C8-027

FIRST PROGRESS REPORT

DEGRADATION OF PLANT CELL WALLS BY CELLULOSOMES OF ANAEROBIC BACTERIA

INSTITUTO CENTROAMERICANO DE
INVESTIGACION Y TECNOLOGIA INDUSTRIAL
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FIRST PROGRESS REPORT

DEGRADATION OF PLANT CELL WALLS BY CELLULOSOMES OF ANAEROBIC BACTERIA

GRANT No. DPE-5544-G-SS-8061-00

PRESENTED BY ICAITI TO

AGENCY FOR INTERNATIONAL DEVELOPMENT
(A I D)

GUATEMALA, JUNE 1989

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1. INTRODUCTION

The agency for International Development (AID) of the United States of America granted ICAITI for a research program entitled "Degradation of Plant Cell Walls by Cellulosomes of Anaerobic Bacteria", under the terms and conditions of Grant No. DPE-5544-G-SS-8061-00. ICAITI subcontracted the Tel Aviv University to obtain technical services set forth in the scope of work of the contract which is enclosed in this report.

The overall aim of the research project is to understand the interaction between resting cell suspensions of cellulolytic bacteria and cell walls of plant cells in suspension culture.

A specific objectives are the characterization of the enzyme complexes ("cellulosomes") produced by Clostridium thermocellum and the study of the equilibria of their absorption to cellulosic plant cell wall degradation. Once the cellulosomes are characterized to the point that enzymatic defficiency for efficient cell wall degradation can be identified, the investigators plan to enhace the cellulosomes by adding the necessary enzymes.

In order to measure the kinetics of cellulosic cell wall degradation without interference from lignin or lignin-removing pre-treatmet, the plant cell used as substrate will be grown from callus in suspension culture.

The present progress report covers the period from August 1, 1988 to June 30, 1989.

2. FIRST COORDINATION MEETING

The first coordination meeting of this project was carried out on five sessions, during March 27 and 28, 1989. The agenda is attached to this report. An extra session was including during the night of the 27, in order to have extended participation of Ed. Bayer, on leave from the Army.

A program of activities for the training stay of Fabiola de Micheo was prepared as a result of the group discussions. A report of this activity in includes in section 3.

Considering the results already obtained by the two israeli groups and the interest of ICAITI in the evaluation of local complex substrates, the substrate variable was selected (cane pith, cane fibers, banana fibers, alfalfa cell walls, fiter paper, microcristaline cellulose, etc.). Another variable will be the presence or absence of added beta-glucosidase (NOVO). The analysis will be performed in at least two time intervals. A sample of each substrate (50-100 grs) will be sent to the Tel Aviv University for parallel assays. Suggested analysis: turbidimety, DNS, glucostat, HPLC and cellulose degradation.

As a complement, the adsorbed % as a function of the substrate concentration will be assayed.

The visit of both israeli participants to ICAITI was schedule for the first semester of 1990, in order to have enough research results for discussion. The same year, a second research stay in Israel of Fabiola de Micheo was planned. Her task will be the collaboration in the construction of the "supercellulosome" with beta-glucosidase activity.

The meeting also included a brief visit to Mr. Raf Elishav, Head, Section for Contracts and Grant Applications. He indicated that the first payment check had already arrived and later on, sent a short note certifying that this research does not present unacceptable environmental hazards (see section 5).

A joint applied research publication is expected as a result of the experiments planned during this meeting.

3. REPORT OF TRAINING STAY

The training stay was carried on by Fabiola de Micheo (ICAITI) from March 27 to April 14, 1989. The activities included the attendance to the First Coordination Meeting (see section 2)

LABORATORY EXPERIENCES

3.1. Preparation of the inoculum of Clostridium thermocellum YS.

Medium and cultivation conditions were followed according to Bayer et al (1). The following additives were used per liter of destilled water:

0.5 g	$MgCl_2.6H_2O$
1.3 g	$(NH4)_2SO_4$
0.5 g	K_2HPO_4
0.5 g	KH_2PO_4
5.0 g	Yeast extract
10.5 g	MOPS (3-[N-Morpholino] propanesulfonic acid)
2.0 mg	Resazurin (1 ml of 0.2 % solution)
8.0 g	Cellobiose Disolved in 950 ml of destilled water and the pH was adjusted to 7.3 with NaOH.
1.0 g	Cysteine-hydrochloride, disolved in 50 ml of destilled water, the pH adjusted to 9 with NaOH 10 N, prepared and added to the medium, just before it was dispensed into 120 ml serum bottles.

The bottles were treated with a stream of nitrogen gas, sealed with butyl rubber septum-type stoppers, and autoclaved for 15 minutes, at 121°C.
6 ml of C. thermocellum YS stock were inoculated in every serum blottle, and the cells were grown anaerobically for 10 hours, at 60°C.

3.2. Separation and purification of the cellulosome.

For purification of the cellulosome, a 12 liter culture of C. thermocellum YS was grown in a fermentor, according to the procedure described by Lamed et al (4). The medium mentioned before was prepared containing proportional additives. Potasium phosphate buffer 1 M (420 ml) pH 7.3, was used instead of Mops, and 5 g of microcrystalline cellulose (Avicel) per liter of medium, was used instead of cellulose. 200 µl of antifoam (Triton X100), diluted 10 fold, were added. The fermentor was autoclaved, and 2 ml of a 3 % solution of cysteine-hydrochloride, (autoclaved separately) were added the day before the inoculation. A volume of 800 ml of the inoculum previously prepared, was introduced in the fermentor. The fermentation was carried under anaerobic conditions, at pH 7.3. Cells were grown for 24 hours at 60°C under constant stirring at 100 rpm.

