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Production of Protease Enzyme from Wheat Straw

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Abstract

Protease enzyme production was studied and optimized as a first step to collect information about solid state fermenter) to produce protease enzyme. A local isolated *Aspergillus niger* was used for this study with constant spores feeding in every experiment at (10^5/g). Experiments carried out in conical flasks with (250 ml) containing (10 g) of wheat straw as a substrate with different conditions included temperature, pH, hydration ratio, and fermentation time, the results comprised by measuring protease activity (u). The results showed that the best activity can be obtained at (T = 32°C, t= 100 hrs, pH= 2.5 and hydration ratio is 1:3). On the other hand the results is courage to proceed to design a solid state protease fermenter from wheat straw.

Keywords: Fermentation, Protease, Straw, pH, Hydration, Solid State Fermenter.

Introduction

Protease enzyme is one of the most important industrial products also it used in many food and pharmaceutical applications. It is used in
1.lether industries in dehairing and bating stages.
2.detergents manufacturing
3.cheese manufacturing
4.protein hydrolysates

Most organisms (i.e. bacteria fungus) produce protease. The important thing to industry is to obtain extracellular enzymes. There are different type (acidic and basic) of this enzyme depending on the organism and the cultivation conditions. The microps produce protease enzyme include type and six of bacteria and fungus differ in their microorganism, composition according to the products and the growing circumstances. Such microps can be isolated from natural sources like soil, crops material food by planting them in a amid contain protein source and preparing the proper circumstances to grow the microps, usually the milk agar is used for this purpose. The primarily isolation may be done by making a farms of raw materials by adding cheep protein materials to soil the amending the pH to produce the demanded enzyme and keep them into convent temperature. There are more than one definition to the substrates of solid states fermentation, one of them it: growing of microps on solid state without presence of water, or its substrate in which growing and producing microps on solid state were found (Michael, 1988, Agosin, 1985, Hameed, 1999, Battaglino, 1991, Cannel, 1980, Chen, 1977).

The aim of this research is to study the operating conditions of producing acidic protease enzyme from wheat straw by solid state fermentation to *Aspergillus Niger and* obtain the optimum conditions which can be used for future study of bioreactor design to produce this enzyme.

Materials and Methods

Wheat straw used as a substrate for solid state fermentation hydrated by sodium acetate buffer, and a local isolated *Aspergillus niger* was used as enzyme production source , sodium acetate, casein, TCA(trichloroacetic acid) were used as a chemicals ,also the following equipments were used for measures and analysis; sensitive balance, pH meter, autoclave (sterilizer), incubator, centrifuge, oven (dryer), spectrophotometer (UV), pipette, micro-pipette, test tubes, conical flasks.

Fermentation Procedure

The experiments were carried out in identical 250 ml conical flasks each containing 10 g of wheat straw substrate which was hydrated by adding 0.02 molar of sodium acetate buffer solution with a certain value of hydration ratio as mentioned in attached (tables, 2, 3 and 4). The flasks were closed with cotton wool plugs. the medium was sterilized by an autoclave operating at 121°C of temperature, and pressure of 1.5 bar for 15 minutes. Proliferation was carried out by adding 1 ml inoculums to the medium and incubated at a certain temperature with respect to variables study as shown in tables (2, 3 and 4) and figures (1 to 8) later. Incubation periods ranged between (28-156 hr.), pH (1-4.5), temperature (24-38°C), hydration ratio (1:1-5.5:1 wt/vol), size of inoclum (spore number) was constant 10^5/g wet weight.

At the end of each of run, the samples from each flasks were prepare to the analytical step.

Analytical Procedure

Enzyme Activity

Protease activity was measured by degradation of casein. 2 mL of culture filtrate was added to 2 mL of 1% (w/v) casein (pH 7.5) and incubated for 10 min at 40°C. The reaction was stopped by adding a protein-precipitating agent, 4 mL of 0.4 molar trichloroacetic acid (TCA). Solutions were centrifuged in speed of 2500 rpm for 20 minutes. The precipitated protein was removed by filtration and the absorption of filtrate was measured at 275 nm. One unit of protease activity (u) was defined as the amount of enzyme that released 1 *micro* gram of tyrosine per min under assay condition (pH 7.5, 40°C). Blank was prepared by the same way except adding TCA before adding enzymes the values will compare with prepared standard absorption tyrosine curve at 275 nm (Liy, 2002, Murachi, 1976).

