PROJECT REPORT FORM

PROJECT NO. 2294
COOPERATOR Institute
REPORT NO. 1
DATE Dec. 22, 1961
NOTE BOOK 2032
PAGE 13 TO 16
SIGNED John R. Peckham

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WOOD FIBER STUDIES

INTRODUCTION

A memorandum, Mathes to Peckham under date of September 7, 1961, describes the background for this work. Essentially, Dr. Mathes proposed to compare a variety of fiber isolation methods with one another and with the micropulping procedure established for Project 2057. The methods which were used are described on pages 3-12, Notebook 2032. Tests which were proposed for the isolated fibers were KAPPA number and zero-span tensile measurements on handsheets prepared from pulps beaten for 15 min. in a Jokro mill.

EXPERIMENTAL

It was determined that it would not be feasible to apply the KAPPA number* test to the samples of fiber submitted by Dr. Mathes. The method calls for ca. 50% utilization of 100 cc. of 0.1 normal potassium permanganate solution, and the pulps were so well delignified that this became impractical. A 25-cc. basis permanganate number test was substituted. These values were so low that the test was discontinued after evaluation of all pulps bearing the suffix "A". The conditions which were used in preparing the fibers and the results of tests on the handsheets are shown in Table I.

* Tappi Tentative Standard T 235 m, Tappi 42, no. 11(1959).

TABLE I

DELIGNIFICATION CONDITIONS AND PHYSICAL PROPERTIES OF PULPS

Sample	Delignification Procedure		Yield	$KMnO_4$ No.	Canadian Standard Freeness	Basis Wt., lb., 25x40/500	Apparent Density	Zero-span tensile,[b] lb./in.
1	Extracted[a] chips chlorited for 24 hr. as per conditions on p. 4-5, Notebook 2032	A	--	3.7	300	39.6	14.1	50.4
		B	--	---	115	39.9	16.6	60.6
		C	--	---	90	40.3	16.8	56.3
2	Extracted[a] chips as per Sample 1, followed by a 1.75-hr. soak in a 0.1 N NaOH soln.	A	--	2.2	440	39.9	15.3	54.3
		B	--	---	430	38.9	15.6	64.3
		C	--	---	450	38.9	15.6	61.1
3	Extracted[a] chips treated with peracetic acid and sodium acetate for 29.5 hr.	A	--	6.4	90	40.1	16.7	56.5
		B	--	---	50	39.6	16.5	63.0
4	Extracted[a] chips pretreated 24 hr. with ethanolamine, chlorited for 8 hr.	A	--	2.2	330	39.2	15.7	58.9
		B	--	---	260	39.3	15.7	58.1
		C	--	---	335	38.5	16.0	58.7
5	Extracted[a] chips pretreated 24 hr. with ethanolamine, leached in tap water for 2 hr., chlorited for 4 hr.	A	--	1.4	435	39.0	15.6	62.9
		B	--	---	440	39.2	15.1	61.0
		C	--	---	280	39.2	15.1	62.5
6	Extracted[a] chips, kraft pulped (Notebook 2032, page 16)	A	43.8	14.6	580	37.7	13.0	62.9
		B	43.4	14.2	590	38.5	13.3	65.9
		C	43.5	13.7	580	38.9	13.4	63.1
7	Unextracted chips, kraft pulped (Notebook 2032, page 16)	A	41.9	14.3	570	38.9	13.4	62.0
		B	42.2	14.2	560	38.9	13.0	62.7
		C	42.6	14.1	580	36.6	13.1	65.5

[a] Alcohol benzene.

[b] Corrected for 45-lb. basis wt.

PROJECT REPORT FORM

PROJECT NO. 2294
COOPERATOR Institute of Paper Chemist
REPORT NO. 2
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NOTE BOOK 2156
PAGE 94 TO 96
SIGNED Martin C. Mathes
Martin C. Mathes

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THE GROWTH OF LARGE AMOUNTS OF ISOLATED TISSUE AS A SOURCE OF PLANT PROTEIN

CULTURE METHODS

The tissue used in this study was isolated from triploid aspen (1) stem sections and grown in liquid cultures designated as No. 303 (2). This tissue was grown on agar medium until October 3, 1962, when the tissue was placed in liquid No. 23 (3). The tissue has been maintained in the dark at 29-30°C. on a rotary shaker (approximately 120 rpm) in liquid No. 23 medium since October 3, 1962. At intervals of 3 to 4 weeks the tissue has been sub-cultured by cutting small pieces of tissue inoculum. These pieces (2-3 mm. cubes) are then maintained in stock cultures as follows: 250 ml. Erlenmeyer, 50 ml. No. 23 medium, approximately 10 pieces tissue inoculum, 29-30°C., dark, rotary shaker (approximately 120 rpm).

