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Xylanase production from an alkalophilic actinomycete isolate *Streptomyces* sp. RCK-2010, its characterization and application in saccharification of second generation biomass

Adesh Kumar, Rishi Gupta, Bhuvnesh Shrivastava, Yogender Pal Khasa, Ramesh Chander Kuhad *

Lignocellulose Biotechnology Laboratory, Department of Microbiology, University of Delhi South Campus, New Delhi 110021, India

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ABSTRACT

Xylanase production by a newly isolated *Streptomyces* sp. RCK-2010 was optimized for varying culture conditions following one factor at a time (OFAT) and response surface methodology (RSM) approaches. An initial medium pH 8.0, agitation 200 rpm, incubation temperature 40 °C and inoculum size 1.0% (v/v) were found to be optimal for xylanase production (264.77 IU/ml), after 48 h of incubation. Among various carbon sources tested, the actinomycete secreted higher level of xylanase on wheat bran. The production medium when supplemented separately with various nitrogen sources, the enhanced xylanase production was observed with beef extract followed by peptone. RSM employing central composite design (CCD) was used to optimize the xylanase production using wheat bran, beef extract and peptone as model factors. The RSM showed that the optimum level of wheat bran (2.5% w/v), peptone (0.2% N_2 equivalent) and beef extract (1.2% N_2 equivalent) resulted in almost 3.0 fold improvement in xylanase production (2310.18 IU/ml). To the best of our knowledge this is the best xylanase volumetric productivity (1155 IU/ml/day) by any *Streptomyces* spp. reported in the literature. The enzyme was most active at 60 °C and pH 6.0 and almost 40% stable after 4 h at optimum temperature. Saccharification of steam exploded rice straw with xylanase (60 IU/g dry substrate) supplemented with cellulase (24 FPU/g dry substrate) and β-glucosidase (60 IU/g dry substrate) resulted in 88% (w/w) saccharification of the cellulosic substrate.

1. Introduction

Economical feasibility of biofuel production from lignocellulosic materials, the second-generation biomass, is a major technological challenge. Currently the major attraction for bioethanol production is cellulose fraction, while, hemicellulose, the second most abundant natural polymer is yet to be tapped. Xylan, the major hemicellulose, consists of 1, 4-β-linked D-xylose units substituted with different side groups such as L-arabinose, D-galactose, acetyl, feruloyl, p-coumaroyl and glucuronic acid residue [1,2]. Depending on its state, xylan can be used for various purposes such as, polymeric xylan as adhesives and emulsifiers [3], arabino-xylooligocaccharides as prebiotics [4,5], whereas monomeric xylose can be fermented to ethanol [6,7].

Among various methods used to breakdown the xylan backbone, its hydrolysis using xylanases is one of the most environmental benign alternative, as xylanases are highly specific in their nature and application [2,8,9]. Xylanases (EC. 3.2.1.8) are also attracting extensive interest due to their wide applications in pulp bleaching, bioethanol production and oligosaccharides production [7,10,11]. Furthermore, tolerance to high pH and temperature are desirable properties of xylanases for their effective use in various industries. The alkalitolerant xylanases reduce the chlorine requirements for pulp bleaching, while the higher temperature will enhance their rate of reaction. Though a variety of microorganisms have been reported to produce xylanolytic enzymes [2,8,10], there are few reports on the production of alkali and thermostable xylanase from actinomycetes. The present study deals with the production of thermostable and alkalitolerant xylanase from an alkalistable actinomycete isolate *Streptomyces* sp. RCK-2010.

Since, the production of xylanases is strongly influenced by their culture conditions and medium constituents, to maintain a balance among process conditions and to minimize the amount of un-utilized components, optimization of medium composition is essential [8]. The optimization process is generally carried out using one factor at a time (OFAT) approach, but it does not consider interaction among variables [12]. While, the optimization using statistical approaches such as response surface methodology (RSM) offers quick screening of large experimental domain and modeling of interactive effects of process variables, which enables each

* Corresponding author. Tel.: +91 11 24112062; fax: +91 11 24115270.
E-mail address: kuhad85@gmail.com (R.C. Kuhad).

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reaction parameters to be optimized incoherence with others for achieving maximum enzyme production [12,13]. Moreover, multivariate experiments in RSM also reduce the number of necessary optimization and give more precise results than those obtained by univariate strategies [13] and results in significant improvement of enzyme production.

In the present study, the optimization of xylanase production from an actinomycete isolate *Streptomyces* sp. RCK-2010 following OFAT and RSM approach was carried out. Moreover, an attempt has been made to evaluate the efficiency of xylanases in improving the hydrolysis of steam exploded rice straw, the abundantly available second generation feedstock, into sugars.

2. Experimental

2.1. Raw material and chemicals

The steam exploded rice straw having 77% (w/w) holocellulose (18% hemicellulose, 59% cellulose) was procured from Dr. A. J. Varma, Polymer Chemistry Division, National Chemical Laboratory, Pune, India. The substrate was washed thoroughly and dried at 60 °C in a hot air oven [14]. Xylan, cellobiose, glucose, carboxymethyl cellulose, D-xylose and 3, 5 di-nitrosalicylic acid were purchased from Sigma (St. louis, U.S.A.). All other media components and chemicals were purchased locally.

