Evaluation of Ca-independent α-amylase production by *Bacillus* sp. KR-8104 in submerged and solid state fermentation systems

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Abstract
This study investigates the production of crude Ca-independent and low pH active α-amylase by *Bacillus sp.* KR-8104 in submerged fermentation (SmF) and solid-state fermentation (SSF) systems. Different parameters were evaluated in each system using "one factor at a time" approach to improve the production of enzyme. The results showed that in the SmF the maximum enzyme production was achieved in culture medium that contained dextrin as a carbon source, as well as yeast extract and meat extract as nitrogen sources incubated at 37ºC and 180 rpm for 48 h. While SSF of *Bacillus* sp. KR-8104 using wheat bran (WB) as a substrate showed that using tap water or distilled water as a moisturizing agent, a substrate-water ratio of 1:1.5 (w/v) and incubation at 37ºC for 48 h gave the maximum α-amylase production. From different extraction medium examined in this study 0.1% (v/v) aqueous mixture of Tween 20 and distilled water illustrated maximum results (~100 U/g).
Keywords: Ca-independent α-amylase; *Bacillus sp.* KR-8104; submerged fermentation; solid state fermentation

INTRODUCTION

Amylases are among the most important enzymes in global market that have potential application in a number of industrial processes such as food, pharmaceuticals, cosmetics, fermentation, textile and paper industries. Although they can be derived from different sources, including plants, animals and microorganisms, microbial enzymes generally meet industrial demands. The performance, economics and feasibility of each α-amylase applications is influenced by important enzyme characteristics, including specificity, stability, optimum temperature and pH performance (Gupta *et al*., 2003). Therefore, selection of the suitable microbial strains which produce enzymes that show good activity and stability at industrially desirable conditions will be valuable.

At present, microbial amylases have almost been completely replaced the chemical hydrolysis of starch in the starch processing industry. In the starch industry, the pH of the starch slurry, which is normally around 4.5, must be adjusted between 5.8 and 6.2 prior to the use of α-amylase in the liquefaction step (Sivaramakrishnan *et al*., 2006) because most of the commercial bacterial α-amylases are unstable at low pH. Hence, the development of an active and stable α-amylase at low pH values would result in omitting the required pH adjustment step (Liu and Xu, 2008; Sajedi *et al*., 2005). Additionally, the stability and/or activity of α-amylases that are generally used in starch hydrolysis are Ca^{2+}-dependent. Since Ca^{2+} ions inhibit glucose isomerase, the added Ca^{2+} must be removed from the product stream using ion-exchangers before the isomerization step from glucose to fructose. Consequently, the demand for Ca^{2+}-independent α-amylases has increased (Antrim, 2007).

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Although, amylase has been produced by submerged fermentation (SmF), SSF has remarkable potential for the production of industrial enzymes in view of its economic and engineering advantages. It can be of particular significance in those processes where a crude product may be used as an enzyme source (Oliveira *et al*., 2010). Agro-industrial residues are generally considered the best substrates for the SSF processes and enzyme production in SSF. The use of SSF for the production of enzymes and other products has many advantages over submerged fermentation (Rose´s and Guerra 2009; Gangadharan *et al*., 2006) and these have been widely discussed in the literature (Pandey *et al*., 2008).

Generally, the microbial production of α-amylase is greatly influenced by the components of the culture medium, especially the carbon and nitrogen sources and physical conditions, such as the pH, temperature, agitation, level of dissolved oxygen and the inoculum concentration (Dey *et al*., 2001; Gigras *et al*., 2002; Dolnik *et al*., 2007; Oshoma *et al*., 2010; Rajagopalan and Krishnan 2010; Rezaei *et al*., 2010).

Bacillus sp. KR-8104 is a local isolate which has produced Ca-independent α-amylase with potential activity and stability at low pH (Sajedi *et al*., 2005). Such interested characteristics illustrate that it is logical to seek both submerged and solid state fermentation systems to improve the production of this enzyme. The purpose of the present study was to investigate the production of amylase under SmF as well as SSF conditions. A number of factors that influence amylase production by *Bacillus* sp. KR-8104 through both of above mentioned systems were studied using one factor at a time approach.

