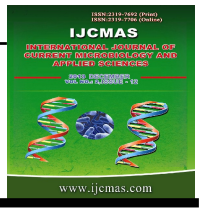
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 12 (2013) pp. 206-218**
http://www.ijcmas.com



Original Research Article

Optimization of nutrition factors for *Ceriporiopsis subvermispora* biomass production

Daniela Chmelová[1] and Miroslav Ondrejovič[2*]

[1]Department of Biochemistry and Biotechnology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic
[2]Department of Biotechnology, Faculty of Natural Sciences,University of Ss. Cyril and Methodius, Nám. J. Herdu 2, 917 01 Trnava, Slovak Republic,
Corresponding author

A B S T R A C T

The main aim of this study was focused on selecting suitable propagation medium, optimizing its components of medium by response surface methodology for biomass production of *Ceriporiopsis subvermispora*. The independent variables were concentration of glucose (carbon source) (3.2, 15, 32.5, 50 and 61.2 g/l), casein hydrolysate (nitrogen source) (4.95, 10, 17.5, 25 and 30 g/l) and manganese ions (0, 0.203, 0.5, 0.797 and 1.0 mmol/l). The dependent variables were dry biomass (g/l of propagation medium), glucose utilization (%) and laccase activity (U/l). A polynomial regression model containing linear, interaction and quadratic terms was used for analysis of the experimental data. The optimal conditions for biomass production were 61.1 g/l glucose as carbon source, 21.8 g/l casein hydrolysate as nitrogen source and 0.03 mmol/l of manganese ions. Secondary, the optimal conditions for effective glucose utilization as a technological parameter were 3.34 g/l glucose as carbon source, 28.0 g/l casein hydrolysate as nitrogen source and 0.56 mmol/l of manganese ions. Finally, the results show that the optimal conditions for the biomass growth of *C. subvermispora* are not suitable for production of laccase. Optimal growth condition of *C. subvermispora* can be used for cultivation of fungal biomass intended for laccaseproduction.

Keywords

White-rot fungus; propagation medium; response surface methodology; laccase.

Introduction

Ceriporiopsis subvermispora is a white-rot basidiomycete which can degrade lignin preferentially before cellulose during degradation of lignocellulose material (Souza-Cruz *et al*., 2004). *C. subvermispora* produces hydrolases such as hemicellulases and the incomplete cellulose – degrading system, and oxidoreductases such as laccase and manganese peroxidases (MnP) (Guerra *et al*., 2003; Souza-Cruz *et al*., 2004). It lacks lignin peroxidase (LiP) activity, although LiP-like genes have been detected (Rajakumar *et al*., 1996). From

the group oxidoreductases, laccase is an interesting enzyme of these fungi, because is able oxidize phenolic and non-phenolic compounds. Therefore, laccase has many applications in various sectors of industry and in environmental biotechnology (Couto and Toca-Herrera, 2006; Shekker *et al*., 2011; Sarria-Alfonso *et al*., 2012).

The production of laccase is usually described as part of secondary metabolism of white-rot fungi (Howard *et al*., 2003), which starts at the negative environmental conditions such as lack of nutrients, the presence of specific organic compounds in cultivation medium (catechol, syringaldazine, 2,5-xylidine, veratryl alcohol), or negative growth conditions (low temperature, pH, pressure, ionic strength) (Couto and Toca-Herrera, 2006; Levin *et al*., 2010). On the other hand, the negative environmental conditions inhibit the effective accumulation of fungal biomass. Therefore, laccase can be produced by white-rot fungi in two-step system. In the first step, the fungal biomass must be produced and consequently this biomass exposed to negative environmental factors can produce target enzymes.

Optimal growth condition for white-rot fungi is pH ranging from 4.5 to 5.5 and temperature varying from 25 to 30 °C (Galhaup *et al*., 2002). For biomass production, easily utilizable carbon and nitrogen sources can be used (Mikiashvili *et al*., 2006). The most commonly used carbon sources are glucose, mannose, fructose and lactose and nitrogen sources are yeast extract, peptone, urea, ammonium sulfate and sodium nitrate (Shekker *et al*., 2011). For optimization of growth medium composition can be successfully employed response surface methodology (RSM) as statistical tool because allows to value the effects of each independent variables but almost the mutually effects of all independent variables to selected dependent variable (Vyas *et al*., 2013).

The aim of this work was to optimize propagation medium composition to reach maximum biomass production of *C. subvermispora* by using RSM system. The results can be subsequently used for preparation of fungal biomass for production of laccase.

Materials and Methods

Microorganism

Culture of *Ceriporiopsis subvermispora* ATTC 90467 was provided from the Centraal bureavoor Schimmel cultures (Netherlands). The culture was maintained on malt agar at 4 °C. In all cases, the suspension of fungal mycelium was prepared by scraping of plaque (1 cm^2) of the growth culture from agar plate using microbiological loop and mixing in sterile deionized water (10 ml).

