SNEATH, P. H. A.: In Bergey's Manual of Systematic Bacteriology, Vol. 2. Ed. by SNEATH, P. H. A., NAIR, N. S., and SHARPE, M. E., The Williams and Wilkins Co., Baltimore (1986), 1104–1139.

STOPLER, T. V., CAMUESCU, V., and VOICULESCU, M.: Broncho-pneumonia with lethal evolution determined by a microorganism of the genus *Bacillus (B. cereus)*. Romanian Med. Rev. **19** (1969), 7–9.

SUGAR, A. M., and MCCIOSKEY, R. V.: *Bacillus licheniformis* sepsis. JAMA **238** (1977), 1180–1181.

TUAZON, C. V., MURRAY, H. W., LEVY, C., SOLNY, M. N., CURTIN, J. A., and SHERGREN, J. N.: Serious infections from *Bacillus spp.* JAMA **241** (1979), 1137–1140.

WAKISAKA, Y., and KOIZUMI, K.: An enrichment isolation procedure for minor *Bacillus* populations. The journal of Antibiotics **XXXV** (1982), 450–457.

Author's address: Dr. A. A. M. SHOREIT and M. A. ISMAIL, Assiut University, Faculty of Science, Botany Department, Assiut, 71516, Egypt.

Zentralbl. Mikrobiol. **147** (1992), 551–556
Gustav Fischer Verlag Jena

[Faculty of Education and Faculty of Science, Ain Shams University, Cairo, Egypt]

Effect of Alkali Pretreatment on Degradation of Some Cellulosic Wastes by *Aspergillus sydowii*

Wirkung einer Alkali-Vorbehandlung auf die Zersetzung einiger cellulosehaltiger Abprodukte durch *Aspergillus sydowii*

MOHAMED GHAREIB, KHIRIYA A. YOUSSEF, and M. M. NOUR EL DEIN

Key words: Cellulose degradation, alkali pretreatment, *Aspergillus sydowii*

Summary

Alkali pretreatment of pods of bean, rice plant straw, wheat bran, sugar-cane bagasse and sawdust enhanced their degradation by *Aspergillus sydowii* (BAINIER & SARTORY) THOM & CHURCH. The fungus could produce 0.153 g dry mycelium when grown on 100 ml of 10% NaOH pretreated sawdust-containing medium. Alkali hydrolysates were assimilated better than the cellulosic pulps of different wastes. Maximal growth amounting to 0.239 g/100 ml dry biomass was produced on hydrolysate of 10% NaOH pretreated sawdust for 30 minutes. Highest accumulation of different cellulases and pectinase was occurred on cellulosic pulps-containing media. Good xylanolytic activity has been achieved on both cellulosic pulps and hydrolysates media. Cellulases, xylanase and pectinase of *A. sydowii* were proved to be an inducible enzymes.

Zusammenfassung

Eine Alkali-Vorbehandlung von Bohnenhülsen, Reisstroh, Weizenkleie, Zuckerrohrbagasse und Sägespänen erhöhte ihre Degradation durch *Aspergillus sydowii* (BAINIER und SARTORY) THOM und CHURCH. Der Pilz konnte 0,153 g Trockenmyzel bilden, wenn er in 100 ml eines mit 10%iger NaOH vorbehandelten Kleinmediums wuchs. Alkalihydrolysate wurden besser assimiliert als Cellulosebrei verschiedener Abprodukte. Bestes Wachstum (bis zu 0,239 g/100 ml Trockenbiomasse) fand auf dem Hydrolysat von Sägespänen statt (Vorbehandlung 10% NaOH, 30 min.).

Most fungi cannot utilize many agricultural and industrial wastes. Proper pretreatments would enhance the bioavailability of these wastes. Alkali pretreatment has been reported to be a convenient method in this regard (DETROY et al., 1982; FARID et al., 1983; GOLOVLYOVA et al., 1986 and TODOROVIC and GUJIC, 1987). TSAO and CHIANG (1983) reported that alkali pretreatment is the best known method due to its multiple effects.

This work was embodied to investigate the degradation efficiency of *A. sydowii* when grown on NaOH pretreated cellulosic wastes.

Materials and Medthods

Organism: *A. sydowii* (BAINIER & SARTORY) THOM & CHURCH was isolated from Egyptian soil and identified by the International Mycological Institute, England. The funguns was maintained on solid Czapek's medium and subcultured whenever required.

