Characterization of the Xylanolytic Activity of *Cellulomonas*

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Abstract: Maximum production of xylanolytic enzymes by *Cellulomonas* CSI-17 required aerobic conditions, a temperature of 30°C and a pH between 6.2 and 7.2. Under these conditions, maximum levels of xylanolytic activity were reached within 48 hr; addition of further mineral salts and yeast extract to the growth medium decreased the time to 24 hr.

At pH values between 5.4 and 8.4 the xylanase was stable up to a temperature of 30°C, for at least 48 hr after harvest; over the same temperature range, the β -xylosidase was stable only at the higher end of this pH range.

The culture filtrate was similar to the growing cells in its ability to hydrolyse xylan whilst the growing cells metabolized the bulk of the hydrolysis products, the latter accumulated as soluble reducing sugars when the culture filtrate was used as the enzyme source.

1. Introduction

A highly cellulolytic *Cellulomonas* mutant CSI-17⁴ has been shown to be improved over the original parent strain CSI-1, with respect to hemicellulolytic (including xylanolytic) activity.^{8, 11} The hemicellulolytic activity was shown to be directly proportional to the xylose content in the hemicellulose substrate. From induction studies during growth on xylan, crystalline cellulose and carboxymethylcellulose, it has been shown that although both cellulolytic and xylanolytic activities have been similarly effected by the mutation, xylanolytic activity is distinct from cellulolytic activity.

Peiris⁸, demonstrated that when the *Cellulomonas* mutant, CSI-17, was grown under aerobic conditions at 30°C in an unbuffered medium containing 0.5% xylan, 1% of each Dubos mineral salt and 0.02% yeast extract, maximum xylanolytic activity was attained within two days. The current investigation sought to improve enzyme production by varying the pH, temperature, aeration and the concentrations of mineral salts and yeast extract.

Choudhury¹, showed that non-growing cultures of *Cellulomonas* CSI-17 are effective in hydrolysing pretreated sugar cane bagasse. In the current investigation

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the hydrolysis of xylan by growing cultures (*in vivo*) and non-growing cultures (*in vitro*) was compared. The xylan used as substrate for digestion was an arabinoxylan obtained from oats and was similar in sugar composition to the hemicellulose fraction of sugar cane bagasse.

2. Materials and Methods

2.1 Bacterial Strain.

The *Cellulomonas* strain was CSI—17, a mutant previously shown to be highly cellulolytic⁴ and xylanolytic.⁸

2.2 Growth and Assay Substrate.

Xylan, a commercial preparation, obtained from oats and supplied by Fluka AG, Buchs SG, Switzerland was used as growth and assay substrate. High performance liquid chromatographic (HPLC) analysis of an acid hydrolysate showed its neutral sugar composition to be xylose, arabinose and hexose in the ratio of 73:12:15. The hexose was found by thin layer chromatography to be mainly glucose with a trace of galactose. (The sugar composition was therefore similar to that of hemicelluloses A and B isolated by Rickard and Peiris¹¹ from sugar cane bagasse. In all three cases xylose was the major component). As a growth substrate it was used as supplied; for enzyme assay the fraction containing particles between 63 and 250 μ in size was used.

2.3 Growth and Fractionation of Cultures.

The cultures were grown as described by Peiris.⁸ They involved growth at 30° C in shake flasks with unbuffered medium (initially pH 7.2) containing 0.5% xylan, 1% of each Dubos mineral salt³ and 0.02% yeast extract. After growth for two days, the cultures were harvested, separated into cellular and extracellular (culture filtrate) fractions and the cellular fraction homogenized.

The following deviations from standard conditions described by Peiris⁸ were made: (a) the shake flask cultures were buffered at various pH values by the inclusion of either 40 mM 3-(N-morpholino)—propanesulphonic acid (MOPS), Calbiochem, San Diego, U.S.A. or 40 mM 2-(N-morpholino) ethanesulphonic acid (MES), Sigma, St. Louis, U.S.A. in the medium and adjusting the pH to the desired value;⁷ (b) shake flasks were substituted with a fermentor as the growth vessel; a stirred tank fermentor⁹ with a working volume of 1 l which was fabricated at the School of Biotechnology, University of New South Wales, Australia, was used; the air supply system consisted of an adjustable pressure reducing valve, a needle valve for control of air flow rate and a flow meter with a range of 100 to 1200 ml/min; the dissolved oxygen tension (DOT) in the medium was measured with a steam sterilizable amperometric probe which was connected to a meter calibrated between 0% and 100% saturation of the medium with oxygen; standard growth medium was employed except for a three-fold increase in the yeast extract and each Dubos mineral salt in one instance, as indicated; except where otherwise specified, temperature was maintained at 30°C and DOT between 60 and