Medium composition

Basic mineral medium contains $MgSO_4$. 7 H_2O 0.5 g/l; NaCl 0.1 g/l; $CaCl_2$ .2 H_2O 0.1 g/l; $CuSO_4$.5 H_2O 0.1 mg/l; $FeSO_4$.7 H_2O 0.2 mg/l; $MnSO_4.H_2O$ 0.02 mg/l and $ZnCl_2$ 0.15 mg/l (Aquiar *et al*., 2006). 50 ml of liquid medium containing basic mineral medium withglucose as carbon source (10 g/l) and casein hydrolysate as nitrogen source (5 g/l) was inoculated with 5 ml fungal mycelium suspension. This liquid culture was maintained shaken (min. 200 RPM) for 15 days at 30 °C and pH 5.0.

The selection of experimental ranges

*C. subvermispora*was cultivated for 15 days at 30 °C and pH 5.0 in basic mineral medium (Aguiar *et al*., 2006) with different concentration of macro-compounds such as glucose (0, 1, 10, 25, 50 and 100 g/l), casein hydrolysate (0, 1, 1.66, 2.5, 5, 10, 25, 50 and 100 g/l), $MgSO_4$. 7 H_2O(0, 0.1, 0.25, 0.5, 1 and 2.5 g/l) and $CaCl_2$.2 H_2O (0, 0.01, 0.05, 0.1, 0.5 and 1 g/l) and micro-compound $MnSO_4$.4 H_2O ($8x10^{-5}$, 0.8, 1.6, 3.2 and 5.0 mmol/l). An effect of different concentrations of the tested macro- and micro-compounds of propagation medium for biomass growth was evaluated on the base of optical density ($OD_{450\ nm}$) compared to the growth of the negative control (non-inoculated sterile medium) (Banerjee *et al*., 1993).

Experimental design

Three factors, five level experiment was carried out with tested, independent variables such as concentration of glucose (carbon source) (3.2, 15, 32.5, 50 a 61.2 g/l), concentration of casein hydrolysate (nitrogen source) (4.95, 10, 17.5, 25 a 30 g/l) and concentration of manganese ions (0, 0.203, 0.5, 0.797 a 1.0 mmol/l). Real variables values were transformed into non-dimensional coded form (Table 1). Measured dependent variables were dry biomass (g/l of propagation medium), glucose utilization (%) and laccase activity (U/l). Experimental data were fit by the polynomial regression of the second order (Eq. 1), and regression coefficients b_i were calculated.

$$Y = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{ii} X_i^2 + \sum_{\substack{i=1 \\ i<j}}^{k-1} \sum_{j=2} b_{ij} X_i X_j \quad (1)$$

where X_i are independent variables responsible for response Y and b_i are regression coefficients, describing relations of the measured properties to coded levels of the selected parameters. For computer and statistical processing, the Statgraphics plus software 5.1 was applied. All experiments were carried out as three parallel attempts.

Analytic methods

Photometrical determination of biomass growth

Sterile propagation medium (200 μl) with different concentration of the selected macro-compounds (glucose, casein hydrolysate, $MgSO_4$. 7 H_2O and $CaCl_2$. 2 H_2O) and micro-compound ($MnSO_4$. 4 H_2O) was pipetted into sterile 96-well microplates. In each well, fungal mycelium suspension of white-rot fungus *C. subvermispora* (20 μl) was added. Microplates with inoculated medium were incubated at 30 °C for 15 days. The biomass growth was monitored by measuring of absorbance at 450 nm (Banerjee *et al*., 1993).

Determination of dry biomass

After filtration and washing of fungal mycelium with distilled water, dry biomass was determined by a moister analyzer IR-35 (Denver Instrument, USA).

Glucose utilization

After 15 days cultivation of white-rot fungus *C. subvermispora*, propagation medium was centrifuged at 4,000 RPM for 10 minutes. The supernatant was used the analysis of residual glucose in a medium by DNS method (Miller, 1959).

Laccase activity

Laccase activity was determined by oxidation of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (Shin *et al*., 1987). Activity of laccase was expressed in unit (U) as the amount of enzymes able to oxidation of 1 mg of ABTS per minute.

Results and Discussion

Selection of optimization ranges for biomass growth

Although extensive research has been done previously on the biochemistry and the enzymatic activities of ligninolytic enzymes of white-rot fungus *Ceriporiopsis subvermispora* (Rajakumar *et al*., 1996; Guerra *et al*., 2003; Aguiar *et al*., 2006; Chmelová *et al*., 2011), very little information is however available on this fungal growth and need for nutrients. And it is necessary to produce fungal biomass in the first step, because ligninolytic enzymes (laccase and peroxidases) are usually products of secondary metabolism (Howard *et al*., 2003). In our opinion, the effective production of ligninolytic enzymes can be realized by two-step process included the phase of fungal biomass production and the phase of enzyme production.

