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Full Length Research Paper

Determination of kinetic parameters of α-amylase producing thermophile *Bacillus sphaericus*

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Studies on the α-amylase-producing thermophilic bacterium isolated and identified from a hot spring in Jordan and designated as *Bacillus sphaericus* were carried out in a laboratory scale fermenter. The growth and enzyme production optimum conditions were pH 7 and 50°C. The kinetic study of cellular growth indicates μ_{max}, K_s, τ_d, $Y_{x/s}$ and k_d were 0.53 h^{-1}, 1.1 g/l, 1.98 h, 0.44 g cell/g starch and 0.4 g/l/h, respectively. The optimum starch concentration for the enzyme production was 32 g/l and higher concentrations show substrate inhibition with inhibition constant K_i 190 mg/l. The kinetic parameters of α-amylase activation V_{max}, and K_m were 263 µmole mg^{-1} enzyme min^{-1} and 0.97 mg/ml, respectively. The effect of different carbon and nitrogen sources on the cellular growth was tested.

Key words: *Bacillus sphaericus*, thermostable α-amylase thermostable enzymes, kinetic parameters, batch fermentation, Jordan hot springs.

INTRODUCTION

Among the starch-hydrolyzing enzymes, α-amylase (1,4-α-D-glucan glucanohydrolase, EC 3.2.11) is of considerable commercial importance especially in the production of sweeteners (Malhotra et al., 2000). However, there are two main requirements in the production process of sweeteners from starch: the temperature should be 50°C or more and pH close to 7 to prevent the browning effects and to reduce the viscosity of starch pastes (Castro et al., 1999). This requirement necessitates the use of thermostable α-amylase in order to withstand the process temperature. Consequently, the use of thermostable α-amylase produced by thermophilic microorganisms of high cellular growth is of major interest (Jin et al., 2001). In addition, the use of thermostable enzymes in biochemical processes has shown many advantages such as the enhancement of the reaction rate constant, increasing the diffusion rate as the medium viscosity decreases with temperature (Milo et al., 1999), Increasing the solubility of the hydrophobic substrates and lowering the risk of contamination by pathogenic microorganisms (Mützel et al., 1996). Furthermore, these thermophilic microorganisms usually produces enzymes of increased shelf-life via enhancement their resistance to chemical denaturation (Becker et al., 1997).

Thermostable α-amylase has been produced by different types of microorganisms such as: *Bacillus thermoleovorans* (Malhotra et al., 2000; Narang and Satyanarayana, 2001), *Bacillus licheniformis* (Castro et al., 1999), *Bacillus* sp. (Jin et al., 2001), *Geobacillus* (former *Bacillus*) *stearothermophilus* (Brigidi et al., 1997; Srivastava, 1987), *Norcardiopsis* sp. (Stamford et al., 2001) and *Bacillus amyloliquefaciens* (Kallio et al., 1987). The present investigation represents another contribution in this context and deals with the characterization of α-amylase producing thermophilic *Bacillus sphaericus* isolated from Jordan hot springs. The growth kinetics of this thermophile various on starch as a limited substrate will be investigated in shake flasks as well as in laboratory-scale batch fermenter. The kinetic parameters of the produced enzyme in addition to the effect of temperature and pH on its stability will be examined.

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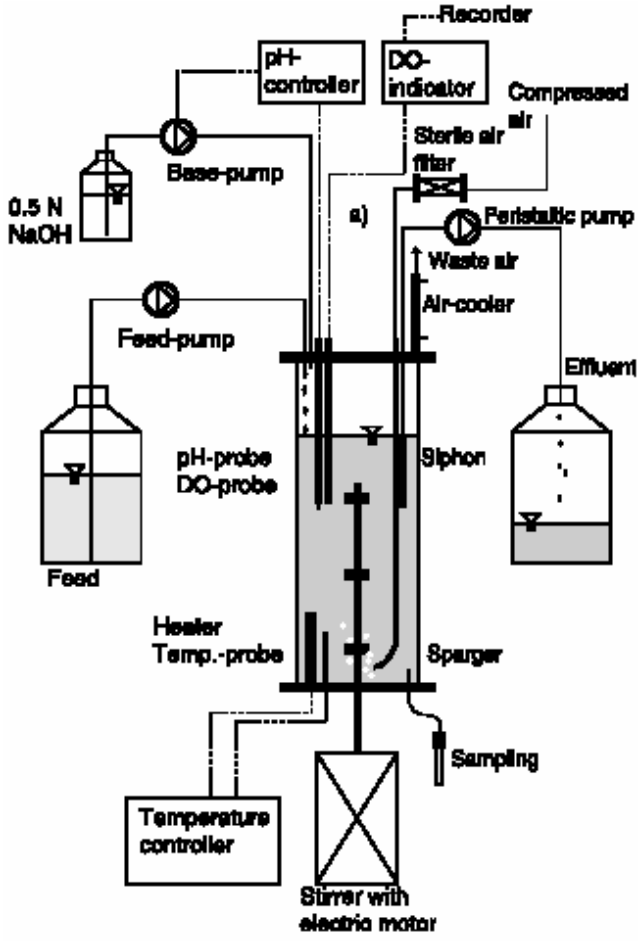

