SCREENING AND CHARACTERISATION OF XYLANASE PRODUCING AQUATIC *STREPTOMYCES* SP. FROM THE SEDIMENTS OF COCHIN BACKWATER SYSTEM.

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ABSTRACT

Enzymes from aquatic actinomycetes could be an innovative tool in various industrial applications. Rising demand for xylanase in several industrial processes necessitates the bioprospecting and manipulation of microbes for novel and greater enzyme productivity. In the present study, actinomycete isolates from the sediment samples of Cochin backwater system were screened for xylanase production and ER1 isolate showed the highest potential. 16SrRNA gene sequence analysis revealed the relatedness of the isolate to *Streptomyces* sp. Xylanase production was optimal at pH 7, incubation temperature 35◦C, agitation speed 50 rpm, potassium nitrate and peptone (nitrogen sources), Ca^{2+} and Mn^{2+} (metal ions). Under optimum conditions, 3986 U/mL of xylanase activity was attained, which was a 2.12 fold increase from unoptimised medium (1277.33 U/mL). The enzyme was purified by cold acetone precipitation and DEAE-cellulose ion exchange chromatography. Molecular weight was determined to be 23 KDa. The enzyme was stable between pH 7-8 and temperatures 40-70°C. The enzyme showed a Km of 0.018 micromoles and Vmax of 11.72 mmol min^{-1}. The enzymatic activity was increased by hexane, Co^{2+}, polyethylene glycol and inhibited by Hg^{2+} and beta mercaptoethanol. Xylanase with profound activity at high temperatures is very beneficial in paper biobleaching and deinking industrial processes.

***Keywords*:** Xylanase; *Streptomyces*; Optimisation; purification; submerged fermentation; characterisation.

INTRODUCTION

Microorganisms from the aquatic environment, owing to its huge genetic, biochemical diversity and unique characteristics, are a promising source of new enzymes with hyperthermostability, salt tolerance and cold adaptation properties for various industrial applications (Sivaperumal *et al.,* 2017). Accumulation of such structurally unique metabolites by the aquatic microorganisms is due to the special conditions involving high salinity, high pressure, low temperature and special lighting conditions in the aquatic habitat that differ from those found in terrestrial habitats (Zhang and Kim, 2010). Aquatic actinomycetes are microorganisms widely distributed in aquatic habitats like estuary, mangroves etc. (Suryavanshi, 2007). They make up large part of microbial population of aquatic system and emerged as rich source for the production of industrially important enzymes (Radhika *et al.,* 2011).

With the growing demand on enzymes with unique properties, research in novel regions of marine ecosystem for unique natural enzymes became indispensable as known compounds from already overexploited terrestrial ecosystems have been exhausted (Basha and Rao, 2017). Estuaries and mangrove forests are unique ecosystems in semi sheltered areas near the ocean coastline and are the most productive ecosystems ("Estuaries, Salt Marshes, and Mangroves - MarineBio.org", 2017).

Cochin backwater system is the longest estuarine system on the southwest coast of India, which forms more or less a northward extension of Vembanadu Lake. This positive tropical estuary is well connected to the rivers and lagoons on one side and to the Arabian Sea on the other (Varma *et al.,* 1981). Mangrove ecosystems are found in isolated patches along the banks of Cochin estuary. They cover an area of 2.6 km^2 (Narayanan, 2006). The microbial source of the Cochin estuary is still untapped and not exploited for production of industrially important enzymes and other bioactive secondary metabolites.

Amongst the various enzymes produced by microorganisms, xylanases occupy an important platform, as they are extensively used in biotechnological applications mainly in paper and pulp industries, food, animal feed as well as for bioconversion of lignocellulosic waste into value-added products covering all the sectors of industrial enzymes market. Xylanases are glycosidases which catalyse the endo hydrolysis of 1, 4-β-D-xylosidic linkages in xylan (Collins *et al.,* 2005). Most of the currently applied xylanase in various industries such as Cartazyme, Panzea, and Nutri Xylanase are harvested from terrestrial microorganisms such as *Thermomonospora fusca*, *Bacillus licheniformis* and *Bacillus subtilis* respectively.

To the best of our knowledge, very few studies have been done on xylanase from aquatic actinomycetes. In the present study, estuarine actinomycetes from the sediment samples of Cochin backwater system, were screened for

xylanase production and identified using molecular methods. Effect of various physical and chemical parameters on the growth and xylanase production by *Streptomyces* sp. ER1, which was to be a prospective strain, was also analyzed. Purification and characterisation of the enzyme produced by the prospective strain was also studied in detail.

Materials and Methods:

Actinomycete isolates and screening:

Actinomycetes were isolated (90 No.s) from the sediment samples of Cochin estaury, as described by Rosmine and Saramma, 2016. Xylanolytic bacteria were qualitatively identified with the help of Congo red dye (Teather and Wood, 1982). The isolates were grown on Nutrient agar medium supplemented with xylan (0.25%) and incubated at room temperature (28 ± 2°C) for 5 days. After incubation culture plates were stained with 0.1% Congo red solution for 15 min and decoloured with 1M NaCl for 10 min. The plates were examined for the appearance of yellow zone of hydrolysis around the colonies. Among the isolates that are capable of producing xylanase, one potent strain was selected which gave the maximum zone of hydrolysis.

Characterisation of the selected isolate:

Biochemical characterisation:

The isolate was identified up to the generic level based on morphological, cultural, physiological and biochemical tests as per the methods of the International *Streptomyces* Project (ISP) (Shirling and Gottlieb, 1966).

Isolation of genomic DNA, 16S rDNA amplification and phylogenetic analysis:

Genomic DNA of the isolate was extracted as per the standard protocol (Sambrook *et al.,* 1989). 16S rDNA gene was amplified using universal primers (27F: 5'-AGA GTT TGATCCTGG CTC AG-3, 149R: 5'-GGT TAC CTT GTTACG ACTT-3'). The PCR reaction conditions included an initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 40 s, annealing at 55°C for 40 s, and extension at 72°C for 1 min 30 s. A final extension at 72°C for 10 min was also included. The PCR product was sent for sequencing at Scigenom, Kochi. The sequence obtained was submitted to the basic local alignment search tool (BLAST; http://www.ncbi.nlm.nih.gov/BLAST) at NCBI (National Center for Biotechnology Information) to determine the percentage similarity with already identified 16S rRNA sequences in the GenBank database. The strain ER1 was used for xylanase production in this study. Multiple alignments of the sequences were also carried out using Clustal W software and the phylogenetic tree was constructed by neighbor-joining method using Mega 6 software.

Inoculum

The inoculum was raised in the xylanase production medium A. The culture was grown in Erlenmeyer flask containing 100 mL of the xylanase production medium on an incubator shaker (100 rpm) at ambient room temperatures (30 ± 2°C). Unless otherwise specified, the inoculum of age 24 h was used at 5% v/v ($452X10^1$ CFU/mL).

Production of xylanase

Production of xylanase was studied in correlation with the growth profile of the culture under SmF. The incubation was carried out for 120 h at ambient room temperature (30 ± 2°C). After the

incubation period, cells were separated by centrifugation (10000 rpm, 15 min, 4°C) and the supernatant served as the extracellular crude xylanase.

Biomass estimation

10 mL of fermented production medium A broth was collected in a pre-weighed centrifuge tube and centrifuged at 8000 rpm for 15 min. Supernatant was discarded and the pellet was washed thrice with sterile distilled water, followed by drying the pellet at 95°C, till attaining constant weight. The biomass was expressed in dry cell weight (mg/mL) (Khusro *et al.,* 2016).

Xylanase assay

Xylanase activity was determined using beechwood xylan (Sigma, Germany) (Bailey, 1992). A 0.2 mL culture supernatant was added to 2 mL xylan solution (1%; pH 7.0; 100 mm sodium phoshate buffer) and incubated at 55°C. After 30 min, 3 mL 3,5-dinitrosalicylic acid reagent was added to stop the reaction, and the amount of reducing sugars released in the reaction was estimated by measuring the absorbance at 540 nm (Miller, 1959). A control was run simultaneously which contained all the reagents but the reaction was terminated prior to the addition of enzyme extract. One unit of xylanase activity was defined as the amount of enzyme catalysing the release of 1 µmol of reducing sugar equivalent to xylose per minute under the specified assay conditions. All the experiments were carried out independently in triplicate and the results presented are mean of the three values.

Selection of production medium for xylanase production:

11 different media, A (Techapun *et al.,* 2001), B (M9 medium) (Roy, 2004), C (Mandel's and Sternburg's medium (Mandel and Sternburg, 1976), D (Ragunathan and Dhas, 2013), E (Kim *et al.,*1985), F (Suneetha *et al.,* 2011), G (Liu *et al.,* 2013), H (M medium) (Obi and Odibo, 1984), I (Ramakrishnan and Narayanan, 2013), J (Tryticase soy broth) (Liu *et al.,* 2013), K (Nutrientt broth with xylan) (Hiremath and Patil, 2011) were used for comparative studies to find the basal nutrient medium for further formulation of optimal medium.