In our work, we selected medium, which is commonly used for cultivation of white-rot fungi (Aguiar *et al*., 2006) containing glucose as carbon source, casein hydrolysate as nitrogen source and mineral components. In this medium, we followed glucose concentration, biomass production and laccase activity. Results are shown in Figure 1.

The results show that growth curve reached the stationary phase after 12 days of cultivation and the concentration of carbon source (glucose) has decreased below 1 g/l after 15 days. Production of laccase was observed when a glucose concentration in the medium declined to critical level (approximately 2 g/l). Whereas after 15 cultivation days of *C. subvermispora* there is no longer a change in the glucose concentration, in the next experiments, we cultivated white-rot fungus for 15 days. Production of laccase reached maximum at 19^{th} cultivation day with a decrease in biomass production (Figure 1). From these results, suitable conditions for laccase production are not suitable for biomass growth. Therefore, for rapid assessment of medium compounds, which can potential to influence growth of biomass, we were applied statistical Plackett-Burman model. This model was used to screen the significant variables that influenced positive or negative biomass growth and it was based on the presence (+) or absence (-) of the selected macro- or micro-compound in cultivation medium (Table 2).

The results obtained in the Plackett-Burman design were consistent (Table 2). This model revealed glucose, casein hydrolysate, magnesium sulfate, calcium chloride and manganese sulfate were significant component for biomass production. In addition, sodium chloride hasn't influence on fungal growth.

Considering the results obtained by using Plackett-Burman design, we proceeded to find optimization range for biomass growth.Significant factor influenced the production of biomass is concentration of macro- and micro-compounds. The concentration effect of selected compounds (glucose, casein hydrolysate, magnesium sulfate, calcium chloride and

manganese sulfate) to fungal growth was monitored for the 15-day by measuring the optical density of cultivation medium (Figure 2). According to Banerjee *et al*. (1993), correlation between fungal biomass growth determined by photometric and gravimetric methods was linear. At the same time, this method is faster and easier to prepare executable.

From results in Figure 2, carbon and nitrogen sources are important for fungal growth. Carbon source is necessary for ensuring of energy and building blocks for synthesis of various compounds and nitrogen source is essential for amino and nucleic acid synthesis. Figure 2a shows that biomass growth of white-rot fungus *C. subvermispora* was stimulated in media with a concentration range of glucose from 10 to 50 g/l. Glucose is as a readily consumed substrate, but it is cheap and easily available carbon source (Galhaup *et al*., 2002). Lowest glucose concentration (1 g/l) was not sufficient for development of fungal biomass and highest glucose concentration (100 g/l) negatively affected biomass production. High concentration of glucose can cause the production of exopolysaccharides such as pullulan and scleroglucans which hinder the growth of biomass (Xiaoyan *et al*., 2007). Based on these results, optimization range for the glucose concentration was set from 15 to 50 g/l. The concentrations of casein hydrolysate varied from 10 to 25 g/l stimulate positive growth of *C. subvermispora* (Figure 2b). Higher concentrations (50 and 100 g/l) have a negative effect on the growth of biomass. At lowest concentrations (1, 1.66 and 2.5 g/l) of casein hydrolysate, the fungal growth was slowed and stopped due to exhaustion of a nitrogen source. Concentrations of casein hydrolysate as a second independent variable of optimization were varied from 10 to 25 g/l. The organic nitrogen substrates such as peptone, casein hydrolysate or malt-extract supported better fungal biomass production and enzyme activities as compared to the inorganic nitrogen substrates (Levin *et al*., 2008; Chmelová *et al*., 2011).

Changes in fungal growth by concentrations of magnesium and calcium ions varied in the ranges 0.1 – 2.5 g/l and 0.01 – 1 g/l respectively. Magnesium and calcium ions are important for biomass growth because these serve as cofactors/ prosthetic groups of different metabolic enzymes (Irshad and Asgher, 2011). Both ions in different concentrations not affected the production of biomass (Figure 2c, 2d). On the other hand, high concentrations of $CaCl_2$ (0.5 and 1.0 g/l) inhibited significantly growth of fungus. Therefore, the concentrations of magnesium and calcium ions in optimization are fixed (0.1 g/l) and not further optimized.

Last compound from Plackett-Burman model with essential function for fungal biomass growth is manganese ions. Manganese is referred as growth stimulator (Manubens *et al*., 2007) and is also regarded as an inductor for the expression of ligninolytic enzymes. It is part of the catalytic site MnP and plays a regulatory role in the expression of LiP (Hammel and Cullen, 2008) and laccase (Manubens *et al*., 2007). In our experiment, effect of five different concentrations of manganese ions ($8x10^{-5}$, 0.8, 1.6, 3.2 a 5 mmol/l) to fungal biomass production was tested. Manganese ions are commonly used in the media for the cultivation of white-rot fungi as a part of a solution of transition metal ($8x10^{-5}$mmol/l)

Table.1 The proposed experimental conditions for the selected parameters.

