Isolation and Partial Characterization of Crude Cellulase- Free Xylanase from Pseudomonas Aeruginosa and Staphylococcus Aureus for Possible use in Paper Industry

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Abstract— The study was to isolate and characterize crude cellulase-free xylanase from bacteria isolated from soil. The bacteria, Pseudomonas aeruginosa and Staphylococcus aureus, secreted extracellular xylanase along side cellulase when grown in submerged media supplemented with glucose. Xylanase and cellulase activity were determined using birch wood xylan and carboxymethylcellulose as substrates respectively at 24hour interval. Optimum xylanase activity of 8.10X10-3 µmol/ml and 6.13x10-3 µmol/ml were obtained after 48 hours of incubation respectively. Also, optimum cellulase activity after 72 hours of incubation for both bacteria was 12.24 mmol/L and 6.35 mmol/L. The two bacteria had optimum xylanase activity at 60oC. Thermostability study showed that crude xylanase from both bacteria retained about 50% residual activity after incubating the enzyme for 210 minutes at the optimum temperature of 60oC. The result revealed that both bacteria are likely potential candidates for production of relatively cellulase-free xylanase that may be beneficial in biobleaching.

Key words: Pseudomonas aeruginosa, Staphylococcus aureus, cellulase-free xylanase, birch wood xylan, carboxymethylcellulose

I. INTRODUCTION

There is no doubt that the present day industries employ more of plants and plant derived materials for their daily activities. There is therefore, the need to readily hydrolyze some of the plant components such as xylans to make them handy. Xylanases have successfully been used in this regard. The enzyme has been categorized or classified as one of the most important industrial enzymes (Collins et al., 2005). Xylanases have attracted considerable research interests of recent mainly due to their potential applications in various industries (Viikari et al., 2001). One of the widely recognized applications of xylanase is in the biobleaching of paper pulp. Until recently, the classic way to achieve the biobleaching was to add chlorine based bleach which generates organo-chlorine pollutants into the environment. The use of xylanase breaks hemicellulose chains that are responsible for the close adherence of lignin to cellulose network, thus reducing the production of organo-chlorine pollutant such as dioxins and enhances strength of the paper.

Application of fungal xylanases in paper and pulp industries is improved by acidic pH, and growth requirement of the fungi themselves. Nevertheless, this is not the case with bacterial xylanases with optimal pH generally slightly higher than the pH optima of fungal xylanases (Bansod et al., 1993). In most industrial applications, the low pH optimum for growth and production of xylanase activity necessitates additional steps in the subsequent stages which make fungal xylanases less suitable. Although, high levels of xylanases are produced by several fungi, the presence of considerable amount of cellulase activity and lower pH optimums make them less suitable (Kang et al., 1996); if one uses an acidic xylanases, it is possible to wash off the alkali out of the pulp (Srinivasan and Rele, 1999). The various limitations associated with the extensive industrial applications of fungal xylanases have necessitated the need for a relatively cellulase-free, alkaliphilic and thermostable xylanase that may meet most industrial processes. It is against this background that this study was carried out to evaluate the production potentials of cellulase-free crude xylanase of Pseudomonas aeruginosa and Staphylococcus aureus isolated from soil.

II. MATERIALS AND METHODS

A. Source Of Bacteria

Pseudomonas aeruginosa and staphylococcus aureus were isolated from soil from at the Crop Pathology Laboratory of Crop Protection Department, University of Maiduguri, Nigeria. The organisms were identified as described in the Berger's manual of determinative bacteriology (Holt et al. 1994). They were maintained on nutrient agar as slants.

B. Fermentation Media

Submerged fermentation method was used in the study and the growth and enzyme production medium was prepared as described by Kim et al., (1985) by dissolving the following salts in distilled water (g/100ml), 0.05 proteous peptone, 0.03 urea, 0.02 KH2PO4, 0.03 CaCl2, 0.14 NaNO3, 0.03 MgSO4.7H2O in 250ml Erlenmeyer flask. This was then autoclaved at 121OC for 15min. After sterilization, trace elements made up of 0.005g FeSO4, 0.0014g ZnSO4.7H2O, 0.002g CaCl2, 0.0016g MnSO4 making 0.1% concentration and 0.3g of glucose was added as a carbon source.

C. Inoculation

The stock bacterial strains were activated by culturing in nutrient broth for 24 hours at 37oC. 1ml of the 10-6 serially diluted bacterial solution was inoculated into the culture medium prepared. The medium was incubated for 192 hours at 38oC.