100% saturation; (c) either soluble or insoluble xylan replaced the mixed product in some media; standard medium containing 0.5% xylan was prepared, autoclaved, cooled and centrifuged at 20,000g for 30 min; the supernatant served as the soluble xylan medium (0.34% carbohydrate); the residue was suspended in fresh medium to yield the insoluble xylan medium (0.4% carbohydrate); both media were resterilized; (d) the cellular fraction of the harvested cultures was not homogenized prior to assay of xylanolytic activity; the cells were collected by centrifugation, washed and suspended in buffer in the standard manner and used as an intact cell preparation.

2.4 Viable Cell Counts.

The culture was diluted 1 in 10^7 by serial dilution. 0.1 ml of each dilution was spread on nutrient agar plates in duplicate and incubated at 30°C until single colonies appeared. Viable cells were determined by counting the number of colonies on plates containing 50 to 200 colonies and taking the average value.

The nutrient agar plates were of the following composition (per l/ of distilled water): 25g nutrient broth No. 2 (Oxoid, CN 67), 5g yeast extract (Oxoid, L 21) and 12g Kobe agar. The diluent used for serial dilutions of the culture was 0.85% (w/v) sterile sodium chloride (B.D.H., A.R.).

2.5 Assay Procedures.

Xylanase, β -xylosidase, reducing sugar and total carbohydrate were assayed as described by Peiris.⁸ All enzyme activities, including those in the cellular fraction, are expressed in milliunits (mu)/ml of culture, where one International Unit releases one μ mole of product/min. Reducing sugar (RS) and total carbohydrate (TC) are expressed in μ g/ml; the percentage hydrolysis was calculated as the $(RC/TC) \times 100$.

3. Results and Discussion

3.1 Effect of Growth Parameters on Xylanolytic Activity

3.1.1 pH.

When the *Cellulomonas* strain was grown for two days in culture media buffered between pH 5.8 and 7.2, maximum levels of both xylanase and β -xylosidase activity were recorded at pH 7.1 (Table 1). The enzyme levels were, however, virtually constant between pH 6.2 and 7.2, the variation being considered to be within the limits of accuracy of the enzymic assays. The xylanase activity was less than that recorded in a standard (unbuffered) culture. Other growth parameters were tested in unbuffered cultures, initially adjusted to pH 7.2.

3.1.2 Temperature.

When the temperature was varied between 23°C and 40°C, maximum enzyme activity at the end of the first day was recorded in the culture grown at 35°C (Table 2). However, at the end of the second day, maximum xylanase activity was recorded in the culture grown at 30°C; similar levels of β -xylosidase activities were recorded in the cultures grown at 23°C and 30°C.

Table 1. Effect of pH on Production of Xylanolytic Activity by CSI-17 Grown for Two Days on 0.5% Xylan

pH	Buffer	Viable Counts/ml (cells x 10 ⁹)	Enzyme Activity (mu/ml of culture)			
			Xylanase		β -Xylosidase	
			Total	% Extra-cellular	Total	% Extra-cellular
5.8	MES	0.7	603	21	1.5	<10
6.2	MES	2.6	11002	87	10.4	3
6.4	MES	1.8	11398	85	9.6	2
6.4	MOPS	2.0	10159	87	11.3	< 1
7.1	MOPS	2.4	13122	87	12.2	3
7.2	MOPS	2.6	12942	88	10.7	3
7.2	(unbuffered)	4.0	18630	90	11.3	5

Table 2. Effect of Temperature on the Production of Xylanolytic Activity by CSI-17 Grown on 0.5% Xylan in a Fermentor

Culture Time (days)	Temperature (°C)	Viable Counts/ml (cells x 10 ⁹)	Enzyme Activity (mu/ml of culture)			
			Xylanase		β -Xylosidase	
			Total	% Extra-cellular	Total	% Extra-cellular
1	23	0.1	48	60	0.9	86
	30	3.4	4716	68	4.5	1
	35	0.9	6660	54	7.2	1
	40	0.03	2250	61	0.8	12
2	23	1.7	8226	51	12.8	1
	30	1.9	14040	83	12.2	2
	35	3.3	10485	88	8.7	2
	40	0.00	1054	78	0.3	28

3.1.3 Dissolved Oxygen Tension (DOT).

Growth and enzymic activity was very low in cultures grown under anaerobic conditions (DOT 0% saturation, maintained by bubbling sterile nitrogen gas into the medium) (Table 3). Although high aeration (DOT 60-100%, maintained by bubbling sterile air into the medium) promoted enhanced growth, xylanase production was maximized at moderate aeration (DOT 10-30% saturation, maintained by regulating the inflow of sterile nitrogen gas and air into the medium). Moderate aeration probably is comparable to standard shake flask conditions. The differences in β -xylosidase production at high and moderate aeration were minimal.

