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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 3 (10): 1762-1768, 2000

Studies on Activation of Amylolytic Enzymes Production by Gamma Irradiated *Aspergillus niger* Using Some Surfactants and Natural Oils under Solid State Fermentation

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Abstract: Active enhanced isolates producing high levels of amylolytic enzymes were obtained by gamma irradiation of the wild type *Aspergillus niger* F-909. The potent isolate No. 44e was derived from 0.8 kGy treated group grown on radicle (a waste of malt beverage industry) containing medium under solid state fermentation (SSF) conditions. The potent isolate exhibited enhanced production of alpha-amylase (EC 3.2. 1.1), glucoamylase (EC 3.2. 1.3) and alpha-glucosidase (EC 3.2.1.20), with relative increase in activities over the parental strain as reaching 111.3, 117.4 and 140% respectively. A study was conducted on this isolate to assess the effect of supplementation of radicle medium with low levels of five surfactants namely Tween 20, Tween 40, Tween BO, Tween 80 and Triton X-100 and four plant oils i.e. cotton seed, maize, soybean and sunflower on the enzymes synthesis under SSF conditions. The enzyme levels were considerably increased in the presence of Tween 80 at level 0.2% (v/v). Several solvent systems were evaluated with respect to their extraction abilities of the fungus amylolytic enzymes grown on the solid state culture. The most efficient extracting solvent proved to be tap water adjusted to pH 3.5. Furthermore, the extraction temperature, contact time and shaking speed affected the extraction process. Purification folds of 10.2, 10.4 and 11.9 for alpha-amylase, glucoamylase and alphaglucosidase were obtained by partial purification through precipitation with 65% saturation ammonium sulphate. Application studies were carried out on the partially purified enzyme of the potent isolate No. 44e which was used to hydrolyze native raw starch as a substrate wherein about 97% saccharification for 20% slurry of raw potato starch was achieved after 14h. Furthermore and in complementary experiments 8.6% (vlv) ethanol yield was produced from 18% (w/v) starch hydrolyzate when -fermentation studies were carried out with *Saccharomyces cerevisiae* after 24 h at 36°C. Yeast biomass of 21.3 g L^{-1} was produced when the culture conditions using the starch hydrolyzate were modified to obtain microbial protein production.

Key words: *Aspergillus niger*, amylolytic enzymes, gamma irradiation, surfactants, natural oils, solid state fermentation

Introduction

Use of mutagens has now been accepted as a routine practice in the amylolytic enzymes fermentation industry with a view to securing high yielding mutants of many microorganisms. Attempts to isolate mutants of some microorganisms that could give high yield of many enzymes have been made and a good number of high yielding mutants have been obtained by using gamma ray as mutagen (Meyrath *et al*., 1971; Tamada *et al*., 1987; Rajoka *et al*., 1998; El-Batal *et al*., 2000). The ability to produce amylases is widespread among microorganisms including thermophlic forms (Kindle, 1983). Microbial amylases found a wide spread applications in many industries (Lonsanc and Ramosh, 1990). The physicochemical properties of enzymes and their yields depend both on the genetic physiological and biochemical peculiarities of the producer (Nunberg *et al*., 1984; Kessim, 1983). Solid state fermentation system (SSF) possess many advantages for producing amylolytic enzyme e.g. short incubation period, high enzymes level, less impurities as well as more economic for purification (Selvakumar *et al*., 1996; Amirul *et al*., 1996).

In a pervious work (Fadel, 1999a) cultivated *A. niger* F-909 in radicle (waste of malt drinks) using SSF technique and high levels of amylolytic enzymes namely alpha amylase, glucoamylase and alpha-glucosidase were maintained under optimal physiological conditions. In the present work the study will be extended to explore the role of gamma irradiation on the productivity of the enzymes by *A. niger* F-909. The effect of surfactants and some of natural oils on the enzymes release is evaluated. Suitable methods for enzymes recovery from the mouldy substrate as well as partial purification of the obtained enzymes are described. The obtained enzymes was used for hydrolyzing raw potato starch.

Materials and Methods

Microorganisms: *Aspergillus niger* F-909 was locally isolated by the first author and identified by Prof. Dr. M. M. Abd El-Kader, Plant Pathology Lab, N. R. C. Egypt, *Saccharomyces cerevisiae*- AFZ-998 was isolated from sugar cane molasses and identified by the first author.

