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MEASUREMENT OF THE DEGREE OF CELLULOSE
DEGRADATION CAUSED BY FUNGI.

A SENIOR THESIS.

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AND PAPER TECHNOLOGY,
WESTERN MICHIGAN COLLEGE,
JUNE 6, 1955

SUBMITTED BY

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ABSTRACT

The objective of this thesis is to obtain a method for determining the degree of cellulose degradation, caused by fungal attack, using testing methods common to the paper industry rather than complex biochemical testing procedures.

LITERATURE SURVEY

INTRODUCTION.

During World War II the necessity of protecting military supplies made it necessary that much research be done on microbiological attack of paper. A large quantity of the materials sent to the South Pacific was wrapped in paper. The high temperature and humidity of the South Pacific readily supported the growth of micro-organisms. Consequently many supplies were damaged because their wrappings were destroyed by microbiological attack.¹

Much work has been done since that time in regard to preventing microbiological attack of paper. This work has been handicapped by the lack of a quantitative method for the comparison of the microbiological decomposition of paper. Many visual evaluations have been used, along with strength comparison tests, but as yet there has been no definite quantitative method of evaluation.

METHODS OF FUNGAL ATTACK AND THE PRODUCTS THAT ARE FORMED.

It is commonly believed that the decomposition of cellulose by bacteria and fungi is caused by enzymes which

these micro-organisms secrete.² In an experiment by Buchner the liquids were pressed out of a growth of fungus and the extract put on the paper. Other portions of the paper were treated with the fungus. Results of the experiment showed almost identical amounts of decomposition in both cases.

The decomposition is thought of as taking place in two stages: first the cleavage of the cellulose chain into sugars, then the fermentation of the sugars to aliphatic acids. At the present time not much is known as to the products formed.

METHODS FOR TESTING AND TYPES OF EVALUATION.

Tappi recently introduced a standard entitled "Fungus Resistance of Paper and Paperboard."³ The sample of paper or paperboard is inoculated with a test organism, after which the incubation period begins. The reporting of the test results is as follows. If the sample supports growth of the organism before the end of one week's incubation, the paper is reported as not being fungus resistant. If there is sparse growth after two week's incubation, the paper is reported as moderately fungus resistant. If no growth is present at the end of two week's incubation, the

paper is reported as fungus resistant.

A more accurate evaluation would seem to be desirable for this test. The method of reporting doesn't distinguish small differences in resistance. Two samples could have the same classification of resistance, and yet they could vary in individual resistance. If it was necessary to measure small differences in resistance, this method could not be used.

Shema used the "penetration method" for the testing of fungus resistance.⁴ This procedure involved cutting out the center of the sample and inoculating the sample with a test organism. Additional layers of the sample were placed over the inoculated area, and the whole sample was incubated. Observations were made at four, seven, fourteen and twenty-one days, and the amount of growth that had penetrated through the layers was reported as none, slight, moderate or heavy.

It was further shown that the porosity of the sheet was directly proportional to the penetration time. It was impossible to take the porosity on the exposed sample, as it was too badly decomposed. This points out the drawbacks of physical testing, the sample is often so decomposed that

it is impossible to run a test on it.

A procedure to give a numerical value to the amount of fungal degradation in textiles has been used by Great-house, Klemmie, and Barker.⁵ The tensile strength of the fibers was taken before and after the fungal attack. The results were interpreted as the difference between the two tensile strengths, which would be the strength loss. A ratio of the two strengths could be calculated, or the percentage of strength lost could be determined. Some of the fibers were destroyed making it impossible for the tensile test to be run.

Work was done at the Institute of Paper Chemistry, on the determination of the bacterial degradation of woolen fibers.⁶ It is believed that some adaptation of this procedure could be applied using fungi and cellulose. The fibers that underwent decomposition were dyed with a 1% solution of safranin. When viewed under a microscope it was noted that the dye had penetrated through the broken portions of the cell wall of the fibers. The heavier the attack on the fiber, the more dye there was absorbed. There was, however, no way quantitatively to determine the amount of dye absorbed by the fiber.