D. Xylanase Assay

The xylanase activity was assayed using 1% birch wood xylan (w/v) as the enzyme substrate. The liberation of reducing sugar xylose was estimated by the Dinitrosalicylic acid (DNS) method (Miller et al., 1959). The assay was at 24hour intervals. One unit of xylanase activity was defined as the amount of enzyme required to liberate 1µmol of xylose equivalent per minute under the assay condition.

E. Cellulase Activity

The cellulase activity was assayed by estimating the amount of glucose released upon hydrolysis of cellulose from carboxymethyl cellulose (CMC) substrate. Glucose released was determined at 24 hour intervals.

One unit of cellulase activity was defined as the amount of enzyme which released 1umol of reducing sugar measured as glucose per minute under the assay condition. Therefore, Glucose concentrations (µmol/L) = Absorbance of test/Absorbance of standard X Concentration of standard. Estimation of glucose released was by the use of Glucose oxidase kits (Randox) obtained from Sigma laboratory ltd, Germany.

F. Effect Of Ph

The working pH of the reaction mixture was varied from pH 4-10 of the sodium acetate buffer used. The optimum pH for each of the bacteria species was evaluated.

G. Effect Of Temperature And Thermostability Assay

The effect of temperature on xylanase activity was measured at pH 5.0 (sodium acetate buffer) from 30o-90oC. Thermal stability of xylanase was determined by assaying the residual activity at the enzyme's optimum temperature determined, and decay equation was used to calculate the half-life of the enzyme.

H. Kinetics Of The Enzyme

The concentration of the substrate (birch wood xylan) was varied from 0.2-1.0mg/ml in 0.1M sodium acetate buffer (pH 5.0) and the activity was evaluated for the determination of the KM and Vmax of the enzyme.

III. Results and Discussion

As the incubation period increases crude xylanase activity increased up to the optimum enzyme activity for both bacteria obtained after 48 hours. Pseudomonas aeruginosa had xylanase activity of 8.10X10-3 umol/ml as against 6.13X10-3umol/ml for staphylococcus aureus (Fig. 1). The optimum cellulase activity of 12.24 mmol/l for Staphylococcus aureus and 6.35 mmol/l for Pseudomonas aeruginosa were obtained on the third day (72hr) of incubation as depicted in figure 2. This may be explained on the fact that microbial strains' metabolism, growth and enzyme production pattern differ with the fermentation environment (Prakasham et al., 2007). The submerged fermentation method employed in this study may be more beneficial as compared to other techniques due to more nutrient availability, sufficient dissolved oxygen supply and less time required for the fermentation. The result from the figures also showed gradual decrease in enzyme activity with increase in incubation period. The reduction in xylanase yield after optimum period was probably due to the depletion of nutrient available to microorganism or due to proteolysis (Uma and Rita, 2008).

Cellulase activity was assayed as a function of glucose production and the result showed that the two bacterial strains produced cellulases along side xylanases. The slightly higher cellulase activity from S. aureus could be attributed to xylanases since negligible cellulolytic activities by some xylanases can also be attributed to the presence of cellulose binding domains (CBDs) in them. A bifunctional enzyme couples the actions of two distinct enzymes into one protein complex to perform two acts that would typically be performed separately, xylanases are an example. The catalytic domains are joined by a peptide linker (Khandeparker and Numan, 2008). Although it is quite unnatural for one enzyme to have a high affinity toward a non specific substrate, CBDs are usually distributed in xylanases (Black et al., 1995). Apart from the hydrolytic activity against xylan, some of the microbial xylanases produced from Streptomyces halstedii JM8 are also reported to contain cellulose binding domain. Some exo-β-1, 4-xylanase from Cellulomonas fini hydrolyzes not only cellulose but also xylan since they share the same catalytic mechanism (White et al., 1994).

Pseudomonas aeruginosa had KM of 0.12 mg/ml and S. aureus, 0.15 mg/ml. The maximum velocity reaction (Vmax) of 2.3x10-4 µmol/min and 8.6x10-4 µmol/min for P. aeruginosa and S. aureus respectively was obtained. The low KM values means high affinity for the substrate but the crude xylanase from S. aureus was higher. Reports have shown most xylanases have KM in the range of 10-2 -10-5. Beg et al., (2001) reported a KM value for microbial xylanases in the range of 0.27-14 mg/ml. However, pH which is the most important factor for the characteristics of the xylanase showed that maximum activity was at pH 7 and pH 8 for P. aeruginosa and S. aureus respectively. Studies by Gawande and Kamat (1999) have shown that optimal pH of bacterial xylanases oscillates between the pH of 5-7.5. In recent studies 100% activity was retained by bacillus areeniciselenatis DSM 15340 xylanase for 2 hours of incubation at pH 10. Stability at the extreme pH value may be due to charged amino acid residues.

