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Comparative study between liquid state fermentation and solid state fermentation for production of endo–1, 4- beta–xylanase by *Bacillus licheniformis*

Sahira Nsayif Muslim [(*)]
Shatha Ali Shafiq
Mohammed Fadhel Khudhair

Abstract: *Bacillus licheniformis isolated from soil was determined as the most efficient xylanase producer, while the isolates from rice and wheat samples did not produce this enzyme. Maximum xylanase production could be achieved in Liquid State Fermentation (LSF) with inoculum size of 1.5 ml and after an incubation period 48 hours, at 40 ºC and pH 7. Birchwood xylan and yeast extract were found to be the best carbon and nitrogen sources respectively, for producing high level of xylanase. Solid state fermentation gave higher xylanase production in comparative with liquid state fermentation. Different fermentation conditions were standardized for xylanase production in Solid State Fermentation (SSF), the optimum being 72 hours growth at pH 6.0, cultivation temperature 50 ° C and substrate to moisture ration of 1:2 (w/v). Among different lignocelluloses substrates a mixture of wheat bran and corn bran was found to be the best substrate for xylanase production. The inoculum size of 2 ml resulted in maximum production of xylanase.*

Key words: *xylanase, Bacillus licheniformis, liquid and solid state fermentation.*

دراسة مقارنة بين تخمرات الحالة السائلة والصلبة لإنتاج انزيم endo–1, 4- beta–xylanase من قبل بكتريا *Bacillus licheniform*

ساهرة نصيف مسلم
شذى علي شفيق
محمد فاضل خضير

الملخص: تعد بكتريا *Bacillus licheniformis* المعزولة من التربة الأكثر كفاءة في انتاجية أنزيم الزايلينيز مقارنة بالعزلات الأخرى المعزولة من الحنطة والرز؛ حيث كانت غير منتجة لهذا الانزيم. تم الحصول على أقصى انتاجية لانزيم الزايلينيز باستخدام طريقة تخمرات الحالة السائلة بحجم 1.5 مل وبمدة حضانه ٤٨ ساعة ودرجة حرارة ٤٠ م ودالة حامضية مقدارها ٧ وأفضل مصادر الكربون والهيدروجين للحصول على أعلى انتاجية لهذا الانزيم عند استخدام زايلين خشب البتولا ومستخلص الخميرة. أعطت تخمرات الحالة الصلبة أعلى انتاجية لانزيم الزايلينيز بالمقارنة مع تخمرات الحالة السائلة، وبظروف تخمر قياسية شملت استخدام اللقاح بحجم ٢ مل وفترة حضانة ٧٢ ساعة وعند دالة حامضية مقدارها ٦ ودرجة حرارة ٥٠ ° م وبنسبة رطوبة تراوحت ٢:١ (وزن / حجم)، كما أظهرت النتائج أفضل مادة اساس لانتاجية انزيم الزيلينيز عند استخدام خليط نخالة الحنطة ونخالة الذرة من بين المواد السليلوزية الملكننه المختلفة.

الكلمات المفتاحية: انزيم الزايلينيز، *Bacillus licheniformis* ، تخمرات الحالة الصلبة والسائلة.

(*) Department of Biology, College of Science, Mustansiryia University, shathaali2007@yahoo.com

1. Introduction

The genus Bacillus is one of the most ubiquitous and diverse, with reprehensive found in the soil as well as associated water sources such as rivers, coastal waters and estuaries' (Dasilva *et al*., 2005). However, it was isolated from foods including meats, dairy products and cereals (Donnarumma *et al*., 2010). *Bacillus licheniformis* is found in most soils and dominate in nutrient poor soils such as moorland (Dasilva *et al*., 2005). Also they are common in foods including natural agricultural products such as cereals which it presumably colonizes from wind – blown dust and soil particles (Dasilva *et al*., 2005). *Bacillus licheniformis* is of special interest with respect to thermo stability as well as chemo stability of enzyme (Bergquist *et al*., 1987). Also the thermophilic Bacillus offer significant potential for biotechnological application α-amylase, protease xylanase (Nazina *et al.,* 2000). Xylan is the second most abundant biopolymer after cellulose and the major hemicellulostic polysaccharide found in the plant cell wall (Timell, 1976).

Xylanase (endo- 1,4- β –D- xylan xylanohydrolase ; EC 3.2.1.8) degrade the xylan backbone into small oligomers .This enzyme is required for many applications such as bleaching of Kraft pulp, increasing the brightness of pulp, improving the digestibility of animal feed and for clarification of fruit juices etc (Biely *et al*., 1985). About 30-40 % of the production cost of many industrial enzymes is accounted by the cost of growth substrate (Hinnman, 1994). The use of low cost substrates for the production of industrial enzymes is one of the ways to greatly reduce production costs. This can be achieved by using solid agricultural waste materials as substrates (Wizani *et al.,* 1980). Solid state fermentation processes can be defined as the growth of microorganisms on moist solid materials in the absence of free flowing water (Cannel and Young, 1980). These processes have been used for the production of food, animal feed and both pharmaceutical and agricultural products (Young *et al*., 1998). Therefore this study aimed to isolate of Bacillus from soil, rice and wheat samples and comparison between liquid state fermentation and solid state fermentation for production xylanase enzymes.

2. Material and Methods

2.1. Collection of samples.

Thirty samples were collected from different sources in sterile containers (10 samples of each soil, rice and wheat).

2.2. Isolation of Bacillus spp.

One gram of each soil, rice and wheat were added separately to 9 ml of sterile water and shake to homogenize and heated to 80 ºC for 10 min in water bath, serial dilutions for each samples were set up by using sterile water from each dilution 0.1 ml was spread on a nutrient agar plates, and incubated aerobically at 37 ° C for 24 hours, morphological characteristic were used to identify Bacillus such as size, color, shape and margin of the colony as well as gram stain according to Lopez *et al*., 1998.

2.3. Xylanase production in LSF

The activated isolates were inoculated in liquid medium (0.5 % Birchwood xylan, 0.2 % yeast extract 0.25 % NaCl, 1.5% KH2PO4, 0.5% NH4Cl and 0.025 % MgSO4.7H2O, pH 7.0) and then incubated at 37 ° C for 48 hours, and then the enzyme was separated by centrifugation at 8000 rpm for 20 min Roy and Rowshonul (2009).

2.3.1. Determination of enzyme activity:

The amount of the produced Xylanase was measured by using 1% Birchwood xylan as the substrate. Xylanase activity was assayed in 2 ml of reaction mixture containing 1 ml of crude extracellular enzyme source and 1 ml of 1% a birch wood xylan (prepared in 50 mM sodium acetate buffer, pH 5). The mixture was incubated at 60 ºC for 10 min, then the reaction was stopped by the addition of 3 ml of 3,5-dinitrosalicylic acid (DNS) and the contents were boiled for 15 min. After cooling the color developed was read at 540 nm. The amount of reducing sugars liberated was quantified using xylose as standard .One unit of enzyme activity is defined as the amount of enzyme which releases 1 µmol of xylose in 1 min. under assay conditions (Miller, 1959). The protein content in the culture supernatant was determined according to the method described by Lowry *et al*. (1951) and performed by using bovine serum albumin as standard.