Figure 1. Fermenter Bioreactor set-up for the continuous and batch experiments.

MATERIALS AND METHODS

Microorganism and medium

The microorganism used in this study was isolated from samples of a Jordanian hot spring in the north area. These samples were collected from points 50 cm below the water surface where the temperature was 60°C, and pH was 7. Samples were cultivated using tryptic soy broth (v/v) and incubated for 7 days at different temperatures. It was found that the microorganism is aerobic, elliptic-shaped, and was identified to be *B. sphaericus*. The optimal conditions for growth were 50°C and pH 7**.**

Precultures of this strain were grown in previosly autoclaved, tightly closed 250 ml Erlenmeyer shake flasks containing 50 ml (OCHs, Bovenden, Germany) of *Bacillus* growth medium. These liquid cultures were incubated for 24 h at 50°C in a shaking incubator at 200 rpm (Infors, Bottimingen, Switzerland). These preculture were used to inoculate four Erlenmeyer shake flasks used to optimize the carbon source. The broth medium used for cells cutivation in these flasks comprising 98% solution A, and 1% solutions B and C. Solution A contains (g/l deionized water) unless stated otherwise: carbon source (soluble starch, glucose, sucrose or olive oil) 10, ammonium sulphate 3, K_2HPO_4 0.6, and KH_2PO_4 0.54. Solution B contains 20 g $MgSO_4.7H_2O$ per 1000 ml deionized water and solution C contains (g/l deionized water): $CaCl_2.2H_2O$ 2, $FeSO_4.7H_2O$ 1, $MnSO_4.H_2O$ 0.5, $CuSO_4.5H_2O$ 0.1, $Na_2MoO_4.2H_2O$ 0.1 and HCl (0.1 M). Solution A was freshly prepared as required and autoclaved before inculating. Solutions B and C were prepared as stock solutions and added to solution A using sterile filter and aseptic conditions. After addition of solutions A and B the pH was adjusted to 7.0 using 0.1 M NaOH. All chemicals and reagents used were analytical grade available in the laboratory. Absorbance of the cell suspensions was measured at regular intervals in cuvettes (light path, 1 cm) at 600 nm. After 24 h incubation, it was found that the fermentation flask containing starch had the highest turbidity, whereas that contining olive oil had very little turbidity. The fermented broth of starch substrate was centrifuged at 13000 g for 5 min, at 4°C. The cell free supernatant gave positive test for α-amylase activity.

Batch fermentation in a Lab-scale fermenter

Batch fermentation experiments were carried out in a 100 mm diameter foil lab-scale fermenter (Type, VSF, Bioengineering, Wald, Switzerland) with a working volume of 1.7 l. The details of the bioreactor design and instrumentation are described in Figure 1. The fermentation was conducted at 50°C and the pH was maintained at 7 by the controlled addition of 1 M NaOH. The agitation speed was set to 1000 rpm and an aeration rate was adjusted to 80 l/h. The dissolved oxygen concentration was monitored using a standard amperometric pO_2 probe (Mettler-Toledo Prozeβanalytic GmbH, Stein-batch, Germany). This flow rate was enough to maintain pO_2 above 10% saturation to avoid oxygen limitation. Each of the batch process continued until the optical density reached a maximum and started to decrease after the stationary phase.

The cell dry weight (CDW) was determined by centrifugation certain volume of the fermentation broth and then washing the pellets twice by deionized water followed by drying of the washed pellets at 105°C for 2 h.