MATERIALS AND METHODS

Microorganism, inoculum, and culture conditions: *Bacillus* sp. KR-8104, which was isolated in previous work (Sajedi *et al*., 2005), was used for α-amylase production. The strain was maintained on agar slant at 4ºC. Inocula were prepared by transferring a loop of fresh culture from the agar slant into a 250 ml Erlenmeyer flask containing 50 ml of inoculum medium consisting of 8 g/l nutrient broth, 10 g/l meat extract, 10 g/l soy meal peptone, 10 g/l soluble starch and 0.5 g/l NaCl. The inocula were incubated for 18 h at 37ºC and 160 rpm. The number of viable colonies in the inocula was found using serial dilution and plating to be ~ 10^9 cfu/ml.

Enzyme production under SmF: The main culture medium for amylase production consisted of: 10.0 g/l soluble starch, 4.0 g/l soy meal peptone, 3.0 g/l meat extract, 1.0 g/l KH_2PO_4, and 0.3 g/l $MgSO_4.7H_2O$. The fermentations started with 5% (v/v) preculture, at initial pH 6.8, 37ºC, and 160 rpm in a rotary shaker incubator for 48 h under uncontrolled pH.

Enzyme production under SSF: Commercial quality wheat bran was purchased from the local market and used as the solid substrate. Ten grams of the substrate were placed in 500 ml Erlenmeyer flasks and moistened with distilled water at a ratio of 1:1.5. After autoclaving at 121ºC for 20 min, the flasks were inoculated with 2 ml of the inoculum. The contents of the flasks were then mixed thoroughly to ensure uniform distribution of the inoculum and incubated at 37ºC for 48 h. The pH was initially neutral and was allowed to follow its natural course throughout the fermentation.

Enzyme extraction: In SmF experiments, the cultures were harvested by centrifugation at 10,000 g for 10 min at 4ºC and the cell-free supernatant was used as the source of the enzyme. While in SSF samples, crude enzyme was extracted by mixing a known quantity of fermented substrate with distilled water in 1:10 ratio on a rotary shaker (180 rpm) for 1 h. The slurry was squeezed through wet cheesecloth. The filtrate was then centrifuged (10000 g, 10 min, 4ºC), and the clear supernatant was used as the enzyme source.

Amylase activity assay: The activity of α-amylase was determined by the Bernfeld (Bernfeld, 1955) procedure using soluble starch (Sigma Chemical Co., USA) as a substrate at pH 6.8 and temperature 55ºC. A reaction mixture containing 500 µl of 1% substrate (w/v) in 0.02 M phosphate buffer (pH 6.8), 100 µl of crude enzyme, and 400 µl of the 0.02 M phosphate buffer (pH 6.8) were incubated for 15 min at 55ºC. The blank contained 400 µl 0.02 M appropriate buffer, 500 µl of a 1% starch solution in the same buffer, and 100 µl crude enzyme. The reaction was stopped by adding 1 ml of 3, 5-dinitrosalicylic acid solution, followed by heating in a boiling water bath for 10 min and cooling to room temperature. The concentration of reducing sugar was measured at 540 nm using maltose as a standard. One unit (U) of α-amylase is defined as the amount of enzyme that releases 1 µmol of reducing sugar as maltose/min under the assay conditions and is expressed as U/l of the fermented medium and U/g of dry fermented substrate in SmF and SSF systems respectively.

Biomass estimation: For biomass estimation in SmF,

the culture filtrates were harvested by filtration using a pre-dried and pre-weighed Whatman filter paper No. 1 and dried at 80ºC until a constant weight was attained. The growth of the organism was determined as dry weight.

Viable cell count: The viable bacterial count in SSF was determined by suspending the fermented substrate in peptone-phosphate buffer at a 1:10 ratio and shaking the suspension for 15 min, followed by serial dilutions and plating onto plate count agar. The number of viable cells in the fermented substrate after 48 h incubation at 37ºC is reported as colony forming unit (cfu/g).

pH and Moisture content: The pH was measured after suspending 2 g of wet fermented solid in 18 ml of distilled water using a pH meter (pH Lab 827, Metrohm Ltd., Switzerland). The moisture contents of the substrate and fermented solid were estimated by drying a known quantity of each to a constant weight at 80ºC for 24 h. The moisture content was calculated based on the weight loss after drying.

All of the experiments were carried out in triplicate and the analyses were duplicated. The mean values are presented in the results.