SCHEMATIC DESCRIPTION OF THE PURIFICATION OF THE CELLULOSOME

CELL CULTURE
(24 h)

|

CENTRIFUGATION

(10,000 rpm, 4°C)

|

SUPERNATANT

Sodium azide 0.05 %
Addition of 120 g of Avicel,
stirring for 1 h at room temperature

|

Separation of cellulose (Avicel) by decantation

|

Cellulose washing in a Buchner funnel with 2 liter of Tris buffer, [Tris(hydroxymethyl)aminomethane], 50 mM, pH 7.7

|

Elution of the cellulosome with 1.5 l of 1 % of triethylamine

|

Precipitation of the cellulosome by the addition of 3 volumes of acetone, centrifugation, 9,900 rpm, 4°C, 15 min.

|

Pool of the cellulosome with Tris buffer, 25 mM, pH 7.7
3 ml. Centrifugation at 17,000 rpm, 4°C for 15 min.

|

SEPHAROSE 4B GEL CHROMATOGRAPHY

Chromatography column, 2.5 by 80 cm
The column was equilibrated and eluted with 50 mM Tris-buffer, pH 7.5 containing 0.05 % of sodium azide.
Flow rate of 40 ml/h and collected fractions of 6 ml

|

Protein, by the absorbance reading of the fractions at 280 nm (Fig 1).

|

Electrophoresis of the fractions from 21 - 43, and of the cellulosome std. Combination of the fractions, precipitation with acetone, and dilution with Tris buffer, 50 mM, pH 7.5 to a concentration of 2 mg of protein/mililitro. A copy of the pattern of older cellulosome on polyacrylamide gel electrophoresis is annexed.

3.3. Determination of Congo Red-Polysaccharide Interactions in the supernatant, 24 hours cell growth of Cl. thermocellum.

Since the use of Congo red as an indicator for B-D-glucan degradation in an agar medium provides the basis for a rapid and sensitive screening test for cellulolytic bacteria, the ability of Cl. thermocellum to degrade B-(1--4)-D-glucan was tested in the supernatant. Carboxymethyl cellulose (0.1 %) was included as substrate in citrate buffer 50 mM, pH 6.7, and 2 % agar on petri plates, following the procedure described by Teather and Wood (7). After 24 hours of incubation at 37° C, the agar medium was flooded with an aqueous solution of 0.1 % of Congo red for 30 minutes, it was then poured off, and the plates were treated by flooding with 1 M NaCl, 5 minutes, three times. Clearing zones due to the hidrolysis were observed.

3.4. Detection of protein in the supernatant, 24 hours cell growth of Cl. thermocellum, by the reaction of lectin bound to peroxidase, on nitrocellulose paper.

0.5 µl of the supernatant and of the cellulosome std. were applied on the nitrocellulose paper. It was flooded with a solution of 2 % of bovine serum albumin in PBS, pH 7.6 to avoid reaction of lectin on the nitrocellulose paper. (PBS is prepared as follows, per liter of destilled water: 8 g of NaCl, 0.2 g of KCl, 0.2 g of KH_2PO_4, and 4.59 of Na_2HPO_4). After 30 minutes the solution was poured off, and 4 ml of 3 ug/ml of lectin in PBS was poured on the paper and shaking slowly for 45 minutes. The paper was washed 5 times with PBS. Substrate : 4.75 ml of 50 mM Tris buffer, pH 7.5 with NaCl brought to 0.9 %, mixed with 0.25 ml of 0.018 % hydrogen peroxide in Tris buffer and 1 ml of 3mg/ml of chloro-naphtol in methanol. Peroxidase substrate was poured on the nitrocellulose paper and shaking slowly until blue spots were observed.

3.5. Degradation of microcrystalline cellulose (Avicel) by: 1.Cellulosome. 2.Cellulosome and B-Glucosidase (Novozym).

The folowing assays were carried out:

1. 1.2 % of cellulose
   Cellulosome: 8 µg/mg cellulose
   B-glucosidase (Novozym): 0.15 units/mg cellulose

2. 1.2 % of cellulose
   Cellulosome: 8 µg/mg cellulose
   B-glucosidase (Novozym): 0.075 units/mg cellulose

3. 3 % of Cellulose

Cellulosome: 8 µg/mg cellulose
B-Glucosidase (Novozym): 0.06 units/mg cellulose

4. 3 % of Cellulose

Cellulosome: 8 µg/mg cellulose
B-Glucosidase (Novozym): 0.03 units/mg cellulose

The assay mixtures contained de following components in 10 ml vials:

	1	2	3	4
	1.2%	1.2%	3%	3%
1- Cellulose 15%	800 µl	800 µl	2000 µl	2000 µl
2- EDTA 200 mM in water	60 µl	60 µl	60 µl	60 µl
3- $CaCl_2$ 1 M in water	60 µl	60 µl	60 µl	60 µl
4- Acetate buffer (0.5 M, pH 5)	600 µl	600 µl	600 µl	600 µl
5- Destilled water	3750 µl	3775 µl	2425 µl	2450 µl
6- Cellulosome 2mg/ml Tris pH 7.5, 50 mM	500 µl	500 µl	1250 µl	1250 µl
7- B-Glucosidase(Novozym) (0.36 units/µl)	50 µl	25 µl	50 µl	25 µl
8- Cysteine-HCl 30* mg/ml	180 µl	180 µl	180 µl	180 µl

* Cysteine-HCl in double destilled water, brought to pH 9 NaOH 5 - 10 N and sealed under a stream of nitrogen gas.