Assay of α-amylase

The activity of α-amylase was evaluated by two methods. In the first method 1.0 ml of substrate solution containing 2% (w/v) soluble starch (Sigma, Germany) in sodium phosphate buffer (pH 7.0) and 1.0 ml of the enzyme solution (cell free supernatant) were incubated at 55°C. After 10 min of incubation the reaction was stopped by 3,5-dintrosaliclic acid procedure (Bernfeld, 1955). One unit of activity was defined as the amount of enzyme that produced 1 µmole of reducing sugar as glucose per min under the conditions of assay. The second method was based on the reduction in blue color intensity resulting from enzyme hydrolysis of starch (Bajpai, 1989; Teodoro and Martins, 2003). In this method the reaction mixture contained 10.0 ml of substrate solution containing 1% (w/v) soluble starch (Sigma-Aldich Chemie GmbH, Germany, Germany) in sodium phosphate buffer (pH 7.0) and 1.0 ml of the enzyme solution were incubated at 55°C for 10 min, then reaction was stopped by adding 10 ml of 0.1 N HCl. One more dilution was made by mixing 1 ml of this acidified solution with additional 10 ml of 0.1 N HCl. Then 1 ml from this solution was added to 10 ml iodine reagent containing 0.05% iodine and 0.5% KI. The absorbance was measured at 660 nm. One unit of activity was defined as the amount of enzyme that reduces the intensity of blue color of starch-iodine solution by 1% at the assay conditions.

Kinetic parameters of α-amylase and its stability under different conditions

The kinetic model of the global amylolytic activities using starch as a limited substrate will be investigated using the simple Michaelis-

Menten kinetics. The effect of temperature, pH and on the enzymatic stability was studied. Various metal ions and inhibitors were studied by incubating the ion of 0.1 mM concentration with the enzyme for 2 h, and then the enzyme activity was measured as before. The ions used were: Na^{+}, Ca^{+2}, Zn^{+2}, Mg^{+2}, Ba^{+2}, Cu^{+2}, Mn^{+2}, Fe^{+2}, Fe^{+3}, and Ni^{+2}. Some of these ions may be found in different proportions in the operational liquid media for the enzyme depending on the application. In addition, the salt tolerance effect was carried out by incubating 10 mM of phosphate buffer (pH 7.0) containing various NaCl concentrations for 24 h at 4°C and in each case activity of the enzyme was measured.

Mathematical model description

There is no information available in the literature about the growth kinetic of thermophilic *B. sphaericus* on starch as a sole carbon source. However, Singh et al. (2004) used the unstructured kinetic model proposed by Kono (1968) for protease production by mesophilic *B. sphaericus* using glucose as a sole carbon source. In addition, this model has successfully been used for kinetic analysis of a number of fermentation processes (Singh et al., 2004). This model assumes that cell growth passes through lag phase, transition phase, exponential phase, declining phase, stationary phases and death phase. On the other hand, enzyme production according to this model starts at the exponential phase and continued in an additional growth phase. For this reason, the kinetic parameters of the cellular growth of thermophilic *B. sphaericus* will be determined using this model and compared by those of Singh et al. (2004) for mesophilic *B. sphaericus* for the production of alkaline proteases.

The growth rate in the exponential growth phase can be expressed by the following first order equation:

$$dX/dt = \mu X \quad (1)$$

Where X is the cell concentration g l^{-1}, μ is the specific growth rate (h^{-1}) and t time (h). Integrating this equation within the range of the exponential growth phase yields the following equation:

$$X = X_C \exp \mu (t - t_C) \quad (2)$$

Where X_C is the cell concentration (g/l) at the boundary point between the exponential and deceleration growth phase, t_C. The growth rate, μ, in this growth phase is equal the slope of the plot between ln (X / X_C) versus ($t - t_C$).

The growth rate in the decline growth phase or the deceleration phase is given by the following equation:

$$dX/dt = \mu X_C (X_M - X) / (X_M - X_C) \quad (3)$$

Where X_M the theoretical maximum cell concentration (g l^{-1})

The integral form of equation 3 can be written as:

$$X = X_M - (X_M - X_C) \exp - \{\mu X_C (t - t_C) / (X_M - X_C)\} \quad (4)$$

Equation 4 is a function of t only and cell concentration at any arbitrary time, t, can be simultaneously calculated.

The growth rate in the stationary phase is zero where as the death rate can be expressed by the first order model:

$$dX/dt = - k_d X \quad (5)$$

Where k_d is the death rate constant (h^{-1})