EXPERIMENTAL PRODUCTION OF TISSUE

Tissue from stock cultures (No. 303) was used as the experimental material. The following table lists the results and conditions employed in the production of large amounts of tissue for protein extraction.

CULTURE NUMBER	INITIAL FRESH WEIGHT (GR)	FINAL FRESH WEIGHT (GR.)	GROWTH INDEX (5)	GROWTH GR./LITER	GROWTH GR/LITER/DAY	CONDITIONS
1	1.98	Contaminated	---	---	---	Growth period -

CULTURE NUMBER	INITIAL FRESH WEIGHT (GR.)	FINAL FRESH WEIGHT (GR.)	GROWTH INDEX (5)	GROWTH GR./LITER	GROWTH GR/LITER/DAY	CONDITIONS
2	2.20	59.1	26.9	56.9	2.4	24 days. Rotary shaker - 120 rpm., dark, 29-30°C.
3	2.32	29.9	12.9	27.6	1.2	
4	2.41	37.7	15.6	35.3	1.5	
5	1.37	38.6	28.2	37.2	1.6	
6	1.25	Contaminated	---	---	---	
7	1.84	55.9	30.4	54.1	2.3	
8	1.75	59.9	34.2	58.2	2.4	
TOTAL	15.12	281.1				

Good fragmentation (6) of the tissue was observed. A number of the smaller pieces of tissue could be used as inoculum in subsequent experiments. Tissues for protein extraction were removed from the medium after 24 days, blotted, placed in a beaker and flooded with distilled water (to prevent freeze drying). The samples were frozen, the plugs of frozen sample removed from the beakers and accumulated in the freezer until sufficient material was available.

REMOVAL OF PROTEINS FROM MEDIUM

The use of low molecular weight medium for the growth of isolated tissue is required in studies dealing with the secretion of high molecular weight materials into the medium. The use of dialysis was investigated as a means of removing the high molecular weight fraction from coconut milk. The use of "low-molecular-weight" coconut milk in No. 23 medium could provide a suitable medium for protein secretion studies.

The growth of isolated aspen tissue (1) on medium containing various combinations of dialyzed coconut milk was observed. Coconut milk (100 ml., after heating

to 60°C., cooling and filtering) was placed in the dialysis tubing and dialyzed against 800 ml. of distilled water for 40 hours (7). The water containing the materials (OUT) which passed through the tubing was used to make 1 liter of modified No. 23 medium (8) while the coconut milk (IN) inside the tubing was used at a concentration of 10% in modified No. 23 medium (9). Good tissue growth (see figure and table) and appearance was obtained on No. 23 medium and modified No. 23 (OUT), while poor growth was observed on No. 23 media in the absence of coconut

CULTURE NUMBER (10)	MEDIUM	INITIAL FRESH WEIGHT	FINAL FRESH WEIGHT	INDEX (5)
1 2 3	No. 23	.097	4.69	48.5
4 5 6	No. 23 - no coconut milk added	.097	0.92	9.4
7 8 9	modified No. 23 + coconut milk outside bag (OUT)	.098	3.74	38.0
10 11	modified No. 23 + coconut milk included in bag (IN)	.103	1.28	12.5

milk or on modified No. 23 (IN). It was concluded that modified No. 23 medium (OUT) could be used to produce tissue for use in studies dealing with the secretion of proteins.

FOOTNOTES

(1) No. 16 tissue, isolated from tree no. T-2-56 on December 26, 1961.

(2) page 48, notebook 2156.

(3) project 2351, chemical components listed appendix table VI. Report No. 3.