Protein Concentration

Dissolving the precipitated protein which was removed as mentioned above by adding 0.05 molar NaOH and the optical density of the solution was measured at 280 nanometer and 235 nanometer. Blank solution of 0.05 molar NaOH was used.

The equation used to estimate protein concentration is (Whiteaker,and Granum 1980):

Protein concen.(mg/ ml) = (light abs. at 235 nm– light abs. at 280nm)/2.5 … (1)

Results and Discussion

Effect of Hydration Ratio

Aspergillus niger was cultured or inoculate in hydrated wheat straw by adding 0.02 molar of sodium acetate buffer in the range of (1:1–5.5:1 wt/vol). The effect of hydration ratios were studied. These studies were conducted at constant temperature (32ºC), pH = 2.5 and cultivation time of 100 hours as shown in table (1) and figs. (1, 2).

Table 1
Hydration ratio effect on enzyme activity and enzyme yield at constant temperature of 32°C, incubation period of 100 hours and pH = 2.

Enzyme yield (×1000) (u/g substrate)	Enzyme activity (×100) (u /g protein)	Hydration ratio (weight/ volume)
0.48	6.30	1.00
0.60	10.00	1.50
0.70	11.90	2.50
0.79	12.40	3.00
0.75	11.50	3.50
0.50	7.70	4.50
0.30	2.80	5.50

From these results, the enzyme activity increased slightly with hydration ratio up to 1:3 and then decreases. The results indicated that at 1:3 hydrations ratio enzymes production with highest yield and activity of 790 units per g substrate and 1240 unit per mg protein respectively. The effect of hydration ratio can be divided in two partitions. In the first partition the increasing of hydration will increase mass transfer media needed for oxygen transformation and cetric acid produced dilution. But when hydration ratios more than (1:3) the concentration of nutrients will be decreased rapidly and that inhibit the yeast growth and also enzyme quality and quantity will be that decrease the activity of protease enzyme.

Effect of pH

Different values of pH medium were studied at constant temperature (32°C), proliferation time of 100 hours and hydration ratio of 1:3 ,as shown in table (2) and figures (3 and 4).

The studied of pH values of growth medium were initially adjusted to pH values of 1, 2, 2.5, 3, 3.5, 4, 4.5 before sterilization.

Table 2
pH values effect on enzyme activity and enzyme yield at constant other variables (temp. =32°C, incubation period of 100 hours and hydration ratio 1:3.

Enzyme yield (×1000) u/g substrate	Enzyme activity (×100), u /g protein	pH
0.41	8.8	1
0.46	9.2	2
0.58	9.8	2.5
0.48	9	3
0.39	7.3	3.5
0.32	5.9	4
0.242	4.4	4.5

The results showed that the activity of enzyme increases from (880 u per g protein to 980 u per g protein) when the pH increases from 1-2.5 respectively and then decreases to reach 440 u per g protein when the pH is 4.5. Also the results showed that the enzyme yield has the same behavior and reach the maximum value of 580 u per g substrate when pH value is 2.5 .The results indicated the best proliferation pH value of 2.5.

Effect of Temperature

The effect of different temperature range between (24 to 38°C) were studied at constant pH 2.5, proliferation time of 100 hours and hydration of 1:3 as shown in table (3) and figs. (5 and 6).

The enzyme activity increased from (1020 to 1200 u per g protein) when temperature increased from 24 to 32°C and then decreased from this value to (620 u per g protein) when temperature reached 38°C.The reason of this behavior can be consider as the relation between organism growth and temperature in one hand and the relation between organism growth and enzyme production in the another hand. Aspergillus Niger can be grown well in middle temperature range around 32°C.

Table 3
Effect incubation temperature on enzyme activity and enzyme yield at constant pH =2.5, incubation period of 100 hours and hydration ratio 1:3.

