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"The Evaluation of Bacterial Cellulose From Acetobacter Xylinum as a Potential Papermaking Material"

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"THE EVALUATION OF BACTERIAL CELLULOSE FROM ACETOBACTER XYLINUM AS A POTENTIAL PAPERMAKING MATERIAL"

by

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ABSTRACT

Bacterial cellulose was biosynthesized by Acetobacter xylinum on an enriched growth medium of glucose, yeast extract and KH_2PO_4. The bacterial cellulose fibers were found to be about one-fourth the size of an average softwood fiber.

Pulp and paper total mill effluents and alfalfa extract were exchanged for the glucose and the yeast extract in the growth medium to see if they could substitute for these materials. Kraft and NSSC total mill effluents were found to substitute for the glucose. The alfalfa extract was found to be a better and cheaper material than the yeast extract. Acetobacter xylinum could not synthesize cellulose on an unsterilized growth medium.

Finally, handsheets were made of the bacterial cellulose at six different levels of addition to a bleached softwood kraft pulp. These sheets were then evaluated for their physical properties. Bacterial cellulose was found to have better tensile, TEA, burst and vastly superior fold compared to a well refined bleached softwood kraft sheet, but had poor tear. Bacterial cellulose had physical properties similar to a well refined wood fiber.

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INTRODUCTION

Throughout the history of the pulp and paper industry people have tried to find new sources of papermaking fibers. In general, these searches have been directed towards sources which are fairly inexpensive and quite abundant. It is evident that these two criteria must be met for the fiber source to be used successfully as a papermaking material. One other important criteria must also be established; the fiberous material must be able to make a sheet with acceptable paper properties.

This investigation will study bacterial cellulose synthesized from Acetobacter xylinum. Essentially it will look at the sheet properties of bacterial cellulose and the use of pulp and paper total mill effluents as a carbon source for the Acetobacter xylinum to synthesize cellulose.

HISTORICAL BACKGROUND

In an attempt to understand the complex structure of higher orders of plants, biologists and microbiologists have studied microorganisms which have a simpler metabolism but contain the same cellular materials. Cellulose is one such material that is found commonly in higher order plants and in some bacteria, fungi and algae. It exists as part of the cellular structure in the fungi (1) and in some filamentous green algae (2). In cellulose producing bacteria, cellulose is found both cellularly and extracellularly.

One particular bacteria, Acetobacter xylinum, synthesizes all of its cellulose extracellularly and therefore, the cellulose is completely disassociated from the structure of the bacteria's own cell (3). As early as 1929 Mark and Von Susich confirmed that the cellulose synthesized by Acetobacter xylinum was identical to that found in cotton by producing x-ray diffraction diagrams of the membranes made by the bacteria (4). Other methods have since been used to show that bacterial cellulose was the same high polymer, native crystalline (Type I) alpha cellulose as found in cotton and in wood. These methods included infrared absorption spectra, solubility studies, electron micrography and the study of its qualitative chemical properties (5). In fact, bacterial cellulose has been found to be one of the purest forms of naturally occurring cellulose. This was brought out by Adams and Bishop when they broke down alpha (I) cellulose from cotton, wood, wheat straw and bacteria,

they found small amounts of other sugars. The two prodominate sugars were xylose and arabinose. Both of these were found in all of the sources of cellulose except that produced by the bacterial which only contained traces of xylose (6).

Acetobacter xylinum has been found to grow in simple mediums of 2% glucose, .27% Na_2HPO_4 and .115% citric acid (7) or 2% glucose, 1% yeast extract and .25% Na_2PHO_4. The optimum pH for synthesis of cellulose was 5-8 and the optimum temperature 30-37 C. (8). Acetobacter xylinum is an aerobic organism and therefore requires oxygen to carry its metabolism. However, Colvin and Webb reported the oxygen uptake by Acetobacter xylinum was variable during cellulose synthesis and not directly proportional to the amount of cellulose produced (9).

The yield of cellulose produced by Acetobacter xylinum follows the normal law of bacterial growth which can be mathematically expressed by the following equation (10):

$$m = kNe^{xt}$$

m = yield of cellulose

N = number of bacteria at inoculum

k = proportional constant

t = time

x = exponential constant

Hestrin and others calculated the cellulose synthesizing activity of a cell to be about 1.2×10^6 anhydroglucose units per minute. This was equivalent to about 500 molecules of cellulose each 2600 glucose residues long (11). At this rate the bacteria would need about 51,134 cubic

feet of space to produce one ton of cellulose per hour. In a study of the degree of polymerization of bacterial cellulose, Husemann and Werner reported that it rose rapidly during the first few days of cultivation, reaching a maximum of about 6000 after 5-6 days, and then decreasing slowly to about 4000 after 25 to 30 days (12). Acetobacter xylinum contains the enzymes responsible for converting UDPG (uridine diphosphate glucose) into cellulose. The building up of the cellulose polymer takes place entirely outside the cell. Gascoigue has offered a probable mechanism of cellulose formation by the enzymes in Acetobacter xylinum. This mechanism starts with a glucose - 1 - phosphate being passed through the cell wall by temporary attachment to a sugar nucleotide lipid. Glucoside then crosses the bacterial cell wall into the extra cellular medium, where the glucose residue is transferred to the growing tip of the microfibril by an extracellular enzyme. Once the glucose has been removed the lipid returns to the cell to pick up more glucose, and the process is repeated until cellulose is formed. This snythesis requires the presence of certain chain initiators or formers, in the medium, produced by bacterial cells and probably consisting of a glucose phosphate-lipid complex (13). Other people have also offered evidence pointing toward chain initiators or primers produced by each bacterium which add on monomer units of glucose to form cellulose (14, 15 and 16).

It has been established that Acetobacter xylinum synthesizes a very pure form of alpha (I) cellulose. However, to be of value as a papermaking

material the cellulose must have suitable fibrill structure. Electron microscopic observations by Mahlethaler showed the cellulose built by Acetobacter xylinum occurs in the form of fibers having a diameter of about 250 A^{o}. This is the same diameter of fibrills of cellulose found in the cell walls of numerous plants (17, 18). The bacterial cellulose forms microfibrills which in turn form longer thread-like fibers. Finally, the fibers entangle with one another producing a tough membrane called a pellicle. Unknown factors induce the initial orientation of many microfibrills in the pellicle, extending for distances of several centimeters. Such microfibrills are joined by others in successive layers to form the final microscopic aggregate (19). The individual microfibrills have been measured to have the following dimensions:

width (lateral)	108 A^{o}
thickness (vertical)	16 A^{o}
length	1-3 microns

The average fiber size was much bigger with a length of greater than 20 microns and a width of 20-300 millimicrons (20, 21).

Finally, the strength of the pellicle and the proof of hydrogen-bonding indicate a probable use as a papermaking material. Several people observed that the fiberous pellicle could be torn apart only with great difficulty (22, 23). Colvin noted it had a high modulus of elasticity which approaches that of mild steel or that of a covalent C-C bond (24). Proof of hydrogen-bonding was indicated by work done

by Ohad who incorporated soluble carboxymethyl cellulose ($I\text{-}C^{14}$) in insoluble cellulose fibers which were synthesized by Acetobacter xylinum cells. The incorporated $I\text{-}C^{14}$ could not be washed out with alkali or exchanged with external $I\text{-}C^{12}$. These indicate that $I\text{-}C^{14}$ was bound to the cellulose fibers by hydrogen bonds and the disassociation of the $I\text{-}C^{14}$ cellulose was very low (25).

EXPERIMENTAL PROCEDURES

This study was divided up into three main areas. First, the growth of Acetobacter xylinum on an enriched growth medium to see if it can biosynthesize cellulose. Second, the growth of bacterial cellulose on different mediums. This portion of the study was specifically aimed at using pulp and paper total mill effluents as a substitute for glucose which supplies Acetobacter xylinum with a carbohydrate source. Finally, the characterization and evaluation of bacterial cellulose as a papermaking material.

Growth on an Enriched Medium

The existence of this study was based on the fact that cellulose can be biosynthesized by Acetobacter xylinum. Therefore, the initial stage of this study was designed to prove or disprove this basic assumption. It was not designed to study the growth parameters for the optimum production of cellulose.