Precedure of pretreatment: Five different cellulosic wastes viz. pods of bean, rice plant straw, wheat bran, sugar-cane bagasse and sawdust were dried and ground until they passed through a 40-mesh screen. Fine powders were treated separately with sodium hydroxide as follows: Certain quantity of a waste was suspended in

5 and 10% solutions of NaOH in a ratio of 1 : 10 (W/V) at 121 °C for 30 and 60 min. The residues were separated by filtration, washed with water until alkali-free, suspended in small volumes of ethanol, filtered and air dried. Alkali hydrolysates were neutralized using solution of HCl.

Media and Cultivation: The dried residues were separately added, in a ratio that litre of the resulting media contained 10 g total carbohydrates, to the following ingredients (g/l): $NaNO_3$, 3; KH_2PO_4, 1; $MgSO_4 \cdot 7H_2O$, 0.5; KCl, 0.5 and $FeSO_4 \cdot 5H_2O$, 0.05. Triplicate sets each of five 250 ml Erlenmeyer flasks were used in this work. Each flask was charged with 20 ml of the fermentation media to ensure a direct physical contact between the fungal mycelia and all constituents of the media. The flasks were sterilized, left to cool, inoculated with equal volumes of spore suspension and incubated statically for 10 days at 30 °C. When the filter-sterilized hydrolysates were used as sole source of carbon, they were added aseptically to the autoclaved inorganic ingredient just prior to inoculation. At the end of the incubation period, each group of five flasks were filtered and their contents mixed and completed to 100 ml with sterilized distilled water. Mixtures of fungal mycelia and residual cellulosic wastes were dried to constant weights. The fungal growth was calculated by the method of NORDSTRÖM (1974) which is based on the increase of nitrogen content of the solid materials.

Analysis of cellulosic wastes: The substrates were analyzed for their contents of pectin, hemicellulose, cellulose and lignin using the methods of JERMYN (1955).

Enzyme assays: Avicelase (C_1), carboxymethylcellulase (C_x) and β-glucesidase (C_2) were assayed colourimetrically using the procedure adopted by MANDELS et al. (1976). The reactions lasted for 30 min at 40 °C. Released reducing sugars were estimated by the dinitrosalicylic acid method (MILLER, 1959). One unit of cellulase activity was defined as the amount of enzyme which liberates reducing sugars equivalent to 1 µmol of glucose per min.

Xylanase activity was determined by a procedure similar to that of cellulase assay with the only difference that xylan was used as substrate. One unit of xylanase activitiy was defined as the amount of enzyme required for the hydrolysis of reducing sugars equivalent to 1 µmol of xylose per min.

Pectinase activity was determined viscometrically by the method of ERIKSSON and GOODELL (1974). One unit of pectinase activity was defined as the amount of enzyme which produces 1 µmol of galacturonic acid per min.

Statistical analysis: Fungal growth was expressed as the mean values of the three replicate along with standard deviation. T-test and least significant difference (L.S.D., $p > 0.05$ = non significant, NS; p: 0.05 to >0.01 = significant, S and $p \leq 0.01$ = high significant, HS) were used in evaluation of fungal growth comparative to the respective controls.

Results and Discussion

Alkali pretreatment of waste cellulosic materials improved their degradation by *A. sydowii*. Pretreatment process changed to great extent the chemical composition of the experimented wastes. Table 1 reveals that alkali pretreatment yielded 74.4−82.7% cellulosic pulps of pods of bean and 62.4−77.3% of the other wastes. 44−56% of hemicelluloses were extracted into the hydrolysates at the time that 4−11% of pectin were lost by alkali pretreatment. Moreover, 55−77% of lignin were removed from the lignified tissues.

TSAO and CHIANG (1983) reported that alkali pretreatment is probably the oldest and best known method of enhancement of microbial degradation of cellulosics. The effectiveness of alkali pretreatment was demonstrated with an increase in both enzyme activities and dry mycelia. HS improvement of fungal growth comparative to the respective control was obtained on alkali pretreated sawdust. An exception of this generalization was the growth of *A. sydowii* on sawdust pretreated for 30 min with 5% NaOH where S increase was occurred. 0.153 g dry mycelium was achieved on 100 ml of medium containing 10% NaOH pretreated sawdust for 30 min. S increase was obtained on media containing 10% NaOH pretreated sugar-cane bagasse and rice plant straw as well as bagasse pretreated with 5% NaOH for 30 min. NS increase of growth was achieved on media with pretreated pods of bean and wheat bran. NS increase was also occurred on 5% NaOH pretreated rice straw.

