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UV mutagenesis for the overproduction of xylanase from *Bacillus mojavensis* PTCC 1723 and optimization of the production condition

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ABSTRACT

Objective(s): This study highlights xylanase overproduction from *Bacillus mojavensis* via UV mutagenesis and optimization of the production process.
Materials and Methods: *Bacillus mojavenis* PTCC 1723 underwent UV radiation. Mutants' primary screening was based on the enhanced Hollow Zone Diameter/ Colony Diameter Ration (H/C ratios) of the colonies in comparison with the wild strain on Xylan agar medium. Secondly, enzyme production of mutants was compared with parental strain. Optimization process using lignocellulolytic wastes was designed with Minitab software for the best overproducer mutant.
Results: H/C ratio of 3.1 was measured in mutant number 17 in comparison with the H/C ratio of the parental strain equal to 1.6. Selected mutant produced 330.56 IU/ml xylanase. It was 3.45 times more enzyme than the wild strain with 95.73 IU/ml xylanase. Optimization resulted 575 IU/ml xylanase, with wheat bran as the best carbon source, corn steep liquor as the best nitrogen source accompanied with natural bakery yeast powder, in a medium with pH 7, after 48 hr incubation at 37˚C, and the shaking rate of 230 rpm. Optimum xylanase activity was assayed at pH 7 and 40˚C. Enzyme stability pattern shows it retains 62% of its initial activity at pH 9 after 3 hr. It also maintains up to 66% and 59% of its initial activity after 1 hr of pre-incubation at 70˚C and 80˚C.
Conclusion: Mutation and optimization caused 5.9 times more enzyme yield by mutant strain. Also this enzyme can be categorized as an alkali-tolerant and thermo-stable xylanase.

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Introduction

The world market for industrial enzymes is estimated to be over billion dollars, and this rate is growing as new enzymes and functions are discovered (1, 2). Xylan hydrolyzing enzymes are important enzymes. Their degrading effect on plant cell wall named these enzymes as the one with numerous applications (1, 3). They perform in paper and pulp bleaching (4, 5), backing industry (6), textile industry (7, 8), animal feeds (9), and food industry (10). Xylanase production have been reported from bacteria, fungi, actinomycetes, and yeasts (11), Yet, the cultivation of actinomycetes and yeasts is often a hard work in large scale due to their slow generation time, high risk for medium contamination, and weak O_2 transfer (10, 12). Hence, bacteria such as *Bacillus* species have been using more vastly in industrial fermentations, to produce robust enzymes (12). *Bacillus* strains continue to be dominant enzyme producing microorganism in microbial biotechnology. These organisms are an important source of extracellular enzymes such as xylanase (2). Due to vast usage of xylanases, high enzyme production amount is of an essence. Different ways have been exploited to reach high xylanase yields. Culture optimization is one of the routine ways (9, 10, 13-15). Strain improvement techniques such as recombinant DNA technology

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(16), advanced cloning, protein recombination engineering (2), and parasexual recombination between overproducing strains (17) have been proved to be among the methods to improve production yield. In many other researches, chemical mutagenic agents like N-methyl N- nitro N-nitroso Guanidine (MNNG) (18, 19) and physical mutagens such as UV radiation with selection techniques resulted in xylanase overproducing strains (7, 20). UV radiation between the ranges of 200 to 280 nm, as a very convenient and fairly safe mutagenic agent by inducing Thymidine dimmers and increasing possibility of deletion during the duplication period, is a kind of classical mutagen (21). In addition, for high yields of enzyme production, the substrate selection is of great paramount (13). In large scale production processes, cheap lignocellulolytic wastes better to be considered to minimize the cost of the substrate and the production process (17). At the present study, *Bacillus mojavensis* persion type culture collection (PTCC) 1723 was used as a parental strain for enzyme production. The primary aim of this study is strain improvement, using UV radiation, in order to isolate a potent overproducing mutant, characterizing the enzyme features, and more enhancements in production yield via optimization. Optimization design was conducted by Minitab software, 16th version. Also, the media compositions were optimized using cheap lignocellulosic materials rather than pure xylan substrate in order to have an economical production process.

Materials and Methods

Materials

Oat spelt xylan (95590) was purchased from Fluka (Darmstadt- Germany). All other media components and chemicals used were obtained from Sigma-Aldrich (Darmstadt-Germany). Agricultural wastes were purchased from local market. Lignocellulosic materials were pre-treated by the method of Kapoor *et al* (22) and Abo-State *et al* (15).

Bacterial strain

B. mojavensis (PTCC 1723) was provided from Iranian Research Organization for Science and Technology, Tehran, Iran. Xylanase producing potentiality of this *Bacillus* was confirmed when it was cultured on Oat spelt Xylan-agar medium plates, pH 7, with the following composition (g/l); Oat spelt xylan 15, peptone 5, yeast extract 5, K_2HPO_4 1, $MgSO_4.7H_2O$ 0,2, and agar 15. Plates were incubated at 37˚C for 24 hr (23). Clear orange xylan digestion halo was appeared around the wild colony when it was subjected for staining with 0.1% w/v Congo-red dye solution for 10 min followed by repeated washing with 1 M NaCl solution (7, 9, 24). All experiments were performed in duplicate.