Acetobacter need three essential material groups for growth and the synthesis of cellulose. They are a carbohydrate source, a source of vitamins, amino acids and trace elements and a source of phosphate. The enriched growth medium used in this study was made up of the following composition:

Carbohydrate - 2% glucose (also called dextrose)

Vitamins, amino acids and trace elements - 2% yeast extract (Bifcobrand)

Phosphate - .1% KH_2PO_4

All materials were percentage by weight and were dissolved in distilled water. (Note: The yeast extract should be added first because of its slow rate of hydration. Also a magnetic stirrer was quite helpful in getting the yeast extract into the solution). The pH was checked with a pH meter and should be between 5.5 and 8.0. In all cases the pH was found to be within this range. However, if it is outside of this range, dilute HCL or NaOH should be used to bring back within the acceptable limits.

1 - 1.5 liters of the growth medium was placed in large Erlenmeyer flasks, 2-4 liter size. The flasks were then covered with aluminum foil and sterilized by autoclaving of about 250^{o}F and 30 psia. The specific conditions vary slightly depending on the type of autoclave used. After the flask and medium cooled down, the flasks were inoculated with approximately 100 milliliters of inoculum. The inoculum was prepared by breaking up a pellicle from a plant or from another flask in a sterilized high speed blender. The flasks were then transferred to a constant temperature and humidity room. The temperature ranged from 30^{o} to 32^{o} degree Centrigrade and the relative humidity from 30% to 40%.

Although Acetobacter xylinum is an aerobic organism and a constant mixing motion is commonly used to increase the dissolved oxygen in the medium for an aerobic organism, the flasks should be left static as any type of corrotant shaking will cause this organism to mutate. A mutation of Acetobacter xylinum is highly undesirable because the mutant strain will not produce hard cellulose. More information on

growth conditions, growth medium composition and the mutation of Acetobacter xylinum can be found in T. Asai's book "Acetic Acid Bacteria" (26) as well as the other literature references.

Growth on Different Mediums

This portion of the study was designed to answer three questions.

1. Can pulp and paper total mill effluents substitute for glucose as a carbohydrate source in the biosynthesis of bacterial cellulose by Acetobacter xylinum?
2. Can alfalfa extract substitute for yeast extract as a vitamin, amino acid and trace elements source for Acetobacter xylinum?
3. Does the effluent have to be sterilized for Acetobacter xylinum to produce cellulose?

Effluents

All of the effluents were total mill effluents from different types of pulp and paper mills.

Kraft - Came from an integrated mill with a C bleaching sequence making 350 TPD. The effluent had an average of BOD_5 of 200 BOD's.

Sulfite - Came from an integrated mill with a bleaching sequence making 140 TPD. The effluent had an average of BOD_5 of 10,000 BOD_5.

Neutral Sulfite Semichemical (NSSC) - Came from an integrated making 330 TPD. The effluent had an average BOD_5 of 2000 BOD_5.

TABLE I

Experimental Design of Growth Medium Substitutions

Everything Sterilized

	Glucose	Kraft Effluent	Sulfite Effluent	NSSC Effluent	Board Effluent	Fine Papers Effluent
Yeast Extract						
Alfalfa Extract						

Everything Sterilized, Except Effluent

	Kraft Effluent	Sulfite Effluent	NSSC Effluent	Board Effluent	Fine Papers Effluent
Yeast Extract					
Alfalfa Extract					

Fine Papers - Came from a non-integrated mill making 300 TPD. The effluent had an average BOD_5 of 125 BOD_5.

Combination Paperboard - Came from a paper board mill making board from recycled fibers. It produced 400 TPD. The effluent BOD_5 was 125 BOD_5.

Alfalfa Extract

The alfalfa extract was prepared by adding 10 gm of alfalfa meal (the kind commonly used as a livestock feed) to 300 ml of .01 N HCl (27) in a round buttoned boiling flask. The flask was refluxed 24 hours at 100°C then filtered through a Buchner funnel with the extract being collected in a clean filtering flask. The extract may be used in this form or concentrated into a solid by evaporation of the water. The latter is suggested because the solid alfalfa extract can be substituted, 1:1 by weight for the yeast extract. The water can be evaporated by boiling the filtrate in an open beaker or by using a rotary vaccum evaporator.

Preparation of the Growth Mediums

Table I illustrates how this portion of the study was set up. In the upper set everything was sterilized and the phosphate kept constant at .1% KH_2PO_4. The first row shows the total mill effluents being substituted for the 2% glucose with the yeast extract constant at 2%. The second row was the same except with 2% alfalfa extract being substituted for the yeast extract. The lower set the control was left out and the effluent was added unsterilized to the rest of the medium just before

inoculation. The rest of the medium in the lower set had been sterilized. Three 25 ml test tubes with screw caps were used for each different set of conditions. The effluents were first adjusted to a pH of 7 with diluted HCl or NaOH. The final volume in each test tube was 17 ml. This included 1 ml of inoculum.

The test tubes were placed in a constant temperature and humidity room at 30 to 32°C. Observations were made daily for bacterial cellulose growth. This portion of the study was only qualative in the fact that proof of bacterial cellulose growth was by finding a pellicle in the test when the experiment was stopped.

EVALUATION OF BACTERIAL CELLULOSE AS A PAPERMAKING MATERIAL

Preparation of Bacterial Cellulose Handsheets

The pellicles of bacterial cellulose were poured out of the flask along with the medium. They were rinsed off with distilled water to get as much of the medium off of the pellicles as possible. The pellicles were then broken up in a Waring Blender at high speed for 1-2 minutes. The borken pellicles were passed through an 40 inch mesh hand screen to screen out any portions of the pellicles which were completely broken apart.

At this stage in the preparation of the pulp slurry, the slurry was still quite colored due to the medium . Therefore, the slurry was centrifuged at 2000 rpm and the medium was poured off. The bacterial cellulose was collected and diluted with more distilled water. The centrifuging and washing with distilled water steps were then repeated

until the bacterial cellulose appeared to be fairly free of the growth medium. At his point the bacterial cellulose was at .25% solids, ready to be used for making handsheets.

The following eight sets of handsheets were made on the Noble and Wood Sheet mold.

Control - 100% Bleached Softwood Kraft
25% Bacterial Cellulose
5% Bacterial Cellulose
10% Bacterial Cellulose
25% Bacterial Cellulose
50% Bacterial Cellulose
75% Bacterial Cellulose
100% Bacterial Cellulose

The bleached kraft softwood fibers used with the bacterial cellulose were refined to a Canadian Standard Freeness of 590 ml in a Valley laboratory beater. The basis weight was 38 g/m^2 or 1.5 gm/Noble and Wood handsheet. To keep the system as simple as possible and to prevent any interference from other sources, no wet end additives were used and only deionized water was permitted in the sheet mold.

It could be seen after making the first few handsheets at higher percentages of bacterial cellulose (greater than 10%) that the freeness of the bacterial cellulose fibers was going to cause a slow draining sheet. Therefore, the following adjustments were made on the usual Noble and Wood Handsheet procedures.

1. Only enough of the pulp slurry was added to the sheet mold to make a 1.5 gm sheet. No extra water was added because of the length of time it would take for it to drain from the sheet.
2. A constant vaccum was applied to the sheet mold to form the sheet and drain the water from it. In the case of the 100% bacterial cellulose sheet this took 4-5 minutes.
3. The sheet was sent through the wet press with a minimal amount of pressure to prevent wet crush of the sheet. This was accomplished by removing all of the weights from the wet press.

The sheets were then dried in the usual manner and placed in a constant temperature and humidty room to condition. The temperature maintained at 72^{o}F and the relative humidity at 50%.

Standard Beater Curve

In order to compare the sheet properties of the bacterial cellulose to that of a wood fiber, a beater curve has run on the same bleached kraft softwood fibers as used in the control. Samples were taken out of the Valley beater at seven different Canadian Standard Freeness. These pulp samples were then used to make handsheets on the Noble and Wood sheet machine following the same techniques used with the bacterial cellulose. These sheets were also conditioned in the constant temperature and humidity room at 72^{o}F and 50% relative humidity.