Culture medium used for optimisation studies

The organism was grown in the selected xylanase production medium A (Techapun *et. al.,* 2001) (Table 1).

Optimisation of nutritional and physical parameters by One Factor at a Time (OFAT) method

In the conventional OFAT method, various nutritional and physical parameters were optimised by maintaining all parameters at a constant level in the production medium A, except the one under study. Each subsequent parameter was examined after taking into account the previously optimised parameter(s). In this study, the biomass of *Streptomyces* sp. ER1 was correlated with its xylanase production for each parameter optimised. Various cultural conditions like incubation period (1–14days), initial medium pH (3-11), incubation temperature (20-40°C (20, 25, 30, 35, and 40)), inoculum age (6, 24 and 30 h) and size (1%, 3%, 5%, 7%, 9% and 11%), agitation (50, 100, 200 rpm), salinity

(0, 5, 10, 15, 20 ppt), and various nutritional conditions such as additional carbon sources (xylose, glucose, sucrose, cellulose, xylan, starch and glycerol), surfactants (Tween 60, Tween 80, SDS and Triton X-100) and other additives (olive oil, EDTA, polyethylene glycol and β-mercaptoethanol), metal ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+}, Fe^{2+}, Cd^{2+} and Hg^{2+} in chloride and phosphate salts), nitrogen sources (tryptone, beef extract, yeast extract, peptone, albumin, gelatin, casein, soya bean meal, phenyl alanine, urea ammonium nitrate, ammonium chloride, di ammonium phosphate, sodium nitrate, ammonium sulphate and potassium nitrate) and substrate (xylan) concentration (0.1-0.5%) were optimized for enhanced production of xylanase by *isolate* ER1 in submerged fermentation process.

Statistical Analysis

All experiments were carried out in triplicates and the results are presented as the mean of three independent observations. Standard deviation for each experimental result was calculated using Microsoft Excel 2003and statistically evaluated using ANOVA at a significance level of $p < 0.05$ by using computer based program SPSS (Version 17.0, Chicago, SPSS Inc.).

Estimation of protein

Estimation of total extracellular protein was performed through Bradford test (Bradford, 1976). Bovine serum albumin (BSA) was used as a standard. Protein content per millilitre of test samples was determined against the standard curve. During the chromatographic steps, proteins were detected by reading absorbance at 280 nm.

Partial purification and characterisation of xylanase:

Partial purification of xylanase was done by cold acetone precipitation and DEAE cellulose ion exchange chromatography. The purity and zymography was tested by SDS and NATIVE PAGE as described by Rosmine *et al.,* 2017.

Characterisation of enzyme

Effect of temperature and pH on enzyme activity and stability

The optimum temperature for enzyme activity was assessed by carrying out the xylanase assay at different temperatures from 20 to 100°C. The optimum pH for xylanase activity was determined in a pH range of 4 to 11 with 1 pH unit increment and expressed in terms of relative activity. The pH stability of the enzyme at the optimum pH and thermal stability was tested by pre-incubating the enzyme in the respective buffer and at optimum temperature, separately, for 6 h and the relative activity (%) was determined in every 1 h.

Effect of metal ions, surfactants, organic solvents and NaCl on enzyme activity

Different metal ions (Zn^{2+}, Co^{2+}, Fe^{2+}, Mn^{2+}, Cu^{2+}, Mg^{2+}, K^+, Ca^{2+}, Mg^{2+}) in their salt form were prepared at a final concentration of 5mM and incubated with the purified enzyme for 30 min at 60°C. The percentage residual activity was calculated by comparing the activity of the treated enzyme with that of the untreated enzyme (in the absence of metal ions) which served as a control and the value was taken as 100%. The

purified enzyme was pre-incubated with the surfactants such as Tween 60, 80, Polyethylene glycol, olive oil, Triton X 100, β- mercaptoethanol and SDS at a concentration of 0.1% for 30 min at 60°C.

The stability of xylanase in different organic solvents such as butanol, chloroform, ethyl acetate, and hexane was checked by pre-incubating the purified enzyme in the solvents for 30 min at 60°C. The purified enzyme was pre-incubated with NaCl in the concentration of 10, 15 and 20% and the mixture was incubated for 30 min at 60°C. After the incubation period, for each factor, activity was calculated in terms of residual activity.

Substrate specificity of xylanase and determination of kinetic constant

The substrate specificity of the purified enzyme was determined by using various substrates in the reaction mixture for xylanase assay. The various substrates used were 1% (w/v) of cellulose, starch, glycerol and xylan. The difference in activity was calculated as percentage relative activity. Km and Vmax values of the xylanase were determined by measuring enzyme activity at various concentrations of xylan (2-6 mg/mL) and were calculated using Lineweaver-Burk equation method.

RESULTS AND DISCUSSION

Selection of xylanase-producer actinomycetes

In all, 10 strains were detected to produce xylanase, as displaying orange-colored xylan digestion halos on xylan agar plates with halo size equal to 7–45 mm in diameter. From this group, one isolate (ER 1) exhibited a zone of clearance of a 45 mm and was selected for optimisation studies since it showed the highest xylanase production.

Characterization of ER 1 isolate and phylogenetic analysis

The actinomycete isolate showed excellent growth and abundant aerial mycelial formation on ISP medium No. 3 (oat meal agar), ISP medium No. 4 (inorganic salts-starch agar) and ISP medium No. 5 (glycerol asparagine agar base) and moderate growth on ISP medium No. 2 (yeast extract-malt extract agar). The aerial mycelium of ER1 is retinaculum type, grey coloured with sparse substrate mycelium. The aerial and substrate mycelium were media dependent. The biochemical characteristics of strain ER1 are presented in Table 1. The BLAST search of 16S rRNA gene sequence against sequences in nucleotide database has shown 99% homology with *Streptomyces* sp. **SJ-UOM-37-09** 16S rRNA gene sequence. The accession number obtained for the identified isolate was deposited under the number KY449279 in GenBank. The phylogenetic tree was inferred from 16S rRNA sequence data by the neighbor-joining method (Figure 1). The tested isolate (ER1) was identified as *Streptomyces* sp.**KY449279**.

Optimisation of fermentation conditions for xylanase production

The data obtained (Supplementary figure 1) clearly showed that the xylanase production was initiated after 24 h of incubation and reached the peak at 120 h. Presence of xylanase activity in the early hours of growth was presumed to be due to the xylanases present in

considerable amounts in the inoculum causing the hydrolysis of xylan in the medium. After 212 hours of growth there was a gradual decline in xylanase activity suggesting the possible action of intracellular proteases from the autolysed cells or the depletion of nutrients in the medium or due to denaturation or decomposition of xylanase by interaction with other components in the medium (Kamble and Jadhav, 2012).

Optimal production of xylanase and biomass was detected at 35°C (Supplementary figure 2). A rise in growth temperature to 40°C lowered xylanase production by 23.68% when compared to that at optimal temperature. *Streptomyces* sp. ER1 could grow at temperatures near 40°C and it indicated that the organism could be classified under thermo-tolerant bacteria (Dubeau *et al.,* 1987). The culture showed maximum xylanase production and growth at pH 7.0 (Supplementary figure 3). Higher production of xylanase was noticed when the pH of the medium was in the range of 7-9, suggesting *Streptomyces* sp. ER1 to be alkali tolerant. Similar findings were reported earlier (Bhosale *et al.,* 2011). Further, there was no growth and xylanase production at pH 3 and 11 as cultivation of the organism at an unfavourable pH may limit the growth, xylanase production and substrate accessibility (Bajpai, 1997).

The highest xylanase production was recorded when 24 h old inoculum was used. This could be due to the logarithmic phase of *Streptomyces* sp. ER1, used as inoculums (Supplementary figure 4). Higher enzyme production at inoculum size of 5% was related to the rapid growth of *Streptomyces* sp. ER1, which resulted in higher degradation of substrates and increased availability of nutrients (Kavya and Padmavathi, 2009) (Supplementary figure 5 and Supplementary table 1). Higher inoculum density might produce too much biomass, which would deplete the nutrients necessary for product formation (Sangeetha *et al*., 2004). This might be the reason for lesser xylanase production at inoculum concentration above 5% even though the biomass production increased from 1 % to 11%.

In the present study, low agitation speed (50 rpm) seemed favourable for xylanase production. There was a slight reduction in the xylanase production at 150 rpm (Supplementary figure 6). This was probably because of the deleterious effect of high shear stress on the actinomycete, which caused cell lyses and release of proteases that could inhibit xylanase production (Fang *et al.,* 2007). Maximum xylanase production and biomass was obtained with the production medium of 15 ppt showing the halophillic character of *Streptomyces* sp. ER1 (Supplementary figure 7). However, xylanase production decreased at 20 ppt, compared to that at 15 ppt. This might be due to the osmotic stress caused by NaCl (Kaur *et al.,* 2015).