In the work at Western Michigan College by H. Parker, two methods of evaluation were used.⁷

One method was a strength comparison of the treated and untreated samples. The paper was tested with the Mullen, tear, and tensile testers, under the Tappi Standard Testing Procedures. The paper was inoculated with a mixture of four fungi which are known to attack paper. The paper was incubated for seven days after which it was again tested with the Mullen, tear, and tensile testers. The results were interpreted as being the percentage of strength lost.

One problem encountered was that the fungi did not attack the paper uniformly. In some cases the fungi completely destroyed portions of the paper, while leaving other areas untouched. This presents a problem that is often encountered when running strength tests, inasmuch as an overall average of the degradation can not be obtained. If strength tests were going to be run, the paper would have to be uniformly attacked by the fungi. This does not happen, however.

The other procedure for evaluation was visual observation. The samples were inoculated with the same mixture of fungi, and incubated. The samples were observed during

the two week's incubation and reported as follows:

1. No growth after fourteen days incubation.
2. Very slight growth, observed with a hand lens.
3. Light growth, up to 25% of the total surface area.
4. Moderate growth, 25-75% of the surface area.
5. Heavy growth, covering over 75% of the surface.
6. Heavey growth with much sporulation.

Almost all of the work done in this field has used visual methods for evaluation. The commonest method involves estimating the area covered by fungal growth in a given number of days.

New methods are now being devised in which the products of the decomposition of paper are quantatively measured.⁸

A method similar to this, using enzymes on cotton fibers, has been tried with success. Samples of the fibers were taken at different stages of incubation, and the products that had been formed were analyzed. From this it was possible to tell how far the fibers had gone toward decomposing. This procedure is very time consuming and complex. Little has been done in this line of research.

CONTROLS OF TESTS BEING RUN.

It has been found when running the above tests, or ones similar to these, that several factors must be controlled in order to get accurate results. The amount of agar in the nutrient and the pH of it must always be kept constant.⁹ Also each fungus has a pH range in which it can obtain its maximum growth.¹⁰

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EXPERIMENTAL OUTLINE

TYPE OF PAPER TO BE USED.

A ordinary kraft wrapping paper, 52 lb. basis wt., 24 X 36 - 500. Forty samples, 3" by 6", are to be used.

ORGANISMS TO BE USED.

A mixture of Chaetomerium globosium, Aspergillus niger, and Aspergillus terreus, all of which are known cellulose destroyers, are to be used.

NUTRIENT MEDIA.

Potato dextrose agar, to be used for growing the test organisms.

Mineral salt agar, to be used during the incubation period.

COMPOSITION

Amonium nitrate	3.0 g.
Dipotassium nitrate	1.0 g.
Potassium chloride	.25g.
Magnesium sulphate	.25g.
Agar	10.0 g.
Distilled water	1,000.0ml.

pH 6.5 to 6.8

PREPARATION OF THE INOCULUM.

30 g. of wheat bran is mixed with 30 g. of 0.1 N HCl and sterilized for 30 minutes at 15 p.s.i. 10 ml. portions of the test organisms are mixed into the bran suspension. The mixture is incubated for 48 hours, then the clumps of bran are broken up and the incubation continues until the fungi have sporulated throughout the wheat bran. The bran is dried and stored in a air tight container.

METHOD OF INOCULATION.

A large mouth jar containg the wheat bran and fungi is throughly shaken up and the sample of paper is immediately inserted into the mouth of the jar for 10 seconds. The sample is then ready for incubation.

METHOD OF INCUBATION.

500 cc. of the mineral salt agar is put into each of five trays, 14" by 17", that contain eight samples apiece. They are incubated for one week at 28°C. and a R.H. of 85-95%.

TESTING PROCEDURE.

After the incubation period is over, the samples will be washed off with distilled water, dried, and weighed. After

the weighing the samples are to be disintegrated in a Waring blender for 5 minutes. Hand sheets are to be made by Tappi Standard T 205m-53, and the following tests will be run:

Zero span tensile test, Tappi Standard T 481sm-52.