Testing of the Handsheets

Both the bacterial cellulose sheet and the sheet from the beater curve were tested for the following physical properties.

TEST	UNITS	TAPPI STANDARD
Basis Weight	g/m^2	T-410
Caliper	.001 in.	T-411
Tensile	Kg	T-494
% Stretch	%	T-494
Tensile Energy Absorption (TEA)	Kg/m	T-494
TEAR (internal)	gm	T-414
M.I.T. Fold	Folds	T-511
Burst (mullen)	psi	T-403

Also, the tensile factor was determined by dividing the tensile by the basis weight to correct for variation in basis weight between groups. Finally, the sheets were first visually inspected on a light table for their formation. An arbitrary point system established the relative meaning to their formation. The point values ranged from 5 to 1, 5 referring to good formation and 1 for poor formation.

RESULTS

Growth on an Enriched Medium

Within two days of inoculation the Acetobacter xylinum had produced pellicles at the air medium interface in all of the flasks. The pellicles continued to grow at the surface until they sank from their own weight or were jarred loose by shaking the flask. In either case a new pellicle proceeded to grow on the surface by the next day. By weighing the wet pellicles when they were pulled out for the handsheet study and finding the pellicles consistency as growth rate was determined. This should be considered a very rough figure because the medium had not been washed from the pellicles at this point. The growth rate for the bacterial cellulose was found to be:

.0496 gm/liter/day on a volume basis

and

.2985 gm/ft.^{2}/day on a surface area basis.

This was the air-medium surface area.

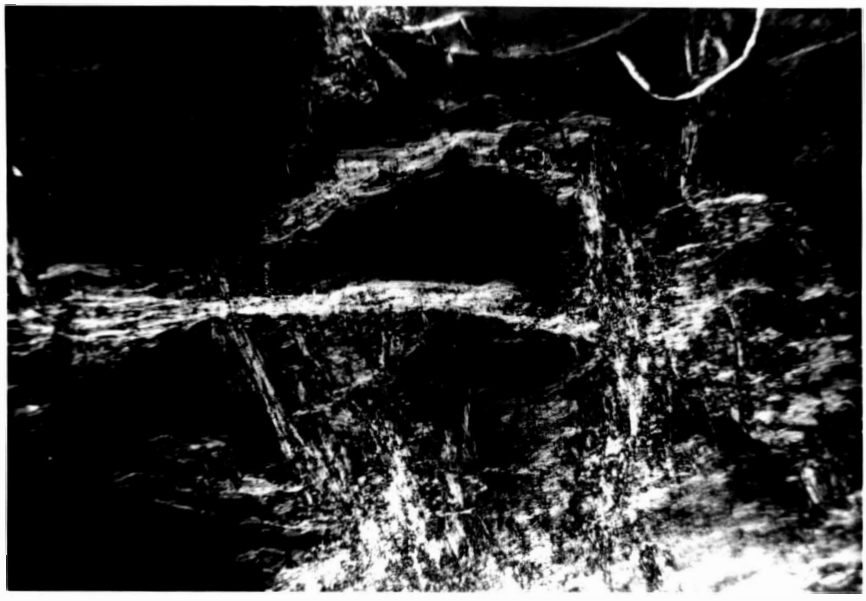
Figures 1 and 2 are photo-micrographs of the bacterial cellulose after some of the pellicles were broken up in a Waring blender. Figure 1 has pictures at 32 X and Figure 2 are at 80 X. The pictures on Figure 3 are of bleached softwood fibers at 32 X for comparison purposes.

From these pictures the length of the bacterial cellulose fiber was estimated to be about 1.0 - 1.25 millimeters and the diameter about .01-0075 millimeters. This means the bacterial cellulose fibers are about 1/4 the size of an average softwood fiber. Also from the pictures one

FIGURE 1

PHOTOMICROGRAPHES OF

BACTERIAL CELLULOSE AT 32X



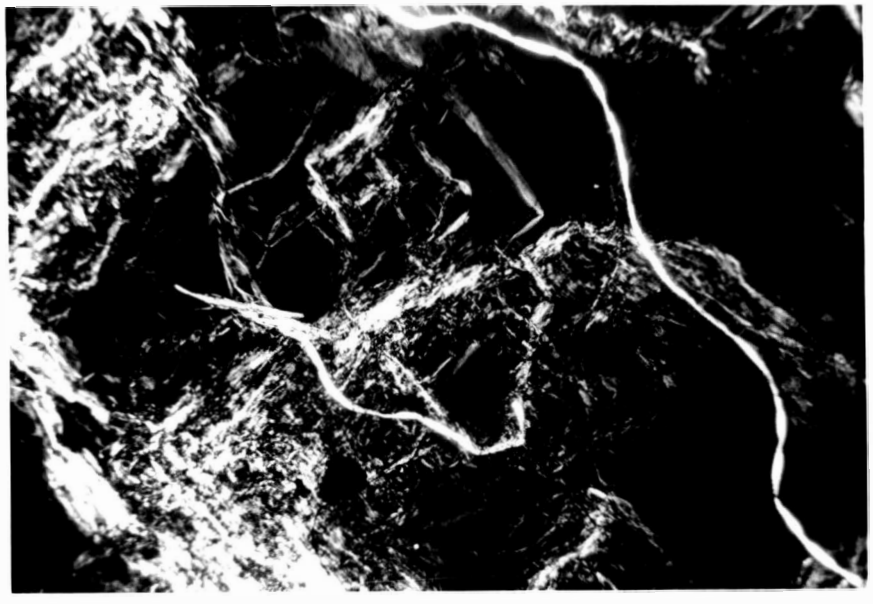


FIGURE 2

PHOTOMICROGRAPHES OF

BACTERIAL CELLULOSE AT 80X

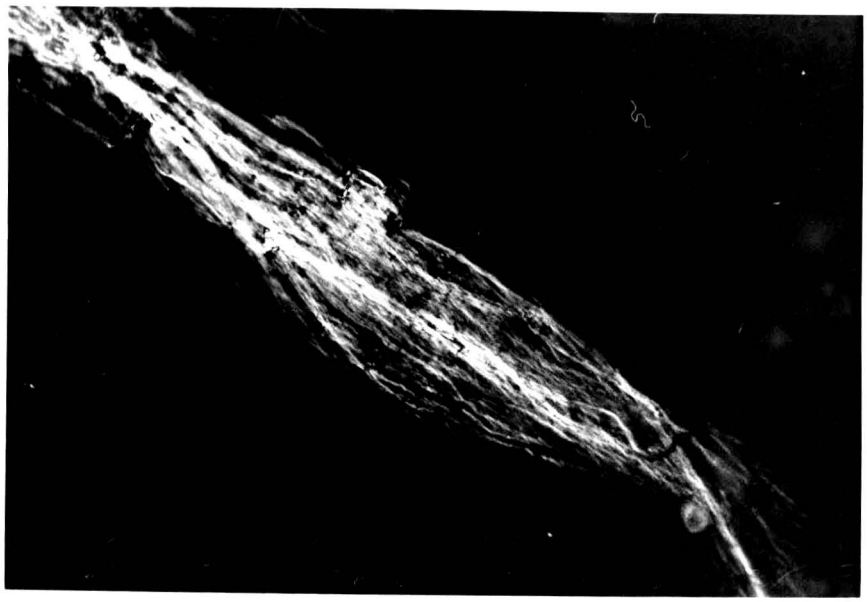
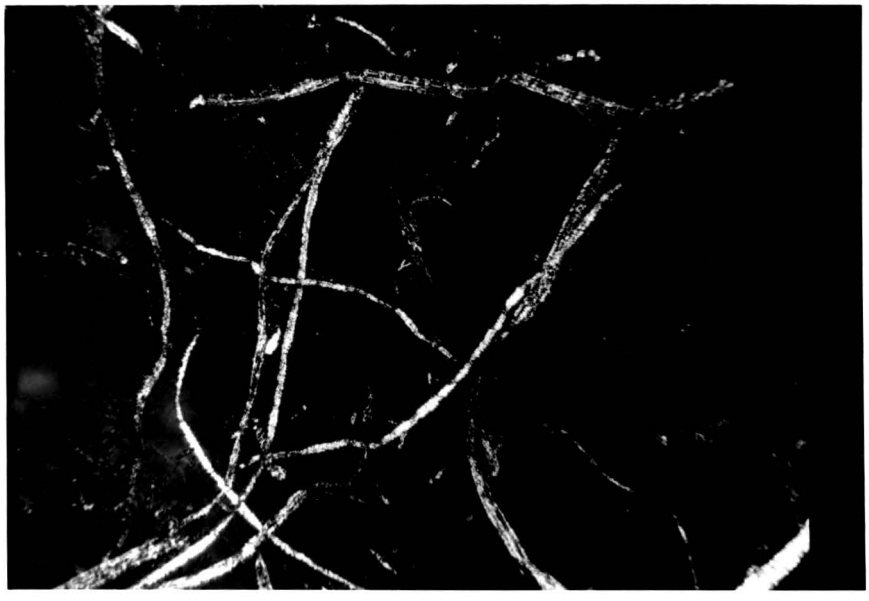


FIGURE 3

PHOTOMICROGRAPHES OF

SOFTWOODS FIBERS AT 32X



can see the bacterial cellulose looks similar to a well refined fiber which has a large surface area for bonding.