Parameter	Coded form				
	-1.682	-1	0	1	1.682
Concentration of glucose [g/l]	3.2	15	32.5	50	61.2
Concentration of casein hydrolysate [g/l]	4.95	10	17.5	25	30
Concentration of manganese ions [mmol/l]	0	0.203	0.5	0.797	1

Table.2 Dry biomass of white-rot fungus *C. subvermispora* produced during 15 days in propagation medium in absence of the one of macro- or micro-compounds.

Sample	Glucose	Casein hydrolysate	$MgSO_4$	NaCl	$CaCl_2$	$MnSO_4$	Dry biomass [g/l of propagation medium]
Run 1	+	+	+	+	+	+	0.251±0.012
Run 2	-	+	+	+	+	+	**0.015±0.005**
Run 3	+	-	+	+	+	+	**0.010±0.003**
Run 4	+	+	-	+	+	+	**0.095±0.025**
Run 5	+	+	+	-	+	+	0.218±0.047
Run 6	+	+	+	+	-	+	**0.128±0.048**
Run 7	+	+	+	+	+	-	**0.096±0.034**

Table.3 Experimental matrix with independent variables in their real and coded form and observed values of the dependent variable (dry biomass [g/l propagation medium], glucose utilization [%] and laccase activity [U/l]) measured during the experiments.

Exp.	Concentration of glucose [g/l]	Concentration of casein hydrolysate [g/l]	Concentration of manganese ions [mmol/l]	Dry biomass [g/l]	Glucose utilization [%]	Laccase activity [U/l]
1	50 (1)	25 (1)	0.203 (-1)	6.90	30.3	4.2
2	50 (1)	10 (-1)	0.797 (1)	4.06	15.8	11.3
3	15 (-1)	10 (-1)	0.203 (-1)	5.76	55.8	11.7
4	15 (-1)	25 (1)	0.797 (1)	6.24	62.5	2.6
5	32.5 (0)	17.5 (0)	0.5 (0)	6.21	38.4	2.8
6	50 (1)	25 (1)	0.797 (1)	5.58	23.1	23.5
7	50 (1)	10 (-1)	0.203 (-1)	5.86	28.2	11.3
8	32.5 (0)	17.5 (0)	0.5 (0)	6.57	21.0	1.9
9	15 (-1)	10 (-1)	0.797 (1)	6.36	66.4	11.6
10	15 (-1)	25 (1)	0.203 (-1)	6.76	71.1	7.6
11	32.5 (0)	4.95 (-1.682)	0.5 (0)	4.30	20.7	91.5
12	32.5 (0)	17.5 (0)	0 (-1.682)	7.37	38.0	7.4
13	32.5 (0)	17.5 (0)	0.5 (0)	6.08	25.9	2.2
14	32.5 (0)	30.0 (1.682)	0.5 (0)	4.34	42.0	8.9
15	32.5 (0)	17.5 (0)	1.0 (1.682)	5.75	25.9	7.6
16	61.8 (1.682)	17.5 (0)	0.5 (0)	5.96	24.2	22.5
17	3.2 (-1.682)	17.5 (0)	0.5 (0)	6.44	83.3	570.3

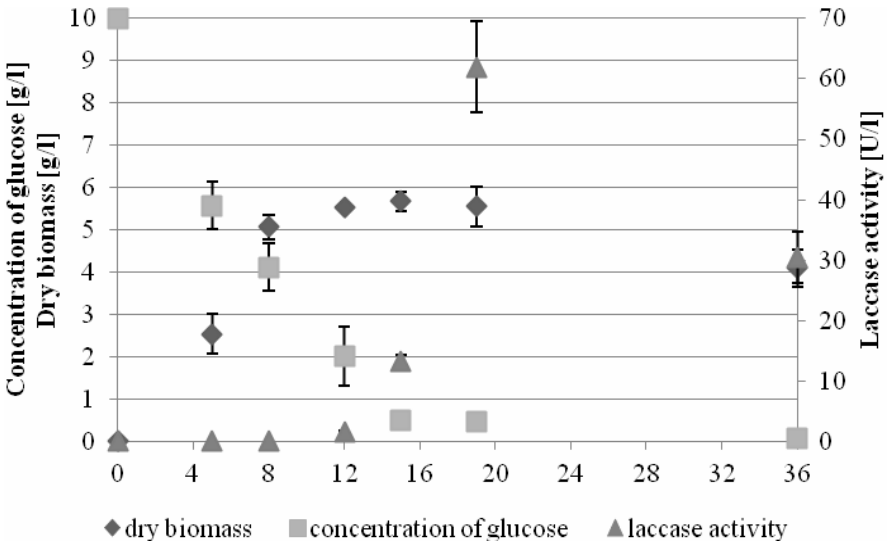
Table.4 Regression coefficients of the model polynomial regression of the second order for dependent variables – dry biomass [g/l] and glucose utilization [%]