2.2. Isolation, screening of xylanase producing actinomycetes and identification of the potent isolate

For isolation of alkalo-tolerant actinomycetes, the soil samples collected from University of Delhi South Campus, New Delhi, India were initially treated with alkali by suspending 1.0 g of each sample in sterile distilled water (pH 9.0) [10]. The samples were then serially diluted and spreaded on actinomycete isolation agar containing (g/L) sodium caseinate 2.0, L-aspargine 0.1, sodium propionate 4.0, K_2HPO_4 0.5, $MgSO_4$ 0.5, $FeSO_4$ 0.001 and agar 15.0 (pH 8.0) and incubated at 37 °C for 96 h. The actinomycetes colonies developed were purified by repeated transfer of cultures and the isolates were screened for xylanase production using congo-red plate assay method and selected on the basis of hydrolysis zone [15].

For identification of the potent xylanase producing actinomycete isolate (R-10), a 500 bp region of 16S rRNA gene was amplified in a thermocycler (G-storm, USA) using the universal primers (CCAGCAGCCGCGGTAATACG) and (ATCGGCTACCTTGTTAC GACTTC). The PCR products were purified and sequenced as described earlier [13] and the nucleotide sequence has been deposited in the GenBank database (accession no. HQ658475). The sequence data was analyzed for the homology with the similar existing sequences available in the data bank of National Center for Biotechnology Information (NCBI) using BLAST search.

2.3. Microorganism and maintenance

Streptomyces sp. RCK-2010 was maintained on xylan agar plates containing (g/L) xylan 5.0, peptone 5.0, yeast extract 5.0, KH_2PO_4 1.0, $MgSO_4$ 0.1 and agar 20.0 at 37 °C, subcultured every fortnight and stored at 4 °C. The modified Horikoshi medium having (g/L) KH_2PO_4 1.0, $MgSO_4$ 0.1, yeast extract 5.0, peptone 5.0, and glucose 5.0, was used for the xylanase production.

2.4. Optimization of xylanase production

In order to screen the effective parameters for the optimization of xylanase production from *Streptomyces* sp. RCK-2010, various process variables such as cultivation time (up to 60 h), temperature (30–42 °C), initial pH (5.0–9.0) of the medium, agitation (100–300 rpm), inoculum size (0.4–1.2% v/v of 18 h old culture), carbon sources and nitrogen sources were studied under submerged fermentation conditions using OFAT approach. The time course of xylanase production was carried out at 37 °C, pH 8.0 and 200 rpm with an inoculum size of 0.4% (v/v). Each factor examined for optimization was incorporated further in the subsequent experiments. All other experiment conditions were kept constant unless otherwise stated.

Further to study the interaction among the three effective parameters selected from OFAT method, i.e., wheat bran (A), beef extract (B) and peptone (C) on xylanase production from *Streptomyces* sp. RCK-2010, experiments were conducted using RSM approach. The maxima (+1) and minima (−1) values for wheat bran were 0.5 and 2.5% (w/v), while for both beef extract and peptone were 0.2 and 1.2% (N_2 equivalent). The statistical software Package Design-Expert 6.0 Stat-Ease, USA was used to analyze the experimental design. The design was used for the determination of the optimum culture conditions for xylanase production. The analysis was used to identify the influence of variables on each other on the xylanase production and to determine the optimum fermentation conditions.

2.5. Characterization of xylanase from Streptomyces sp. RCK-2010

The xylanase produced under optimized conditions was purified partially with ammonium sulphate precipitation. The optimal pH for partially purified xylanase activity was determined using different buffers (0.2 M) (citrate–phosphate, pH 3.0–6.5; phosphate buffer, pH 6.0–7.5; Tris–HCl, pH 7.5–9.2; glycine–NaOH, pH 8.5–10.5 and carbonate–bicarbonate, pH 9.0–10.0) and the optimum temperature for xylanase activity was determined between 30 and 85 °C. The thermostability of the partially purified enzyme was studied by incubating the enzyme at 55 and 60 °C up to 4 h at pH 6.0, and the residual activities were estimated periodically.

2.6. Application of xylanase in saccharification of steam exploded rice straw

Enzymatic hydrolysis of steam exploded rice straw (10.0 g) was carried out at 5% (w/v) substrate concentration in 50 mM citrate phosphate buffer (pH 5.0). The substrate suspension was pre-incubated at 50 °C on a rotatory shaker (Innova-40, New Brunswick Scientific, Germany) at 150 rpm for 2 h. Thereafter, the slurry was supplemented with enzyme cocktail; cellulase (24 FPU/g), β-glucosidase (60 IU/g) and crude enzyme extract containing varied dosages of xylanase (20–80 IU/ml). The enzymatic reaction without xylanase served as control. Tween 80 (1% v/v) was also added in the reaction mixture to facilitate the enzyme action. The enzymatic hydrolysis was performed at 50 °C and 150 rpm for 36 h. Samples of enzymatic hydrolysate were withdrawn regularly and analyzed for amount of glucose released.

2.7. Analytical methods

All the analyses have been carried in triplicates and the data presented here is the mean of the triplicates along with the standard deviation.