Growth on Different Mediums

Table II summarizes the results of this portion of the study. An XX indicates growth occurred for that particular growth medium composition. Both the kraft and NSSC effluents had growths and therefore it appears they do have a high enough carbohydrate content to support growth of the bacterial cellulose. Although the sulfite effluent did not show any growth, the bacterial cellulose on the kraft and NSSC effluents indictes that total mill effluents from integrated mills have a high enough carbohydrate content for Acetobacter xylinum to synthesize cellulose.

When alfalfa extract was substituted for yeast extract bacterial cellulose was found in all of the effluent growth mediums, therefore, the alfalfa extract must have supplied the Acetobacter xylinum with vitamins, amino acids and trace elements as well as a carbohydrate source. This can be explained from the fact that 3-5% of alfalfa is souble carbohydrates and must have been extracted by the .01 NHCl.

Even though this study was not concerned with the economics of the production of bacterial cellulose, the substitution of alfalfa extract for yeast extract does represent a substantial savings on the cost of the growth medium. Yeast extract costs approximately 10.50/lb. while alfalfa extract costs only .25/lb if a .30% conversion from alfalfa to alfalfa extract is used.

TABLE II

Results of Growth Mediums Substitutions

Everything Sterilized

	Glucose	Kraft Effluent	Sulfite Effluent	NSSC Effluent	Board Effluent	Fine Papers Effluent
Yeast Extract	XX	XX		XX		
Alfalfa Extract	XX	XX	XX	XX	XX	XX

XX - indicates growth occurred

Everything Sterilized, Except Effluent

	Kraft Effluent	Sulfite Effluent	NSSC Effluent	Board Effluent	Fine Papers Effluent
Yeast Extract					
Alfalfa Extract					

Finally, Table II shows no bacterial cellulose was found on the growth mediums where the effluents were not sterilized. However, other microgranism did flourish on these growth mediums. These two facts indicate that Acetobacter xylinum is not a very fast growing bacteria and cannot compete against other microorganism for the food source. Therefore it appears the growth medium must be sterilized before Acetobacter xylinum can grow and synthesize cellulose.

Evaluation of Bacterial Cellulose as a Papermaking Material

Although no major difficulties were encountered in making and testing the bacterial cellulose handsheets, the handsheets at high percentages (10%) of bacterial cellulose did drain very slowly. This was evidenced by the changes that were made in the usual Noble and Wood procedure for making handsheets in order for the sheets to drain at all. The fine bacterial cellulose fibers immediately plugged the screen and slowed down any further drainage.

The plugging of the screen could be seen as the sheets were pulled off of the screen. This plugging forced the screens to be cleaned much sooner than usual.

Figures 4-10 show the effect of bacterial cellulose on the strength properties of paper. The strength of parameter was plotted along the y-axis while increasing percentages of bacterial cellulose were plotted along the x-axis. Also on each respective figure are the beater curves. They are plots of the strength parameter verus decreasing Canadian Standard Freeness which is a reflection of increased refining time. It should be

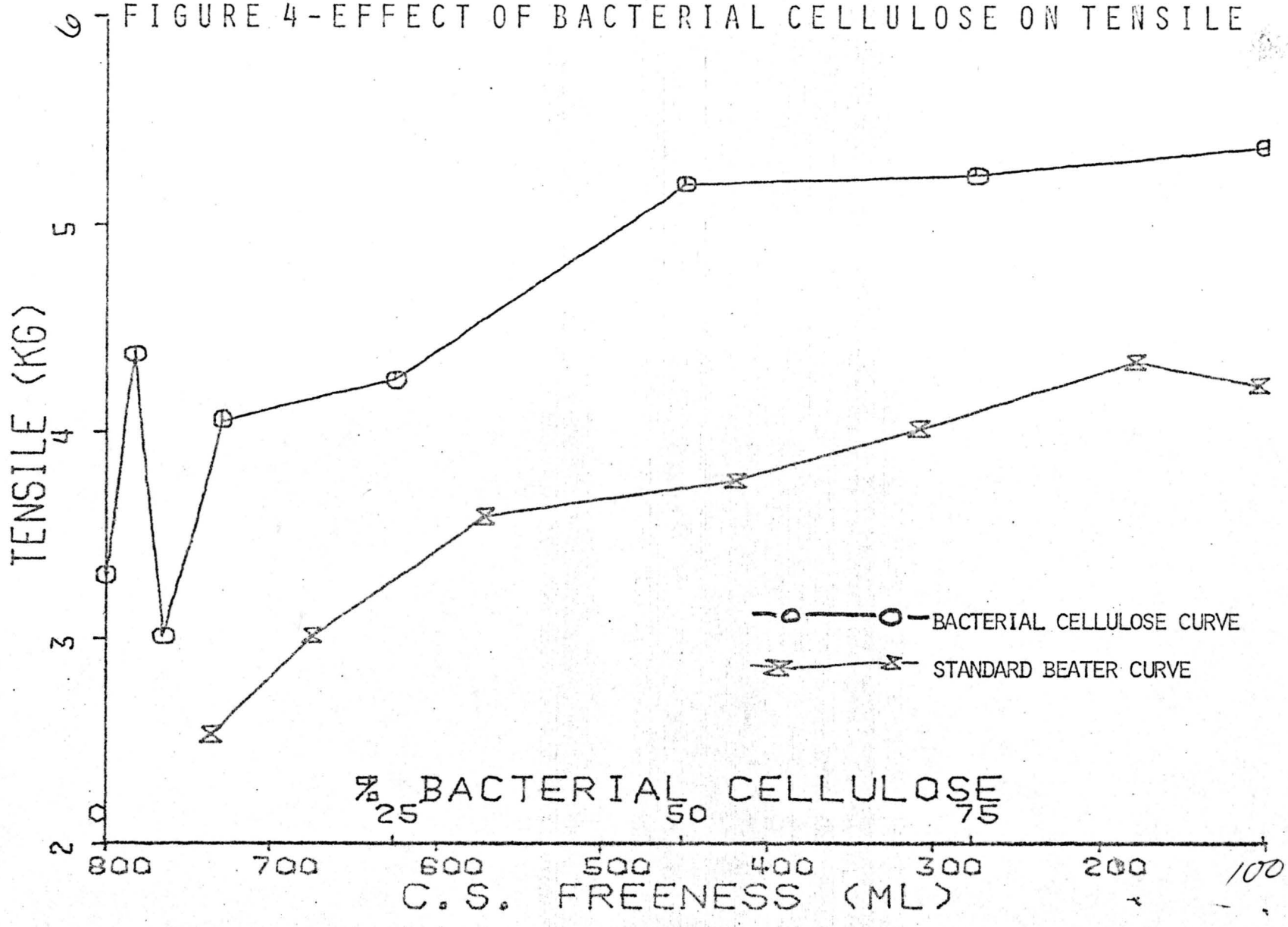


FIGURE 4-EFFECT OF BACTERIAL CELLULOSE ON TENSILE
TENSILE (KG)
6
5
4
3
2
BACTERIAL CELLULOSE CURVE
STANDARD BEATER CURVE
% BACTERIAL CELLULOSE
25
50
75
800
700
600
500
400
300
200
100
C.S. FREENESS (ML)