Model parameter		Dry biomass	Glucose utilization
Constant effect		3.33735	94.248
Linear effect	Concentration of glucose [g/l] (A)	-0.0116502	**-2.91819**
	Concentration of casein hydrolysate [g/l] (B)	0.396637	-0.2591
	Concentration of manganese ions [mmol/l] (C)	**-0.138135**	-4.25506
Quadratic effect	A x A	0.000112722	**0.0327723**
	B x B	**-0.0113228**	0.0362049
	C x C	1.84286	25.5168
Interaction effect	A x B	0.0016	-0.00190476
	A x C	**-0.0769601**	-0.519481
	B x C	-0.0359147	-0.785634

Table.5 Optimal concentration of glucose, casein hydrolysate and manganese ions for dry biomass and glucose utilization and the comparison with predicated and experimental values of dependent variables

Optimal composition of propagation medium		
Concentration of glucose [g/l]	61.1	3.34
Concentration of casein hydrolysate [g/l]	21.8	28.0
Concentration of manganese ions [mmol/l]	0.03	0.56
	Dry biomass [g/l]	Glucose utilization [%]
Predicted values	8.42	98.0
Experimental values	8.59	97.1

Figure.1 The dependence of the concentration glucose, laccase activity and dry biomass on time of cultivation of white-rot fungus *C. subvermispora* in propagation medium with glucose as carbon source (10 g/l) and casein hydrolysate as nitrogen source (5 g/l) at 30 °C at an initial pH 5.0.



(Kaal *et al*., 1995; Aquiar *et al*., 2006). From our results (Figure 2e), increasing of manganese concentration increased production of biomass production of *C. subvermispora*. Manubens *et al*., (2007) found that the presence of manganese in the propagation medium encourages the growth of white-rot fungus *C. subvermispora* and simultaneously inhibits *lcs*, gene encoding laccase. Based on the measured results, the concentration of manganese ions was selected as a third independent variable of optimization with the variable range from 0.203 to 0.797 mmol/l.

Optimization of propagation medium by RSM method

From these results, it was determined that glucose, casein hydrolysate and manganese ions had significant effect on biomass production. Consequently, we carried out three factors, five level experiment with tested, independent variables (concentration of glucose, casein hydrolysate and manganese ions). The optimal composition of propagation medium was calculated by the software Statgraphics Plus 5.1., processed by RSM approach. In Table 3, experimental matrix with independent and dependent variables are presented. Dependent variables were dry biomass, glucose utilization and laccase activity. Glucose utilization was evaluated as a secondary parameter characterizing the efficiency of technological process of biomass production of *C. subvermispora* and laccase activity was evaluated to confirm the hypothesis of two-step production of laccase.

Multiple linear regression

For the purpose of the fitting the presented results in Table 3, polynomial regression of the second order (Eq. 1) with regression coefficient $R^2 = 0.95$ for dry biomass as parameter Y1,

Figure.2 Effect of propagation medium with the different concentration of a - glucose, b - casein hydrolysate, c - $MgSO_4$, d - $CaCl_2$ and e - $MnSO_4$ to white-rot fungus *C. subvermispora* biomass growth expressed by absorbance at 450 nm during 15 days at 30 °C and pH 5.0.

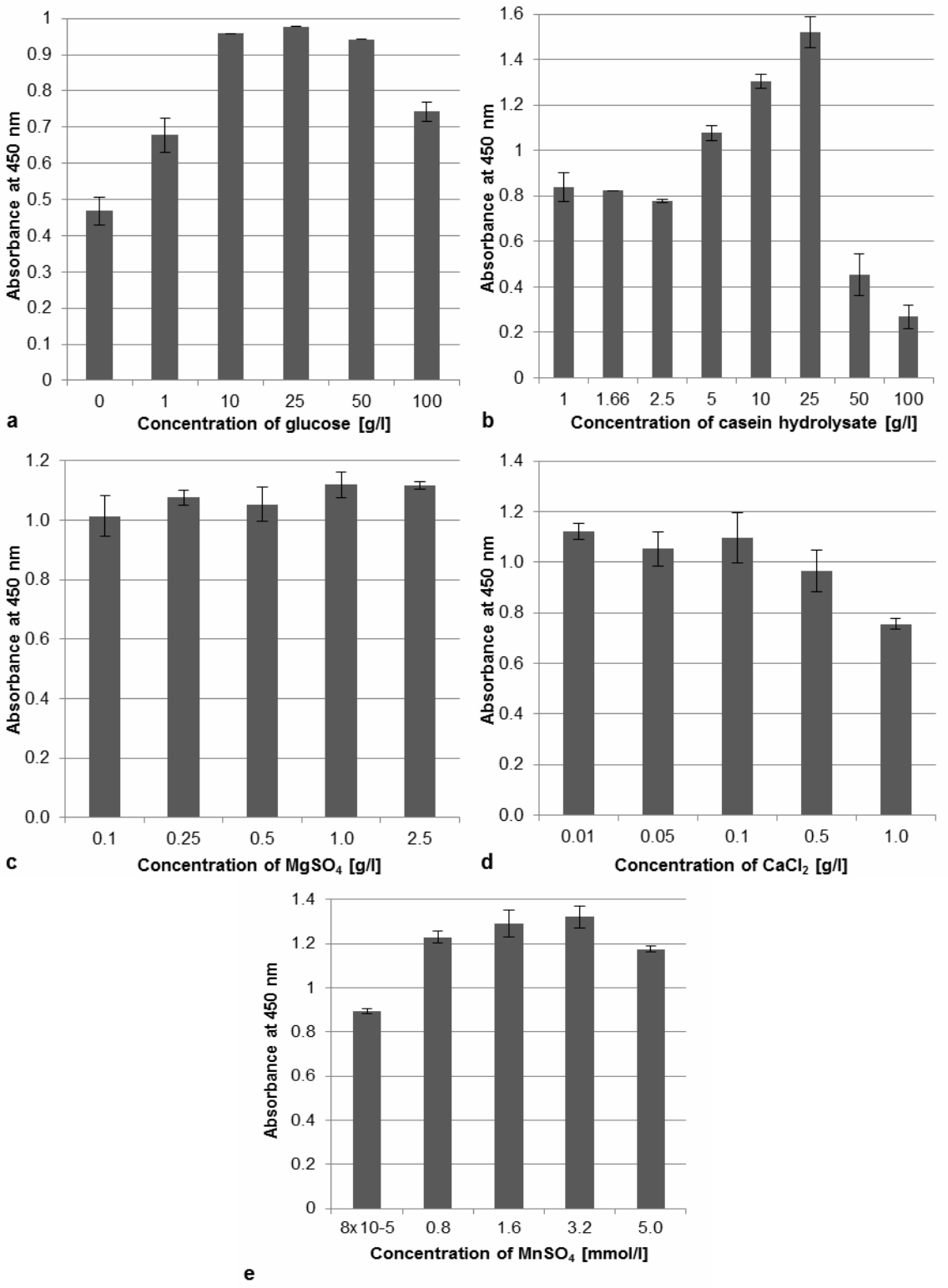
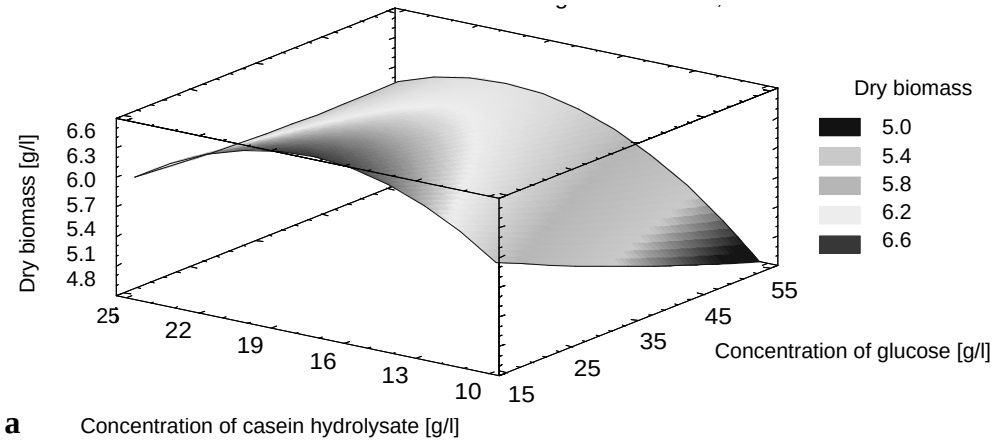
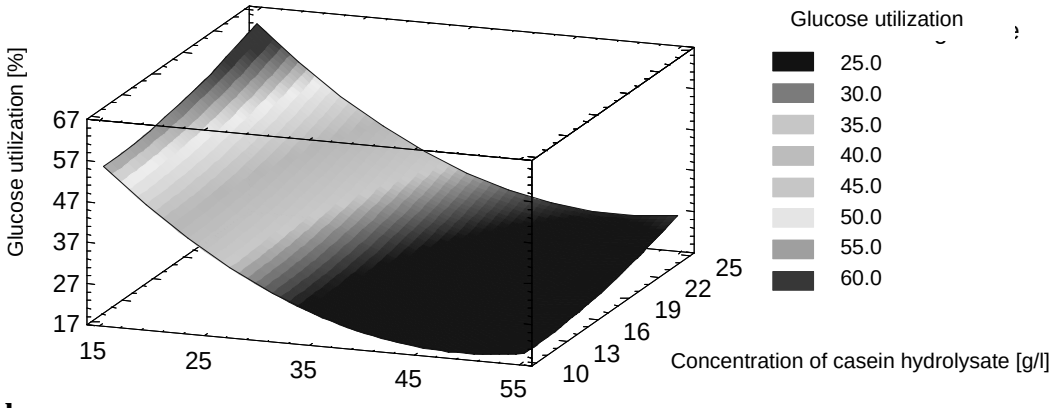