Enzyme yield (×1000) u/g substrate	Enzyme activity (×100), u /g protein	Temp.(°C)
0.6	10.2	24
0.68	11.5	28
0.7	12	32
0.64	10.4	34
0.49	8.5	36
0.28	6.2	38

Effect of Cultivation Time

Proliferation of *Aspergillus niger* for several incubation time were studied at constant temperature (32°C), pH 2.5 and hydration ratio of 1:3 in order to produce highest enzyme activity with highest yield .the results indicated that when proliferation time increases that will cause an increase in enzyme activity and yield from 180 and 130 to reached its maximum values of 1200 and 780 respectively at time equal to 100 hours during increasing in cultivation time from 28 to 100 hours and then decreases to 700 and 600 at time equal to 156 hours .The results indicated the highest yield enzyme activity and highest enzyme activity obtained at incubation period of 156 hours as shown in table (4) and figures (7 and 8).

Table 4. Effect of incubation time on enzyme activity and enzyme yield at constant other variables (temperature of 32°C, pH of 2.5 and hydration ratio of 1:3.

Enzyme yield (×1000) u/g substrate	Enzyme activity (×100), u /g protein	Time (hours)
0.13	1.8	28
0.3	6.2	52
0.6	11.3	76
0.78	12	100
0.74	11.1	124
0.68	9	148
0.6	7	156

Conclusion

From this study it can be recommended that it is good to produce protease enzyme with high activity using wheat straw substrate plants as a solid state fermentation of *Aspergillus Niger.* So it is important to go ahead in studying other parameters such as hydrodynamic of bioreactor to produce this enzyme according to the parameters mentioned in this study. The results showed that the best activity can be obtained at (T=32°C, t=100 hrs, pH= 2.5 and hydration ratio is 1:3).

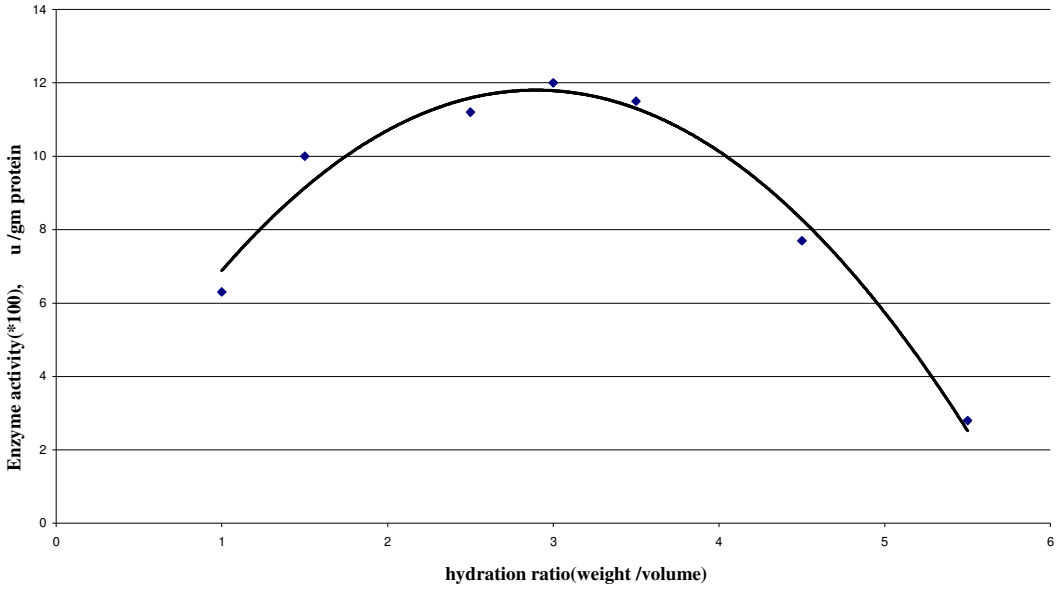

Fig.1 Hydration ratio effect on enyzme activity at constant temperature of 32 ,incubation time of 100 hours and pH of 2.5.