The crude xylanases from the two bacteria increased with temperature until optimum activity is reached at 60oC. As with most enzymes as the temperature was raised the activity of the xylanases decreased. Reports have shown that most microbial xylanases are more active at 50o-60oC (Kang et al., 2004). The changes induced by high temperature during the enzyme production may be attributed to reduced synthesis of proteins essential for growth and other physiological processes (Gawande and Kamat, 1999). The thermostability studies of the enzyme from bacteria revealed that 50% of residual activity was retained after 210 minutes of incubation at the optimum temperature of 60oC. The crude xylanase from P. aeruginosa and S. aureus has half-life (t1/2) of 1.37 hours and 1.5 hours respectively. Corall et al, (2002) reported that xylanase from an Aspergillus niger remained stable up to 100oC, yet lost about 50% of its activity after 15 min at this temperature.

IV. Conclusion

The xylanase from S. aureus is slightly alkaliphilic and both enzymes show considerable thermostability. The result revealed that both bacteria are likely potential candidates for production of relatively cellulase-free xylanase that may be beneficial in biobleaching. Commercial applications of xylanases demand the identification of highly stable enzymes that remain active under routine handling conditions.

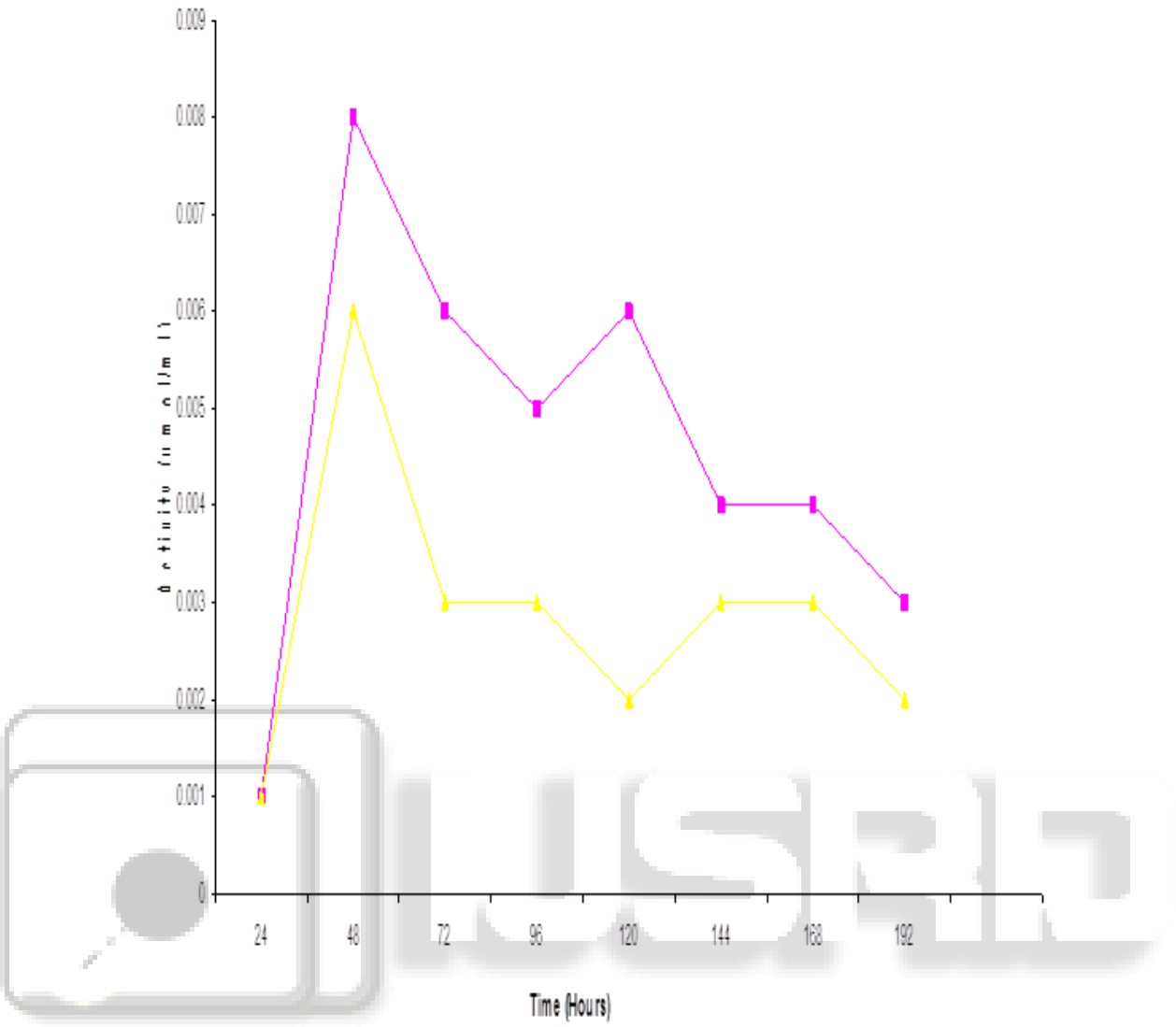

Fig. 1: Time Course For Production of Crude xy Lanse by Pseudomonas Aerugiosa and Staphylococcus Aureus.

