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EFFECT OF CULTURE CONDITIONS ON *BACILLUS* SP. (STRAIN RL1) XYLANASE PRODUCTION

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Abstract:

The production of the xylanase enzyme was examined in this study using *Bacillus* sp. (Strain RL1) and five different carbon sources (starch, mannitol, xylose, pine apple, and sucrose) and six different nitrogen sources (yeast extract, beef extract, urea, sodium nitrate, calcium nitrate, and tryptone). The process of assaying dinitrosalicylic acid was used to measure xylanase activity. The crude enzyme was most active at pH 7 and 40 °C. The highest enzyme activity was found when xylose, with a concentration of 3.9913 U/ml, was used as a carbon source. By employing beef extract as a nitrogen source, the enzyme xylanase was generated in the maximum concentration (1.7335 U/ml). *Bacillus* sp. (Strain RL1) was able to produce a significant amount of xylanase with high levels of activity across a range of pH and temperature conditions by using xylose and beef extract as carbon and nitrogen sources.

Keywords: xylanase enzyme, *Bacillus* sp. RL1, xylose, Beef extract.

INTRODUCTION: According to numerous studies, *Trichoderma* spp., including those by (Wong *et al*., 1988 ; Gomes *et al*., 1992 ; Li *et al*., (2000), and Beg *et al*., (2001), several bacteria and fungi, including *Bacillus*, *Aspergillus*, *Penicillium*, *Schizophyllum*, *Aureobasidium*, and *Thermomyces*, are capable of producing xylanase. The versatility of xylanase enzymes, which include pulp preparation and bleaching, has attracted a lot of interest (Buchert *et al*., 1993). Since most agricultural wastes contain xylan, xylanolytic enzymes hold great potential for digesting agricultural and forestry wastes (Gomes *et al*., 1992). Thus, in this regard, the impact of extra carbon and nitrogen sources on the xylanase synthesis by *Bacillus* sp. Strain RL1 was investigated.

MATERIALS AND METHODS

Media components, chemicals, and media Sigma Chemicals Ltd. in the USA provided all of the analytical-grade chemicals, media, and media components, while Shanghai Chemicals Ltd. in China provided the remaining chemicals.

Microorganism and culture : In order to isolate the xylanase-producing bacterial strain (*Bacillus* sp. RL1) from soil (Jin Yun mountain, 1800 m), pour plate and serial dilution up to 10-9 procedures were applied. The single colonies on the plates were isolated and purified by transferring them five times onto CMC agar plates.For primary screening, the isolate was grown at 30°C for 24 hours on a medium containing (g/L), KH2PO4, 2, (NH4) 2SO4, 4, MgSO4, 0.5, Peptone, 10, agar agar, 20, and distilled water, supplemented with 1% carboxy methyl cellulose (CMC).

Identification of the isolates: On CMC agar slants, the bacterial isolates discovered during the primary screening were kept in pure culture. Until usage, each agar slant was kept chilled at 4 °C. Gram's staining and endospore staining were done after studying the colony morphology of the isolated culture. Standard techniques were used to perform physical and biochemical evaluation on the isolated colonies (Kannan, 2002). Burges' Manual, seventh edition, was used for identification.

Secondary screening: Cellulolytic activity was screened for again using the Congo red test. To encourage the secretion of xylanase, the bacteria were grown on consecutive CMC agar plates with (g/L), KH2PO4, 2, (NH4)2SO4, 4, MgSO4, 0.5, Peptone, 10, agar agar, 20, and distilled water, supplied with 1% CMC, at 30°C for 24 hours. Following incubation, an aqueous Congo red solution solution (1% w/v) was poured over the agar substrate and left to sit for 15 minutes. The plates were then washed with 1M NaCl for 20 minutes after the stain was removed, and the solution was then drained off. The emergence of a unique hydrolysis zone revealed that cellulose was being broken down (Teather and Wood, 1982).

Confirmation of xylanase activity: To evaluate the xylanase synthesis in our laboratory, we developed this approach. Supernatant free cell was treated with 0.5 ml of 1% substrate (CMC) in 0.05 M citrate buffer (pH 4.8) for 30 min at 50°C. After 15 minutes at 100 °C, the reaction was stopped by adding 0.1 ml of naphthol (1%) and gently mixing the mixture. When 0.5 ml of concentrated H2SO4 is added, the solution's color should shift to brown, signifying a successful test.