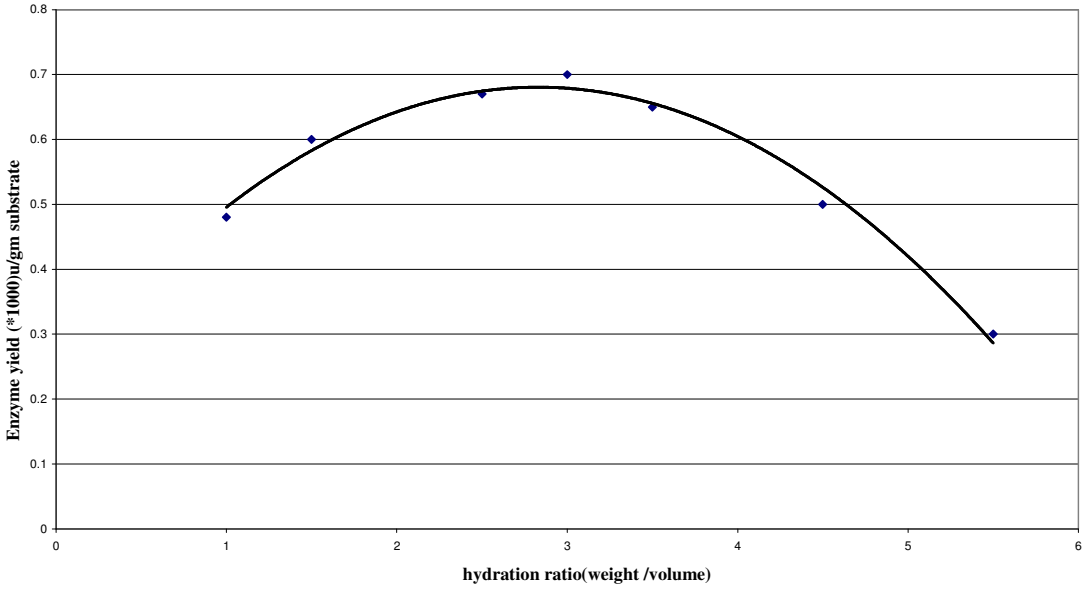

Fig.2 Hydration ratio effect on enzyme yield at constant temperature of 32 ,incubation time of 100 hours and pH of 2.5.

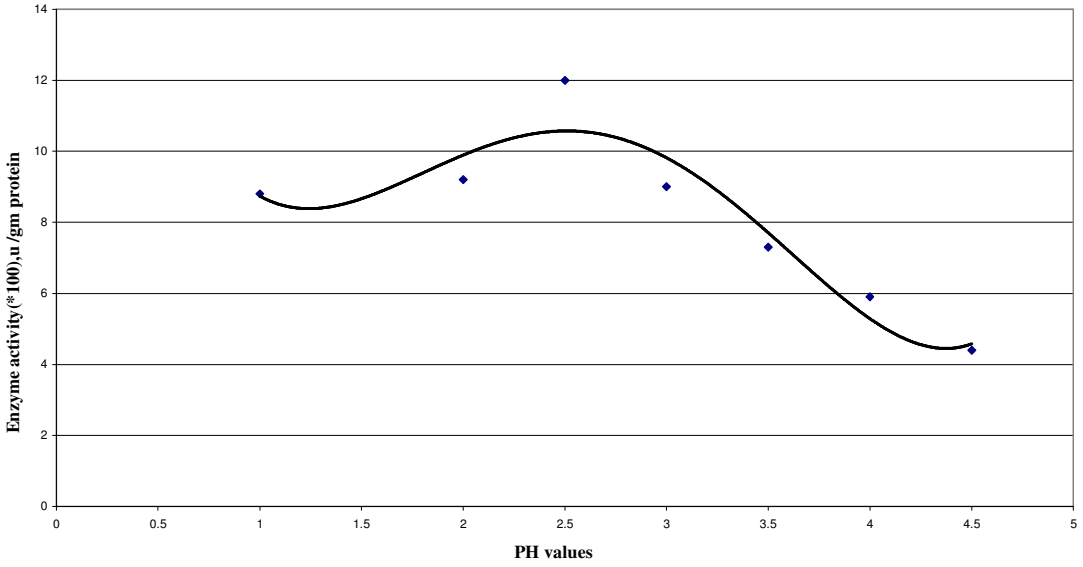

Fig .3 pH values effect on enzyme activity at constant other variables (temperature of 32 ,incubation time of 100 hours and hydration ratio of 1:3.

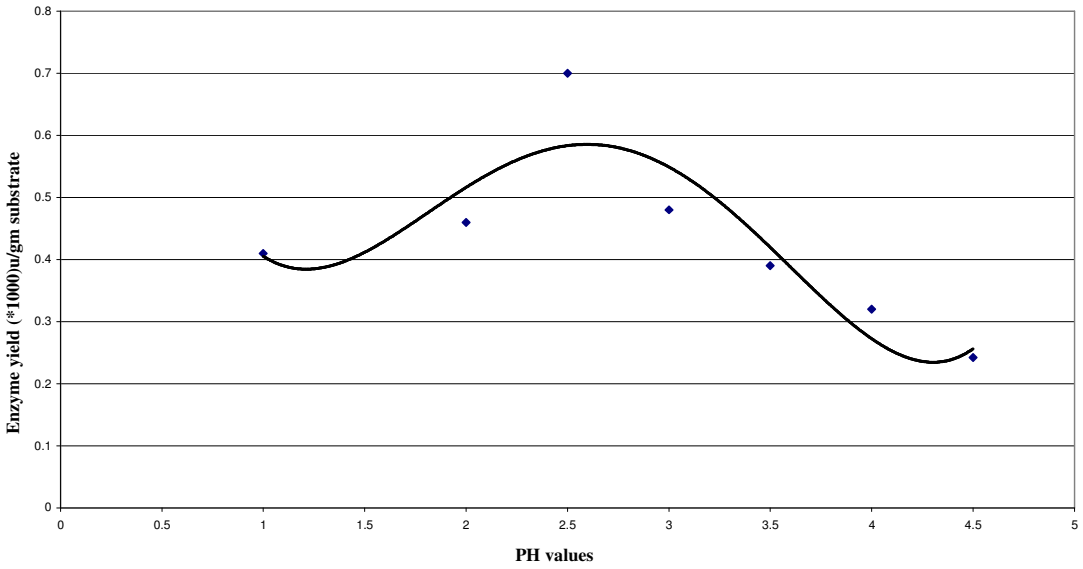

Fig 4.pH values effect on enzyme yield at constant other variables (temperature of 32 ,incubation time of 100 hours and hydration ratio of 1:3.

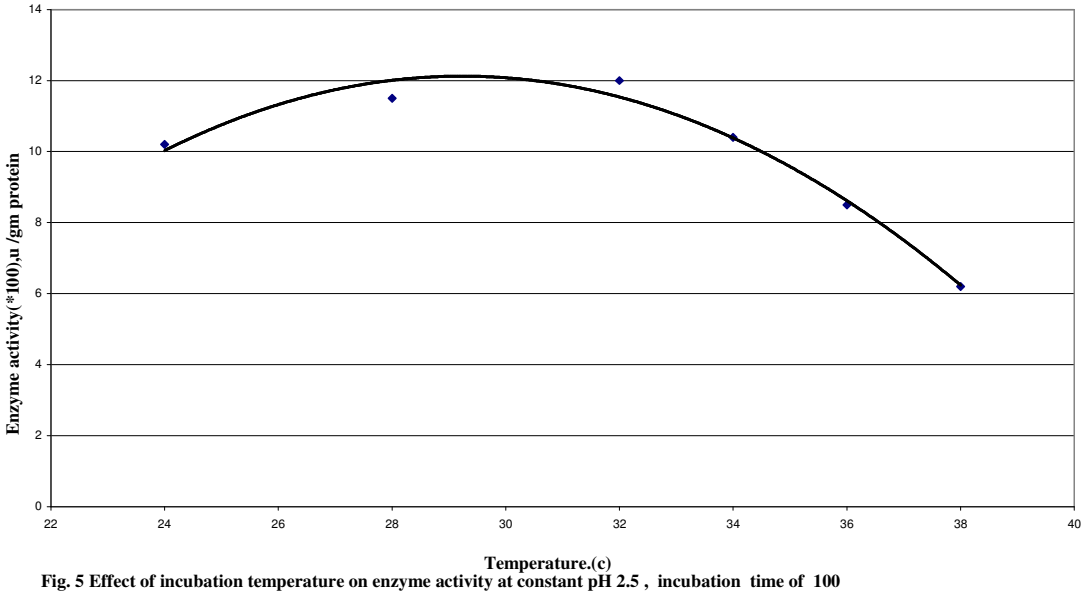

Fig. 5 Effect of incubation temperature on enzyme activity at constant pH 2.5 , incubation time of 100 hours and hydration ratio of 1:3 .

