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UTILIZATION OF EGYPTIAN CANE-SUGAR BAGASSES FOR THE PRODUCTION OF CELLULASES BY ASPERGILLUS TERREUS THOM

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Cane-sugar bagasses contains about 60% cellulose and 0.2% reducing sugars. The spectroscopic detection of elements present in the ash of cane-sugar bagasses revealed that it contained sodium, potassium, magnesium, manganese iron, calcium and other trace elements. *Aspergillus terreus* Thom utilized cane-sugar bagasses as carbon sources. The presence of cane-sugar bagasses in the fermentation medium induced the organism for the production of the cellulolytic enzymes (cellulases.) Cellulases were successfully precipitated and purified by cooled acetone from the fermentation broth. The hydrolytic activities of the precipitated crude cellulases were tested on sodium carboxymethylcellulose (CMC or Modocol M) and decreasing the percentage viscosity of Modocol M and appearance of reducing sugars which were taken as criteria for the cellulytic activities of cellulases showed that these enzymes could be produced by *Aspergillus terreus*, when cane-sugar bagasses were included in the fermentation medium as carbon source.

Cellulose occurs abundantly in Nature, notably as the principle constituent of the cell walls of most plants. It may be present in relatively pure state, as in the cellulose of the cotton plant, or in close association with many other compounds, such as the hemicellulose and lignin of wood and bast fibres.

The fermentation of natural material to give useful sugars, solvents, enzymes, antibiotics, fertilizers and even gases is operated on a large scale.

In the present paper, trials were devoted for the utilization of cane-sugar bagasses for the production of cellulases by an isolated organism from Egyptian cotton, namely, *Aspergillus terreus* Thom.

Materials and Methods

Isolation and Maintenance of the Cellulose Decomposing Fungus.—*Aspergillus terreus* Thom was isolated from Egyptian native cotton and was identified through the kind help of the Central Bureau Voor Schimmal Cultures Baarn, Holland. The organism was maintained on the following ingredients (g/l): sucrose 20.0, $NaNO_3$ 2.0, KH_2PO_4 1.0, $MgSO_4.7H_2O$ 0.5, KCl 0.5, $FeSO_4.7H_2O$ 0.005 and agar 20.0. The slants were stated in a refrigerator at 5°C.

The medium used for the production of cellulases was similar to the above-mentioned ingredients except that sucrose was preplaced by Egyptian cane-sugar bagasses.

Preparation of Cane-sugar Bagasses.—Cane-sugar bagasses were thoroughly ground and washed with tap water. It was soaked in tap water and autoclaved at 15 lb/in² for 20 min to ascertain that all reducing sugars were get off. The fine pieces of cane-sugar bagasse were used as carbon sources for the growth of *Aspergillus terreus* Thom. In the liquid medium 50 g/l cane-sugar bagasses were used as carbon sources. Erlenmeyer flasks of 1000-ml, each containing 200 ml liquid medium, were sterilized at 22.5 lb/in² for 15 min. The initial pH of the medium was 6.0 before sterilization. One ml of standard spore suspension of *Aspergillus terreus* Thom was introduced into each flask under aseptic conditions. The inoculated flasks were inserted on a shaker of 200 rev/min at 30°C for 10 days. On completion of the incubation period, the fermented medium was filtered and filtrates were treated for the precipitation of cellulases.