Experimental design

SmF: The 'one variable at a time' approach was used in this study. Amylase production was optimized by altering various physicochemical conditions and the culture medium components, then observing the effects after 48 h of incubation at 160 rpm and 37ºC, unless otherwise stated. The process parameters that were varied at this stage included: the incubation period (0-72 h), the shaking speed (140-200 rpm), the concentration of the preculture (1-5 % v/v), the use of synthetic surfactants (0.1% (w/v) of Tween 20, Tween 80, and SDS), and the use of different carbon and nitrogen sources.

To optimize the medium composition, different carbon sources namely; starch, dextrin, cellulose, methyl cellulose, glucose, maltose, lactose, sucrose, fructose, glycerol, molasses and permeate, all at 1% (w/v), organic nitrogen sources (soybean meal, yeast extract, malt extract, casein peptone, meat peptone, peptone and corn steep liquor), and three kinds of inorganic nitrogen salts (NH_4Cl, NH_4NO_3 and urea, all at (0.4% w/v) in addition to 0.3% w/v meat extract) were individually evaluated for their performance in α-amylase production.

SSF: Different agro-industrial by-products including; wheat bran, rice bran, rice husk, soybean oil cake, canola oil cake, okara, alkaline treated sugar cane bagasse, potato peel and wheat bran with sugar cane hydrolysate or date seed hydrolysate were used as solid substrate and their effect on the production of α-amylase was determined. The most promising substrate was selected and used in subsequent experiments.

The production α-amylase under SSF was optimized by altering various physicochemical conditions and observing the effects after 48 h of incubation at 37ºC, unless otherwise stated. The other qualitative and quantitative process parameters, which were optimized for maximal enzyme production, were: the type of the moisturizing agent e.g., tap water, distilled water, basal medium solution, phosphate buffer (pH 6.8), and acetate buffer (pH 5), the ratio (w/v) of the solid substrate to the moisturizing agent (1:0.5, 1:1, 1:1.5, 1:2, 1:2.5, 1:3, 1:3.5, and 1:4), the incubation period (0-120 h), the particle size (500-1400 μm), the concentration of the preculture (10-40 % v/w) and the enzyme extraction solution e.g., distilled water, tap water, acetate buffer, phosphate buffer, glycerol 10% (v/v), and Tween 20 (0.1%).

RESULTS

Production of α-amylase under SmF: Initial studies showed that *Bacillus* sp. KR-8104 produces the maximum α-amylase and grows best at neutral pH. Therefore, in all cases the pH of the culture medium was adjusted to 6.8-7.0 before autoclaving.

The production of α-amylase in SmF was studied over a period of 72 h. The cellular growth, the production of α-amylase and the pH profile versus incubation time is shown in Figure 1. The α-amylase production pattern indicates that the induction of the enzyme took place during the growth phase, that lasted approximately 16 h, and the maximum yield (2450 U/l) was obtained after 48 h of incubation. Continuing the incubation beyond 56 h resulted in a slight decline of the enzyme yield, and 2395 U/l was achieved after 72 h. The pH of the culture medium increased after the growth phase until the end of the incubation time. The effect of the inoculum concentration and the shaking speed on the production of α-amylase are presented in Figure 2. The optimum conditions for α-amylase production were found to be: neutral pH (before autoclaving), 2% v/v inoculum, and shaking at 180 rpm at 37ºC

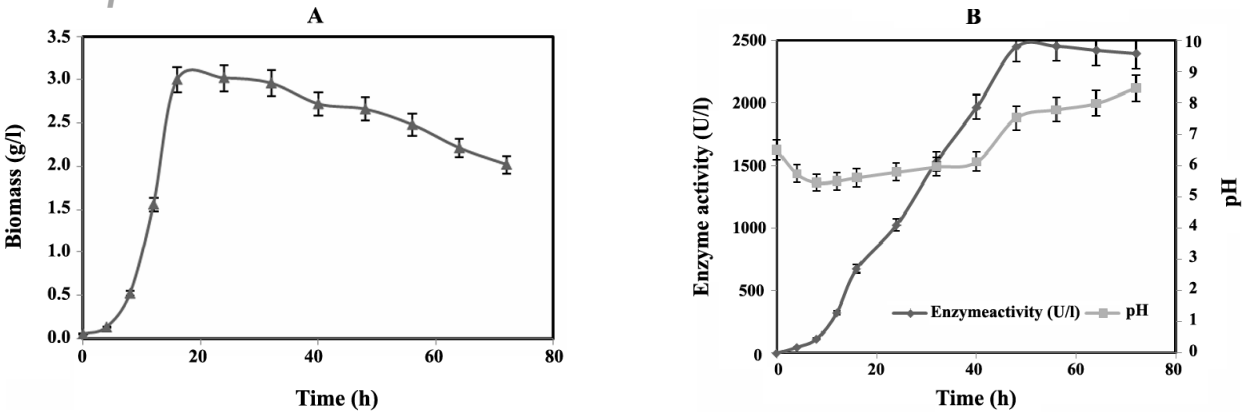

Figure 1. The effect of the incubation period A: on biomass and B: production of α-amylase and the pH profile in SmF at 37°C, 160 rpm with the main culture medium, and 5% inoculums.