The yield coefficient $Y_{x/s}$ (g cells/g substrate) and the doubling time τ_d (h) were calculated by the following equations:

$$Y_{x/s} = \Delta x / \Delta s \quad (6)$$

$$\tau_d = \ln 2 / \mu_{max} \quad (7)$$

Since starch when used as a limited substrate showed inhibition at relatively high concentration the substrate inhibition kinetics expressed by the following equation could be assumed to describe the growth kinetics of *B. sphaericus* on starch as a limited substrate:

$$\mu = \mu_{max} S / (k_s + S + (S^2 / K_I)) \quad (8)$$

Where K_I is the equilibrium constant of the inhibition constant, g/l, k_s is the half-saturation constant of growth kinetics, g/l and S is the substrate concentration, g/l. At low substrate concentration the term (S^2/K_{SI}) can be neglected and equation 9 reduces to Monod equation as follows (Feitkenhauer et al., 2001, 2003):

$$\mu = \mu_{max} S / (k_s + S) \quad (9)$$

On the other hand, at high substrate concentration the term (k_s /S) can be neglected and equation 8 reduces to:

$$\mu = \mu_{max} / (1 + S / K_I) \quad (10)$$

The kinetic model of the global enzymatic activity using starch as a substrate could be expressed using the simple Michaelis-Menten kinetics expressed by the following equation:

$$v = v_{max} S / (k_m + S) \quad (11)$$

Where v_{max} is the maximum reaction rate constant (h^{-1}) and k_m is the saturation constant g/l. The double reciprocal plot (Lineweaver-Burk) plot will be used to determine the values of k_m and v_{max}.

RESULTS AND DISCUSSION

Characterization and identification of the microorganism

B. sphaericus bacterial strains were isolated from Al-Shoneh hot springs in Jordan. Colony counting revealed that each species was present in water sample with a concentration ranged from 1.0 – 5.0 x 10^3 cells/ml. After identification and characterization, the isolates were screened for thermostable α-amylase enzyme production at pH 7.0. Strains were cultured on different types of agar supplemented with 2% soluble starch and those of higher enzyme activity were selected according to the clearance area around the colonies.

The organism *B. sphaericus* grows on blood agar producing circular and adherent colonies at pH 7.0 and 55°C. This organism has a gram-positive type cell wall as determined by gram staining and electron microscopy. The cells have ellipsoidal shape and adhere to each other to form longitudinal chains containing two or more cells. This highly motile bacterium was identified as *B. sphaericus* by the API test reactions. Since this strain is the first α-amylase producing thermophile isolated from Al-Shoneh hot springs in Jordan it is named as *B. sphaericus* JT3.

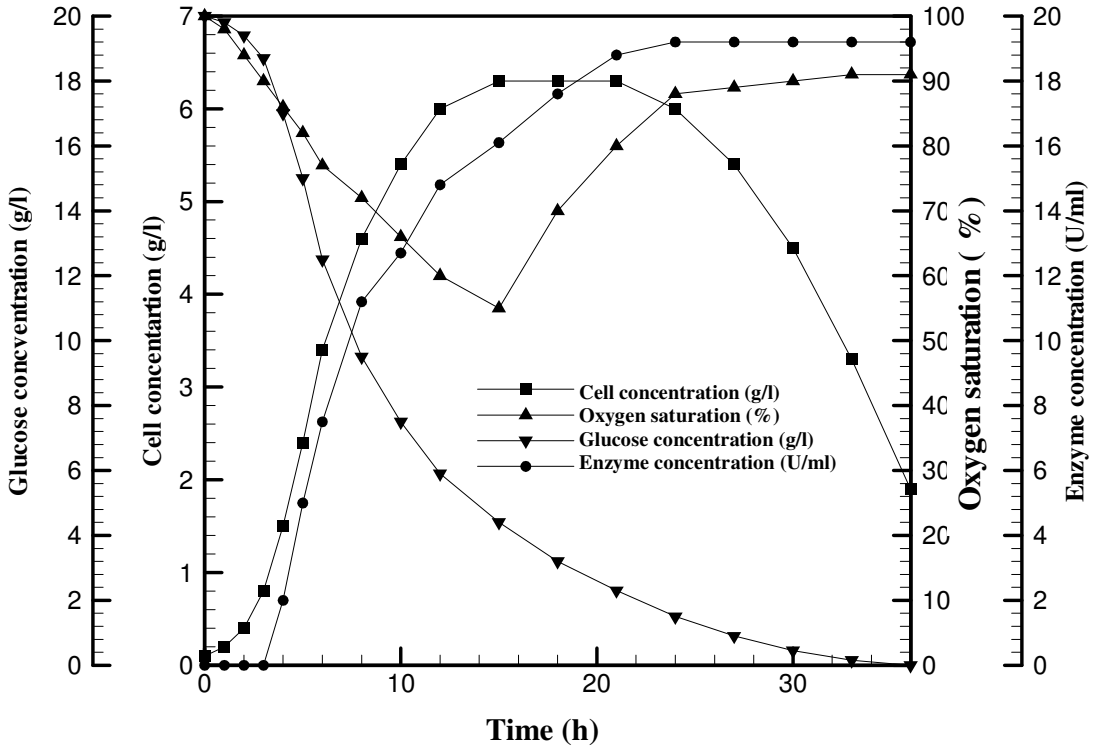

Figure 2. Batch production of α-amylase by *B. sphaericus*. The temperature of the fermenter was controlled at 50°C, pH 7.0, air flow rate 80 l/h and agitation speed 1000 rpm.