Figure.3 The relation between the independent variables (glucose and casein hydrolysate concentration) and dependent variables: a - dry biomass (g/l), b - glucose utilization (%) at constant concentration of manganese ions (0.5 mmol/l).



a



b

$R^2 = 0.96$ for glucose utilization as parameter Y2 and $R^2 = 0.67$ for laccase activity as parameter Y3.

Regression coefficient analysis

Regression coefficients of the model for dry biomass and glucose utilization obtained by multiple polynomial regression are presented in Table 4. Regression coefficients for laccase activity are not shown because the results not confirm the possibility of optimizing medium by RSM. Independent variable in coded form (Table 3) allow direct interpretation of the effect (linear, quadratic and interaction) of the independent variables to dependent variables and visualization by 3D surface plots (Figure 3) assisted visualization of the statistically important factors (marked as bold in the Table 4) obtained from statistical analysis. Biomass production influenced positive linear effects of glucose and casein hydrolysate concentrations and positive quadratic effects of glucose and manganese ions concentrations. Whereas the

glucose utilization influenced negative linear and positive quadratic effects of glucose concentration.

Determination and experimental validation of the optimal conditions

Optimal concentration of glucose, casein hydrolysate and manganese ions are presented in Table 5. These were experimentally verified. Dependent parameters were comparable with experimentally measured values at the level of the statistical significance at $p < 0.05$. These results confirmed the possibility of optimizing propagation medium by RSM approach.

Sarria-Alfonso *et al*. (2012) found that the optimal medium composition for biomass production of *Pleurotus ostreatus* consisted of glucose 25 g/l, yeast extract 5 g/l, Tween 80 0.38 % (v/v), rice husk 10 g/l, $CaCl_2$ 1 g/l, and pH 4.88 ± 0.2. In comparison with our results, 25 g/l of glucose was insufficient for high biomass yields of *C. subvermispora* (61.1 g/l). Similarly, in the case of nitrogen source, *C. subvermispora* needs higher concentration (28.1 g/l) of nitrogen source in comparison with *P. ostreatus* (5 g/l). Despite the fact that the manganese ions are stimulant for biomass production (Manubens *et al*., 2007), our results suggest that higher concentration of Mn^{2+} ions have a negative effect on biomass growth. Although, white-rot fungus *C. subvermispora* utilized only 24.2 % from 61.1 g/l of glucose during 15 cultivation days but the rest glucose can be recycled for further cultivation which will focus on the production of laccase.

The optimal conditions for the production of biomass not correlate with optimal composition of a medium suitable for the efficient utilization of the carbon source provided. Laccase activity in the propagation media after cultivation of white-rot fungus *C. subvermispora* was confirmed assumptions about the disproportion between the optimum conditions for production of fungal biomass and laccase. The highest level of laccase activity was detected in experimental media no. 11 (91.5 ± 0.5 U/l) and no. 17 (570.5 ± 71.0 U/l) containing the boundary concentration of casein hydrolysate (4.95 g/l) and glucose (3.2 g/l). The lowest production of fungal biomass was evaluated in the same media. This evidence shows the necessity of two-step production of laccase. The first step is the production of biomass and subsequent production of enzymes.

Acknowledgment

This contribution is the result of the project implementation: Centre of excellence for white-green biotechnology, ITMS 26220120054, supported by the Research & Development Operational Programme funded by the ERDF and by the grant No. FPPV-08-2012.

References

Aquiar, A., Souza-Cruz, P.B., Ferraz, A. 2006. Oxalic acid, Fe^{3+} - reduction activity andoxidative enzymes detected in culture extracts recovered from *Pinustaeda* wood chipsbiotreated by *Ceriporiopsis subvermispora*. Enzyme Microb. Tech.38, 873–878.

Banerjee, C., Chisti, Y., Moo-Young, M. 1993. Spectrophotometric determination of mycelial biomass. Biotechnol. Tech.7, 313–316.