The xylanase activities were determined by measuring the amount of reducing sugars released from xylan (1% w/v in citrate phosphate buffer; pH 6.0) at 60 °C for 10 min, as described earlier [4]. One international unit (IU) of xylanase activity was defined as amount of enzyme required to release 1 μM of reducing sugars as xylose from xylan per min under reaction conditions. The protein concentrations were measured by the Lowry's method with BSA (Bovine serum albumin) as standard [16]. The reducing sugars

were estimated following DNS method of Miller [17] and the saccharification efficiency was calculated as follows:

$$\text{Saccharification (\%)} = \frac{\text{Amount of sugar released in enzymatic saccharification}}{\text{Amount of carbohydrate present in the substrate}} \times 100$$

3. Results and discussion

3.1. Selection and molecular identification of potent xylanase-producing actinomycete

Out of the 57 alkalo-tolerant actinomycetes isolated from soil samples, 13 isolates showed the ability to produce xylanase. Among these isolates, R-10 was found to exhibit the largest zone of hydrolysis on xylan agar plate flooded with congo-red [15]. Moreover, the identification of the actinomycete isolate R-10 was done by 16S rRNA gene sequences. The nucleotide BLAST similarity search analysis based on 16S rRNA gene sequence revealed that the isolate R-10 was closely related to the genus *Streptomyces* (Fig. 1) and the organism was termed as *Streptomyces* sp. RCK-2010.

3.2. Effect of pH, temperature, agitation and inoculum size on xylanase production

Time course of xylanase production by *Streptomyces* sp. RCK-2010 showed the maximum xylanase production (158.2 IU/ml) after 48 h of incubation and thereafter it gradually declined (Fig. 2). The reduction in xylanase yield could be due to nutrients depletion or due to proteolysis [18]. Among various incubation temperatures studied, the maximum xylanase production (170.72 IU/ml) with specific xylanase activity of 73.59 IU/mg protein was achieved at 40 °C. The enzyme production was observed to be decreased by 12.3% on increasing the incubation to 42 °C (Table 1). The optimum temperature of 40 °C for xylanase production is also in correlation with our previous report on *Streptomyces cyaneus* [19].

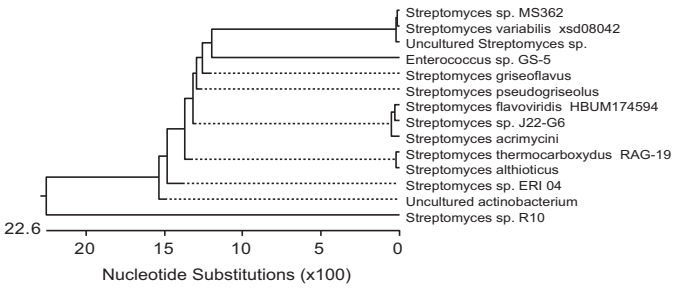

Fig. 1. Dendrogram of *Streptomyces* sp. RCK-2010 (*Streptomyces* sp. R-10) showing the similarity with other *Streptomyces* cultures.

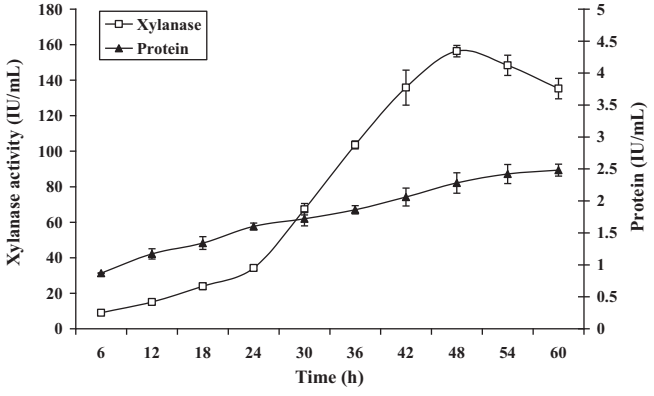

Fig. 2. Time course of xylanase production from *Streptomyces* sp. RCK-2010 under submerged cultivation conditions.

The actinomycete isolate when grown under variable shaking conditions, the optimum xylanase of 170.79 IU/ml with specific activity 73.51 IU/mg protein was observed at 200 rpm after 48 h of incubation (Table 1). Increase or decrease in the agitation rates beyond 200 rpm resulted in low xylanase yields. The lower enzyme level under low agitation conditions may be attributed to the dissolved oxygen (DO) limitation, improper mixing of media components and cell clumping [20]. However, the decrease in enzyme activity on increasing the agitation might be as a result of the cells shearing [13].

Among wide range of initial pH of the production medium tested, pH 8.0 was found to be more effective in production of maximum xylanase (178.67 IU/ml; specific activity 82.17 IU/mg protein), which drastically decreased beyond pH 8.0 (Table 1). Earlier reports on xylanase production by several fungi and bacteria have also been shown to be markedly dependent on the initial pH of the medium [21,22]. Interestingly, the preference of higher pH (8.0) by *Streptomyces* sp. RCK-2010 qualified it as a alkalophilic actinomycete.

