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Enhanced production of xylanase by a newly isolated *Aspergillus terreus* under solid state fermentation using palm industrial waste: A statistical optimization

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ABSTRACT

Xylanase production by a newly isolated *Aspergillus terreus* MTCC 8661 was optimized using palm fiber in solid state fermentation (SSF). Different fermentation parameters such as incubation temperature, moisture content, medium pH, particle size, incubation time, inoculum, xylose and sodium nitrate concentrations were investigated at the individual and interactive level by the Taguchi methodology. All selected fermentation parameters influenced xylanase production. Moisture content, incubation time and inoculum concentration were the major [~85%] influential parameters on xylanase production at the individual level. At the interactive level, inoculum concentration was important and accounted for more than 50% of the severity index with particle size and incubation temperature. Xylanase production improved from 41,000 to 115,000 U/g indicating 227% improvement after optimization suggesting that this fungal strain, *A. terreus* MTCC 8661, has the commercial potential for hemicellulosic enzyme production.

1. Introduction

Xylan is an integral part of lignocellulosic structure, which is the most abundant and renewable biomass available on earth [1]. It is an heterogeneous polysaccharide consisting of β-1,4-linked D-xylosyl residues along with a small fraction of arabinose, glucuronic and arabinoglucuronic acids linked to the D-xylose back bone [2]. Several industrial processes have been developed that utilize agro-industrial biomass residues as raw materials for the production of bulk chemicals such as ethanol and single-cell protein and enzymes. In fact, use of agro-industrial residues in bioprocesses is effective as alternative bio-substrates, and may reduce pollution problems caused by their disposal [3]. Xylanases [EC.3.2.1.8] are responsible for hydrolysis of xylan; they first attack the internal main-chain linkages and subsequently releasing xylosyl residues by endwise attack of xylooligosaccharides [4]. These enzymes have recently attracted considerable attention due to their application potential in hydrolysis or bioconversion of lignocelluloses to sugars. In addition, xylanases also have application potential in industries involved in clarification of juices, extraction of plant oils and extracellular polymeric substances [EPS]. They have been known to improve the nutritional value of silage, green feed, coffee, starch and as bleaching agents in pulp and paper industry [5–8].

A variety of microorganisms including bacteria, yeast and filamentous fungi have been reported to produce xylanolytic enzymes [9–11]. The research indicated that xylanase production differed with different strains and was regulated by the physiological, nutritional, and biochemical nature of the microbes employed [4,8–10]. Notable environmental and fermentation factors that influence metabolism-mediated production yields include pH, temperature, aeration, agitation, carbon and nitrogen sources, metal ion requirement, incubation time, initial inoculum size, etc., [12–15]. Hence, for commercial production, optimization of medium composition is one of the essential steps to minimize the amount of unutilized components for a cost-effective yield. In general, no defined medium has been established for the best production of any metabolite because the genetic diversity present in different microbial sources causes each organism or strain to have its own special conditions for maximum yield of production [15]. Therefore, it is highly imperative to optimize all fermentation parameters including medium composition, which further facilitates economic design of the full-scale operation system for newly isolated microbial strains. However, it is impractical to optimize all fermentation parameters in conventional methodology to establish the optimum conditions by understanding the interactions of all parameters, as this involves numerous experiments if all possible combina-

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tions are to be investigated [12,16,17]. In contrast statistically planned experiments effectively reduce the number of experiments by developing a specific design of experiments which also minimizes the error in determining the values for significant parameters [17,18].

In the present study, the objective was to elaborate the best conditions for the production of extracellular xylanase by isolated *Aspergillus terreus* strain in solid state fermentation [SSF] to take the advantage of SSF specially in resisting bacterial contamination, ease of purification and the use of inexpensive natural agro-industrial materials. SSF was carried out through a stepwise optimization strategy including, verification of the model and monitoring the production pattern.

2. Materials and methods

2.1. Microorganism and maintenance

The fungal strain, *A. terreus*, was isolated from soil samples collected from Rajamandry palm oil industrial area was used in this investigation. The strain was identified as *A. terreus* by Microbial Testing and Collection Center [MTCC], Institute of Microbial Technology, Chandigarh, India. The fungal strain was maintained on Potato Dextrose Agar slants and stored at 4 °C with periodic [30 days] sub-culturing.

