Egypt. J. Food Sci. **32**, No. 1-2, pp. 117- 130 (2004)

Nutrients Requirements for *Trichoderma reesei* and *Sclerotium cepivorum* as Effective Factors on Value Adding Wastes

Nagwa, M.A. El Shimi, A.M. Khalaf Allah, M.A.M.* Saleh, and Hala, M.Z.A.* Mohamed

*Food Technology Dept. Faculty of Agriculture, Cairo University, Special Food & Nutrition Dept.*Food Technology Research Institute, Giza, Egypt.*

THE *Trichoderma reesei* and *Sclerotium cepivorum* requirements for cellulases production from beet pulp and olive cake were investigated with respect to their affinity to cellulases activities. The appreciated nutrients were $(NH4)_2$ SO_4 (1.6 g/l) KH_2 PO_4 (4.0 g/l), Ca CL_2 (0.4 g/l), Mg SO_4 (0.6 g/l), and Tween 80 (2.0 g/l) for *T. reesei*. However, these concentrations, in the same order, were 1.4, 3.0, 0.3, 0.3, and 2.0 g/l for *S. cepivorum* with substitution of $(NH_4)_2SO_4$ by NH_4 H_2 PO_4. Vitamin B mixture seemed to be more suitable for *T. reesei*, whereas, sodium acetate was the best for *S. cepivorum*. Addition of 5.0 % filter paper in the presence of 2.0% beet pulp, resulted in the highest values of cellulase enzymes production and activities for both organisms.

Keywords: Nutrients requirements, cellulases production and activities, beet pulp, olive cake, *Trichoderma reesei, Sclerotium cepivorum*

Research efforts are being devoted to the conversion of food processing wastes to feed ingredients, single cell protein and enzyme production (Nagodawithana and Reed, 1993). Cellulase enzymes possess an important role in bio-industry such as macerating of vegetables, digestion, vegetable oil and hydrolysis of cellulosic materials to be used for special cases (Haska, 1997). Cellulase enzymes could also be used in food processing and fermentation; for instance, saccarification and fermentation process to produce lactic acid from cellulosic biomass (Chen and Lee,1997), and to convert lignocellulosic wastes into carbohydrate components for the production of ethanol (Ingram *et al.* 1997) and 2,3 butanediol (Gong *et al.* 1997). Therefore, many strains of microorganisms were selected by Cuevas *et al.* (1991) for cellulase enzymes production by using rice straw and rice bran medium. *T. reesei* and *T. viride* were also used for production of β-glucanase which was utilized in brewing applications (Nagodawithana and Reed, 1993). Khalaf Alla *et al.* (1993) used *T. viride* for the production of cellulases on soy bean hulls. Furthermore, Azab and Ammar (1994) used sugar cane bagasses to produce the cellulase enzymes.

The nutrients requirement of the growth medium (carbon source, nitrogen source, mineral salts, activators and growth factors) for any microorganism is included in the environmental conditions under which the microorganism was cultivated. It could be considered one of the most important variables affecting the cultivation and production of microbial enzymes and consequently the activity of such enzymes. Thus, it is of importance to put the nutrients content of the growth media under control and study.

The filter paper and cellulose powder as carbon sources are the most relevant for the maximum fungal growth and cellulase enzymes production (Tamada *et al.* 1988). Fadel *et al.* (1994) found that production of cellulases was more sensitive to nitrogen sources in the medium. On the other hand, some minerals and their adequate amount are required by different microorganisms for growth and cellulases synthesis (Sachslehner *et al.* 1998). Addition of some activators and stimulators was found to enhance the celluases production and activity (Lejeune and Baron, 1995).

The objectives of the present study were to investigate the suitability of some Egyptian food industries wastes, beet pulp and olive cake, as good media for *T. reesei* and *S. cepivorum* growth and cellulase enzymes production and activity. Moreover, this investigation was extended to identifying the optimum type and amount of the nutrient to accomplish such purposes.

Material and Methods

Materials

Beet pulp waste was obtained from Delta beet sugar company, EL-Hamol, Kafer EL-Sheikh Governorate, Egypt. Olive press cake (the compressed cake retained after olive oil extraction by up to 650 Bar using the hydraulic compressor Toscana Engologica, Mori, Alfa Oil 200 type, Italy) were obtained from the oil processing unit, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt. *T. reesei* (3653) and *S. cepivorum* (Locally isolated) were obtained from the microbiological unit of Soil, Water & Environment Research Institute and Department of Onion Disease, Plant Disease Institute, respectively, Agricultural Research Center, Giza, Egypt.

