OPTIMIZATION OF LIGNINOLYTIC ENZYMES PRODUCTION FROM COCULTURE OF *Pleurotus opuntiae* AND *Paecilomyces carneus* USING PLACKETT-BURMAN DESIGN

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Introduction. Cocultures of different micro-organisms may be advantageous for the production of enzymes. One example is the production of laccase and manganese peroxidase (MnP) (1), the genera *Pleurotus* has been studied widely for the production of these enzymes; however the specie *P. opuntiae* up till now has not been studied. Previously we found (data unpublished) that the coculture of *P. opuntiae* with *Paecilomyces carneus* (a soil borne filamentous microfungi) resulted in 1.5 and 0.2 increase fold of laccase and MnP production respectively, in a Sivakumar liquid medium (2). Therefore the aim of this work was to optimize the production of laccase and MnP in the coculture of *P. opuntiae* and *P. carneus* using a Plackett-Burman design.

Methods. Eleven variables were assessed for laccase and MnP production (U = IU/mg of protein), the factors were the components of the culture medium (10 elements) and the amount of inoculum, each variable was evaluated at two levels high (+1) and low (-1), and four central points (0) were used for a total of 16 experiments.

Results. The variables that were significant ($p<0.05$) for laccase production were: glucose, KH_2PO_4, $(NH_4)_2SO_4$, $FeSO_4$, $MnSO_4$, $CuSO_4$ and inoculum (Figure 1), with a correlation between the mathematical model and experimental results (R^2=0.98, p=0.01).

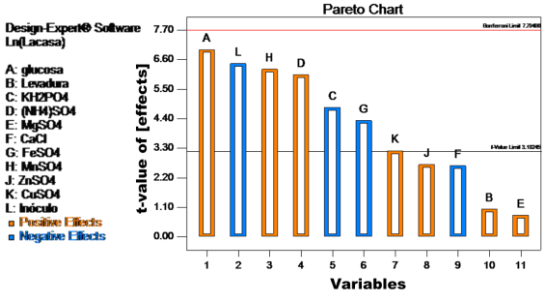

Fig. 1. Pareto diagram showing the effect of the factors evaluated on the laccase production.

For the production of MnP variables that were significant ($p<0.05$) were: Glucose, Yeast, $MgSO_4$, CaCl, $MnSO_4$ and inoculum, with a correlation between the model and the variables (R^2=0.94, p=0.04) (Figure 2). The table 1 shows the laccase and MnP activity in all experiments.

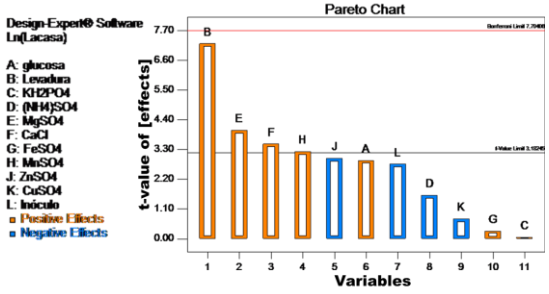

Fig. 2. Pareto diagram showing the effect of the factors evaluated on the MnP production.

Table 1. Laccase and MnP production in a Placket-Burman design.

Stan. order	Treatment	Laccase (U)	MnP (U)
1	8	33331.3	638.4
2	10	1785.9	434.8
3	15	16022.9	260.7
4	13	25461.6	328.8
5	4	13373.9	220.9
6	14	7037.8	50.7
7	1	14621.1	216.4
8	5	16625.1	371.8
9	2	12513.6	211.5
10	6	13098.4	177.4
11	12	13085.8	84.1
12	7	8082.7	88.4
13	3	12113.4	155.3
14	16	11942.8	147.9
15	11	13547.4	217.8
16	9	9292.4	128.5

Conclusions. Laccase and MnP production in treatment 8 under Plackett-Burman design increased 2.1 and 1.7 fold in contrast of normal coculture. The common variables that affected the production of laccase and MnP were glucose, $MnSO_4$ and inoculum.

References.

1. Bade J, Mast GE, Popovik MK, Bajpai R, Stahl U. (2010). *J. Appl. Microbiol*. 109: 371-387.
2. Sivakumar R, Rajendran R, Balakumar C, Tamilvendran M. (2010). *Int. J. Engine. Scien. Tech*. 2(12): 7133-7141.