Culture conditions

Bacterial suspension was primed with the method described by Varalakshmi *et al* (25). Briefly, it was prepared with inoculation of a loopful colony into Luria-Bertani broth medium (L.B) , pH 7 (10 g Tryptone, 5 g yeast extract, 5 g NaCl in 1L of distilled water) and incubated at 37˚C for 18 hr in a shaking incubator (IRC-I-U,Clim-O-Shake, System Kuhner, Switzerland) at 150 rpm. 50 ml sterile oat spelt xylan broth medium without agar, in 250 ml flasks, were inoculated with 2% v/v *Bacillus* suspension (1.5×10^8 CFU/ml) equal to 0.5 degree of McFarland index (15). During 96 hr incubation at 37˚C and pH 7 under 160 rpm shaking speed, the enzyme production of parental strain was assayed at 24 hr intervals.

Xylanase activity assay

Sampling from flasks was performed in sterile conditions for xylanase assay. Taken samples were centrifuged in a microfuge at 8500* g for 5 min. And the clear cell-free culture supernatant was used as the crude enzyme source (10). Enzyme activity was determined by dinitrosalicylic acid stopping method. The reaction mixture contained 500 µl of 0.5% w/v respective homogenized substrate prepared in phosphate buffer, pH 7.0 and 500 µl appropriately diluted enzyme solution. The reaction mixture was incubated at 40˚C for 15 min (26, 27). D-xylose standard calibration curve was employed to determine the amount of reducing sugar released. One unit of xylanase activity was defined as the amount of enzyme required to release 1 micromole of D-xylose from its substrate in one min under the assay conditions (9).

UV irradiation

UV irradiation method was conducted based on a protocol described by Zarif *et al* (28), Aly *et al* (29), and Shafique *et al* (30). All cultured xylan agar plates with bacterial suspension were exposed to 280 nm short wavelengths UV light from a distance of 20 cm, using a 30 W germicidal Philips lamp. Irradiation time stopped at every 30 sec and finished when a survival cell rate of about % 0.01 was obtained. Effectible UV dose was that in which the survival cell rate of 0.01% observed (31). Survival curve was drawn based on colony counting on plates with survivals (30).

Mutants' selection

After UV irradiation, all the plates were incubated 24 hr in dark environment at 37˚C. Then, they were stained as described before to display xylan digestion haloes around the colonies (7, 9, 23, 24). H/C ratio measurement (Halo diameter zone / Colony diameter ratio) was used as a criterion to screen the mutant colonies. Those colonies showing

Table 1. Design table for the first phase of optimization (Factors and their levels)

Multilevel factorial design			
Factors	3	Replicates:	2
Base runs	60	Total runs	120
Base blocks	1	Total blocks	2
Number of levels	5, 4, 3		
Factor	Type	Levels	Values
Carbon source	Fixed	5	Wheat bran, Oat bran, Rice bran, Molasses, Bagasse
Nitrogen source	Fixed	4	Corn steep Liquor powder, Fish protein, Soy protein, Natural bakery yeast powder
Time course production profile	Fixed	3	24 hr, 48 hr, 72 hr

the H/C ratio bigger than that of the wild strain were selected (21, 31, 32).

Secondary screening

Screened colonies from first step were assayed for enzyme activity in oat spelt xylan containing liquid cultures. 50 ml of liquid culture media, as described above, but without agar in 250 mL erlenmeyer flasks, were inoculated with 0.2% v/v of each mutated bacterial suspension (15, 25). Flasks were incubated on a shaking-incubator at 37˚C for 96 hr with shaking rate of 160 rpm. Enzyme activity for mutated colonies were determined every 24 hr (9, 10, 26, 27). Finally, a mutant colony with highest enzyme activity was selected as a superior mutant and used for the optimization step.

Production medium optimization

Optimization process, designed with Minitab software 16th version, was conducted in 3 phases. At first phase of optimization, effects of 3 factors were assessed based on a full factorial design. Details of this design are shown in Table 1. Liquid culture composition was as xylan broth medium substituted with cheap indicated carbon sources, with constant 20 g/l concentration, and nitrogen sources with constant 10 g/l concentration. Enzyme production level was assayed at 24 hr intervals for 72 hr.

As it has been displayed in Table 3, last phase of optimization was designed based on the results obtained from first and second phase using response surface method (RSM) and central composite design (CCD).

Optimum pH and temperature of xylanase activity

Effects of pH and temperature on enzyme activity were determined based on a full factorial design, using Minitab software-16th version. Detail of this design is shown in Table 4. 0.05 M sodium phosphate buffer (pH 7 and/or 8) and 0.05 M Glycine-NaOH buffer (pH 9, 10, and/or 11) were used for assaying enzyme activity (23).

pH and temperature stability pattern of xylanase

Effect of pH on enzyme stability was determined by incubating the crude enzyme extract without substrate in different buffers at pH ranging from 8 to 11 for 4 hr. Residual xylanase activity was assayed at 1 hr intervals in 0.05 M sodium phosphate buffer and pH 7 at 40˚C. Thermostability for this enzyme was determined by assaying residual xylanase activities after incubation of the crude xylanase in the absence of the substrate at 40, 50, 60, 70, and 80˚C for 4 hr. Residual enzyme activity was assayed at 1 hr intervals in 0.05 M sodium phosphate buffer, pH 7, at 40˚C. All measurements were performed in duplicates.

Statistical analysis

The results were presented as means value for presented points. Statistical analysis for obtained data were performed automatically using one way analysis of variance (ANOVA) with Minitab software, 6th version. The impact of factors on each data was evaluated by paired t-test for all optimization steps. Significance of obtained results was accepted at $0.00<P\text{-value}<0.05$. Analysis of result's validity were checked considering *P*-value of factors in each experiment ranging from *P*-value> 0.00 to *P*-value<0.05.

Results

Effects of UV radiation on surviving cells and their frequency were measured as presented in Figure 1. The best time of irradiation has been calculated as ratio of surviving cells to the total number of cells at the beginning of the treatment, which were observed after 210 sec exposure to UV light. This is the point at which 99.99% of the initial cells have been killed.

Table 2. Experimental range and levels of independent variables in second phase of optimization

Wheat bran (g/ l)	Corn steep Powder (g/l)	Inoculum % (v/v)	K_2HPO_4 (g/l)	KH_2PO_4 (g/L)	$MgSO_4.7H_2O$ (g/ l	pH	Tem	Rpm	Malt extract (g/l)	Natural bakery yeast (g/l)	Tween 80 % (v/v)	Trace elements solution %(v/v)
20.0	5.0	10	1.0	0.0	0.0	7	30.0	200	0.0	5.0	0.0	0.2
20.0	10.0	2	1.0	1.0	0.0	7	30.0	160	5.0	0.0	0.2	0.0
15.0	10.0	10	0.0	1.0	0.2	7	30.0	160	0.0	5.0	0.0	0.2
15.0	5.0	10	1.0	0.0	0.2	9	30.0	160	0.0	0.0	0.2	0.0
20.0	5.0	2	1.0	1.0	0.0	9	37.0	160	0.0	0.0	0.0	0.2
20.0	10.0	2	0.0	1.0	0.2	7	37.0	200	0.0	0.0	0.0	0.0
20.0	10.0	10	0.0	0.0	0.2	9	30.0	200	5.0	0.0	0.0	0.0
20.0	10.0	10	1.0	0.0	0.0	9	37.0	160	5.0	5.0	0.0	0.0
15.0	10.0	10	1.0	1.0	0.0	7	37.0	200	0.0	5.0	0.2	0.0
20.0	5.0	10	1.0	1.0	0.2	7	30.0	200	5.0	0.0	0.2	0.2
15.0	10.0	2	1.0	1.0	0.2	9	30.0	160	5.0	5.0	0.0	0.2
20.0	5.0	10	0.0	1.0	0.2	9	37.0	160	0.0	5.0	0.2	0.0
15.0	10.0	2	1.0	0.0	0.2	9	37.0	200	0.0	0.0	0.2	0.2
15.0	5.0	10	0.0	1.0	0.0	9	37.0	200	5.0	0.0	0.0	0.2
15.0	5.0	2	1.0	0.0	0.2	7	37.0	200	5.0	5.0	0.0	0.0
15.0	5.0	2	0.0	1.0	0.0	9	30.0	200	5.0	5.0	0.2	0.0
20.0	5.0	2	0.0	0.0	0.2	7	37.0	160	5.0	5.0	0.2	0.2
20.0	10.0	2	0.0	0.0	0.0	9	30.0	200	0.0	5.0	0.2	0.2
15.0	10.0	10	0.0	0.0	0.0	7	37.0	160	5.0	0.0	0.2	0.2
15.0	5.0	2	0.0	0.0	0.0	7	30.0	160	0.0	0.0	0.0	0.0
17.5	7.5	6	0.5	0.5	0.1	8	33.5	180	2.5	2.5	0.1	0.1
20.0	5.0	10	1.0	0.0	0.0	7	30.0	200	0.0	5.0	0.0	0.2
20.0	10.0	2	1.0	1.0	0.0	7	30.0	160	5.0	0.0	0.2	0.0
15.0	10.0	10	0.0	1.0	0.2	7	30.0	160	0.0	5.0	0.0	0.2
15.0	5.0	10	1.0	0.0	0.2	9	30.0	160	0.0	0.0	0.2	0.0
20.0	5.0	2	1.0	1.0	0.0	9	37.0	160	0.0	0.0	0.0	0.2
20.0	10.0	2	0.0	1.0	0.2	7	37.0	200	0.0	0.0	0.0	0.0
20.0	10.0	10	0.0	0.0	0.2	9	30.0	200	5.0	0.0	0.0	0.0
20.0	10.0	10	1.0	0.0	0.0	9	37.0	160	5.0	5.0	0.0	0.0
15.0	10.0	10	1.0	1.0	0.0	7	37.0	200	0.0	5.0	0.2	0.0
20.0	5.0	10	1.0	1.0	0.2	7	30.0	200	5.0	0.0	0.2	0.2
15.0	10.0	2	1.0	1.0	0.2	9	30.0	160	5.0	5.0	0.0	0.2
20.0	5.0	10	0.0	1.0	0.2	9	37.0	160	0.0	5.0	0.2	0.0
15.0	10.0	2	1.0	0.0	0.2	9	37.0	200	0.0	0.0	0.2	0.2
15.0	5.0	10	0.0	1.0	0.0	9	37.0	200	5.0	0.0	0.0	0.2
15.0	5.0	2	1.0	0.0	0.2	7	37.0	200	5.0	5.0	0.0	0.0
15.0	5.0	2	0.0	1.0	0.0	9	30.0	200	5.0	5.0	0.2	0.0
20.0	5.0	2	0.0	0.0	0.2	7	37.0	160	5.0	5.0	0.2	0.2
20.0	10.0	2	0.0	0.0	0.0	9	30.0	200	0.0	5.0	0.2	0.2
15.0	10.0	10	0.0	0.0	0.0	7	37.0	160	5.0	0.0	0.2	0.2
15.0	5.0	2	0.0	0.0	0.0	7	30.0	160	0.0	0.0	0.0	0.0
17.5	7.5	6	0.5	0.5	0.1	8	33.5	180	2.5	2.5	0.1	0.1

After repeated treatment of wild strain by UV rays, obtained colonies underwent screening steps.

The H/C ratio equal to about 1.6 mm was measured for the wild strain, and UV treated colonies with bigger H/C ratio than that of the parental strain were selected as the mutant colonies (31, 32). At first screening step, among numerous colonies obtained after UV treatment, 36 colonies showed the H/C ratio≥1.6. As it has been drawn in Figure 2-A, the mutant colony number 17 has shown the biggest H/C ratio equal to 3.1 among the other screened colonies. In Figure 2-B, the frequency of mutant colonies with bigger H/C rate than that of the parental strain is drawn. In secondary screening step, the enzyme activity of screened colonies measured in liquid culture base on Bailey and Miller method. Data in Figure 2-C revealed that the mutant number 17, producing 330.56 IU/ml enzyme activity, has the potentiality to produce 3.4 times more enzyme than that of the parental strain with 95.73 IU/ml initial enzyme production. In Figure 2-D, the frequency of mutated colonies with higher enzyme activity has been drawn. Based on statistical analysis, it was found that the H/C ratio measurements outcome is mighty compatible with the results adapted from determining the enzyme activity amounts of selected mutants. Indeed, the correlation coefficient between results from the first and secondary screening step is about 0.853 with $0.00 < P\text{-value} < 0.05$. This implies a strong correlation between the semi-qualitative and quantitative experiments. The coefficient of determination (r^2) of these 2 experiments have been calculated about 0.0727%, This reveals that in 72.7% cases, the enzyme production level of mutants can be estimated according to the results obtained from the first screening step just by calculation of H/C ratio of mutated colonies.

Table 3. Central composite experimental design chart at 3rd phase of optimization

Central composite design (CCD)	
Factors : 5	Replicates :2
Base runs: 32	Total runs: 64
Base blocks : 1	Total blocks:1
Two-level factorial: Half fraction	
Cube points:	32
Center points in cube:	12
Axial points: 20	
Center points in axial: 0	

Finally, the mutant number 17 was selected as the potent mutant colony and underwent optimization step.

Optimization results

As it has been indicated in Figure 3, wheat bran as the best carbon source accompanied with corn steep liquor powder as the best nitrogen source enhanced enzyme activity to about 412.23 IU/ml after 48 hr incubation.

In second phase of optimization, effects of other parameters for enzyme production has been evaluated. For example, increasing the amount of main carbon source, wheat bran, is not suitable for enhanced enzyme production. Also, the presence of KH_2PO_4 and malt extract does not affect enzyme production, so these factors can be ignored in culture composition. In fact, 2nd phase of optimization disclosed the best inducers for enhanced xylanase production in culture medium. Data analysis of 2nd phase of optimization reveals five main factors in which their presence have major effect on increasing enzyme yield. Figure 4 depicts important factors which have been shown on right hand side of the vertical standardized line of Pareto chart. They are significant factors in which have led the increase in enzyme production amount in culture composition.

At 3rd phase of optimization, the exact level and materials concentration of factors (g/l) were optimized. Briefly, wheat bran as the best carbon source with 7.5 g/l concentration, corn steep liquor powder as the best nitrogen source with 6 g/l concentration, natural bakery yeast powder with 12 g/l concentration, K_2HPO_4 as the best additive with 0.5 g/l concentration, and optimum shaking speed of

Table 4. Full factorial design for determination of optimum pH and temperature of enzyme activity. Variables and their levels

Multilevel factorial design			
Factors: 2		Replicates: 2	
Base runs: 30		Total runs: 60	
Base blocks: 1		Total blocks: 1	
Number of levels: 5, 6			
General linear model: Enzyme activity (IU/ml) versus pH, Temperature			
Factor	Type	Levels	Values
pH	Fixed	5	7, 8, 9, 10, 11
Temperature	Fixed	6	30, 40, 50, 60, 80 (°C)

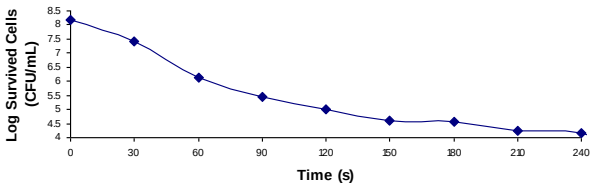

Figure 1. The effect of UV light irradiation on survival of *Bacillus mojavensis* PTCC1723. Data points have been expressed as means= 2)

230 rpm induced the enzyme activityto 575.109 IU/ml after 48 hr of incubation at 37˚C, and pH 7. Interaction effects of factors on enhancement of xylanase production in liquid culture could be observed in contour plots in Figure 5.

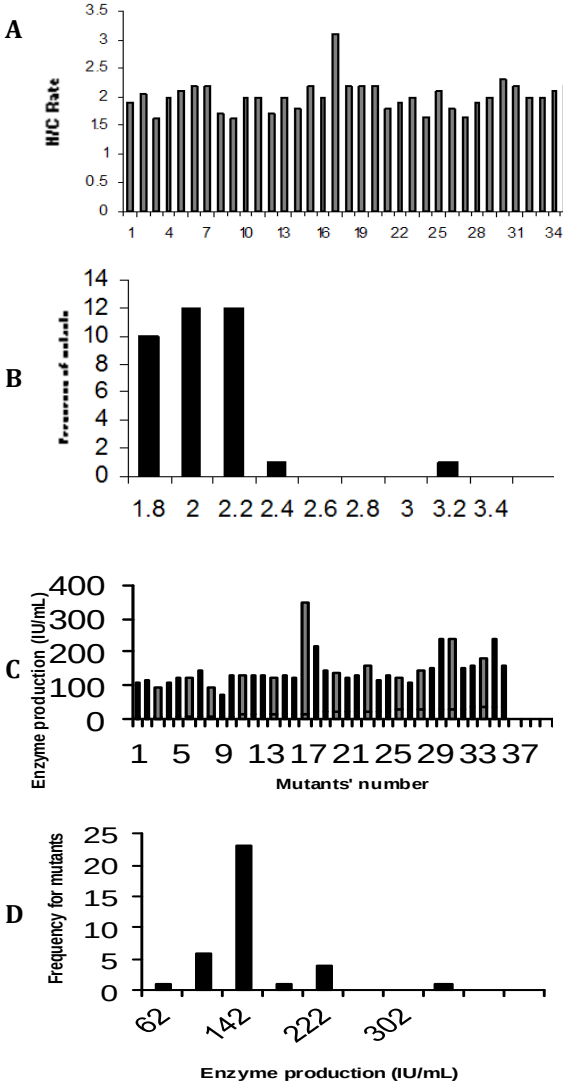

Figure 2. Results for first and secondary screening step: A: Comparison of H/C ratio among mutant colonies treated with UV rays B: Frequency of mutants with bigger H/C ration than that of the parental strain C: Comparison of enzyme production level among mutant strains D: Frequency of mutants with higher enzyme production level than that of the parental strain. Data points have been expressed as means (n=3)

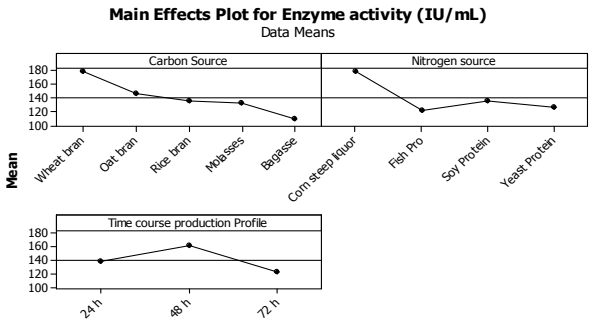

Figure 3. Main effects of 3 chief factors on enzyme production at first phase of optimization

A final verification test, given by Minitab software, was conducted in order to acknowledge the accuracy of the optimization model. According to verification test (Table 5), 575.109 IU/ml was observed as the highest experimental value for enzyme productivity under the desired condition. Predicted value for highest enzyme activity by software was about 600.144 IU/ml. However, the accuracy of the model, designing process, and obtained results was verified, since the difference between predicted value and the experimental result was not significant.

Enzyme characterization

According to Figure 6, in section A and B, optimum xylanase activity from the mutant strain was found to be at 40˚C, and pH 7. All the experiments were tested in duplicates and the results shown are the mean values.

The alkali-stability pattern of xylanase reveals that this enzyme can easily tolerate high pH ranges. Noticing Figure 6-C, it is understandable that this enzyme could at least hold about 50% of its maximum activity at pH 8, 9, and 10 during 4 hr pre-incubation. Therefore, produced xylanase is an alkaline stable enzyme.

As it is presented in Figure 6-D, the enzyme has acquired a good stability at 50 and 60˚C, since there was no drastic failure in xylanase activity during4 hr of pre-incubation at these temperatures. It retained 66% and 59% of its maximum activity after 1 hr pre-incubation at 70 and 80˚C. Also, after 4 hr pre-incubation of crude enzyme solution at 60˚C, residual enzyme activity up to 62% was assayed.

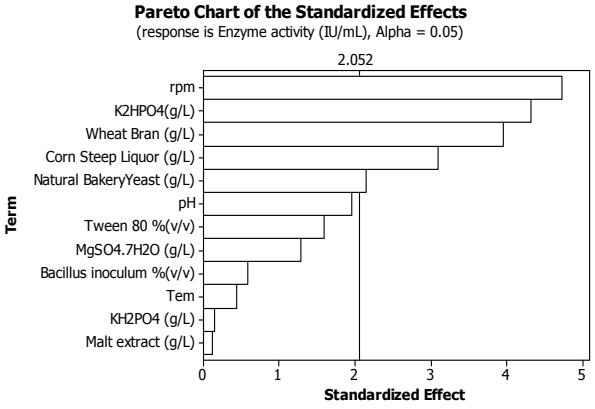

Figure 4. Significant factors that affect high xylanase production in liquid culture

Discussion

Owing to industrial applications of xylanases, the enzyme's high yield is demanding, and researchers have fulfilled this aim by testing different methods. Tork *et al* (16), in 2013, enhanced xylanase production from *Bacillus subtilis* XP10 by 5 fold using recombinant techniques. Desphpande *et al* (33), in 2008, rendered a mixed culture of *Aspergillus niger* and *Trichoderma reesei* QM, mutant number 414, to increase xylanase production in the fermentation medium. For better efficiency, random mutagenesis is an applicable technique for strain improvement (34). It can enhance enzyme productivity several fold in comparison with the wild strains. It is presumed that this rate can be increased by repeating further random mutagenesis and can be continued considering the fact that this kind of mutation is an accidental event (18, 19). UV mutation, for example, led to 161% increase in xylanase production by a mutant strain of *Streptomyces pseudogriselus* (7). In similar research, xylanase production from initial 115 unit boosted to 215 IU/ml (1.7 fold increase) when *A. niger* GCBT-35 treated with chemical mutagenesis agent such as N-methyl N- nitro N-nitroso guanidine (19). Similarly, Rahim *et al* (20), in 2009, induced a mutation at *Neurospora crassa* with UV rays at 254 nm. Optimization of the production process, on the other hands, plays a crucial role in enhancement of enzyme production amount (18). As a case in point, Porsuk *et al* (9) increased this rate by 5 times when medium optimization caused 255 IU/ml xylanase production from *Streptomyces* sp. CA24. Many researchers use a combination of mutation and culture optimization techniques in order to elevate xylanase yields. For example, *A. niger* RH12 produces 1.65 times more enzyme (172.31 IU/ml) after treatment with chemical mutagenesis agent in comparison with its initial production ability, which equal to 104.59. This rate increased to 2.7 fold to final 289.86 IU/ml xylanase production when medium is optimized with wheat bran as the best carbon source and meat extract as the best nitrogen source (18).

Table 5. Verification test chart designed with Minitab software

Response optimization						
Actual response: Xylanase activity = 575 IU/ml						
Parameters						
	Goal	Lower	Target	Upper	Weight	Import
Enzyme activity	Maximum	430	580	580	1	1
Global solution						
Wheat bran= 7.5						
Corn steep liquor powder= 6						
K_2HPO_4 (g/l)= 0.5						
Natural bakery yeast powder= 12.5						
rpm= 230						
Predicted responses						
Enzyme activity= 600.144 , desirability = 1.000000						
Composite desirability= 1.000000						

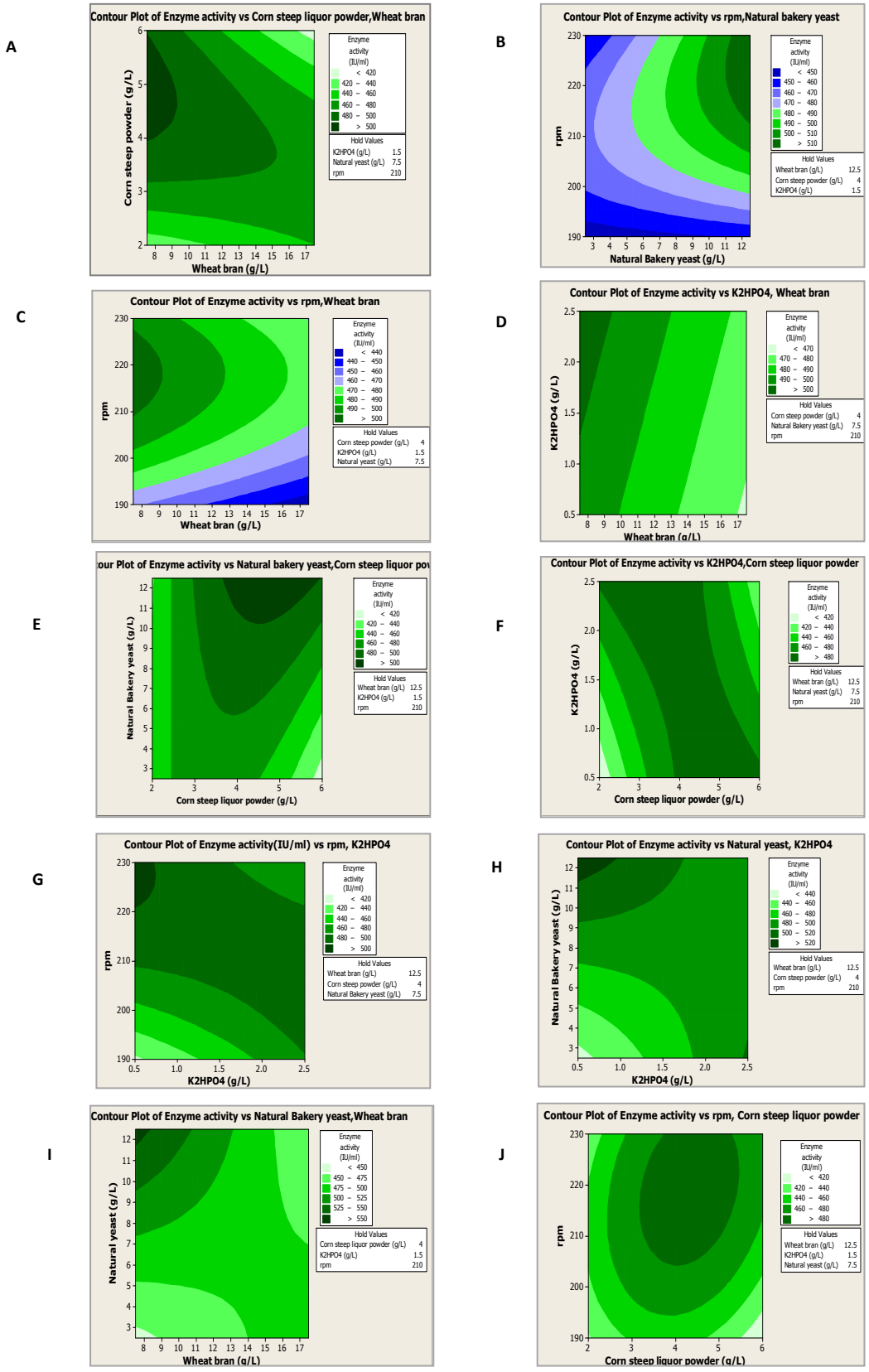

Figure 5. Contour plots of xylanase production from mutant number 17, and interactions between variables two by two: A: Interaction of corn steep liquor and wheat barn. B: Interaction of rpm and natural bakery yeast powder. C: Interaction of rpm and wheat barn. D: Interaction of K_2HPO_4 and wheat barn. E: Interaction of natural bakery yeast powder and corn steep liquor. F: Interaction of K_2HPO_4 and corn steep liquor. G: Interaction of rpm and K_2HPO_4. H: Interaction of natural bakery yeast powder and K_2HPO_4. I: Interaction of natural bakery yeast powder and wheat barn. J: Interaction of rpm and corn steep liquor

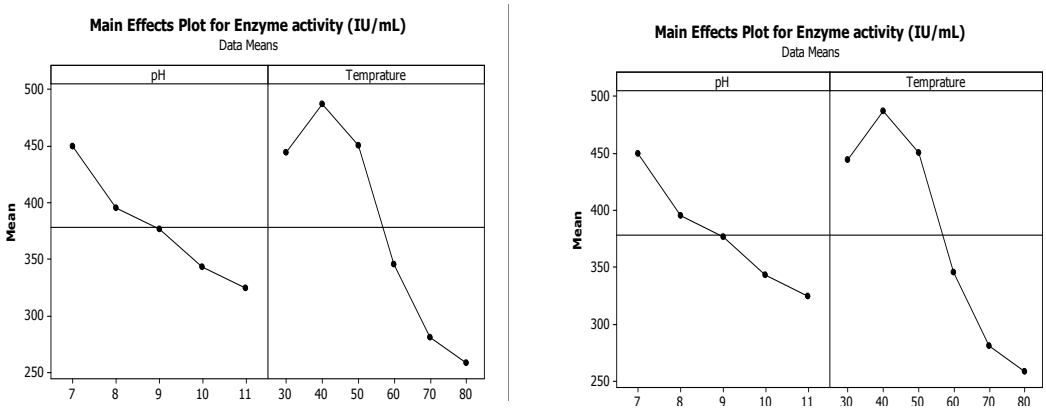

Figure 6. Xylanase characterization produced from mutant strain**:** A: Optimal pH and temperature of xylanase activity. B: pH and temperature profile of xylanase activity from mutant strain. C: pH stability pattern of xylanase from mutant strain. D: Thermal stability pattern of xylanase from mutant strain

Dwivedi *et al* (8), in 2009, used cheap lignocellulosic material such as congress grass as carbon source for medium optimization cultured with *Penicilium oxalicum* mutant SAUE3.510. First, mutagenesis with UV and Ethidium bromide together resulted an overproducer mutant. Finally, optimization of the production medium led to 475.2 IU/ml xylanase yield, which was 1.87 times higher than the xylanase level from parental strain.

Accordingly, presented results in this article, for increasing xylanase yield via mutation, optimization, and using lignocellulosic material such as wheat bran as the best carbon source are in comply with other researches. Similarly, Coman and Bahrim (14) used wheat bran as carbon source and as a powerful xylanase inducer in fermentation medium inoculated with *Streptomyces* spp. P12-137. Abo-state *et al* (15) reported 373 IU/ml xylanase production from *Bacillus* isolate strain MAM-29 using rice straw for medium optimization. Similar results have been reported by Sharma *et al* in 2013 (12). They reduced the enzyme production cost utilizing low cost and abundantly available forest agro residuals as substrate for xylanase production from *Bacillus coagulans* B30. Sugumaran *et al* (10), in 2013, reported about 7.5 IU/ml xylanase from *Bacillus subtilis* after optimizing culture medium with cassava bagasse as carbon source and yeast extract as nitrogen source. Also Gupta *et al* (13) reported wheat straw as carbon source in his optimization research for xylanase production with *Melanocarpus albomyces*. Therefore, results in this research is in agreement with other researches, in which wheat bran as carbon source, corn steep liquor as nitrogen source and natural bakery yeast powder were logical and low cost inducers for high xylanase production from mutant *B. mojavensis* strain, whilst the cost of fermentation process and medium preparation was too low.

It is noteworthy that, considering a vast review among the xylanase related researches; this is the first report among the printed articles that cheap lignocellolytic materials in combination with cheap nitrogen sources such as corn steep liquor powder and natural bakery yeast powder have been rendered. In all optimization researches for xylanase production, mineral or organic nitrogen sources such as yeast extract (22), peptone, tryptone (23), meat extract (18, 35), ammonium nitrate , sodium nitrate (11), ammonium sulfate (36), and so on (37) have been used. Thus, this finding indicates the possibility of xylanase production cost cut down by considering the other main xylanase inducers such as nitrogen sources in fermentation medium. The enzymes characteristics depend on their origin from which they have been isolated. There are many reports for thermostable and alkaline stable xylanases produced from different microorganism (1, 2, 4). Xylanase produced from mutant *Penicillium oxalicum* showed a reasonable stability at 80˚C for about 2 hr. This enzyme also was stable at pH 9 for 24 hr, so the produced enzyme named as an alkali-tolerant and thermo stable enzyme (8). Also Knob and Carmona (38), in 2008, reported a thermo stable xylanase from *Penicillium sclerotiorum*, which was stable at 40˚C for 72 min. In this research, according to Figure 6-C and Figure 6-D, the produced xylanase can tolerate alkaline and high temperature conditions. Accordingly, xylanase from mutant strain number 17 can be named as an alkali-tolerant and thermo stable enzyme. These findings can be useful for enzymes' industrial applications.

Conclusion

In this investigation, UV radiation at 280 nm increased xylanase production to 3.45 times from a novel mutant strain number 17 derived from *B. mojavensis* PTCC 1723. In addition, optimization process caused 1.7 fold more enzyme production amounts. The outcome strongly supports that mutation and optimization of the production medium in combination not only reinforced xylanase

overproduction up to 5.9 times, but also diminished the cost of the production process using cheap nutritional sources. Given results indicated that it is feasible to apply both cheap carbon and nitrogen sources to induce high xylanase yields. Furthermore, chosen model for the optimization was efficient, relatively simple, and time and material saving. This study also concluded that using hybrid techniques such as mutation and culture optimization together can result better and faster for having overproducer strains in industrial microbiology and biotechnology.

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