Biocatalysis and Agricultural Biotechnology 6 (2016) 51–57

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Insight on xylanase from *Aspergillus tubingensis* FDHN1: Production, high yielding recovery optimization through statistical approach and application

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ARTICLE INFO

Article history:
Received 23 May 2015
Received in revised form
3 October 2015
Accepted 30 January 2016
Available online 13 February 2016

Keywords:
Aspergillus tubingensis FDHN1
Xylanase production
Solid state fermentation
Statistical methodology
Enzyme recovery
Optimization
Saccharification

ABSTRACT

The present study focused on xylanase production from *Aspergillus tubingensis* FDHN1 under solid state fermentation at different flask levels and its high yield recovery process. The maximum xylanase production, 4061.32 U/g, was achieved in different sized flasks. The study further aimed at higher yield recovery in a single step from fermented sorghum straw through statistical methodology. For optimizing recovery conditions with respect to extractant volume (8–16 mL/g), extraction time (30–150 min), extraction speed (50–250 rpm) and extraction temperature (30–50 °C), central composite design (CCD) in 27 runs was employed. Maximum xylanase yield under validated recovery conditions was 5177.23 U/g with 90% recovery efficiency. Next, the crude xylanase, commercial cellulase and combination of both were subjected for saccharification of various untreated and pretreated agro-residues. The studied enzymes yielded much higher sugar content in combination, rather than their utilization as alone. Overall, xylanase from *Aspergillus tubingensis* FDHN1 can be produced and recovered at high level under solid state fermentation, exhibiting potential applications.

1. Introduction

To establish a viable enzyme production process, the recent research focuses on selection of the most suitable fermentation technique and its optimization for better productivity and enzyme recovery. Recently, among several fermentation techniques, solid state fermentation (SSF) technique has earned special attention that enables microbial growth on moist solid agro-residues. The SSF technique has several advantages over submerged fermentation (SmF) technique, such as utilization of cheaper agro-industrial byproducts as a solid support, small volumes of liquid required for product recovery, low energy demand and thus offers cost-effective process (Beg et al., 2001). Among the microbial community, filamentous fungi have been widely explored for the production of various extracellular hydrolytic enzymes, especially xylanases using SSF technique. Xylanases have extensive applications in the paper and bakery industries, in xylose and xylo-oligosaccharide production from biomass and in juice clarification (Polizeli et al., 2005; Battan et al., 2006; Dhiman et al., 2008).

In recent years, xylanase production under SSF has been widely studied by several researchers (Lakshmi et al., 2009; Pal and Khanum, 2010; Rodriguez-Fernandez et al., 2011; Panwar et al., 2014). Apart from high productivity of xylanases, special attention has also given to their properties. Especially, thermostable xylanases could be used in industries where a cooling step would be uneconomical, while acidophilic/alkaliphilic xylanases could be used where constant adjustment of pH to neutral would not be feasible. In accordance to this an interest to find novel xylanases is still in progress.

Despite of producing novel xylanases from newly isolated fungal sources, a very next goal should be establishment of simple and high yield recovery process with minimum enzyme loss. Till date, only a handful of reports that show xylanase optimization and efficient recovery have been available (Pal and Khanum, 2010; Panwar et al., 2014). The major factors affecting the enzyme recovery process include the type of an extractant, its volume, extraction period, extraction temperature and speed. An improper optimization of these variables could adversely affect to the overall production cost by increasing the time and steps for total enzyme recovery, generating poor enzyme extract, increasing enzyme loss, decreasing yield and hence results in poor downstream processing. An ideal extraction process would generate highly concentrated enzyme extract within a short time span.

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http://dx.doi.org/10.1016/j.bcab.2016.01.014
1878-8181/

Table 1
Experimental plan of the four variable Central Composite design in coded and selected levels and the corresponding experimental and predicated responses.

