INOCULUM EFFECTS ON THE PRODUCTION OF PIGMENTS BY *Penicillium purpurogenum* GH2 IN SUBMERGED FERMENTATION

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Key words: pigments, inoculum, factorial design.

Introduction. The worldwide demand for natural food and textile colorants is rapidly increasing. It has been reported that some microorganisms have the ability to produce pigments in high quantities (1). The production, extraction, the diversity in microorganisms and sophistication of technology has made their choice more feasible (2). In industrial fermentation processes the inoculum or seed cultured played an important role. Depending on the desired product, the optimal inoculum for a given bioprocess varies and cannot be generalized (3). Seed cultured is influenced by the inoculum concentration, spore viability, pH value, temperature, dissolved oxygen concentration, and mechanical stress (4). This study aims to identify the factors affecting seed culture preparation for the production of pigments by *Penicillium purpurogenum* GH2.

Methods. *Penicillium purpurogenum* GH2 (DIA-UAdeC collection) was used for the pigment production in this study. The medium Potato Dextrose Broth (ATCC medium: 336) was used for the seed culture preparation. The individual and combined effects of initial pH, agitation speed and inoculum concentration were investigated through a 2^3 full factorial design (Table 1). Production, recovery and analysis of pigments were made according to the methodology reported by Morales-Oyervides et al. 2011. Analysis of variance (ANOVA) and Tukey's multiple-comparison method at the 95% significance were performed using Statistica 8.0 (StatSoft Inc., USA).

Table 1. Experimental levels of the independent variables according to the 2^3 full-factorial design.

Independent variable	Symbol	Levels	
		-1	+1
pH	X_1	5	7
Inoculum concentration, espmL^{-1}	X_2	5x10^4	5x10^6
Agitation, rpm	X_3	100	200

Results. The experimental results obtained for the production of pigments by *Penicillium purpurogenum* GH2, under different seed cultured preparation conditions according to a 2^3 full factorial design, are shown in Table 2.

Table 2. Experimental matrix and results of biomass (B), pigments production (P) and pigments per biomass yield ($Y_{P/B}$) with coded levels of the variables.

Runs	Independent Variables			Responses		
	X_1	X_2	X_3	B (gL^{-1})	P (OD_{500nm})	$Y_{P/B}$ (ODLg^{-1})
1	-1	-1	-1	6.33	8.68	1.48
2	-1	-1	+1	6.07	19.08	3.04
3	-1	+1	-1	6.52	13.86	2.05
4	-1	+1	+1	6.25	18.03	2.65
5	+1	-1	-1	8.63	9.66	1.10
6	+1	-1	+1	6.34	14.51	2.25
7	+1	+1	-1	7.48	12.55	1.61
8	+1	+1	+1	6.16	18.47	2.88

It can be noted that *Penicillium purpurogenum* GH2 was able to growth and produce pigments under all the evaluated seed culture conditions, however, the production strongly varied according to the levels employed for the independent variables. According to the statistical analysis, in the studied range of values, the inoculum concentration and agitation are the variables that had the greatest influence on this process. The highest production of pigments (19.08 OD) was obtained when using an initial pH of 5, inoculum concentration of 5x10^4 espmL^{-1} and at 200 rpm (Run 2). Under these same conditions the pigment per biomass yield also achieved the highest value (3.04 ODLg^{-1}).

Conclusions. The study shows that seed culture preparation conditions have considerable influence on the fermentative process of pigments production by *Penicillium purpurogenum* GH2.

References.

1. Mapari S, Nielsen K, Larsen T, Frisvad J, Meyer A, Thrane U. (2005). *Curr. Opin. Biotechnol.* 16(2):231–238
2. Juzlova P, Martinkova L, Lozinski J, Machek F. (1994). *Enzyme Microb. Technol.* 16(11), 231-235.
3. Gibbs P, Seviour R, Schmid F. (2000). *Crit. Rev. Biotechnol.* 20(1):17–48
4. Deckwer W, Jahn D, Hempel D, Zeng A. (2006). System biology approach to bioprocess development. *Eng. Life Sci.* 6(5): 455–469.
5. Morales-Oyervides, L. (2011). Tesis de Licenciatura. Universidad Autonoma de Coahuila.