

Using Different Concentrations Of Some Agricultural Wastes as a Carbon Source For The Production of Xylanase Enzyme

Raed S. Khudhair

Department of Biology, College of Education for Pures Science, Univesity of Thi-qar, Iraq.
Email: dr.raedsaeed@utq.edu.iq

Abstract. xylan extracted by dilute acid treatment from papyrus was used as a carbon source to produce xylanase enzyme in five concentrations of 0.1, 0.3, 0.5, 0.7 and 0.9%. the highest production of xylanase was at a concentration of 0.5%, with an enzyme activity of 2800 U/ml, then a concentration of 0.7% with an enzymatic activity of 2520 U/ml, then concentrations of 0.9, 0.3 and 0.1% with an enzymatic activity Of 2360, 2280 and 1280 U/ml, respectively. Under production conditions, the rotor was incubated in front of the vibrating incubator at 150 rpm and at a temperature of 37 C for 48 hours.

Keywords: Agricultural Wastes, Carbon, Xylanase Enzyme

Introduction: xylan, after cellulose, is the most available polysaccharide in wood. many agricultural and industrial wastes consist of a number of major chain heterogeneous sugars that contain different or branched alternatives (Shallom and Shoham, 2005). the alternatives include arabinofuranosyl, glucuronyl and acetyl group, which show a clear effect on the chemical properties. structural and also on the enzymatic hydrolysis of xylan in lignocellulose (Collins et al., 2005).

Xylanase is produced by many fungi and bacteria. Industrially, filamentous fungi that produce these enzymes are important due to the secretion of xylenes and their ease of use (Dekker, 2003)

These commercial enzymes are used in the pulp and paper industry, food and beverage, textile and animal feed industries (Polizeli et al., 2005)

Also, xylanase enzymes show a great potential to increase the production of many useful products such as xylitol and ethanol in an economic way (Beg et al., 2001)

Most commercial xylanase enzymes are produced by *Penicillium*, *Aspergillus*, *Bacillus*, *Trichoderma*, *Taloromyces* and *Aureobasidium* (Li et al., 2000).

Keyword: Agricultural waste, xylanase enzyme, submerged fermentation

Materials and Methods

Extraction of xylan from papyrus: xylan was extracted from papyrus by using the dilute acid treatment method for the purpose of recovering the largest amount of xylan present in the biomass

Dilute acid treatment: the dilute acid treatment method was used to extract xylan mentioned in Yang et al., (2005)

Quantification of extracted xylan: the xylan extract was estimated according to the method reported by Dubois et al. (1956) based on the phenol-sulfuric acid method for the determination of total sugars

Microorganism: *Bacillus subtilis* bacteria, which produce xylanase enzyme.

Production medium: the nutritional medium mentioned in Gowdhaman et al., (2014) was adopted consisting of : Xylan 0.5, Peptone 0.5, NaCl 0.1, K_2HPO_4 0.2, $CaCl_2$ 0.01, $MgSO_4 \cdot 7H_2O$ 0.01, Yeast extract 0.1 (g/l).

Production of xylanase: the 250 ml flasks and the container on the production medium were inoculated with 100 ml / flask with 1 ml of bacterial suspension and 1×10^7 cells / ml of pure *Bacillus subtilis* bacteria and incubated in the vibrating incubator at a temperature of 37 C at a number of 150 rpm for 48 hours (Gowdhaman et al., 2014).

Enzyme extraction: xylanase was extracted by centrifuging the production medium at a speed of 12000xg for 10 minutes at a temperature of 4 C. The precipitate was discarded and the filtrate was counted as the crude extract in the enzyme (Roy and Rawshanul, 2002).

Estimation of enzyme activity: it was followed as described by Miller, (1959) in estimating the activity of the enzyme according to the method of estimating reducing sugars.