for 48 h.Therefore, Tween 80 and Tween 20 as nonionic surfactants, and sodium dodecyl sulfate (SDS) as an anionic surfactant at 0.1% (w/v) were added to improve the production of extracellular α-amylase. As illustrated in Figure 3, no significant difference in the enzyme concentration was detected between the control and treatments with Tween 20 or Tween 80. However, SDS inhibited growth and production of the α-amylase completely.

The results of screening different carbon and nitrogen sources are presented in Table 1. Among the various carbon sources, dextrin, with soy meal peptone and meat extract as the nitrogen source, resulted in the highest α-amylase activity (2804 U/l) after 48 h. Regarding the

Table 1. The effect of different carbon and nitrogen sources on the biomass and α-amylase production.

Additions	Biomass(dry weight g/l)	α-amylase activity (U/l)
Carbon sources (1%)*		
Starch	2.87	2422±43
Dextrin	2.78	2804±51
Maltose	1.76	2092±38
Glucose	1.68	1132±41
Fructose	1.26	1104±26
Sucrose	1.84	981±56
Lactose	2.71	2303±37
Glycerol	0.32	259±22
Methyl Cellulose	1.73	2269±33
Cellulose	ND	ND
Permeate	2.74	2371±45
Molasses	2.40	480±28
Nitrogen sources**		
0.4% Soy Meal Peptone+ 0.3%Meat Extract	3.20	2458±19
0.4% Yeast Extract + 0.3%Meat Extract	2.57	2986±34
0.4% Malt Extract + 0.3%Meat Extract	2.20	1850±41
0.4% Meat Peptone+ 0.3%Meat Extract	1.93	1243±46
0.4% Casein Peptone+ 0.3%Meat Extract	1.68	646±29
0.4% Peptone + 0.3%Meat Extract	1.73	1789±44
0.4% Corn Steep Liquor + 0.3%Meat Extract	1.80	1908±52
0.4% NH4NO3 + 0.3%Meat Extract	2.29	947±47
0.4% NH4Cl + 0.3%Meat Extract	1.82	775±39
0.4% Urea + 0.3%Meat Extract	1.96	312±35
0.4%Yeat Extract	2.36	1207±38

*Main medium culture formula, by replacing soluble starch with different C-sources using soy meal peptone and meat extract as N-source. **Main medium culture formula, by replacing soy meal peptone with different nitrogen sources using soluble starch as C-source. ND: Not Detected. (This test was done at 160 rpm, 5% inoculums, 37°C and 48 h).