2.3.2. Optimization of growth conditions in LSF medium.

2.3.2.1. Effect of carbon sources: Liquid production medium was inoculated with selected isolate at different carbon sources (dextrose, xylems, mannose, lactose, sucrose, CM- cellulose, birch wood xylan, oat spelt xylan and fructose) and incubated at 37 ° C for 48 hours at pH 7. Then the specific activity was assayed as described above.

2.3.2.2. Effect of nitrogen sources: Liquid production medium was inoculated with selected isolate at different nitrogen sources (ammonium sulfate, ammonium nitrate, peptone, Treptone, urea, ammonium chloride, potassium nitrate and yeast extract) and incubated at 37 ° C for 8 hours at pH 7. Then the specific activity was assayed.

2.3.2.3. Effect of incubation temperature: Liquid medium was incubated with selected isolate at different temperatures (50, 55, 60, 65) ° C for 48 hours, then the activity was measured.

2.3.2.4. Effect of pH: Liquid medium was prepared at different pH values (2- 9) medium was inoculated and inoculated and incubated at 40 ºC for hours, then the specific activity was assayed.

2.3.2.5. Effect of incubation period: Liquid production medium was inoculated with selected isolate of inoculums (0.5, 1, 1.5, 2 and 2.5 ml of selected isolate and incubated at 40 ° C for 48 hours, and then the specific activity was assayed.

2.4. Xylanase production in SSF.

Ten gram of wheat bran as solid substrate was hydrate with 1: 1.5 (w/v) phosphate buffer (0.02 M at pH 7), after 48 hours of fermentation at 40 ° C, solid

substrate was removed and suspended in the same buffer, vortex to extract the xylanase. The sample was filtrated through a type of cloth; the filtered was centrifuged at 8000 rpm for 20 min, for the supernatant. The specific activity was assayed (Abou- Dobara *et al*., 2011).

2.4.1. Optimization of growth conditions in SSF medium:-

2.4.1.1. Effect of various complex organic solid substances: Various solid substrates (wheat bran, corn bran and rice bran) and mixed two substrates together in the same time were hydrated with 1: 1.5 (w/v) of phosphate buffer and inoculated with selected isolate then incubated at 40 ° C for 48 hours . The specific activity was measured.

2.4.1.2. Effect of incubation temperature: The mixture of wheat bran and corn bran were hydrated with phosphate buffer at ratio 1:1.5 (w/v) and inoculated with 2 ml of isolate, then incubated at different temperatures (37, 40, 45, 50, 55, 60) ° C for 48 hours, the specific activity was assayed.

2.4.1.3. Effect of pH: The mixture of wheat bran and corn bran were hydrated with buffers at different pH values (4-9) including acetate buffer, phosphate buffer and glycine buffer at ratio 1: 1.5 (w/v) and incubated at 50° c for 48 hours, and then the specific activity was assayed.

2.4.1.4. Effect of incubation period: The mixture of wheat bran and corn bran was hydrated with phosphate buffer pH 6 at ratio 1: 1.5 (w/v) and inoculated with 2 ml of selected isolate, then incubated at 50 ° C for different periods (24, 48, 72, 96, 120) hours and the specific activity was assayed.

2.4.1.5. Effect of moisture level: The mixture of wheat bran and corn bran was hydrated with phosphate buffer pH 6 at different ratios (1, 1:5, 1:2 and 1:3) (w/v) and inoculated with 2 ml of selected isolate, then incubated at 50 ° C for 72 hours, and specific activity was assayed.

2.4.1.6. Effect of inoculum size: The mixture of wheat bran and corn bran was hydrated with phosphate buffer pH 6 at ratio 1: 2 (w/v) and inoculated with different inoculum sizes (0.5, 1, 1.5, 2, 2.5 and 3) of selected isolate, incubated at 50 ° C for 72 hours, the specific activity was assayed.

3. Result and Discussion

3.1. Isolation and identification of Bacillus spp.

Sixteen bacterial isolates were obtained and identified as Bacillus spp. as shown in figure (1). The result showed that the isolation percentage from soil samples which reached to 100%, while it was 30% of each wheat and rice samples. Watanabe and Hayano (1993) insured that Bacillus genera are wide spread among bacteria in soil. Moreover, Harwood (1989) mentioned that the common habit for Bacillus spp. is soil.

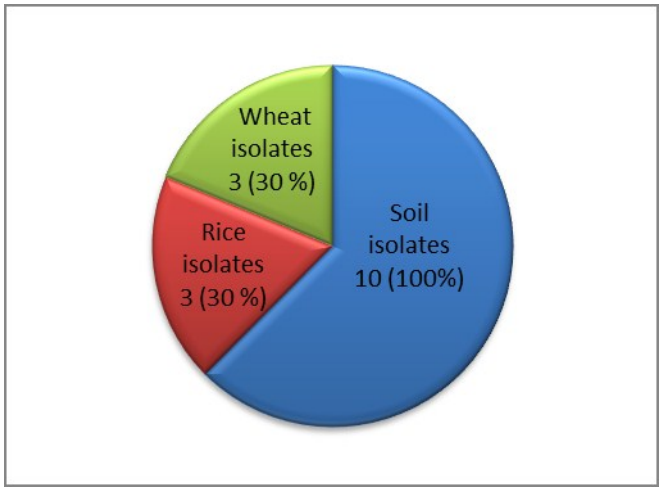


Figure (1): *Bacillus* spp. isolated from different sources

3.2. Detection of xylanase producer isolates.

The results showed that all the Soil Bacillus sp. isolates were able to produce xylanase with a range of specific activity starting from 12.7 to 35.6 U/mg, while all other rice and wheat samples did not produce this enzyme. Among the soil isolates Bacillus isolate (Bs8) was the most efficient xylanase producer with specific activity reached to 35.6 U/mg, Figure (2) shows the Specific activity of the bacterial isolates after incubation at 37 ° C for 48 hours.

Based on that results, (Bs8) was selected to determine the optimization conditions of Xylanase production, and then the morphological and biochemical tests which performed showed that isolates is *Bacillus lichenoformis* according to Li *et al.,* (2009).