FIGURE 5-EFFECT OF BACTERIAL CELLULOSE ON TENSILE FACTOR

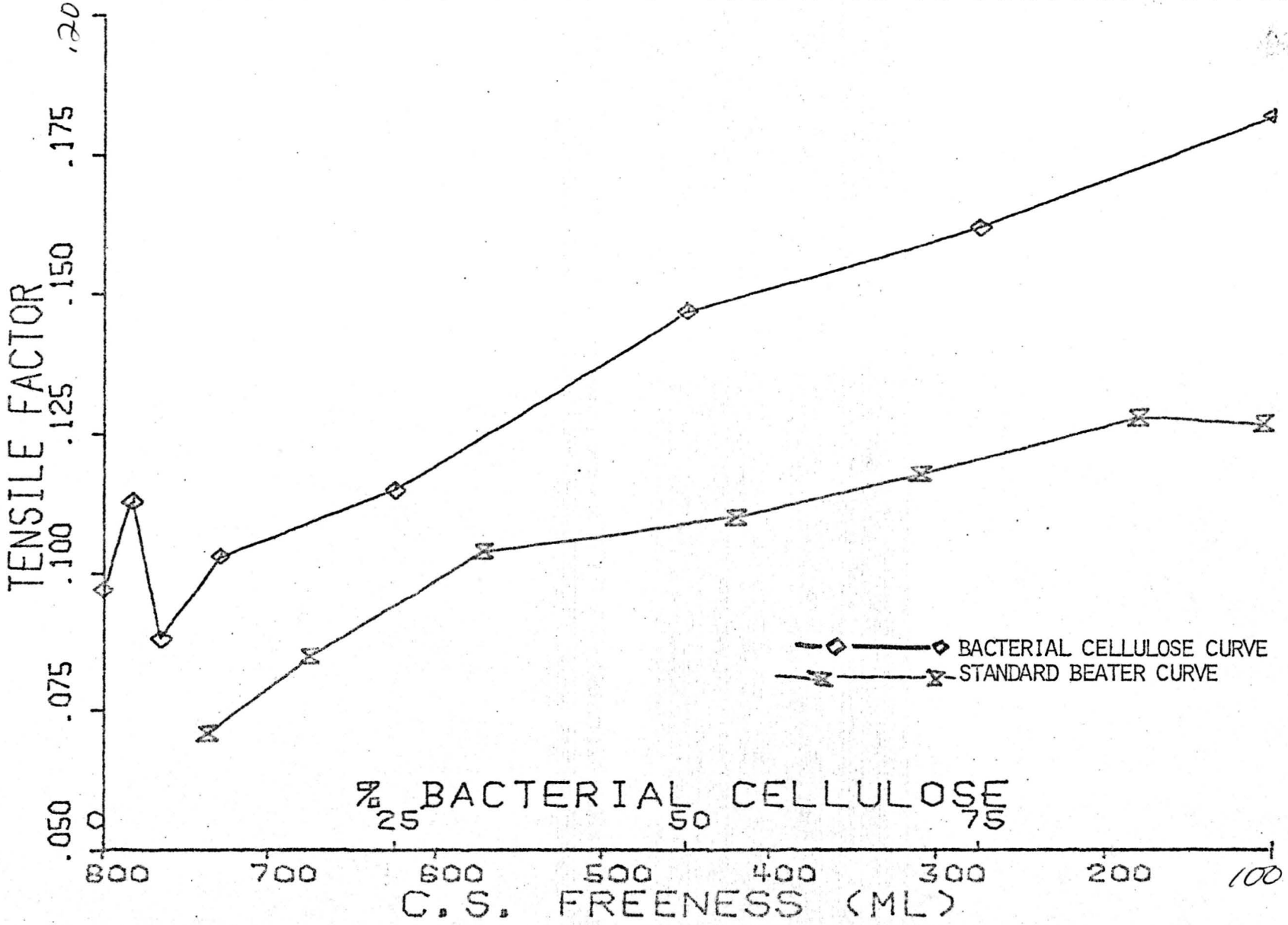


FIGURE 6- EFFECT OF BACTERIAL CELLULOSE ON MULLEN

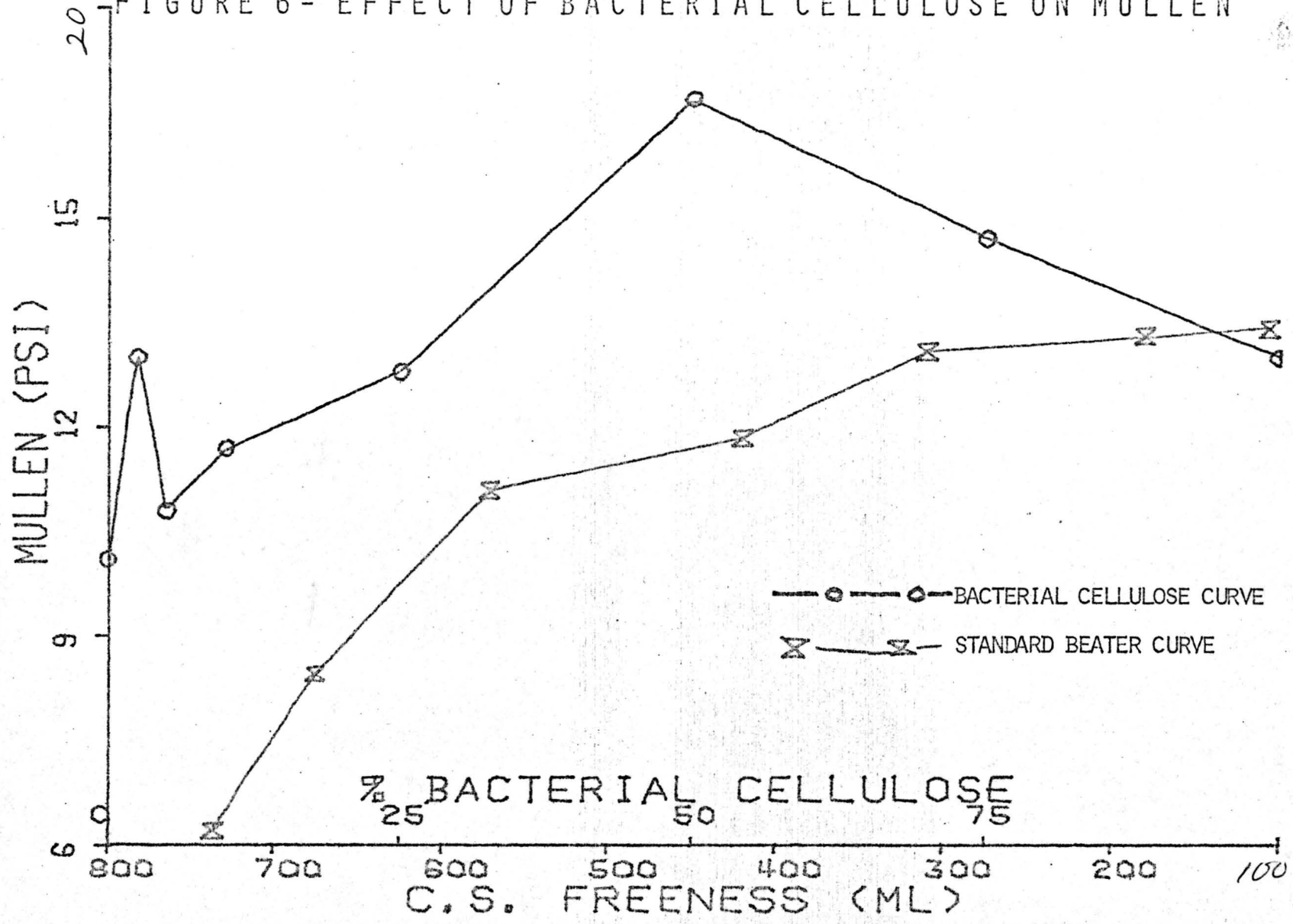


FIGURE 7 - EFFECT OF BACTERIAL CELLULOSE ON FOLD

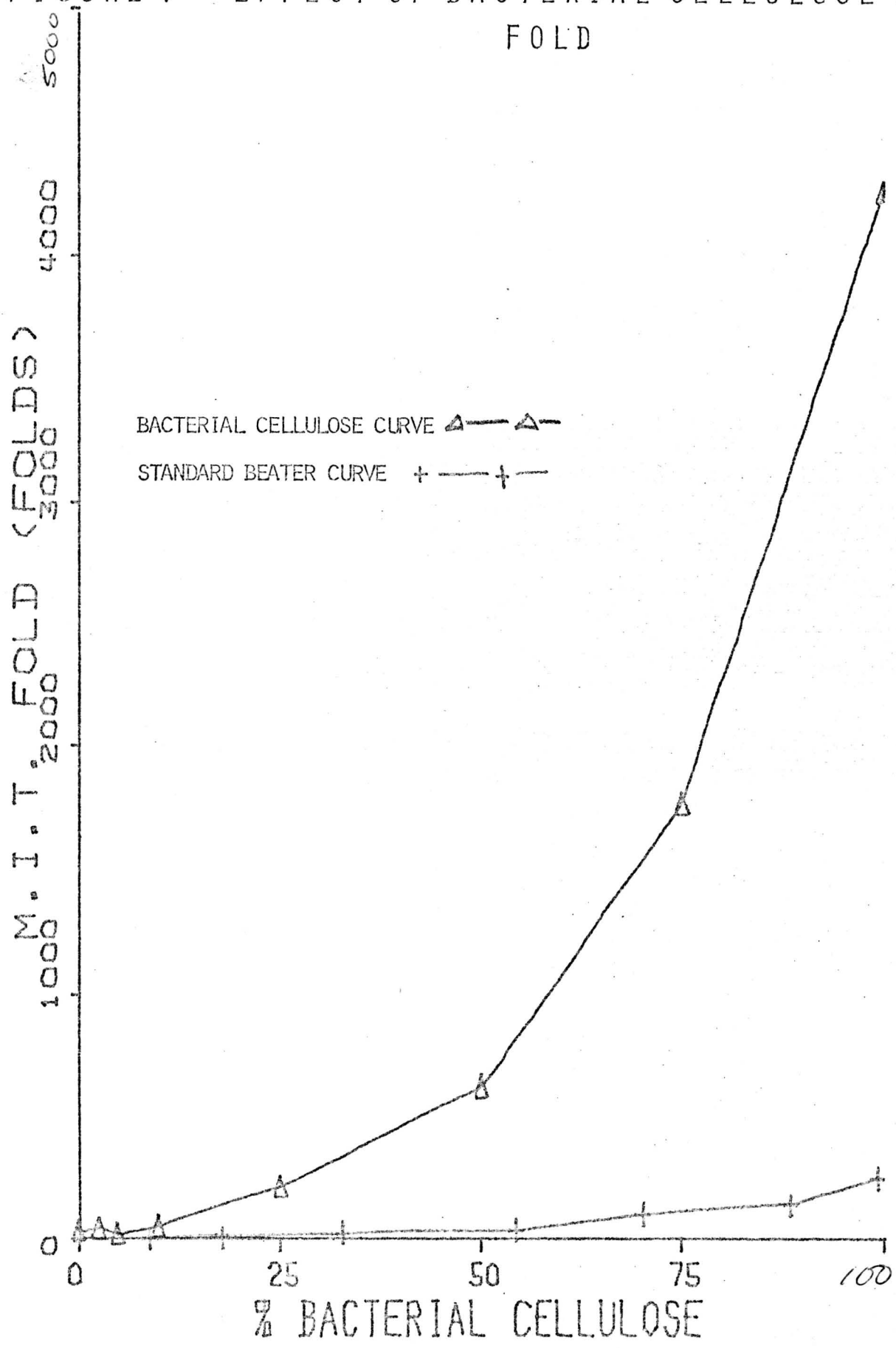