Methods

Material pretreatments and enzyme production methods

The waste materials were ground in a Perten laboratory Mill and alkali pretreated as described by El Shimi *et al.* (2002). The ground wastes were boiled for 15 min. in 4% NaOH at 1:5 solid: liquid ratio. The insoluble fraction was collected by filtration on cheese cloth, continuously washed with tap water to remove the residual of NaOH. The excess liquid was removed by squeezing the slurry through two layers of chease cloths and dried in the oven at 70°C. for 24 hr. The used methods and the basal media for growth, cultivation and production of cellulase and hemicelluloytic enzymes by the tested fungi were prepared as suggested by Mandels and Weber (1969).

The most favorable conditions for cultivation and cellulase enzymes production as well as activities, by growing on the alkali pretreated of the tested waste materials, were used. Therefore, bacterial culture was maintained on nutrient agar slant ingredients medium and other environmental conditions were applied as recommended by Khalaf Allah *et al.* (2002). Incubation period, pH value, incubation temperature and the inoculum size of *T. reesei* were 12 days, 5.0, 30°C and4%, while the corresponding values for *S. cepivorum* were 16 days, 4.5, 21°C and 2%, respectively. *T. reesei* was incubated at 200 rpm whereas, *S. cepivorum* was incubated without shaking.

Microorganisms nutrient requirements

The most favorable growth nutrients and their amounts for cultivation and production of cellulase enzymes by cellulase fungi (*T. reesei* and *S. cepivorum)* were studied.

The tested carbon sources (beet pulp and olive cake) were added to the medium individually or as mixed from 1.0 up to 7.5%. Five nitrogen sources, $(NH_4)_2SO_4$, $(NH_4)H_2PO_4$, $(NH_4)_2HPO_4$, $(NH_4)_3PO_4$ and Corn steep liquor, were separately added to give a final nitrogen concentration of 0.297 g/l of media. The most favorable source was used in amount of 1.2 to 1.8 g/l to determine the optimum amount. KH_2PO_4 (2 to 4 g/L) was used as a potassium source for the most suitable fungi, $CaCl_2$ and $MgSO_4$ were added from 0.2 to 0.6 g/l. Tween 80 was added as a surfactant from 0.2 to 2.0 g/l. The vitamin β mixture activator (0.05%) was added individually but the other activators, sodium acetate (Na-acetate, 0.1%) and sodium citrate (Na-citrate, 0.1%), were added individually or as a mixture (2% in a ratio of 1:1). Filter paper was used as an inducer (up to 5.0%).

The identified favorable nutrient and its optimum amounts for each tested nutrient was consequently applied in the next nutrient assayed.

Analytical methods

Enzymes assay

The produced enzymes, *i.e.* Carboxymethyl cellulase (CMC-ase), filter paper cellulase (FP-ase) and cellobiase activities were determined by Takao *et al.* (1985), whereas, hemicellulase (HC-ase) activity was determined by Ghose and Kundu (1980). One unit of each enzyme activity is defined as the amount of enzyme that release one μ mole of reducing sugars (determined as described by Miller *et al.*1960) from the substrate per minute under the tested conditions. The specific activity was calculated by dividing the resulted units of activities by mg protein (determined by the method of Lowry *et al.* 1951) of the produced enzyme.

Results and Discussion

It is of importance to apply the best pretreatments and microorganisms which possess the highest affinity to waste materials to achieve the maximum production of cellulase enzymes and their activities (El Shimi *et al.* 2002 and Khalaf Allah *et al.* 2002).

The nutrients requirement

Effect of beet pulp and olive cake concentration

The changes in soluble protein content of cellulases produced by the tested fungi and the cellulases activities production as affected by substrate concentration are shown in Table (1). The maximum production of cellulases in a culture filtrate of *T. reesei* was observed at the concentrations of 2 and 5% of beet pulp and olive cake, respectively. These results were in agreement with those found by Fadel and Abd El-Kader (1994) and Khalaf Allah *et al.* (1993). It is also evident that the maximum values of soluble protein, cellulolytic enzymes activity and specific activity in the enzyme filtrate of *T. reesei* were obtained by cultivation of the fungus in a medium containing 2.5% beet pulp and 2.5% olive cake. However, these values were lower than those obtained by using beet pulp alone. On the contrary, olive cake waste showed the lowest amount for such parameters. It was also noticed that HC-ase enzyme recorded the highest value of activity (unit/ml) and specific activity (unit/mg protein) compared to CMC-ase, while filter paper cellulase and cellobiase enzymes gave the lowest value. Beet pulp concentration of 2% seemed to be more favorable for maximum enzyme formation by *S. cepivorum,* while, maximum enzymes production was achieved by growing this strain on olive cake medium at 1% concentration.

It could be concluded that beet pulp (as a carbon source for *S. cepivorum* growth) gave the maximum values of soluble protein, cellulases enzymes activities and specific activities compared to olive cake and their mixtures, while olive cake waste gave the lowest values.