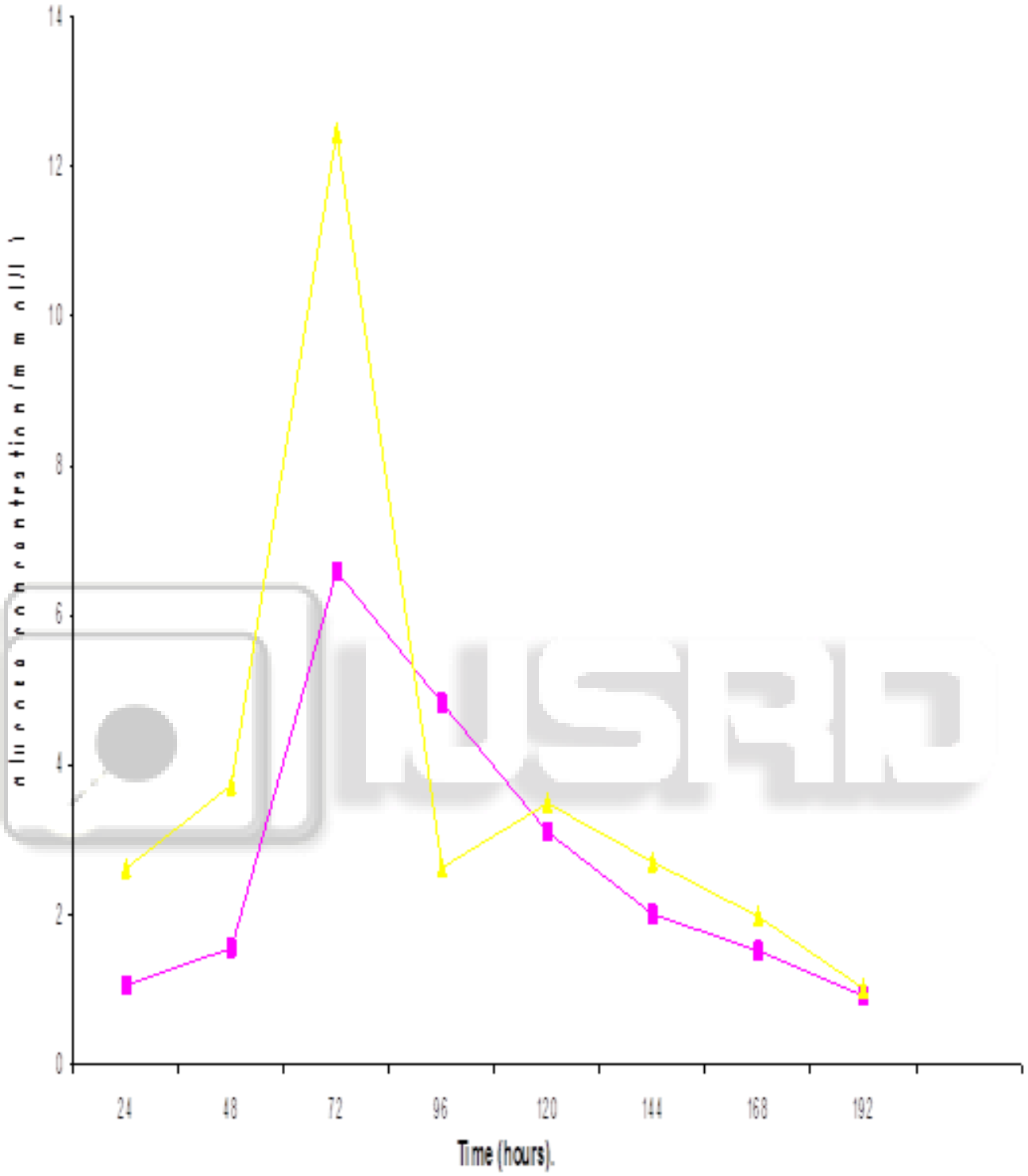

Fig. 2: Time Course for Cellulase Production Aeruginosa and Staphylococcus Aureus.