Analysis of growth kinetic parameters

The results of batch α-amylase fermentation by *B. sphaericus* grown on starch as a limited carbon source in batch fermenter are shown in Figure 2. Cell growth was limited in the first 2 h after inoculation and cell mass increases from 0.1 to 0.4 g/l in this period where as the substrate concentration decreases from 20 to 19.4 g/l. In addition, the increase in the enzymatic activity in the same period was negligible and the dissolved oxygen concentration, PO_2 decreases from 100 to 96%. This behavior is common in most batch fermentation processes where a lag phase is required for adaptation to the new environment. Then the lag phase was followed by the exponential growth phase, which continues 6 h. In this period cell density increases from 0.4 to 4.6 g/l., the enzyme activity increases from 0 to 11200 U/l. The rapid cellular growth in this phase was accompanied by a sharp decrease in both the dissolved oxygen concentration, PO_2, which decreases from 96 to 78% and the substrate concentration which decreases from 19.4 to 9.5 g/l. The exponential growth phase was followed by the decline growth phase. This phase started after 8 h from the inoculation time and continues 7 h. In this phase the cell density increases from 4.6 to 6.3 g/l, the enzymatic activity increases from 11200 to 16200 U/l, the dissolved oxygen concentration decreases from 78 to 55% and the substrate concentration decreases from 9.5 to 3.2 g/l.

After the decline growth phase, a stationary growth phase of constant cell mass was observed over a period of 6 h. However, the enzymatic activity in this period continues to increase from 16100 to 18800 U/l, indicated partly growth associated product. In addition, the dissolved oxygen concentration increased rapidly from 55 to 80% indicting that the rate of oxygen consumption is lower than the rate of oxygen transfer from the gaseous phase to the fermentation broth. Subsequently, the substrate concentration decreased slowly until it reached 2.3 g/l. This decrease in the substrate is consumed as a source of energy for the non growing cells. At the end of the stationary phase the death phase of the old cells started. The cell density after 36 h from the inoculation decreased to 1.9 g/l and while the enzymatic activity was constant and equal 19200U/l. In this death phase the dissolved oxygen concentration continued to increase until it reached 92% and the substrate was completely depleted. Appreciable amount of the foam was formed in this phase indicating the endogenous metabolism characterizing this phase.

Figure 3 shows the variation of cell growth rate, dX/dt, substrate utilization rate, dS/dt, and the amylase production rate, dP/dt, with the fermentation time, t. It is evident from Figure 3 that the cell growth rate increased until it reached it's maximum after 8 h from the inoculation time, then it continuously decreased in the decline growth phase until its value becomes zero in the stationary pha-

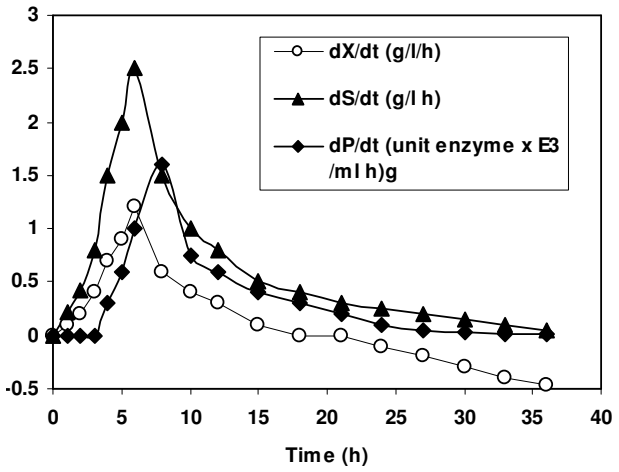

Figure 3. Rate profiles of growth (dX/dt), starch utilization (dS/dt) and enzyme production (dP/dt).

se. Subsequently, in the death phase the rate of cell mass change is negative as a result of the endogenous metabolism. Similarly, the substrate consumption rate increased and its maximum occurred after 8 h from the inoculation time which is the same time where the cell mass maximum rate occurred. However, substrate consumption continues even when the cell growth rate was zero or negative. This is attributed to the energy requirements of the living cells. Furthermore, the enzyme production rate was observed to start after 3 h from the inoculation time and its maximum was lagging behind the maximum growth rate by 2 h or occurred in the decelerating growth phase. The enzyme production rate then started to decrease until it reached zero when the substrate concentration approaches zero, indicating that the presence of starch is required for post growth enzyme synthesis. These results are in agreement with those of Singh et al. (2004), for the production of proteases using mesophilic *B. sphaericus*.