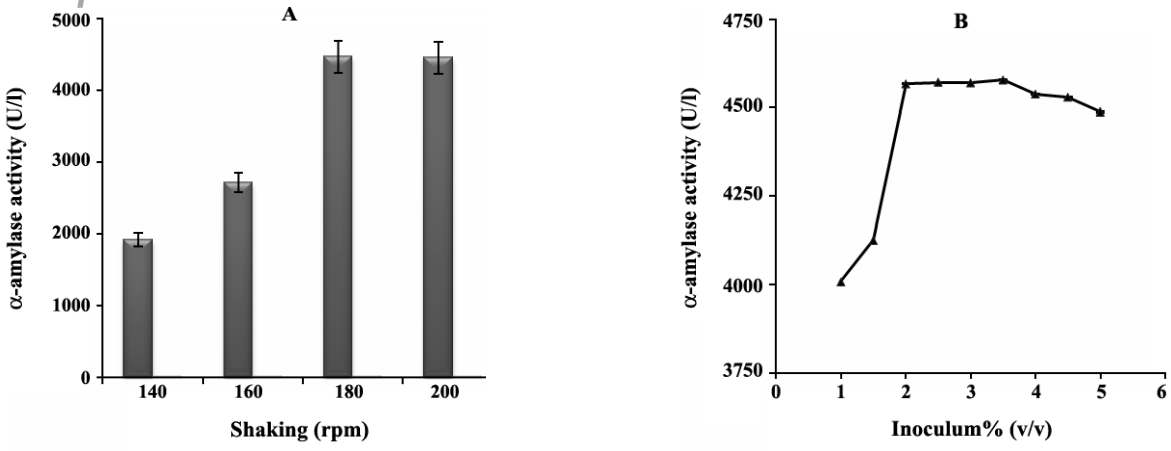

Figure 2. The effect of A: the shaking speed and B: the inoculums concentration on the α-amylase production of in SmF at 37ºC for 48 h with the main culture medium, A: 5% inoculums; B: 180 rpm.

nitrogen sources, a mixture of yeast extract and meat extract with starch as the carbon source increased the α-amylase production to 2986 U/l (Table 1).

Production of α-amylase under SSF: The ability of *Bacillus* sp. KR-8104 for α-amylase production on wheat bran as a common substrate in SSF was evaluated. It colonized well on the solid media and was capable of producing good titers of the enzyme.

Selection of the suitable substrate: Preliminary screening was performed in order to select the most suitable substrate in term of enzyme production. Although all of the substrates used in this study were capable of supporting the growth of microorganism, there was a clear difference in enzyme synthesis. As indicated in Table 2 wheat bran proved superior to the other substrates. The order of substrate suitability in terms of enzyme synthesis was wheat bran > soybean oil cake > malt spent brewing > potato peel > canola oil cake > okara > alkaline treated sugar cane bagasse and there was no enzyme synthesis in the case of using rice bran or husk as substrate. Therefore, in subsequent experiments wheat bran was used as the substrate for the production of α-amylase.

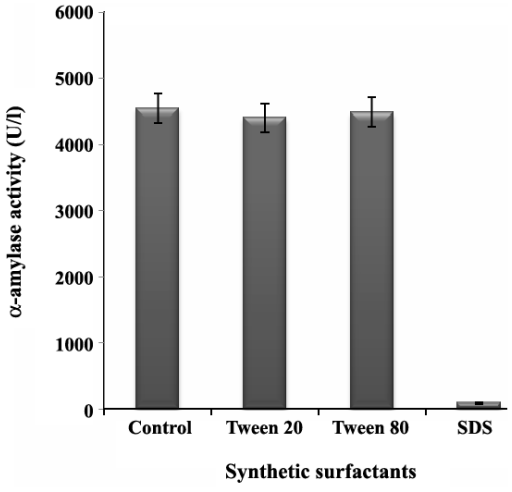

Figure 3. The effect of synthetic surfactants on α-amylase production in SmF at 37°C and 180 rpm for 48 h with the main culture medium and 2.5% inoculums.

The effect of moisturizing agents: Variations in the levels of enzyme production were observed when six different moisturizing agents were used to moisten the WB (Fig. 4). Of the various agents used in this study, tap water and distilled water were found to be the best agents to moisturize WB, whereas the use of acetate buffer (pH 5.0) as a moisturizing agent inhibited the production of α-amylase.

The effect of moisture levels: A substrate-water ratio of 1:1.5 (w/v) was found to be the most favorable for enzyme synthesis (93.45 U/g), when compared to other ratios that were tested (Fig. 5).

The effect of particle size: Substrate particle sizes, ranging from 500 μm to 1400 μm, were investigated with a view towards optimization (Fig. 6). The results showed that a particle size of 900 μm was the most effective for the production of α-amylase, followed by a particle size of 700 μm, which resulted in slightly lower enzyme levels.

The effect of preculture concentration: No significant

Table 2. Screening of agro-industrial by-products for the production of α-amylase by *Bacillus* sp. KR-8104; temperature: 37ºC; moisturizing agent: basal solution; solid substrate to moisture ratio: 1:2, incubation period: 48 h.

Substrate	Cell number (cfu/g)	Enzyme activity (U/g DDB)
Wheat bran	16.5×10^8	94±5
Rice bran	$<10^4$	ND
Rice husk	$<10^4$	ND
Soybean oil cake	14.4×10^8	79±7.5
Canola oil cake	16.4×10^8	44±3.4
Okara	13.2×10^8	38±3.2
Sugar cane bagasse (alkaline treated)	40×10^8	33± 2.9
Potato peel	120.5×10^8	65± 4.3

ND: Not Detected

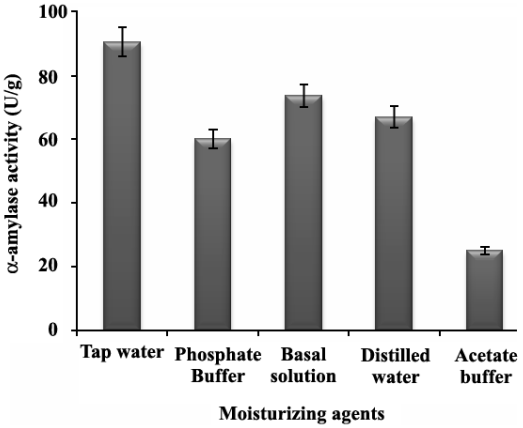

Figure 4. The effect of moisturizing agents on the production of α-amylase by *Bacillus* sp. KR-8104 in SSF; temperature: 37°C; particle size: mixed; solid substrate to moisturizing agent ratio: 1:1.5, incubation period: 48 h.

difference was observed in the enzyme production when the amount (%v/w) of the inoculation was enhanced from 10% to 25%. As illustrated in Figure 7, there was a negligible variation in the production of enzyme when the amount of inoculation varied within that range.

Solvent extraction selection: Different solvents were tested for this study were tap water, distilled water, 0.1M acetate buffer pH 5.0, 0.1 M phosphate buffer pH 6.8, 0.1% (v/v) aqueous mixture of Tween 20, and 10% (v/v) aqueous mixture of glycerol. The medium used for the extraction of crude enzyme from the fermented matter was found to have a profound effect on the enzyme yield. From Figure 8 it is clear that among all the solvents used, 0.1% (v/v) aqueous mixture of Tween 20 and distilled water gave the best result.

DISCUSSION

Although the production of α-amylase in large scale has a long history, introducing new microbial strains

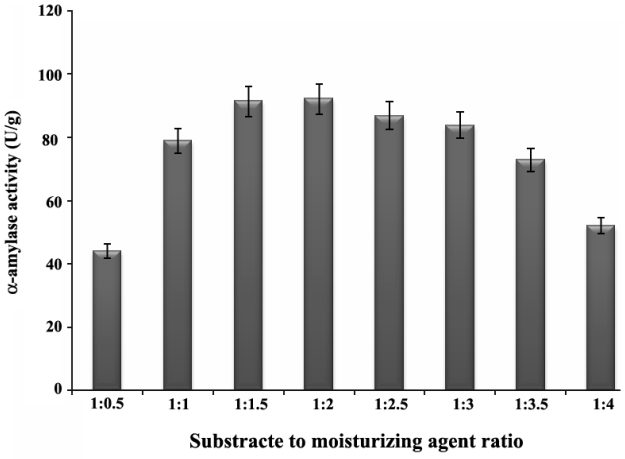

Figure 5. The effect of substrate to moisturizing agent ratio on the production of α-amylase by *Bacillus* sp. KR-8104 in SSF; temperature: 37°C; particle size: mixed, incubation period: 48 h.

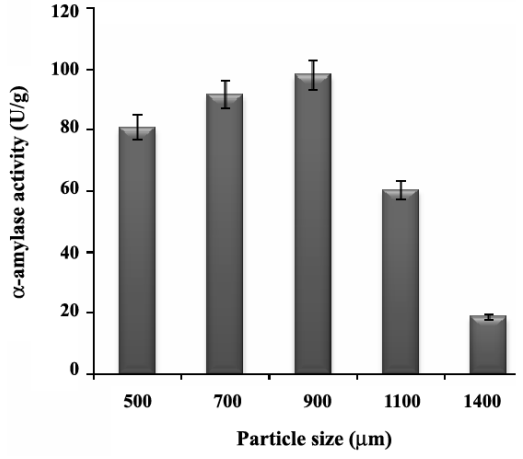

Figure 6. The effect of wheat bran particle size on the production of α-amylase by *Bacillus* sp. KR-8104 in SSF; temperature: 37°C; solid substrate to moisturizing agent ratio: 1:1.5, incubation period: 48 h.