Fermentation experiment: In a 50 ml Erlenmeyer flask containing 30 ml of CMC medium made up of (g/L) KH2PO4, 2, (NH4)2SO4, 4, MgSO4, 0.5, Peptone, 10, distilled water, and 1% carboxy methyl cellulose (CMC) without agar, the culture was grown aerobically for 72 hours at 35 °C and pH 7.0. The culture was centrifuged for 10 minutes at 10,000 rpm after the incubation phase. The activities of xylanase, FPaes, and extracellular protein were then assessed in the culture supernatant. This data represents the average of three replicates plus the standard error. **Xylanase assay:** Xylanase activity was determined using a 1% solution of oat spelt xylan as the substrate, and the amount of reducing sugars released was quantified using the dinitrosalicylic acid method (Miller,1959), according to Bailey *et al*. (1992). Under the specified conditions, one unit of enzyme activity was defined as one mole of xylose equivalent produced every minute.

Improving the culture conditions for the synthesis of enzymes:

Effect of carbon source : the effect of different carbon sources on the production of xylanase enzyme, namely starch, mannitol, xylose, pine apple and sucrose.

Effect of nitrogen source : the production medium was prepared using different nitrogen sources included : yeast extract, beef extract, urea, sodium nitrate, calicium nitrate and tryptone.

pH optimum: By altering the pH of the CMC broth to 4.0, 6.0, 7.0, 8.0, 9.0, and 10.0 before inoculating the bacteria, it was possible to determine the impact of the initial medium pH on the production of xylanase and FPase. Culture broths were then centrifuged at 10,000 rpm for 10 minutes after 72 hours of incubation at 35°C to yield supernatants from which xylanase and FPase activity as well as extracellular protein were later determined.

Temperature optimum: The chosen bacterial isolate was cultured in CMC broth and incubated at 30, 40, 50, 60, 70, 80, and 90°C for 72 hours to ascertain the impact of temperature on xylanase and FPase synthesis as well as extracellular protein. Then, culture broths were centrifuged at 10,000 rpm for 10 minutes to extract supernatants, and xylanase, FPase, and extracellular protein activities were evaluated in the supernatants.

RESULTS AND DISCUSSION

Trichoderma spp. specifically utilised *Bacillus*, *Aspergillus*, *Penicillium*, *Schizophyllum*, *Aureobasidium*, and *Thermomyces* in the production of xylanase (Wong *et al*.,1988 ; Gomes *et al*.,1992 ; Li *et al*., 2000 ; Beg *et al*.,2001).

Effect of the carbon source: According to Figure 1, the growth of *Bacillus* sp. RL1 varies according to the carbon source that is employed in the medium. The xylose compound produced the most xylanase enzyme (3.9913 U/ml yield), followed by starch, pine apple, and mannitol as carbon sources. This demonstrates how xylan has a strong stimulatory effect on xylanase synthesis. Sucrose, however, had the lowest dry biomass content. Among the established carbon sources, the medium made with xylose produced considerably (P 0.05) more xylanase enzyme, which may be because easily metabolizable substrates may prevent the synthesis of enzymes (Bocchini *et al*., 2008).

One of the crucial and variable components of the medium used for microbial fermentation is the carbon supply. The varied xylan sources, which may contain nutrients that stimulate bacterial growth and enzyme production, or the equivalent amount employed in the production medium may be to blame for the difference in effectiveness. The varied xylan between the two carbon sources or the distinct physiology of the isolated bacteria may be to blame for the ratio of these nutrients. which may allow them to produce a high yield of the enzyme (Gowdhaman *et al*., 2014).