After the addition of Cysteine-HCl the vials were sealed under a stream of nitrogen, and were incubated with intermittent shaking at 60°C. Samples were taken at 0, 2, 4, 24, 48, and 96 hours. Turbidity was measured making dilution of 1:33 and reading the absorbance at 660 nm. Glucose by Glucose Oxidase method from Sigma was assayed in the supernatant up to 24 hours of incubation.

Table No 1 shows the residual turbidity as percentage of the original during incubation time, the highest turbidity decrease (residual subtracting from 100 %) was observed in the samples containing 1.2 % of cellulose. The turbidity was the same after 48 hours of incubation in the four assays.

The increase of sugars measured up to 24 hours of incubation is presented in Fig. No 2, corresponding to the percentages of solubilization of cellulose presented in table No 2. Higher percentages of solubilization in both 1.2 % and 3 % of cellulose, were obtained with higher concentrations of Novozym. The degradation of cellulose depended on the

concentration of Novozym, since the purified cellulosome is not able to complete the degradation of cellulose to glucose.

3.6. DEGRADATION OF ALFALFA CELL WALLS (UNTREATED), BY: 1. CELLULOSOME. 2. CELLULOSOME WITH B-GLUCOSIDASE.

The composition of alfalfa cell walls was proportioned by Dr. Lamed in the department of Biotechnology at the University of TelAviv.

Glucose	49.00 %
Xilose	17.00 %
Arabinose	3.20 %
Galactose	1.70 %
Mannose	1.70 %
Uronic	5.80 %
amino-acids	10.00 %
Lignine	18.0 %

It was assumed that glucose came only from cellulose, thus, giving a 49 % of original cellulose.

The following assays were carried out:

1. Cellulosome: 8 µg/mg of alfalfa (16.80 µg/mg cellulose).

2. Cellulosome: 8 µg/mg of alfalfa
   B-glucosidase (Novozym): 0.27 units/mg of alfalfa (0.55 units/mg of cellulose)

The reaction mixture contained the following components in 150x13 mm test tubes:

	1 Control	2 Alfalfa + Cs.	3 Alfalfa + Cs. + Novozym
1- Alfalfa	20 mg	20 mg	20 µl
2- EDTA 200 mM in water	10 µl	10 µl	10 µl
3- $CaCl_2$ 1 M in water	10 µl	10 µl	10 µl
4- Acetate buffer (0.5 M, pH 5)	60 µl	60 µl	60 µl
5- Destilled water	890 µl	810 µl	795 µl
6- Cellulosome 2mg/ml Tris pH 7.5, 50 mM	---	80 µl	80 µl
7- B-glucosidase(Novozym) (0.36 units/µl)	---	---	15 µl
8- Cysteine-HCl 30 mg/ml	30 µl	30 µl	30 µl

The tubes were sealed with rubber stoppers under a stream of nitrogen gas, and were incubated with intermittent shaking at

60°C. The samples were taken out at 0, 4, 24, and 48 hours and controls were carried for every incubation time.
The samples were centrifuged, the supernatants were boiled for 10 minutes and analyzed for reducing sugars with dinitrosalicylic acid reagent (DNS) (5), for pentoses with orcinol (3), and for glucose by Glucose-Oxidase technique from Sigma. Every residue was washed with destilled water after centrifugation, and dried at 60°C to obtain the dry weight.

Figures No 3 and No 4, show the increase of reducing sugars (µmoles of glucose/ml), pentoses (µmoles of ribose/ml), and glucose (µmoles of glucose/ml), at different incubation hours for the experiment with cellulosome and cellulosome plus Novozym respectively. In the first experiment, increase of glucose was not observed; there was no solubilization of alfalfa cell walls by purified cellulosome, since it is only able to degrade the cellulose to cellobiose. In the second experiment with cellulosome and Novozym, the following values as percentage of solubilization of cellulose from alfalfa cell walls were obtained: 5.67 %, 14.90 %, and 15.97 % at 4, 24, and 48 hours of incubation.

In table No 3, the loss in dry weight as percentage of the original alfalfa cell walls is presented.

3.7. DETERMINATION OF CELLULOSOME ADSORBED BY ALFALFA CELL WALLS.

Adsorption assay was followed according to Bayer et al (2) The cellulosome was added in two concentrations:

1) 8 µg/mg alfalfa
2) 16 µg/mg alfalfa

The same components as in the case of degradation of alfalfa cell walls were additioned, without cysteine-HCl, and the samples were shaking a 4° C for 1 hour. The samples were centrifuged and the supernatants were examined for endoglucanase activity (Carboxymethylcellulase, CAMase) (6), including 1 control without cellulosome, and 1 sample for initial CAMase activity. This was determined as follows:

0.5 ml of diluted samples in test tubes.
1.5 ml of carboxymethylcellulose (2 % CMC, low viscosity in water, with 0.5 M buffer acetate, pH 5, 50:20 volumes).

The samples were incubated 1 hour at 60°C, and the reaction was stopped by cooling in ice water. 3.0 ml of dinitrosalicylic acid reagent were additioned, boiled for 10 minutes, and after cooling in ice water, the absorbance was measured at 640 nm.

