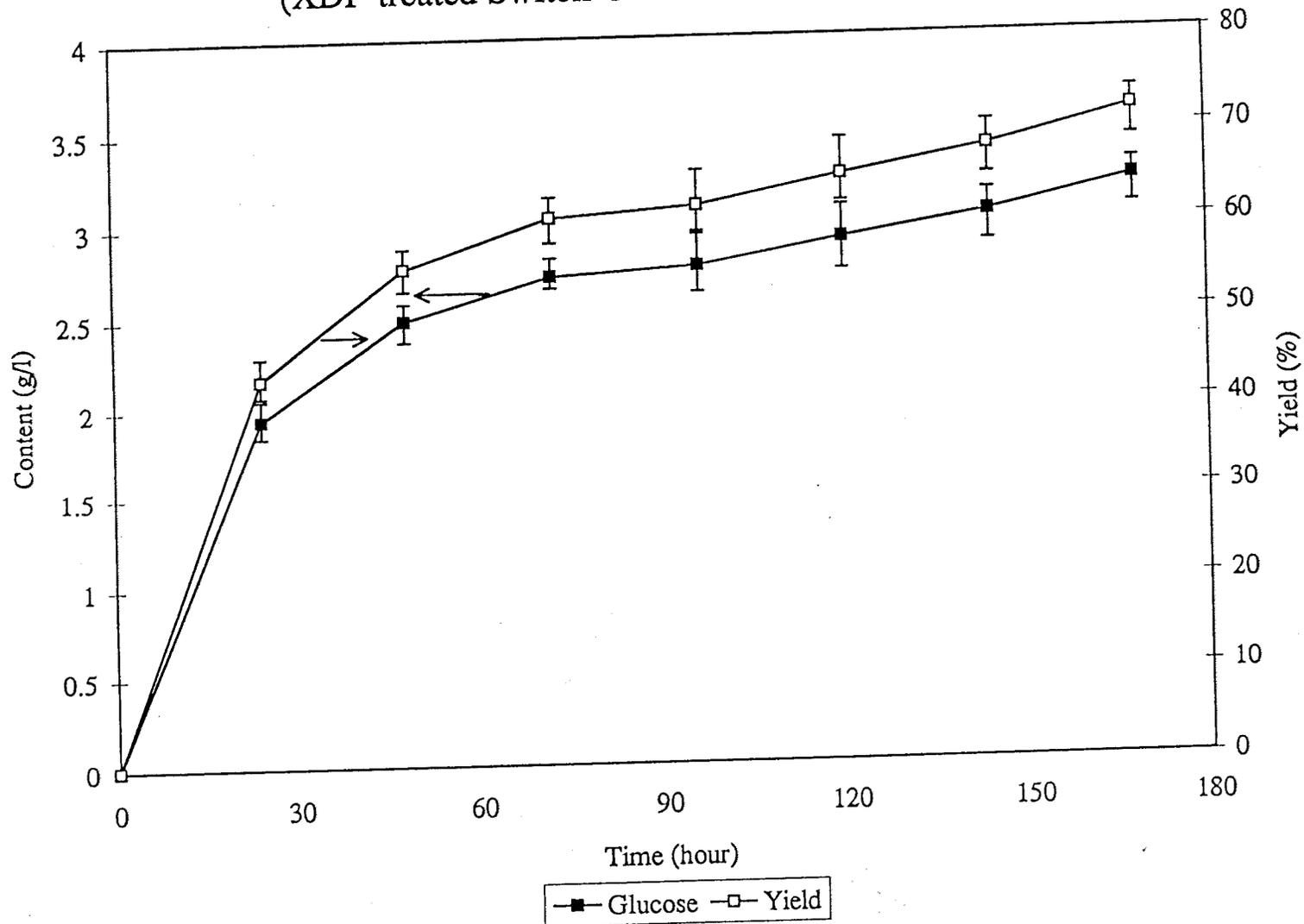


Figure 7. α -Cellulose Hydrolysis
(No Nutrition)

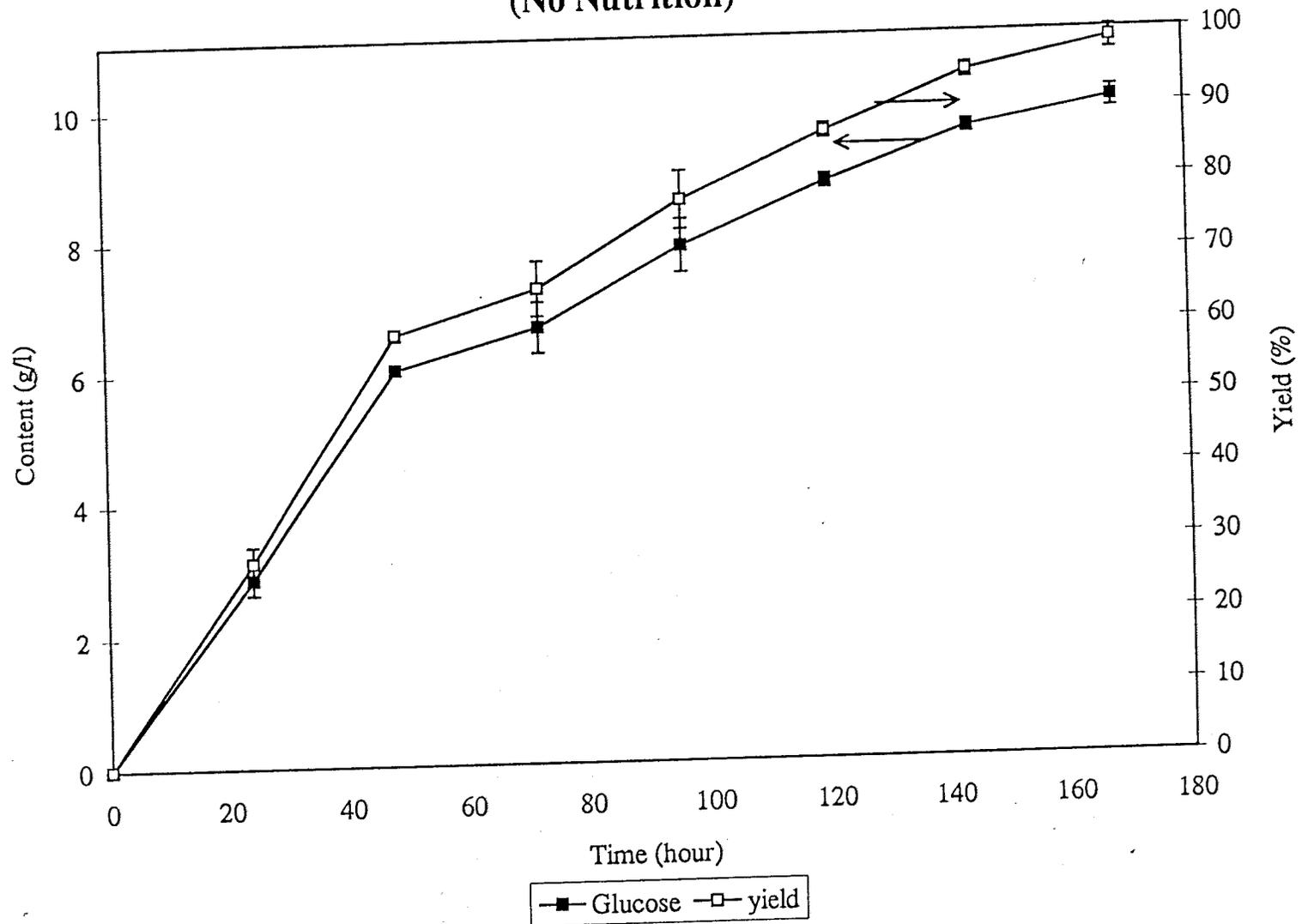


Figure 8-A. Comparison of Biomass Hydrolysis
(With Nutririon)