Substrate: Radicle, waste of malt beverages manufacture was obtained from Al-Ahram of Beverages Company, Egypt.

Inoculum preparation: The stock culture of the fungus was maintained on potato- dextrose agar (PDA) and subcultured at 5 days time intervals at 30°C. The fungal spores was scraped into conical flask containing 25 ml sterilized water, shaked for 10 minutes. The spore suspension was used to inoculate the fermentation flasks.

Irradiation technique: Mutagenic treatment with gamma

rays was carried out on *A. niger* F 909. Spores (10^5-10^6 spores ml^{-1} of culture - 7 days old) were suspended in sterile saline solution containing Tween 80 (0.1% v/v) and exposed to increasing doses of gamma irradiation namely 0.0 (unirradiated), 0.1, 0.2, 0.4,, 0.6, 0.8, 1.0, 1.25, 1.5, 1.75 and 2.0 kilo gray (k Gy) at ambient temperature. The process of irradiation was carried out at the National Center for Radiation Research and Technology (NCRRT). The irradiation facility used was the Cobalt - 60 GAMMA CHAMBER 4000A - INDIA. The average dose rate was 33.3 Gy min^{-1} at the time of experiments. Selection of hyper producing, gamma irradiated survivors isolates, for amylolytic enzymes activity was done as the ability of formation of a clear zone with a weak iodine solution 10.3% (w/v) I_2 in 3% (w/v) on the isolation medium (PDA) containing 2% soluble starch (Uguru *et al*., 1997). The selection of high producers of amylolytic activity was determined by measuring the ratio of the diameter of the clear zone formed to that of the colony.
The colonies showing higher amylolytic activity were picked up, purified with the single colony isolation technique and maintained on suitable culture medium and were used for enzymes production on solid fermentation medium.

Culture conditions: The fungus isolate No. 44e was cultivated on moistened radicle (70% (v/w) tap water initial pH 4.5). The fermentation flasks were inoculated with 10% (v/w), incubated at 36°C for 48 h (Fadel, 1999a).

Effect of addition of surfactant and natural oils on enzyme production: Tween 80, 60, 40, 20 and Trition X-100, as well as cotton seed, soybean, maize and sun flower oils (0.1% v/w) were sterelized individually, then added to the fermentation flasks. Control treatments (no additives) were run simultaneously.

Effect of type of solvent on efficiency of enzyme extraction: At the end of the growth period different solvents including, tap water, distilled water 0,1M acetate buffer (pH 5.0), 0.1M citrate buffer (pH 5.0), 0.1M citrate phosphate buffer (pH 5.0), 0.1M and 0.2M of NaCl were used for enzymes recovery from the mouldy substrate. This process was carried out with shaking at a rate of 100 rpm on a rotary shaker for 1 hour at 30°C. Using tap water as an extracting agent, it was adjusted at different pH ranges between 2.5 to 6.0 to asses the suitable pH for enzymes leaching. Different solid: solvent ratios ranged from 1:2.5 to 1:12 were used to report the suitable rate for enzymes leaching.

Effect of contact time, extraction pH and temperature on enzyme recovery: The elution was carried out at intervals ranging between 30 till 150 minutes to determine the suitable contact time. Different temperatures (20, 30, 40 and 50°C) for 1 h using the above shaker speed to study the influence of temperature of leaching on the enzymes activity production. Different shaking speeds (25, 50, 75, 100, 125, 150, 175, 200 rpm) were used and compared to control treatment (without shaking) were conducted at 40°C for studying the suitable shaking speed for enzymes leaching.

Analytical Procedures: Alpha- amylase, glucoamylase and alpha-glucosidase were assayed by the methods described by Castro *et al.* (1993) and Amirul *et al*. (1996).

Protein determination: The protein was determined by Folinphenol reagent according to the method described by Lowry *et al*. (1951).

Purification of enzymes: The partial purification of enzymes was carried out with ammonium sulfate according to the procedure described by Ramesh and Lonsane (1987) and Minagawu and Hamaishi (1962) after treatment of the fungal culture with tap water (pH 3.5) at solid ratio rate equal to 1: 10 (w/v).
The obtained enzyme precipitate was dissolved in 10 ml 0.1 M citrate buffer (pH 5.0).