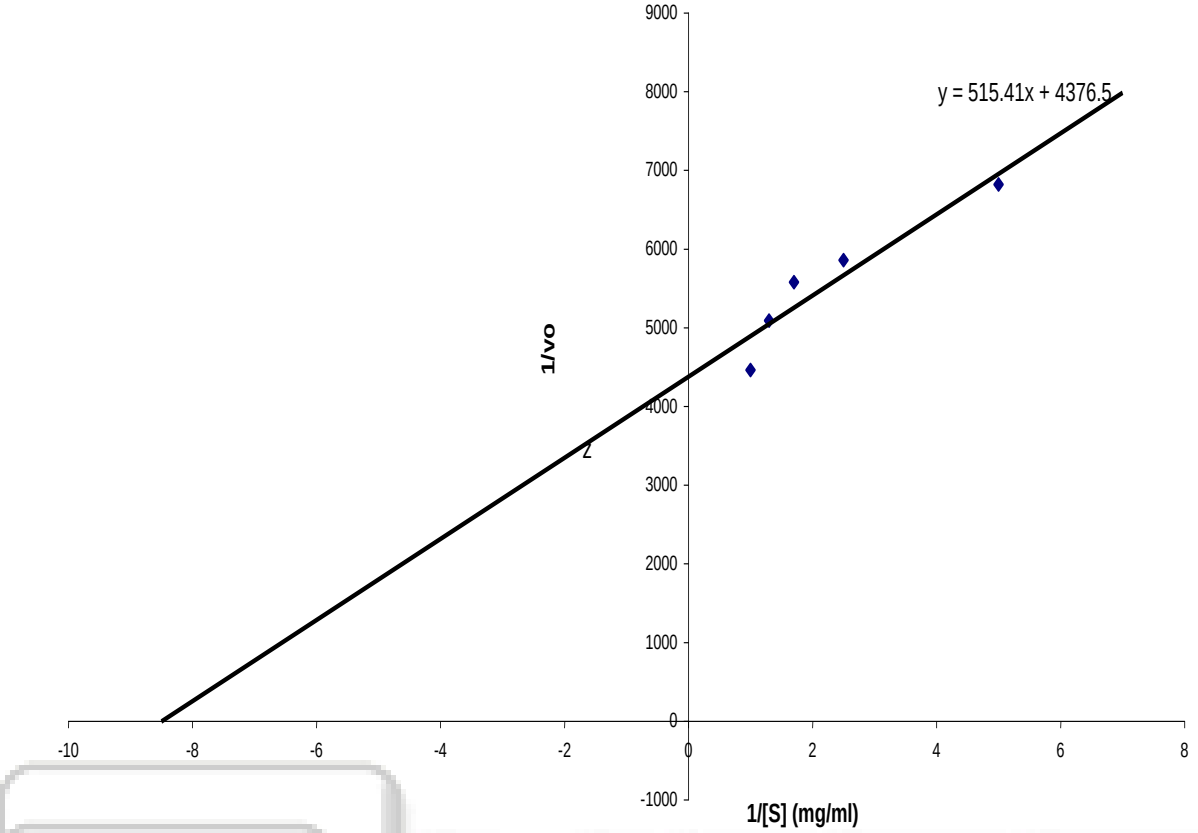

Fig. 3: Detemination of KM and VMax of xy lanase from Pseudomonas Aeruginosa

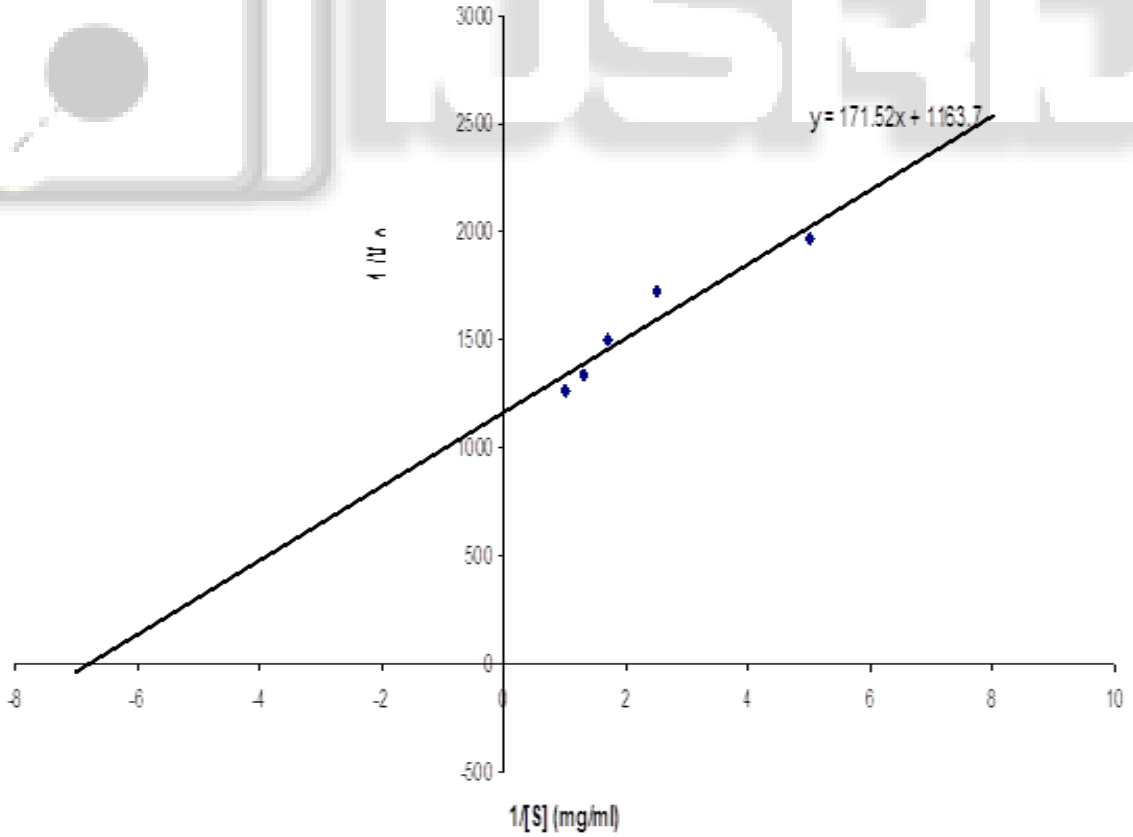

Fig. 4: Determination of km and vmax XY Lananse Produced by Staphylococcus Aureus