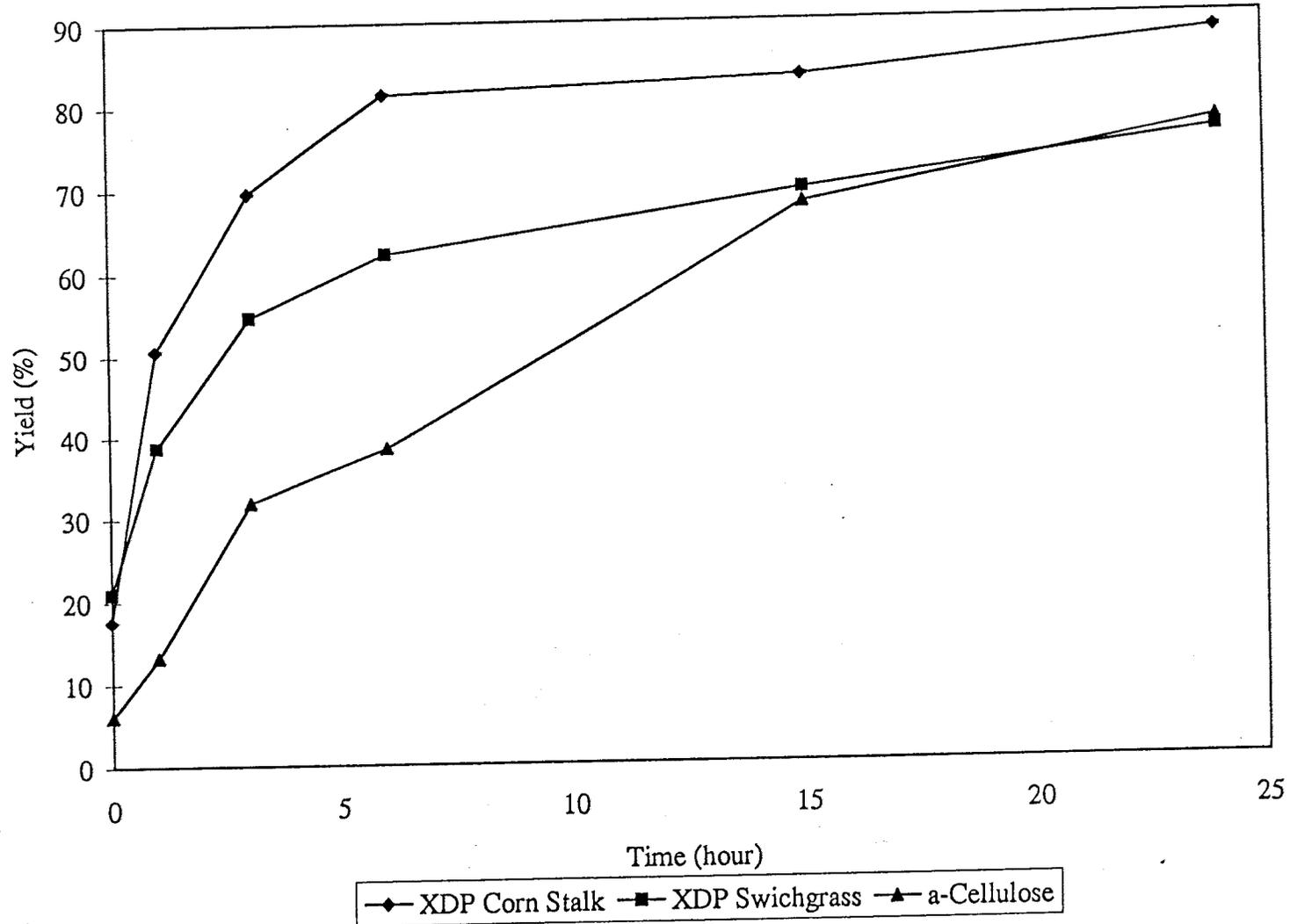


Figure 8-B. Comparison of Biomass Hydrolysis
(Without Nutrition)

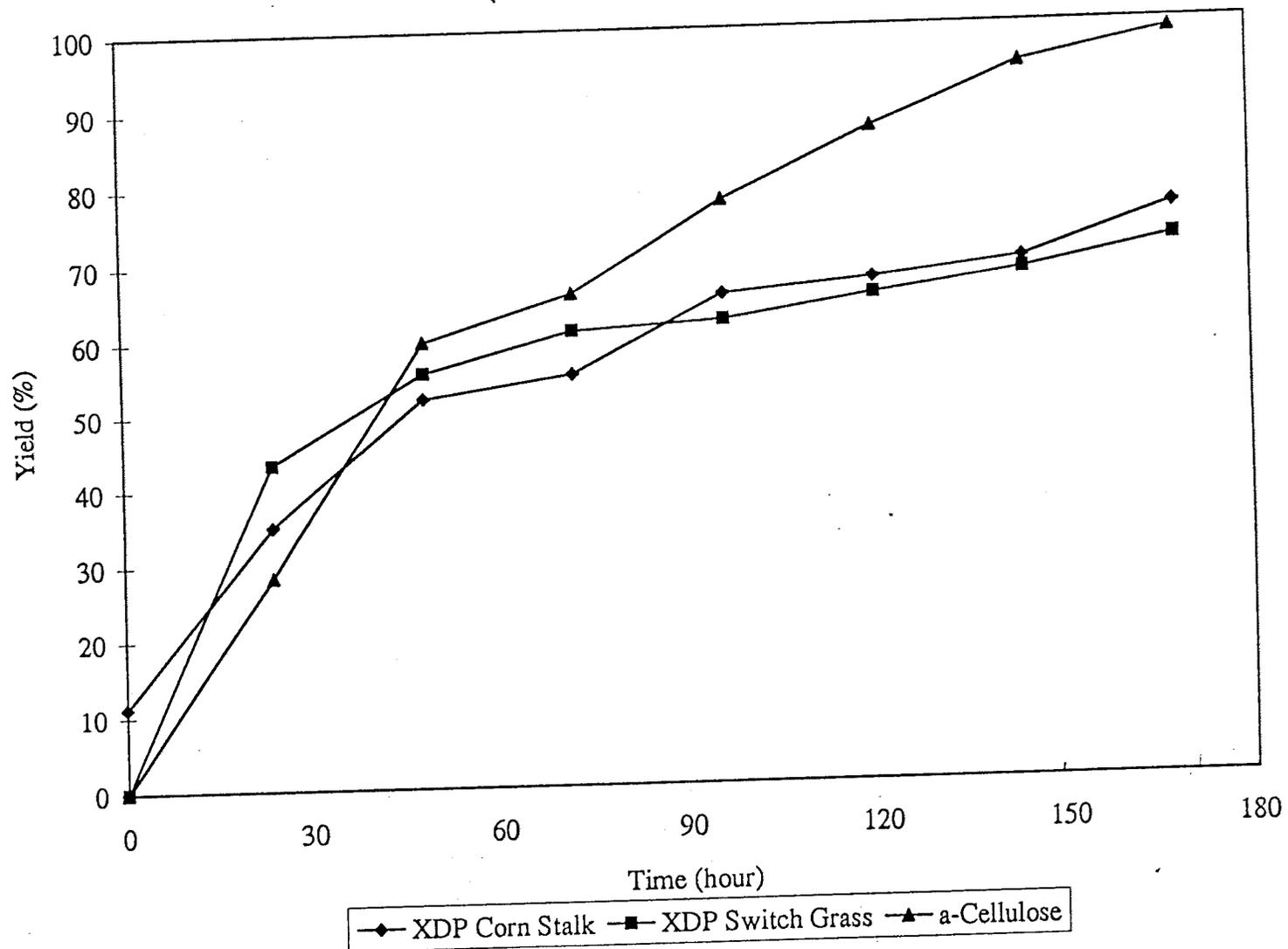


Figure 9. SSF of Biomass
(XDP-treated Corn Stalk; PH Initial=5.03, Final=4.88)