Enzymatic starch hydrolysis: Raw potato starch was chosen as a substrate for hydrolysis by partially purified enzymes of gamma irradiated *A. niger* cultures as follows: Sixty g of raw potato starch was introduced in 500m1 capacity conical flask containing 295 ml tap water and 5 ml of solubilized precipitate of 66% ammonium sulphate. The pH value was adjusted to 4.5, then incubated in shaking water bath at 65°C. Samples were taken at intervals of two hours to determine the percent of saccharification. The obtained data compared by the data obtained using culture extract.

Ethanol production: Fermentation of enzymatic starch hydrolyzate for ethanol production by *Saccharomyces cerevisiae* AFZ- 998 was carried out under the optimal culture conditions described (Fadel, 1999b). The ethanol productivity was estimated by the method.

Fermentation efficiency: Fermentation efficiency was calculated after the sugar was determined by method of Herbert *et al*. (1971) by the equation.

Yeast production (Fadel, 1997): The yeast *S. cerevisiae* AFZ-998 was cultivated in 50 ml portions of the starch enzymatic hydrolyzate (1% total sugars) supplemented with 0.2% $(NH_4)_2HPO_4$ and 0.1% fodder yeast. The flasks were incubated on a rotary shaker (150 rpm) for 24 h at 30°C. The growing yeast culture was used to inoculate the experimental vessels containing a medium composed of enzymatic hydrolyzate solution (5% sugars), pH 5.0, urea 0.125%, H_3PO_4, 0.2%, (inocuium size 8% (v/v) shaking rate 250 rpm at 32°C for 36 h.

Biomass analysis: The yeast culture was centrifuged at 4,000 rpm for 5 m inutes. The biomass was oven dried at 70°C to constant weight to determine the dry weight. Crude proteins were determined by Mirco Kjeldahel (A. 0. A. C., 1980) Total carbohydrates and total lipids were determined by the method described by Herbert *et al*. (1971). Ash content was calculated by ignition at 750°C for 8 h.

Results and Discussion

Selection of the potent isolate: The fungal spores were irradiated by gamma- rays and those giving clear zones in the starch plates were selected as previously described. Among the survivors Isolates ten enhanced isolates proved

Table 1: Amylolytic enzymes production by-enhanced isolates of gamma irradiated A. Niger F-909 on modified starch agar plates and on radicle waste under SSF system

Strains	Gamma Irradiation dose kGy	On plates	On radicle waste		
		Zone ratio of amylolytic enzymes	Enzyme activity U g^{-1} waste		
			Alpha Amylase	Gluco Amylase	Alpha-gluco sidase
Parent *A. niger* F. 909	0.0	0.87	5520	184	160
Enhanced isolates No.					
4a	0.1	0.90	5520	184	165
130c	0.4	0.86	5775	190	175
18c	0.4	0.97	5786	193	172
32d	0.6	1.04	5817	196	177
44e	0.8	1.15	6144	216	224
16f	1.0	1.13	5972	217	225
25f	1.0	1.12	5790	218	192
12f	1.0	1.11	5937	215	188
15g	1.25	1.09	5917	212	189
19h	1.50	1.07	5878	184	190

Zone ratio equal the value of the diameter amylolytic enzymes (mm) per the diameter of the colony (mm).

Table 2: Partial purification of amylolytic enzymes produced by *A. niger* 44e cultivated under SSF technique

Step	Total protein mg ml^{-1}	Total activity U ml^{-1}			Specific activity U ml^{-1} protein			Purification fold			% Enzyme recovery		
		A*	G**	AG***	A	G	AG	A	G	AG	A	G	AG
Crude extract	6.1	70.7	26	25	11.6	4.3	4.2	-	1.14	1.17	100	100	100
Claritied extract	4.9	68.3	24	24	13.9	4.9	4.9	1.20	1.1	1.1	96.6	92.3	96
Ammonium sulphate 65% fraction	0.56	49.5	18.3	17.9	118.8	44.6	50.0	10.2	11.9	11.9	70.0	70.4	71.3

*Alpha-amylase **Giucoamylase ***Alpha-glucosidase

Table 3: Saccharification of different concentrations of native potato starch using partially purified amylases of *A. niger* 44e cultured under SSF system