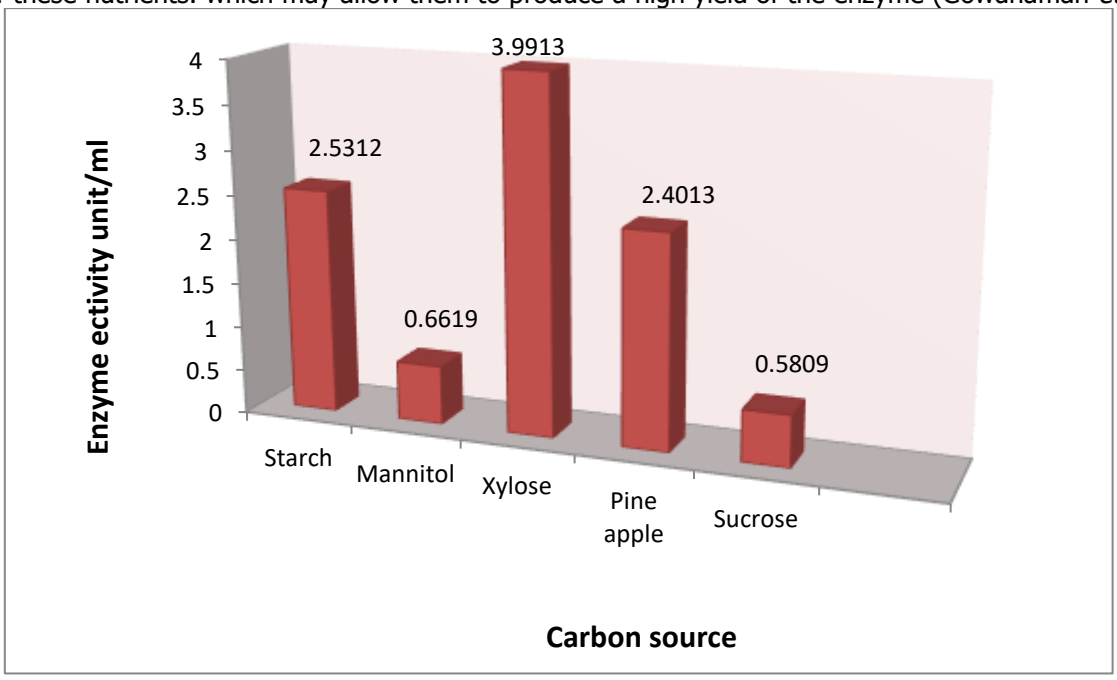


Figure 1 : The effect of the carbon source on the production of xylanase enzyme.

The greatest activity of the xylanase enzyme was discovered to be 2,350 U/ml utilizing starch, followed by ammonium sulfate, and it reached to 2,480 U/ml, according to Haq *et al*., (2002). This enzyme was created using several carbon

sources from a mutagenic strain of the mold *Aspergillus niger* GCBMX-45. According to research by Ninawe and Kuhad, (2005), which used a variety of waste agricultural crops that were added to the breeding medium at a rate ranging from 1-2 (w/v), the activity of the enzyme produced by *Streptomyces cyaneus* SN32 reached 632 U/ml when wheat bran was added to the production medium at a rate of 2%. In a different investigation, *Melanocarpus* sp. generated xylanase with an efficiency of 264.2 units/ml from birch wood xylan (Ghatora *et al*., 2007). According to Annamalai *et al*., (2009), the usage of oat xylan resulted in the maximum synthesis of the enzyme using *Bacillus subtilis*, with an efficiency of 128 U/ml. According to Gowdhaman *et al*. (2014), employing birch wood xylan resulted in a maximum production of the xylanase enzyme from the bacteria *Bacillus aerophilus* KGJ2, reaching 113.83 U/ml.

Effect of the nitrogen source : The result are shown in Figure 2. the nitrogen source, beef extract, gave the highest production of xylanase enzyme, which amounted to 1.7353 U/ml, then the nitrogen source of sodium nitrate, which gave 1.0385 unit/ml of xylanase enzyme, then nitrogen source of urea, which gave 0.9613 U/ml, then the nitrogen source is yeast extract, which obtained a productivity of 0.9484 U/ml of xylanase enzyme, and then nitrogen source of calicium nitrate, which provided 0.4682 U/ml.