The results gave the following CMCase activity and the percentage of cellulosome adsorbed:

	µmoles glucose/ mg protein/min	Adsorbed cellulosome µm glucose/ mg prot/min.	Percentage adsorbed
Cellulosome 8µg/mg alfalfa :	1.01	4.77	82.67 %
Cellulosome 16µg/mg alfalfa:	3.05	2.72	47.14 %
Initial activity:	5.77		

In the second case, it can be assumed that the cellulosome was highly in excess, thus the lower adsorption.

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1) Bayer, E. A., Kenig, R., and Lamed, R. 1983. Adherence of Clostridium thermocellum to cellulose. J. Bacteriol. 156: 818-827.

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3) Keleti, G., and Lederer, W. H. 1974. Pentoses (orcinol). Handbook of Micromethods for the Biological Sciences. pp. 79.

4) Lamed, R., Setter, E., and Bayer, E. A. 1983. Characterization of a cellulose-binding, cellulase-containing complex in Clostridium thermocellum. J. Bacteriol. 156:828-836.

5) Miller, G.L. 1959. Use of Dinitrosalicylic acid reagent for determination of reducing sugars. Analytical Chemistry Vol 31, No 3 426-428.

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7) Teather, R. M. and Wood, P. J. Appl. Environ. Micriobiol. 1982. Use of congo red polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. 43:777-780.

Table No 1 RESIDUAL TURBIDITY (PERCENTAGE)

	0 h	2 h	4 h	24 h	48 h	96 h
1.2 % cellulose Novozym 0.15 U/mg	100 %	100 %	75 %	45 %	43 %	43 %
1.2 % cellulose Novozym 0.075 U/mg	100 %	100 %	83 %	50 %	43 %	43 %
3 % cellulose Novozym 0.06 U/mg	100 %	100 %	81 %	67 %	62 %	63 %
3 % cellulose Novozym 0.03 U/mg	100 %	100 %	73 %	52 %	52 %	51 %

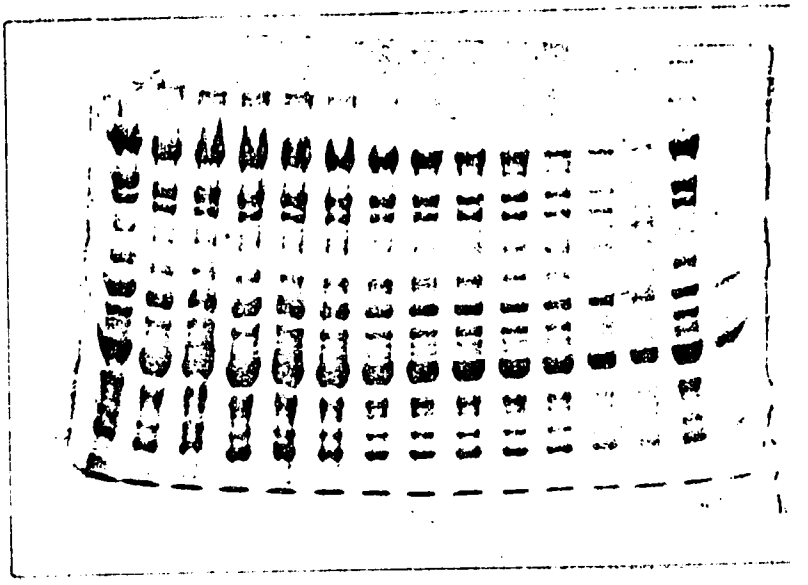
Cellulosome was added in a concentration of 8 ug/mg of cellulose.

Table No 2 SOLUBILIZATION OF CELLULOSE (PERCENTAGE)

	0 h	2 h	4 h	24 h
1.2 % cellulose Novozym 0.15 U/mg	0 %	15.00 %	29.58 %	95.70 %
1.2 cellulose Novozym 0.075 U/mg	0 %	16.47 %	28.23 %	70.27 %
3 % cellulose Novozym 0.06 U/mg	0 %	12.22 %	21.14 %	69.49 %
3 % cellulose Novozym 0.03 U/mg	0 %	10.27 %	21.20 %	60.42 %

Table No 3 DRY WEIGHT LOSS (PERCENTAGE)

	0 h	4 h	24 h	48 h
2 % alfalfa cell walls cellulosome 8ug/mg	0 %	0.00 %	10.50 %	15.80 %
2 % alfalfa cell walls cellulosome 8 ug/mg Novozym 0.27 U/mg	0 %	10.50 %	10.50 %	15.80 %



Photograph of polyacrylamide gel electrophoresis results from cellulosome.
(Courtesy: Tel Aviv University)