3.1.4 Mineral Salts and Yeast Extract.

Supplementation of the culture medium with a three-fold increase in the yeast extract and all the Dubos mineral salts had a marked effect on the rate of production of the xylanolytic enzymes, which were increased approximately three-fold over their levels in the culture containing the standard medium by the end of the first day (Table 4). By the end of the second day, however, the activities in the standard culture had virtually equalized with those in the supplemented culture.

Choudhury,¹ found that increased concentrations of Dubos salts and yeast extract in a growth medium containing 2% pretreated bagasse enhanced the viable count and the hydrolytic activity of CSI-17 cultures. They suggested that the increased activity was a direct result of the increased biomass. In the current study, where the growth substrate was 0.5% xylan, the effect of the salts and yeast extract on the rate of production of xylanolytic activity over the first day appeared not to be dependent on increased biomass but rather a direct effect on enzyme production per unit cell number.

It appears that, the addition of yeast extract and Dubos mineral salts to the growth medium had been effective in improving xylanolytic enzyme production by CSI-17 beyond that obtained in standard cultures. The variation of temperature, pH and DOT virtually had no effect on the enzyme production.

Under all the growth conditions tested, xylanase activity (at its maximum levels) was mainly present in the culture filtrate (extracellular fluid), whilst the β -xylosidase remained associated with the homogenized cells.

3.2 β -Xylosidase Location

It was conceivable that the association of the β -xylosidase in the cellular fraction of the cultures was the result of its adsorption to unutilized insoluble xylan substrate present in this fraction. In order to eliminate this possibility, an experiment was conducted where soluble xylan replaced the mixture of soluble and insoluble xylan as the growth substrate. After growth for two days by the standard procedure, the culture was fractionated into its cellular and extracellular (culture filtrate) fractions. The cells were washed twice with saline, resuspended in 25ml of dilute (1:10) McIlvaine's buffer² and a sample (intact cells) taken for assay. The remainder was homogenized according to the standard procedure and a sample taken for assay. The remainder of the

Table 3. Effect of Dissolved Oxygen Tension (DOT) on the Production of Xylanolytic Activity by CSI-17 Grown on 0.5% Xylan in a Fermentor

Culture Time (days)	DOT (%saturation)	Viable Counts/ml (cells x 10 ⁹)	Enzyme Activity (mu/ml of culture)			
			Xylanase		β -Xylosidase	
			Total	% Extra-cellular	Total	% Extra-cellular
1	60 - 100	3.4	4716	68	4.5	1
	10 - 30	0.6	4608	41	2.3	0.1
	0	0.4	211	34	0.7	4
2	60 - 100	7.8	14040	83	12.2	2
	10 - 30	2.9	18396	78	11.3	2
	0	0.1	217	31	0.7	13

Table 4. Effect of Increased Concentrations of Dubos Salts and Yeast Extract on the Production of Xylanolytic Activity by CSI-17 Grown on 0.5% Xylan in a Fermentor

Culture Time (days)	Dubos Salts and Yeast Extract Concentration	Viable Count/ml (cells x 10 ⁹)	Enzyme Activity (mu/ml of culture)			
			Xylanase		β -Xylosidase	
			Total	% Extra-cellular	Total	% Extra-cellular
1	Standard	3.4	4716	68	4.5	1
	x 3	3.7	13086	63	17.6	0.5
2	Standard	7.8	14040	83	12.2	2
	x 3	11.8	15732	80	13.8	1

homogenate was centrifuged at 17,000g for 15 min and the supernatant was designated the intracellular fraction. The pellet, after resuspension in 10ml of dilute McIlvaine's buffer, was designated the cell wall fraction. The intact cells, homogenized cells, the intracellular fraction, the wall fraction and the culture filtrate were all assayed for β -xylosidase activity. Results (Table 5) were all corrected for volume changes and in common with other results, are reported in m units/ml of original culture.