An inoculum concentration ranging from 0.4 to 1.20% (v/v) revealed 1.0% (v/v) as optimal inoculum level for maximum xylanase yield (264.77 IU/ml) and specific xylanase activity (115.11 IU/mg protein) (Table 1). However, lower enzyme yield (226.68 IU/ml) at higher inoculum level (1.2% v/v) could be the result of faster nutrient consumption. Hence, an optimal inoculum level is necessary for maintaining balance between the proliferating biomass and available nutrients to produce maximum enzyme level.

3.3. Effect of carbon and nitrogen sources on xylanase production

Among various carbon sources used *Streptomyces* sp. RCK-2010 exhibited clear preference for lignocellulosic agro-residues as compared to pure sugars (Table 2) and wheat bran was observed to be the optimum substrate for maximum xylanase production (761.37 IU/ml). This may be because that wheat bran acts as a complete nutritious feed containing various soluble sugars, which are helpful for the initiation of growth and replication of microorganisms and remains loose even under moist conditions providing a large surface area [8]. In addition, higher xylanase production on wheat bran may be due to low lignin and silica content [11]. Wheat bran has been reported as an ideally suitable substrate for xylanase production for other microorganisms as well [23,24]. Interestingly, when monosaccharides and disaccharides were used as carbon sources, xylanase production ranged from 82 to 260 IU/ml. This implies that the enzyme is not specific to xylan-rich substrates and can be produced constitutively up to a certain level. But the use of pure sugars as substrate is uneconomical for large-scale production of xylanases, while agricultural residues are cost-effective substrates for xylanase production [25]. Further different concentrations of wheat bran ranging from 1.0 to 3.0% (w/v) were tested for xylanase production and the maximum xylanase production (805.12 IU/ml) was obtained when the actinomycete was grown on 2.5% (w/v) wheat bran (Fig. 3)

To optimize the effect of nitrogen sources for xylanase production, different organic and inorganic nitrogen sources were added by replacing the yeast extract and peptone from the production medium. The maximum activity of xylanase (617.58 IU/ml) with specific activity of 263.92 IU/mg protein was obtained with beef extract and followed by peptone (340.69 IU/ml) (Table 3). The enhanced production of xylanase in presence of beef extract as well as peptone may be attributed to organic nitrogen source mediated regulation of microbial growth and metabolism, as has

Table 1
Physiological parameters optimization for xylanase production from *Streptomyces* sp. RCK-2010 under submerged cultivation conditions following OFAT method.

Variables	Temperature (°C)				
	30	35	37	40	42
Activity (IU/ml)	90.11 ± 1.80	98.14 ± 3.93	156.34 ± 11.36	170.72 ± 0.68	149.71 ± 9.66
Protein (mg/ml)	1.92 ± 0.12	2.07 ± 0.08	2.28 ± 0.13	2.31 ± 0.16	2.36 ± 0.14
Specific activity (IU/mg protein)	46.86 ± 2.41	47.36 ± 0.48	68.52 ± 1.24	73.59 ± 1.11	63.42 ± 0.44
Variables	Agitation (RPM)				
	100	150	200	250	300
Activity (IU/ml)	49.25 ± 0.52	146.38 ± 2.37	170.79 ± 5.17	161.03 ± 2.27	121.59 ± 3.98
Protein (mg/ml)	2.02 ± 0.09	4.03 ± 0.11	2.32 ± 0.18	2.46 ± 0.15	2.05 ± 0.12
Specific activity (IU/mg protein)	24.31 ± 0.52	36.61 ± 0.65	73.51 ± 2.57	65.31 ± 5.41	59.19 ± 4.12
Variables	pH				
	5	6	7	8	9
Activity (IU/ml)	110.42 ± 5.41	139.19 ± 2.68	161.03 ± 3.37	178.67 ± 5.26	116.45 ± 4.29
Protein (mg/ml)	2.07 ± 0.07	4.00 ± 0.06	2.13 ± 0.19	2.17 ± 0.12	2.13 ± 0.20
Specific activity (IU/mg protein)	53.26 ± 2.54	34.74 ± 1.47	75.39 ± 3.38	82.17 ± 4.29	54.43 ± 3.14
Variables	Inoculum size (v/v)				
	0.4	0.6	0.8	1.0	1.2
Activity (IU/ml)	169.6 ± 2.63	189.66 ± 2.94	226.97 ± 4.77	264.77 ± 3.97	226.68 ± 4.52
Protein (mg/ml)	2.07 ± 0.08	2.28 ± 0.13	2.03 ± 0.16	1.85 ± 0.09	2.21 ± 0.04
Specific activity (IU/mg protein)	81.85 ± 5.24	83.13 ± 2.59	111.38 ± 4.74	115.11 ± 8.59	102.19 ± 6.47

Table 2
Effect of carbon sources on xylanase production from *Streptomyces* sp. RCK-2010 at 40 °C with shaking (200 rpm) after 48 h of incubation following OFAT method.