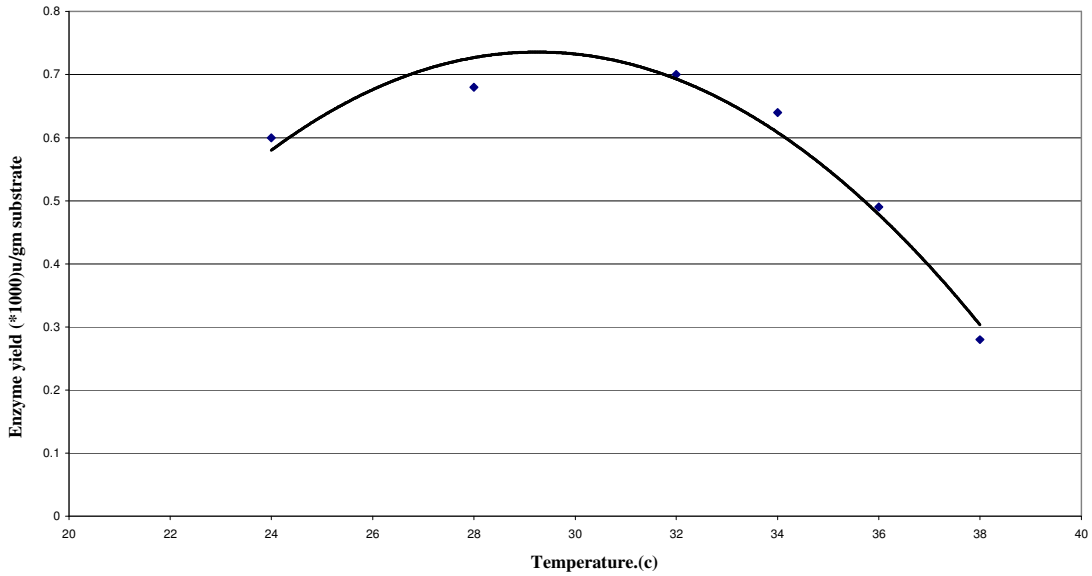

Fig. 6 Effect of incubation temperature on enzyme yield at constant pH 2.5 , incubation time of 100 hours and hydration ratio of 1:3

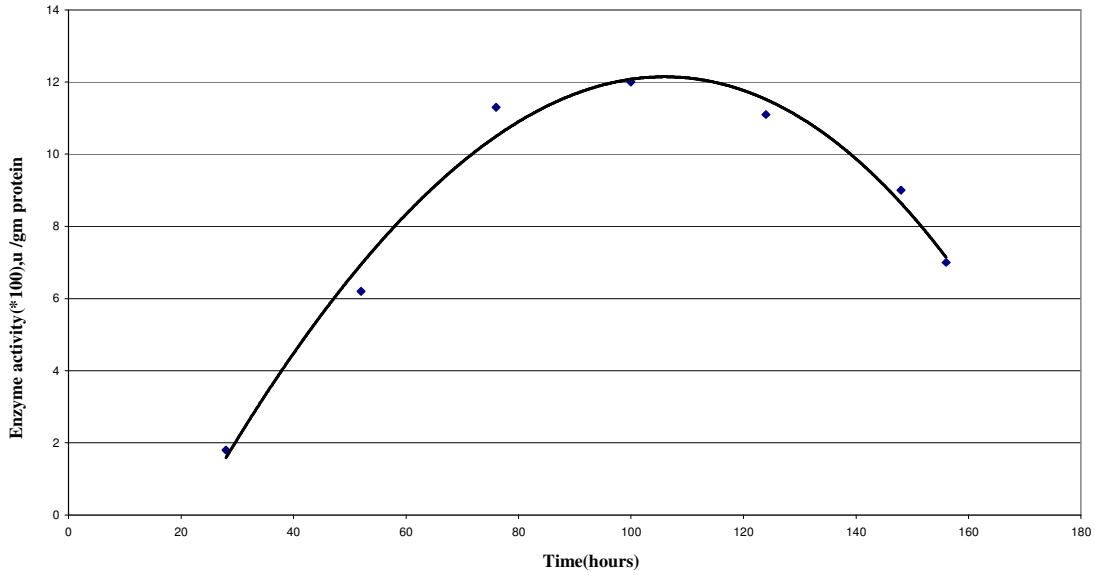

Fig.7 Effect of incubation time on enzyme activity at constant other variables (temperature of 32 ,pH of 2.5 and hydration ratio of 1:3)