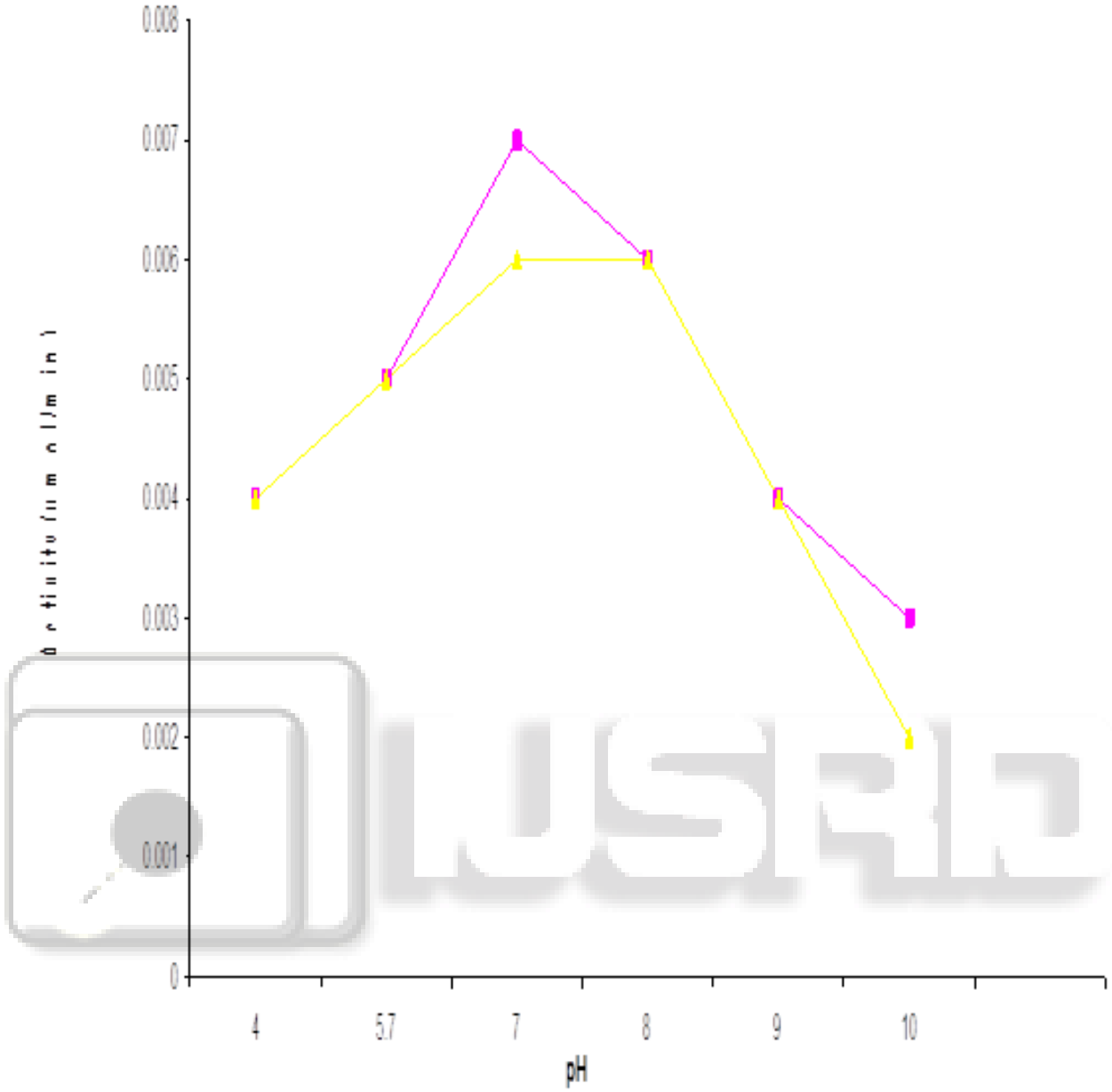

Fig. 5: Effect of ph on xylanase pseudomonas aeruginosa and Staphylococcus aureus