Result and Discussion

The optimum concentration of the carbon source: the carbon source is one of the necessary and variable components in the fermentation medium of microorganisms, as important and vital for the growth and metabolism of cells (Gowdhaman et al., 2014). xylan extracted by acidic method was used for the agricultural residues was added to the production medium instead of the carbon source in the same quantity. The obtained results showed in Figure (1) that xylan extracted from the papyrus plant gave the highest activity to the enzyme xylanase produced by *Bacillus subtilis* with an efficiency of 2800 U/ml. the reason for the difference in effectiveness, may be due to the difference in the concentration of extracted xylan, which may contain nutrients that support bacterial growth and enzyme production. Thus, the presence of physiological differences may enable the bacteria to give a high productivity of the enzyme.

In order to determine the optimal concentration of the carbon source in the production of xylanase enzyme from *Bacillus subtilis* strain bacteria, the effect of different concentrations of xylan produced from sedge was studied, including concentrations (0.1%, 0.3%, 0.5%, 0.7%, and 0.9%) with a difference of 0.2% between the concentration. Finally, the results shown in Figure 1) showed that the enzymatic activity reached a maximum of 2800 U/ml at a concentration of 0.5% compared with the rest of the concentrations.

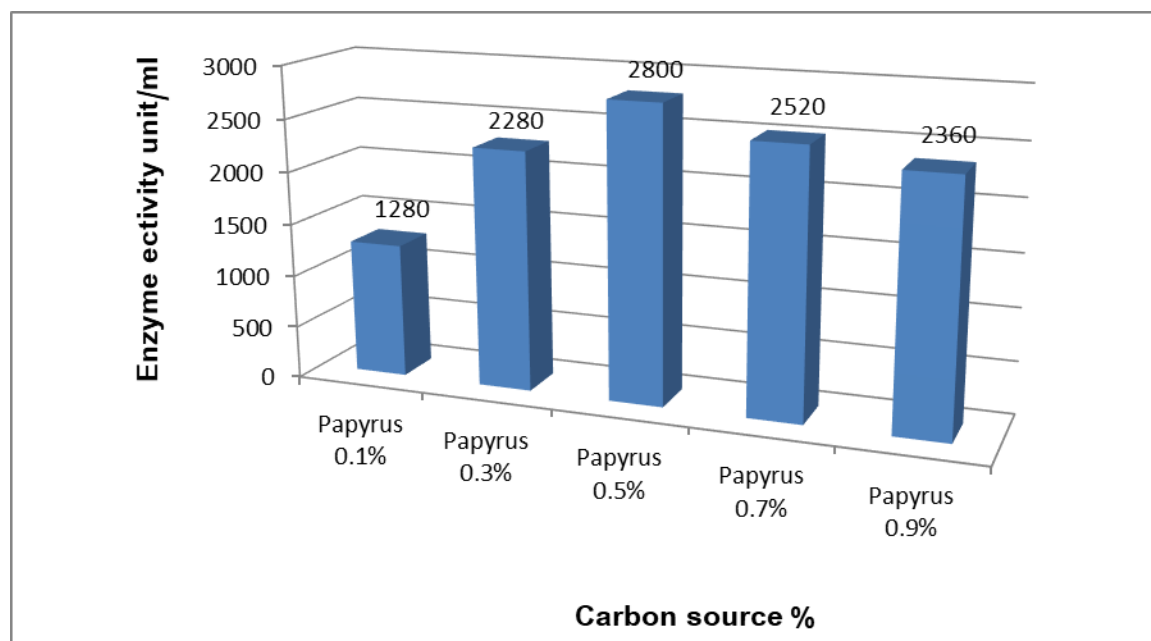


Fig (1) : effect of xylan concentration produced from papyrus on the productivity of xylanase enzyme from *Bacillus subtilis* bacteria.

The type and concentration of carbon sources required to be provided in the production medium varies for the development of different types of microorganisms intended for the production of industrial enzymes.