Effect of nitrogen sources

Supplementing the growth medium with a suitable nitrogen source is essential for fungal growth and cellulase enzymes production. Data presented in Table (2) reveal that the maximum activity of cellulases (CMC-ase, FP-ase and cellobiase) as well as hemicellulase in enzyme filtrate of *T. reesei* were obtained in the presence of ammonium sulphate, $(NH_4)_2SO_4$, followed by diammonium phosphate, $(NH_4)_2HPO_4$. However, when corn steep liquor was used by such fungus, the yield of cellulases enzymes was minimum. Such a trend was also observed by Fadel and Abd-el Kader (1994).

The best nitrogen source for enzymes production by *S. cepivorum* was diammonium phosphate followed by ammonium sulfate, mono ammonium phosphate, triammonium phosphate and corn steep liquor which showed the lowest cellulases and hemicellulase production. Diammonium phosphate achieved the maximum values of carboxymethyl cellulase, filter paper cellulase, cellobiase and hemicellulase production. These results are in agreement with those obtained by Hong *el al.* (1988).

TABLE 1. Effect of beet pulp, olive cake and their mixture concentrations on the production of cellulases by:*Trichoderma reesei*.

Substrate Concentration (%)		Soluble protein (mg/ml)	Enzyme activity (unit/ml)				Specific activity (unit/mg protein)			
			CMC-ase	FP-ase	Cellobiase	HC-ase	CMC-ase	FP-ase	Cellobiase	HC-ase
Beet pulp	1.0	1.130	1.456	0.284	0.085	3.084	1.288	0.251	0.075	2.730
	2.0	1.250	1.787	0.392	0.101	3.930	1.430	0.314	0.081	3.144
	2.5	1.235	1.719	0.360	0.099	3.676	1.392	0.291	0.080	2.976
	5.0	1.230	1.707	0.344	0.091	3.646	1.388	0.280	0.074	2.964
	7.5	1.224	1.689	0.338	0.089	3.594	1.380	0.276	0.073	2.936
Olive cake	1.0	0.949	1.110	0.215	0.063	1.556	1.170	0.227	0.066	1.640
	2.0	0.967	1.133	0.220	0.076	1.992	1.172	0.228	0.079	2.060
	2.5	0.999	1.242	0.229	0.080	2.252	1.243	0.229	0.080	2.254
	5.0	1.023	1.278	0.237	0.082	2.656	1.249	0.232	0.080	2.596
	7.5	1.013	1.156	0.234	0.079	2.620	1.141	0.231	0.078	2.586
Beet Pulp & Olive cake Mixtures	0.5 + 0.5	1.037	1.078	0.260	0.070	2.548	1.040	0.234	0.068	2.458
	1.0 + 1.0	1.108	1.310	0.280	0.081	2.856	1.182	0.253	0.073	2.578
	1.25 + 1.25	1.117	1.391	0.286	0.084	2.924	1.245	0.256	0.075	2.618
	2.5 +2.5	1.126	1.480	0.291	0.086	3.084	1.314	0.258	0.076	2.738
	3.75 + 3.75	1.118	1.419	0.286	0.084	3.048	1.269	0.256	0.075	2.726
Sclerotium cepivorum										
Beet pulp	1.0	1.189	1.575	0.322	0.071	3.344	1.325	0.271	0.060	2.812
	2.0	1.281	2.070	0.387	0.074	3.588	1.616	0.302	0.058	2.800
	2.5	1.279	2.065	0.367	0.066	3.530	1.614	0.287	0.052	2.760
	5.0	1.273	2.051	0.365	0.065	3.506	1.611	0.287	0.051	2.754
	7.5	1.261	2.015	0.359	0.064	3.470	1.598	0.285	0.051	2.752
Olive cake	1.0	1.021	1.286	0.206	0.053	1.858	1.260	0.202	0.052	1.820
	2.0	1.020	1.252	0.205	0.051	1.740	1.227	0.201	0.050	1.706
	2.5	1.018	1.249	0.204	0.051	1.726	1.227	0.200	0.050	1.695
	5.0	1.015	1.225	0.203	0.049	1.706	1.207	0.200	0.048	1.681
	7.5	1.012	1.225	0.201	0.048	1.674	1.210	0.199	0.047	1.654
Beet Pulp & Olive cake Mixtures	0.5 + 0.5	1.115	1.355	0.262	0.061	2.638	1.215	0.235	0.055	2.366
	1.0 + 1.0	1.150	1.631	0.289	0.063	2.680	1.418	0.251	0.055	2.330
	1.25 + 1.25	1.141	1.615	0.275	0.058	2.634	1.415	0.250	0.051	2.309
	2.5 +2.5	1.136	1.581	0.264	0.056	2.610	1.392	0.250	0.049	2.298
	3.75 + 3.75	1.129	1.525	0.262	0.055	2.556	1.351	0.250	0.049	2.264

TABLE 2. Effect of nitrogen sources on the production of cellulases from 2% beet pulp by: *Trichoderma reesei* .