Time (hours)	Saccharification%				
	Native potato starch slurry % (w/v)				
	5	10	15	20	20*
0	0.00	0.00	0.00	0.00	0.00
2	29.4	25.1	19.3	14.3	7.6
4	64.5	56.4	46.3	27.5	16.1
6	96.4	89.2	65.2	48.2	26.4
8	98.1	98.0	81.7	64.3	39.5
10	98.1	98.0	94.9	79.3	54.2
12	98.1	98.0	97.7	95.9	73.6
14	98.1	97.8	97.7	97.1	82.08
16	98,1	97.5	97.7	97.1	91.8
18	97.6	97.4	97.7	96.3	95.2
20	97.6	96.9	97.5	96.2	96.8
22	97.3	96.4	97.1	96.1	96.80

*saccharification by culture extract before clarification

Table 4: Production of ethanol from enzymatic potato starch hydrolyzate (18% total sugars) obtained by action of amylolytic enzymes of gamma irradiated *A. niger* (44e1 by Saccharomyces cerevisiae AFZ-998 under static fermentation at 36°C after 24h

Time	Ethanol riches (v/v)	Consumed sugars (%)	Fermentation Efficiency
0	0	0	
8	3.4	38	33.3
16	8.1	86	79.9
24	8.6	96	84.8
36	8,5	97	83.8

to be promising. These were selected and tested for their enzymes activities on quantitative basis.

Data presented in Table 1 clearly indicate that isolate No. 44e, derived from the 0.8 kGy treatment group, exhibited the highest of amylolytic activity as compared to the parent strain *A. niger* F-909. Moreover, the active enhanced isolate afforded a poor growth which may be of advantage in handling the mashes at the end of the fermentation period (Meyrath *et al*., 1971).

It is obvious in present study, that those doses with moderate degree of kill are more suitable for selecting enhanced isolates (0.8 up to 1.25 k Gy). Therefore, the highest potent isolate No. 44e of *A. niger* was selected in the subsequent investigations.

In harmony was our results gamma-ray treatments induced mutagenesis and yield stable and viable mutants for hyper production of many enzymes (Meyrath *et al*., 1971; Tamada *et al*., 1987; Kumakura, 1993; El-Batal *et al*., 2000). These enhancement by gamma-ray may be due either to an increase in the gene copy number or to improvement in gene expression, or both (Rajoka *et al*., 1998).

Table 5: Summary for the production of single cell protein from enzymatic hydrolyzate of raw potato starch by amylolytic enzymes of *A. niger* 44e cultivated on radicle under SSF technique by *Saccharomyces cerevisiae* AFZ998 at 35°C after 36h

Component	Amount
Dry weight (g/l)	21.3
Total carbohydrates %	38.1
Crude protein %	42.1
lipids %	2.7
Ash content %	8.4

Effects of surfactant and natural oils: Figure 1 illustrates that among the different Tweens additive, Tween 80 enhanced enzymes biosynthesis considerably. Moreover, minor

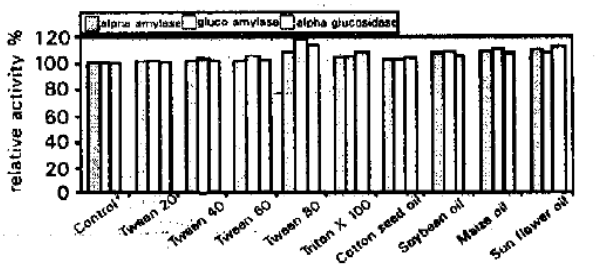

Fig. 1: Influence of some surfactants and natural oils on yields of amylolytic enzymes by *A. niger* 44e cultivated on radicle waste under 5SF system. The growth was allawed for 48h at 35°C. •6144. 216 and 224 Wig substrate for alpha-amylase, giucoamylase and alpha-glucosidase

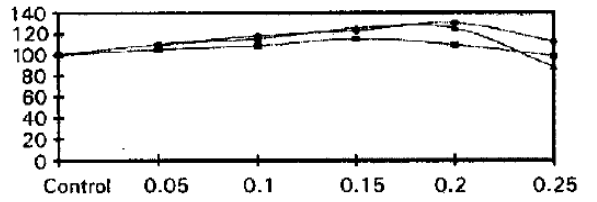

Fig. 2: Effect if Tween 80 concentration on the biosynthesis of amylolytic enzymes by *A. niger* 44e cultivated on radicle waste under 55F system after 48h at 36°C
6144, 216 and 224 IU/g substrate for alpha-amylase, glucoamylase and alpha-glucosidase