Concerning enzyme activities of *A. sydowii* on cellulosic pulps-containing media, the results (Table 2) demonstrates the production of 1.19 units/ml of C_1 on wheat bran pretreated with 10% NaOH for 30 min. 1.16 units/ml was obtained on 10% NaOH pretreated sawdust.

Table 1. Chemical composition of cellulosic biomasses after alkali pretreatment

Waste	NaOH conc. (%)	Residual yield (%)	(%)			
			Cellulose	Hemi-cellulose	Pectin	Lignin
Pods of bean	–	100	35.2	35.0	20.7	–
	5	82.7	42.6	23.7	24.1	–
		80.1	43.9	22.2	24.1	–
	10	76.2	46.2	22.0	24.9	–
		74.4	47.3	20.7	25.0	–
Rice plant straw	–	100	34.3	33.1	4.6	15.4
	5	70.4	48.7	26.2	6.2	9.8
		67.1	51.1	25.2	6.4	8.2
	10	65.0	52.7	24.5	6.5	7.1
		62.4	54.9	23.2	6.6	5.6
Wheat bran	–	100	38.0	30.9	16.0	–
	5	77.3	49.1	22.4	20.1	–
		74.9	50.7	20.9	20.1	–
	10	73.2	51.6	20.2	20.2	–
		71.4	53.2	19.0	20.3	–
Sugar-cane bagasse	–	100	41.7	23.8	5.2	21.0
	5	75.8	55.0	17.5	6.4	12.6
		72.5	57.5	16.7	6.6	10.4
	10	70.1	59.4	16.2	6.7	8.9
		67.5	61.7	15.5	6.8	7.1
Sawdust	–	100	47.3	24.1	1.1	25.2
	5	74.2	63.6	18.2	–	15.2
		70.7	66.8	17.4	–	12.9
	10	68.2	69.3	16.8	–	11.1
		65.2	72.5	16.2	–	8.8

– For each concentration of NaOH the first line represent the results after pretreatment for 30 minutes, the second line are those after 60 minutes.

= All values of chemical analysis are average of two estimations.

10% NaOH treated pods of bean for 30 min offered a convenient carbon source for accumulation of 1.16 units/ml of C_x. Treatment of wheat bran with 10% NaOH for 60 min enabled the fungus to excrete 1.12 and 0.95 units/ml of C_x and C_2, respectively.

Xylanase accumulation reached the maximum i.e. 1.42 units/ml when the organism was grown on medium containing pods of bean pretreated with 10% NaOH for 30 min. A content of 1.29 units/ml of xylanase was obtained on 10% NaOH treated rice straw for 60 min.

Pods of bean pretreated with 10% NaOH for 60 min improved the pectolytic activity of *A. sydowii* to the rate of 11.93 units/ml.

The previous results reveals that the cellulosic pulps were degraded by *A. sydowii* more easily than the untreated cellulosics. This can be attributed to the multiple effects of alkali pretreatment i.e. removal of great part of lignin, an increase in surface area by swelling and an alteration of crystalline and amorphous structure of cellulosics (Tsao and Chiang, 1983). Needless to say that lignin barriers and crystalline structure are the two major deterrents to cellulosics hydrolysis (Tsao et al., 1978).

Table 2. Growth and enzyme activities of *A. sydowii* on media containing alkali pretreated cellulosic wastes

Waste	NaOH conc. (%)	Dry weight (g/100 ml)	Enzyme activities (units/ml)				
			C_1	C_x	C_2	X	P
Pods of bean	–	0.133 ± 0.006	0.92	0.61	0.44	0.69	3.86
	5	0.137 ± 0.009 NS	1.09	0.99	0.58	0.99	10.64
		0.143 ± 0.007 NS	1.16	1.12	0.65	1.22	11.00
	10	0.148 ± 0.007 NS	1.16	1.16	0.82	1.42	11.85
		0.148 ± 0.006 NS	1.16	1.16	0.88	1.35	11.93
Rice plant straw	–	0.114 ± 0.004	0.48	0.56	0.44	0.76	3.47
	5	0.116 ± 0.005 NS	0.78	0.82	0.56	1.09	6.42
		0.124 ± 0.006 NS	0.85	0.92	0.65	1.22	6.61
	10	0.136 ± 0.006 S	0.92	1.05	0.78	1.29	7.11
		0.140 ± 0.007 S	0.92	1.09	0.85	1.19	7.11
Wheat bran	–	0.140 ± 0.007	0.95	0.71	0.65	0.73	4.26
	5	0.147 ± 0.007 NS	1.05	0.92	0.78	1.02	10.60
		0.150 ± 0.006 NS	1.05	1.05	0.85	1.16	10.81
	10	0.151 ± 0.007 NS	1.16	1.12	0.92	1.16	10.94
		0.151 ± 0.009 NS	1.19	1.12	0.95	1.12	11.00
Sugar-cane bagasse	–	0.098 ± 0.006	0.56	0.58	0.34	0.53	3.96
	5	0.109 ± 0.006 NS	0.75	0.65	0.48	0.96	5.40
		0.115 ± 0.005 S	0.92	0.71	0.58	1.12	5.64
	10	0.117 ± 0.005 S	1.05	0.78	0.71	1.06	5.73
		0.117 ± 0.005 S	1.09	0.82	0.78	0.99	5.80
Sawdust	–	0.104 ± 0.006	0.56	0.34	0.41	0.42	4.30
	5	0.125 ± 0.005 S	0.85	0.48	0.56	1.06	4.80
		0.143 ± 0.005 HS	0.99	0.61	0.75	1.09	4.61
	10	0.153 ± 0.005 HS	1.16	0.65	0.78	0.99	4.55
		0.151 ± 0.005 HS	1.16	0.75	0.82	0.83	4.55

– All values of enzyme activities are average of two estimations.

It was found suitable to study the behaviour of *A. sydowii* on media containing alkali hydrolysates of different wastes (Table 3). The fungus utilized the extracted hemicelluloses to form a considerable yield of biomass. 0.239 g dry mycelium was produced on 100 ml of medium containing hydrolysate of 10% NaOH treated sawdust for 60 min as a sole source of carbon. 0.22 and 0.215 g/100 ml dry weights were achieved on hydrolysates of 10% NaOH pretreated pods of bean and wheat bran for 60 min, respectively. These results accord completely with the findings of DETROY et al. (1982) who found that alkali treatment of lignocellulosics yielded hemicellulose fraction available for utilization by fungi.

Low content of different cellulases was detected in culture filtrates of *A. sydowii* grown on the alkali hydrolysates. This can be explained on the basis of the fact that cellulases are inducible enzymes (REESE and MANDELS, 1963). Xylan which is the principal component of alkali hydrolysates is not an inducer of cellulase (ERIKSSON and GOODELL, 1974). In view of the previous interpretation, increase for xylanase in the same culture filtrates was not contrary to expectation since it is an inducible enzyme also (KING, 1966; VARADI, 1972 and SANDHU et al., 1983). However, there are some examples of constitutive xylanases (REESE and MANDELS, 1963). *A. sydowii* produced the highest content of xylanase on alkali hydrolysates amounting to 1.78 units/ml when the carbon source was 10% NaOH treated sawdust for 60 min.

Table 3. Growth and enzyme activities of *A. sydowii* on alkali hydrolyzate-containing media

Waste	NaOH conc. (%)	Dry weight* (g/100 ml)	Enzyme activities (units/ml)				
			C_1	C_x	C_2	X	P
Pods of bean	–	0.133 ± 0.006	0.92	0.61	0.44	0.69	3.86
	5	0.168 ± 0.006 S	0.68	0.65	0.48	0.73	2.41
		0.185 ± 0.004 HS	0.71	0.65	0.48	1.12	2.70
	10	0.191 ± 0.005 HS	0.71	0.61	0.48	1.32	2.94
		0.220 ± 0.006 HS	0.71	0.68	0.48	1.45	3.10
Rice plant straw	–	0.114 ± 0.004	0.48	0.56	0.44	0.76	3.47
	5	0.116 ± 0.004 NS	0.48	0.51	0.37	0.92	3.17
		0.142 ± 0.005 HS	0.51	0.51	0.37	1.22	3.51
	10	0.158 ± 0.005 HS	0.51	0.51	0.37	1.32	3.87
		0.167 ± 0.005 HS	0.55	0.51	0.34	1.52	3.90
Wheat bran	–	0.140 ± 0.007	0.95	0.71	0.65	0.73	4.26
	5	0.194 ± 0.004 HS	1.09	0.78	0.56	1.02	3.66
		0.209 ± 0.006 HS	1.09	0.82	0.56	1.29	3.89
	10	0.211 ± 0.005 HS	1.12	0.82	0.56	1.29	4.00
		0.251 ± 0.005 HS	1.12	0.82	0.56	1.39	4.38
Sugar-cane bagasse	–	0.098 ± 0.006	0.56	0.58	0.34	0.53	3.96
	5	0.122 ± 0.006 S	0.44	0.48	0.00	0.89	3.60
		0.135 ± 0.004 HS	0.44	0.44	0.34	1.19	3.74
	10	0.139 ± 0.005 HS	0.48	0.44	0.34	1.25	3.80
		0.153 ± 0.005 HS	0.48	0.44	0.34	1.32	3.85
Sawdust	–	0.104 ± 0.006	0.56	0.34	0.41	0.42	4.30
	5	0.194 ± 0.007 HS	0.71	0.61	0.34	1.12	4.17
		0.201 ± 0.005 HS	0.71	0.58	0.37	1.52	4.40
	10	0.237 ± 0.006 HS	0.75	0.61	0.37	1.75	4.72
		0.239 ± 0.006 HS	0.75	0.61	0.37	1.78	4.72