Nitrogen source	Soluble protein (mg/ml)	Enzyme activity (unit/ml)				Specific activity (unit/mg protein)			
		CMC-ase	FP-ase	Cellobiase	HC-ase	CMC-ase	FP-ase	Cellobiase	HC-ase
$(NH_4)_2SO_4$	1.250	1.787	0.392	0.101	3.930	1.430	0.314	0.081	3.144
$(NH_4)H_2PO_4$	1.222	1.732	0.352	0.074	3.804	1.417	0.288	0.060	3.113
$(NH_4)_2HPO_4$	1.236	1.765	0.369	0.090	3.276	1.428	0.299	0.073	2.650
$(NH_4)_3PO_4$	1.224	1.737	0.364	0.082	3.134	1.419	0.297	0.067	2.560
Corn steep liquor	1.173	1.604	0.330	0.070	2.892	1.367	0.281	0.060	2.465
Sclerotium cepivorum									
$(NH_4)_2SO_4$	1.281	2.070	0.387	0.074	3.588	1.616	0.302	0.058	2.800
$(NH_4)H_2PO_4$	1.279	1.998	0.356	0.066	3.286	1.562	0.278	0.052	2.570
$(NH_4)_2HPO_4$	1.319	2.142	0.419	0.077	4.284	1.624	0.318	0.058	3.248
$(NH_4)_3PO_4$	1.274	1.931	0.330	0.064	3.414	1.516	0.259	0.050	2.680
Corn steep liquor	1.234	1.712	0.289	0.055	2.996	1.387	0.234	0.045	2.428

Effect of nitrogen level using ammonium sulphate, and di-ammonium phosphate

During the fermentation course, the cellulase enzymes production was gradually increased as the ammonium sulphate level increased up to 1.6 g/l, then slightly decreased (Table 3). However, 1.4% concentration could be used as an optimal concentration for cellulase enzymes production by *T. reesei* (Nasr, 1994).

It was also found that the soluble protein content in the culture filtrate of *T. reesei* and consequently the specific activities (unit/mg protein) of cellulase enzymes values increased as the $(NH_4)_2SO_4$ concentration increased to 1.6 g/1. *T. reesei* showed the highest activity and specific activity of hemicellulase enzyme, but was relatively low in cellobiase production. These results are in agreement with those found by Nasr (1994). The same Table shows also, that the use of lower or higher concentration than 1.6 g/l $(NH_4)_2HPO_4$ decreased the production of cellulase enzymes produced by *S. cepivorum* as well as their specific activity (Table 3).

The behavior of the cellulase enzymes (carboxy methy cellulase , filter paper cellulase and cellobiase) and hemicellulase which were produced by cultivating *T. reesei* or *S. cepivorum* on 2% beet pulp medium containing 1.6 g/l $(NH_4)_2 SO_4$ or 1.4 g/l $(NH_4)HPO_4$, respectively, showed a similar trend (Table 3). Hemicellulase and carboxy methyl cellulose enzymes had the higher values of enzyme activities and specific activities than the filter paper cellulose and cellobiase enzymes.

Effect of mono potassium phosphate level

The results in Table (3) revealed that a very low cellulase enzymes productivity was obtained by *T. reesei* at the low level of KH_2PO_4 (g/1). The increase in mono potassium phosphate concentration was subtended by a gradual increase in enzymes formation. The highest productivity of CMC-ase, FP-ase, cellobiase and HC-ase was obtained when KH_2PO_4, was used at a concentration of 4 g/1 which also resulted in the highest amount of soluble protein as well as the highest specific activities of cellulases enzymes were produced . A similar trend was also obtained by Chrapkowska *et al.* (1977).

Effect of calcium chloride level

Data presented in Table (4) indicated that increasing $CaCl_2$ concentration in the medium led to a parallel increase in the activities of cellulase and hemicellulase enzymes obtained by the both fungi. The results showed also that, increasing the concentration of $CaCl_2$ (0.4 g/1) above the optimum levels inhibited the production of cellulase enzymes and decreased the amount soluble protein in the culture filtrate of both investigated fungi.

Effect of magnesium sulphate level

Addition of $MgSO_2$ at level 0.6 g/1 basal media greatly enhanced the cellulase enzymes production by *T. reesei*. This could be attributed to the higher supplementation of Mg which increases production and activity of cellulases

TABLE 3. Effect of $(NH_4)_2SO_4$, $(NH_4)_2HPO_4$ and KH_2PO_4 concentration on the production of cellulases by: *Trichoderma reesei*.