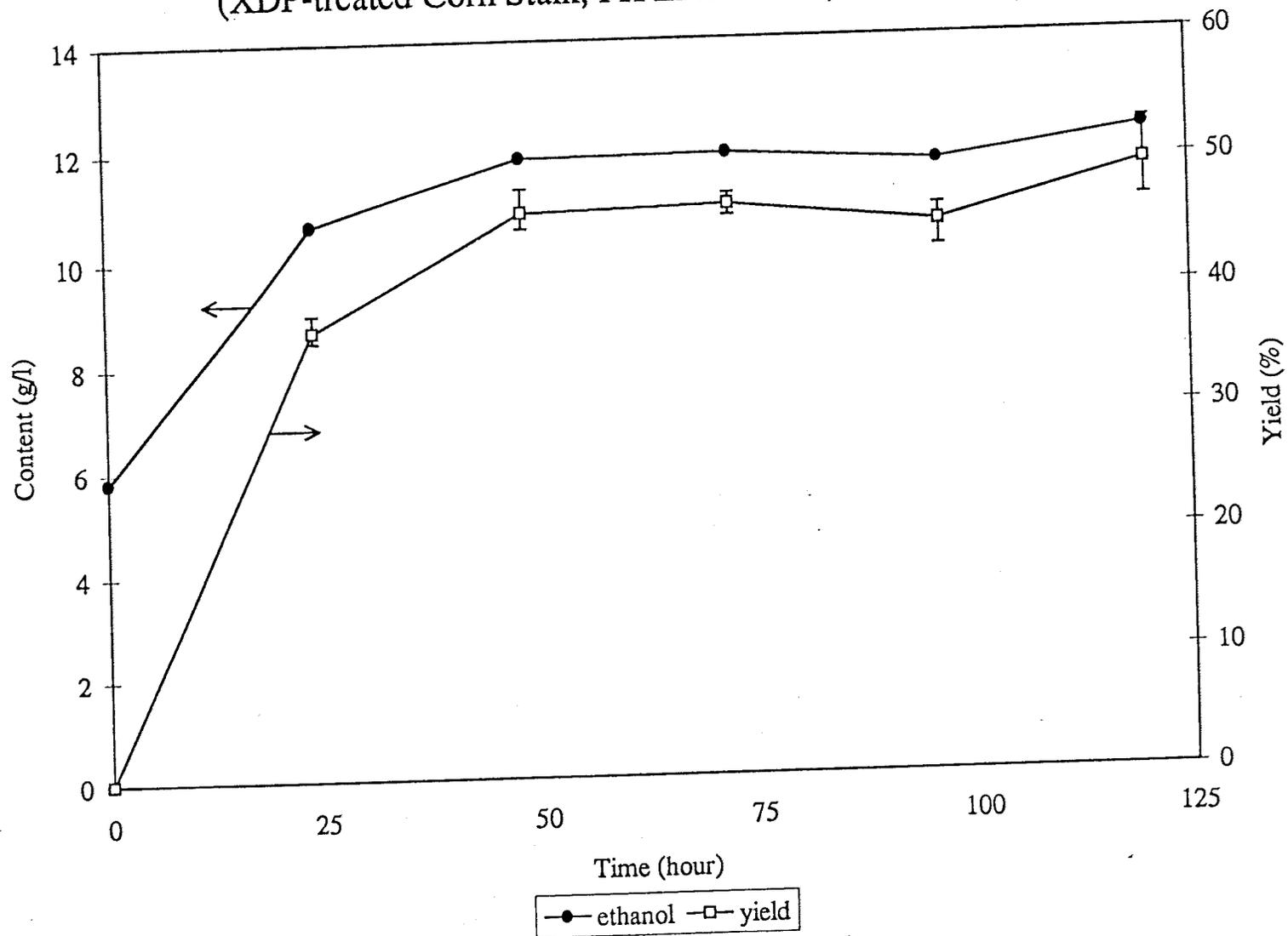


Figure 10. SSF of Biomass
(XDP-treated Switch Grass; PH Initial=5.05, Final=5.15)