(4) Each 3 liter Erlenmeyer contained approximately 80 pieces of tissue and 1 liter of liquid No. 23 medium.

(5) Growth Index = Final Weight/Initial Weight of Inoculum.

(6) Approximately 180 pieces of tissue obtained from the original inoculum.

(7) Placed in 1 liter graduate with magnetic stirring.

(8) No coconut milk was added. Chemicals added to the 800 ml. and made up to 1 liter.

(9) 100 ml. of dialyzed coconut milk and chemicals added to distilled water and made up to 1 liter.

(10) Each culture contained 5 pieces of No. 16 tissue. Growth period - 4 weeks.

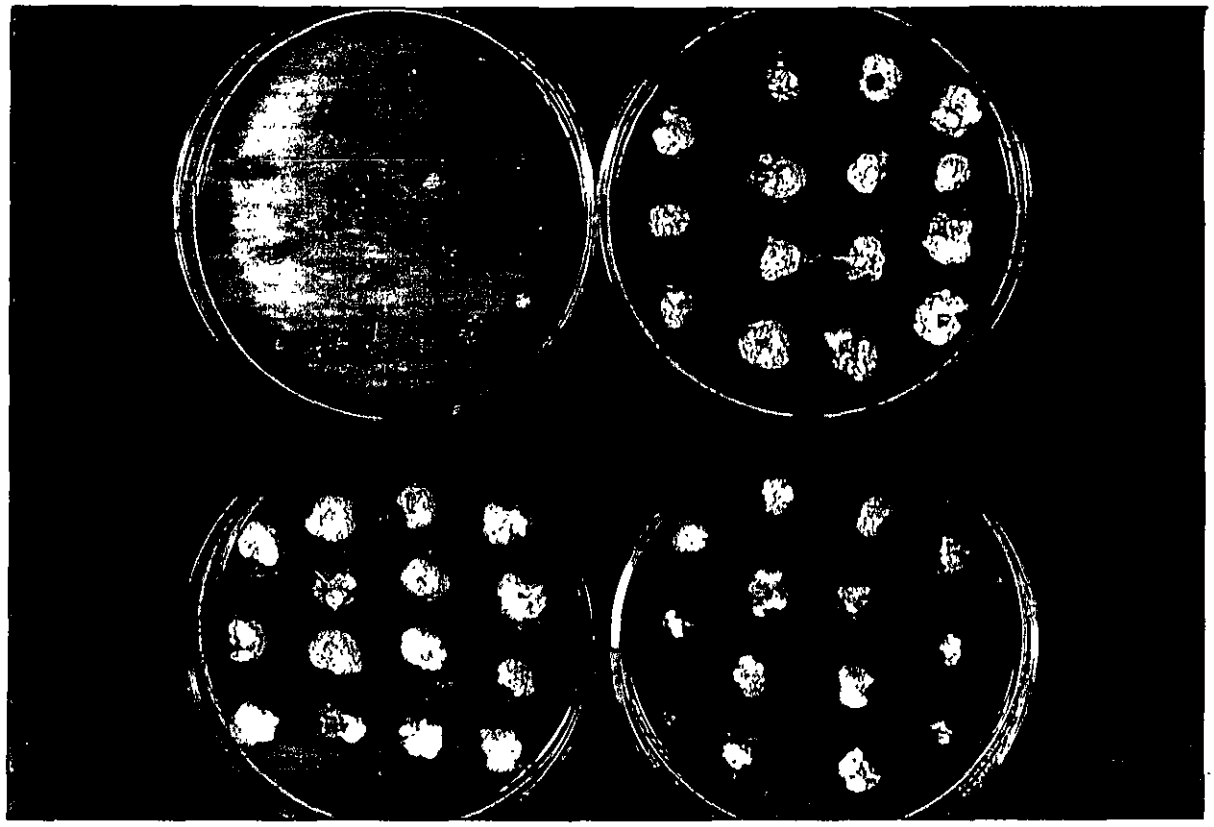

FIGURE. The growth of isolated aspen tissue on modified No. 23 medium.

UPPER: left - No. 23 medium in the absence of coconut milk
right - No. 23 medium

LOWER: left - modified No. 23 medium (OUT)
right - modified No. 23 medium (IN)