The kinetic parameters of α-amylase production by thermophilic *B. sphaericus* are presented in Table 2. These parameters were evaluated from Figures 1 and 2 and from the set of equations used in the model. The differences between the values obtained in this investigation and those of Singh et al. (2004) are referred to growth conditions such as substrate, temperature, pH and type of enzyme produced.

The effect of starch concentration on the relative cell mass of *B. sphaericus* growth is shown in Figure 4. It is evident from Figure 6 that the cellular growth increases as starch concentration increases until it reaches a maximum at a starch concentration of 32 g/l then the cell mass starts to decrease until it reaches 50% of the maximum at a starch concentration of 60 g/l. These results agree with those of Molhatra et al. (2001) for the production of α-amylase by an extreme thermophile *B. thermooleovorans* at 70°C and pH 7.0. This behavior is attributed to the fact that at high concentration of carbon source, the consumption of the carbon-energy substrate is rapid (Shuler and Kargi, 1996), then the release of toxic wastes is more likely due to energy-spilling reactions.

As mentioned above, the growth kinetic model at relatively low substrate concentration or 32 g/l starch in the present investigation can be represented by Monod equation described by equation (9). The values of the model parameters k_s and μ_{max} evaluated by constructing the double reciprocal (Lineweaver-Burk) plot were 1.5 g/l and 1.05 h^{-1}, respectively. On the other hand, at relatively high substrate starch concentration (>32 g/l in the present investigation) starch shows inhibition. The value of the substrate inhibition constant K_I was determined from a plot of 1/μ versus ($S/(K_I\mu_{max})$ which will produce a straight line of slope 1/ μ_{max} and intercept of (1/ K_I K_s). the value of K_I. The value of K_I obtained from the intercept was 190 mg/l.

Effect of temperature and pH

The variation enzyme activity as a function of temperature is shown in Figure 5. The enzyme activity increases as temperature increases until it reaches the maximum activity at 65°C then it decreases until complete deactivation was recorded at 100°C. α-amylase activity was 64% of the maximal activity at 35°C. Moreover, the thermal stability of the enzyme was measured at 64°C and was found 3.5 h. These results are reasonable compared to those found in the literature like those of Molhatra et al. (2000) who reported production of α-amylase by *B. thermooleovorans* at 70°C and optimum pH of 8.0 with a half-life of 3 h at 100°C.

Figure 6 shows the relative activity of α-amylase at different pH values. It is evident that the optimum activity was obtained at pH 7.0 and it retained relatively high activity in the pH range 7 - 10. For instance, at pH 10 the enzyme retained about 80% of its maximal relative activity. However, steep reduction in α-amylase activity in acidic media. This activity is 30% at pH 5.5 and complete deactivation occurred at pH 4.5. This behavior is attributed to the structure of functional groups in the active site which seem to be basic and react with the hydrogen ion and the result was deformation of the enzyme active site. These results are in agreement with those of Srivastava and Baruah (Srivastava and Baruah, 1986) for the growth of *G. sterothermophilus* where complete deactivation occurred at pH 4.0. However, there are other α-amylases that have optimum pH in the acidic range. Stamford et al. (2001) reported that the optimum pH for the α-amylase produced by *Nocardiopsis* sp. at 70°C was 5.0.

Effect of metal ions

Table 3 shows the residual activity of α-amylase after incubation with different ions of 0.1 mM concentration of

Table 1. Effect of carbon and nitrogen sources on the growth of *G. sterothermophilus* JT2 and amylase activity after incubation for 24 h.

Carbon source	Biomass (A 600)	Enzyme activity (U/l)	Nitrogen source	Biomass (A 600)	Enzyme activity (U/ml)
Sucrose	1.92	15950	None	0.20	0
Maltose	1.91	14021	Peptone	1.75	13960
Lactose	1.61	12860	Yeast extract	1.84	14520
Fructose	1.7	13800	Beef extract	1.71	13690
Starch	1.97	16060	Casein	1.79	13980
Citric acid	2.12	16900	$NaNO_3$	2.19	15360
Olive oil	1.12	2300	KNO_3	1.79	14280
Glycerol	1.60	3700	NH_4Cl	1.20	1700
D-Galactose	1.42	10100	Urea	1.10	2100

Table 2. Kinetic parameters of batch α-amylase production process.