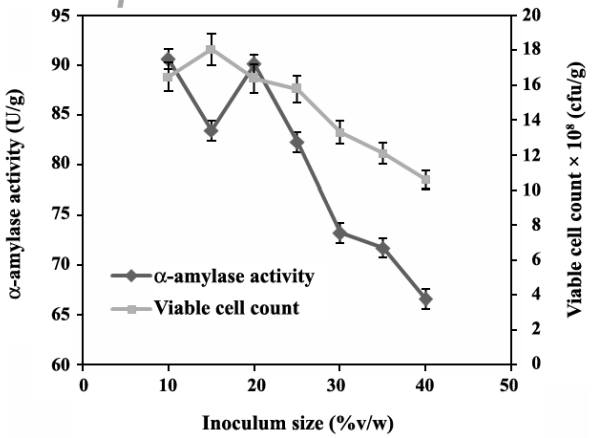

Figure 7. The effect of inoculum size on the cell growth and the production of α-amylase by *Bacillus* sp. KR-8104 in SSF; temperature: 37°C; particle size: mixed; solid substrate to moisturizing agent ratio: 1:1.5, incubation period: 48 h.

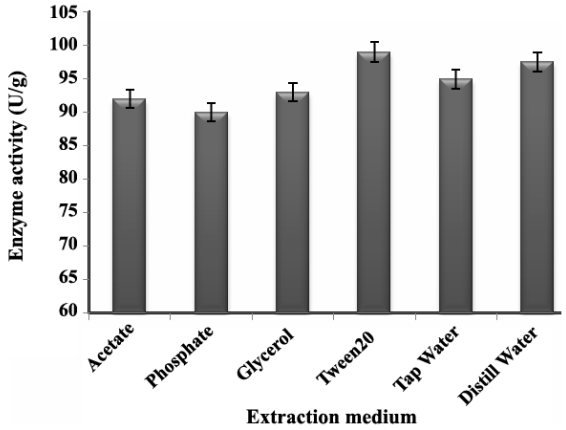

Figure 8. The effect of extraction medium on the cell growth and the production of α-amylase by *Bacillus* sp. KR-8104 in SSF.

(Mahdavi *et al*., 2010) with potentially interested characteristics is still welcomed by related industries due to the importance of enzyme performance in different environmental conditions. On this base, the Ca-independency as well as low pH activity of the *Bacillus* sp. KR-8104 α-amylase is seemed as an appropriate alternative for industrial application, especially in starch hydrolysis industry. Thus, investigation of the production of α-amylase by *Bacillus* sp. KR-8104 in submerged and solid state fermentation systems as two main approaches in the production of enzymes and determination of the optimum conditions is main concern in the initial phase of studying the possibility of large scale production of α-amylase by *Bacillus* sp. KR-8104.

In SmF, a pH reduction trend in the first stage of the fermentation process is due to the consumption of carbon source as the main substrate and accumulation of organic acids, but after the exhaustion of the carbon source or any metabolic change that made the carbon substrate unavailable, the cells shifted to the consumption of nitrogen compounds. So, by producing ammonium and urea as byproducts which resulted from the nitrogen substrate metabolism, the pH is increased.

Surfactants are known to increase the secretion of proteins by increasing the permeablity of the cell membrane. Goes and Sheppard reported that addition of Tween 80 and Tween 20 and SDS at concentrations of 0.05% and 0.10% (v/w) increased the production of α-amylase significantly (Goes and Sheppard 1999). Another study showed that the addition of Tween 80 (1.3%) to the fermentation medium increased the production of α-amylase 2-fold in *Thermomyces lanuginosus* (Sivaramakrishnan *et al*., 2006). Rao and Satyanarayana compared the effects of various surfactants in the production media of Ca^{2+} independent α-amylase by *Geobacillus thermoleovorans* (Rao and Satyanarayana, 2003). Cholic acid and Tween 80 gave 2-fold enzyme yields at a concentration of 0.03% mass per volume ratio, while a mixture of PEG 8000 and SDS showed an inhibitory effect on enzyme production. The effects of representative surfactants on the growth of *Saccharomyces cerevisiae* WSH-J701 and glutathione production were studied (Wei *et al*., 2003). The results showed that the SDS had critical inhibitory concentrations of 0.5 g/l, to cell growth during cultivation. Above these concentrations, cell growth was greatly inhibited or even ceased. Nonionic surfactants such as Tween 80 could also affect cell growth only when their concentrations reached much higher levels. Our results showed no significant difference in the α-amylase production in presence of Tween 20 or Tween 80. While the cell growth and production of the α-amylase by *Bacillus* sp. KR-8104 were inhibited completely in presence of SDS in submerged fermentation system. Thus, it could be concluded that the effect of synthetic surfactants is dependent upon the applied microbial strain characteristics.