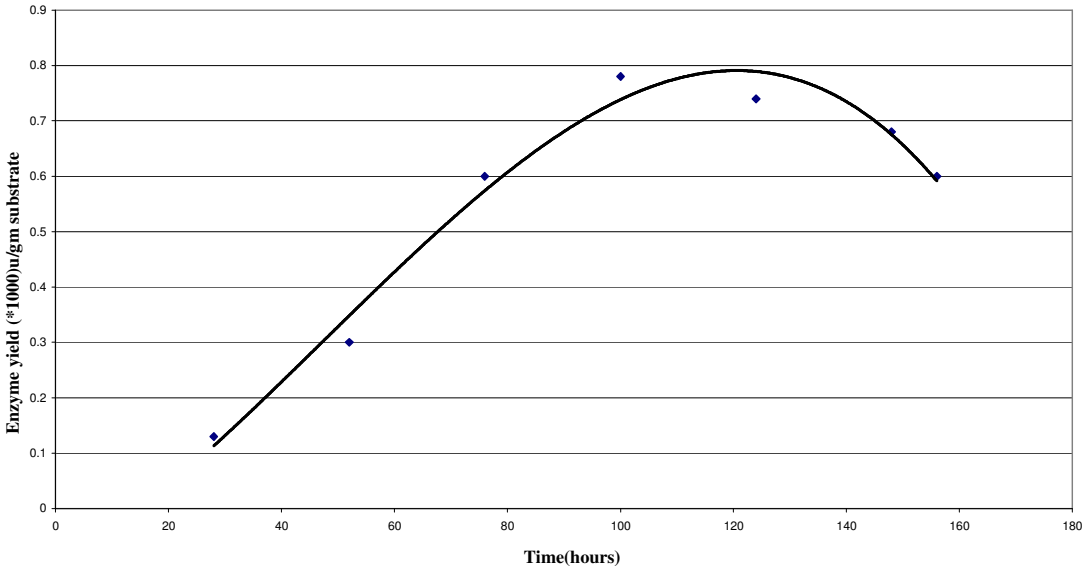

Fig.8 Effect of incubation time on enzyme yield at constant other variables (temperature of 32 ,pH of 2.5 and hydration ratio of 1:3.

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انتاج انزيم البروتيز من نخالة الحنطة

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الخلاصة

تم دراسة عملية إنتاج انزيم البروتيز كخطوة اولى من أجل وضع المعايير الاساسية وإيجاد الظروف التشغيلية لغرض تصميم المخمر fermenter في الحالة الصلبة من خلال تنمية كائنات حية مجهرية وهي نوع من الاعفان تسمى (*Aspergills niger*)، على وسط زرعي هو نخالة الحنطة . تتكون مراحل البحث من تغيير الحامضية للوسط الزرعي حيث نقوم بترطيبه بمحاليل قاعدية وحامضية واخرى متعادلة بنسب ترطيب مختلفة حيث توضع باوعية حجم ٢٥٠ مل حيث يوضع بها الوسط الزرعي وهو نخالة الحنطة ويتم اللقاح بالكائن الحي المعد سلفا" بعدد من الخلايا عددها $١٠^{٥}$ / غرام ثم يتم اخذ النماذج ودراسة تاثير الحامضية ونسبة الترطيب،زمن التخمير، ودرجة الحرارة على الفعالية للانزيم المنتج وكميته حيث يتم تثبيت احدى المتغيرات السابقة وتثبيت المتغيرات الاخرى وايجاد الظروف المثلى لهذه المتغيرات.

أظهرت النتائج إن الظروف التشغيلية المثلى لغرض تصميم المخمر هي درجة الحرارة تساوي ٣٢°م، والزمن يساوي ١٠٠ ساعة، والحامضية تساوي ٢,٥ ونسبة الترطيب تساوي ١:٣.