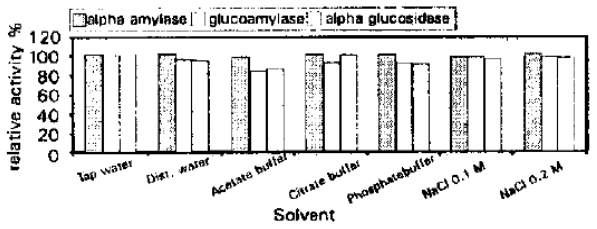

Fig.3: Effect of type of solvent on the leaching of amylolytic enzymes of *A. niger* 44e cultivated on radicle waste under SSF (Relative activity 100% equal 6348.293 and 200 IU of alpha amylase, glucoamylase and alpha-glucosidase respectively/g substrate)

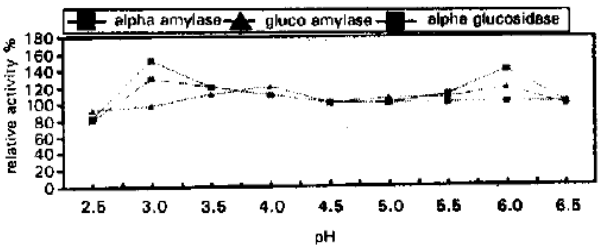

Fig.4: Influence of p14 variations on the recovery of amylolytic enzymes from fermented radicle (Relative activity 100% equal 6348.293 and 200 IU of alpha amylase, glucoamylase and alpha•glucosidase respectively/g substrate)

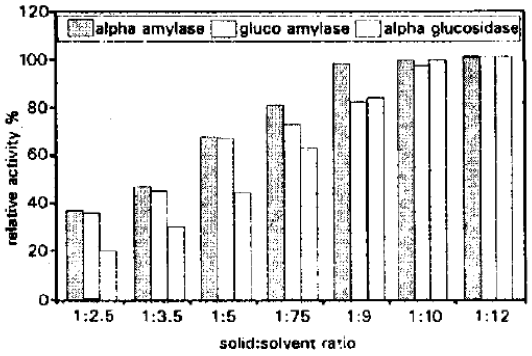

Fig. 5: Effect of solid: solvent ratio on the arnylolytic enzymes leaching (100% relative activity means the highest level of enzymes can be obtained by leaching)

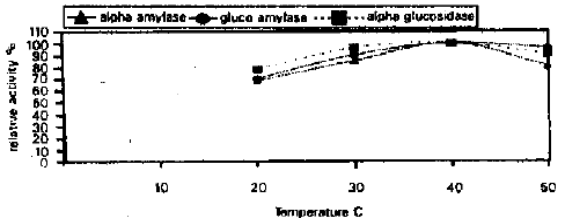

Fig. 6: Effect of the extraction temperature on the amylolytic activity levels produced by *A. niger* 44e cultivated on radicle by SSF system after 48h at 36°C
(100% relative activity means the highest level of enzyme scan be obtained by using 40°C)

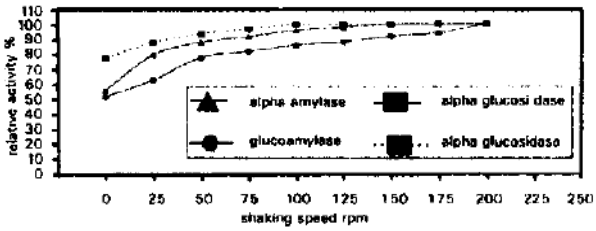

Fig. 7: Effect of mixing rat eon the amylolytic enzymes recovery from *A. niger* 44a cultivated on radicle waste by 5SF system after 48h at 36°C
(100% relative activity means the highest level of enzymes can be obtained by action of shaking)

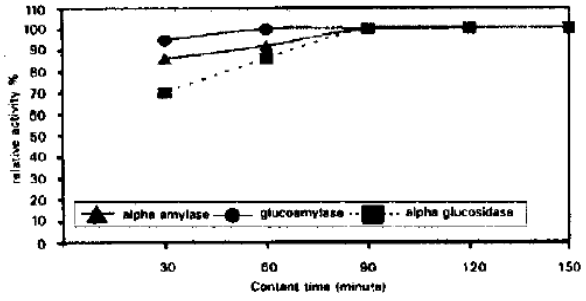

Fig. 8: Influence of contact time on the enzymes level activity produced by *A. niger,* 44e cultivated on radicle waste by SSF system after 48h at 36°C
(100% relative activity means the highest level of enzymes can be obtained by action of contact time 90 minutes)

stimulatory effects were detected in the presence of the tested natural oils. Maize oil, however, was more effective in increasing alpha-amylase and glucoamylase production. Thus Tween 80 showed more stimulating effect on synthesis of the tested enzymes.