Model constants	Value
μ_{max} (h^{-1})	0.53
τ_d (h)	1.98
$Y_{x/s}$ (g cells/g starch)	0.44
X_C (g/l)	3.4
X_M (g/l)	7.5
k_d (g/l/h)	.04

Table 3. Effect of various metal ions on amylase activity.

Metal ions	Residual activity (%)
None	100
Ag^{+1}	89
Ca^{+2}	98
Mn^{+2}	92
Mg^{+2}	98
Fe^{+2}	77
Fe^{+3}	55
Zn^{+2}	70
Cu^{+2}	92
Ba^{+2}	40
Ni^{+2}	94
Pb^{+2}	89

the ion chloride for 2 h. It is clear in the table that some ions such as Ca^{+2}, Mn^{+2}, Ni^{+2} and Cu^{+2} retain enzymatic activity by 98, 98, 94 and 92%, respectively. On the other hand Ba^{+2} ions have retains only 40% of the enzyme activity. Similar results were obtained by Srivastava (1987) who studied the effect of different cations and inhibitors on the activities of α-amylase and β-amylase produced from *G. sterothermophilus* grown at 50°C.

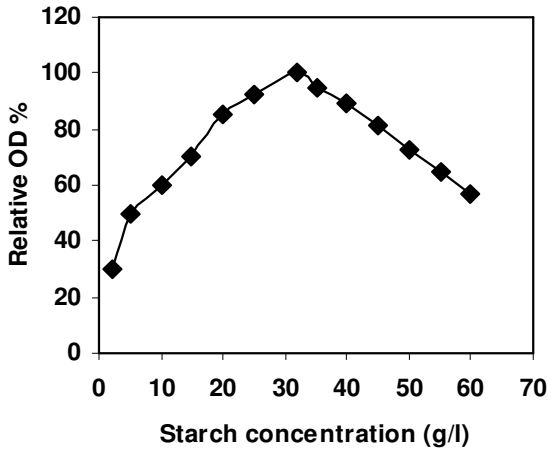

Figure 4. The effect of starch concentration on the microbial growth at 55°C and pH 7. The maximum (100%) optical density (600 nm) of the fermentation broth was 6.4.

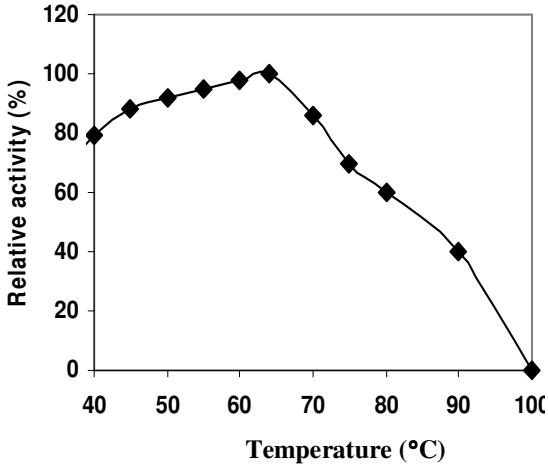

Figure 5. Effect of temperature on the stability of α-amylase at pH 7.0, (100% of enzyme activity = 19.2 U/ml).

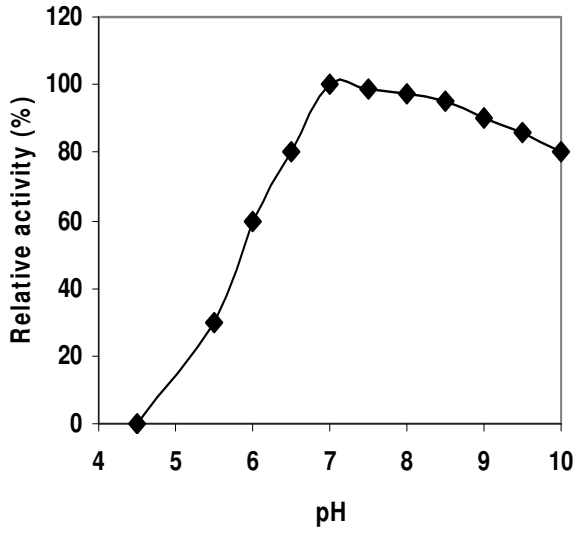

Figure 6. Effect of pH on the relative activity of the α-amylase at 50°C, (100% of enzyme activity = 19.2 U/ml).