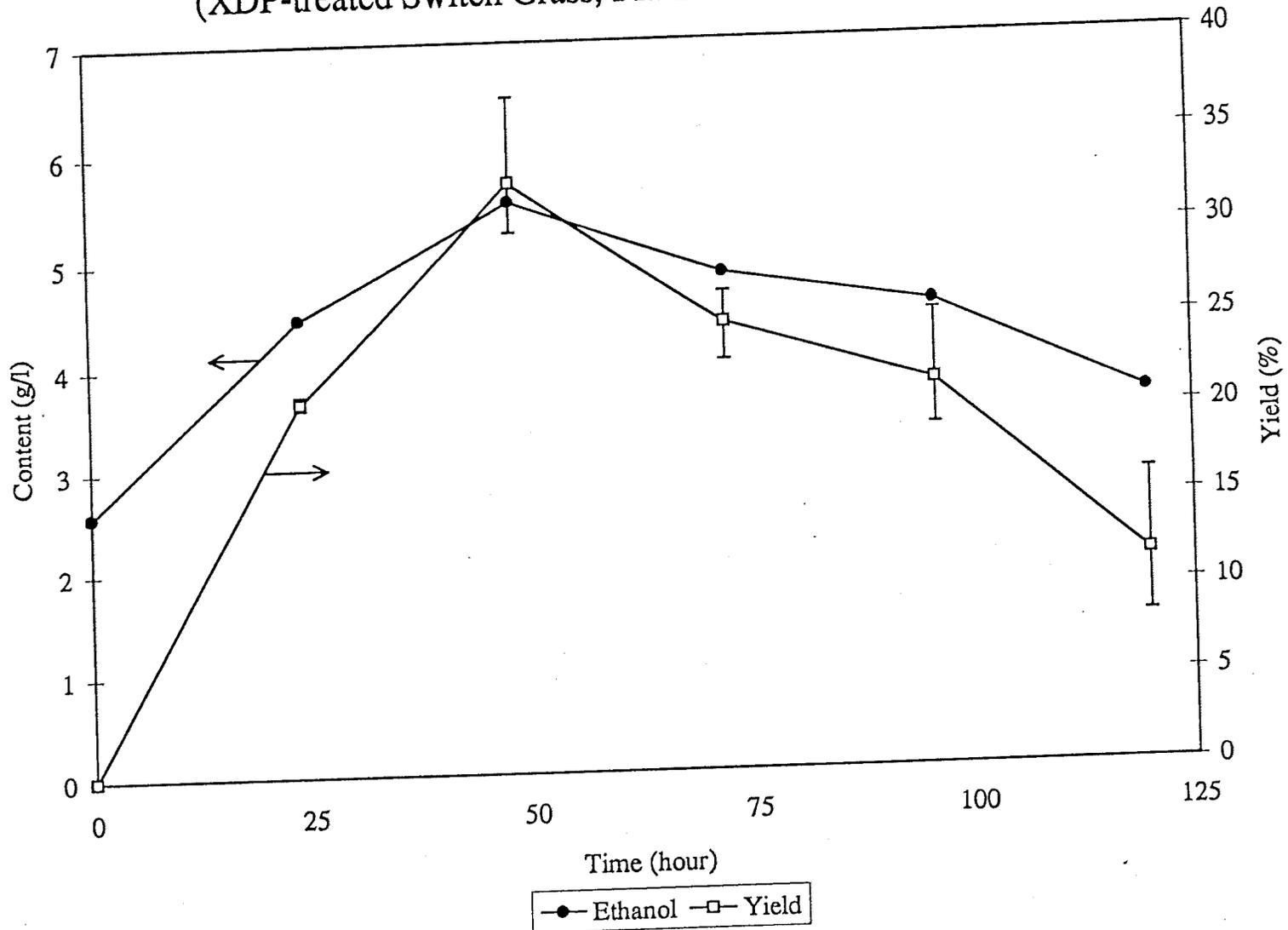


Figure 11-A. SSF of α -Cellulose

(PH Initial=5.02, Final=4.81)

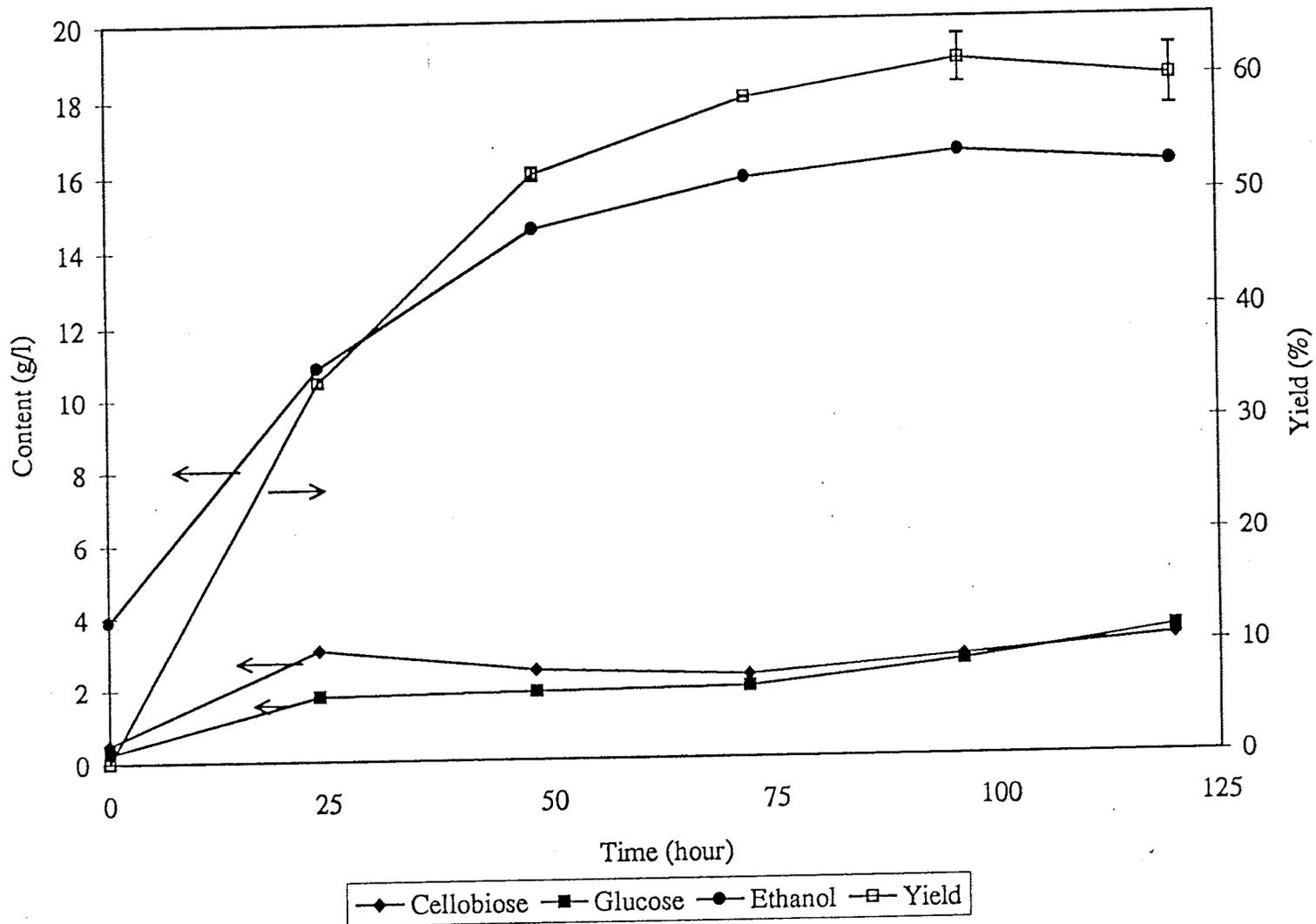


Figure 11-B. SSF of α -Cellulose

(PH Initial=5.06, Final=4.89)

