ELSEVIER

Process Biochemistry 35 (1999) 127–133

PROCESS BIOCHEMISTRY

www.elsevier.com/locate/procbio

Effect of substrate composition on the mycelial growth of *Pleurotus ostreatus*. An analysis by mixture and response surface methodologies

Oscar Soto-Cruz [a], Gerardo Saucedo-Castañeda [a,*], José Luis Pablos-Hach [b], Mariano Gutiérrez-Rojas [a], Ernesto Favela-Torres [a]

[a] *Departamento de Biotecnología, Universidad Autónoma Metropolitana, Iztapalapa, A.P. 55-535, C.P. 09340, Mexico D.F., Mexico*
[b] *Facultad de Medicina Veterinaria y Zootecnia, UNAM, Ciudad Universitaria, Mexico D.F., Mexico*

Received 23 September 1998; received in revised form 26 February 1999; accepted 19 March 1999

Abstract

The effect of the composition of a mixture containing, oat straw (OS), oat bran (OB) and copra cake (CC), on the mycelial growth of *Pleurotus ostreatus* was studied using mixture and response surface methodologies. The applied constraints to the mixtures were: moisture content higher than 70%, C/N ratio less than 30 and total mixture cost less than 2% of the retail cost of fruiting bodies of *P. ostreatus*. The maximum observed value of apical growth rate (0.50 ± 0.02 cm day^{-1}) was obtained using 0.633, 0.284 and 0.083 (g g^{-1} mixture, dry basis) for OS, CC and OB mass fractions, respectively. Under these conditions the C/N ratio was 22.4–23.2. Loss of dry matter decreased from 16.9 to 8.5% as the OS fraction (lignin and cellulose source) was increased from 0.55 to 0.80 (g g^{-1} mixture, dry basis). The utilisation of mixture and response surface methodologies was an useful approach to evaluate the relationship between substrate composition and mycelial development of *P. ostreatus*.

Keywords: *Pleurotus ostreatus*; Solid substrate cultivation; Mycelial growth; Mixture methodology; Response surface methodology

1. Introduction

Pleurotus ostreatus is an edible fungi with a nutritional value similar to milk and meat [1]. Large scale production of this fungi has been attempted in various developing countries including India, Brazil and Mexico [2]. *Pleurotus* world production has increased from 7.7 in 1986 to 24.2% in 1990 [3]. In the case of Mexico, production was estimated at 1825 ton in 1997 [4].

Mushroom cultivation is a simple, low cost and environmentally friendly technology for the utilisation of rural and agro-industrial residues [5]. The cultivation of edible fungi on agricultural by-products like sugar cane bagasse, coffee pulp and straws could be considered as a valuable approach for the production of protein enriched food [6]. Additionally, fermented residues could be used as animal feed after mushroom cultivation [7,8]. The final product, fruiting bodies of *P. ostreatus*, is obtained after solid substrate cultivation (SSC) in two stages. In the first stage mycelia development requires 2 weeks incubated at 28°C in the darkness. Once the substrate has been totally invaded, the second stage involves the production of fruiting bodies taking a further 2–3 weeks. The second stage requires humid saturated air and alternated periods (12 h) of light and darkness [7].

The biological efficiency (fresh weight of fruiting bodies per dry weight of the substrate) of the final product depends on the development of mycelia in the first culture stage. Contaminations can decrease biological efficiency from 40 to 100% [9]. The risk of contamination is maximal at the beginning of the process and it could be inversely related to *Pleurotus* mycelia development [10]. In this sense, as long as the duration of mycelia development is reduced, the risk of substrate contamination could also be reduced.

* Corresponding author. Tel.: +52-5-7244999; fax: +52-5-7244712.
E-mail address: saucedo@xanum.uam.mx (G. Saucedo-Castañeda)

0032-9592/99/$ - see front matter
PII: S0032-9592(99)00043-6

In the mixture methodology, experiments are carried out in order to find the component proportions giving an optimal response for a selected variable [11], such as the growth rate of *Pleurotus*. In contrast, response surface methodology (RSM) is defined as a tool to analyse the effect of a selected response of independent variables and for modelling of complex systems [12,13]. RSM is an useful approach for analysing biological processes and has been used widely in food science and technology [14,15], microbiology [16] and enzyme applications [17]. Mixtures methodology and RSM could be associated by studying the physical characteristics of the measured response surface such as the shape, slope or the highest point [11].