Surfactants have been reported to affect the growth rate and enzyme production of many fungi. Similar findings have been recorded with respect to the action of surfactant on different microbial enzymes (Reese and Maguire, 1969 and 1971; Sukan *et al*., 1988). The mechanisms by which detergents enhance extracellular enzyme production was reported to be due to increased cell membrane permeability, change in lipid metabolism and stimulation of the release of enzymes are among the possible modes of the action (Omar *et al*., 1987; Lestan *et al*., 1990).

The favorable effect of natural oils on growth and product formation can not be ascribed entirely on the antifoaming activity. Since natural oils are metabolizable, the resulting product from their hydrolysis is a mixture of fatty acids. Thus, the drift towards acidic pH, during the course of the fermentation is due to the release of fatty acids from natural oils. Among these, linoleic, linolenic and oleic acids have been proven to have significant effects on the membrane permeability of microorganisms (Greenway and Dyke, 1979).

Effect of Tween 80 concentrations: Figure 2 illustrates the influence of various concentrations i.e. 0.05. 0.1, 0.15, 0.2 and 0.25 (v/w) of Tween 8 0 on the biosynthesis of alpha-amylase, glucoamylase and alpha- glucosidase by tim selected potent isolate *A. niger* 44e. The obtained results indicate that the enzymes differ in their response to the concentration of the surfactant. Thus at 0.2% level considerable increase in glucoamylase activity reaching about 30% was maintained. Similarly about 25 and 15% *increase* in alpha-glucosidase and alpha- amylase activities. Sukan *et al.* (1988) demonstrated that the stimulating effect of surfactant may be due to change into an inhibitory effect of enzyme synthesis, as the concentration of surfactant increases above certain levels. In addition, surfactant have been reported not affect the biosynthetic pathway but promote the utilization of metabolites (Matosic *et al*., 1998). Huot *et al.* (1996) indicated that the stimulatory effect of Tween 80 in increasing enzyme yields varies from organism to organism and for different enzymes of the same organism.

Effect of solvent on enzymes leaching: Figure 3 shows the influence of various solvents on enzymes level extraction. The leaching agent affects considerably the enzymes activities. Tap water is more suitable than other tested agents. Glucoamylase was more extractable than alpha -amylase and alpha-glucosidase. Citrate buffer was found to be more suitable for eluting alpha-amylase and alpha-glucosidase, whereas distilled water and sodium chloride was more suitable to elute glucoamylase. Various solvents such as water, dilute salt solution, buffer, dilute solution of glycerol, tap water, aqueous solution of ethanol and toluene saturated water were used by many workers for extraction of the amylases. Distilled or tap water alone or with 0.5-1% glycerin or NaCl was found to be best (Ramakrishna *et al*., 1982; Lonsanc and Ramosh, 1990). Enzyme extraction by an alcoholic solvents was found to be less efficient than the aqueous solvents. Qadeer *et al*. (1980) used phosphate buffer pH 7.0 for extraction of alpha-amylase from wheat bran fermented by a bacterial strain of *Bacillus* sp. The effect of the use of phosphate buffer at pH 5.9 and 7.7, acetate buffer at pH 5.9, or water for extraction of alpha-amylase were compared by Feniksova *et al.* (1960). They found that phosphate buffer of pH 5.9 was more suitable for alpha- amylase extraction. Fernandez-Lahore *et al.* (1998) found that 0.5 M NaCl was more suitable for recovery of acid protease from the fermented mass than distilled water or surfactants.

Factors affecting enzymes recovery: In solid state fermentation (SSF) systems the product (starch-hydrolyzing enzymes in our case) must *be* initially leached from the fermented solids, which comprises, non-utilized solid substrate, microbial mass, the product for which fermentation was carried out and a number of metabolites formed during the course of fermentation (Ramesh and Lonsane, 1987). The product needs to be leached out with a suitable solvent. The leaching efficiency is however affected by a number of factors such as the ratio of solids to solvent, contact time, temperature and pH of the system (Lonsanc and Ramosh, 1990; Thakur *et al*., 1990).