Carbon source (0.5% w/v)	Xylanase yield (IU/ml)	Protein mg/ml (mg/ml)	Specific activity (IU/mg protein)
Amylose	181.01 ± 12.36	2.59 ± 0.14	69.78 ± 2.44
Glucose	260.03 ± 17.22	2.02 ± 0.07	128.71 ± 8.41
Pectin	115.31 ± 8.96	2.28 ± 0.06	91.84 ± 5.62
Mannose	203.61 ± 15.63	2.22 ± 0.02	97.81 ± 6.57
Lactose	151.63 ± 9.87	1.55 ± 0.07	103.0 ± 8.35
Fructose	158.42 ± 11.84	1.54 ± 0.05	74.42 ± 6.21
Sucrose	119.27 ± 9.14	1.60 ± 0.08	90.51 ± 4.58
Maltose	152.34 ± 9.21	1.68 ± 0.10	47.45 ± 2.10
Arabinose	82.81 ± 3.52	1.94 ± 0.08	42.57 ± 1.57
Xylose	225.33 ± 13.25	1.80 ± 0.11	125.46 ± 10.25
Wheat bran	761.37 ± 2.24	2.51 ± 0.14	303.33 ± 8.54
Wheat straw	505.11 ± 36.33	2.31 ± 0.12	216.93 ± 12.73
Prosopis juliflora	381.05 ± 19.22	1.91 ± 0.14	199.47 ± 14.37
Lantana camara	373.35 ± 27.13	1.76 ± 0.08	212.13 ± 15.49
Corn cob	325.19 ± 24.66	1.55 ± 0.13	184.85 ± 15.62
Corn stover	289.86 ± 22.63	1.89 ± 0.14	153.36 ± 7.58
Sugarcane bagasse	423.15 ± 36.51	1.63 ± 0.07	259.60 ± 5.36
Birchwood xylan	215.27 ± 13.11	1.53 ± 0.08	140.69 ± 10.24

Table 3
Effect of nitrogen sources on xylanase production from *Streptomyces* sp. RCK-2010 at 40 °C with shaking (200 rpm) after 48 h of incubation following OFAT method.

Nitrogen source (0.55% N_2 equivalent)	Xylanase yield (IU/ml)	Protein (mg/ml)	Specific activity (IU/mg protein)
Organic			
Peptone	340.69 ± 19.74	2.21 ± 0.12	154.15 ± 5.47
Beef extract	617.58 ± 36.98	2.34 ± 0.15	263.92 ± 3.65
Yeast extract	281.69 ± 22.49	2.22 ± 0.08	126.89 ± 7.46
Tryptone	258.34 ± 23.30	2.15 ± 0.10	120.16 ± 7.25
Inorganic			
NH4Cl	88.35 ± 5.26 (2.43 36.35)	2.43 ± 0.21	36.35 ± 2.54
$NaNO_2$	122.38 ± 6.93(2.13)	2.13 ± 0.15	57.45 ± 4.29
$NaNO_3$	205.08 ± 18.73	2.95 ± 0.09	69.51 ± 1.58
$(NH_4)_3HPO_4$	210.13 ± 18.73	2.37 ± 0.11	88.66 ± 3.65
Control[a]	78.26 ± 5.42	1.87 ± 0.07	41.85 ± 2.17

[a] The control medium used was devoid of nitrogen source.

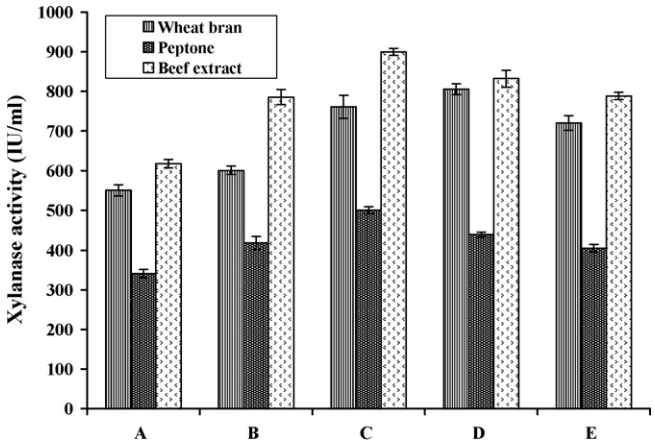

Fig. 3. Effect of different dosages of carbon and nitrogen sources on xylanase production from *Streptomyces* isolate RCK-2010. Where, A, B, C, D and E are 0.5, 0.6, 0.7, 0.8, 0.9% N_2 equivalent of beef extract and peptone; and 1.0, 1.5, 2.0, 2.5 and 3.0% (w/v) wheat bran concentration, respectively.

been reported that the complex nitrogen is an essential requirement for growth and enzyme production [26]. Moreover, it has also been observed that nitrogen can significantly affect the pH of the medium during the course of fermentation [27], thereby influences the microbial metabolism. Our results are very much in agreement with the earlier reports, where actinomycetes were found to produce higher xylanase on organic nitrogen sources [24,28,29]. In order to estimate the optimum dosage of nitrogen sources, the xylanase production was carried out at different dosage (0.5–0.9% N_2 equivalent) of both peptone and beef extract were carried out. Irrespective of the nitrogen sources tested, the maximum xylanase production was observed at 0.7% N_2 equivalent. The xylanase production with optimal level of beef extract and peptone as nitrogen sources was observed to be 889.21 and 503.24 IU/ml, respectively (Fig. 3).