$(NH_4)_2SO_4$ (g/L)	Soluble protein (mg/ml)	Enzyme activity (unit/ml)				Specific activity (unit/mg protein)			
		CMC-ase	FP-ase	Cellobiase	HC-ase	CMC-ase	FP-ase	Cellobiase	HC-ase
1.2	1.245	1.759	0.364	0.095	3.884	1.413	0.292	0.076	3.120
1.4	1.250	1.787	0.392	0.101	3.930	1.430	0.314	0.081	3.144
1.6	1.255	1.809	0.413	0.104	4.032	1.441	0.329	0.083	3.212
1.8	1.243	1.776	0.398	0.102	3.908	1.429	0.320	0.082	3.144
$(NH_4)_2HPO_4$ (g/L)	***Sclerotium cepivorum***								
1.2	1.304	2.059	0.387	0.072	3.476	1.576	0.297	0.055	2.666
1.4	1.319	2.142	0.419	0.077	4.284	1.624	0.318	0.058	3.248
1.6	1.301	1.981	0.397	0.074	3.862	1.523	0.305	0.057	2.968
1.8	1.279	1.825	0.379	0.070	3.650	1.427	0.296	0.055	2.854
KH_2PO_4 (g/L)	***Trichoderma reesei***								
2	1.255	1.809	0.413	0.104	4.032	1.441	0.329	0.083	3.212
3	1.274	1.943	0.465	0.104	4.162	1.525	0.365	0.082	3.266
4	1.286	2.015	0.478	0.105	4.294	1.567	0.372	0.082	3.339
	Sclerotium cepivorum								
2	1.319	2.142	0.419	0.077	4.284	1.624	0.318	0.058	3.248
3	1.407	2.670	0.688	0.080	5.474	1.898	0.489	0.057	3.890
4	1.380	2.514	0.648	0.077	5.354	1.822	0.470	0.056	3.880

TABLE 4. Effect of $CaCl_2$, $MgSO_4$ and Tween 80 concentration on the production of cellulases by *Trichoderma reesei* .

$CaCl_2$ (g/L)	Soluble protein (mg/ml)	Enzyme activity (unit/ml)				Specific activity (unit/mg protein)			
		CMC-ase	FP-ase	Cellobiase	HC-ase	CMC-ase	FP-ase	Cellobiase	HC-ase
0.20	1.276	1.943	0.434	0.102	4.136	1.523	0.340	0.080	3.242
0.30	1.286	2.015	0.478	0.105	4.294	1.567	0.372	0.082	3.339
0.40	1.401	2.991	0.560	0.115	4.784	2.135	0.400	0.082	3.415
0.60	1.351	2.681	0.509	0.108	4.010	1.984	0.377	0.080	3.177
				Sclerotium cepivorum					
0.20	1.379	2.586	0.429	0.069	4.482	1.875	0.311	0.050	3.250
0.30	1.407	2.670	0.688	0.080	5.474	1.898	0.489	0.057	3.890
0.40	1.367	2.481	0.453	0.075	4.272	1.815	0.331	0.055	3.126
0.60	1.356	2.425	0.433	0.074	4.238	1.788	0.319	0.055	3.125
$MgSO_4$ (g/L)				***Trichoderma reesei***					
0.30	1.401	2.991	0.560	0.115	4.784	2.135	0.400	0.082	3.415
0.40	1.443	3.114	0.585	0.120	4.950	2.158	0.405	0.083	3.430
0.60	1.501	3.391	0.611	0.126	5.174	2.259	0.407	0.084	3.447
0.80	1.430	2.836	0.434	0.097	4.406	1.983	0.303	0.068	3.118
				Sclerotium cepivorum					
0.20	1.382	2.586	0.656	0.075	4.774	1.871	0.475	0.054	3.454
0.30	1.407	2.670	0.688	0.080	5.474	1.898	0.489	0.057	3.890
0.40	1.374	2.581	0.651	0.076	4.784	1.878	0.474	0.055	3.482
0.60	1.340	2.359	0.630	0.073	4.346	1.760	0.470	0.054	3.244
Tween 80 (g/L)				***Trichoderma reesei***					
0.2	1.338	2.531	0.447	0.094	3.492	1.892	0.334	0.070	2.610
0.5	1.410	2.919	0.564	0.109	4.618	2.070	0.400	0.077	3.276
1.0	1.501	3.391	0.611	0.126	5.174	2.259	0.407	0.084	3.447
2.0	1.542	3.891	0.689	0.131	5.320	2.523	0.447	0.085	3.450
				Sclerotium cepivorum					
0.2	1.378	2.542	0.601	0.068	5.154	1.845	0.436	0.049	3.740
0.5	1.386	2.609	0.646	0.075	5.330	1.882	0.466	0.054	3.846
1.0	1.407	2.670	0.688	0.080	5.474	1.898	0.489	0.057	3.890
2.0	1.429	2.814	0.744	0.085	5.786	1.969	0.521	0.059	4.048

(Chrapkowska *et al.* 1977). However, increasing Mg SO_4 concentration to 0.8 g/l decreased the production of celluloiytic enzymes and inhibited the enzymes activity and specific activity. Excess Mg in the medium seemed to inhibit the biosyntheseis and cellulase enzymes activity (Tamada *et al.* 1988).