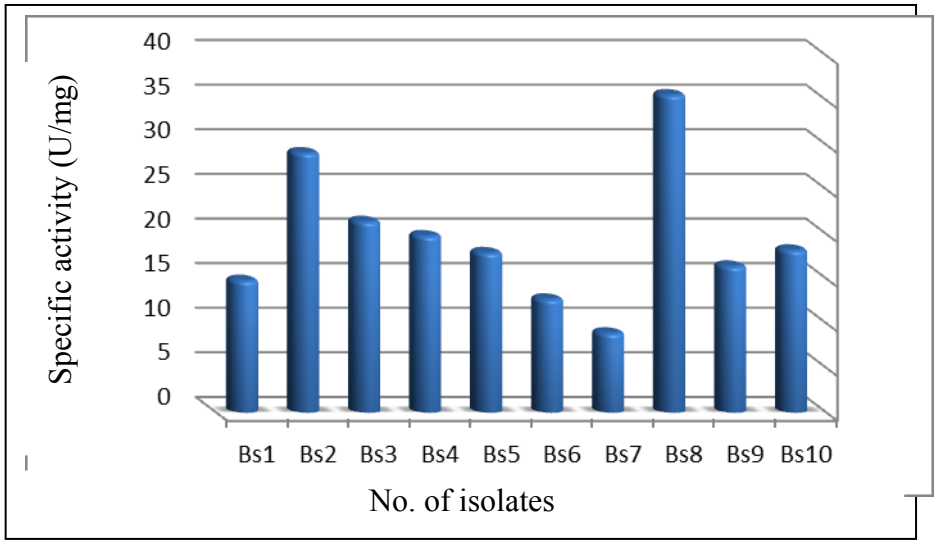


Figure (2): Production of xylanase by Bacillus isolates in liquid medium.

3.3. Optimum conditions for xylanase production by (LSF).

3.3.1. Effect of carbon source: Among all nine carbon sources which tested as a sole source for carbon and energy, the results revealed that the maximum production of xylanase achieved by using birch wood xylan as a sole source for carbon with specific

activity of Xylanase reached to 35.6 U/mg after incubation at 37 ° C for 48 hours, while all dextrose, sucrose and fructose were less effective as shown in figure (3). The carbon sources are considered as the most important factor for the bacteria which provide required energy for a growth and a production of enzyme (Ali, 2006). Kachlishvili *et al.,* (2006) demonstrated that the selection of an appropriate carbon source was critical in xylanase production, since substrate induction and catabolite repression controlled it.

Irfan *et al.,* (2012) indicated that selection of suitable medium and fermentation conditions plays a vital role in xylanase production and is a pre-requisite to make the process cost effective. Xylanase is known to induce xylanase production. Mohamed *et al.,* (2011) found that the best carbon source for xylanase production *from Bacillus coagulans* and *Bacillus licheniformis* was found to be xylan and the activity of xylanase is much lower in the presence of sugars compared with xylan.

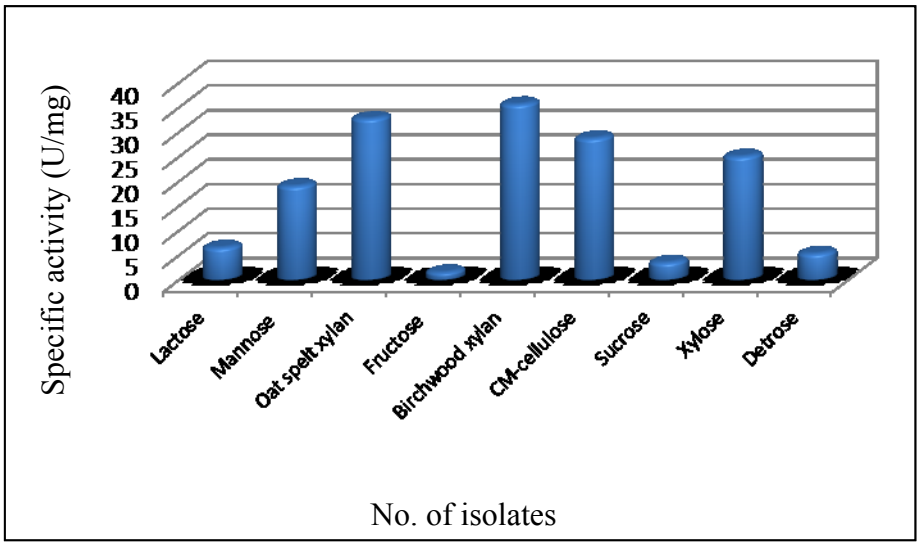


Figure (3): Xylanase production by Bs8 in liquid medium at different carbon sources.

3.3.2. Effect of nitrogen source: Eight nitrogen sources were used to determine the optimum nitrogen source for xylanase production. The results showed a highest specific activity was recorded 35.6 U/mg protein when yeast extract was used as a sole source of nitrogen, on the other hands the activity of xylanase was lower in the presence of additive such as ammonium chloride and potassium nitrate as reported in figure (4). Nitrogen source is one of the important factors that affected bacterial growth due to the bacteria requires nitrogen to complete the metabolic pathways. Also nitrogen enhances and increases bacterial growth these increase in bacterial growth may be caused increasing in xylanase production (Nair *et al.,* 2006). The result agreed with result showed by Rajashri *et al.* (2012) which reported that enzyme production by Bacillus sp. was stimulated by the addition of yeast extract and casein. Sindhu *et al*., (2006) reported that yeast extract, peptone and casein supported improvement in xylanse production by Bacillus megaterium, while ammonium chloride and ammonium nitrate markedly reduced xylanase production. Mohamed et al. (2011) found that peptone was the best nitrogen source for producing high level of xylanase by *Bacillus licheniformis.*

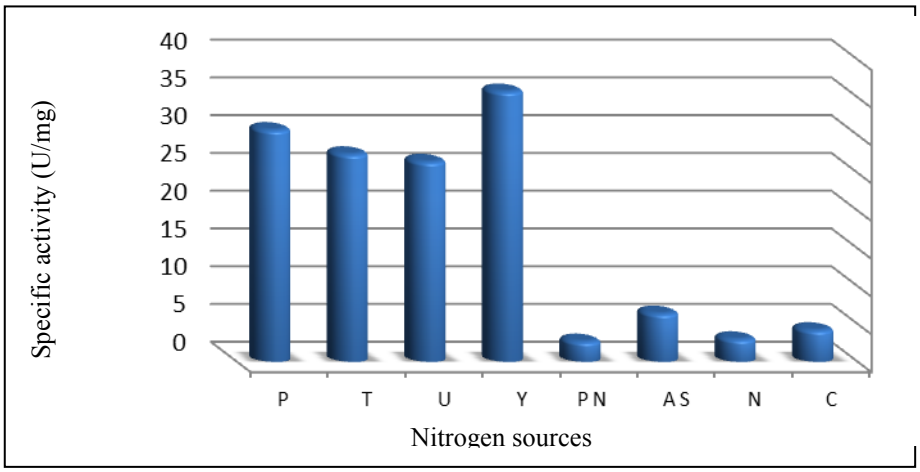
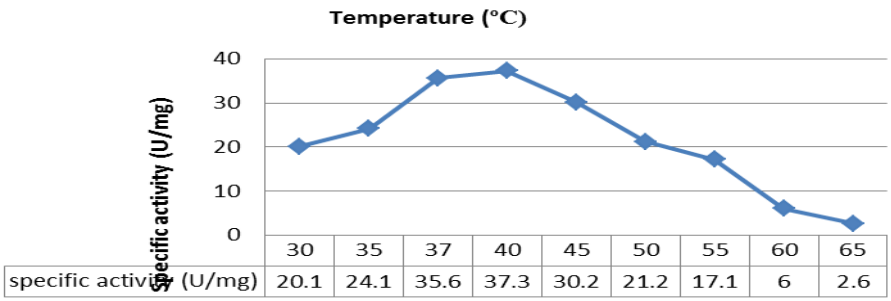