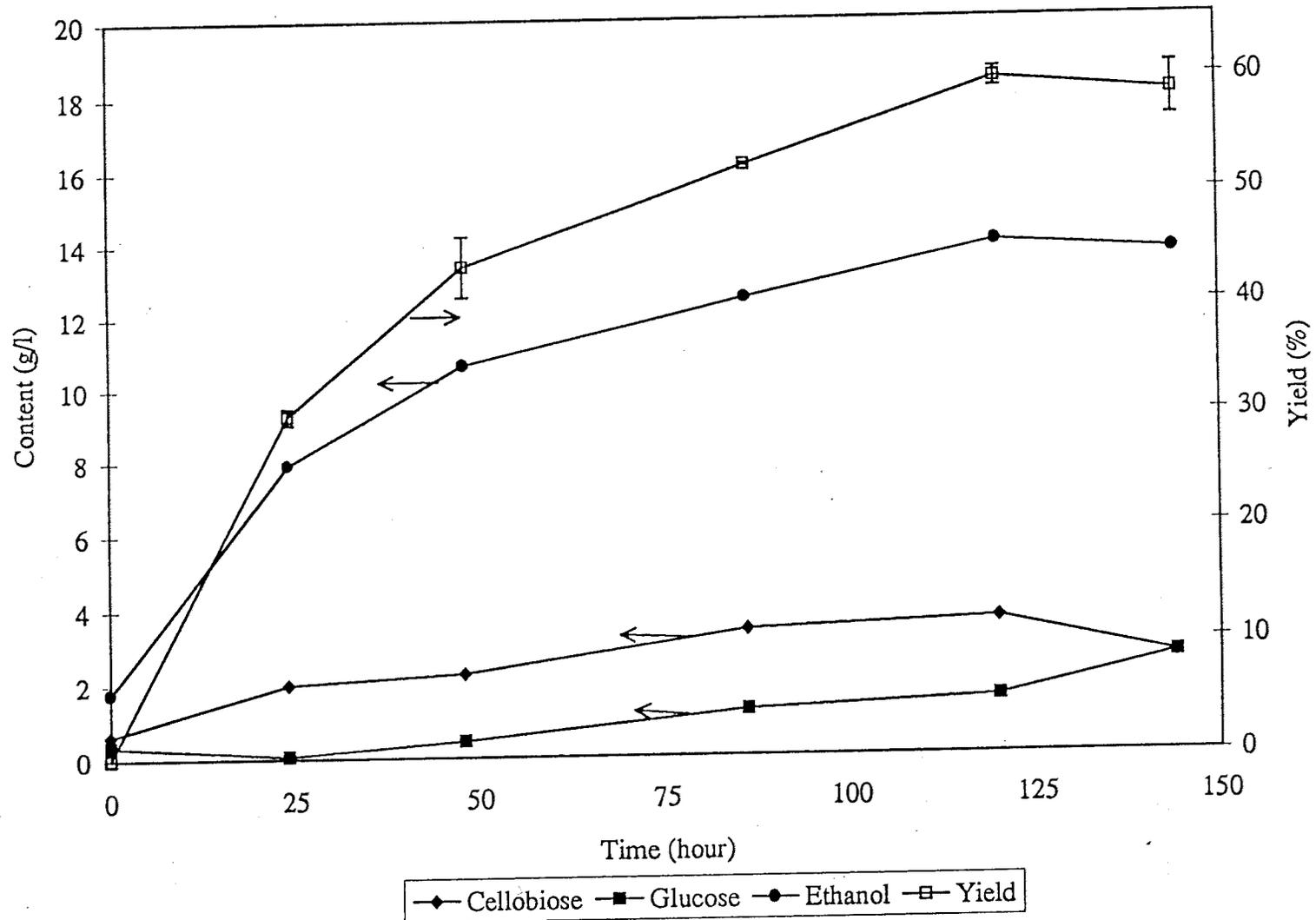


Figure 11-C. SSF of α -Cellulose

(PH Initial=5.09, Final=4.24)

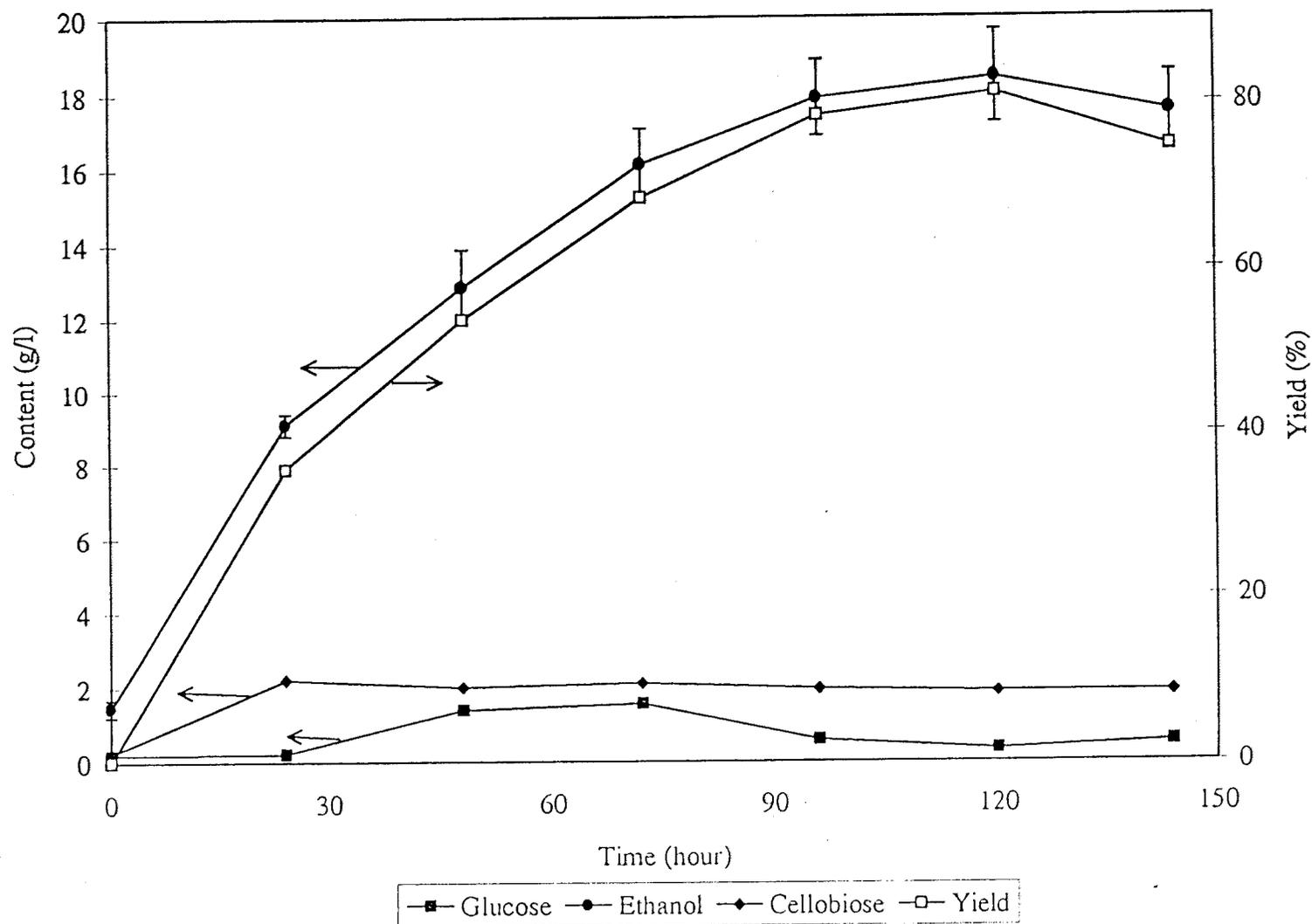


Figure 12. Comparison of SSF
(Different Biomass)