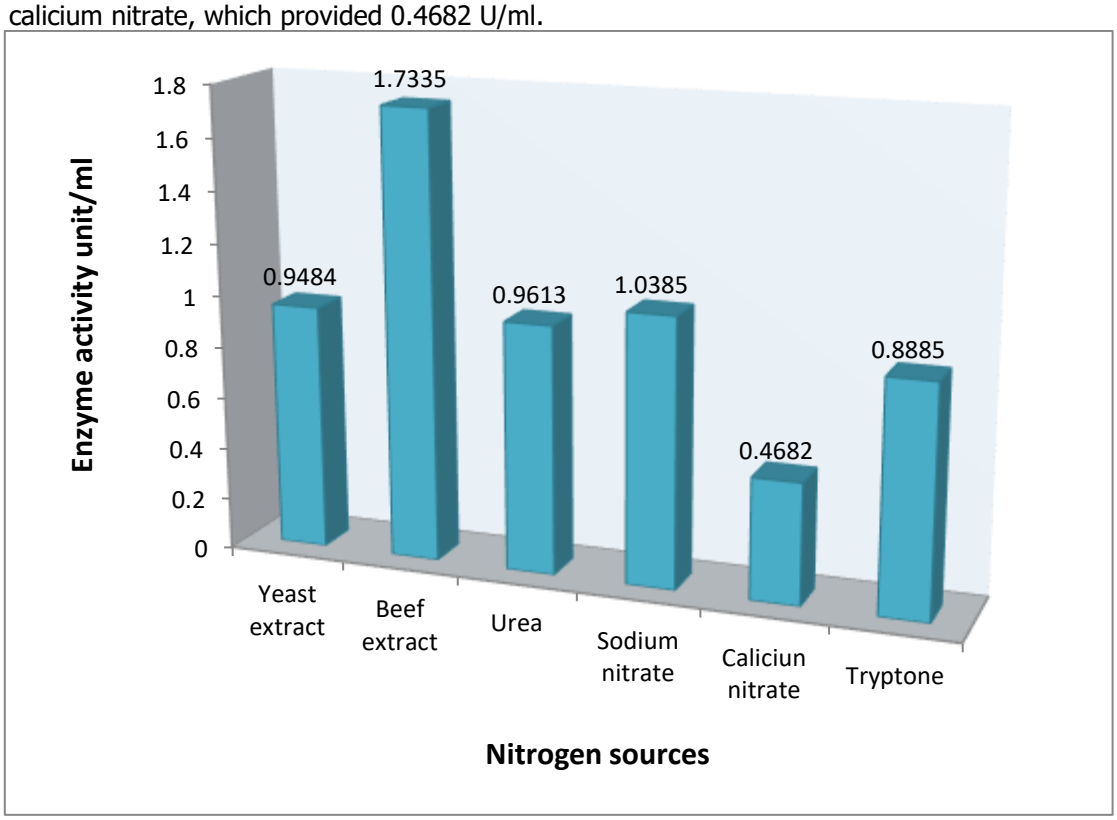


Figure 2 : The effect of the nitrogen source on the production of xylanase enzyme

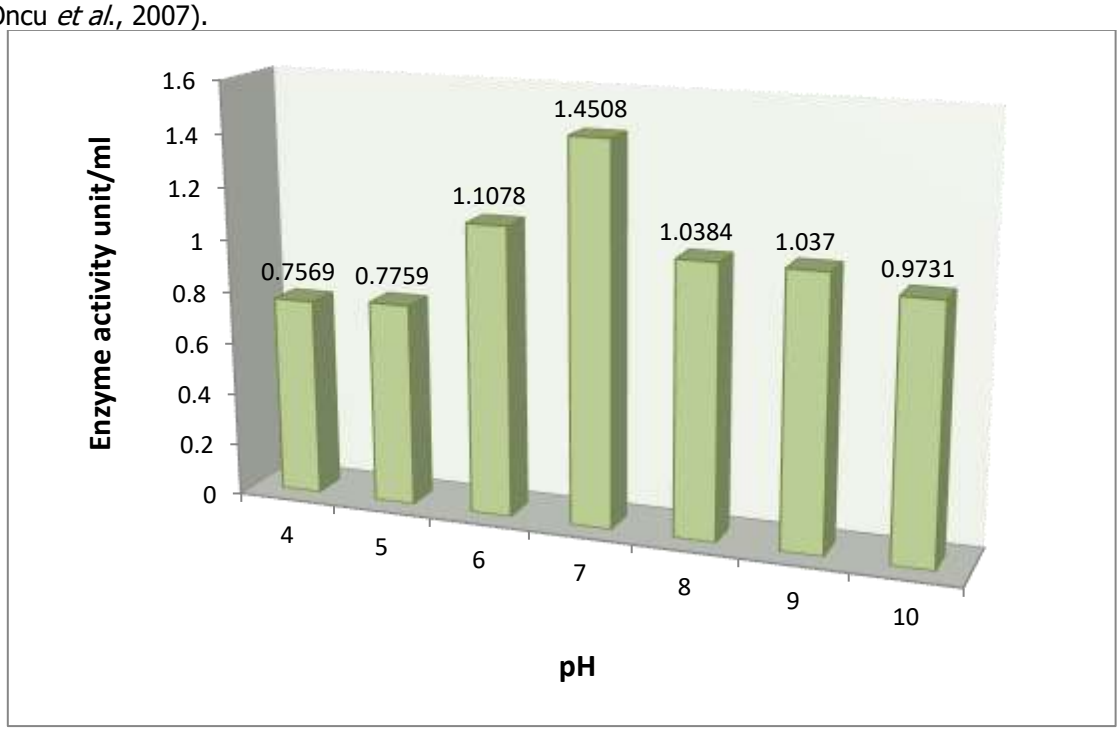
One of the key elements in the growth of the microorganism and the synthesis of numerous organic compounds, such as amino acids and proteins, is the source of nitrogen in the production medium. The components of the medium are balanced, and vice versa, it leads to an increase in the growth rate in the production process