3.4. Statistical optimization of xylanase production using RSM

The result of RSM experiment for studying the effect of three independent variables; wheat bran (A), beef extract (B) and peptone (C), are presented along with the mean predicted and observed responses in Table 4. The table showed the maximum and minimum levels of variables resulted in the RSM and also depicted that total four center points were set up at runs of 7, 12, 13 and 19 and almost similar xylanase activities (~1100 IU/ml) were observed. However, the maximum xylanase production (2310.18 IU/ml) was achieved at run no. 18 containing (% w/v); wheat bran 2.5, beef extract 1.2 and peptone 0.20. While, the minimum xylanase (439.9 IU/ml) was observed in run no. 16 containing minimum amount of wheat bran (0.50% w/v), beef extract (0.20% w/v) and peptone (0.20% w/v). From multiple regression analysis, it was observed that the second-order polynomial equation can explain xylanase production regardless of the significance of the coefficients:

$$Y = 65.69 + 235.79\,A + 801.24\,B + 406.87\,C + 240.83\,AB - 794.90\,BC$$

where Y is the response value. In current experiment, Y value is the level of xylanase production (IU/ml), A, B and C represent the coded levels of wheat bran, beef extract and peptone, respectively.

The statistical significance of the regression model was checked by F-test. The model was highly significant, as manifested by the F-value and the probability value [(P_{total} model > F) = >0.0001] was achieved. The goodness of fit was manifested by the determination coefficient (R^2). In this case the R^2 value of 0.9775 indicated that the response model can explain 97.75% of the total variations. The value of the adjusted determination coefficient (AdjR^2) was also high enough (0.9695) to indicate the significance of the model. The parameter coefficient and the corresponding P-value suggested that among the independent variables, A (wheat bran) and B (beef extract) have a significant effect on xylanase production.

The 3D response surfaces plots were employed to determine the interaction of the medium components and the optimum levels that have the most significant effect on xylanase production. Fig. 4a describes the effects of wheat bran and beef extract on xylanase production, when peptone was fixed at its middle level (0.70 N_2 equivalents). The xylanase production yield increased with increase in both the components and the increase in xylanase activity as a function of increasing levels of wheat bran seems very similar to the increase as a function of increasing beef extract concentration (Fig. 4a). High levels of xylanase production with increase in wheat bran concentration could be due to the fact that wheat bran is nutrient reservoir for xylanolytic microorganism and acts as carbon and nitrogen source. While, the interaction between beef extract and peptone (wheat bran at middle level)

Table 4
Experimental design and results of the RSM for the production of xylanase from *Streptomyces* sp. RCK-2010.

Run	Factor-A wheat bran	Factor-B beef extract	Factor-C peptone	Response xylanase activity (IU/ml)	
				Predicted value	Actual value
1	0.5	1.2	0.2	1134.85	1106.9
2	2.5	1.2	0.7	1956.23	2015.21
3	1.5	0.7	0.2	1203.21	1192.65
4	0.5	0.7	0.7	724.06	677.78
5	2.5	0.2	0.2	1030.74	969.52
6	2.5	0.2	1.2	1187.97	1262.03
7	1.5	0.7	0.7	1128.42	1190.55
8	0.5	0.2	1.2	710.71	692.22
9	1.5	0.2	0.7	825.40	801.87
10	1.5	0.7	1.2	1053.64	944.98
11	0.5	1.2	1.2	678.49	753.11
12	1.5	0.7	0.7	1128.42	1190.25
13	1.5	0.7	0.7	1128.42	1101.52
14	2.5	1.2	1.2	1637.40	1553.59
15	2.5	0.7	0.7	1532.79	1559.31
16	0.5	0.2	0.2	372.17	439.90
17	1.5	1.2	1.2	1157.94	1223.35
18	2.5	1.2	0.2	2275.06	2310.18
19	1.5	0.7	0.7	1128.42	1093.25
20	1.5	1.2	0.7	1431.45	1347.64