This study was designed to assess the effects of oat straw (OS), oat bran (OB) and copra cake (CC) in mixtures used as substrates during mycelial development in SSC of the edible mushroom *P. ostreatus*. In order to achieve this goal, the methodologies of mixtures and response surface were applied.

2. Materials and methods

2.1. Microorganism and raw materials

P. ostreatus strain IE8 was obtained from the Institute of Ecology of Jalapa, Veracruz, México and grown on malt extract agar (MEA) at 25°C. Mycelia was developed on a mixture containing oat straw (OS), copra cake (CC) and oat bran (OB) at different proportions as indicated below. Carbon and nitrogen were analysed in order to determine the carbon–nitrogen ratio (C/N) of the mixture. The carbon content was estimated from the ash content of the substrate and the nitrogen content was determined by the Kjehldal method [18].

2.2. Inoculum preparation

Heat resistant bags with 500 g of sorghum seeds (moisture 35%) were sterilised at 121°C for 60 min (initial pH 6.5) and inoculated with two MEA plugs containing mycelial mat (1 cm diameter) and incubated for 10 days at 28°C (primary inoculum). Secondary inocula were prepared using 10% (w/w) of primary inoculum, as starter culture, on sorghum seeds and incubated as indicated Zadrazil and co-workers [7].

2.3. Experimental design

The effect of three components (OS, OB and CC) of a mixture upon mycelial development in SSC was studied using the methodology of mixtures, described by Cornell [11]. In the preparation of the mixtures the following constraints were applied; the moisture content was maintained greater than 70%, the C/N ratio was less than 30 and the mixture price was less than 2% of the *Pleurotus* fruiting bodies price in the Mexico city market. These constraints were applied and an experimental region was defined. The experimental region was delimited by the range values of each component as follows (g g^{-1} mixture, dry basis): $0.55 \leq OS \leq 0.8$; $0.0 \leq OB \leq 0.25$; $0.2 \leq CC \leq 0.45$. The justification is given in Section 3.

The experimental composition of mixtures were calculated by simplex centroid design [11,19]. The resulting compositions and experimental region are shown in Table 1 and Fig. 1 (shaded area), respectively. Fermenting material for SSC was prepared as follows; a 10 g sample of dry matter mixture was blended with enough Na_2CO_3 (0.12 g dm^{-3}) solution in order to fix the initial pH at 6.5 and the moisture content at 72%. Material was packed at an apparent density from 0.33 to 0.38 g cm^{-3} in 125 cm^3 glass bottles, 11 cm length

Table 1
Influence of the mixture composition on the apical growth rate (AGR, cm day^{-1}) of *P. ostreatus* and the loss of dry matter (LDM, g per 100 g) during solid cultivation of *Pleurotus ostreatus*

Substrate composition*			AGR** (cm day^{-1})	LDM** (g per 100 g initial dry matter)
Oat straw	Copra cake	Oat bran		
0.800	0.200	0.000	0.464^{ab}	8.52^{a}
0.550	0.200	0.250	0.463^{ab}	18.32^{b}
0.550	0.450	0.000	0.431^{a}	17.43^{b}
0.675	0.200	0.125	0.472^{ab}	10.99^{ab}
0.550	0.325	0.125	0.446^{ab}	15.09^{ab}
0.675	0.325	0.000	0.455^{ab}	15.81^{ab}
0.633	0.284	0.083	0.502^{b}	14.94^{ab}
0.717	0.241	0.042	0.486^{ab}	9.97^{ab}
0.592	0.241	0.167	0.461^{ab}	10.86^{ab}
0.592	0.366	0.042	0.454^{ab}	14.27^{ab}

* Expressed in dry matter fractions.
** Values with different letters are significantly different ($P<0.05$).