2.2. Optimization methodology

Taguchi design methodology was performed using a modified version in four phases [12] [with various steps], viz., planning, experimentation, software analysis, and validation of results. Taguchi design of experiments [DOE] was used to set up the critical fermentation factors: temperature, pH, particle size, moisture content, xylose, sodium nitrate, inoculum level, and incubation time that have a significant influence on the xylanase production. The next stage involved selecting the appropriate Taguchi design structure for data analysis [12]. L-18 Othogonal Array was selected for above control parameters with three levels of factor variation (Table 1). All of these factors were assigned with three levels, except incubation temperature which was assigned with two levels. Xylose and sodium nitrate were dissolved in distilled water, the pH was adjusted using 0.1N NaOH or 0.1N HCl solutions and this solution was used for moisturizing the substrate.

Table 1
Selected fermentation factors and their assigned levels for xylanase production with *Aspergillus terreus*.

S. no.	Factor	Level 1	Level 2	Level 3
1	Incubation temperature (°C)	35	40	
2	Moisture 100 × (g water/g substrate dry weight)	2.0	2.5	3.0
3	pH	5.0	6.0	7.0
4	Xylose (g xylose/g substrate dry weight)	0.4	0.6	0.8
5	$NaNO_3$ (g $NaNO_3$/g substrate dry weight)	0.4	0.5	0.6
6	Inoculum (ml spore solution/g substrate dry weight)	1.0	2.0	3.0
7	Particle size (mm)	2.1–2.8	0.7–2.0	0.3–0.7
8	Incubation time (h)	36	48	60

2.3. Solid state fermentation [SSF]

Solid state fermentation experiments were performed for xylanase production with *A. terreus* employing the selected 18 experimental trails (Table 2) in combination with eight factors at selected levels (Table 1). All experiments were conducted in 250 ml Erlenmeyer flask containing 5 g of medium [selected size of palm fiber] with relative moisture content, inoculated with selected levels of spore solution containing 1×10^8 spores/ml under sterile conditions. Prior to use, the palm fiber material was dried in a hot air oven [50 °C; 24 h] and subsequently sieved using a metal mesh of required size. The sterilized flasks were then incubated at different temperatures [30 or 33 °C] in an incubator [DK-SI010] for 36, 48 and 60 h and the entire contents of the flask were used for enzyme extraction and estimation. The xylanase activities presented in this study are the average values of three individual determinations.

2.4. Analysis of experimental data and prediction of performance [AEDPP]

Qualitek-4 software [Nutek Inc., MI] for automatic design of experiments using the Taguchi approach was used in the present study. The obtained experimental data was processed using Qualitek-4 software with bigger is better quality characteristic for the determination of the optimum culture conditions for the fermentation. The analysis was used to identify the influence of individual factors on the xylanase production and to determine the optimum fermentation conditions.

Table 2
Experimental setup [L-18 orthogonal array) for xylanase production with *Aspergillus terreus*.

Exp no.	Factor levels								Xylanase production (U/g solid biomass)		
	1	2	3	4	5	6	7	8	Experimental	Predicted	Error
1	1	1	1	1	1	1	1	1	61,993	62,367	374
2	1	1	2	2	2	2	2	2	85,772	86,146	374
3	1	1	3	3	3	3	3	3	77,269	77,643	374
4	1	2	1	1	2	2	3	3	112,211	106,061	−6150
5	1	2	2	2	3	3	1	1	88,438	82,288	−6150
6	1	2	3	3	1	1	2	2	89,490	83,340	−6150
7	1	3	1	2	1	3	2	3	91,379	97,154	5775
8	1	3	2	3	2	1	3	1	63,271	69,046	5775
9	1	3	3	1	3	2	1	2	101,712	107,487	5775
10	2	1	1	3	3	2	2	1	63,838	63,463	−375
11	2	1	2	1	1	3	3	2	79,380	79,005	−375
12	2	1	3	2	2	1	1	3	66,467	66,092	−375
13	2	2	1	2	3	1	3	2	76,103	82,252	6149
14	2	2	2	3	1	2	1	3	93,157	99,306	6149
15	2	2	3	1	2	3	2	1	66,382	72,531	6149
16	2	3	1	3	2	3	1	2	89,657	83,881	−5776
17	2	3	2	1	3	1	2	3	102,878	97,102	−5776
18	2	3	3	2	1	2	3	1	80,880	75,104	−5776