For *S. cepivorum*, magnesium sulphate concentration of 0.39 g/l gave the highest cellulase enzymes activities and specific activities. While, the use of $MgSO_4$ levels within the range of 0.4 g/l inhibited enzymes production (Sachslehner *et al.* 1998).

Comparing the two tested fungi, the previous results indicated that at the optimum $MgSO_4$ concentration *S. cepivorum*, gave the maximum activity and specific activity for both hemiecellulase and filter paper cellulase enzymes . On the other hand, the maximum activity and specific activity of carboxy methyl cellobiase and cellobiase were obtained by *T. reesei* (Naser, 1994).

Stimulators and activators factors

Effect of Tween 80 concentration

The presence of surfactants in culture medium was found to be effective for enzymes biosynthesis. Data presented in Table (4) show a gradual increase in soluble protein content as well as cellulase enzymes activity and specific activity in the culture filtrate of *T. reesei* and *S. cepivorum* as the Tween 80 concentration reached 2.0 g/l, where the maximum values of all the tested parameters were accomplished. This trend was in agreement with that found by Lejeune and Baron (1995). Tween 80 increases the cell permeability, allowing for more rapid release of the cellulase which in turn lead to greater enzyme synthesis (Reese and Magiure 1971).

Effect of activators

The effect of different activators on stimulating fungal growth and cellulases production is reported in Table (5). Results generally indicate that, addition of various activators (such as sodium acetate and sodium citrate at 1:1 ratio and vitamin β mixture) to the growth medium favored the enzymes production and activity by either *T. reesei* or *S. cepivorum* compared to that occurred with the control samples (without activators). Vitamin β mixture markedly increased the activity and specific activity of the produced cellulase enzymes by *T. reesei*. This increment may be due to the effect of vitamin β mixture as a growth factor for *T. reesei* (Tasphpulatove *et al.* 1977).

It could be noticed that all the activators increased the enzyme production by *S. cepivorum*. Wherein, addition of sodium acetate and sodium citrate mixture (1:1 ratio) to the cultivating medium greatly enhanced the cellulases induction by such strain, however, the obtained yield was lower than that achieved in the presence of vitamin B mixture. Addition of sodium acetate or sodium citrate individually to the medium recorded the lower values. These results are in accordance with those found by Gupta *et al.* 1972 and Kandil, 1981).

TABLE 5. Effect of different activators on the production of cellulases by: *Trichoderma reesei*.

Activators (%)	Soluble protein (mg/ml)	Enzyme activity (unit/ml)				Specific activity (unit/mg protein)			
		CMC-ase	FP-ase	Cellobiase	HC-ase	CMC-ase	FP-ase	Cellobiase	HC-ase
Without activators	1.542	3.891	0.689	0.131	5.320	2.523	0.447	0.085	3.450
Na-acetate at 0.1	1.602	4.136	0.783	0.137	5.566	2.581	0.489	0.086	3.474
Na-citrate at 0.1	1.615	4.362	0.804	0.141	5.638	2.701	0.498	0.087	3.491
Na-acetate.+ Na-citrate at 0.2 (1:1)	1.636	4.607	0.844	0.148	5.821	2.816	0.516	0.090	3.558
Vit. B mix..at 0.05	1.714	4.940	0.940	0.157	6.406	2.882	0.548	0.092	3.737
Sclerotium cepivorum									
Without activators	1.429	2.814	0.744	0.085	5.786	1.969	0.521	0.059	4.048
Na-acetate at 0.1	1.521	3.269	1.167	0.095	7.356	2.149	0.767	0.62	4.836
Na-citrate at 0.1	1.447	2.892	0.860	0.086	6.064	2.00	0.594	0.059	4.190
Na-acetate.+ Na-citrate at 0.2 (1:1)	1.476	2.991	1.027	0.091	6.954	2.026	0.696	0.062	4.712
Vit. B mixt.at 0.05	1.466	2.969	0.984	0.088	6.536	2.025	0.671	0.060	4.458

TABLE 6. Effect of filter paper on the production of cellulases by: *Trichoderma reesei*.