Only 2% of the activity was located in the extracellular fraction, the remainder being associated with the intact cells. 37% of the cellular activity was 'lost' during homogenization, presumably as a result of enzyme inactivation. 69% of the residual activity in the homogenized cells was associated with the wall fraction while 31% was intracellular. The activity of the intact cells suggests that either the β -xylosidase is located on the outside of the cells or that the synthetic substrate used in this investigation readily passes into the cells and the nitrophenol product is readily excreted. The fact that the cell wall fraction possessed more than twice the activity of the intracellular fraction supports the concept of the enzyme's location on or in the cell wall.

As soluble xylan was the substrate, the results minimize the possibility that the location of the enzyme in the standard cellular preparations (containing homogenized cells plus unutilized insoluble xylan) is due to its adsorption on to the insoluble xylan.

3.3 Enzyme Stability

This study was part of an overall aim to maximize the ability of preparations from *Cellulomonas* cultures to hydrolyse pretreated bagasse. It was therefore necessary to ensure that the enzymes, including the xylanolytic enzymes, were stable after harvest. The enzymes tested were the β -xylosidase of intact cell preparations and the extracellular xylanase of a culture of CSI-17 which had been grown for two days on 0.5% xylan under standard conditions.

In order to test the effect of pH on storage stability, portions of the culture filtrate and intact cell preparations were adjusted to specified pH values by the addition of dilute (1:10) McIlvaine's buffer.² These were then stored at specified temperatures and the extracellular xylanase and cellular β -xylosidase activities

Table 5. Distribution of β -Xylosidase in a CSI-17 Culture Grown for Two Days on Soluble Xylan

Fraction	β -Xylosidase Activity (μ /ml of culture)
Extracellular (culture filtrate)	0.2
Cellular	
Intact cells	10.1
Homogenized cells (standard preparation)	6.4
Intracellular fraction	2.0
Wall fraction	4.4

determined at zero time and after one and two days storage. 0.2% azide was present, as a preservative and metabolic inhibitor, in all portions. Just prior to assay of each portion for enzyme activity the pH was adjusted to 7.0 by the addition of either 0.1 M citric acid or 0.2 M disodium hydrogen phosphate, the components of McIlvaine's buffer. Volume changes were accounted for in the calculation of activity per ml of original preparation.

Initial pH values had been adjusted to 5.0, 6.0, 7.0 and 8.0. During storage, however, pH changes occurred, presumably as a result of certain metabolic reactions which had not been inhibited by 0.2% azide. In all cases mean values between initial and final pH are recorded.

The results, recorded in Table 6, revealed that, at least for two days, the extracellular xylanase was stable at pH values between 5.4 and 8.4 and temperatures up to and including 30°C. At 42°C, it was unstable at all pH values tested and lost at least 50% of its initial activity within one day.

The stability of the cellular β -xylosidase was pH and temperature dependent. At all pH values tested, it was relatively stable at 4°C, but at likely process temperatures (25°C, 30°C and 42°C) it lost more than 50% of its activity during storage for two days at pH 5.3 and 6.1. At the higher pH values tested (6.9 and 7.4) it retained at least 70% of its activity at 25°C and 30°C and 50% at 42°C. This suggests that to maintain this enzyme in an industrial process the pH should not be allowed to decline below 6.9 (or perhaps a lower, untested, value between 6.1 and 6.9).

3.4 Extent of Hydrolysis of Xylan

In order to determine whether non-growing cultures of *Cellulomonas* were as effective as growing cultures in hydrolysing xylan, *in vivo* and *in vitro* tests were conducted. Both soluble and insoluble xylan were used as substrates for digestion in each case. The extent of hydrolysis during growth over two days under standard conditions was compared with that obtained over a further two days after addition of fresh substrate.

Each *in vitro* digest contained 15 ml of enzyme (culture filtrate from the standard culture) plus 15 ml of fresh xylan medium and 0.2% sodium azide. Incubation was carried out at 40°C. Zero time samples were prepared by mixing enzyme which had been boiled for half an hour with an equal volume of fresh xylan medium.

In the case of both the *in vivo* and the *in vitro* tests, the zero time samples and those collected after incubation for two days were boiled for 15 min and reducing sugar (RS) and total carbohydrate (TC) measured. The percentage hydrolysis of the residual carbohydrate was calculated from the values for the two day samples, using the expression $(RS/TC) \times 100$. In addition, the difference between the initial and final TC values was considered to be metabolized and therefore fully hydrolysed carbohydrate. Its percentage of the initial TC was added to the percentage hydrolysis of the residual carbohydrate (corrected for the fraction of the initial carbohydrate which it represented) to give the total percentage hydrolysis. The initial RS was comparatively high in the *in vitro* tests due to 'carry over' with the enzyme preparation.