Results indicated that xylan was the optimum carbon source inducing the highest level of xylanase production (Supplementary figure 8). Similar results were obtained in earlier studies (Nour El Dhein, 2014). In the present study, addition of olive oil and polyethylene

glycol, Tween 60 and Tween 80 individually to the basal medium stimulated the xylanase production (Supplementary figure 9). These results were in line with earlier report (El-Gendy and El-Bondkly, 2014). Such compounds presumably increased the permeability of the cell membrane and cause rapid secretion of the xylanase and also provided superior permeability of oxygen and extracellular enzyme transport through the cell membranes of microorganism (El-Batal *et al.,* 2015). The addition of surfactants like SDS, Triton X-100 and EDTA completely inhibited the growth of *Streptomyces* sp. ER1, by solubilising the cell membrane or might be ascribed to the change in the nature of the substrate by decreasing the availability of reaction sites and hence no xylanase production was observed (Kapoor *et al.,* 2008).

Among metal ions, Mn^{2+}, Fe^{2+}, Co^{2+} and Zn^{2+} enhanced the xylanase production compared to that of control. However, Cu^{2+} and Hg^{2+} inhibited xylanase production by *Streptomyces* sp. ER1 (Supplementary figure 10). Similar results were obtained earlier *(*Gaur *et al.,* 2015). Potassium nitrate was found to be the optimum inorganic nitrogen source for xylanase production. Among organic nitrogen sources, peptone was found to be the optimum which could be due to the presence of enormous amount of amino acids, peptides, vitamins, trace elements and mineral salts (Pham *et al.,* 1998) (Supplementary figures 11 and 12).

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0.4% xylan in the production medium A showed -the highest xylanase production followed by 0.3% (Supplementary figure 13). Higher concentration of xylan (0.5%) gradually declined xylanase production, which may be due to the increased viscosity of fermentation medium, that eventually caused negative effect on the uniform circulation of nutrient and oxygen and reduction of microbial growth (Karim *et al.,* 2014).

Effect of temperature and pH on xylanase activity and stability from *Streptomyces* sp. ER1

The optimum temperature for xylanase activity was 60°C (Figure 2). Many investigators reported optimum reaction temperature of 55 to 75°C for xylanase activity (Goswami and Pathak, 2013). The optimum pH for xylanase activity was found to be 7.0 (Figure 3). Xylanase was quite steady in a wide range of pH from 5 to 9. The decline in enzyme activity above 9.0 and below 5.0 may be due to alteration of substrate binding and catalysis, which is often affected by charge distribution on both the substrate and particularly enzyme molecules (Shah and Madamwar, 2005). Characterisation of several actinomycete xylanases showed the neutral nature of xylanase (ElGendy and ElBondkly, 2014).

Recent research in the area of xylanase application, especially in the paper industry, has been focused on working at high temperature (approximately 60°C) as it improves the degree of delignification and the brightness of pulp (Bajpai, 1997). In the present study, the relative xylanase activity at pH 8 was 98.69 % with that at pH 7 and the enzyme retained 84.82% of xylanase activity after 6 h of incubation at 60°C (Figures 4 and 5). Thus, this study reveals that the

enzyme is robust and maintains its properties for long duration for their applicability, especially in paper and pulp bleaching.