Tensile breaking strength, Tappi Standard T 404m-47.

Bursting strength, Tappi Standard T 403m-53.

Internal tearing resistance, Tappi Standard T 414m-42.

Alpha cellulose test, Tappi Standard T 203m-44.

1% caustic soda solubility, Tappi Standard T 212m-44.

METHOD OF REPORTING.

All of the above tests will also be run on the untested paper and a comparison of the results from the tested and untested paper will be made. In addition the weights of samples, before and after testing, will be used for comparison.

EXPERIMENTAL WORK.

The experimental procedure follows the experimental outline, given previously, with these modifications: The period of incubation was changed from seven to ten days. The weight loss of the samples wasn't used.

PROCEDURE.

Forty samples, 3" by 5", of the test paper were inoculated by the spore cloud method. A large jar, containing a mixture of finely ground dried wheat bran and the mixture of dried fungi, was shaken to produce a cloud of dust. A sample of the paper was inserted in the mouth of the jar and held there for ten seconds.

The inoculated samples were placed in the nutrient media and incubated at 28° C. and a R.H. of 85-95%.

At the end of the incubation period the samples were placed in hot water, 80-90° C. for five minutes, to aid in the removal of the nutrient remaining on the sample.

After further washing with tap water to remove the remaining particles of nutrient and fungi, the paper was disintegrated in a Waring Blender for 30 seconds, after which hand sheets were made by Tappi Standard T205m-53.

Mullen, tear, tensile, and zero span tensile, were run, and the data recorded in Table II. The results for these tests in Table II are not the actual readings, but factors, corrected to the standard basis wt., as given in Tappi Standard T205m-53.

The alpha cellulose and the one percent caustic soda tests were run, the results of these appear in Tables III, and IV.

Another sample, to be used as a standard, of the test paper was treated exactly in the same manner, except it was not inoculated with the fungi mixture. The results of the tests upon these samples are in Table I. The values obtained are also factors corrected for a constant basis wt. The alpha cellulose and one percent caustic soda tests were also run. The results are in Tables III, and IV.

EVALUATION OF THE DATA.

A comparison of the results of Tables I, II, III, and IV, relates the following information:

Mullen. The average value for the exposed samples was found to be 2.47 lbs. per sq. inch less than the average for the standard samples. The exposed sample has only 81.5% of the original bursting strength left.

Tensile. The tensile of the exposed sample shows a loss of 1.335 lbs. per inch from the original sample. It has only 77.9% of the original tensile strength left.

Tear. The exposed portion lost 34.39 grams resistance as compared to the standard sample. There is only 43.4% of the original tearing resistance now remaining.

Zero span tensile. A loss of 5.84 lbs. per inch upon exposure. 59.1% of the original zero span tensile strength remaining.

Alpha cellulose test. The exposed portion exhibited a loss of 3.3%, as compared to the standard. The sample still contained 96.7% of its original alpha cellulose content.

One percent caustic soda test. Shows a increase upon exposure of 4.65% in the amount of extractives. The exposed sample is now 163% higher than before exposure.

SUMMARY OF EXPERIMENTAL RESULTS.

All of the physical tests run on the paper gave losses in strength upon exposure to fungal attack. The greatest loss of strength appeared in the tearing resistance, followed by the zero span tensile test, the tensile test, and with the mullen test showing the smallest loss. This would indicate there is a breakdown in the fiber.

The alpha cellulose test gave a very small change in results, thus indicating that it is not the alpha cellulose the fungi attack.

In the one percent caustic soda test a substantial difference in content was noted. There was a definite increase in the amount of solubles present. It could, therefore be assumed that the products of degradation are mostly all in the extractive portion of the cellulose fiber.

CONCLUSION.

It would seem possible to relate the amount of cellulose degradation by fungi by using papermaking tests. The physical tests by which the best results were obtained, are the tearing resistance test and the zero span tensile test.

For chemical tests the one percent caustic soda test gave very favorable results. It seems there is a direct relation between degradation and the amount of extractives.