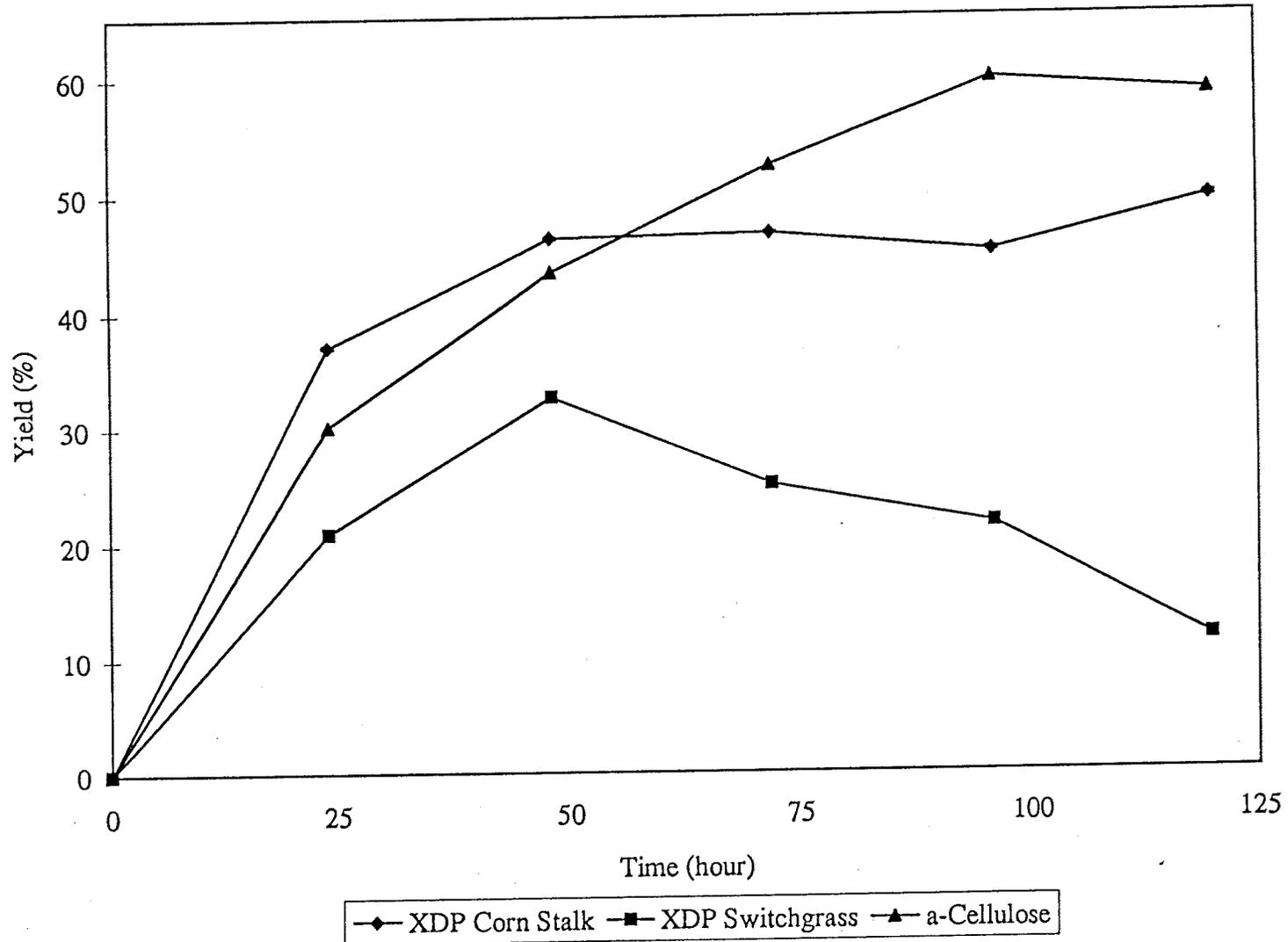


Figure 13.SSF With NRRL 11878
(XDP Treated Corn Stalk; PH Initial=5.03, Final=5.10)

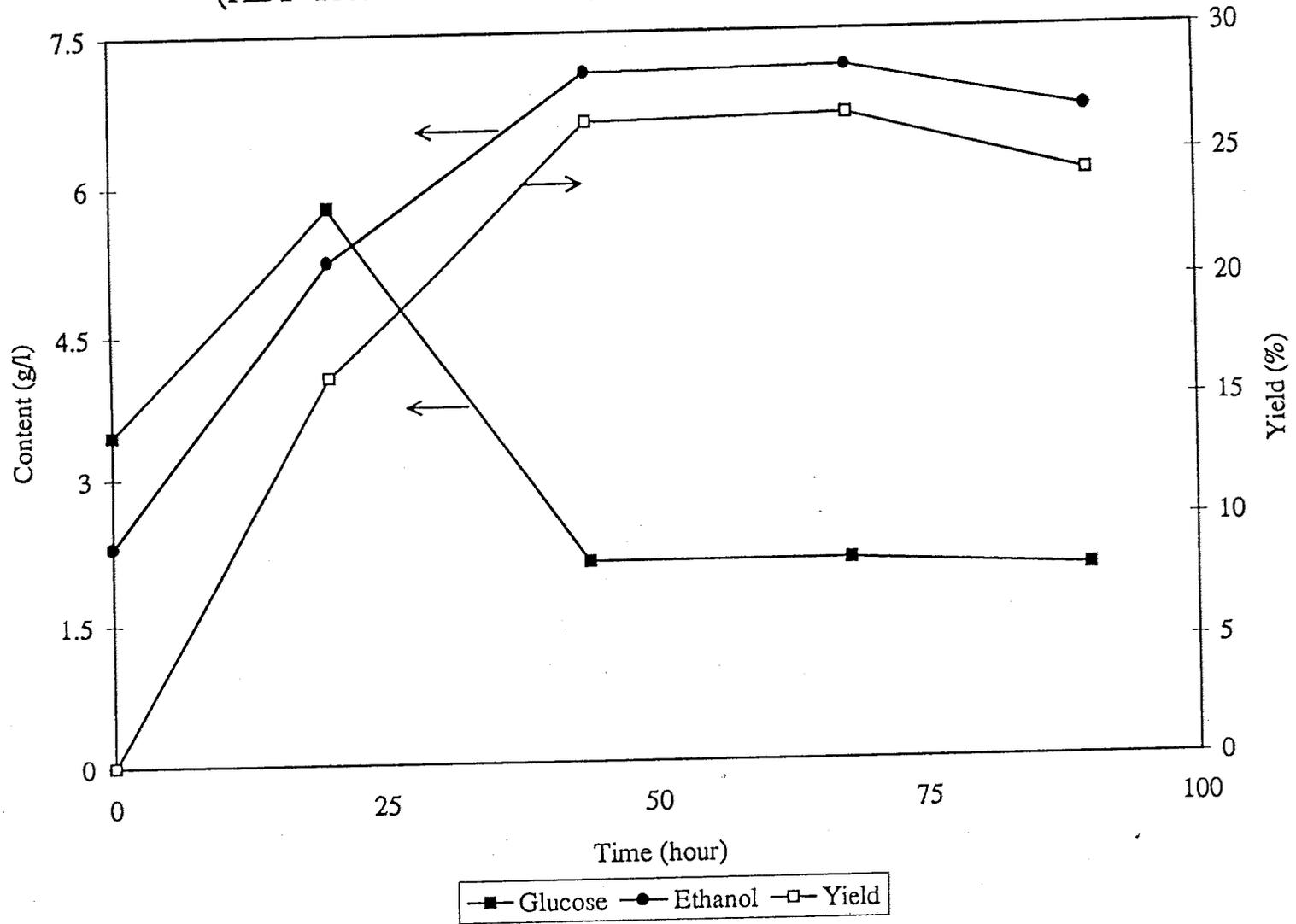


Figure 14.SSF With NRRL 11878 & 11545

(XDP Treated Corn Stalk; PH Initial=5.05, Final=5.00)