Figure (4): Xylanase production by Bs8 in liquid medium at different nitrogen sources.(Pepton, T: Trepton, U: Urea, Y: Yeast extract, P N: Potassium nitrate, A S: Ammonium sulfate, N: Nitrate, C: Chloride.)

3.3.2. Effect of incubation temperature: The results which reported in figure (5) showed that maximum production of xylanase observed at 40 °C when the specific activity reached 37.3 U/mg, while the higher temperatures were not suitable for enzyme production, the specific activity decreased to 2.6 U/mg at 65 °C. The temperature is imported effect on enzyme stability and production, the high temperature affect the metabolism of living cells including protein synthesis (Bull and Bushneh, 1976). *Bacillus licheniformis* produced xylanase at 50 °C with maximum activity (Mohamed *et al.,* 2011). The enzyme production by *Bacillus* sp. was better at temperature close to 40 ºC (Wahyuntari *et al.,* 2009,), in contrast John et al., (2006) reported that xylanase production by *Bacillus subtilis* 168 was best at 37 °C .



	30	35	37	40	45	50	55	60	65
specific activity (U/mg)	20.1	24.1	35.6	37.3	30.2	21.2	17.1	6	2.6

Figure (5): Xylanase production by Bs8 in liquid medium at different incubation temperatures.

3.3.3. Effect of pH value.: The results showed that the maximum xylanase production obtained when pH the value of the production medium adjusted to 7. The specific activity was reaching to 37.4 U/mg, reduction in enzyme activity was observed at pH lower or higher than pH 7 as shown in figure (6).

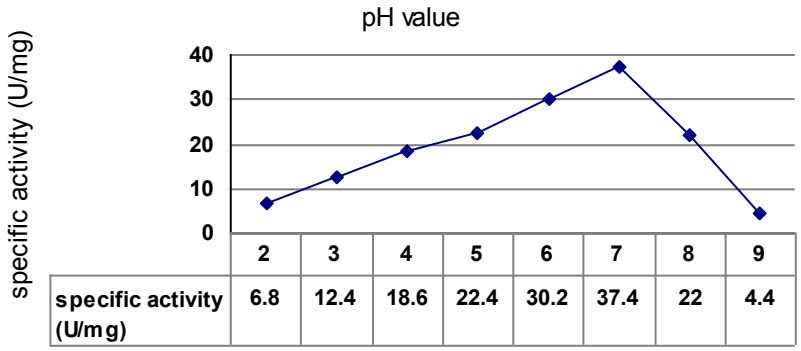


Figure (6): Xylanase production by Bs8 in liquid medium at different pH values at 40 °C for 48 hours.

Many researchers reported that pH represents one of the most factors influencing xylanase production, Ashger *et al.* (2007) showed that among the physical parameters, the pH of the growth medium plays an important role in inducing morphological changes in the organism and in enzyme secretion. Any change in pH affect the protein structure and causes decline in enzyme activity and do affect the affinity of enzyme for substrate, especially whenever active site has been altered (Battestin and Macedo, 2007). Growth and enzyme production condition of most *Bacillus subtilis* was reported at pH 6-8 and temperature 37-70 °C (Oakley *et al.,* 2003). Also *Bacillus pumilus* produced xylanase with demonstrating maximal activity at pH 7 (Monisha *et al.,* 2009).

3.3.4. Effect of incubation period: The results showed that the enzyme production was initiated after 24 hours of incubation with gradual increasing in productivity with increasing in incubation period figure (7). The specific activity reached the maximum 37.3 U/mg after 48 hours incubation, followed by decline in specific activity reached 3.3 U/mg after 120 hours incubation at 40 °C.

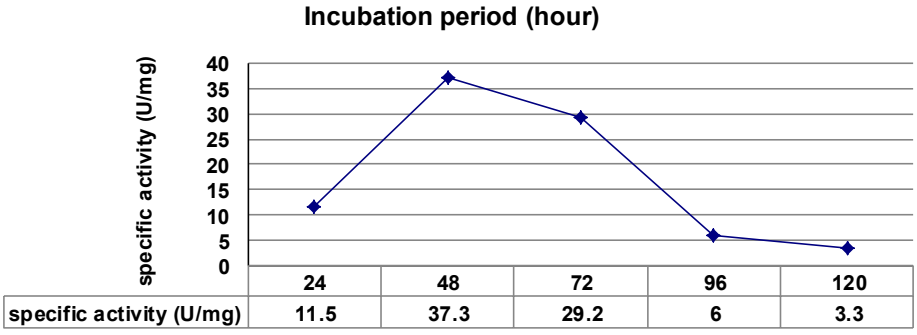


Figure (7): Xylanase production by Bs8 in liquid medium after incubation at 40 °C for different periods..

This decline was probably due to denaturation or decomposition of the substrate as a result of interactions with other components in the medium (Soares *et al*., 1999). Maximum xylanase production could be achieved after an incubation period of 48 hours for *Bacillus coagulans* and after an incubation period of 60 hours

for Bacillus licheniformis (Mohamed *et al.,* 2007). In addition, *Bacillus arseniciselenatis* produced xylanase after 48 hours of incubation (Kamble and Jadhav, 2011).

3.3.5. Effect of inoculum size: The results showed that xylanase production by *Bacillus licheniformis* Bs8 was variable at different inoculum sizes. Xylanase activity slightly increased with the increase of the inoculum size until 1.5 ml with specific activity reached to 38.4 U/ mg, and then decreased to 15.2 U/mg with increase in inoculum size to 2.5 ml as illustrated in figure (8). The lowest enzyme production at lower inoculum level might be due of less number of viable cells in the production medium require more time to grow to an optimum number to utilize the nutrients in substrate and for enzyme production (Kashyap *et al.,*2002) . In addition, less enzyme production at higher inoculum size might be due to either decrease nutrient availability for the large number of viable cells, or rapid accumulation of toxic metabolites (Zhang *et al.,* 2002).