RECOMMENDATIONS FOR FURTHER EXPERIMENTAL WORK.

It would seem desirable to run test while exposing the paper for different lengths of time. In this way a curve for degradation might be obtained. More chemical tests should be run to learn what portion of the cellulose the fungi attack.

Original Strength Characteristics.

Individual Test Results.

<u>No.</u>	<u>Wt.</u>	<u>Basis Wt.</u>	<u>Mullen</u>	<u>Tensile</u>	<u>Tear</u>	<u>Zero Span.</u>
1.	1.17	41.6	13.9	6.53	-	12.1
2.	1.15	40.9	12.8	5.04	-	15.2
3.	1.16	41.3	12.9	5.61	-	12.8
4.	1.22	43.4	13.3	4.98	48.3	14.9
5.	1.19	42.3	12.6	5.42	-	13.1
6.	1.48	51.7	12.8	5.23	-	16.1
7.	1.21	43.1	14.1	6.29	-	13.5
8.	1.21	43.1	13.2	5.73	65.7	14.1
9.	1.16	41.3	13.2	4.68	-	14.9
10.	1.23	43.8	12.0	6.02	-	13.8
11.	1.21	43.1	14.4	5.87	-	15.4
12.	1.25	44.5	12.3	5.56	60.1	15.4
13.	1.23	43.8	13.2	5.29	-	14.2
14.	1.19	42.3	14.1	6.02	-	14.6
15.	1.18	42.0	13.2	5.21	-	14.7
16.	1.11	39.5	15.1	5.67	-	14.3
17.	1.20	42.7	12.0	5.30	68.9	14.2
	Average values.		13.24	5.565	60.75	14.32

Strength Characteristics of Exposed Paper.

Individual Test Results.

<u>No.</u>	<u>Wt.</u>	<u>Basis Wt.</u>	<u>Mullen</u>	<u>Tensile</u>	<u>Tear</u>	<u>Zero Span</u>
1.	1.23	43.8	12.2	4.05	-	8.35
2.	1.25	44.5	12.0	4.99	-	8.04
3.	1.16	41.3	10.1	4.55	-	10.75
4.	1.17	41.6	10.8	4.34	23.9	8.76
5.	1.24	44.1	10.2	4.13	-	9.10
6.	1.17	41.6	9.5	3.69	-	8.31
7.	1.20	42.7	12.3	3.95	-	7.75
8.	1.19	42.3	10.6	5.02	25.3	9.24
9.	1.24	44.1	9.9	4.14	-	8.19
10.	1.22	43.4	12.3	4.60	-	8.27
11.	1.25	44.5	10.5	4.63	-	8.46
12.	1.24	44.1	13.3	3.99	25.2	8.27
13.	1.14	40.6	10.0	4.66	-	8.31
14.	1.18	42.0	10.4	3.13	-	8.33
15.	1.17	41.6	5.4	1.72	-	7.45
16.	1.22	43.4	12.1	4.64	29.9	8.55
17.	1.18	42.0	12.7	4.53	-	8.07
18.	1.20	42.7	10.5	5.05	-	8.35
19.	1.19	42.3	10.1	4.52	27.5	8.60
Average Values.			10.77	4.23	26.36	8.48

TABLE III

Results of Alpha Cellulose Test.

T203m-44

Original paper:

Trial 1.	Trial 2.	Trial 3.
83.12%	83.53%	83.45%

Average value: 83.37%

Exposed paper:

Trial 1.	Trial 2.	Trial 3.
80.42%	80.33%	79.81%

Average value: 80.18%

This shows a loss of 3.3% in the alpha cellulose content of the exposed paper in relation to the original paper.

TABLE IV

Results of 1% Caustic Soda Solubility Test.

T212m-54

Original paper:

Trial 1.

5.77%

Trial 2.

5.85%

Average value: 5.77%

Exposed Paper:

Trial 1.

9.36%

Trial 2.

9.47%

Average value: 9.42%

There is a increase of 4.65% in the exposed paper from the original paper.

PHOTOGRAPH OF GROWTH ON PAPER