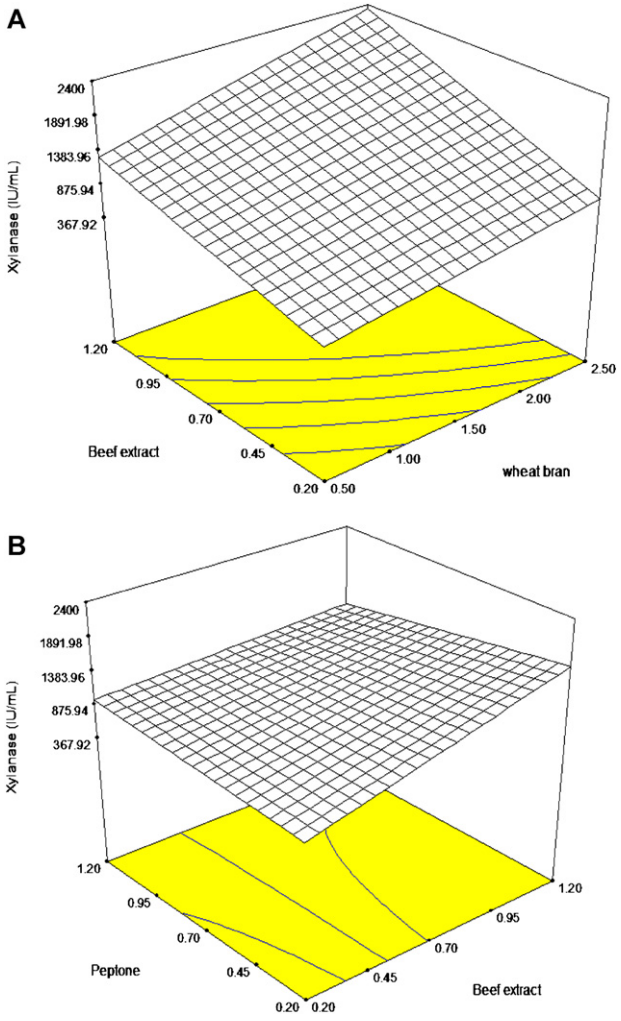

Fig. 4. Response curves of the RSM experiments for the production of xylanases from *Streptomyces* sp. RCK-2010.

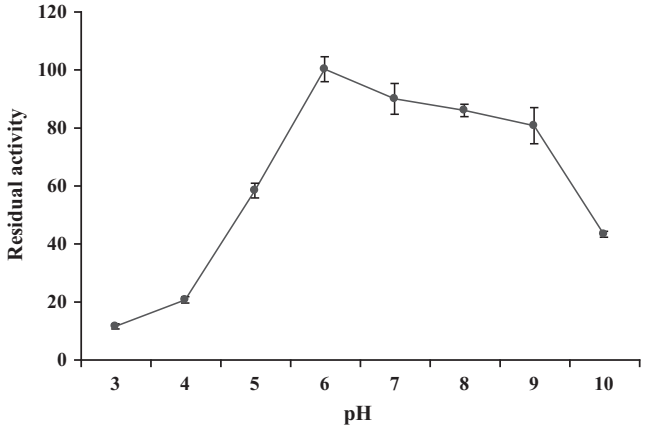

Fig. 5. Effect of pH on xylanase activity assayed at 60 °C for 10 min The xylanase activity was assayed in pH range 3.0–10.0 using different buffers (citrate–phosphate buffer, Tris–HCl and glycine–NaOH). 100% xylanase activity was equivalent to 2310 IU/ml.

demonstrated that the xylanase production increased with increase in beef extract concentration, but the enhancement in peptone concentration did not significantly increased the xylanase production (Fig. 4b).

The xylanase production capability of *Streptomyces* sp. RCK-2010 has been compared with the other *Streptomyces* strains grown under optimized conditions (Table 5). To the best of our knowledge *Streptomyces* sp. RCK-2010 has been found to produce fairly good amount of xylanase (2310 IU/ml) compared to the majority of the *Streptomyces* strains reported in the literature (8–2360 IU/ml) and comparatively exhibited maximum volumetric productivity (1155 IU/ml/day) under submerged fermentation conditions (Table 5).

3.5. Effect of pH and temperature on the activity and stability of xylanase

Among various pH values (3–10) tested, the partially purified xylanase was active over a wide range of pH 5–9 with more than 80% of residual enzyme activities. The optimal pH for *Streptomyces* sp. RCK-2010 xylanase was 6.0 (Fig. 5). Although, most xylanases known today are active at either acidic or neutral pH [30–32], recently several alkalotolerant xylanases have also been reported in efficient bleaching of paper pulp [4,33]. Moreover the alkaline xylanases, which are operationally stable at higher temperature, are more beneficial because of savings in cooling cost and time [4]. The xylanase from *Streptomyces* sp. RCK-2010 exhibited its temperature optima of 60 °C at pH 6.0 (Fig. 6). Similar temperature optima of 60–65 °C have been reported earlier for other xylanases [4,31,33]. While the profiles obtained for thermostability (at 55 and 60 °C) of partially purified xylanase from *Streptomyces* sp. RCK-2010 revealed that the enzyme was thermostable with approximately 50 and 40% residual activity at 55 and 60 °C, respectively, after 4 h of incubation (Fig. 7).

Table 5
Comparison of xylanase production by different species of *Streptomyces*.

Culture	Production (U/ml)	Time (days)	Volumetric productivity (IU/mL/day)	Substrate	Reference
Streptomyces sp. QG-11-3	84.26	2	42.1	Xylan	[36]
Streptomyces sp. QG-11-3	2360	5	472.0	Wheat bran	[36]
Streptomyces galbus ATCC3005	12	6	2.0	Oat splet xylan	[37]
Streptomyces sp. Ab106	15	5	3.0	Cane bagasse	[38,39]
Streptomyces sp. AMT-3	28	10	2.8	Wheat bran	[40]
Streptomyces sp. AMT-3	70	10	7.0	Larchwood xylan	[40]
Streptomyces sp. Ab106	15	6	2.5	Corn hulls	[41]
Streptomyces actuosus A-151	10.3	4	2.6	Rice bran	[42]
Streptomyces olivaceoviridis E-86	1653	5	330.6	Corncob	[43]
Streptomyces galbus NR	8.75	5	1.8	Wheat bran	[44]
Streptomyces cyaneus SN32	730	2	365.0	Wheat bran	[25]
Streptomyces violaceoruber	1500	1.5	1000.0	Wheat bran	[13]
Streptomyces sp. RCK-2010	2310	2	1155.0	Wheat bran	This study