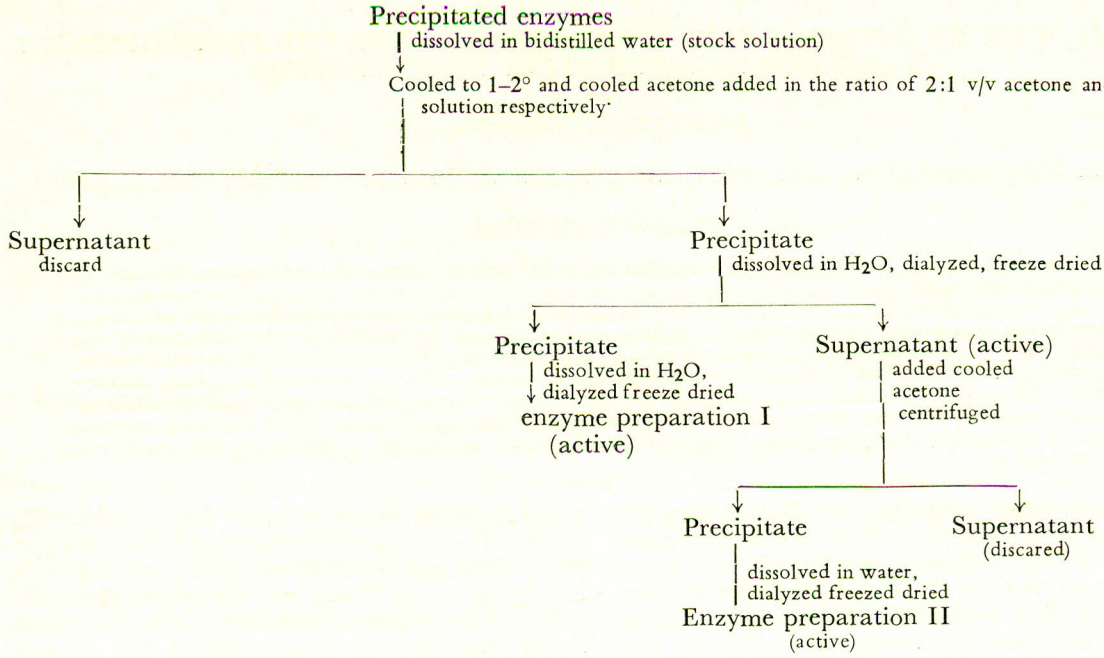
Precipitation of Cellulases.—Different techniques were used for the precipitation of cellulases. The cellulytic enzymes were precipitated from the fungal filtrates either by inorganic salts, e.g. ammonium sulphate, or by organic solvent such as acetone and alcohol–ether mixture according to the method described by Taha *et al.*[1]

Purification of the Precipitated Cellulases.—The precipitated enzymes served as the starting material for the purification of the enzymes, which involves a series of acetone fractionations. The scheme of purification is shown in Chart 1. After the purification of the cellulytic enzymes, they were standardised and kept in phosphate buffer of pH 4.0 for measuring their cellulytic activities.[2–6]

Measurement of Cellulolytic Activities.—The cellulolytic activity of the enzyme was estimated by the measurement of the percentage decrease in viscosity of a soluble cellulose derivative when treated with the enzymes preparation. Sodium carboxymethylcellulose (Modocol M obtained from Mooch Damjo Aktlebolag Omskoldsvik, Sweeden) was used as the soluble substract. Viscosimetric measurement was done according to the method described by Taha *et al.*[1] The cellulolytic activity was also measured iodometrically as the increase in the reducing sugars.[6–8]

Spectroscopic Determination of Elements of Cane-sugar Bagasses.—The spectrum detection of elements in the ash of cane-sugar bagasses were carried out in the spectroscopic laboratories of the

CHART I



After the purification of the cellulolytic enzymes, they were standardised and kept in phosphate buffer of pH 4.0 for measuring their cellulolytic activities.[2-6]

National Research Centre, Cairo. Quantitative determinations of calcium, magnesium, manganese, zinc and iron were carried out.[9,10]

Separation of Reducing Sugars.—The reducing sugars, produced by the cellulolytic enzymes, were separated by using Whatman No. 1 chromatographic paper. The moving phase was *n*-butanol–pyridine–water (6:4:3 v/v) and aniline phthalate as spraying agent.

Results and Discussion

Growth of Aspergillus terreus Thom *on the Medium Using Cane-sugar Bagasses as Carbon Source.*—The organism grew luxuriantly on the medium supplemented with cane-sugar bagasses as carbon source and, as the cellulolytic enzymes are adaptive ones, the organism began to excrete these hydrolytic enzymes from the first day of incubation. The cellulolytic enzymes, i.e. cellulases, convert the cellulosic substratum into compounds of lower molecular weights, especially cellobiose and the latter was utilized by the organism intracellulary.

From the spectroscopic analyses of cane-sugar bagasses ash (Table 1), it can be concluded that the ash contains the essential elements which could influence and cover the requirements of the growing organism.

Precipitation and Purification of Cellulases.—The precipitated enzymes were further purified by dissolving the precipitates in bidistilled water and cooled to 1–2°C. Enzyme preparation–cooled acetone (1:2 v/v) were mixed and left in a refrigerator at 5°C overnight. The precipitates were dissolved in water and dialyzed (enzyme preparation I). The supernatant was cooled and the addition of cooled acetone was repeated. The solution was centrifuged. The precipitates obtained were dialyzed, dissolved in water and kept in phosphate buffer of pH 4.0 (enzyme preparation II). The activities of enzyme preparation II was more active than enzyme preparation I. Therefore, enzyme preparation II was added on the soluble cellulose derivative for studying their hydrolytic activities.

Action of the Purified Enzymes in Modocol M.—2.5 ml purified enzymes at pH 4.0 phosphate

TABLE 1.—ANALYSES OF ASH OF CANE-SUGAR BAGASSES.

Spectroscopic* detection of elements in ash	Na	M	Fe	FT
	K	M	Al	FT
	Mg	M	Si	T
	Ca	M	Zn	T
	Mn	L	Ba	VVFT
Quantitative determination of elements in % ash	Ca	2.50		
	Fe	0 01		
	Mn	0.90		

*VVFT=0.0001%, FT=0.001–0.001%, T=0.01–0.1%, L=0.1–1.0% and M=1.00–1.0%.

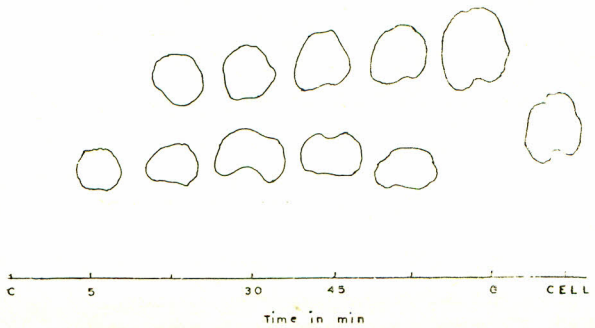


Fig. 1.—Degradation of CMC by the action of fungal cellulases. C, control; G, glucose; Cell, cellobiose. Moving solvent: n-butanol–pyridine–water 6:4:3 (v/v). Spraying agent: aniline phthalate.

buffer were added to 5 ml 1.0% sodium carboxymethylcellulose. The buffered Modocol M containing cellulytic enzymes was incubated in a water bath at 40°C. Measurement of decreasing the percentage in viscosity and quantitative determination of reducing sugars were evaluated at different times of intervals.

TABLE 2.—ELUCIDATION OF % DECREASE IN VISCOSITY AND REDUCING SUGARS DURING THE ACTION OF THE PRECIPITATED ENZYMES ON SODIUM CARBOXYMETHYLCELLULOSE.

Time min	% Decrease in viscosity	Reducing sugars mg/ml
5	20	0.01
10	50	0.10
15	60	0.30
20	70	0.40
30	75	0.45

The data indicated that the precipitated enzymes acted directly, when added to the soluble cellulosic substratum and with increasing time, both percentage decrease in viscosity and appearance of reducing sugars increased. The proper time of the cellulolytic actions was about 20 min. The decrease in the viscosity was about 75% which indicated that the prepared enzymes were of high purity and this was substantiated by measuring the

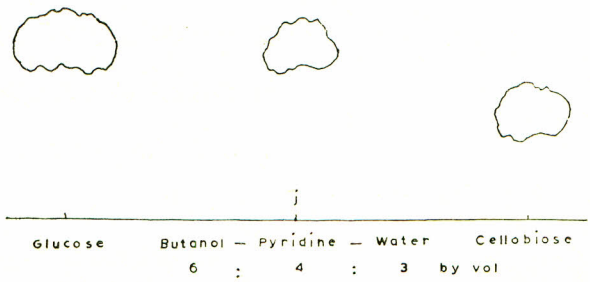


Fig. 2.—Glucose as the main end product at the end of incubation. I. J. sample containing cellulases. Moving phase:n-butanol–pyridine–water 6:4:3 (v/v). Spraiyng agent: aniline phthalate.

reducing sugars. At 20 min incubation about 0.4 mg/ml was liberated.

Paper chromatographic separation of reducing sugars (Fig. 1) shows that cellobiose appeared after 5 min from the addition of the enzymes, but glucose appears after 10 min. Good separation of both sugars was achieved at 20 min (Fig. 2).

Cellulases produced by *Aspergillus terreus* Thom. when cane-sugar bagasses were used as carbon source proved to be of high quality.

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