several researchers have studied the effect of carbon source concentration on the production of xylanase from different microorganisms, as Yasinok et al., (2008) mentioned that the best production of xylanase enzyme from *Bacillus pumilus* is by using corn grain powder at a concentration of 3%, as found Annamalai et al., (2009). that the optimum concentration of oat xylene for producing xylanase from *Bacillus subtilis* bacteria was 5 g/l, and Roy and Rowshanul, (2009) found that the production of xylanase enzyme from *Bacillus cereus* was using oat xylan at a concentration of 0.5%, while Krishnaveni indicated, (2011) indicated that the use of xylan and glucose together gives the best production of xylanase enzyme from *Bacillus cereus* at a concentration of (0.5-3%) for each, while (Sharma et al., 2013) indicated that the highest production of xylanase enzyme from *Paenibacillus macquariensis* was by using Birch wood xylan at a concentration of 2%, and Gowdhaman et al., (2014). showed that the best concentration of birch wood xylan for producing xylanase enzyme from *Bacillus aerophilus* KGJ2 is 0.5%, and Amit and Nikul, (2014) found that a concentration of 0.3 g/100 ml of xylan pine wood give The highest yield of xylanase was obtained by using bacterial isolate from garden soil.

References

1. Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Robers. P. A. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28: 350-356.
2. Shallom, D. and Shoham, J. (2003). Microbial hemicellulases. *Current Opinion in Microbiology*, 6: 219-228.

3. **Collins, T.; Gerday, C. and Feller, G. (2005).** Xylanases, xylanases families and extremophilic xylanases. **FEMS Microbiology Reviews**, 29: 3-23.
4. **Dekker, M. (2003).** Handbook of Fungal Biotechnology, Dilip K. Arora ed., New York. 10.
5. **Polizeli, M. L. T. M.; Rizzatti, A. C. S.; Monti, R.; Terenzi, H. F. Jorge, J. A. and Amorim, D. S. (2005).** Xylanases from fungi: properties and industrial applications. **Applied Microbiology and Biotechnology**, 67: 577-591.
6. **Beg, Q.K.; Kapoor, M.; Mahajan, L. and Hoondal, G. S. (2001).** Microbial xylanases and their industrial applications: A review. **Applied Microbiology and Biotechnology**, 56: 326-338.
7. **Li, K.; Azadi, P. R. Collins, R.; Tolan, J.; Kim, J. S. and K. E. L. (2000).** Relationships between activities of xylanases and xylan structures. **Enzyme Microbiology and Technology**, 27: 89-94.
8. **Yang, R.; Xu, S.; Wang, Z. and Yang, W. (2005).** Aqueous extraction of corncob xylan and production of xylooligosaccharides. **LWT-Food Sci Technol**, 38:677-682.
9. **Gowdhaman, D.; Jeyalakshmi, G.; Sugumaran, K.; Subramanian, N. S.; Santhosh, R. S. and Ponnusami, V. (2014).** Optimization of the xylanase production with the newly isolated *Bacillus aerophilus* KGJ2. **Turkish Journal of Biochemistry**, 39(1): 70-77.
10. **Roy, N. and Rawshanul, H. M. (2009).** Isolation and characterization of Xylanase producing strain of *Bacillus cereus* from soil. **Iranian Journal of Microbiology**, (2): 49-53.
11. **Miller, G. L. (1959).** Use of dinitrosalicylic acid reagent for determination of reducing suger. **Analytical Chemistry**, 31(3): 426-429.
12. **Yasinok, A. E.; Sahin, F. L. and Haberal, M. (2008).** Isolation of Endopfytic and Xylanolytic *Bacillus pumilus* Strain from *Zea mays*. **TARIM BILIMLERI DERGISI**, 14(4): 374:380.
13. **Annamalai, N.; Thavasi, R.; Jayalakshmi, S. and Balasubramanian, T. (2009).** Thermostable and alkaline tolerant xylanase production by *Bacillus subtilis* isolated from marine environment. **Indian Journal of Biotechnology**, 8: 291-297.
14. **Krishnaveni, M. (2011).** PRODUCTION AND OPTIMIZATION OF XYLANASE FROM ESTUARINE *BACILLUS CEREUS*. **International Journal of Pharma and Bio Sciences**, 2
15. **Sharma, M.; Mehta, S. and Kumar, A. (2013).** Purification and Characterization of Alkaline Xylanase Secreted from *Paenibacillus macquariensis*. **Advances in Microbiology**, 3: 32-41.
16. **Amit, D. and Nikul, C. (2014).** Screening and Kintic study of xylan Enzyme Production Bacteria from green soil. **The world Journal of Engineering and Applied Sciences**, 1(1): 1-3.