Worthy of mentioning that higher growth of *A. sydowii* was mostly achieved on hydrolysate-containing media. This may be due to the ease of assimilation of hemicelluloses than cellulose. Production of maximal yields of cellulases and pectinase on cellulosic pulps-containing media, in addition to the high content of xylanase in both residue and hydrolysate-containing media confirmed that these enzymes are inducible. The results accumulated throughout this work manifested the possibility of exploiting all products of alkali pretreatment (cellulosic pulps and hydrolysates) for growth and enzyme production by *A. sydowii*.

References

Detroy, R. W., Cunningham, R. L., Bothast, R. J., Bagby, M. O., and Herman, A.: Bioconversion of wheat straw cellulose/hemicellulose to ethanol by *Saccharomyces uvarum* and *Pachysolen tannophilus*. Biotechnol. Bioeng. **24** (1982), 1105–1113.

Eriksson, K.-E., and Goodell, E. W.: Pleiotropic mutants of the wood-rotting fungus *Polyporus adustus* lacking cellulase, manannase and xylanase. Can. J. Microbiol. **20** (1974), 371–378.

Farid, M. A., Shaker, H. M., and El-Diwany, A.: Effect of peracetic acid, sodium hydroxide and phosphoric acid on cellulosic materials as a pretreatment for enzymatic hydrolysis. Enzyme Microb. Technol. **5** (1983), 421–424.

GOLOVLYOVA, L. A., GREKOVA, G. A., CHERMENSKY, D. N., and GAVRILOV, A. V.: Bioconversion of straw and sawdust pretreated by different physical and chemical methods. Prikl. Biokhim. Mikrobiol. **22** (1986), 715.

JERMYN, M. A.: Cellulose and hemicellulose. In: Modern methods of plant analysis, vol. 2 (K. PEACH & M. V. TRACEY, eds.) 1955, 197–224.

KING, N. J.: The extracellular enzymes of *Coniophora cerebella*. Biochem. J. **100** (1966), 784–792.

MANDELS, M., ANDREOTTI, R., and ROCHE, C.: Measurement of saccharifying cellulase. In Biotechnol. Bioeng. Symp. **6** (1976), 21–34.

MILLER, G. L.: Use of dinitrosalicylic reagent for the determination of reducing sugars. Anal. Chem. **31** (1959), 426–428.

NORDSTRÖM, U. M.: Bark degradation by *Aspergillus fumigatus:* growth studies. Can. J. Microbiol. **20** (1974), 283–298.

REESE, E. T., and MANDELS, M.: Enzymatic hydrolysis of β-glucans. In: Advances in enzymic hydrolysis of cellulose and related materials (edited by E. T. REESE. Pergamon Press). Oxford 1963, 197–234.

SANDHU, D. K., KAUR, B., SIDHU, M. S., and SINGH, S.: Association of *Aspergillus fumigatus* with sugarcane bagasse, biochemical and physiological stydies. Trans. Br. Mycol. Soc. **81** (1983), 213–219.

TSAO, G. T., and CHIANG, L.-C.: Cellulose and hemicellulose technology. In: The filamentous fungi, vol. 4-Fungal technology (E. S. JOHN et al., eds.). EDWARD ARNOLD (publishers) LTD, London 1983, 296–326.

TSAO, G. T., LADISCH, M. R., LADISCH, C., HSU, T. A., DALE, B., and CHOU, T.: Fermentation substrates from cellulosic materials: Production of fermentable sugars from cellulosic materials. Anual Reports on Fermentation Processes **2** (1978), 1–21.