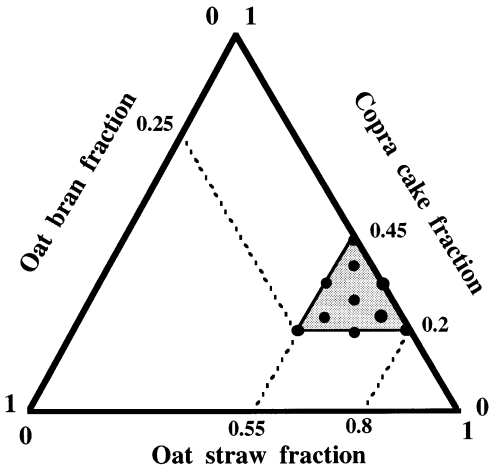

Fig. 1. Experimental region (shaded area) and substrate compositions (●) of the mixtures containing (g g^{-1} mixture, dry basis): oat straw (OS), oat bran (OB) and copra cake (CC) during mycelial cultivation of *P. ostreatus*. Lower and upper limits for the mass fraction of each component are shown.

and 4 cm diameter. They were cotton plugged, sterilised at 121°C for 60 min and further inoculated with 3.45 g of secondary inoculum (wet basis) on top of the substrate (Fig. 2b). Cultures were prepared in triplicate.

The following response variables were measured: (i) Apical growth rate (AGR) in terms of mycelia length in cm day^{-1}. Four measurements from equidistant points around the bottle circumference were taken every 24 h as indicated in Fig. 2(a). Mycelial length was measured by using a vernier (Fig. 2b) in each point and the mean value was plotted against time and the slope calculated. (ii) The loss of dry matter (LDM) of the substrate was calculated taking into account the initial and final dry matter of the substrate and expressed in terms of g per 100 g initial dry matter, as described by Sato and co-workers [20].

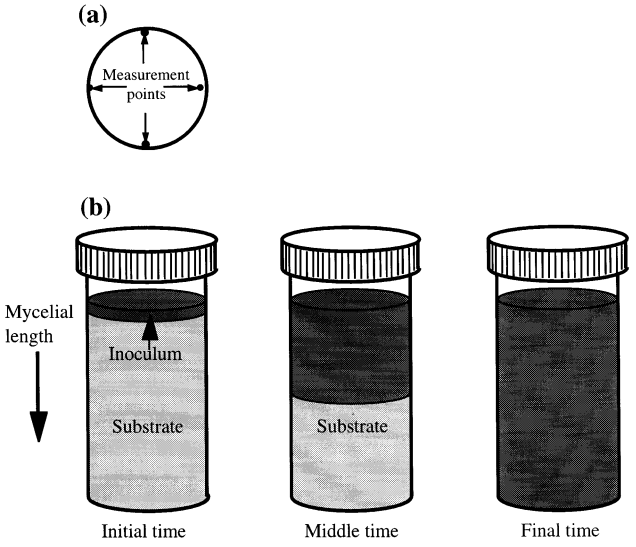

Fig. 2. Schematic representation of the experimental system. Cross view showing the four measurements points for mycelial length (a) and lateral view showing the inoculation point (b).

2.4. Statistical analysis

Statistical analysis was performed using the program Statistical Analysis System®, significant differences ($P < 0.05$) were confirmed by ANOVA analysis. The general form of the Scheffé model [11,19]:

$$\eta = \sum_{i=1}^{p} \beta_i X_i + \sum\sum_{i<j}^{p} \beta_{ij} X_i X_j + \sum \sum_{i<j<k}^{p} \sum \beta_{ijk} X_i X_j X_k \quad (1)$$

was applied to the response variables, for the case three components the model (Eq. (1)) can be written for apical growth rate as follows:

$$\mathrm{AGR} = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3 \quad (2)$$

and for loss dry matter as:

$$\mathrm{LDM} = \lambda_1 X_1 + \lambda_2 X_2 + \lambda_3 X_3 + \lambda_{12} X_1 X_2 + \lambda_{13} X_1 X_3 + \lambda_{23} X_2 X_3 + \lambda_{123} X_1 X_2 X_3 \quad (3)$$

X_1, X_2 and X_3 correspond to the dry matter fractions of OS, CC and OB, respectively. The coefficients β_i and λ_i were calculated by multiple regression analysis by using Stat Graphics®.

In the case of a mixture of three components the addition of the mass fractions (g g^{-1} mixture, dry basis) of each component must be equal to 1, that is 100% of the mixture.

3. Results and discussion

Cornell [11] has pointed out: in the general mixture problem, the response that is measured is a function only of the proportions of the ingredients present in the mixture and is not a function of the amount of the mixture. Depending on the knowledge of the experimental systems, some constraints can be applied to the mixture components to reduce the experimental region as indicated below.