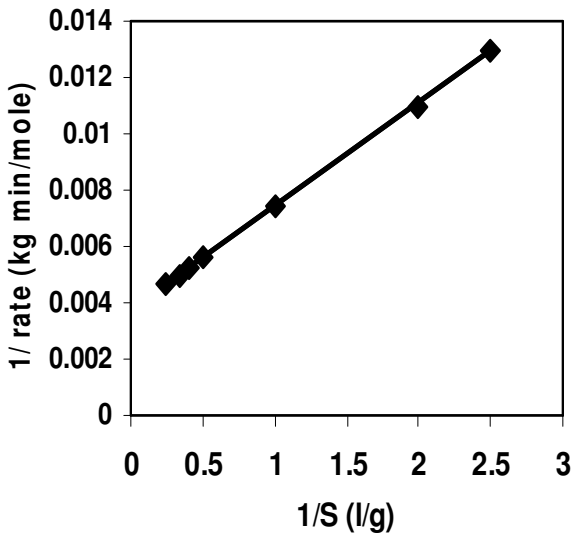

Figure 8. Line weaver-Burk plot for estimation of α-amylase kinetic parameters at pH 7 and

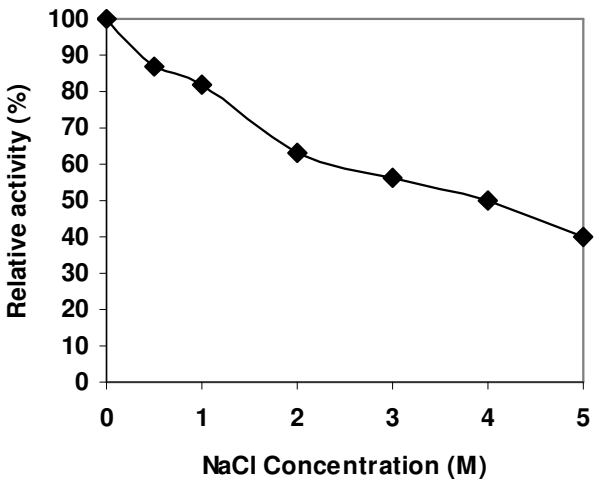

Figure 7. Effect of NaCl concentration on the stability of α-amylase at 50°C, pH 7.0, (100% of enzyme activity = 19.2 U/ml).

Salt tolerance test

This test is important in treatment of effluent with high salinity containing starch or cellulosic residues in pollution control mechanism (Cordeiro et al., 2002). The enzyme α-amylase produced by *G. sterothermophilus* was incubated in 1.0 M and 5 M NaCl. Figure 7 shows the effect of NaCl concentration on the enzyme activity. It can be seen from Figure 7 that the enzyme activity decreases as NaCl concentration increases. However, the enzyme retains 63% and 40% of its original activity after 24 h at 4ºC, when NaCl concentration was the 3 and 5 M, respectively.

Enzymatic kinetic parameters

The kinetic model of the global enzymatic activity using starch as a reaction limited substrate could be expressed using the simple Michaelis-Menten kinetics expressed by equation (11). The resulted plot has a slope equal K_m/V_{max} and an intercept equal $1/V_{max}$. The values of V_{max}, and K_m were 260 µmole mg^{-1} enzyme min^{-1} and 0.96 mg/ml (Figure 8).

Conclusion

A thermophilic bacterium designated *B. sphaericus* JT3 was isolated from Al-Shoneh hot springs in Jordan. This strain was grown on mineral medium with starch as carbon source and at different operational conditions in a lab scale fermenter. The optimum conditions for growth were temperature of 50°C and a pH 7.0. The kinetic parameters of cellular growth in the different phases were estimated. The result indicates that the values of μ_{max}, K_s, τ_d, $Y_{x/s}$ and k_d were 0.53 h^{-1}, 1.1 g/l, 1.98 h, 0.44 g cell/g starch and 0.4 g/l/h, respectively, respectively. The kinetic parameters of α-amylase activation V_{max}, and K_m were 260 µmole mg^{-1} enzyme min^{-1} and 0.98 mg/ml, respectively. The characterization study showed that the enzyme activity was retained in the presence of 0.1 mM of Ca^{+2}, Mn^{+2}, Ni^{+2} and Cu^{+2} retain enzymatic activity by 98, 98, 94 and 92% respectively. On the other hand Ba^{+2} ions have retains only 40% of the enzyme activity. The salt tolerance test indicates that the enzyme activity decreases as Na^{+1} concentration increases. The above results indicate that the isolated thermophilic bacterium has potential for the production of α-amylase of relatively improved stability at different conditions.

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