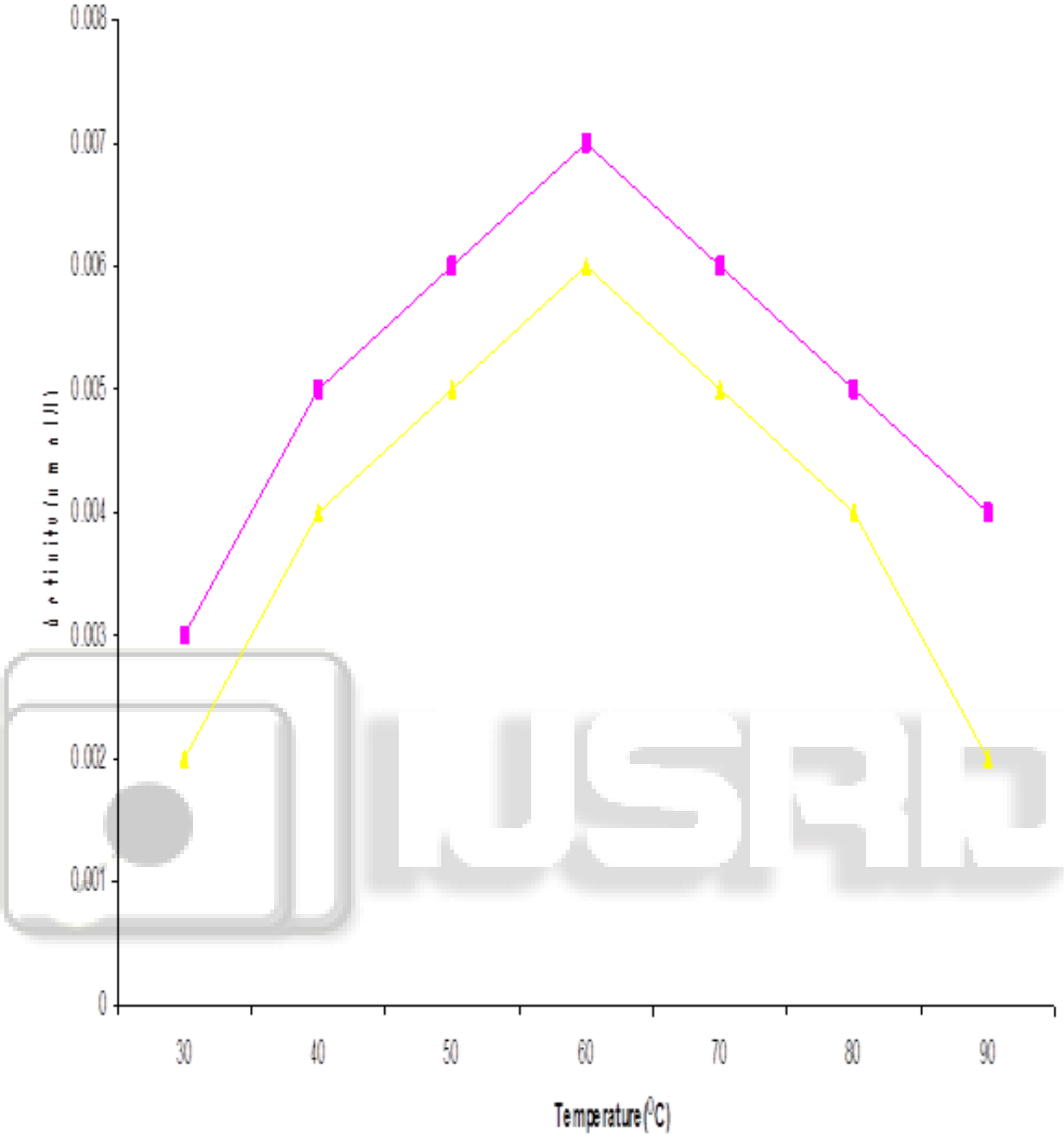

Fig. 6: Effect of temperature on xylanase production pseudomonas aeruginosa and staphylococcus arureus