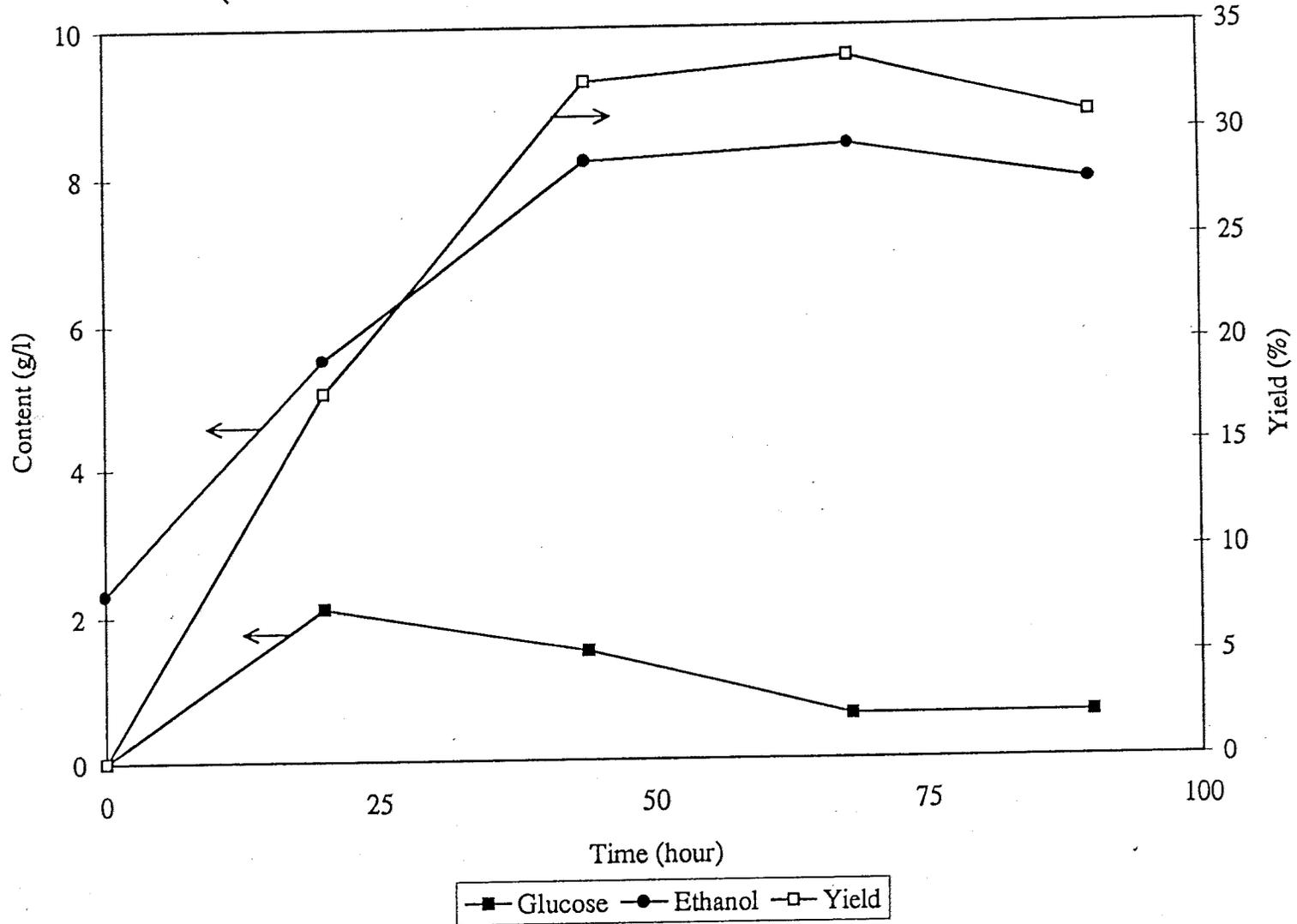


Figure 15. Comparison of SSF
(Different Strains and Temperature)

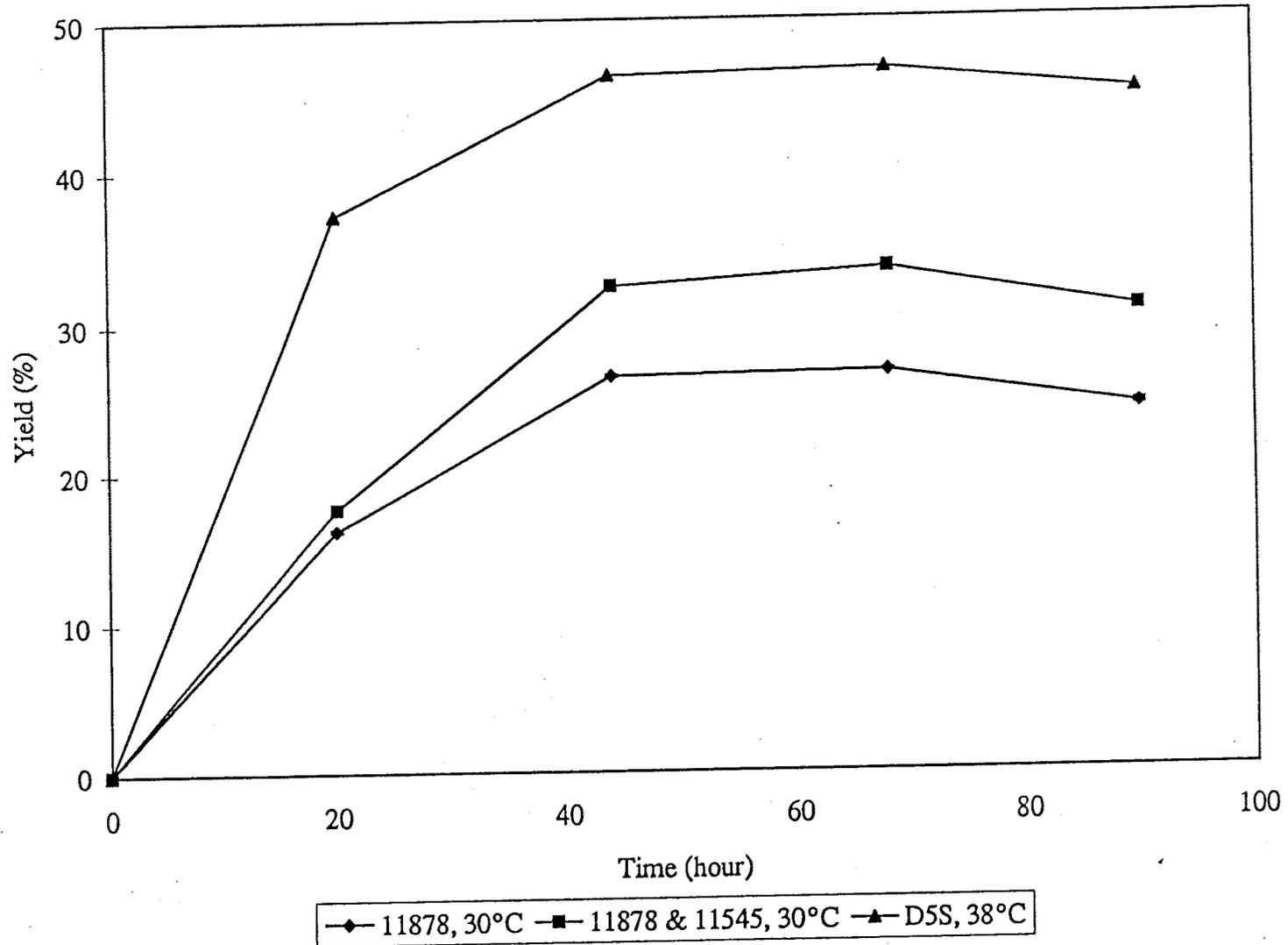


Figure 16. Hydrolysis of Extracted Liquid
(XDP-treated Wheat Straw II)