Fig. 1 SEPHAROSE 4B GEL CHROMATOGRAPHY

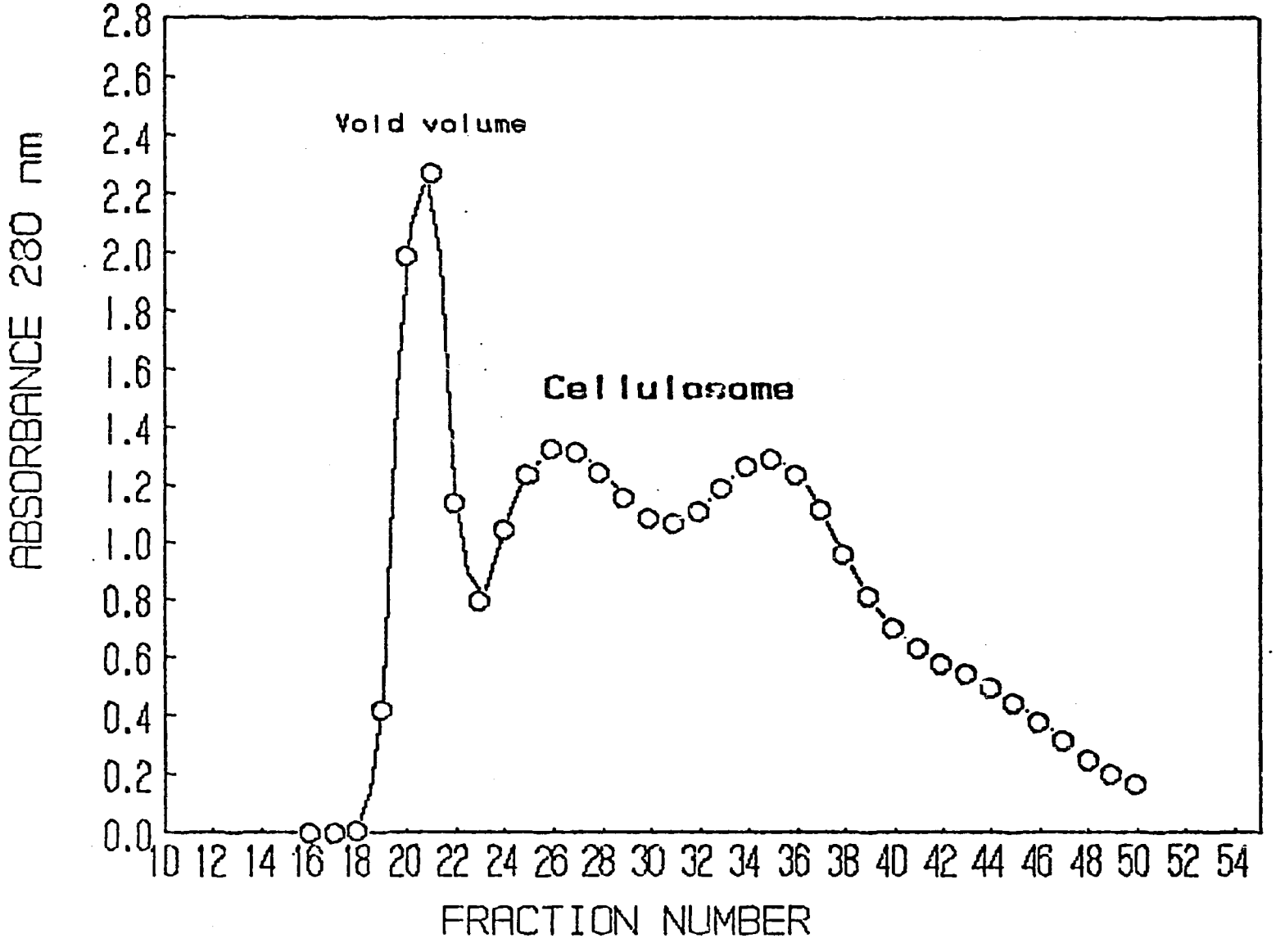


Fig 2 DEGRADATION OF CELLULOSE

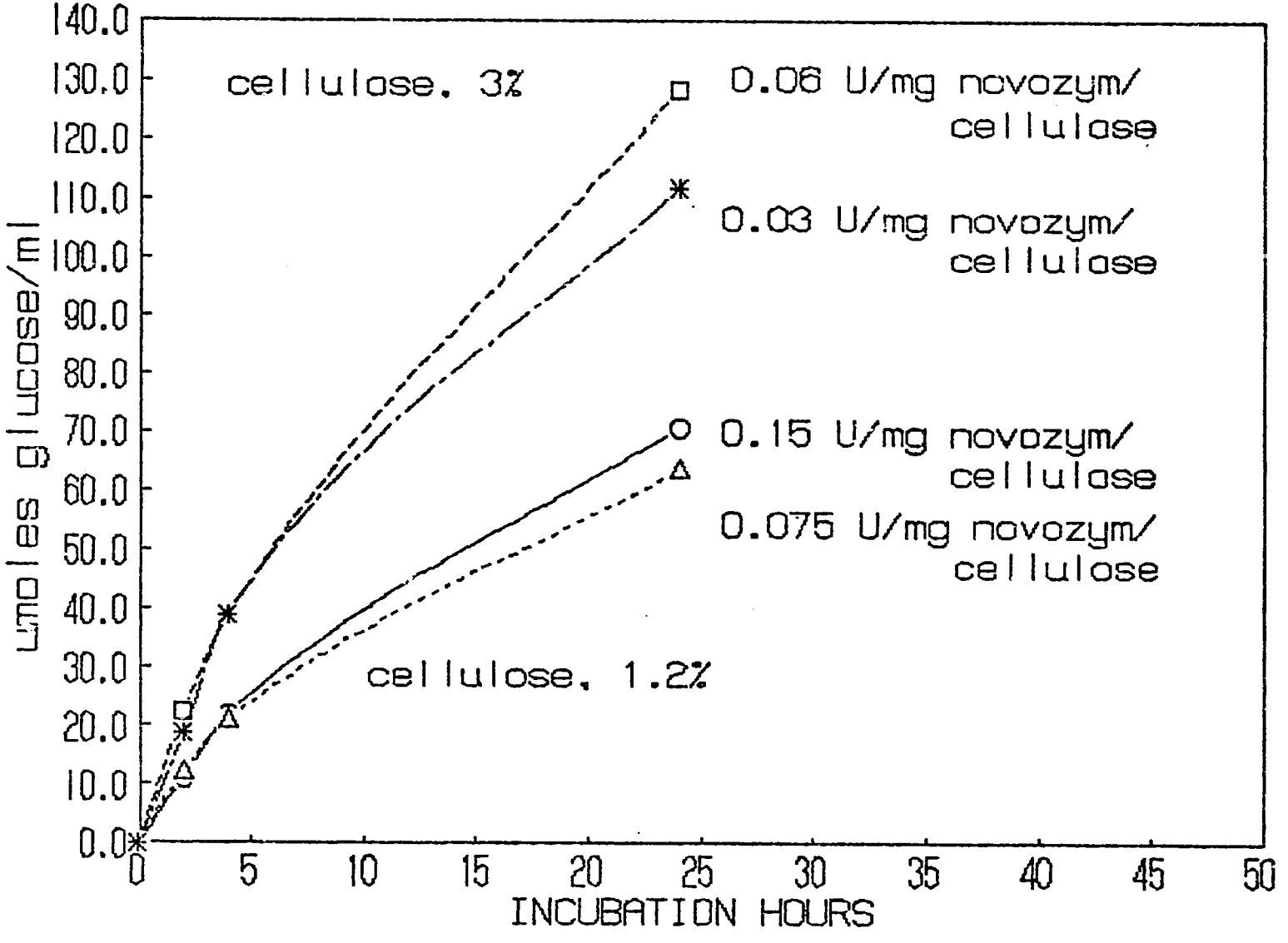


Fig 3 DEGRADATION OF ALFALFA CELL WALLS

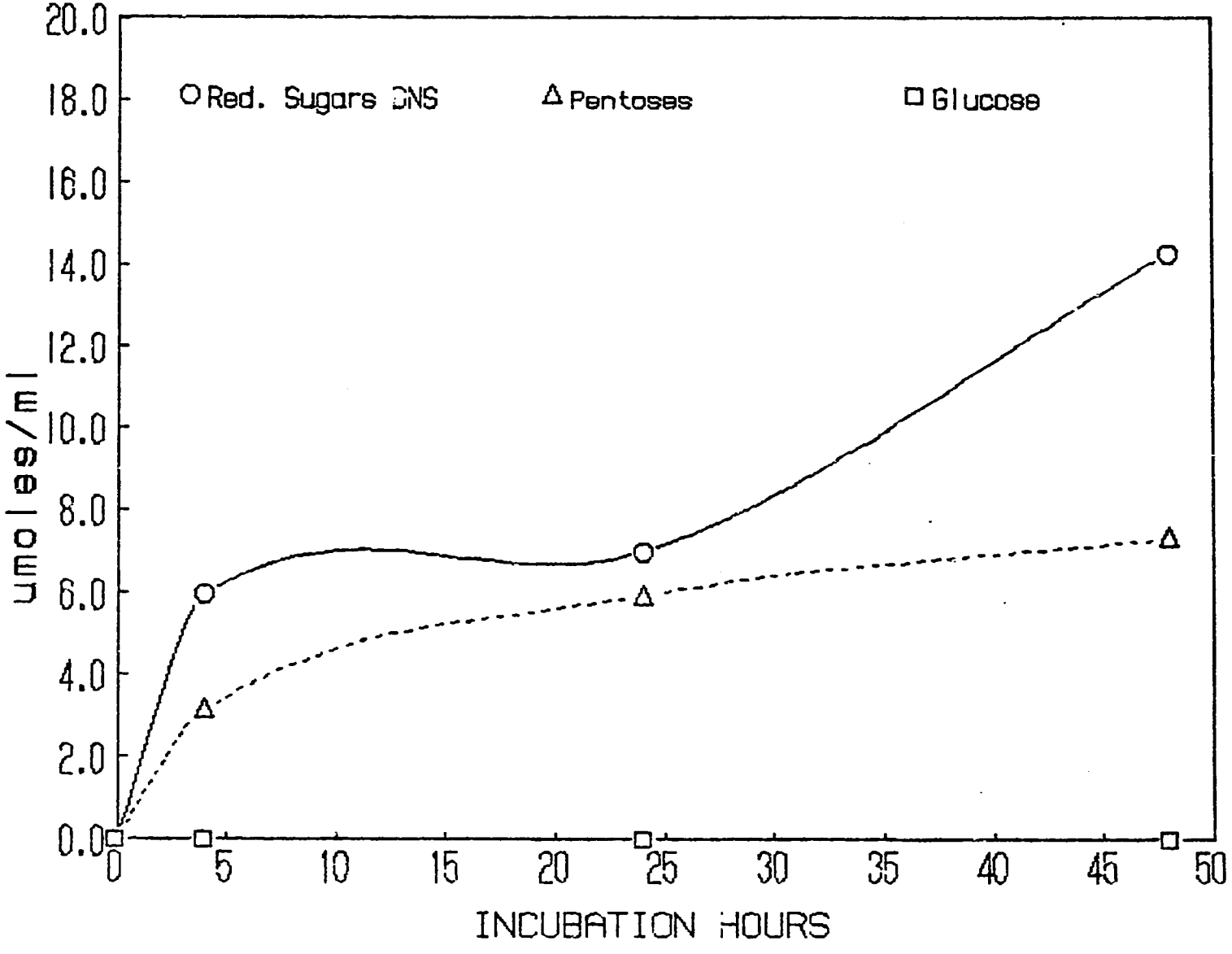
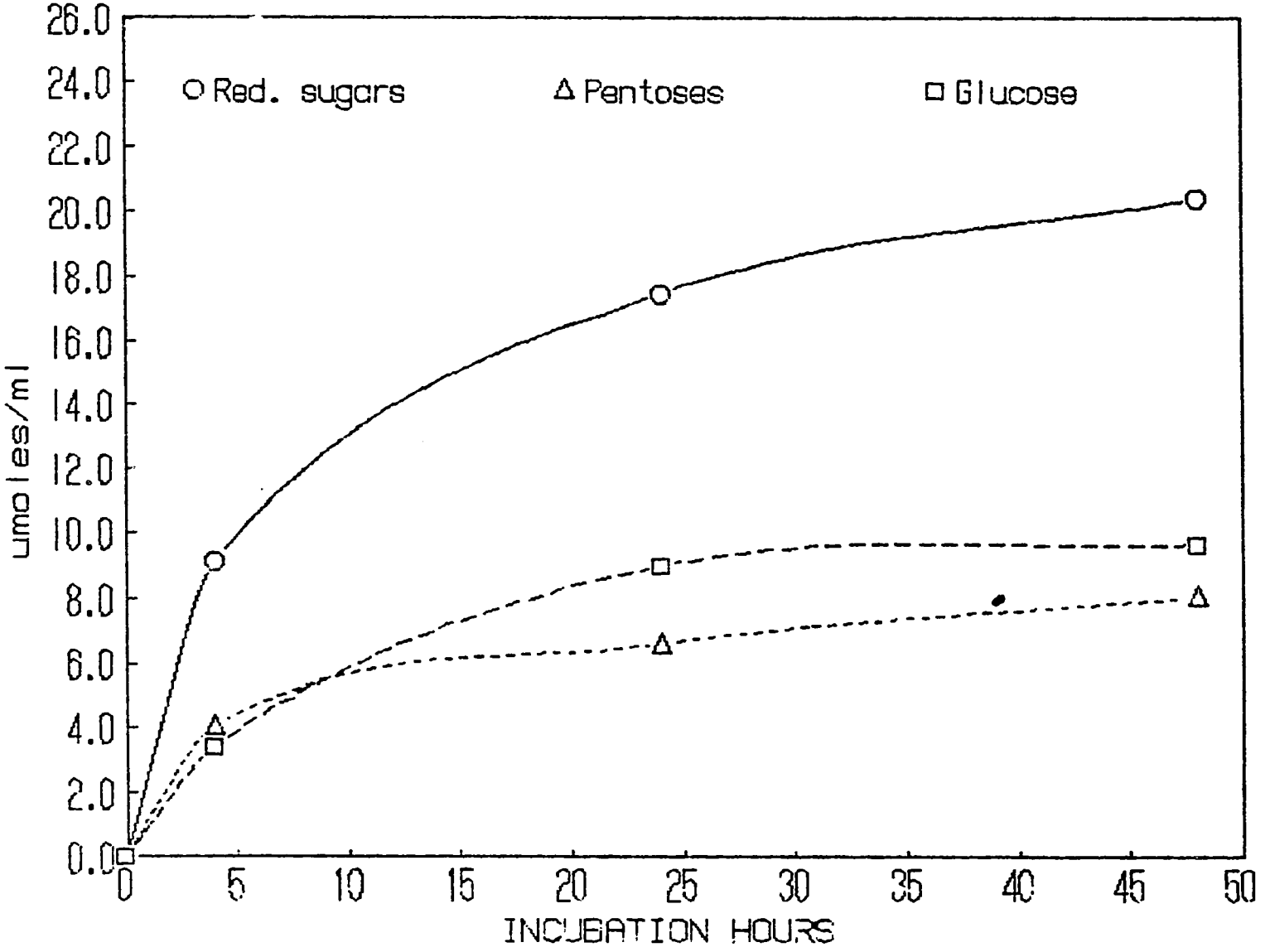


Fig. 4 DEGRADATION OF ALFALFA CELL WALLS



4. CONTRACT

CONTRACT BETWEEN

Central American Research Institute for Industry (ICAITI)

and

Tel Aviv University

This contract entered this first day of January, 1989, between the Central American Research Institute for Industry (hereinafter referred to as ICAITI) and the Tel Aviv University (hereinafter referred to as the University) whose address is: Ramat Aviv, Israel.

Witnesseth that

Whereas, ICAITI pursuant of Grant No. DPE-5544-G-SS-8061-00 "Degradation of Plant Cell Walls by Cellulosomes of Anaerobic Bacteria", desires to obtain technical and scientific services set forth in the scope of work herein and whereas, the Center for Biotechnology of the George S. Wise Faculty of Life Sciences, Tel Aviv University, represent that it is qualified to provide such services, and it is willing to undertake performance as set forth in Article 1 below.

Now, therefore, the parties hereby agree as follows:

ARTICLE 1: SCOPE OF WORK: The University will perform the following tasks:

1.1 Certification by the appropiate institutional committee that the research does not present unacceptable environmental hazards.

1.2 Description of environmental safeguards and plans for containment as pertinent to the project.

1.3 Characterization of the cellulosomes of Clostridium thermocellum and of at least two other meso- or thermophilic anaerobes previously selected by ICAITI.