Table 6. Storage Stability of Xylanolytic Activity of CSI-17 Preparations

Storage Temperature	Storage	pH	Storage Time (days)	Relative Xylanase (culture filtrate)	Relative β -Xylosidase (Intact cell preparation)
	Culture Filtrate	Intact cell Preparation			
40°C	5.4	5.3	0	100	100
			1	130	110
			2	120	100
	6.2	6.1	1	95	96
			2	94	78
	7.1	6.9	1	84	103
			2	101	101
	8.4	7.4	1	120	80
			2	118	81
25°C	5.4	5.3	1	108	80
			2	105	43
	6.2	6.1	1	85	77
			2	85	35
	7.1	6.9	1	84	96
			2	84	99
	8.4	7.4	1	105	84
			2	102	72
30°C	5.4	5.3	1	117	58
			2	109	22
	6.2	6.1	1	89	63
			2	88	28
	7.1	6.9	1	84	96
			2	94	90
	8.4	7.4	1	98	80
			2	98	81
42°C	5.4	5.3	1	43	13
			2	34	3
	6.2	6.1	1	43	17
			2	46	13
	7.1	6.9	1	44	75
			2	34	54
	8.4	7.4	1	40	58
			2	11	52

The values for percentage hydrolysis can be considered as approximations only. Use of the DNS method for measurement of reducing equivalents was based on the fact that it is widely used for the determination of reducing sugars liberated by α -amylase¹³ and by carboxymethylcellulase.⁶ Since the reducing power (on a molar basis) of an oligomeric series increases with chain length, falsely high values are given for percentage hydrolysis when dimers and oligomers are present in the digest. The discrepancy does however decrease with chain length; this is evident when the ratio (1.0:1.4) of reducing powers of glucose and maltose⁵ is compared with the ratio of (1.0:1.95) of reducing powers of maltose and maltoheptaose.¹² The method therefore provides a relatively simple semi-quantitative means of comparing the abilities of different enzyme preparations to hydrolyse polysaccharides.

The results (Tables 7 and 8) reveal that neither the *in vivo* nor the *in vitro* system was capable of hydrolysing soluble or insoluble xylan completely. The total percentage hydrolysis values indicated that *in vitro* the cell free culture filtrate was just as effective as a living culture in hydrolysing about 75% of the soluble xylan. Only when the cell free system was obtained from a culture grown on insoluble xylan was there an apparent decrease (from 67% to 50%) in the ability of the culture filtrate to match that of the living culture to hydrolyse insoluble xylan. (This may perhaps indicate that a factor (or factors) required for effective hydrolysis of insoluble xylan remained associated with the residual insoluble xylan of the culture; this was a significant proportion of the initial carbohydrate and was discarded when the cell free culture filtrate was separated and used as the enzyme source). Minimization of carbohydrate metabolism *in vitro* resulted in the products of hydrolysis accumulating as soluble reducing sugars.

A further experiment was undertaken to determine whether inclusion of cells in the *in vitro* enzyme digest would improve the rate and extent of soluble xylan hydrolysis. A standard culture of CSI-17, grown on soluble xylan, was divided into two portions; one was centrifuged and the cell-free culture filtrate collected, whilst the other was left untreated. Both preparations served as enzyme preparations for *in vitro* digestion of a soluble xylan. In contrast to the previous test, the reaction mixture was buffered at pH 7.0 to ensure maximum enzyme stability (see above) and enzyme activity.¹⁰ Enzyme stability was also improved by incubating the digests at 30°C, rather than at the optimum (40°C) for enzyme activity¹⁰ as in the previous test.

Flasks containing 40ml of soluble xylan, 40ml of McIlvaine's buffer (pH 7.0), 40 ml of enzyme and 0.2% sodium azide, were incubated at 30°C for two days. Samples, taken at various time intervals, were boiled for 15 min and the reducing sugar (RS) and the total carbohydrate (TC) determined (after removal of cells by centrifugation, where appropriate). Controls were prepared using enzyme sources which had been boiled previously for 1hr before mixing them with soluble xylan solution and buffer in the ratio 1:1:1. The controls were assayed in the same manner as the samples. In all cases, the approximate degree of polymerization (DP) was

calculated by dividing the TC by the RS; the same limitations in accuracy apply to calculation of DP as to calculation of percentage hydrolysis (see above).