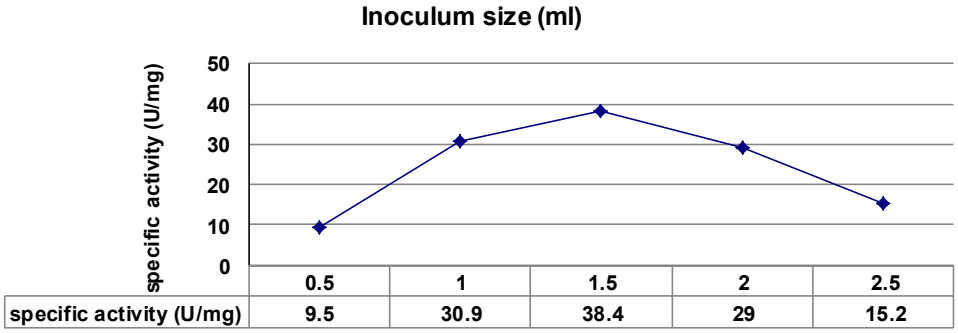


Figure (8): Xylanase production by Bs8 in liquid medium and different sizes of inoculum after incubation at 40 °C for 48 hours at pH.

3.4. Optimum conditions for xylanase production by SSF.

3.4.1. **Effect of various complex organic solid substrates:** Agricultural waste including wheat bran, rice bran, and corn bran were used as solid substrate. The enzyme gave highest specific activity 64.8 U/mg in media containing mixed of wheat bran and corn bran as substrate in SSF as shown in figure (9). Whereas the media containing corn and rice gave the lowest specific activity of xylanase 39.9 U/mg. This suggested xylan degradation, stimulating xylanase synthesis and xylose production, took place by adding complex carbon sources wheat bran and corn bran. Agricultural residues have been reported to induce xylanase synthesis efficiently (Pandey, 2003). The wheat bran and corn bran contained xylan and protein, which were served as substrates as well as carbon and nitrogen sources for microorganisms respectively (Kamble and Jadhav, 2012). The results of (Thiago and Kellaway, 1982) were reported for xylanase production from Bacillus species used wheat and corn as the best substrates and gave optimum enzyme activity . Other study showed a high level of xylanase production from *Bacillus licheniformis* using corn as substrate (Gupta and Kar, 2009; Gupta and Kar, 2008).

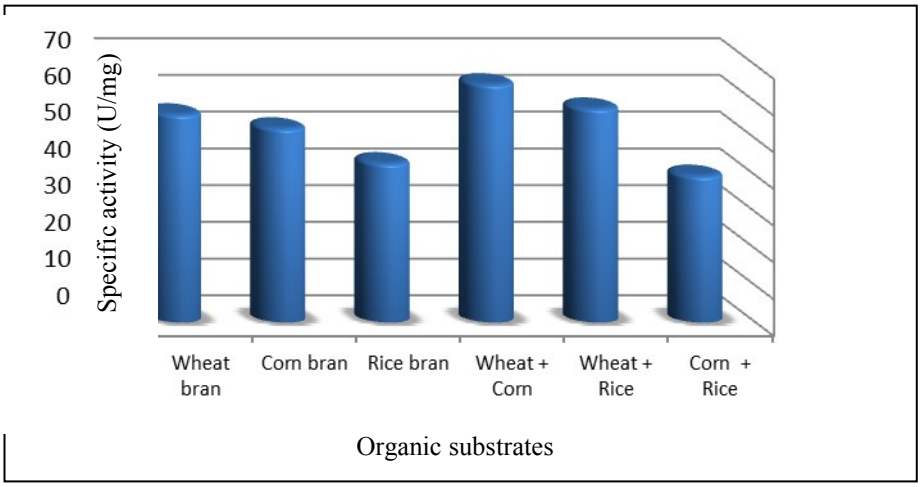


Figure (9): Production of xylanase by Bs8 with various organic solid substrates at ratio 1:1.5 (w/v), at pH 7, at 40 °C for 48 hours.

3.4.2. **Effect of incubation temperature:** The results showed that the optimum temperature for xylanase production was 50 °C then the production of enzyme was decreases with increasing of incubation temperature to reach 38.2 U/mg at 60 °C (Figure (10)). The fermentation temperature is very important for SSF since growth and production of enzymes or metabolites are usually sensitive to temperature (El-Ahwanya and Youssef, 2007). SSF temperature increases as a consequence of the metabolic activity when the heat removed is not enough. This affects directly spores germination, growth and product formation. The temperature level reached a function of the type of microorganism, the porosity, the particle diameter and the depth of the substrate (Sodhi *et al*., 2005). Similar findings have been reported with *Bacillus licheniformis* and Bacillus coagulans where enzyme production reached maximum level at 50° c (Mohamed *et al.,* 2011). While Gevais and Molin (2003) revealed that the maximum xylanase production by Bacillus sp. was obtained at incubation temperature ranging from 55 to 60 °C.

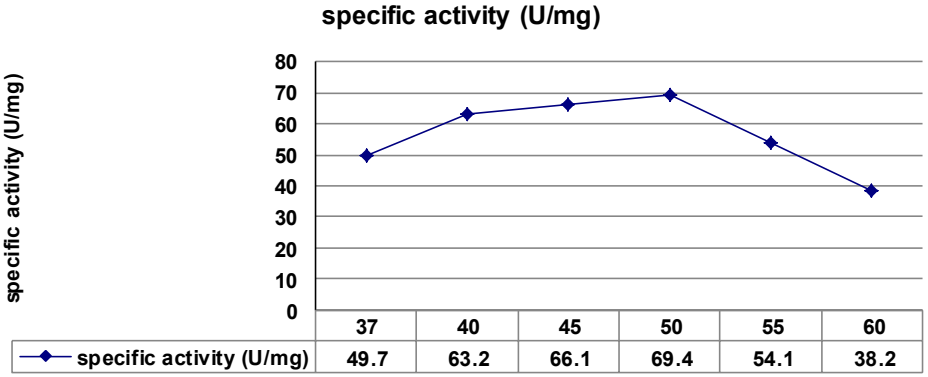


Figure (10): Production of xylanase by Bs8 in SSF, hydrated at ratio 1:1.5 (w/v), at pH 7, at different incubation temperatures for 48 hours .

3.4.3. **Effect of pH value:** The results illustrated in figure (11) showed that the maximum production obtained when the pH value of the production medium adjusted to 6 at this level, the specific activity was 74.8 U/ mg. The pH of growth medium plays an important role by inducing morphological changes microbes and in enzyme secretion. The pH level changes observed during growth of microorganism also affect the products stability in the medium (Hiremath and Patil, 2011). Gupta and Kar (2009) determined that optimum xylanase production from Bacillus sp. was observed at pH 6. While Gupta *et al.,* (2003) reported that the maximum activity was achieved, when Bacillus sp. was grown on synthetic medium of pH 10 at 45 °C.

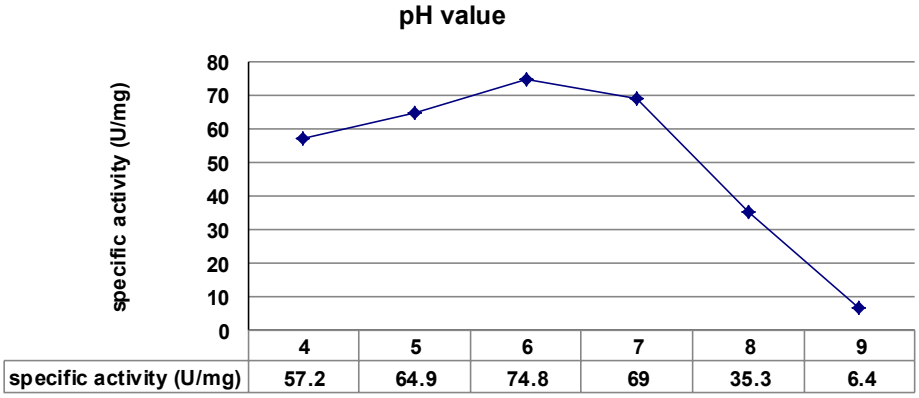


Figure (11): Production of xylanase Bs8 under SSF, hydrated at ratio 1:1.5 (w/v), with different pH values at 50 °C for 48 hours .

3.4.4. **Effect of incubation period**: The results showed that the production of enzyme increased with increasing the incubation period till 72 hours. The maximum level reached 85.2 U/ mg (figure (12)), and then decreasing of production was observed at 12 U/mg after 120 hours. It can be concluded that enzyme production begins in early stage of bacterial growth and continuous during the period of incubation, also the production of enzyme during the early stag may help the bacteria in degradation of polysaccharide xylan to oligosaccharide which are utilize as carbon source for the growth of bacteria (Kumar *et al*., 2011). The reduction in xylanase yield after optimum period was probably due to the depletion of nutrients available to the microorganism or due to proteolysis (Ramesh and Lonsane, 1990). Similar observations have been reported with *Bacillus licheniformis* where enzyme production maximum level of enzyme production after 72 hours in wheat bran medium (Mohamed *et al.,* 2011). In contrast (Flores *et al*., 1997) revealed that maximum xylanase production by *Bacillus subitlis* was recorded in stationary phase 36 hours of the culture.

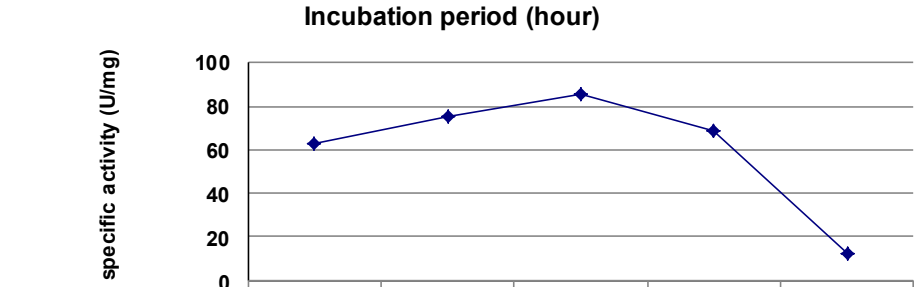


Figure (12): Production of xylanase by Bs8 under SSF, hydrated at ratio 1:1.5 (w/v), with different incubation time at 50 °C.

3.4.5. Effect of moisture level.

Mixture of wheat bran and corn bran was hydrated with different ratio to determine the optimum hydration ratio for enzyme production. The results revealed that the optimum hydration ratio was 1: 2 (w/v). The specific activity reached 94.7 U/mg, while it decreased to 57.3 U/mg when the hydration ratio increased to 1:3 (w/v) (figure (13)). Moisture is one of the most important parameters in SSF that influences the organism and thereby enzyme production .Moreover, higher moisture level decreases porosity, promotes development of stickiness, altering particle structure, increase the change of contamination and resulting in low oxygen transfer rate (Annamalai *et al*., 2009). Previous studies provided production of xylanase by *Bacillus licheniformis* and *Bacillus coagulans* with 1:2 (w/v), however, the optimum condition for the production of xylanse by *Bacillus megaterium* 1:1.5 (w/v) (Francis *et al*., 2003 Francis).

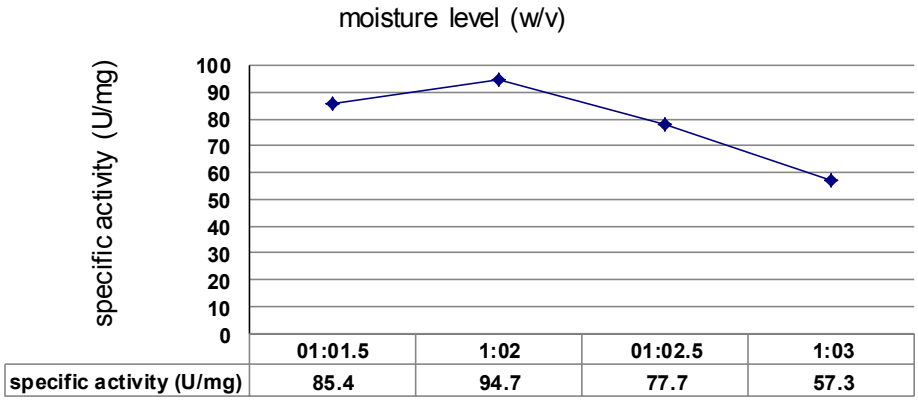


Figure (13): Production of xylanase by Bs8 under SSF, hydrated wth different ratios of phosphate buffer pH 6, at 50 °C c for 72 hours .

3.4.6. Effect of inoculum size.

From results that shown in figure (14), the optimum inoculum size for xylanase production was 2 ml with specific activity 95 U/mg, while it decreased 23.1 U/mg when the inoculum size reached to 3 ml. The decrease in the enzyme production at lower inoculum level less than 2 ml might because of less number of viable cells in the production medium require more time to grow up an optimum number to utilize

the nutrients in substrate and for enzyme production (Mohamed *et al.,* 2011). Francis *et al.,* (2003) revealed that the highest xylanase production by *Bacillus megaterium* was observed at inoculum level of 10 % size.