Filter paper (%)	Soluble protein (mg/ml)	Enzyme activity (unit/ml)				Specific activity (unit/mg protein)			
		CMC-ase	FP-ase	Cellobiase	HC-ase	CMC-ase	FP-ase	Cellobiase	HC-ase
0.0	1.714	4.940	0.940	0.157	6.406	2.882	0.548	0.092	3.737
2.5	1.776	6.000	1.013	0.182	6.920	3.378	0.570	0.102	3.896
5.0	1.790	6.177	1.023	0.191	6.978	3.451	0.572	0.107	3.898
Sclerotium cepivorum									
0.0	1.521	3.269	1.167	0.095	7.356	2.149	0.767	0.062	4.836
2.5	1.697	4.867	1.537	0.123	8.608	2.868	0.906	0.072	5.073
5.0	1.716	4.984	1.587	0.127	8.708	2.904	0.923	0.074	5.075

Comparing the two tested fungi strains, it could be noticed from the same Table that *S cepivrum* possessed the highest values of cellulase enzymes activity and specific activity for hemicellulase and exoglucanase enzymes. On the other hand, *T. reesei* gave the maximum values of such parameters for endo glucanase and cellobiase.

Effect of filter paper

Addition of filter paper to the cultivation medium markedly increased the cellulases enzymes production by *T. reese* and *S. cepivorum* in relative to control samples. The highest enzymes activity and specific activity for both fungi were obtained at 5% filter paper. At such concentration the activity and specific activity of all the tested enzymes as well as soluble protein were progressively correlated. Similar results were obtained by Warzywoda *et al.* (1983).

Consequently, it could be recommended to apply all the optimum aforementioned nutrients content in the media for the producing of cellulase enzymes by the tested strains to raise the value of specified waste materials.

References

Azab, M.S. and Ammar, M.S. (1994) Microbial enzyme from raw baggase and utilization of the end product as a biosorbent. *Egypt. J. Microbiol*, **29** (1), 1.

Chen, R. and Lee, Y.Y. (1997) Membrane mediated extractive fermentation for lactic acid production from cellulosic biomass. *Applied Biochemistry and biotechnology* (USA), **63/65**, 435.

Chrapkowska, K.J., Janicki, J.K. and Zofia, (1977) Role of magnesium and potassium in cellulase biosynthesis during submerged fermentation of fungi. Pr. Kom. Navk Roln Kom Nouk lesn, Poznan. Tow. Przyj. Navk, **43**, 21-8. C. F. C.A. 87, 114276 W.

Cuevas, V.C., Culiat, C.T. and Manaligod, R.L. (1991) Cellulases of improved strains of *Trichoderma harzianum* Rifai. *Philippine J. of Biotechnology* (philippines), **2** (1), 1.

El Shimi, N.M.A., Khalaf Allah, A.M., Saleh, M. A. M. and Zaki, H. M. (2002) Impact of some pretreatments in upwarding the value of some food processing wastes. The Third Scientific Conference of Agricultural sciences. 20-22 October, 2002. Assiut- Egypt.

Fadel, M. and Abd El-Kader, M.M. (1994) Production of cellulases and β-glucosidase by new isolate of *Aspergillus niger* F-92. *Egypt. J. Microbiol.*, **29** (2), 175.

Ghose, B.S. and Kundu, A.B. (1980) Introduction of cellulases and hemicellulases by Tamorined (Tomarinds Indica) Kernel Polysccharide. *J. Ferment. Technol.*, **58** (2), 135.

Gong, C.S., Gao, N. and Tsao, G.T. (1997) Biological production of 2,3-butanediol from renewable biomass. Fuels and chemicals from biomass. Washington, DC (USA). American Chemical Society, 280-293.

Gupta, J.K., Das, N.B. and Gupta, Y.P. (1972) Effect of cultural conditions on cellulase formation by *Trichoderma viride*. *Agric. Biol. Chem.*, **36** (11), 1961.

Haska, N. (1997) Determination on condition of optimal reaction and cellulase stability produced from Penicillium brunneum No. 24. Majalah-Bppt (Indonessia), (71), 41.

Hong, J.S., Kim, D.H., Kim, M.K., Lee, K.R., Kim, Y.S. and Kim, M.S. (1988) Studies on improving the nutritive value of rice straw by fermentation with *Lyophyllum decastes*, 1. Cultural condition of cellulase production and feeding value under fermentation periods. *Korean J. of Mycology*, **16** (3), 128.

Ingram, L.O., Lai, X., Moniruzzaman, M., Wood, B.E. and York, S.W. (1997) Fuel ethanol production from lignocellulose using genetically engineered bacteria. Fuels and chemicals from biomass. Washington, DC (USA) American Chemical Society, 57-73.