According to Zadrazil and co-workers [7] the moisture content of the substrate has to be greater than 70% to guarantee the mycelial growth of *Pleurotus*. On the other hand, the water retention capacity (g water per g dry matter) of components was 4, 1.5 and 1.5 for OS, OB and CC, respectively. As consequence the mass fraction of OS must be at least 0.55 ($L_{OS} = 0.55$, g g^{-1} mixture, dry basis) to obtain a moisture content greater than 70%. This was the first constraint.

Coffee pulp gives the best biological efficiency among all single agro-industrial residues used in Mexico [21].

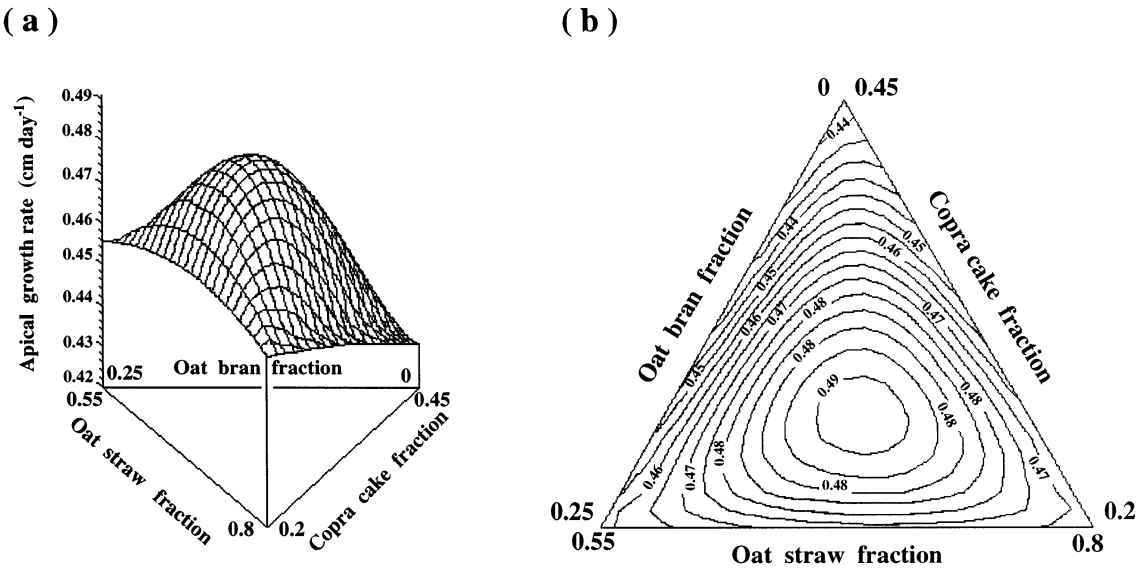

Fig. 3. Effect of oat straw, copra cake and oat bran mass fractions (g g^{-1} mixture, dry basis) on the apical growth rate (AGR, cm day^{-1}) during solid substrate cultivation of *Pleurotus ostreatus*. Response surface (a) and contours (b).

Coffee pulp C/N ratio was measured and it was nearly 30. The C and N concentrations determined for OS, OB and CC were (g per 100 g, dry basis): 45.54 ± 0.03 and 0.87 ± 0.01; 48.95 ± 0.13 and 1.87 ± 0.12; 45.96 ± 0.01 and 3.69 ± 0.09, respectively. The C/N ratio of the mixtures was maintained lower than 30 in order to obtain nitrogen concentration higher than that supplied by coffee pulp. According to this criterion the mass fraction of CC should be greater than 0.05. However, considering the third constraint (see below) the L_{CC} must be 0.2 (g g^{-1} mixture, dry basis). This was the second constraint.

The third constraint was defined taking into account the cost of raw materials. That is, the mixture cost should be less than 2% of the price of *P. ostreatus* fruiting bodies in the retail market of Mexico City. This constraint was satisfied working with a oat bran mass fraction lower than 0.25 (g g^{-1} mixture, dry basis), given a $L_{OB} = 0$. The experimental region, showed in Fig. 1 (shaded area), was delimited combining these three constraints.