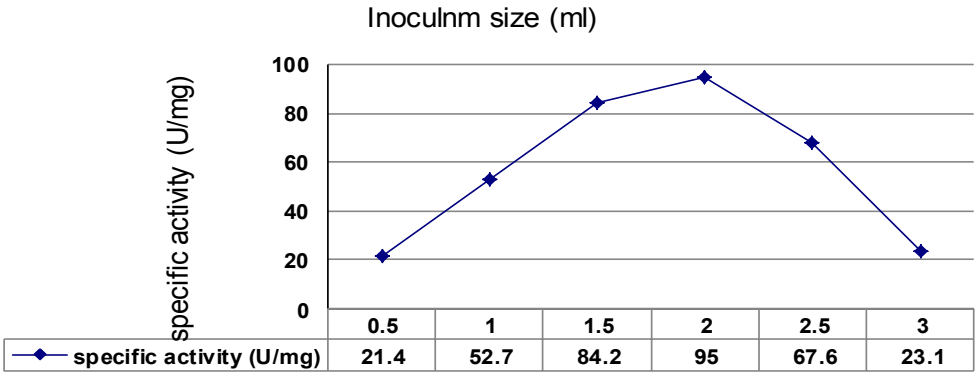


Figure (14): Production of xylanase by Bs8 under SSF, hydrated at ratio 1:2 (w/v) at pH 6, inoculated with different inoculum size of bacterial suspension at 50 °C for 72 hours.

3.5. Comparison between SSF and LSF medium for the production of xylanase enzyme.

LSF medium in the optimum conditions was compared with SSF medium. The results showed that SSF was more efficient in xylanase production than LSF. The highest specific activity (56.8 U/mg) was obtained when used SSF technique, while highest specific activity with LSF was 38.5 U/mg. The solid substrate in SSF provides a rich and complex source of nutrient that may be sufficient or sometimes insufficient and compete with respect to the overall nutritional requirement of that particular microorganism which is cultivated on the substrate (Pandey *et al.,* 2001). SSF preferred to LSF because of simple technique, low capital investment, lower levels of catabolic repression and end product inhibition, low waste output, better product recovery, and high quality production (Mrudula and Kokila, 2010). The solid substrate acts as a source of carbon, nitrogen, minerals and growth factors and has a capacity to absorb water, necessary for microbial growth. Also the microorganisms in SSF are growing under conditions similar to their natural habitats; they may be able to produce certain enzymes and metabolites more efficiently than in submerged fermentation (Pandey, 2003). According to previous results SSF medium was selected instead of LSF medium for xylanase production by *Bacillus licheniformis* Bs8.

References

1-Dasilva T, Reis A, Kent A, kosseva M, Roseiro J C, Hewitt C J (2005). Stress Induced physiological responses to starvation periods as well as glucose and lactose pulse in *Bacillus licheniformis* CCMI 1034 continuous aerobic fermentation processes as measured by multiparameter flow cytometry .Biochem. Eng.J.,V(24): 31-41 .

2-Donnarumma F, Paffetti D, Statzky G, Giannim R, Vettor C (2010). Potential gene exchange between *Bacillus thuringiensis* sub sp. Kurstaki and *Bacillus* spp. in soil in situ soil ., Biol.Biochem.,V(42): 1329-1337.

3-Bergquist,P.; Love,D.;Croft,S.;Streiff,M.,Daniel,R.,Morgan,W.(1987). Genetics and potential biotechnological application of thermophilic and extremely thermophilic microorganisms, Biotechnol.Genet.Rev.,V(5):199-244.

4-Nazina F, Peiars A, Groudieva T, Antranikian G (2000). Cloning expression and biochemical characterization of thermostable xylan hydrolyzing enzyme from the hyperthermophilic archaeon Thermococcus aggregans . FEMS microbiology Leiters :190-223.

5-Timell T (1967). Recent progress in the chemistry of wood hemicelluloses wood,Sci.Technol.,V(1) : 45-70.

6-Biely P, Markovic O, Mislovicova D (1985).Sensitive detection of endo-1,4-beta-glucanases and end0-1,4-beta-xylanases in gels .Anal.Biochem.,V(144): 147-151.

7-Hinnman R L (1994).The changing face of the fermentation industry,Chem.Technol.,V(24):45-48.

8-Wizani W, Esterbaner H, Steiner W, Gomes J (1990). Exo-and endo-cellulose xylanase .Apotent. 1030l-90.

9-Cannel E, and Young M M (1980). Soild state cultivation systems .process Biochemistry, V(15) :24-28.

10- Young M, Moo A, Moreira R, Tengerdy P (1983). In the filamentous fungi. V(4). J.E.Smith, De.R. Berry, B.Kristiansen,eds.Edward Arnold, London,117-144.

11-Lopez C, Blanco A, and Paston F (1998). Xylanase production by a new alkali-tolerant isolate of *Bacillus,* Biotechnology letters,Vol(20): 243-246.

12- Roy N, and Rowshonul H N (2009).Isolation and characterization of xylanase producing strain of *Bacillus cereus* from soil . Iranian J. Microbiol.,1(2) :49-53.

13-Miller, G.L. 1959. Use if dinitrosalicylic acid reagent for determination of reducing sugars, Anal . Biochem. 31 : 426-428.

14- Lovry H, Rosebrough N J, Farr A L, Randaj R J (1951). Protein measurement with the folin phenol reagent,J. Biol.Chem., 193 (1) : 267-275.

15-Abou- Dobara M I, El-Sayed A K, and El- Fayoumy R A (2011). Optimization and physiochemical properites of xylanase from *Bacillus coagulans* and *Bacillus licheniformis*, J. American Science, 7(12): 661-670.

16- Watanabe K, Hayano K (1993). Dirstribtion and identification of proleolytic *Bacillus* species in Paddy Field soil under rice cultivation, Can. J.Microbiol.,V(41) : 674-680.

17- Harwood C (1989). Bacillus Biotechnology Handbooks 2, Plenum Press, New York.

18-Godic K, Smole S, Mozina K (2003). Bacteriocin produced by *Bacillus cereus* from milk, Food Technol., V(41) : 121-129.

19- Li S, He B, Bai Z, Ouyang P (2009). Anoval organic solvent stable alkaline protease from organic solvent tolerant *Bacillus licheniformis* YPLA. J. Mol. Catal.B:Enzymatic, 56 : 85-88.

20-Ali H, (2006). Purification and characterization of thermostable alpha- amylase from local isolate mutant *Bacillus licheniformis*, Msc. Thesis, College of Science, Baghdad university, Iraq.

21- Kachlishvili E, Michel J P, Nino T, and Vladimir E (2006). Effect of nitrogen source on lignocellulolytic enzyme production by white rot basidiomyctes under solid state cultivation, World Journal of Microbiol. And Biotechnol . Vol(22) : 391-397.

22- Irfan M, Nadeem M, Sayed Q, Baig Sh, (2012). Effect of medium composition on xylanase production by *Bacillus subtilis* using various agricultural wastes, Merican- Eurasian J. Agriculture and Environ. Sci., 12(5): 561-565.

23- Mohamed I, Abou-Dobara A, Kasem E, Reham A (2011).El FayoumyOptimization and Physiochemical Properties of Xylanase from *Bacillus Coagulans* and *Bacillus Licheniformis*,Journal of American Science, 7(12) : 661-670.