FIGURE 8 - EFFECT OF BACTERIAL CELLULOSE ON TEAR

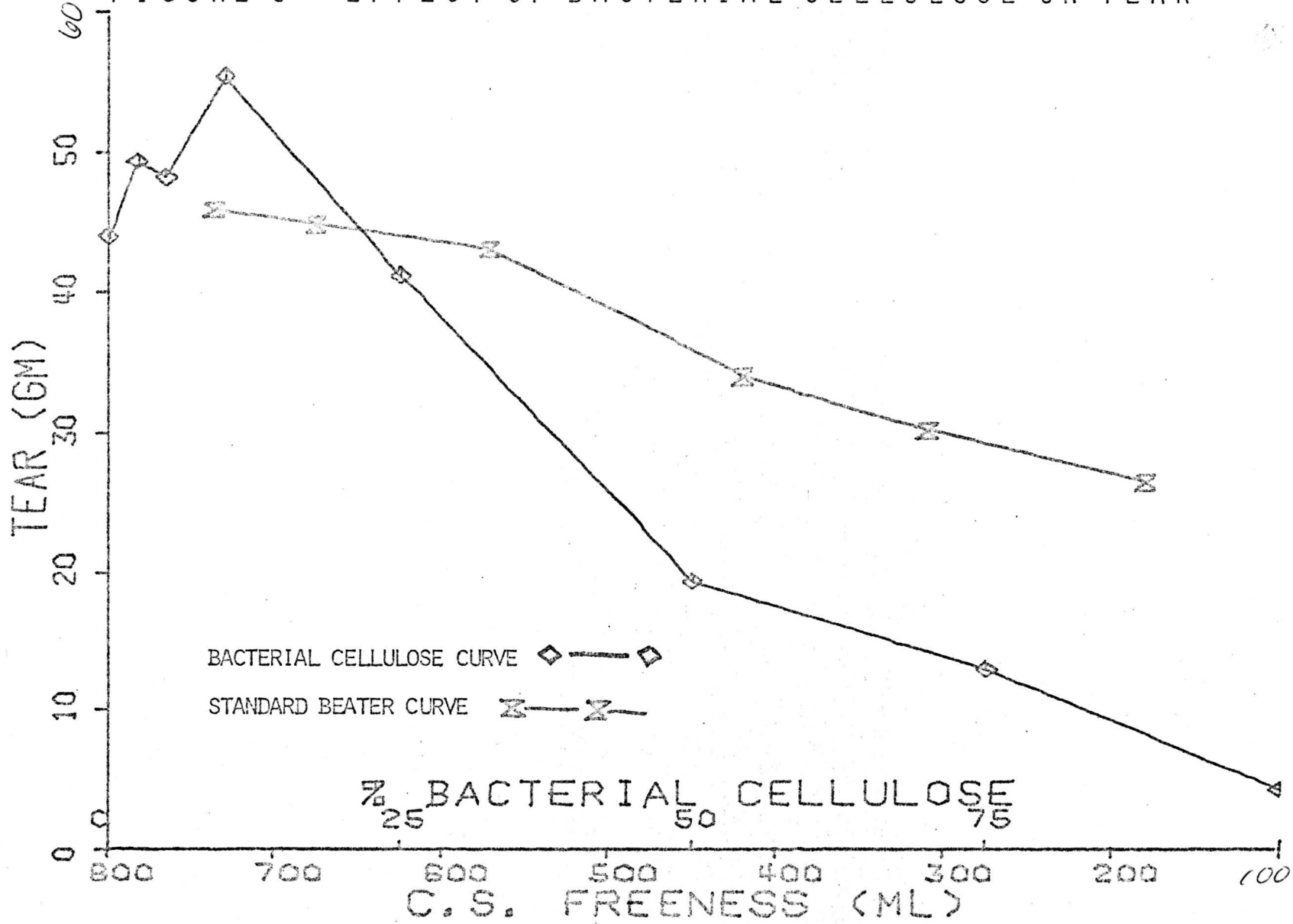


FIGURE 9 - EFFECT OF BACTERIAL CELLULOSE ON TEA

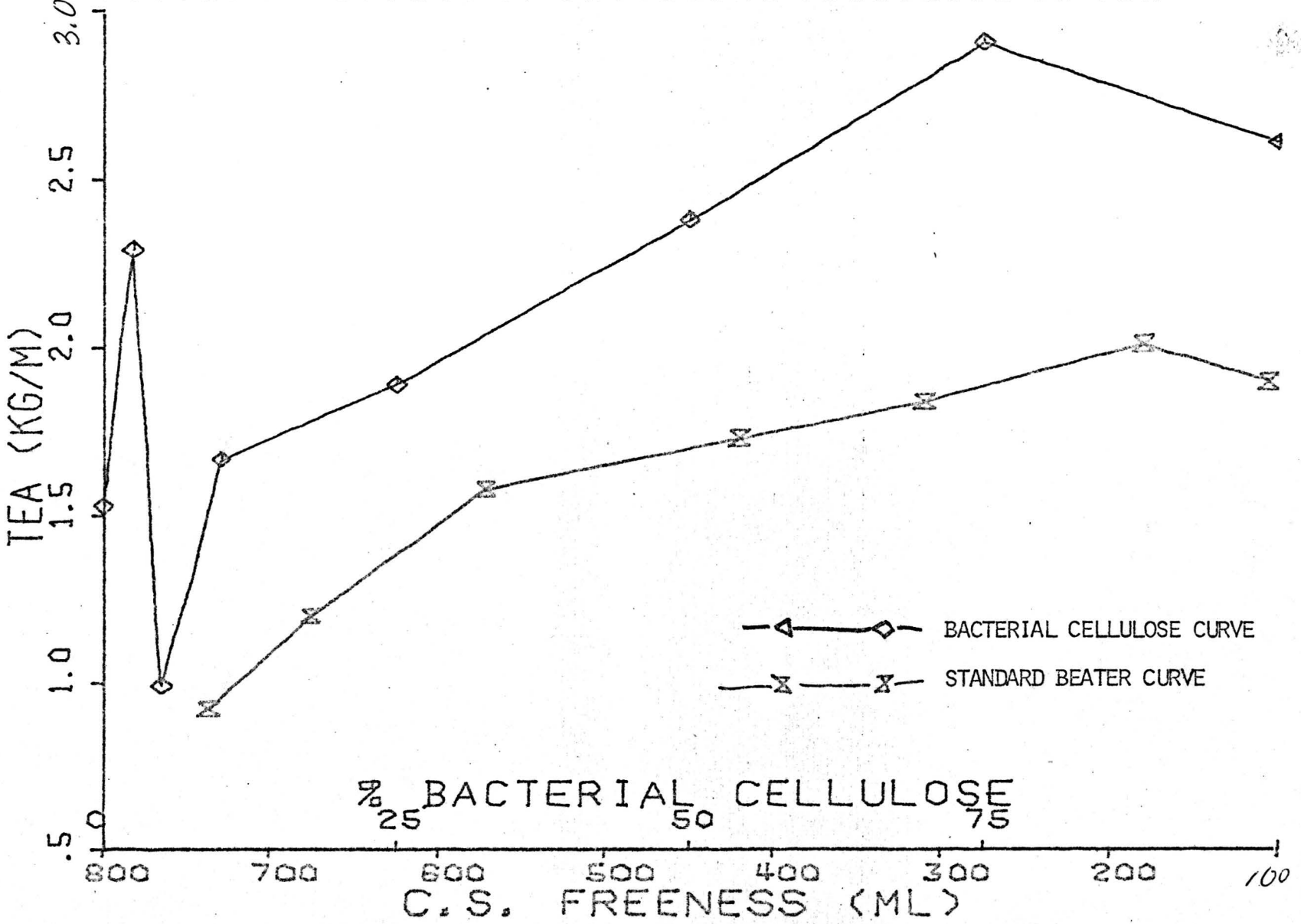


FIGURE 10- EFFECT OF BACTERIAL CELLULOSE ON STRETCH

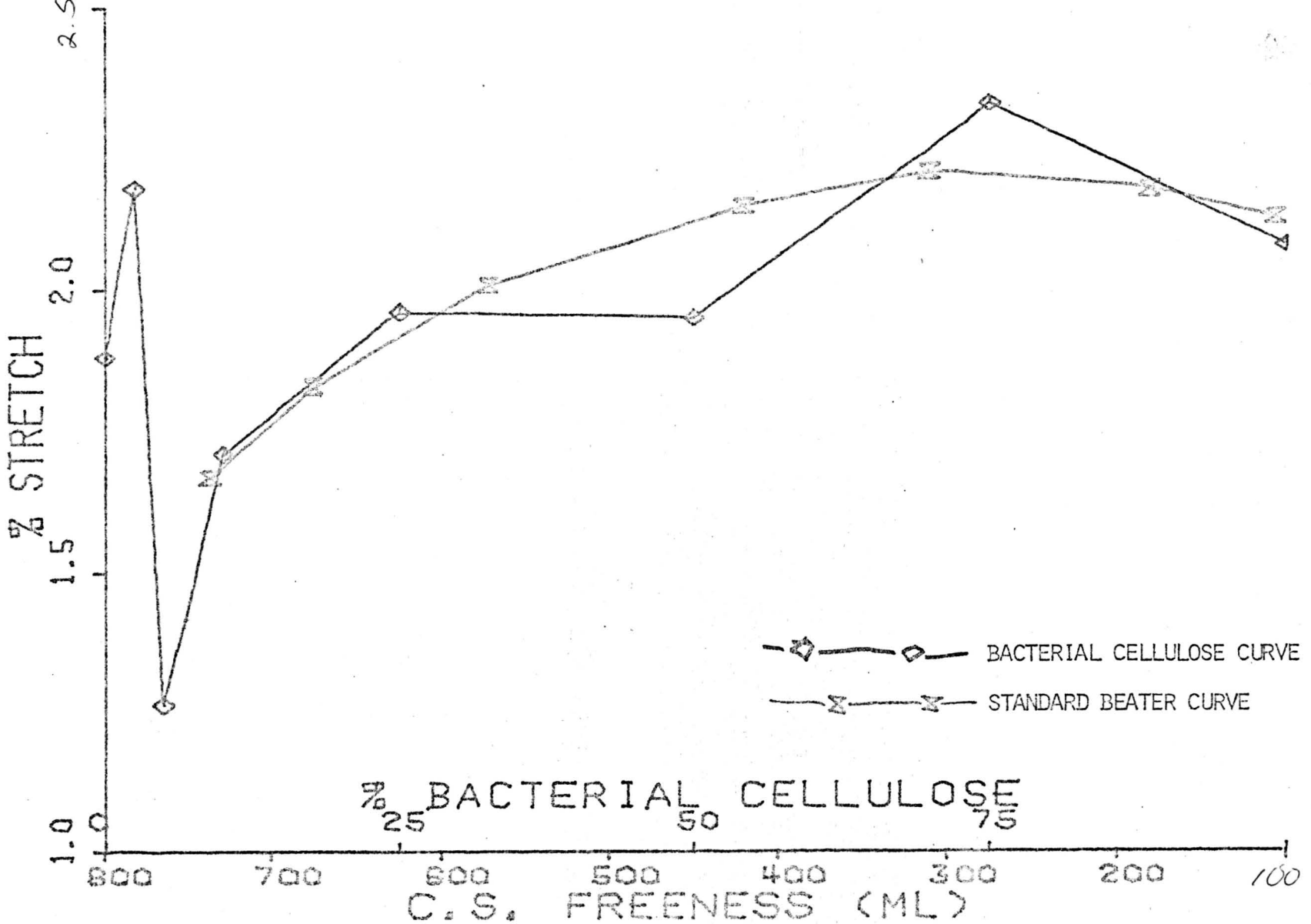


TABLE III

EFFECT OF BACTERIAL CELLULOSE ON FORMATION

% of Bacterial Cellulose	Formation
0	5
2.5	5
5	1-2
10	1-2
25	1-2
50	2.5-3.5
75	4.8-5.0
100	4.8-5.0

5 - good formation

1 - poor formation

noted that in every case except stretch the beater curve is the lower of the two curves.