Figure 4 shows the level of enzymes obtained by leaching with tap water adjusted at different pH values. High level activity of both alpha-amylase as well as alpha-glucosidase was obtained using tap water adjusted to pH 3.0 - 6.0. The ratio of solid to solvent seems to be more important (Fig. 5), as a gradual increase in enzymes activity was attained with increasing amount of the solvent used. The highest level of alpha-amylase was leached with 1:9 solid: solvent ratio, whereas, the optimum ratio was 1: 10 for recovery of both glucoamylase and alpha-glucosidase. Ramesh and Lonsane (1987) indicated that the degree of extraction of the enzyme increased with an increase in the ratio of solid to solvent up to 1:9 when extracted bacterial alpha-amylase from bacterial bran using mixing rate of 180 rpm.

Figure 6 illustrates that at 20°C about 68, 70 and 78% of alpha amylase glucoamylase and alpha glucosidase were respectively extracted after 30 min. The highest recovery yields were obtained at 40°C (Ramesh and Lonsane, 1987).

Figure 7 illustrates the effect of shaking rate on the leaching process. The yields of the enzymes were increased with increasing the shaking speed. The highest enzymes yields were achieved at 200 rpm. Ramesh (1988) obtained better extraction of alpha-amylase from bacterial bran using shaking rate of 180 rpm.

Figure 8 shows that the contact time between solvent and solid is important in recovery of enzymes from fungal culture. The activity of enzymes were increased with increasing of the contact time reaching maximum at 90 minutes contact time.

Purification of the amylases: As shown in Table 2 about 20% of proteins was removed by the clarification techniques adopted. The specific activity was increased to about 1.20, 1.14 and 1.17 fold for alpha-amylase, glucoamylase and alpha glucosidase, respectively. Precipitation with ammonium sulphate resulted in obtaining about 11.4% of the total proteins in the clarified enzymes extract. The ammonium sulphate precipitated fraction gave 10.2, 10.4 and 11.9 purification fold for the tested three enzymes respectively as compared the crude enzyme preparation. It was stressed by Beckord *et al.* (1945) that

clarification of the enzymes extract is necessary because the suspended matter resists-removal by centrifugation or filtration.

Starch saccharification by the fungal enzymes preparation: Table 3 shows the saccharification of different slurries concentration of raw potato starch by amylases precipitated from our potent *A. niger* isolate culture by 65% ammonium sulphate. Data showed that the rate of saccharification was accelerated by using partially purified enzymes than crude extract. Thus the highest saccharification percent reached 97% after 14 h by using ammonium sulfate precipitated enzymes. Janse and Pretorius (1995) could hydrolyze starch in one step using recombinant strain of *S. cerevisiae* producing alpha-amylase, glucoamylase and puilulanase. Also Saha and Bothast (1993) could convert starch by amylases from *Aureobasidum pullulans* to glucose.

Ethanol production: In the present experiment, the potato starch was hydrolyzed by the precipitated enzymes of the *A. niger* 44e as previously described and subsequently inoculated with *S. cerevisiae* to study its suitability for ethanol production. The data in Table 4 show that about 8.6% (v/v) ethanol can be obtained after 24 h incubation at 36°C. The fermentation efficiency was about 87% and the residual sugars (after yeast growth) did not exceed 0.91%. Many authors employed enzymatic starch hydrolyzate for ethanol production as a cheap source of fermentable sugars (Hariantono *et al*., 1991; Pirselova *et al*., 1993; Ryu *et al*., 1994). Moreover, starch hydrolyzate was mixed with whey permeate to attain economic ethanol produCtion (Fadel, 1998).

Production of yeast biomass:
Table 5 summaries the data obtained from cultivation of *Saccharomyces cereisiae* AFZ-998 on enzymatic raw starch hydrolyzate under the optimum conditions described before. High yeast biomass (21.3 g dry weight L^{-1}) can be obtained. The yeast strain could utilize 94% of the total sugars present in the cultivation medium with a utilization efficiency of 0.42 dry yeast g^{-1} sugars. The chemical composition of the biomass exhibited a moderate ash content which reveals its suitability for the purposes of feeding or food preparations in the view of its high protein content (42.1%) (Table 5).

Acknowledgment

The authors wish to express their deep gratitude to Prof. Dr. A. H. El- Refaei Prof of microbiology and biotechnology and Prof. Dr. M. S. Foda. Prof. of applied microbiology, N. R. C. Egypt for their kind help in scientific and language revisions of the article.

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