1.4 Training of a person from ICAITI in the techniques of characterization and handling of cellulosomes of anaerobic microorganisms.

1.5 Construction and characterization of an improved cellulosome for the degradation of plant cell walls.

1.6 Training of a person from ICAITI in the techniques of construction and characterization of such improved cellulosome.

1.7 Participation of two research members from the University in one coordination meeting at ICAITI.

1.8 Reception of the visit(s) of the ICAITI personnel involved in the project to program the joint activities, exchange technology and evaluate results.

1.9 Preparation of three progress reports during the lifespan of the project, dealing with the activities 1.1, 1.2, 1.3 and 1.5 described above.

ARTICLE 2: CONTRACT TERMS: Three years, starting 1st of January, 1989.

ARTICLE 3: STAFFING: Professors Raphael Lamed and Edward Bayer will participate as collaborators to the project. They

will also have the freedom to add workers to the staff according to needs and budget limitations.

ARTICLE 4: LOGISTIC SUPPORT: ICAITI will provide the coordination services for the project, including:

4.1 The purchase and sending of travel tickets for the Israeli participants.

4.2 Per diem and other travel expenses for the same, during the coordination trips, according with the rate used by ICAITI on each visited country.

4.3 Sending sample materials to be need in the project, when required by the activities.

ARTICLE 5: TERMINATION FOR CONVENIENCE OF ICAITI: ICAITI by written notice in 60 days in advance, may terminate this contract in whole or in part when it is the best interest of ICAITI. If so terminated, ICAITI shall be liable only for payment in accordance with the compensation provisions of this contract for services rendered prior to the effective date of termination.

ARTICLE 6: REPORTS: The University will submit to ICAITI three reports on the research work carried on in Israel, according with the ARTICLE 1. They are:

Report 1: Including activities 1.1 and 1.2, due two months after the signing of the contract.

Report 2: Activity 1.3, due April 1990.

Report 3: Activity 1.5, due October 1991.

ARTICLE 7: COMPENSATION AND PAYMENT PROVISION:

7.1 Cost of technical services: This is a contract for services contemplated under ARTICLE 1, and according to the

provisions of US-AID Grant DPE-5544-6-55-8061-00, ICAITI will pay U.S. $ 84,450 on the basis of specific invoices from the first of January, 1989, for services performed and expenses incurred for amounts hot to exceed those authorized by ICAITI in accordance with 7.2 and the following distribution:

- First disbursment: U.S.$ 30,150 upon reception of the signed contract.
- Second disbursment: U.S.$ 25,300 upon reception of progress report (April 30, 1990).
- Third disbursment: U.S.$ 20,000 upon reception of progress report (October 31, 1991).
- Fourth disbursment: U.S.$ 9,000 in december 1991.

7.2 The budget is distributed as follows:

- Salaries	U.S.$	34,300
- Equipment	U.S.$	26,200
- Materials and Supplies	U.S.$	16,000
- Overhead	U.S.$	7,950
T O T A L	U.S.$	84,450

This budget sets limitations for reimbursement of dollar costs for individual line items. Without the prior written consent from ICAITI, the University may not exceed the grand total set forth in the budget attached, nor may the University exceed the dollar costs for any individual line item by more than 15 percent of such line item.

7.3 Total obligation: The total cost of services and material reimbursed hereunder shall not exceed the amount of US$ 84,450.00.

ARTICLE 8: GENERAL PROVISIONS: The provisions set forth in Appendix A, when applicable are incorporated as an integral part of this contract.

In witness whereof, the parties hereto execute this contract on the nineth day of November 1988.

FOR THE UNIVERSITY

Date:

Raphael Lamed

Raphael Lamed

Principal Investigator

Date:

FOR ICAITI

W. Ludwig Ingram, Jr.

Director a. i.

Date:

José F. Calzada

Principal Investigator

Date:

5. CERTIFICATIONS

TEL AVIV UNIVERSITY אוניברסיטת תל-אביב
RESEARCH AUTHORITY רשות המחקר

March 23, 1989
LP

Carlos Rolz
Head Technical Department
Instituto Centroamericano de
Investigacion Tecnologia
Industrial (ICAITI)
Avenida La Reforma 4-47, Zone 10
Guatemala, CA

Dear Sir,

re: Dr. Raphael Lamed
DPE-5544-G-SS-8061-00
Your No. IA 54/7/01/948
our No. 5478

We would like to certify that the above research does not present unacceptable environmental hazards whatsoever.
Therefore all the experiments will be conducted under the standard safeguards of Tel Aviv University through their microbiological and biochemical laboratories.

Rafi Elishav

Rafi Elishav
Head Section for Contracts
and Grant Applications

Raphael Lamed

Dr. Raphael Lamed
Principal Investigator

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317466

Guatemala, June 22, 1989

We have examined the proposed activities of Grant Number DPE-5544-6-SS-8061-00, entitled "Degradation of Plant Cell Walls by Cellulosomes of Anaerobic Bacteria".

Considering that the research will use genetically unmodified biological materials, that the bioconversion of lignocellulosic material by Clostridium thermocellum occur in natural habitats and are currently in use, we therefore certify that the above research does not present unacceptable environmental hazards whatsoever.

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FIRST PROGRESS REPORT

DEGRADATION OF PLANT CELL WALLS BY CELLULOSOMES OF ANAEROBIC BACTERIA

GRANT No. DPE-5544-G-SS-8061-00

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