Selection of a suitable strain, substrate and process parameters are crucial in the success of solid state fermentation process (Sodhi *et al*., 2005; Pandey *et al*., 2000). In SSF, substrate serves both as the nutrient to the microbial culture and as an anchorage for the cells. The cost and availability of the substrate play an important role for enzyme production in SSF. Consequently, the screening of different agro-industri-

al residues for microbial growth and product formation is worth investigating. Our results imply that improvements in enzyme synthesis with other substrates may be possible with further research.

Moreover, moisture level is of critical importance in the use of SSF media, and its influence on the biosynthesis of enzymes has been attributed to the interference of moisture with the physical properties of the solid particles. An optimum moisture level has to be maintained, as lower moisture levels tend to reduce the diffusion of nutrients, microbial growth, stability of the enzymes and distension of the substrate. The existence of optimal moisture levels has been emphasized earlier, as it has profound effects on the growth kinetics and on the physicochemical properties of the solids, which in turn affect the productivity (Krishna, 2005). Babu and Satyanarayana showed that the production of α-amylase using wheat bran with tap water as the moistening agent was comparable to that using a salt solution (S3), which resulted in maximum enzyme production (Babu and Satyanarayana, 1995). Similar reports are available on the use of tap water as a moistening agent in SSF processes (Sodhi *et al*., 2005). Lower moisture levels reduce the solubility of nutrients provided to the organism by the solid substrate and will cause a lower degree of swelling and higher water tension. On the other hand, higher moisture levels lead to particle agglomeration and gas transfer limitation (Krishna, 2005).

In SSF, the particle size of the substrate influences the growth rate and thereby affects the production of enzymes. With smaller particles, the surface area for growth is greater, but the interparticle porosity is less. Conversely, the porosity is greater for larger particles; however, the saturated surface area is less. These two opposing factors i.e., the decrease in surface area and the increase in porosity, interact to determine the optimal growth and enzyme production environment (Muniswaran *et al*., 1994). According to Shukla and Kar (2006), 1000 µm is the optimum particle size for enzyme production in *B. subtilis*, while *B. licheniformis* produces maximal enzyme levels when using particles of 500 µm size and potato peel as the substrate (Shukla and Kar, 2006).

Generally, an increase in the amount of inoculation improves the growth of the microorganisms up to a certain point, however, beyond this point, there can be a reduction in the microbial activity due to nutrient limitations, as observed in our results (Fig. 6). Obviously, lower amount of inoculation resulted in a lower number of cells in the production medium. Mukherjee *et al*. (2009) observed a strong influence of the amount of the inoculation on the production of enzyme. The production of α-amylase by *B. subtilis* DM-03 was enhanced as the concentration of the inoculum was increased from 10 to 100% (v/w), but beyond 100% α-amylase production steadily declined (Mukherjeea *et al*., 2009). The optimum inoculation amount level for the production of α-amylase by *P. chrysogenum* under mixed substrate fermentation was 20% (V/W) (Ertan *et al*., 2006).

In SSF the products are formed at or near the surfaces of the solid materials. So it is necessary to select an efficient solvent for extraction of the product from the fermented material. Due to the lower dielectric constants of organic solvents, the force of interaction between the enzymes and such solvents shall be higher. Even though some of organic solvents may possess a lower dielectric constant than the others, they may exhibit some inhibitory effect on enzyme activity.

The amount of Ca-independent and low pH active α-amylase excreted by *Bacillus* KR-8104 was improved using certain operative and nutritional conditions in both SmF and SSF systems. Among the parameters evaluated in SmF, high shaking speed (high oxygen) and suitable carbon and nitrogen sources enhanced the production of enzyme considerably, whereas addition of three kinds of synthetic surfactants demonstrated different effects on the production of α-amylase by *Bacillus* sp. KR-8104. On the other hand, SSF of *Bacillus* sp. KR-8104 showed good potential to improve the growth and production of α-amylase on the inexpensive and economically feasible substrate, WB. The effects of different parameters including solid substrate, moisturizing agents, solid substrate to moisture ratio, particle size, inoculum concentration (v/w) and extraction medium on enzyme production were determined in SSF system. Such studies are essential before conclusions can be drawn about the optimal method for the production of this enzyme.

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