24- Nair S, Singhal R., Kamat M (.2006). Enhanced production of thermostable pullulanase type 1 using Bacillus cereus FDTA 13 and its mutant, Food Technol. And Biotechnol. Vol(44) : 275-282.

25- Rajashri D Kamble and Anandrao R. Jadhav (2012). Optimization and Scale Up of Cellulase-Free Xylanase Production in Solid State Fermentation on Wheat Bran by *Cellulosimicrobium* sp. MTCC 10645, Jordan Journal of Biological Sciences,5(4): 289-294.

26-Sindhu I, Chhibber S, Capalash N, Sharma P (2006). Production of cellulose free xylanase from *Bacillus megaterium* by solid state fermentation for biobleaching of pulp. Curr Microbiol., **853**:167-172.

27-Bull A, Bushneh M (1976). Environmental control of fungal growth . In: The Filamentous Fungi. Edited by Smith, J. and D. Berry . Vol(2) : 1-26.,Edward,Arnold .

28-Wahyuntari B, Rachamania N, Setyahad S (2009). Effect of pH, Temperature and Medium Composition on Xylanase Production by *Bacillus* sp. AQ-1 and Partial Characterization of the Crude Enzyme, Microbiol Indones,3(1):17-22.

29- John FJ, Rice JD, Preston F (2006). Characterization of Xylanase from *Bacillus subtilis* subsp *subtilis* strain 168 and analysis of its role in depolymerization of glucoronoxylan. J Bacteriol 188: 8617-26.

30- Asgher M, Javaid A M, Rahman S, Legge R (2007). A thermostable α- amylase from a moderately thermophilic *Bacillus subtilis* DR 8806 of a hot mineral spring process, Biochemistry. 47-806.

31- Battestin V, Macedo G (2007). Effect of temperature, pH and additives on the activity of tannase produced by Paecilomyces variotii, Electronic J. Biotechnol., Vol(10) : 191- 199.

32-Oakley AJ, Heinrich T, Thompson CA, Wilce MCJ (2003).Characterization of a family 11 from *Bacillus subtilis* B230 used for paper bleaching. Acta Cryst D59:627-36.

33-Monisha R, Uma M V, Murthy V K (2009). Partial purification and characterization of *Bacillus pumilus* xylanase from soil source, Kathmandu university J. Science, Engineering and Technol., 5(2): 137-148.

34- Soares M, Desilva R, Gomes E (1999). Screening of bacterial strains for pectinolytic activity : characterization of the polygalactronase production by *Bacillus* sp., Rev. Microbiol., Vol(30): 299-303.

35- Kamble R D, and Jadhav A R (2011). Xylanase production by an alkalo- thermophilic *Bacillus areseniciselenatis* DSM S 340 in submerged fermentation, J. Biotech. And Biosci. 1(1) : 132-134.

36- Kashyap P A, Sabu A, Pandey G, Soccol C, (2002). Extracellular glutaminase production by *Zygosaccharomyces rouxii* under solid state fermentation, Process Biochem., Vol(38): 307-312.

37- Zhang H, Wu Y, He Y (2002). Effect of inoculum size and age on biomass growth and paclitaxel production of elicitor treated *Taxus yunnanensis* cell culture, Appl. Microbil. Biotechnol.,Vol(60): 296-402.

38-Pandey A, Soccol C, Rodriguez-Leon J, Nigan P (2001). Factors that influence on solid state fermentation In: Pandy,A. editor . Solid state fermentation in biotechnology : Fundamentals and applications .New Delhi: Asiatech publishers INC . 21-29.

39- Mrudula S and Kokila R (2010). Production of thermostable α-amylase by *Bacillus cereus* MK in solid state fermentation : partial purification and characterization of the enzyme . The internet J. Microbiol., Vol(8): 1937-2019.

40- Pandey A (2003). Solid state fermentation, Biochemical Engineering journal, Vol(13): 81-84.

41- Kamble R D and Jadhav A R (2012). Isolation, purification and characterization of xylanase produced by a new species of *Bacillus* in SSF., International J. Microbiol., : 1-8.

42-Thiago L R and Kellaway R C (1982). Botanical composition and extent of lignification affecting digestibility of wheat and oat straw and pastalum hay. *Anim Feed Sci Technol*., **7**:71-81

43-Gupta U and Kar R (2009). Xylanase production by a thermotolerant *Bacillus* species under solid state fermentation and submerged fermentation, Brazilian Archieves of Biology and Technology, 52(6): 1363-1371.

44- Gupta U and Kar R (2008). Optimization and scale up of cellulase free endo xylanase production by solid state fermentation on corn cob and by immobilized cells of thermotolerant bacterial isolate, Jordan J. Biological Sciences, 1(3): 129-134.

45-El-Ahwanya M D and Youssef A S (2007). Xylanase production by *Bacillus pumilus* : optimization statistical and immobilization methods, Research J. Agricultures and Biological Sciences, 3(6): 727-732.

46- Sodhi H, Sharmak K, Gupta J, Soni S (2005). Production of thermostable α- amylase from Bacillus sp. PS-7 by solid state fermentation and its synergistic use in the hydrolysis of mult starch for alcohol production, Process Biochem . Vol(40): 525-534.

47-Gevais P and Molin P (2003). Biochemistry . Eng-J., Vol(31): 85-101.

48-Hiremath K S and Patil C S (2011). Isolation, production and charaterization of alkalo thermostable xylanase from newly isolated Bacillus sp., International J. Biotechnol. Application, Vol(3): 48-51.

49-Gupta R, Gigras P Mohapatra H, Goswami V, Chanhan B (2003). Microbial α-amylase : a Biotechnological perspective . Process Biochem., Vol(38): 1599-1616.

50-Kumar S S, Panday D D, Naik G R (20110. Purification and molecular characterization of low molecular weight cellulase –free xylanase from thermoalkalophilic *Bacillus* spp. JB99, World J. Sci. and Technol., 1(2):9-16.

51- Ramesh M and Lonsane B (1990). Critial importance of moisture content of the medium in alpha- amylase production by *Bacillus licheniforms* M27 in a solid state fermentation system, Appl. Microbiol. Biotechnol.,Vol(33): 501-505.

52-Flores M E, Perez R, Huitron C (1997). β-Xylosidase and xylanase characterization and production by *Streptomyces sp.* CH-M-1035. Lett Appl Microbiol., **24**:410-416.

53-Annamalai N, Thavasi R, Jayalakshmi S, Balasubramanian T (2009). Thermostable and alkaline tolerant xylanase production by *Bacillus subtilis* isolated from marine environment, Indian J. Biotechnology, Vol(8): 291-297.

54-Francis A, Sabu K, Ampoothiri S, Ghosh G (2003). Use of response surface methodology for optimizing process parameters for the production of α-amylase by *Aspergillus oryzae*, Biochem . Eng. J., Vol(15): 107-115.

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