Chmelová, D., Ondrejovič, M., Ondáš, V., Šturdík, E. 2011. Influence of cultivation conditions on production of

lignocellulolytic enzymes by *Ceriporiopsis subvermispora*. Biologia. 66, 748-754.

Couto, S.R., Toca-Herrera, J.L. 2006. Laccase production at reactor scale by filamentous fungi. Biotech. Adv. 25, 558–569.

Galhaup, C., Wagner, H., Hinterstoisser, B., Haltrich, D. 2002.Increased production of laccase by the wood-degrading basidiomycete *Trametes pubescens*.Enzyme Microb. Tech. 30, 529–536.

Guerra, A., Mendonça, R., Ferraz, A. 2003. Molecular weight distribution of wood components extracted from *Pinus taeda* biotreated by *Ceriporiopsis subvermispora*. Enzyme Microb. Tech. 33, 12–18.

Hammel, K.E., Cullen, D. 2008. Role of fungal peroxidases in biological ligninolysis.Curr.Opin. Plant Biol. 11, 349–355.

Howard, R.L., Abotsi, E., Jansen van Rensburg, E.L., Howard, S. 2003. Lignocellulose biotechnology: issues of bioconversion and enzyme production. Afr. J. Biotechnol. 2, 602–619.

Irshad, M., Asgher, M. 2011. Production and optimization of ligninolytic enzymes by white rot fungus *Schizophyllum commune* IBL-06 in solid state medium banana stalks. Afr. J. Biotechnol. 10, 18234–18242.

Kaal, E.E.J., Field, J.A., Joyce, T.W. 1995.Increasing ligninolytic enzyme activities in several white-rot basidiomycetes by nitrogen-sufficient media. Bioresour. Technol. 53, 133-139.

Levin, L., Hermann, C., Papinutti, V.L. 2008. Optimization of lignocellulolytic enzyme production by the white-rot fungus *Trametes trogii* in solid-state fermentation using response surface methodology. Biochem. Eng. J. 39, 207–214.

Levin, L., Melignani, E., Ramos, A.M. 2010. Effect of nitrogen sources and vitamins on ligninolytic enzyme production by some white-rot fungi. Dye decolorization by selected culture filtrates. Bioresour. Technol. 101, 4554–4563.

Manubens, A., Canessa, P., Folch, C., Avila, M., Salas, L., Vicuňa, R. 2007. Manganese affects the production of laccase in the basidiomycete *Ceriporiopsis subvermispora*. FEMS Microbiol. Lett. 275, 139–145.

Mikiashvili, N., Wasser, S.P., Nevo, E., Elisashvili, V. 2006. Effects of carbon and nitrogen sources on *Pleurotus ostreatus* ligninolytic enzyme activity. World J. Microl. Biot. 22, 999–1002.

Miller, G.L. 1959. Use of dinitrosalicylic reagent for the determination of reducing sugar. Anal. Chem.31: 426–428.

Rajakumar, S., Gaskell, J., Cullen, D., Lobos, S., Karahanian, E., Vicuňa, R.1996. Lip-like genes in *Phanerochaete sordida*, and *Ceriporiopsis subvermispora*, white rot fungi with no detectable lignin peroxidase activity. Appl. Environ. Microbiol. 62, 2660–2663.

Sarria-Alfonso, V., Sánchez-Sierra, J., Aguirre-Sarmiento, M., Gutiérrez-Rojas, I., Moreno-Sarmiento, N., Poutou-Piňales, R.A. 2012. Culture media statistical optimization for biomass production of a ligninolytic fungus for future rice straw degradation. Indian J.Microbiol. 53, 199–207.

Shekker, S.R., Sehgal, S., Kamthania, M., Kumar, A. 2011. Laccase: Microbial sources, production, purification, and potential biotechnological applications. Enzyme Res. 2011, 1–11.

Shin, T., Murao, S., Matsumura, E. 1987. A chromogenic oxidative coupling reaction of laccase: application for laccase and angiotensin I converting enzyme assay. Anal. Biochem.166: 380–388.

Souza-Cruz, P.B., Freer, J., Siika-Aho, M., Ferraz, A. 2004. Extraction and determination of enzymes produced by *Ceriporiopsis subvermispora* during biopulping of *Pinus taeda* wood chips. Enzyme Microb. Tech. 34, 228–234.

Vyas, P., Rahi, P., Chadha, B.S., Gulati, A.2013. Statistical optimization of medium components for massproduction of plant growth-promoting microbial inoculant *Pseudomonas trivialis* BIHB 745 (MTCC5336). Indian J. Microbiol. In press.

Xiaoyan, Z., Yianghua, W., Yan, F. 2007. Influence of glucose feeding on the ligninolytic enzyme production of the white-rot fungus *Phanerochaete chrysosporium.* Front. Environ. Sci. Eng.1: 89–94.