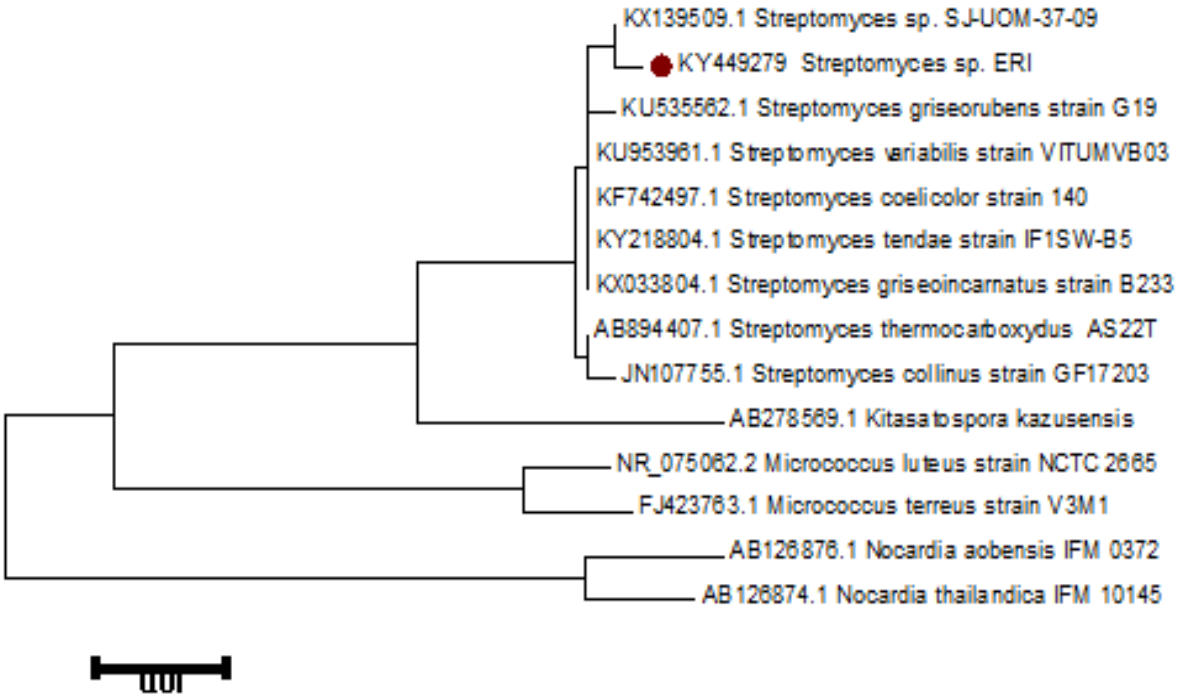


Figure 1. The neighbor-joining tree based on 16S rRNA gene sequences

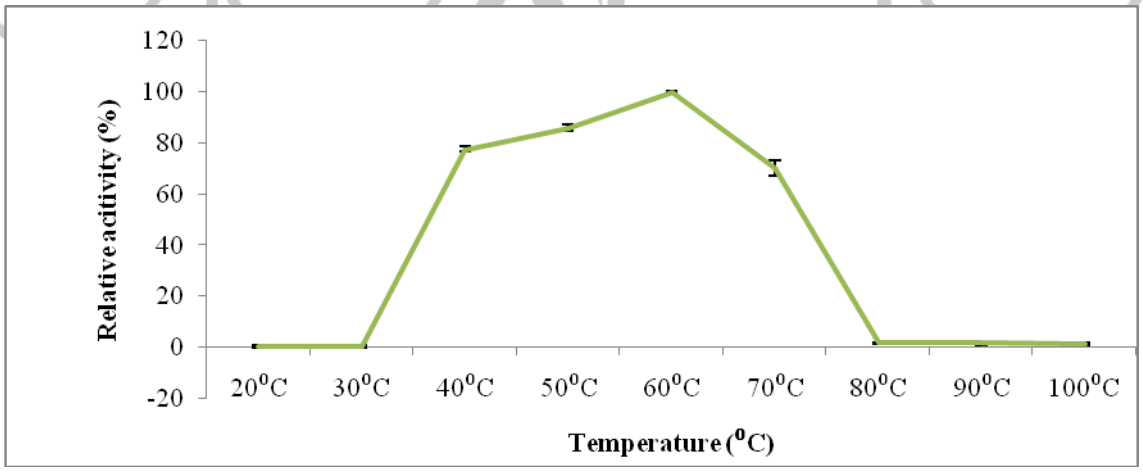


Figure 2: **Effect of temperature on xylanase activity**

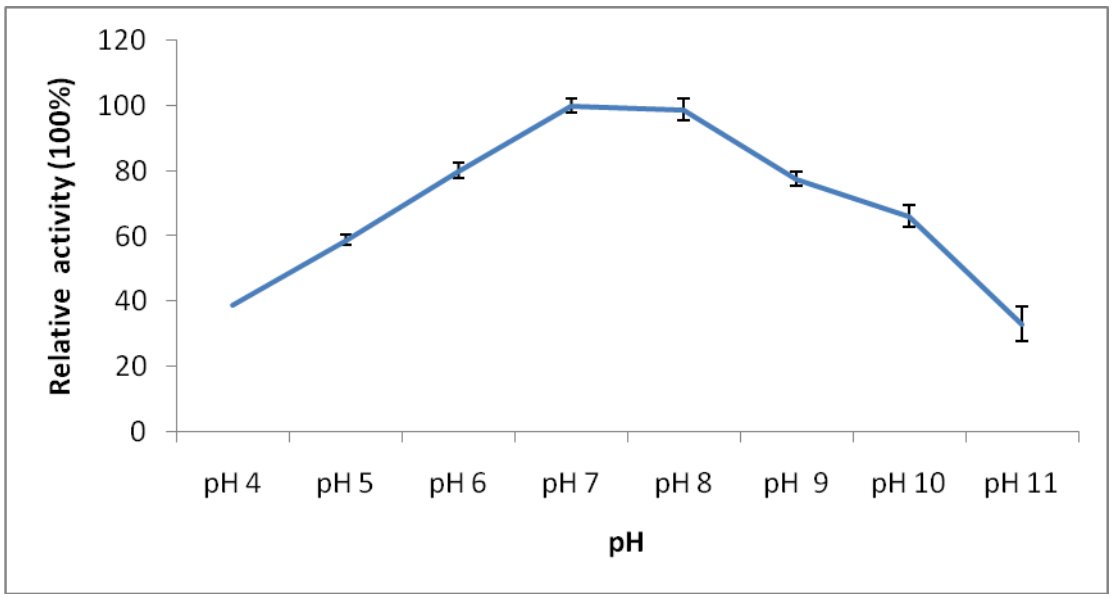


Figure 3: **Effect of pH on xylanase activity**

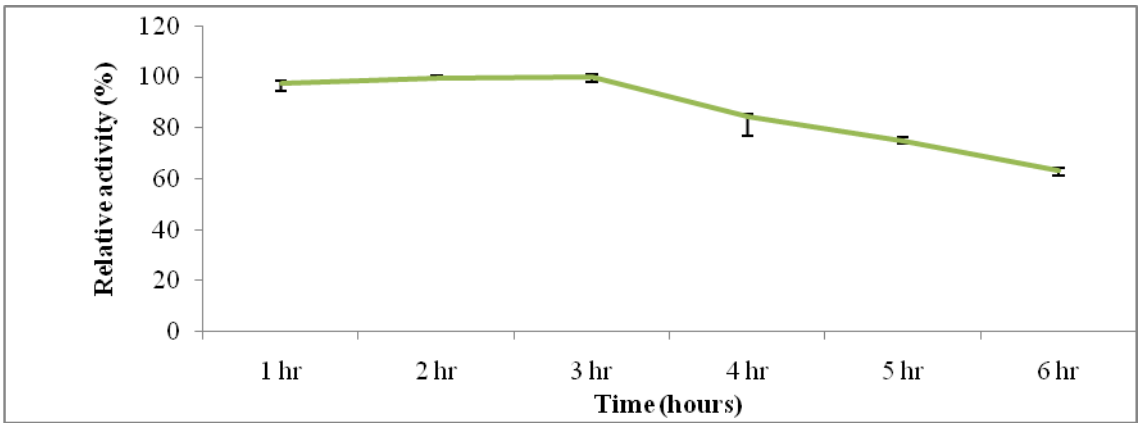


Figure 4: Effect of pH on stability of the xylanase

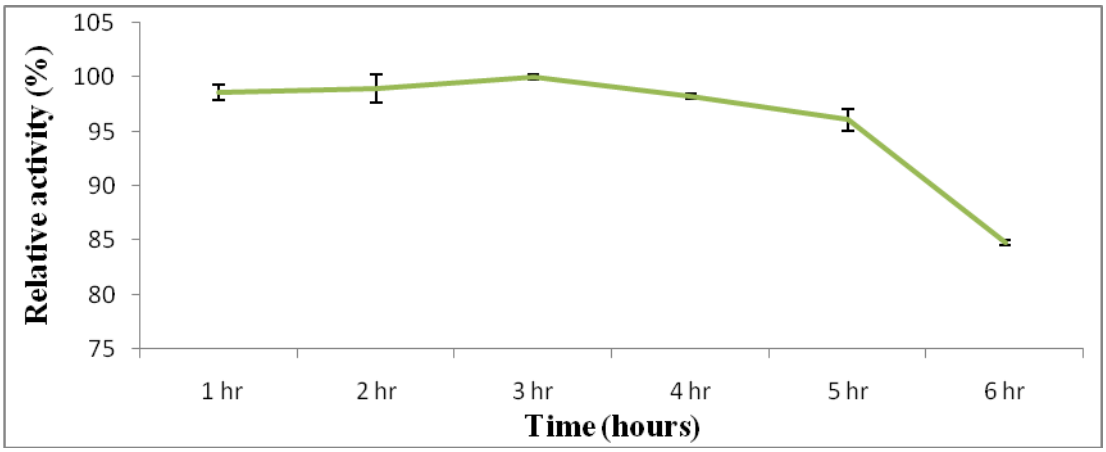


Figure 5: Thermo stability of xylanase

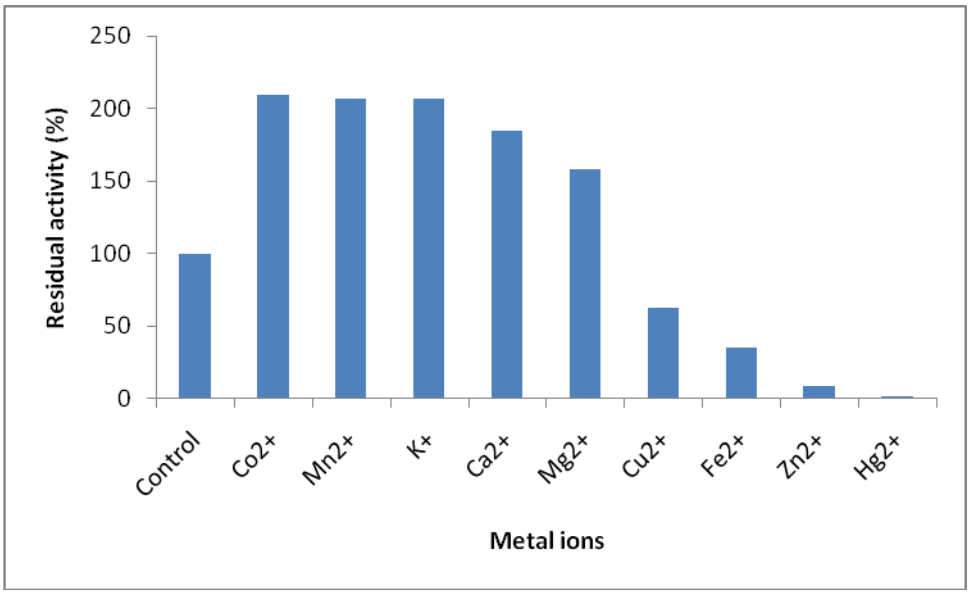