TODOROVIC, R., and GUJIC, S.: Effect of pretreatment and reaction products on hydrolysis of wheat straw by *Trichoderma harzianum* C_1R_1 enzymes. Microbios Lett. **34** (1987), 71–78.

VARADI, J.: The effect of aromatic compounds on cellulase and xylanase production of fungal *Schizophyllum commune* and *Chaetomium globosum*. In: Biodeterioration of materials, vol. 2 (edited by A. H. WALTERS and E. H. HUCK-VAN DER PLAS. Appl. Sci. Publ.). London 1972, 129–135.

Author's address: Dr. MOHAMED GHAREIB and MAHMOUD M. NOUR EL DEIN, Biology Department, Faculty of Education, Ain Shams University, Roxy, Cairo, Egypt; Dr. KHIRIYA A. YOUSSEF, Botany Department, Faculty of Science, Ain Shams University, Abbasiah, Cairo, Egypt.

Zentralbl. Mikrobiol. **147** (1992), 557–561
Gustav Fischer Verlag Jena

[Department of Botany, University of Sagar, Sagar, India]

Influence of Native Carbon Sources on the Production of Pectolytic and Cellulolytic Enzymes by *Fusarium oxysporum* and *Fusarium moniliforme*

Der Einfluß natürlicher Kohlenstoff-Quellen auf die Bildung von pectolytischen und cellulolytischen Enzymen aus *Fusarium oxysporum* und *Fusarium moniliforme*

A. Mehta, S. Chopra, V. Kare and P. Mehta

Key words: Fusarium oxysporum, F. moniliforme, bectolytic, cellulolytic enzyme

Summary

Pectin in the case of *F. oxysporum* and sodium polypectate in the case of *F. moniliforme* were proved to be the best native carbon sources for polygalacturonase (PG) production. Maximal pectinmethyl-galacturonase (PMG) production was recorded with pectin in both the test pathogens. Cotton supported maximal cellulase production in *F. oxysporum* whereas CMC in *F. moniliforme*. Sucrose which served as control was inferior to all the native carbon sources for the production of pectic and cellulolytic enzymes. Both the species of *Fusarium* were capable of producing various pectic and cellulolytic enzymes constitutively and the increased production in presence of certain native (pectic/cellulosic) substances can be considered merely the stimulation of these enzymes synthesis rather than actual induction. No exact correlation could be drawn between biomass and enzyme production. All the native carbon sources used in the present studies were found to be inferior than sucrose for biomass production.

Zusammenfassung

Pectin bei *Fusarium oxysporum* und Na-Polypectat bei *Fusarium moniliforme* offenbarten sich als die besten natürlichen C-Quellen für die Polygalacturase-Produktion. Eine maximale Pectinmethylgalacturonase-Bildung zeigte sich bei beiden Pathogenen mit Pectin. Baumwolle veranlaßte die höchste Cellulase-Produktion bei *F. oxysporum*, während CMC dies bei *F. moniliforme* bewirkte. Saccharose war unbedeutend für die Produktion von pectolytischen und cellulolytischen Enzymen. Beide Fusariumarten waren fähig, verschiedene pectinolytische und cellulolytische Enzyme konstitutiv zu produzieren, und die ansteigende Produktion in Gegenwart von gewissen nativen (petinolytischen und cellulolytischen) Substanzen kann mehr als eine Stimulation als eine aktuelle Induktion angesehen werden. Eine exakte Korrelation zwischen einer Biomasse- und Enzymproduktion war nicht vorhanden. Allerdings hatten die untersuchten natürlichen Substrate einen unbedeutenden Einfluß auf die Biomassebildung gegenüber dem Einsatz von Saccharose.

Cell wall degrading enzymes (PG, PMG and Cx) play an important role during pathogenesis (Albersheim et al. 1969). Influence of native carbon sources on the production of pectolytic and cellulolytic enzymes have been studied by a lot of workers. Nitrogen and native carbon sources can affect the production of these enzymes (Chopra and Mehta 1985). Pectic substances were found to be good carbon sources for the production of pectolytic enzymes by *Xanthomonas malvacearum* (Abo-El-Dohab 1964) and *Rhizopus stolonifer* (Trescott and Tampion 1974). Baldwin (1970) also reported that *X. malvacearum* could produce PMG, PG or PGTE on any of the carbon sources glycerine, pectin, sodium polypectate or galacturonic acid. Pectic substances were also good carbon sources for the