According to Annamalai *et al*., (2009), employing polypeptone and yeast extract at a concentration of 10,5 g/l resulted in the maximum synthesis of the xylanase enzyme from *Bacillus subtilis*. Sharma *et al*. (2013) discovered that the maximum xylanase enzyme synthesis occurred when peptone and yeast extract, each at a concentration of 5,5 g/l by *Paenibacillus macquariensis*, the maximum enzyme production from *Bacillus aerophilus* KGJ2 was found utilizing beef extract as a nitrogen source, followed by the usage of peptone and yeast extract (Gowdhaman *et al*., 2014).

Effect of pH on the synthesis of xylanase : The pH of enzyme production differs from one microorganism to another depending on the type of enzyme produced and the type of microorganism used in the production process, and there are many microorganisms whose growth pH approaches the pH of enzyme production, but there are some cases in which the difference is significant, and the reason may be due to factors related to the cell inself, especially the expression of genetic traits and the physiological aspects of the growth of the microorganism and the production of enzymes (Crueger and Crueger, 1990).

The ability of the bacillus isolate to produce the xylanase enzyme was selected using the production medium with different pH numbers ranging from (4-10), as shown in Figure (3) that the highest productivity of the enzyme was at a pH of 7.0, as its value was 1.4508 U / ml, and the enzyme had good activity between cultures the pH is (6-8) as it reached,1.1078, 1.9731 U/ml, noting that the activity of the enzyme continues to decrease at the rise and fall from the optimal pH, as the change in the range led to a change in the nature of the vital activities, which affects the nature of the produced materials, as the pH affects the microorganisms at the molecular level, the increase in the pH in the microorganisms leads to the removal of amino acids from tRNA, which participates in the process of protein synthesis, which leads to stopping it, the pH of the medium, which affects many of the vital activities carried out by the

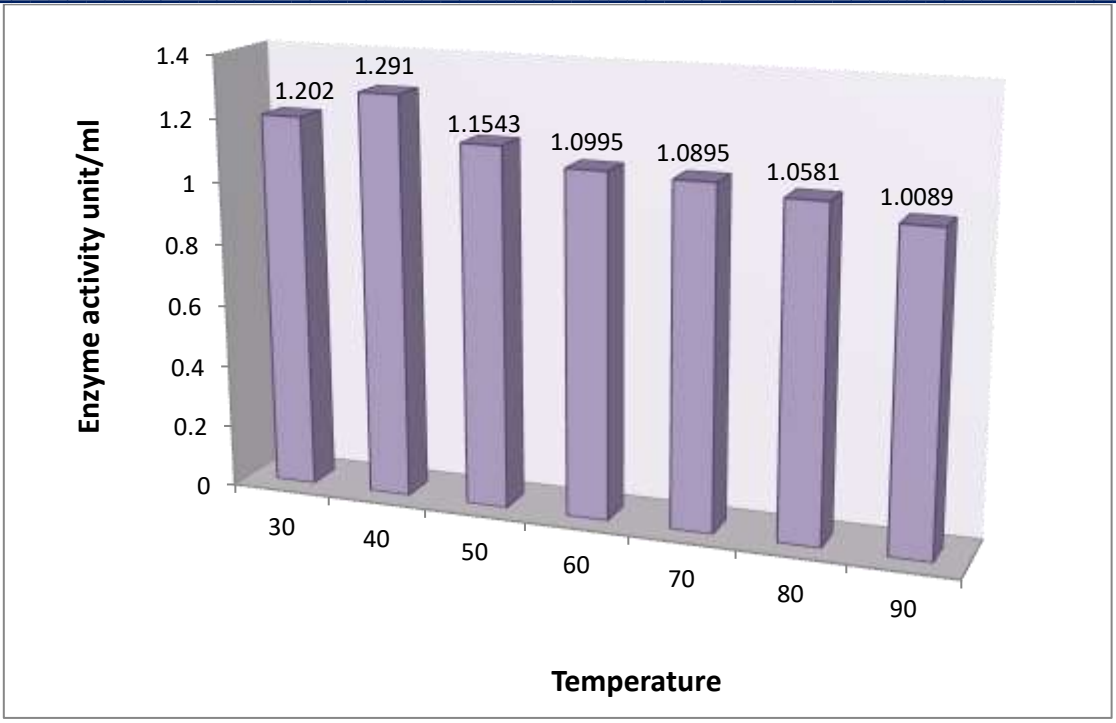
microorganism in the production medium, such as growth, reproduction and production of metabolic compounds, and the pH is one of the environmental factors that should be controlled to achieve the highest production of microbial enzymes (Oncu *et al.*, 2007).