Results are recorded in Table 9. Their control values reveal that the introduction of relatively low molecular weight fragments with the enzyme preparations made interpretation difficult. They do demonstrate however that an initial rapid rate of hydrolysis, detected even at zero time and continuing over 5 hr was followed by minimal further activity. Neither digest was capable of completely hydrolysing the soluble xylan; even at the end of two days, the DP was still 1.5 (67% hydrolysis) in both cases.

Addition of fresh xylan at 5 hr to the culture filtrate resulted in it too being hydrolysed to the same extent as the original material. This indicated that the cessation of activity was not due to enzyme inactivation but rather to resistance of the substrate to complete hydrolysis by the enzyme preparation. A possible explanation is that CSI-17 produces xylanolytic activity which is inversely proportional to chain length and which fails to hydrolyse the low molecular weight xylose oligomers.

The investigation suggested that cell-free preparations of *Cellulomonas* CSI-17 are similar to growing cultures of the strain in their ability to hydrolyse xylan. In comparison to the *in vivo* conditions, where the products were metabolized, relatively high levels of reducing sugars accumulated in the soluble fraction of the *in vitro* digests. This suggests that the latter system is one which is appropriate for saccharification of the hemicellulose fraction of lignocellulose.

Table 7. *In Vivo* Hydrolysis of Soluble and Insoluble Xylan by CSI-17 Cultures

Xylan Medium	Time (days)	Soluble Reducing Sugar ($\mu\text{g/ml}$)	Total Carbohydrate $\mu\text{g/ml}$			% Hydrolysis of Residual Carbohydrate	% Total Carbohydrate Metabolized	% Total Hydrolysis
			Soluble	Insoluble*	Total			
Soluble	0	110	3350	66	3416			
	2	340	1020	177	1197	28		
	Δ	+230	-2330	+111	-2219		65	75
Insoluble	0	70	1600	2400	4000			
	2	50	155	1222	1377	4		
	Δ	-20	-1445	-1178	-2623		66	67

* Includes microbial cells

Table 8. *In Vitro* Hydrolysis of Soluble and Insoluble Xylan by a CS1-17 Extracellular Enzyme Preparation (Culture Filtrate)

Xylan Substrate	Extra-cellular Enzyme Source	Incubation Time (days)	Soluble Reducing Sugar ($\mu\text{g/ml}$)	Soluble	Total Carbohydrate ($\mu\text{g/ml}$) Insoluble	Total	% Hydrolysis of Residual Carbohydrate	% Total Carbohydrate Metabolized	% Total Hydrolysis
Soluble	Soluble Xylan	0	225	1900	33	1933			
	Xylan Culture	2	1495	2050	0	2050	73		
		Δ	+1265	+150	-33	+117		0	73
	Insoluble Xylan	0	80	1700	33	1733			
	Xylan Culture	2	1260	1650	0	1650	76		
		Δ	+1180	-50	-33	-83		5	77
Insoluble	Soluble Xylan	0	205	980	1200	2180			
	Xylan Culture	2	1080	1220	580	1800	60		
		Δ	+875	+240	-620	-380		17	67
	Insoluble Xylan	0	60	720	1020	1740			
	Xylan Culture	2	840	1080	640	1720	49		
		Δ	+780	+360	-380	-20		1	50

Table 9. *In Vitro* Time Course of the Decrease in the Degrees of Depolymerization (DP) of Soluble Xylan (DP.24.4) by CSI-17 Preparations

Time	DP*	
	Total Culture	Culture Filtrate
Control - 0 min	4.7	5.8
0 min	3.2	4.0
7 min	2.4	2.7
18 min	2.0	2.4
22 min	2.0	2.0
45 min	1.7	2.2
1 hr	1.9	2.0
2 hr	1.8	1.9
5 hr	1.7	1.6
20 hr	1.5	1.6
30 hr	1.5	1.5
48 hr	1.5	1.6

* Degree of polymerization = TC/RS

Although the initial *in vitro* rate was rapid, the reaction ceased before complete hydrolysis was reached. Even when the digestion was carried out under conditions which maximized enzyme stability, only about 67% hydrolysis was attained by the cell-free extracts. As the β -xylosidase activity is located in the *Cellulomonas* cells and since this enzyme could be necessary for hydrolysis of the oligomers released by xylanase activity, it was considered that inclusion of cells in the digest may improve the degree of hydrolysis; this proved not to be the case.

Investigations are in progress to identify the products of enzymic digestion of xylan and to improve conditions for their further hydrolysis.

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