Figure 6: Effect of metal ions on xylanase activity

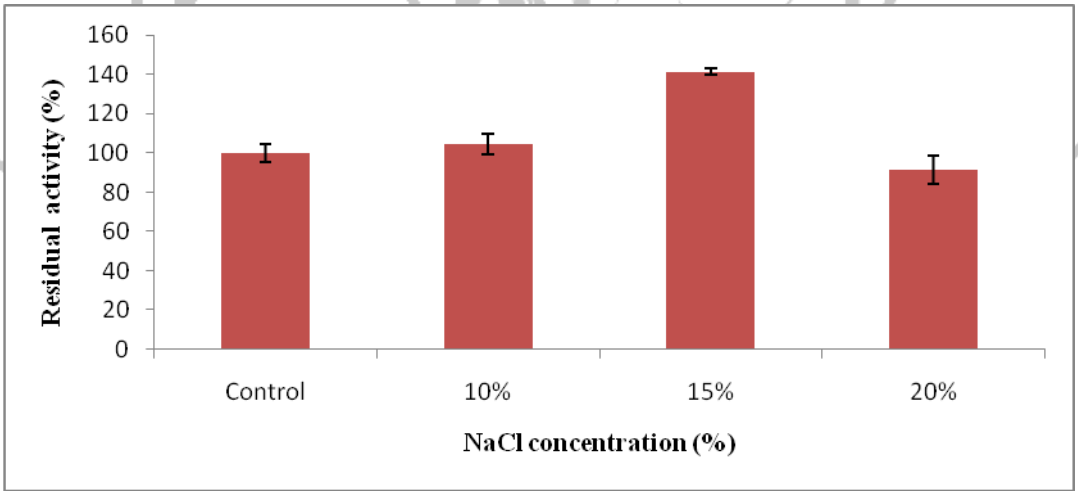


Figure 7: Effect of NaCl on xylanase activity

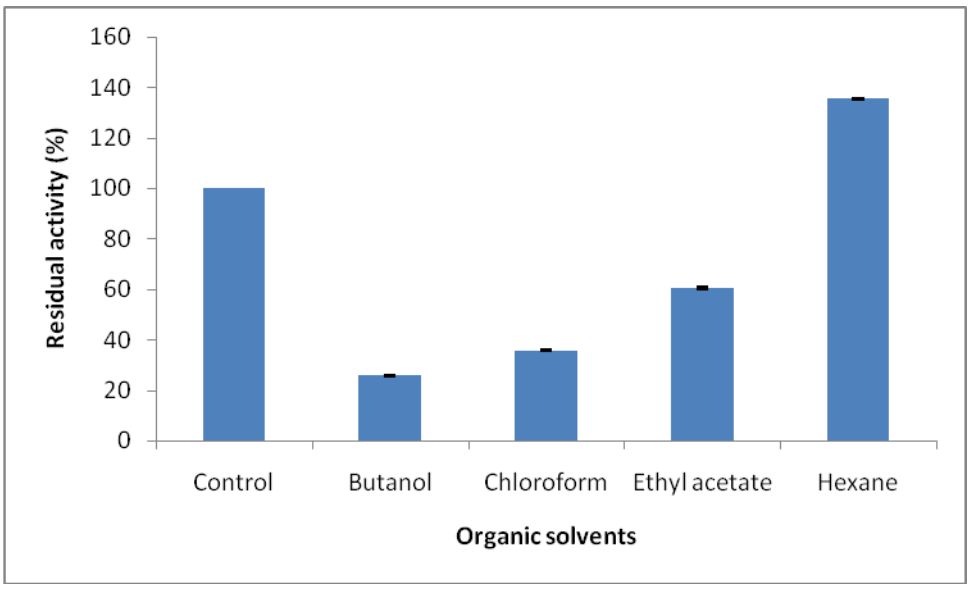


Figure 8: Effect of organic solvents on xylanase activity

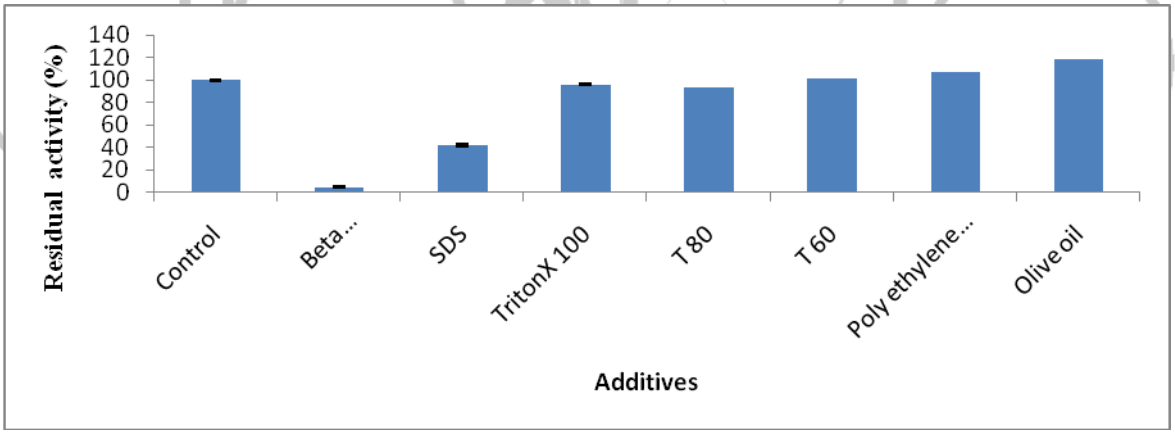
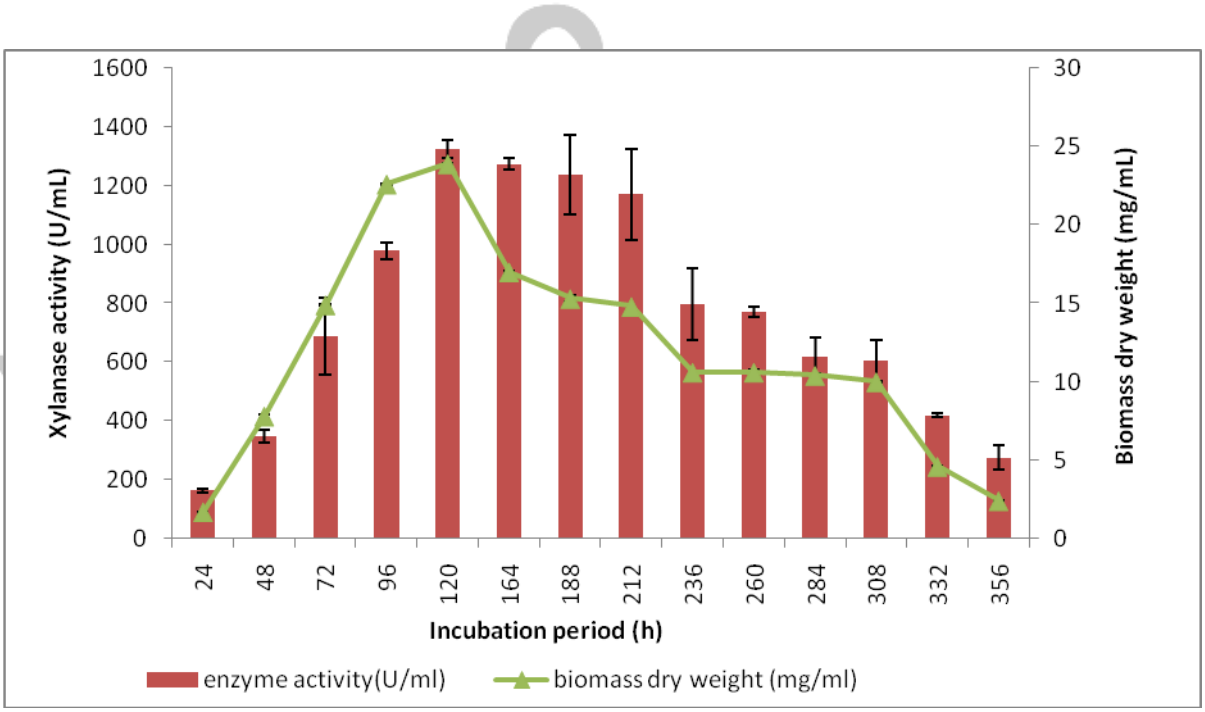
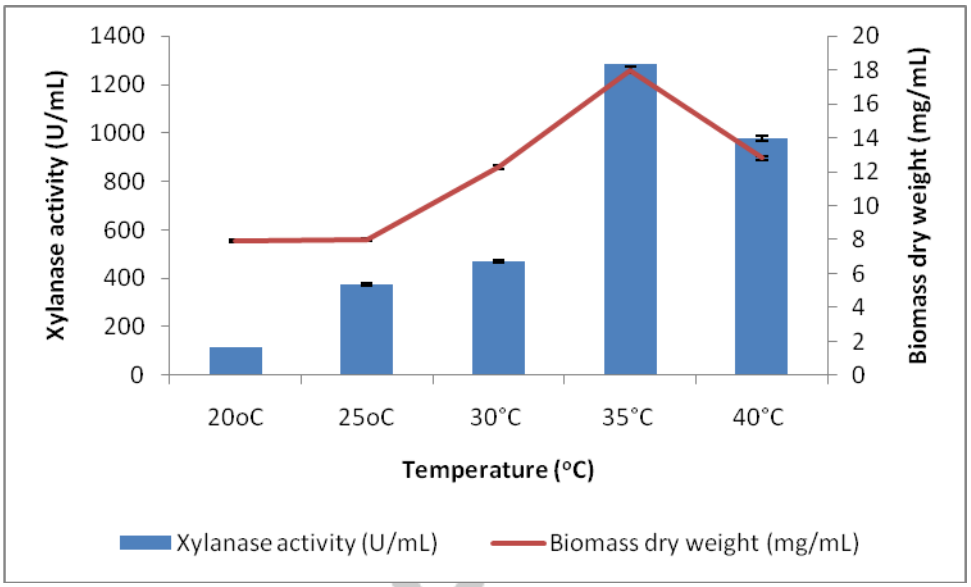


Figure 9: Effect of surfactants on xylanase activity

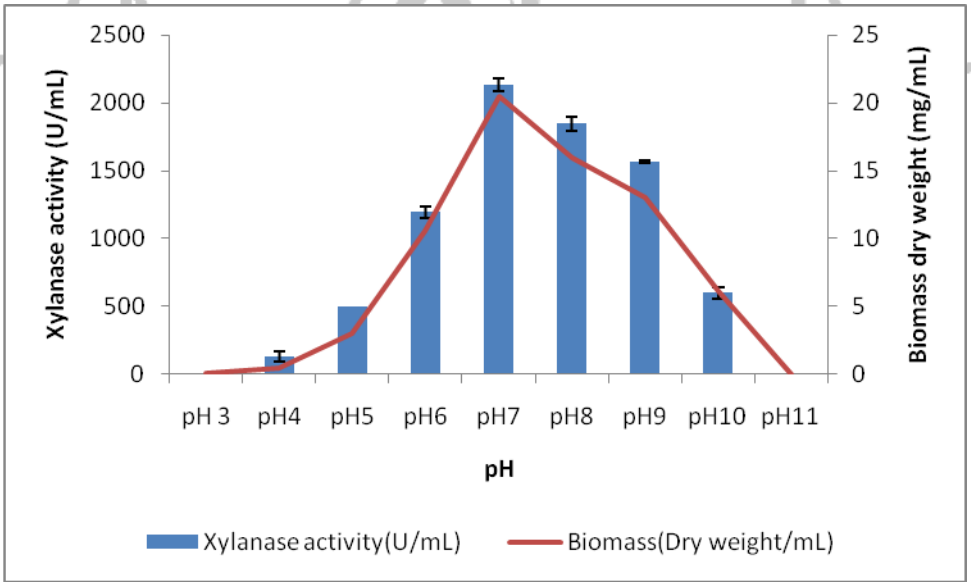
Figure 10: Substrate specificity of xylanase