Fiqure 3 : Effect of pH on xylanase enzyme production by Bacillus sp. (strain RL1).

The highest synthesis of the xylanase enzyme from *Paenibacillus macquariensis* was at pH 9.0, according to Sharma *et al.*, (2013), however Roy and Rowshanul, (2009) claimed that the ideal pH for the development of the enzyme from *Bacillus cereus* is 7.0. The optimal pH of the medium for the generation of xylenase from *Bacillus subtilis*, according to Annamalai *et al.*, (2009) At an initial pH of 8.0, *Bacillus subtilis* XP10 optimally generates the xylene enzyme, according to Tork *et al.*, (2013). Pithadiya *et al.* (2016) found that *Bacillus circulans* can manufacture xylanase at a pH of 7.0.

Effect of temperature on the generation of xylanase : Due to its effect on the solubility of oxygen and the kinetic energy of the molecules, which increases the kinetic energy of the enzyme and the base material and accelerates the chemical reaction, temperature is a key factor in determining the activity of microorganisms, growth rate, metabolism, physiological and functional characteristics of the microorganism. The ideal temperature for the synthesis of each type of enzyme varies. The xylenase enzyme was produced by the microorganism at a variety of temperatures, ranging from (30-90) C, and the findings presented in Figure (4) indicated that the best temperature was thirty, where the enzyme activity reached 1.291 unit/ml. , noted that the enzyme maintained good efficacy at temperatures over 30 C, as evidenced by the enzyme's enzymatic activity at those temperatures being (1.202,1.1543,1.0995,1.0895,1.581,1,0089) units/ml, respectively.



Fiqure 4 : The effect of temperature on the production of xylanase enzyme by Bacillus sp. (strain RL1).

The optimum temperature used for the production of enzymes varies according to the microorganisms, as the optimum temperature and its ranges are corcponding with environmental conditions, so all studies that dealt with the production of different enzymes from microorganisms, whether by submerged fermentation method or by the solid state fermentation method, have been evaluated. This aspect pays special attention, in order to draw thermal limits for production and the necessity of adhering to them in order to obtain the enzyme with high efficiency and productivity (Bocchini *et al*., 2002)

The rise in temperature stimulates all biochemical reactions of microorganisms to reduce to the optimal level, then the rise leads to the irreversible disruption of proteins, including enzymes, nucleic acids and other cell components, and explains the resistance of bacterial cells to the influence of high temperatures until the damage that occurs to the protein does not include the enzymatic protein of the compensating and repairing processes that helps the cells to resume their activity if the appropriate temperature is restored for their growth. Low temperatures also affect all vital activities and make them very slow to increase the viscosity of the protoplasm and decrease the kinetic energy of the enzymes, which leads to a decrease in its activity (Bocchini *et al*., 2002).

Yasinok *et al*. (2008) found that *Bacillus pumilus* produces xylanase enzyme at a temperature of 30 C, Roy and Rowshanul, (2009) found that *Bacillus cereus* produces xylanase enzyme at a temperature of 37 C, Annamalai *et al*. (2009) found that *Bacillus subtilis* produces xylanase enzyme at a temperature of 55 C. and Sharma *et al*, (2013) found that the best temperatyre for xylanase production was at a temperature of 37 C from *Paenibacillus macquriesis*.

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