ORIGINAL PAPER

Submerged cultivation of*Fomesfomentarius* mushroomand increase of biomass yieldby statistical design of experiments and mathematical modeling

Received for publication, November, 25, 2015
Accepted, July, 6, 2016

VICENTIU BOGDAN HORINCAR, ANA-MARIA POPA, GEORGIANA HORINCAR, GABRIELA BAHRIM*

Dunarea de Jos University of Galati, Faculty of Food Science and Engineering, Galați, 800008, România

*Address correspondence to:*Dunarea de Jos* University of Galati, Faculty of Food Science and Engineering, Galați, 800008, România, *Tel: +40336130184, E-mail:*gabriela.bahrim@ugal.ro

Abstract

Submerged cultivation of mushrooms is promising for biomass production, which constitutes a source of biological active compounds. This study proposes a mathematical model for producing maximum biomass yield of Fomes fomentarius in submergedculture controlled conditions.The effect of seven biotechnological parameters (independent variables) with influence on F. fomentarius biomass yield was analyzed by Plackett-Burman factorial design method (PBD). Three variables with different ranges of positive influence (6.36-60 g/l concentration of dextrose, 0.8-7.5 g/lconcentration of yeast extract and time of cultivation of 5-15 days) were selected and their correlative effect on mushroom multiplicationwas established by applying Central Composite Design (CCD) and Response Surface Methodology (RSM). ANOVA analysis of obtained mathematical model showed that it is significant (F value of 14.25). In optimized conditions of submerged cultivation, the yield of dry weight biomass was 23.74 g/l after 11day of submerged cultivation.

Keywords:*Fomes fomentarius*, submerged cultivation, Plackett-Burmandesign of the experiments,central composite design, response surface methodology

1. Introduction

The beneficial properties of several mushrooms are widely known.For many years,they have been consumed and appreciated due to their nutritional value and medicinal properties. However, only recently, starting with the last decade of the 20th century it has been possible to isolate and partially characterize some biological compounds with bioactive effects from mushrooms(KIDD [1]). Modern medicine in eastern countries, such as China, Japan, Korea, and several other Asian countries, still uses mushrooms to treat major diseases (ZAKHARY& al. [2]). The fungal kingdom has a long historical tradition in which higher *Basidiomycetes* mushrooms represent a dominant branch owing to their production of biologically active compounds (SMIDERLE&al. [3]).

Fomes fomentarius, a basidiomycete fungus,originated since the 5000-year-old Iceman, this polypore being used (in past) to make and preserve fire, as first aid kit, as insect repellent or

ORIGINAL PAPER

for spiritual purposes (GRIENKE& al. [4]). Since then, *F. fomentarius*, has been exploited as a traditional Chinese and Korean medicine for many centuries for the treatment of various diseases, including oral ulcers, gastroenteric disorders, hepatocirrhosis, inflammation, and various cancers. Recent studies have shown that *F. fomentarius* has antioxidant, anti-inflammatory, and anti-diabetic activities (LEE [5]; PARK& al. [6]). The traditional mushroom cultivation is time-consuming, hencethe necessity for exploring biotechnology methods (HABIBI [7]).

Recently, mushrooms cultivation in controlled biotechnological conditions has proved to be effective for increasing the yield of biomassand, consequently, the bioactive compounds production (HOU & al. [8]; BURNS& al. [9]; WASSER& al. [10]; HSIEH& al. [11]; JONATHAN& al. [12]). Therefore, the objective of this work was the adjustment of the most important biotechnological parameters in the submerged cultivation process of*F. fomentarius* in order to increase the biomass production.In this purpose, mathematical modeling and statistical analysis tools associated to Plackett-Burman factorial design (PBD), Central composite design (CCD)and Response surface methodology (RSM) were used to evaluate the main and correlative effects of some biotechnological parameters on biomass yield.

2. Materials and Methods

2.1. Mushroom strain

F. fomentarius strain was obtained from Culture Collection of Laboratory for Research of fungi with role in the ecological reconstruction of heavy metals polluted soils-RECOSOL of "Alexandru Ioan Cuza" University of Iasi, Romania. Stock culture was preserved by cultivation on agar nutritive medium containing 15 g/l malt extract and 15 g/l agar, pH=5.5, in Petri dishes with conservation at 4°C.

2.2. Inoculum preparation

The vegetative inoculum was obtained by stationary cultivation for 4 days at 26°C in liquid medium containing (g/l): glucose 40, yeast extract 5, peptone 5 and pH = 5.5. From pure culture grown on solid medium on the Petri dishes were cut three square pieces of mycelium with 0.5 cm in diameter. These were used as start inoculum for inoculation of 100 mL of fermentation medium (HORINCAR & al. [13]).