Run	Design matrix				Experimentally coded levels and values in design matrix				Xylanase activity (U/g)	
	X_1	X_2	X_3	X_4	Extractant volume (mL/g)	Extraction time (min)	Extraction speed (rpm)	Extraction temperature (°C)	Experimental	Predicted
1	−1	−1	−1	−1	10	60	100	35	2566.03	2762.08
2	1	−1	−1	−1	14	60	100	35	3132.37	3185.25
3	−1	1	−1	−1	10	120	100	35	3311.88	3344.58
4	1	1	−1	−1	14	120	100	35	3716.39	3646.94
5	−1	−1	1	−1	10	60	200	35	3021.16	2943.42
6	1	−1	1	−1	14	60	200	35	3273.94	3366.59
7	−1	1	1	−1	10	120	200	35	3855.41	3745.16
8	1	1	1	−1	14	120	200	35	4070.33	4047.51
9	−1	−1	−1	1	10	60	100	45	2641.90	2623.37
10	1	−1	−1	1	14	60	100	45	2937.73	3046.54
11	−1	1	−1	1	10	120	100	45	3273.97	3205.87
12	1	1	−1	1	14	120	100	45	3380.08	3508.22
13	−1	−1	1	1	10	60	200	45	3046.41	3042.89
14	1	−1	1	1	14	60	200	45	3468.59	3466.06
15	−1	1	1	1	10	120	200	45	3918.63	3844.63
16	1	1	1	1	14	120	200	45	4246.97	4146.98
17	0	0	0	0	12	90	150	40	4778.22	4916.59
18	0	0	0	0	12	90	150	40	4947.84	4916.59
19	0	0	0	0	12	90	150	40	5023.70	4916.59
20	−2	0	0	0	08	90	150	40	2875.16	2944.89
21	2	0	0	0	16	90	150	40	3756.23	3670.42
22	0	−2	0	0	12	30	150	40	3514.34	3348.34
23	0	2	0	0	12	150	150	40	4461.84	4611.76
24	0	0	−2	0	12	90	50	40	2896.40	2723.19
25	0	0	2	0	12	90	250	40	3386.15	3543.29
26	0	0	0	−2	12	90	150	30	3119.18	3080.20
27	0	0	0	2	12	90	150	50	3018.06	3040.96

Previously, xylanase production ability of *Aspergillus tubingensis* FDHN1 under SSF and its detailed characterization has been reported (Adhyaru et al., 2015). The present study revealed efficiency of *A. tubingensis* FDHN1 to produce xylanase at different flask levels using sorghum straw as a sole substrate. Another main goal of the present study was to achieve high xylanase recovery with minimum xylanase loss during extraction processes. The study shows the importance of statistically optimized extraction conditions for maximum xylanase recovery from fermented sorghum straw. Central composite design (CCD) was employed to understand the relationship among various extraction parameters (an extractant volume, extraction time, extraction speed and temperature) that affect xylanase recovery. Finally, an attempt was made to saccharify various un-treated and chemically treated agro-residues using crude xylanase, commercial cellulase and cocktail of crude xylanase-commercial cellulase.

2. Materials and methods

2.1. Fungal culture and inoculum preparation

Aspergillus tubingensis FDHN1 (GenBank accession number **KF971693**) was used for xylanase production. Periodic reactivation of the strain was made in Potato dextrose agar (PDA) slants and incubated for 5 days at 35 °C. The spore inoculum was prepared from 5 days grown culture on PDA slants. The fungal spores were suspended from slants by adding sterile distilled water to give a final count of $\sim 1 \times 10^6$ spores/mL.

2.2. Xylanase production at different flask levels by A. tubingensis FDHN1 under SSF

Xylanase production by *A. tubingensis* FDHN1 under SSF was attempted in different sized flasks i.e. 250, 500, 1000 and 2000 mL, containing 9, 18, 36 and 72 g of dry sorghum straw, respectively. The sorghum straw (~3 to 5 mm particle size) was moistened at 1:5 (w/v) ratio using medium, containing (g/L): xylose 3.0; KH_2PO_4 3.0; $NaNO_3$ 2.5; $MgSO_4 \cdot 7H_2O$ 1.0; $CaCl_2$ 0.05; urea 10; (mg/L): $FeSO_4 \cdot 7H_2O$ 7.5; $MnSO_4 \cdot H_2O$ 2.5; $ZnSO_4 \cdot 7H_2O$ 3.6; and 0.1% Tween-80 (v/v), pH 6.0, prior to autoclaving. The sterilized preparations were then cooled, inoculated with 1 mL of spore suspension per nine gram of substrate and incubated at 35 °C for 6 days. The flasks were gently tapped intermittently to mix the content.

After 2 days (48 h) of incubation, at a regular interval of 12 h, up to 6th day (144 h) the whole content of the flasks was extracted in 0.05 m citrate buffer (pH 5.0) at an extractant volume of 10 mL/g fermented sorghum straw. The mixture was agitated at 120 rpm for 60 min at 35 °C. Later, the mixture was centrifuged at 10,000*g* for 15 min at 37 °C and used to analyse the xylanase activity.

2.3. Central composite design for improving xylanase extraction

Initially, xylanase was extracted from fermented sorghum straw using 0.05 m citrate buffer (pH 5.0) as described earlier in Section 2.2. Optimization of the extraction variables by statistical approach may provide the most favorable conditions of extraction by identifying or considering simultaneous interactions among them. Response surface modeling is a set of statistical techniques for designing experiments, building models, evaluating effects of variables and searching for optimum conditions for specific responses. Xylanase from newly isolated *A. tubingensis* FDHN1 found to possess important characters (Adhyaru et al., 2015). Therefore, central composite design (CCD) was employed to achieve its maximal yield in single extraction step.

In CCD, four variables at five levels were used to understand the interactive influence of variables on xylanase recovery. Four independent variables selected were X_1: extractant volume (mL/g), X_2: extraction time (min), X_3: extraction speed (rpm) and X_4: