THERMOSTABLE XYLANASE PRODUCTION BY *Rhizomucor pusillus* UNDER SOLID STATE FERMENTATION: A STATISTICAL OPTIMIZATION

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Keywords: Xylanase, Taguchi methodology, corn cob

Introduction. Xylanases are hydrolytic enzymes, which can completely hydrolyze xylan. A variety of microorganisms have been reported to produce xylanolytic enzymes. The production differed between strains and can be regulated by the physiological, nutritional and biochemical nature of the microbes employed (1). On this way, to search for optimal production conditions is needed.

The aim of this work was to explore the best conditions for the production of extracellular xylanase by isolated *Rhizomucor pusillus* strain using the Taguchi methodology.

Methodology. The xylanase production ability of *Rhizomucor pusillus* (DIQ-UAdeC collection) was analyzed in solid state fermentation (SSF) using corncon (CC) as support-substrate. Taguchi design of experiments (DOE) was employed to conduct the enzyme production optimization (1) (Table 1). SSF experiments were performed employing the selected eighth experimental trials in tray bioreactors. All experiments were conducted at 50 °C. Qualitek-4 software was used for data analysis. Xylanase activity was measured according to the analytical method of Bailey *et al*., (2). One unit of xylanase activity defined as the amount of enzyme required to liberate 1 µmol of xylose/mL under experimental conditions, and expressed as units per gram of dry support (U/gds).

Table 1 Selected fermentation factors and their assigned levels for xylanase production with *Rhizomucor pusillus*.

S. no.	Factor	Level 1	Level 2
1	Moisture 100 x (g water/g substrate dry weight)	2.5	3.5
2	Nitrate (g $NaNO_3$/g substrate dry weight)	0.3	0.6
3	Inoculum (mL spore solución/g substrate dry weight)	0.15	0.6
4	Incubation time (h)	72	96

Results. According to the results, all factors under study influenced xylanase production. Incubation time was the major (~37 %) influential parameter on xylanase production at the individual level. At the interactive level, nitrate was important and accounted for more than 67 % of the severity index with inoculum and more than 32 % with moisture interaction. Xylanase production improved from 8,992 U/gds to 15,635 U/gds indicating an improvement of 74 % after optimization comparison with control experiments.

Table 2 Xylanase production optimum conditions and performance by *Rhizomucor pusillus* under solid state fermentation using corn cob as substrate-support.

S. no.	Factor	Level description	Level	Contribution
1	Moisture	3.5	2	1643.12
2	Nitrate	0.3	1	539.58
3	Inoculum	0.65	2	793.99
4	Incubation	96	2	1990.30
Total contribution from all factors				4966.98
Current grand average of performance				8947.58
Expected results at optimum condition				13914.56

Conclusion. Statistical optimization technique was successfully employed for enhancement of xylanase production. The experimental design determined the influence of individual factors and helped in establishing the relationship between variables and operational conditions as well as the optimal levels for best performance. Validation of the findings of the experimental design demonstrated a significant improvement in enzyme production.

Acknowledgement. Author Robledo thanks to the CONACYT for the financial support.

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