2.3. Submerged cultivation

The fermentative basal medium used for submerged mushroom cultivation consisted of (g/l): glucose 40, peptone 3, yeast extract 5, $KH_2PO_4 \cdot H_2O$ 0.5, $MgSO_4 \cdot 7H_2O$ 0.5, pH=5.5. This medium was inoculated with 1.2 g/l vegetative inoculum. The cultivation took place in Erlenmeyer flasks placed in a rotary shaker (Lab Companion, Korea) with controlled temperature, for 10 days, at 26°C and 150 rpm (HORINCAR & al. [13]; CHEN & al. [14]).

2.4. Biomass yield

ORIGINAL PAPER

Biomass formed in submerged multiplication was determined by assessment of the dry weight, expressed as g/l. The measurement was performed by collecting wet biomass by vacuum filtering sample through reweighed 0.45µm cellulose acetate filter (Sartorius Stedim Biotech, GmbH, Germany). The filtered biomass was then washed twice with distilled water and filters and dried at 105°C (Drying Oven Sanyo, Japan) until achieving a constant weight.

2.5. Optimization of biotechnological process

For mathematical modeling and statistical analysis of biotechnological process there are used the modern tools, as follow [15]:

Plackett-Burman factorial design

The PBD was applied in the early phase of the experiment to identify the most important factors that impact on the biotechnological process. This method provides a screening way for efficient identification of the active factors that influence the process (PAN& al. [16]).

For mathematical modeling a first-order polynomial model (Eq. 1) was used:

$$Y = \beta_0 + \sum \beta_i \chi_i \quad (1)$$

where Y is the predicted response (dry weight biomass, g/l), β_0 is the model intercept, $\beta_i$is the linear coefficient and $\chi_i$is the level of the independent variable.

The effects of seven most important independent variables with influence on the biomass production in submerged cultivation condition were studied. The central point value for each independent variable was chosen based on literature data regarding the submerged cultivation of mushrooms, as follows: glucose concentration 40.00 g/l, yeast extracts concentration 5.00 g/l, peptone concentration 5.00 g/l, inoculum concentration 1.20 g/l, time of cultivation 10 days, pH 5.5 and agitation speed 150 rpm.

The PBD consisted of 12 experiments in agreement with the data presented in Table 1. All experiments were carried out in triplicate in 500 ml Erlenmeyer flasks. The response consisted on dry weight biomass, expressed in g/l.

Table 1.Levels of variation of independent variable (χi) in PBD

Symbol	**Parameter**	**Levels of variation of independent variable (χi)**		
		-1	**0**	**+1**
A	Dextrose concentration, g/l	20.0	40.0	60.0
B	Yeast extract concentration, g/l	2.5	5.0	7.5
C	Peptone concentration, g/l	2.5	5.0	7.5

D	pH	5.0	5.5	6.0
E	Time, days	5	10	15
F	Agitation speed, rpm	100	150	200
G	Inoculum concentration, %	1.0	2.0	3.0

Central composite design of experiments and response surface methodology

After selecting the significant independent variables by PBD, the CCD and RSM were employed to collect more information on the significant effects of the independent variables and their interactions on fungal multiplication that would allow obtaining maximum yield of *F. fomentarius* biomass.

The response (Y, biomass yield, expressed in g/l) was established by using a second-order polynomial model and the data were fitted by multiple regression procedure. The mathematical relationship between the response (Y) and the significant independent variables is given by the following quadratic polynomial equation (Eq. 2):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 \quad (2)$$

where Y is the response (yield of dry weight biomass, g/L); X_1, X_2, X_3 are the codes of the significant independent variables; β_1, β_2, β_3 are the linear regression coefficients; β_{11}, β_{22}, β_{33} are quadratic regression coefficients; β_{12}, β_{13}, β_{23} are interactive regression coefficient estimates, while β_0 plays the role of scaling constant.

2.6. Statistical analysis

For mathematical modeling and statistical analysis was used the software Design Expert version 6.0.8 (Stat-Ease Inc., MN, USA). Analysis of variance (ANOVA) of the models was performed through Fisher test (*F*-test), by determining its associated probability *P(F)* and the coefficient of determination (R^2) which measures the goodness of the regression model fitting. For each variable, the quadratic models were represented as contour plots and response surface curves.

3. Results and Conclusions

3.1.Biotechnological parameters with positive effect on F. fomentarius biomass production

Seven biotechnological parameters such as: concentration of some carbon and nitrogen sources (dextrose, yeast extract, peptone), inoculum concentration, pH value, agitation speed and

ORIGINAL PAPER

the time of cultivation were investigated in order to establish their effect on *F. fomentarius* growth and multiplication ability in liquid medium in submerged cultivation system.

The experimental values for the variation of biomass yield based on independent variables variation are presented in Table 2.

In Fig.1 are represented the responses for *F. fomentarius* biomass yield.

After applying the ANOVA statistical analysis, the following polynomial model was obtained (Eq.3):

$$Y = 7.22 + 0.90A + 0.20B - 0.34C + 0.087D + 1.61E + 0.30F - 2.53G \quad (3)$$

where *Y* was the predicted *F. fomentarius* dry weightbiomass production (g/L), *A* the concentration of dextrose (g/l), *B* the concentration of yeast extract (g/l), *C* the concentration of peptone (g/l), *D* the pH, E the time of cultivation (days), *F* the agitation speed (rpm) and *G* the inoculum concentration (% v/v).

Table 2.The Placket-Burman factorial design of experiments and biotechnological responses

Run	Coded levels of variable							Yield of dry weight biomass (g/l)
	A	B	C	D	E	F	G	
1	-1	1	1	1	-1	1	1	2.02
2	-1	1	1	-1	1	-1	-1	10.25
3	1	-1	-1	-1	1	1	1	9.32
4	-1	-1	-1	-1	-1	-1	-1	8.39
5	-1	-1	-1	1	1	1	-1	6.35
6	1	-1	1	1	-1	1	-1	12.65
7	1	1	1	-1	1	1	-1	2.75
8	-1	-1	1	1	1	-1	1	5.95
9	1	-1	1	-1	-1	-1	1	10.03
10	-1	1	-1	-1	-1	1	1	8.07
11	1	1	-1	1	-1	-1	-1	2.62
12	1	1	-1	1	1	-1	1	7.95

ORIGINAL PAPER

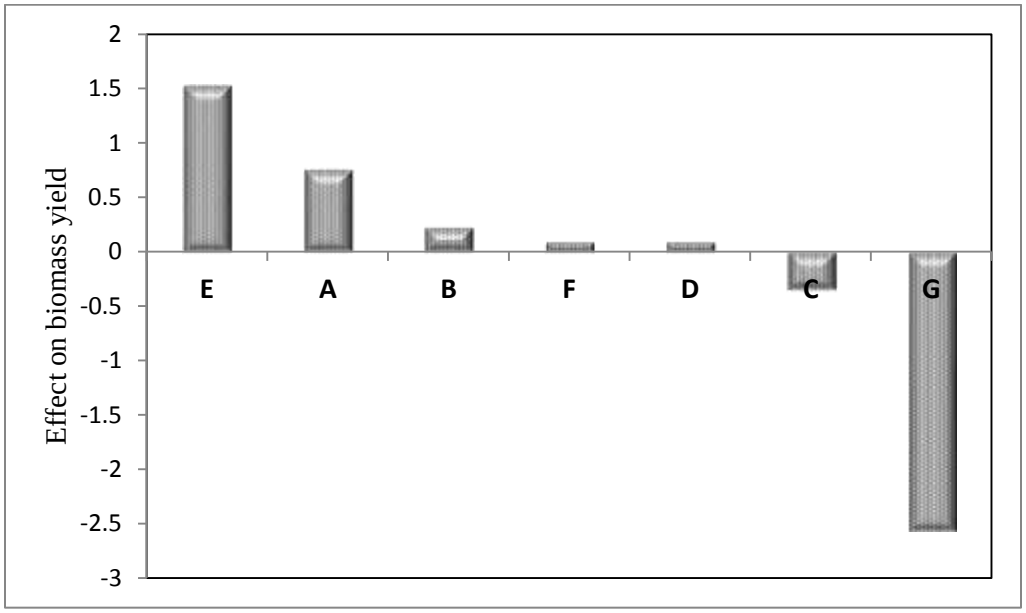

Fig. 1 The effect of the most important independent variables that influence *F. fomentrarius* biomass production by cultivation in submerged cultivation conditions (28°C, 150 rpm, 15 days)

Based on these results has been decided which biotechnological parameters have high positive influence on the biomass yield. As presented in Fig. 1, high mean values, in case of either positive or negative effects indicate that each factor has a large impact on biomass production. On the other hand, mean values close to zero indicate that the factor has little or no effect on biomass production.

Three factors were identified to have significantly influence forbiomass production; i.e dextrose and yeast extract concentrations as well as the time of cultivation. Time of cultivation (*E*) seems to have the highest effect on biomass production, followed by the dextrose concentration (*A*). The yeast extract concentration (*B*) could also have a positive influence in correlation with carbon and nitrogen ratio in the fermentation medium composition. The other three parameters, pH (D), agitation speed (F) and inoculum concentration (G) demonstrated a negative influence on fungal cells growing, and they were not considered in the further studies.

These results are according results reported by to FIDLER& al. [17] which established the optimized medium composition for submerged cultivation of *Flammulina velutipes* strain.

Table 3.Statistical analysis of biotechnological process by the independent variables variation

Source	**Sum of Squares**	**DF**	**MeanSquare**	**F Value**	**Prob > F**
Model	120.44	7	17.21	29.22	0.0028

A	9.74	1	9.74	16.54	0.0153
B	0.49	1	0.49	0.84	0.4142
C	1.39	1	1.39	2.37	0.1987
D	0.082	1	0.082	0.14	0.7285
E	31.01	1	31.01	52.66	0.0019
F	1.06	1	1.06	1.80	0.2504
G	76.66	1	76.66	130.19	0.0003
Residual	2.36	4	0.59		
Cor Total	122.79	11			

C.V.% =10.63; R^2=0.9808; adjusted R^2=0.9473

According to the data presented in Table 3, the Model *F*-value of 29.22 certifies that the model is significant. There is only a 0.28% chance that a "Model *F*-Value" could occur due to noise. The R^2 values (multiple correlation coefficients) closer to 1 proved high agreement between the experimental and predicted responses, and indicate that the mathematical model is very reliable to the present study. The coefficient of variation (CV) indicated the degree of precision with respect to the experimental data. A lower reliability of the experiment is usually indicated by high value of CV. In case of the present study the low value of CV (10.63) showed that conducted experiments were precise and reliable.

Previously reported studies have considered different nitrogen and carbon sources (i.e. yeast extract and dextrose) when cultivating *Pleurotus ostreatus* strain,in submerged condition, in order to determine their influence the biomass production(HORINCARet al. [13]). The results obtained in this study have proved some similarities which indicate the strong influence of medium composition, especially of the C/N ratio on mycelium multiplication in submerged condition. Also, the obtained results comply with previous findings which showing that time of cultultivation has a significant effect on some mushroom biomass production. In addition, our results concerning *F. fomentarius* biomass yield are in agreement with data reported in the literature (TAVARES& al. [19]; BOLLA& al. [20]; RAHMAN& al. [21]; CHEN& al. [19]).

3.2. Mathematical modeling for process optimization

All variables showing a confidence level higher than 95% in the PBD were considered to have a significant effect on the biomass yield and were selected for further optimization studies by means of CCD method. The numerical levels of the variables chosen were set based on PBD

ORIGINAL PAPER

analysis. These are presented in Table 4. Each variable was analyzed at five coded levels (-α, -1, 0, +1, + α) and were decided considering a central point coded value of zero.

Table 4.Experimental matrix of ranges and levels of variation of independent variables

Independent variables	**Coded levels**					
	Symbol	**-α**	**-1**	**0**	**+1**	**+ α**
Concentration of dextrose, g/l	A	6.36	20	40	60	73.64
Concentration of yeast extract, g/l	B	0.8	2.5	5	7.5	9.2
Time of cultivation (days)	C	4.95	7	10	13	15.05

The results of ANOVA*F*-test for the response surface of the quadratic polynomial model were presented in Table 5. By applying multiple regression analysis on the experimental data, the following second-order polynomial model was established to describe the *F. fomentarius* dry weight biomass (Eq.4):

$$Y = 21.52 + 0.11A + 5.37B + 3.18C – 3.06A^2 - 0.69B^2 – 1.33C^2 + 2.18AB – 0.32AC – 0.40BC \quad (4)$$

where Y was the predicted *F. fomentarius* dry weight biomass production (g/l), A the concentration of dextrose (g/l), B the concentration of yeast extract (g/l), and C the time of cultivation (days).

Table 5. ANOVA for the response surface of the quadratic polynomial model

Source	**Sum of squares**	**Degree of freedom**	**Mean square**	**F value**	**P value prob>F**
Model	723.38	9	80.38	14.25	0.0001
A	0.16	1	0.16	0.029	0.8690
B	393.40	1	393.40	69.76	0.0001
C	138.27	1	138.27	24.52	0.0006
A^2	134.59	1	134.59	23.87	0.0006
B^2	6.88	1	6.88	1.22	0.2953

C^2	25.52	1	25.52	4.53	0.0593
AB	38.19	1	38.19	6.77	0.0264
AC	0.85	1	0.85	0.15	0.7068
BC	1.28	1	1.28	0.23	0.6440
Residual	56.39	10	5.64		
Lack of Fit	53.30	5	10.66	17.24	0.0036
Pure Error	3.09	5	0.62		
Cor Total	779.77	19			

C.V.% =13.16; R^2=0.9277; adjusted R^2=0.8626

The model F value of 14.25 implied that the model was significant and also showed that there was only 0.01% chance that a "Model F-value" could occur due to noise. The "Lack of Fit" value of 53.30 and the *P values* are used as a tool to check the significance of each parameter. Thus, *P values* lower than 0.050 denotes the significance of the variables on *F. fomentarius* biomass yield. In this case B, C, A^2, AB are significant model terms. It means that the significant parameters are the concentration of yeast extract (B), the time of cultivation (C) followedby the interaction between the concentration of dextrose and the concentration of yeast extract. There was a high agreement between the experimental and the predicted responses. The CV indicated the degree of precision resulted by comparing the experiments. A low reliability of the experiment is usually indicated by high value of CV; the low value on CV (13.16) in the present study implies that all the experiments were conducted correctly and the results are reliable.

To investigate the correlative effects between the significant variables and the biomass yield, the contour and three-dimensional response surface plots were then analyzed. In Fig.2, 3and 4 are illustrated the responses regarding the influence of each pair of selected parameters while keeping the other three factors constants at their control levels. The three-dimensional plots and their respective contour plots provided visual interpretation of the interaction between two factors and facilitated the identification of the optimum conditions for achieving a high biomass yield.

ORIGINAL PAPER

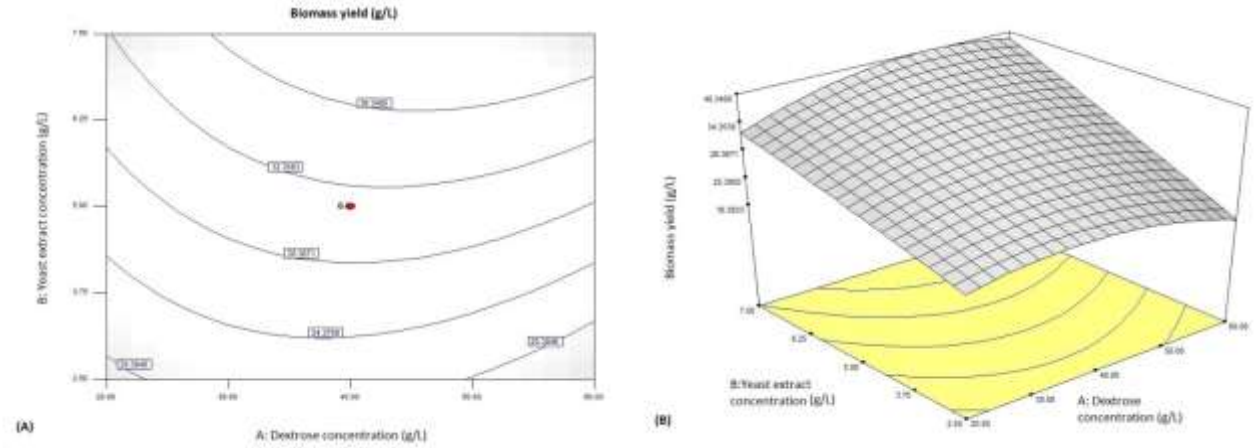

Fig. 2 Contour plot (a) and three-dimensional surface plot (b) showing interactions between the dextrose and yeast extract concentration on *F. fomentarius* biomass production in submerged cultivation system

Fig. 2 describes the interaction between concentration of dextrose (A) and the yeast extract concentration (B). A biomass yield of 23.74 g/l was obtained when the nutritive media contains 43.00 g/l dextrose and 6.50 g/l yeast extract. BOLLA& al. [20] showed that yeast extract has the strongest effect on mushroom's biomass production. Similar with our study, their results are comparable in terms of biomass yield, when lower concentrations of yeast extract were used in the culture medium composition.

In Fig. 3 are represented the interactions between the time of cultivation and the concentration of dextrose (A). It demonstrates that by using a concentration of 42.00 g/l dextrose for a cultivation time of 12 days a biomass yield of 23.74 g/l can be obtained.

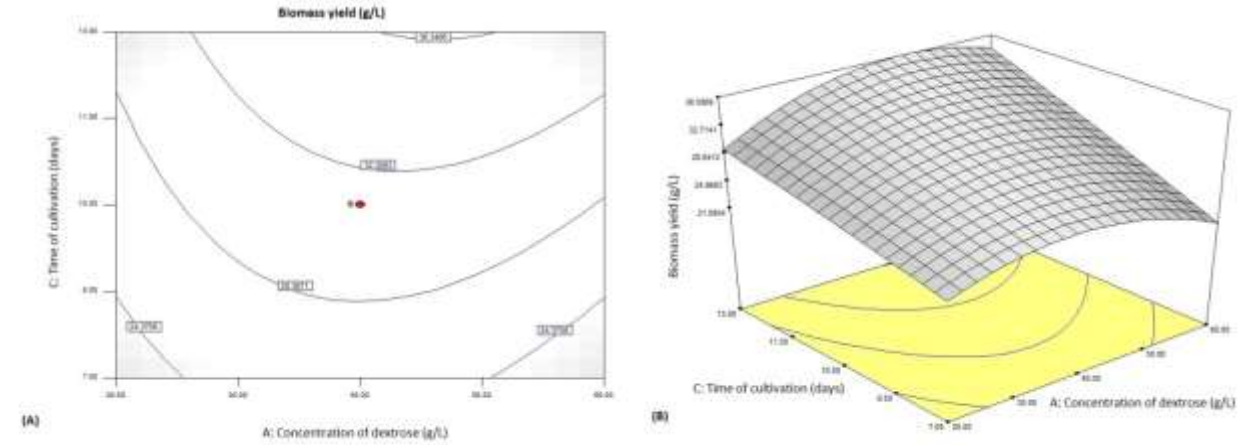

Fig. 3 Contour plot (a) and three-dimensional surface plot (b) showing interactions between the dextrose concentration and time of cultivation on *F. fomentarius* biomass production in submerged cultivation system

ORIGINAL PAPER

In Fig. 4 are represented the correlations between the time of cultivation (C) and the concentration of yeast extract (B). The maximum biomass yield of 23.74 g/l was obtained by using a concentration of 6.2 g/l yeast extract and a time of cultivation of 11 days. BOLLA& al. [20] and RAHMAN [21] also reported that 11 days of submerged cultivation of mushrooms was an adequate time of cultivation for obtaining a high yield of biomass.

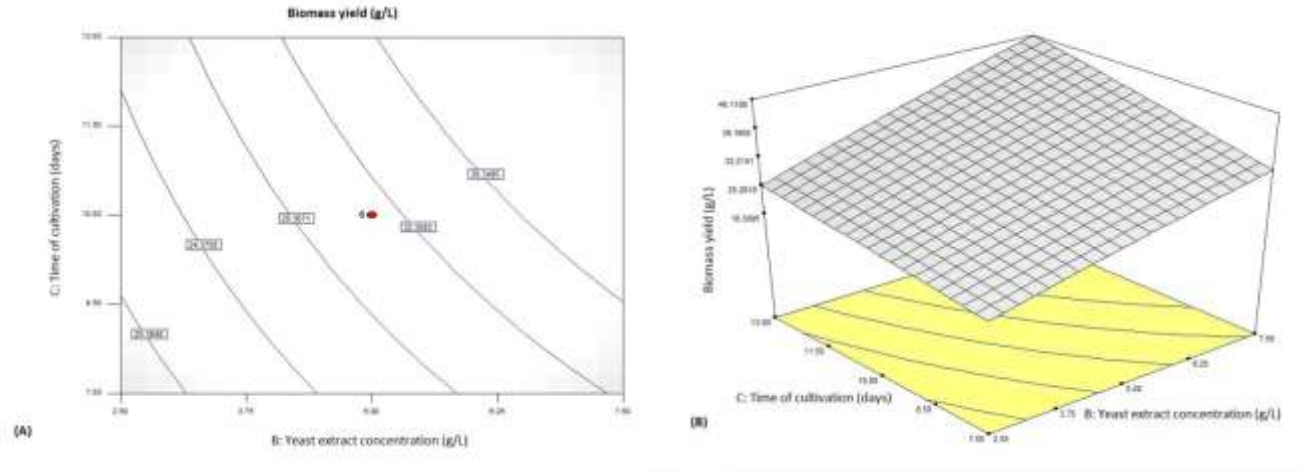

Fig. 4 Contour plot (a) and three-dimensional surface plot (b) showing interactions between the yeast extract concentration and time of cultivation on *F. fomentarius* biomass production in submerged cultivation system

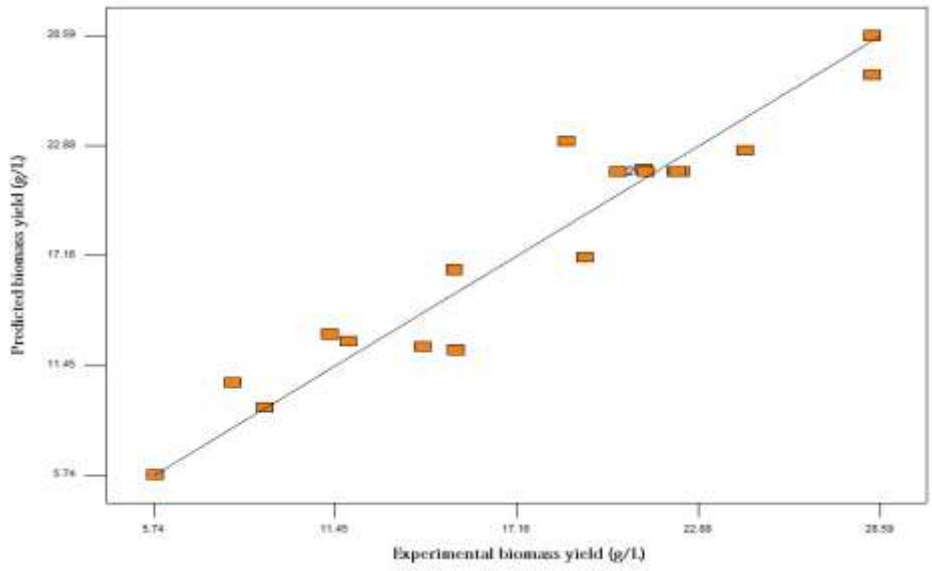

Fig. 5 Parity plot presenting the distribution of experimental vs. predicted values of *F. fomentarius* biomass production on submerged cultivation conditions (CCD)

ORIGINAL PAPER

Good correlation between the experimental and the predicted values of dry weight biomass yield is demonstrated by the parity plot graph (Fig. 5), wherein the points cluster around the diagonal line indicated an optimal fit of the model. Also, the deviation between the experimental and the predicted values is minimal what demonstrates the adequacy of the chosen model.

By using modern strategy of process optimization, based on design of experiments, mathematical modeling and statistical analysis,this study made possible a quick identification of the variables that positively influence the *Fomes fomentarius* biomass production in submerged cultivation system. Thus, it is possible to increase the economic efficiency of the cultivation process of mushrooms by adjusting the principal biotechnological parameters in order to increase the yield of biomass. Taking into account the high economic value of *Fomes fomentarius* strain due to its potential for yielding biological active compounds with great impact in increasing the quality of life, the present study attempts to generate a new route for further researches in this field.

4. Acknowledgements

Support for this research by Project POSDRU/159/1.5/S/138963 – PERFORM is gratefully acknowledged. The authors thank to prof. dr. Cătălin Tănase and dr. Tiberius Balaes from "Alexandru Ioan Cuza" University of Iasi for mushroom culture provided.

References

1. P.M. KIDD, The use of mushroom glucans and proteoglycans in cancer treatment, Altern. Med. Rev., 5: 4(2000).
2. J. ZAKHARY, T.M. ABO-BAKR, A.R. EL-MAHDY, S.A. EL-TABEY, Chemical composition of wild mushrooms collected from Alexandria, Egypt, *Food Chem*., 11: 31 (1983).
3. F.R. SMIDERLE, L.M. OLSEN, A.C. RUTHES, P.A. CZELUSNIAK, A.P. SANTANA-FILHO, G.L. SASSAKI, P.A.J. GORIN, M. IACOMINI, Exopolysaccharides, proteins and lipids in *Pleurotus pulmonarius* submerged culture using different carbon sources, *Carb. Polym*., 87: 368 (2012).
4. U. GRIENKE, M. ZOLL, U. PEINTNER, J.M. ROLLINGER, European medicinal polypores – A modern view on traditional uses, *J. Ethnopharmacol*., 154: 564 (2014).
5. J.S. LEE, Effects of *Fomes fomentarius* supplementation on antioxidant enzyme activities, blood glucose, and lipid profile in streptozotocin-induced diabetic rats, *Nutr. Res.* 25: 187 (2005).
6. Y.M. PARK, I.T. KIM, H.J. PARK, J.W. CHOI, K.Y. PARK, J.D. LEE, B.H. NAM, D.G. KIM, J.Y. LEE, K.T. LEE, Anti-inflammatory and anti-nociceptive effects of the methanol extract of *Fomes fomentarius*, *Biol. Pharm. Bull.*, 27: 1588 (2004).
7. E. HABIBI, S.E. SADAT-EBRAHIMI, S.A. MOUSAZADEH, Y. AMANZADEH, Mycochemical Investigation of the Turkey Tail Medicinal Mushroom *Trametes versicolor* (Higher Basidiomycetes): A

Potential Application of the Isolated Compounds in Documented Pharmacological Studies, *Int J Med Mushrooms*, 17: 255 (2015).

8. X. HOU, W. CHEN, Optimization of extraction process of crude polysaccharides from wild edible *Ba Chu* mushroom by response surface methodology, *Carbohyd. Polym*., 72: 67 (2008).
9. P.J. BURNS, P. YEO, T. KESHAVARZ, S. ROLLER, C.S. EVANS, Physiological studies of exopolysaccharide production from the Basidiomycete *Pleurotus florida*. *Enzyme Microb. Tech*., 16: 566 (1994).
10. S.P. WASSER, I. VLADIMIR, V. ELISASHVILI, K.T. KOK, Effects of Carbon and Nitrogen Sources in the Medium on *Tremella mesenterica* Retz: Fr. (Heterobasidiomycetes) Growth and Polysaccharide Production, *Int. J. Med. Mushrooms*, 5: 49 (2003).
11. C. HSIEH, M.J. TSAI, T.H. HSU, D.M. CHANG, C.T. LO, Medium optimization for polysaccharide production of *Cordyceps sinensis*, *Appl. Biochem. Biotechech*., 120: 145 (2005).
12. G. JONATHAN, S. AYODELE, A. DAMILOLA, Optimization of submerged culture conditions for biomass production in *Pleurotus florida* (mont.) Singer, a Nigerian edible fungus, *Afr. J. Biotechnol*., 1464 (2005).
13. V.B. HORINCAR, A. POPA, G. PARFENE (HORINCAR), G. BAHRIM, Evaluation of some biotechnological parameters influencing the *Pleurotus ostreatus* biomass production by submerged cultivation. *The Annals of the University Dunărea de Jos of Galati, Fascicle Food Technology* (VI), 39: 55 (2015).
14. W. CHEN, Z. ZHAO, S.F. CHEN, Y.Q. LI, Optimization for the production of exopolysaccharide from *Fomes fomentarius* in submerged culture and its antitumor effect in vitro. *Biores. Tech*., 99:3187 (2008).
15. *e-Hand book of Statistical Methods*, http://www.itl.nist.gov/div898/handbook.
16. C.M. PAN, Y.T. FAN, Y. XING, H.W. HOU, M.L. ZHANG, Statistical optimization of process parameters on biohydrogen production from glucose by *Clostridium* spp. Fanp 2, *Biores.Technol*. 99: 3146 (2008).
17. G. FIDLER, S. RODINO, A. BUTU, M. BUTU, G. POPA, C.P. CORNEA, Optimization of submerged culture conditions for *Flammulina velutipes* on SBD culture medium, *J.Biotechnol*., 208 (Supplement), S103 (2015).
18. B.C. ADEBAYO-TAYO, E.E. UGWU, Influence of Different Nutrient Sources on Exopolysaccharide Production and Biomass Yield by Submerged Culture of *Trametes versicolor* and *Coprinus* spp. *AU J. Technol.*, 15: 63 (2011).
19. A.P.M. TAVARES, M.S.M. AGAPITO, M.A.Z. COELHO, J.A. LOPESDASILVA, A. BARROS-TIMMONS, J.A.P. COUTINHO, A.M.R.B. XAVIER, Selection and optimization of culture medium for exopolysaccharide production by *Coriolus (Trametes) versicolor*, *World J. Microb. Biot.*, 21: 1499 (2005).
20. K. BOLLA, B.V. GOPINATH, S.Z. SHAHEEN, M.A. SINGARA CHARYA, Optimization of carbon and nitrogen sources of submerged culture process for the production of mycelial biomass and exopolysaccharides by *Trametes versicolor*. *Int. J. Biotech. Molecular Biol Res*., 1: 15 (2010).
21. N.A. RAHMAN, F. DAUD, M.S. KALIL, S. AHMAD, Tiger milk mushroom cultivation by using submerged culture technique. *Wseas Trans. Biol. Biomed*., 3: 83 (2012).