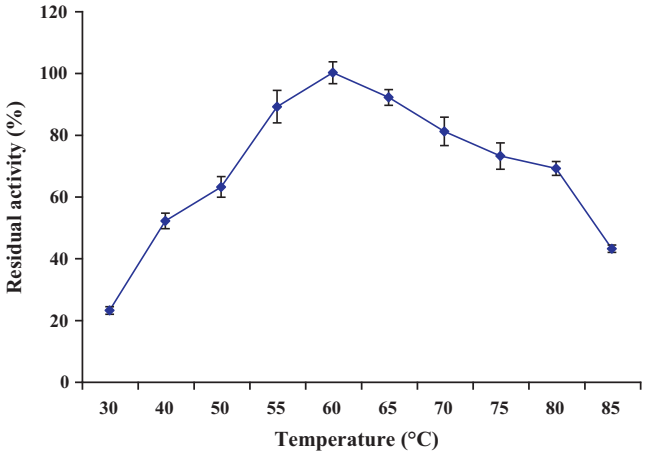

Fig. 6. Effect of temperature on xylanase activity assayed at different temperature range for 10 min. 100% xylanase activity was equivalent to 2310 IU/ml.

3.6. Application of *Streptomyces* sp. RCK-2010 in saccharification of steam exploded rice straw

The time course of enzymatic saccharification of steam exploded rice straw with a combination of cellulase and xylanase revealed that the sugar yield increased continuously till 48 h (Fig. 8). Among various dosage of xylanase used along with cellulases, the enzyme dosage of 60 IU/g dry substrate resulted in maximum increase in saccharification (208 mg/g dry substrate) and thereafter it did not result in any significant improvement (Fig. 8). Though the xylan fraction in steam exploded rice straw was only 18%, the higher release of sugar might be due to the loosening of xylan backbone in the steam exploded substrate. The higher sugar yield may also be attributed to the release of glucan monomers bound tightly to the xylan backbone. The synergistic action of cellulases and xylanases led to a total saccharification efficiency of 88%. Our results are consistent with the earlier works [7,34,35] and indicate higher saccharification of lignocellulosic substrates with combined action of cellulases and xylanases.

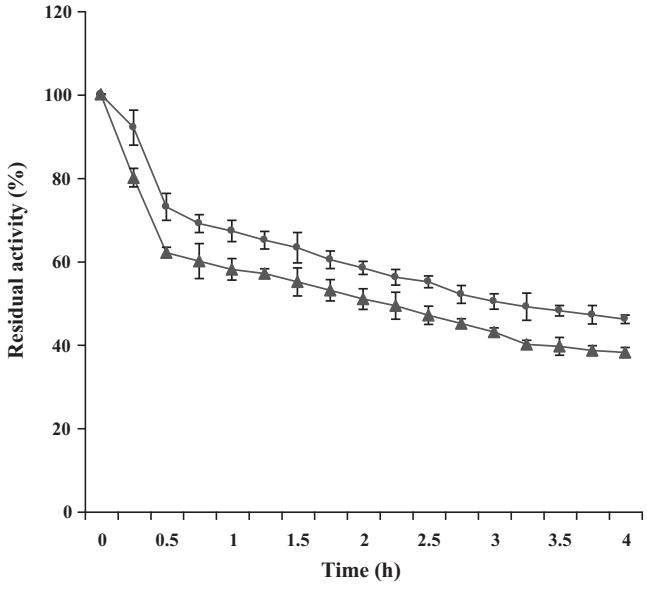

Fig. 7. Thermo stability profile of xylanase activity assayed at 55 °C (●) and 60 °C (▲) temperature for 240 min. 100% xylanase activities was equivalent to 2310 IU/ml.

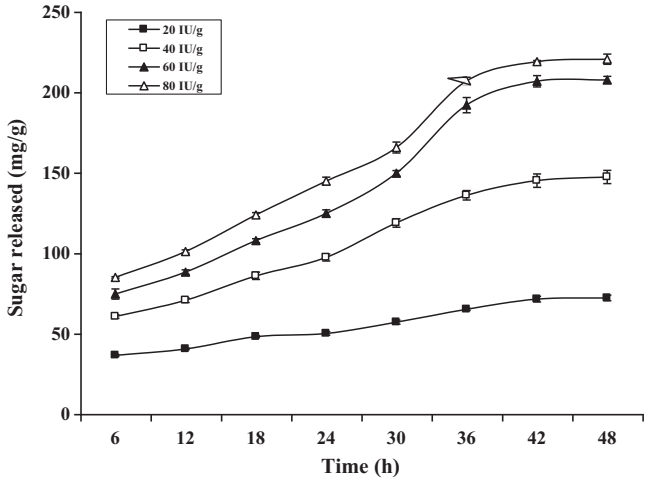

Fig. 8. Effect of different dosage of xylanase (20–80 IU/g substrate) along with 24U FPase, 60U β-glucosidase and 1% (v/v) Tween 80 on the enzymatic saccharification of steam exploded rice straw.

4. Conclusion

The xylanase from *Streptomyces* sp. RCK-2010 shows potential in saccharification of second generation feedstocks into sugars for their eventual fermentation to ethanol.

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