To evaluate the strength properties of bacterial cellulose, the 100% bacterial cellulose sheet should be compared to the beater curve. In doing so the bacterial cellulose was found to have sheet with greater tensile and TEA. The mullen and stretch were about the same for both fibers. The fold was vastly superior to the softwood fiber while its tear was very poor. These strength properties relate fineness of the bacterial cellulose fibers as seen in the microphotographies. The tremendous surface area of the small fibers provide many surfaces for bonding to occur. These strength properties are similar to a well refined pulp, however, the bacterial cellulose clearly has a much greater tensile and fold strength.

There were certain areas of curves which seem to occur on all of the Figures 4-10. The first of these was the initial increase in strength after only 2.5% bacterial cellulose was added to the softwood fibers. In all of the tests the 2.5% bacterial cellulose sheet was stronger than the control of 100% bleached softwood kraft. Therefore, it appears that even small amounts of bacterial cellulose can improve the strength of a bleached softwood sheet.

The second trend that was found in most of the bacterial cellulose curves was the dramatic drop in strength from 2.5% to 5% bacterial cellulose and the gradual upswing back to the higher strength values. The drop in strength was caused by poor formation in the 5, 10, and 25 percent bacterial cellulose sheets. This poor formation can be seen in the samples

attached and from the results in Table III. The values in Table III are from an arbitrary formation comparison which assigns high values for good formation and low values for poor formation. The flocculation of the fibers occurred when the two pulp slurries were mixed together before put in the sheet mold. At the lower and higher percentages of bacterial cellulose the floccing did not take place and good formation occurred. Although it appeared that some type of charge attraction may be causing the flocculation, no explanation can be offered at this time as to why it occurs.

The final trend which seemed to prevail among the curves on Figures 4-10 were the way the bacterial cellulose curve stayed fairly parallel to the beater curve. This suggests that bacterial cellulose behaves similarly to other fibers which are currently being used for papermaking. Increasing the percentage of bacterial cellulose acted in the same way as more refining to the softwood fiber would help in improving the strength of a sheet of paper.

The last trend is important from the standpoint of using bacterial cellulose in the future as a papermaking material. It suggests that bacterial cellulose behaves much like a well refined wood fiber and therefore could possibly be used as a source of fiber by present day papermaking methods.

CONCLUSIONS

1. Acetobacter xylinum can synthesize cellulose on an enriched growth medium of 2% glucose, 2% yeast extract and .1% KH_2DO_4
2. The length and diameter of the bacterial cellulose fibers is about one-fourth that of a softwood fiber.
3. Kraft and NSSC total mill effluents can substitute for glucose as a carbohydrate source for Acetobacter xylinum.
4. Alfalfa extract is a better source of vitmins, amino acids and trace elements for Acetobacter xylinum than yeast extract.
5. Acetobacter xylinum cannot biosynthesize cellulose on unsterilized growth medium.
6. Bacterial cellulose fibers drain very slowly because of the plugging action caused by the smallest of the fiber.
7. Bacterial cellulose has better tensile, TEA, mullen and vastly superior fold to a well refined bleached softwood kraft sheet, but has poor tear compared to the latter.
8. Bacterial cellulose has physical properties very similar to a well refined wood fiber.
9. Poor formation occurs in handsheets with 5-25% bacterial cellulose.

SUGGESTIONS FOR FURTHER WORK

1. In depth study of growth parameters for Acetobacter xylinum to optimized synthesis of bacterial cellulose.
2. Expanded study of growth on total mill effluents. This would include such things as concentrating the effluent, adding small portions of weak black liquor to increase carbohydrate content or looking at different methods of sterilizing the effluent.
3. Work with wet end additives to help improve formation of sheets in the 5-50% bacterial cellulose range.
4. Look at methods of breaking up the bacterial cellulose pellicles, to see if different methods alter the fiber structure.
5. Look at methods of washing the growth medium out of the bacterial cellulose after the pellicles have been broken up.
6. Seed the Acetobacter xylinum with different types of fibers to see if the fiber dimensions of bacterial cellulose could be improved.

LITERATURE CITATIONS

1. Bartnicki-Garia, S., Ann. Rev. Microbiology 22:87 (1968).

2. Colvin, J.R. and Dennis, D.T., Canadian J. Microbiology 10:763 (1964).

3. Colvin, J.R., J. Polymer Science 4:747 (1966).

4. Muhlethaler, K., Biochem Biophys Acta 3:527 (1949).

5. Hestrin, S., and Schram, M., Biochemical J. 58:345 (1954).

6. Adams, G.A., and Bishop, C.T., Tappi 38:672 (1955).

7. Colvin, Bayley, and Beer, Biochem Biophys Acta 23:652 (1957).

8. Hestrin, S., and Schram, M., Biochemical J. 58:345 (1954).

9. Colvin, J.R., and Webb, T.E., Canadian J. Microbiology 10:11 (1964).

10. Brown, A.M., J. Polymer Science 59:155 (1962).

11. Ohad, I., Danon, D., and Hestrin, S., J. Cellular Biology 12(1):31 (1962).

12. Dillinghan, E.O., Jose, A.G., and Kunth, A., Bacteriology Proc. 14:67 (1961).

13. Gascoigne, J.A., Chemistry and Ind. 1580)1963).

14. Brown, A.M., J. Polymer Science 59:155 (1962).

15. Colvin, J.R., and Millman, B., Canadian J. Microbiology 7:383 (1961).

16. Colvin, J.R., and Beer, M., Canadian J. Microbiology 6:631 (1960).

17. Colvin, Bayley, and Beer, Biochem Biophys Acta 23:652 (1957).

18. Nieduszynski, I., and Preston, R.D., Nature 225:273 (1970).

19. Colvin, J.R., Canadian J. Microbiology 12(2):909 (1966).

20. Colvin, J.R., J. Cellular Biology 17:105 (1963).

21. Ohad, I., Danon, O., and Hestrin, S., J. Cellular Biology 12(1):31 (1962).

22. Colvin, J.R., and Beer, M., Canadian J. Microbiology 6:631 (1960).

23. Muhlethaler, K., Biochem Biophys Acta 3:527 (1949).

24. Colvin, J.R., J. Polymer Science 4:747 (1966).

25. Ohad, I., Bulletin of Research Council of Israel Sec. A. No. 4: 279 (1963).

26. Tsai, A., "Acetic Acid Bacteria", Tokyo, University of Tokyo Press (1968).

27. Fulmer, Bantz, and Underkofler, Iowa State College J. Science 18:369 (1944).

APPENDIX I

Data for Figures 4-10

A. Bacterial Cellulose Sheets

	% Bacterial Cellulose							
	0	2.5	5	10	25	50	75	100
Basis Weight (gm/M^2)	34	38.6	34.2	39.3	36.9	35.2	32.2	29.5
Caliper (.001 in.)	4.7	5.1	4.8	5.0	5.1	5.0	4.3	4.3
Tensile (Kg)	3.31	4.37	3.01	4.06	4.25	5.19	5.23	5.37
Tensile Factor (M^{-2})	.097	.113	.088	.103	.115	.147	.162	.182
Burst (psi)	10.1	13.0	10.8	11.7	12.8	16.7	14.7	13.0
Fold (folds)	33	43	24	57	215	619	1769	4254
Tear (gm)	44	49.4	48.2	55.4	41.2	19.3	13	4.2
TEA (Kg/m)	1.5	2.3	1.0	1.7	1.9	2.4	2.9	2.6
% Stretch (%)	1.9	2.2	1.25	1.7	1.95	1.95	2.3	2.1

Data for Figures 4-10

B. Beater Curve Sheets

	Canadian Standard Freeness (ml)						
	737	675	572	420	310	180	105
Basis Weight (gm/M^2)	35.6	35.6	34.6	34.1	33.9	33.8	33.1
Caliper (.001 in.)	-	-	-	-	-	-	-
Tensile (Kg)	2.53	3.01	3.59	3.76	4.01	4.33	4.22
Tensile Factor (M^{-2})	.071	.085	.104	.110	.118	.128	.127
Burst (psi)	6.2	8.4	11.1	11.8	13.1	13.3	13.4
Fold (folds)	3.4	8.6	27	37	101	139	250
Tear (gm)	45.9	44.8	43	34	30	26.5	33
TEA (Kg/m)	.92	1.2	1.6	1.7	1.8	2.0	1.9
% Stretch (5)	1.7	1.8	2.0	2.15	2.2	2.2	2.1