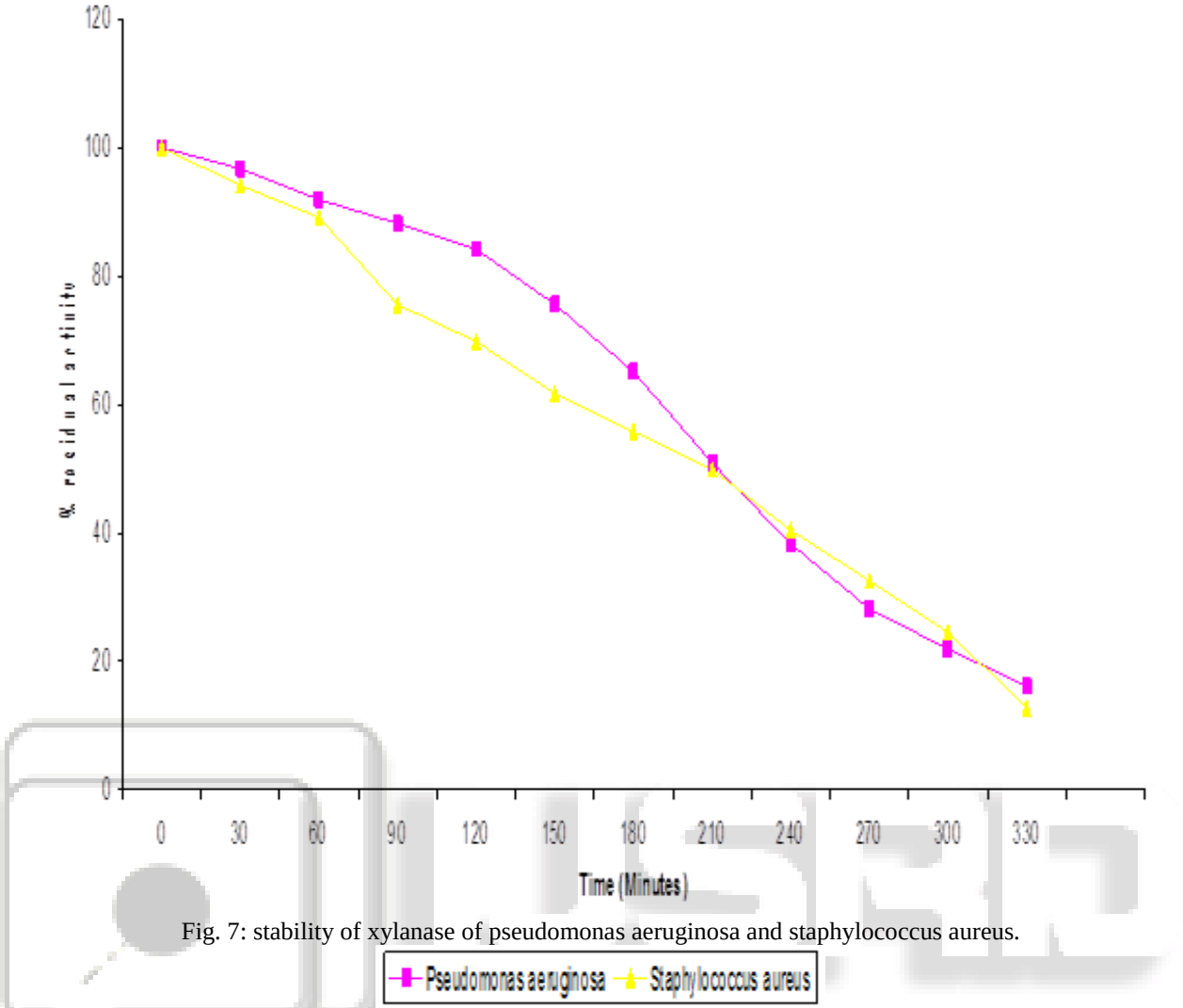

Fig. 7: stability of xylanase of pseudomonas aeruginosa and staphylococcus aureus.

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