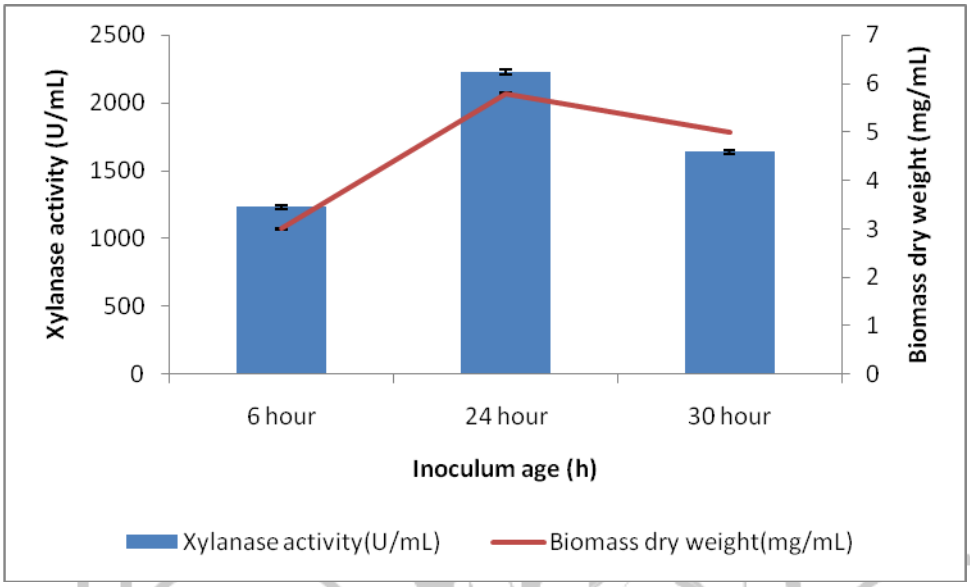
Supplementary Figure 1 : Effect of incubation period on biomass and xylanase production



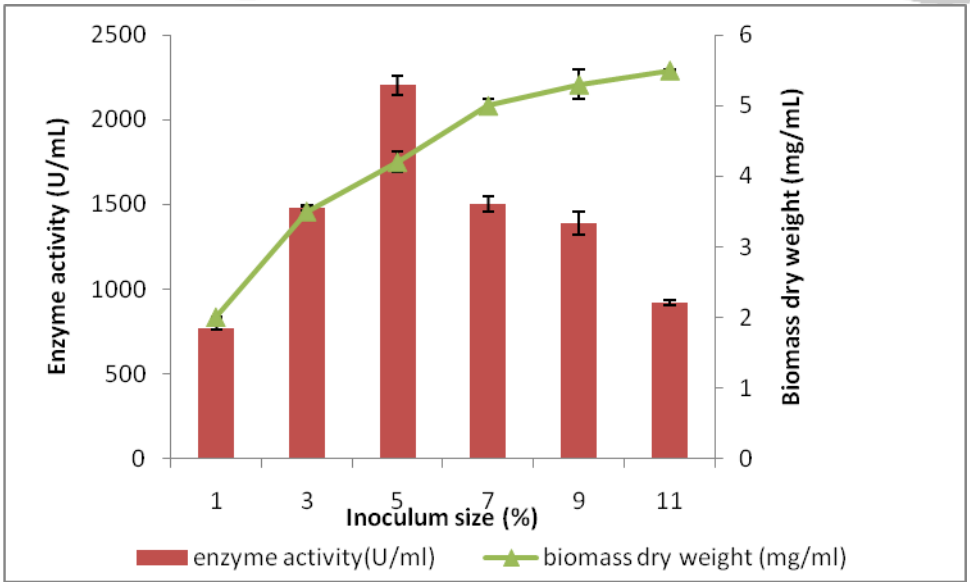
Supplementary figure 2: **Effect of temperature (^{o}C) on biomass and xylanase production**



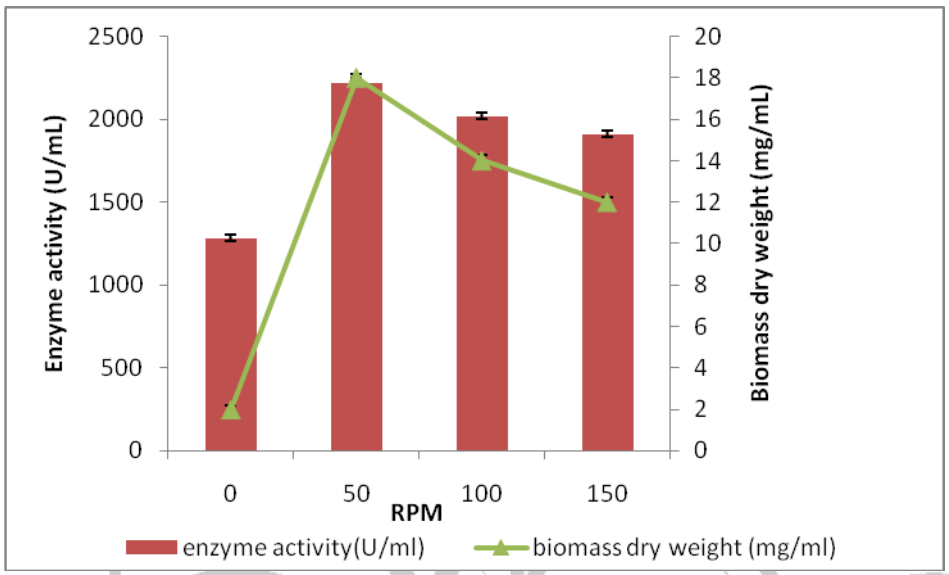
Supplementary figure 3: Effect of pH on biomass and xylanase production



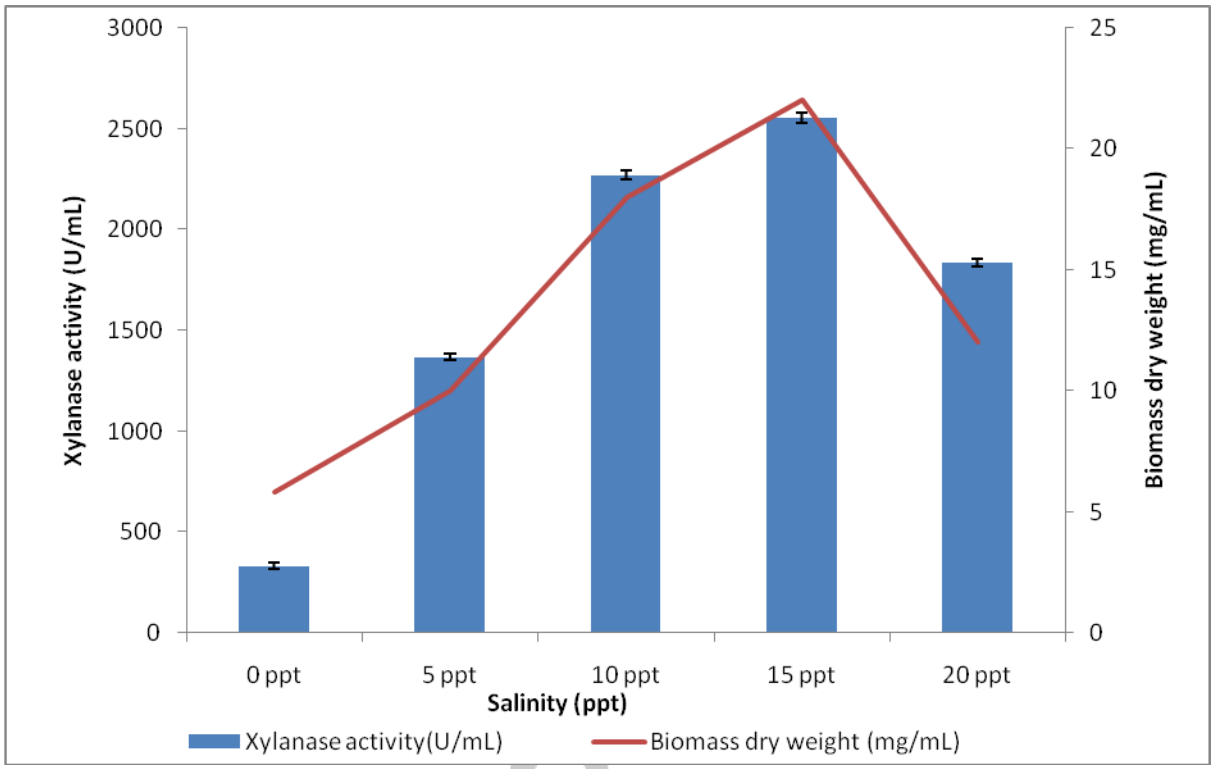
Supplementary figure 4: **Effect of age of inoculum on biomass and xylanase production**



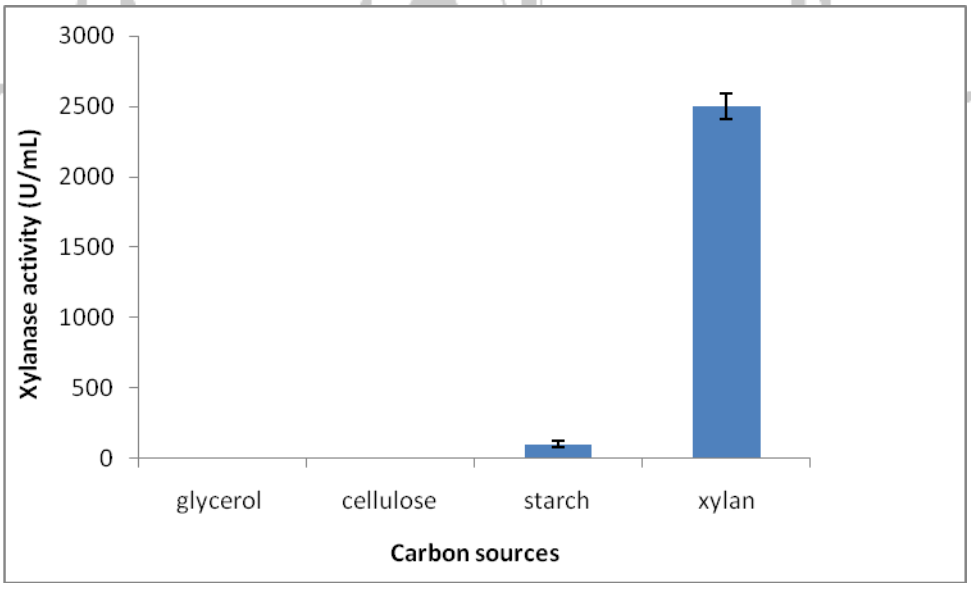
Supplementary figure 5: **Effect of inoculum concentration (%) on xylanase activity and biomass of the culture**



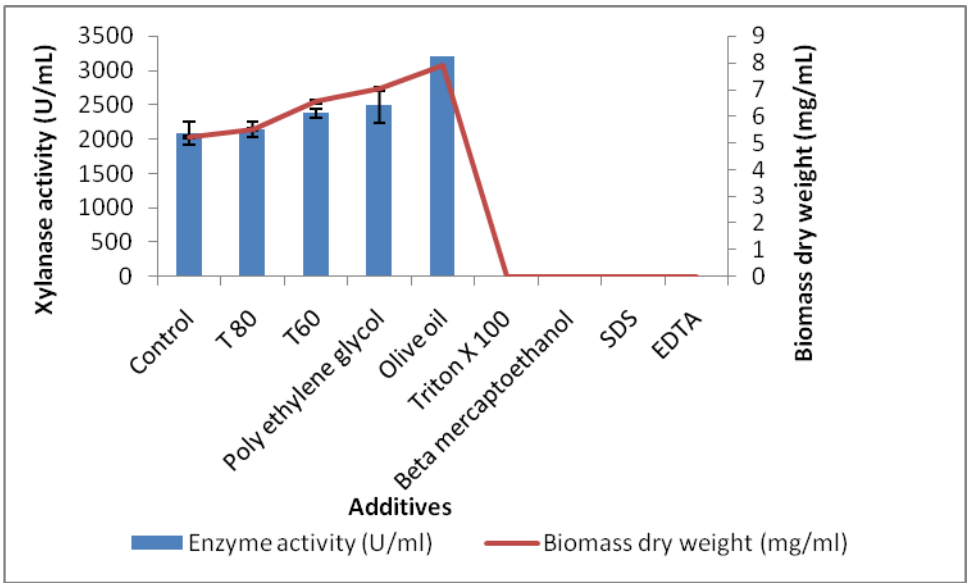
Supplementary figure 6: **Effect of agitation on biomass and xylanase production**



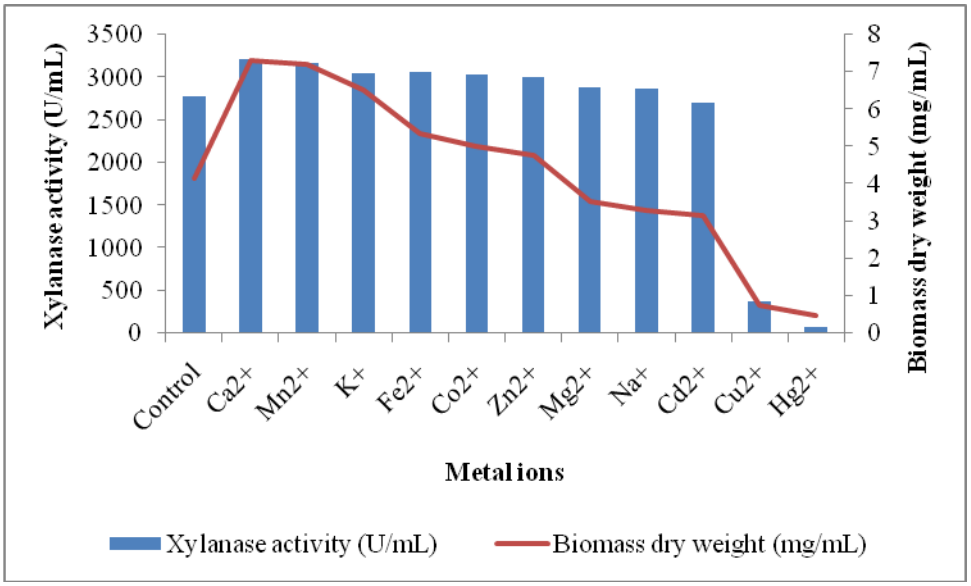
Supplementary figure 7: **Effect of salinity (ppt) on biomass and xylanase production**



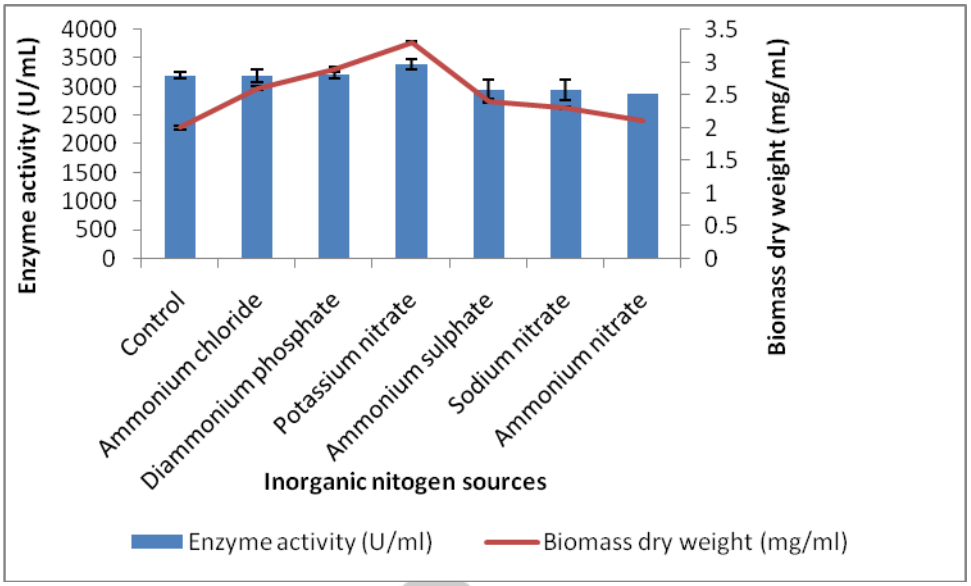
Supplementary figure 8: **Effect of different carbon sources on xylanase production by *Streptomyces* sp. ER1**



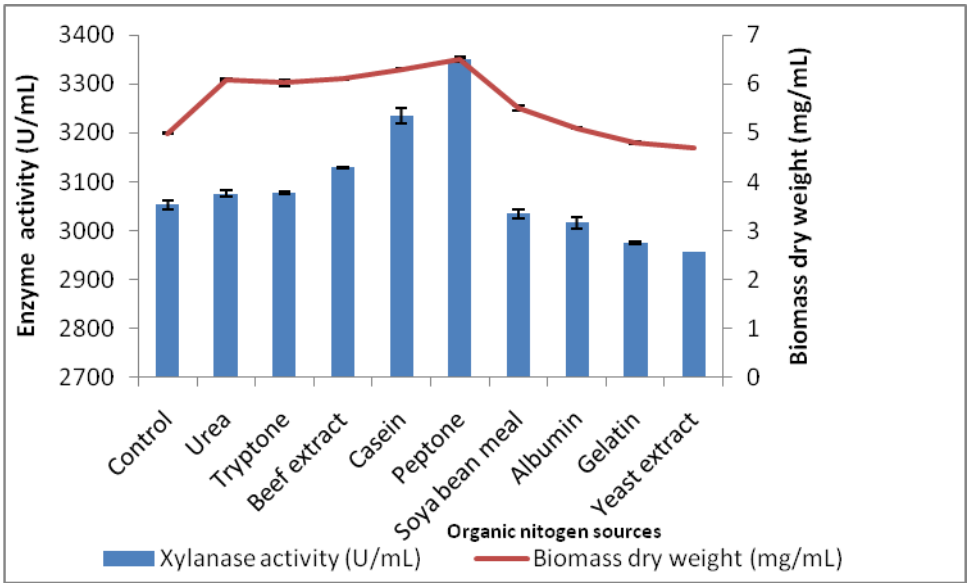
Supplementary figure 9: Effect of different surfactants and other additives on biomass and xylanase production



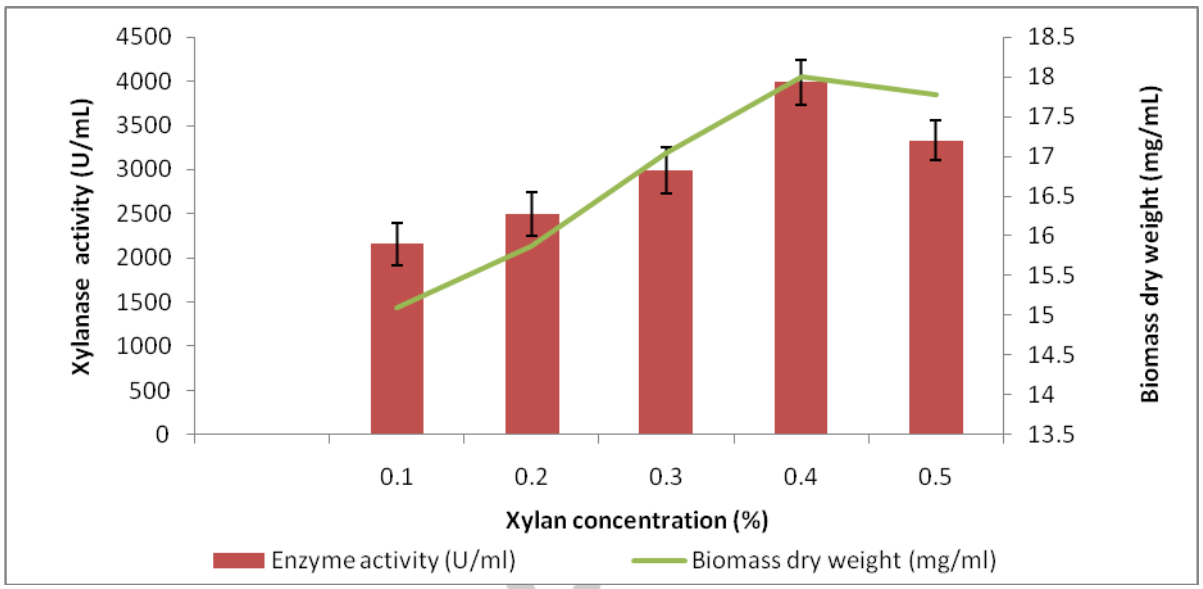
Supplementary figure 10: Effect of different metal ions on biomass and xylanase production



Supplementary figure 11: Effect of different inorganic nitrogen sources on biomass and xylanase production



Supplementary figure 12: Effect of different organic nitrogen sources on biomass and xylanase production



Supplementary figure 13: **Effect of xylan concentration (%) on biomass and xylanase production**

Production medium	**Xylanase activity (U/mL)**
A (Techapun *et al.*, 1991)	**1277.33**±39.971
B (M9 medium), (Roy, 2004)	568.5±35.224
C (Mandel's and Sternburg's medium) (Mandels and Sternburg, 1976)	59.91±7.628
D (Raghunathan and Padma, 2013)	624.5±13.04
E (Kim *et al.*, 1985)	360.3±30.714
F (Suneetha *et al.*, 2011)	135.73±19.641
G (Liu *et al.*, 2012)	782.6±48.397
H (M medium) (Obi and Odibo, 1984)	50.49±5.01
I (Jayapradha and Mahesh, 2013)	539.7±27.18
J (Tryptic soy broth) (Liu *et al.*, 2012)	258.1±31.93

K (Nutrient broth with xylan) (Hiremath and Patil, 2011)	522.4±25.045

Table 1. Effect of Production media on xylanase production by *Streptomyces* sp. ER1

Supplementary Table

Inoculum size (%)	**CFU/ 0.1 mL**
1	101
3	297
5	390
7	452
9	480
11	485

Supplementary table 1: Cell count (CFU) in each inoculum size

Effect of metal ions, NaCl, organic solvents, metal ions and surfactants on xylanase activity from *Streptomyces* sp. ER1

The metal ions such as Co^{2+}, Mn^{2+}, K^{+}, Ca^{2+} and Mg^{2+} were found to stimulate the xylanase activity (Figure 6). Similar findings were reported in previous studies (Yin *et al.*, 2010). $Hg^{2+,}$ Cu^{2+}, Fe^{2+} and Zn^{2+}, significantly reduced the xylanase activity compared to that of control due to combination of Hg^{2+} with sulfhydryl groups (Nakamura *et al.,* 1984), or the auto oxidation of cysteine molecules by Cu^{2+}which lead to the formation of intra and intermolecular disulphide bridges or to the formation of sulfenic acid (Vieille and Ziekus, 2001). 10 and 15% of NaCl enhanced the xylanase activity, as the residual activity was greater than 100%, compared to that of control (Figure 7). However, there was

a slight decrease in the residual activity with 20% of NaCl (91.5%) (Fig 4.10).

The residual xylanase activity increased to 135.37 %, after incubation with n-hexane and the presence of ethyl acetate reduced the residual activity to 60% (Figure 8). Similar reports were found in the previous studies (El-Gendy and El-Bondkly, 2014). However, the presence of butanol and chloroform, dropped enzyme activity to <60 % of residual activity. The increase in xylanase activity with non-polar solvents (hexane) might be due to their hydrophobic properties and decrease in activity with ethyl acetate, butanol and chloroform could be attributed to the high polarity of these solvents that stripped the water layer surrounding the enzyme causing enzyme inactivation (Woldesenbet *et al.,* 2012). Stability of enzyme in organic solvents is considered to be significant in industries such as fine chemical synthesis (Gargouri *et al.,* 1984).

Surfactants such as Triton X 100, Tween 80, Tween 60, polyethene glycol and olive oil increased xylanase activity (Figure 9). However, SDS and β-mercaptoethanol reduced xylanase activity. Similar results were observed by El-Gendy and El-Bondkly (2014). Addition of Tween 80 and polyethylene glycol prevents nonspecific binding of the enzyme to substrate, allowing more enzymes to be available for the conversion of substrate and results in a higher conversion rate (Palmer, 2000).The decrease in xylanase activity in the presence of β-mercaptoethanol indicated the relationship between the reduced form of the cysteine residue and the reduced xylanase activity and that by SDS might be due to conformational changes in the tertiary secondary structure of the protein (Bataillon *et al.,* 2000). Surfactant stable enzymes are used in industries such as detergents and dishwashers (Joseph *et al.,* 2007).

Substrate specificity and determination of kinetic parameters of purified xylanase

The enzyme was specific for beechwood xylan (Figure 10). No activity was shown in presence of other substrates. To determine the Vmax and Km of the xylanase enzyme, its activity was assayed using varying concentration of beechwood xylan (2-6 mg/mL). From the Lineweaver-Burk plot, the K_m value obtained was 0.018 µmol and the V_{max} was 11.72 µmol/min.

Conclusion

The present study established a simple strategy for optimisation and purification of xylanase from *Streptomyces* sp. ER1 under SmF. The biochemical properties of the purified xylanase indicated its possible application in processes operated at high temperatures and alkaline pH. Thus the enzyme could be potent in many industrial applications, like the bio-bleaching of kraft pulp, detergent industrial applications and saccharification of agro-residues to produce fermentable sugars for bioethanol production.

Conflict of interest statement:

We, authors, declare that we have no conflict of interest.

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