Kandil, S.H. (1981) Studies on some enzymes capable of hydrolyzing some food industries wastes. *Ph.D. Thesis*, Fac. of Agric., Ain Shams Univ.

halaf Allah, A.M., El Shimi, N.M.A., Saleh, M.A.M. and Zaki, H. M. (2002) Cellulases production from wastes under different environmental conditions. In press.

Khalaf Allah, A.M., El-Wakeil, F.A. and El-Farra, S.A. (1993) Production of cellulase by *Aspergillus* and *Trichoderma spp.* On soybean hulls. *Res. Bull. Home Econ. Menoufia Univ.*, **3**(1), 33.

Lejeune, R. and Baron, G.V. (1995) Effect of agitation on growth and enzyme production of *Trichoderma reesei* in batch fermentation. *Appl. Microbiol Biotechnol.*, **43**, 249.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Rondall, R.J. (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **193**, 265.

Mandels, M. and Weber, J. (1669) The production of cellulase. In cellulases and their application. *Adv. Chem. Ser.*, **35**, 391.

Miller, G.L., Blum, R., Glennon, W.E. and Burton, A.L. (1960) Measurement of carboxy methyl cellulose activity. *Anal. Biochem.*, **2**, 127.

Nagodawithana, T. and Reed, G. (1993) Enzymes in food processing. Copyright by academic press, Inc. 144.

Nasr, A.A.M. (1994) Biochemical studies on utilization of rice straw as agricultural waste. *Ph. D. Thesis*, Fac. of Agric. Cairo Univ.

Reese, E.T. and Maguire, A. (1971) Increase in cellulase yield by addition of surfactants to cellobiose cultures of Trichoderma viride. Develop . *Indust. Microbiol.*, (12) 212.

Sachslehner, A., Haltorich, D., Qulitz, G., Nidetzky, B. and Kulbe, K.D. (1998) Efficient production of mannan-degrading enzymes by the basidiomycete *Sclerotium rolfsii*. Appli. *Biochem. and Biotech.* (USA), **70** (72), 939.

Takao, S., Komagata, Y. and Sasaki, H. (1985) Cellulose production by Penicillium purpurogenum. *J. Ferment. Technol.*, **36** (2), 127.

Tamada, M., Kasai, N. and Kaetsu, I. (1988) Estimation of cellulase activity based on glucose productivity. *Biotechnology and Bioengineering*, **32**, 920.

Tashpulatove, Zh, Yvsupoval. Kh., Dzhalalova, Kh, and Abdullaev, T. (1977) Effect of some amino acid and vitamins on the cellulolytic activity of *t. Lignorum* 19. Uzb. Biol Zh. (3), 12-15 (Russ) cf. CA., **87**, 136163n.

Warzywoda, M., Ferre, V. and Pourquie, J. (1983) Development of a culture Medium for large-scale production of celluloytic enzymes by *Trichoderma reesei*. *Biotechnology and Bioengineering*, **25**, 3005.

(*Received* 25/2/2003)

احتياجات فطريات *Sclerotium cepivorum &Trichoderma reesei* من العناصر المغذية لرفع قيمة بعض المخلفات.

نجوى محمد أحمد الشيمى ، عبد الرحمن محمد خلف الله ، محمود عبد الله محمد صالح ، هالة محمد زكى

*قسم الصناعات الغذائية – كلية الزراعة – جامعة القاهرة ، و * قسم الاغذية الخاصة و التغذية – معهد بحوث تكنولوجيا الاغذية – الجيزة – مصر.*

تم دراسة إحتياجات فطريات *Trichoderma reeesei & Sclerotium cepivorum* للنمو على مخلفات البنجر والزيتون وقابليتها لاعطاء أعلى نشاط. وقد وجد أن العناصر المغذية لفطر *Trichoderma reesei* هى كبريتات الامونيوم (١,٦جم/ لتر) ، فوسفات ثنائية البوتاسيوم (٤ جم / لتر) ،كلوريد الكالسيوم (٠,٤ جم / لتر) ،كبريتات الماغنيسيوم (٠,٦ جم / لتر)،توين ٨٠ (٢جم / لتر) .بينما كانت الكميات المثلى لفطر *Sclerotium cepivorum* هى ١,٤ ، ٣،٠,٣ ، ٠,٣، ٢، جم /لتر على الترتيب مع إستبدال كبريتات الامونيوم بفوسفات الامونيوم. وقد لوحظ أن مخلوط فيتامين B كان أكثر ملائمة لفطر *Trichoderma reesei* بينما كان ملح خلات الصوديوم هو الأفضل فى حالة *Sclerotium cepivorum*. وكذلك فأن اضافة ٥% سيليلوز (من ورق الترشيح) فى وجود ٢% مخلف البنجر أدى إلى ظهور أعلى نشاط للانزيمات المحللة للسيليلوز الناتجة من كلا الفطرين.