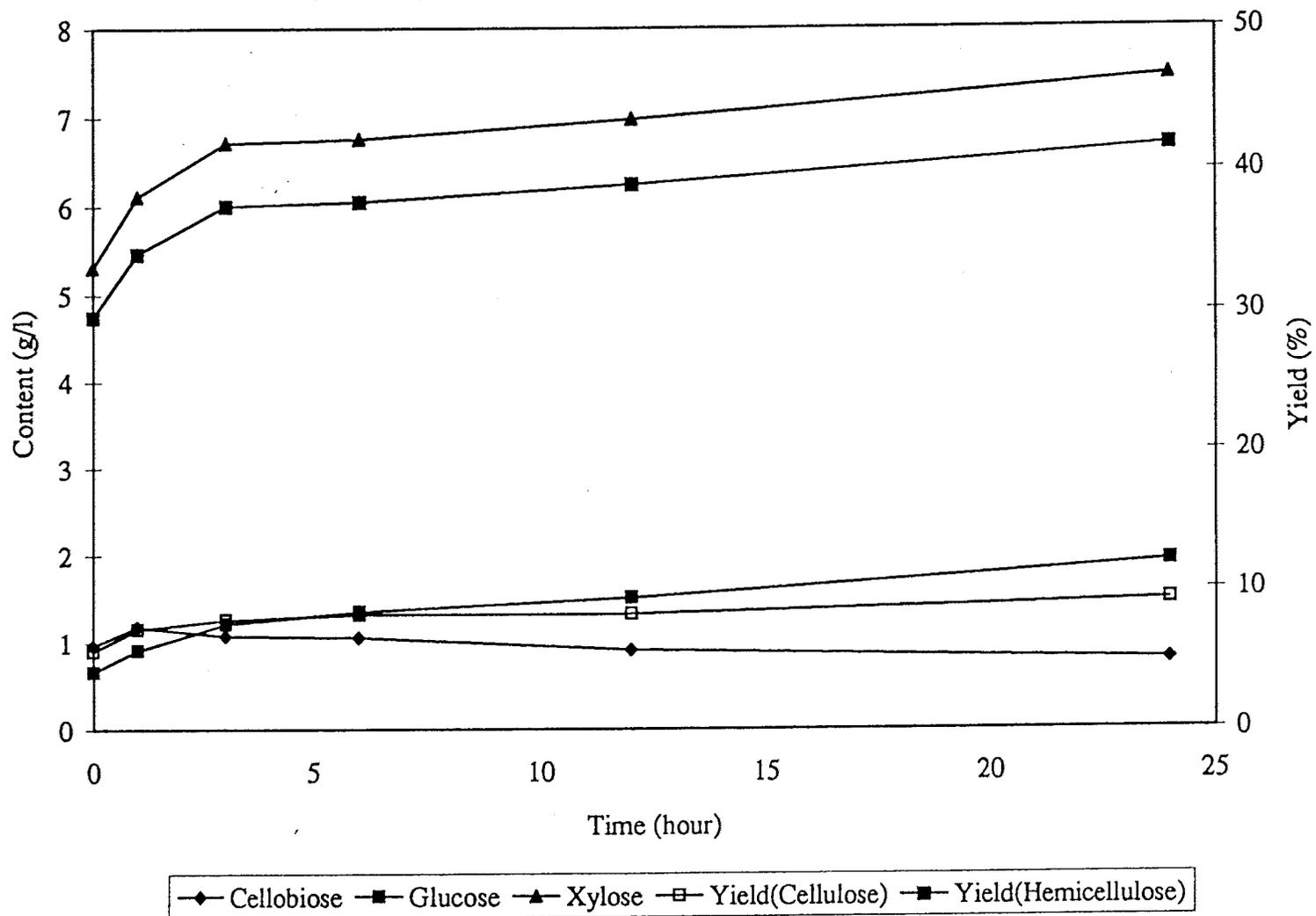


Fig 17. Hydrolysis of Straw(II) Soild
 (XDP-treated Wheat Straw with Nutrition)

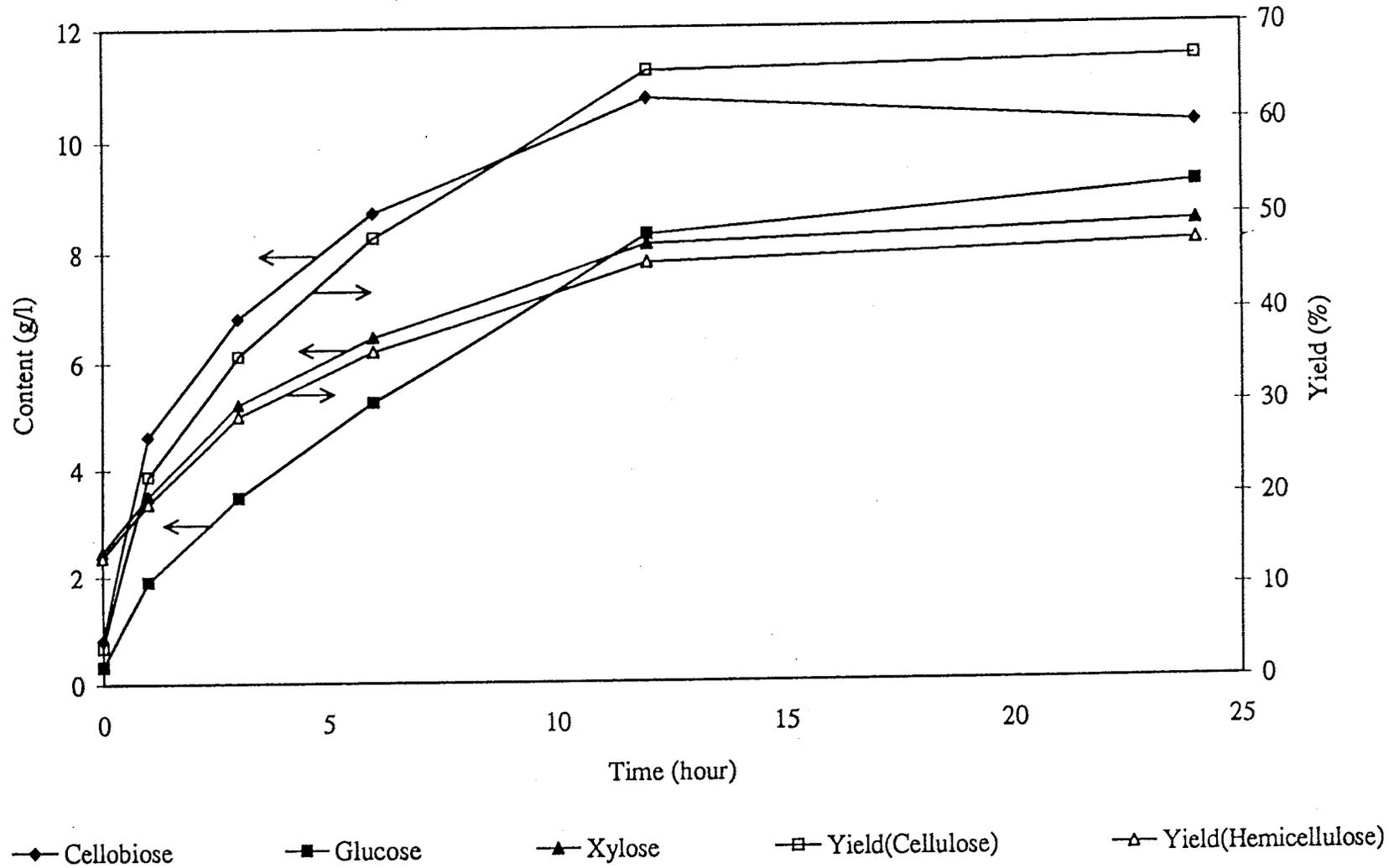


Fig 18. Acetic Acid in Extracted Solution
(XDP-treated Straw (II))