Cellulose and lignin are the main components of oat straw, 44 and 16.5%, respectively [22]. According to Kent [23] oat bran is 63% starch. The nitrogen content of CC was twice and three times greater than the nitrogen content of OS and OB, respectively. The components of the mixture were considered to be sources of cellulose and lignin (OS), starch (OB) and nitrogen (CC).

Table 1 shows the compositions of the experimental mixtures and the observed values of AGR and LDM after mycelial growth of *P. ostreatus*. The sample coefficient of variation ([standard deviation/average] × 100) of experimental results were less than 8.6%, in all cases. The maximum AGR value was observed at the dry matter fractions of 0.633 (OS), 0.284 (CC) and 0.083 (OB). The minimum AGR value was observed at the dry matter fractions of 0.55 (OS), 0.45 (CC) and 0.0 (OB). These points correspond to the centre and the upper point in the experimental shaded region indicated in Fig. 1, respectively. ANOVA analysis indicated significant differences ($P < 0.05$) between maximum and minimum responses for AGR (Table 1), showing that mixture composition has effect on AGR.

AGR data were fitted to the Scheffé model [19] and coefficients estimated by multiple regression, given:

$$\mathrm{AGR} = 0.45\,X_1 + 0.11\,X_2 + 7.11\,X_3 + 0.55\,X_1\,X_2 - 11.88\,X_1\,X_3 - 34.27\,X_2\,X_3 + 61.9\,X_1\,X_2\,X_3 \quad (4)$$

where the correlation coefficient (R^2) obtained was 0.999.

It is important to point out, that the calculated values of the responses are valid only within the defined experimental zone. The response surface and contours of AGR were plotted using Eq. (4) and are shown in the Fig. 3. It shows that the maximum value is located at the base of the triangle. The fit to experimental data was high, according to the results of multiple regression analysis. The calculated maximum forAGR (0.49 cm day^{-1}) was located at the dry matter fractions of 0.65, 0.27 and 0.08 for OS, CC and OB, respectively, which was close to the observed maximum (0.502 cm day^{-1}). In this sense, it was found that the mycelial growth rate in SSC could be maximised by selecting appropriate fractions of different materials in mixtures. These results of AGR are slightly lower to that reported for different strains of *P. ostreatus* in Petri dishes cultures

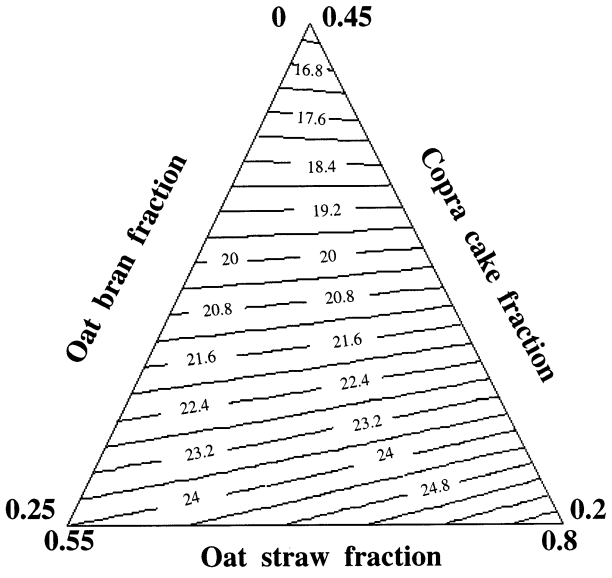

Fig. 4. Effect of oat straw, copra cake and oat bran mass fractions (g g^{-1} mixture, dry basis) on the C/N ratio of the mixtures used as substrates for the cultivation of *Pleurotus ostreatus* mycelia.

using glucose (0.64 cm day^{-1}) and starch (0.57 cm day^{-1}) as carbon sources. This can be explained by the differences in the experimental systems used [24].

The maximum calculated value for AGR is located in a region where the C/N ratio was close to 22.4–23.2 (Fig. 4). Using mixtures with nutritional value higher than residues traditionally used, as substrate in SSC of *Pleurotus*, it is possible to obtain greater biological efficiencies [25,26].

The maximum observed value of LDM was obtained by using the dry matter fractions of 0.55 (OS), 0.2 (CC) and 0.25 (OB) and the minimum LDM value was obtained at dry matter fractions of 0.8 (OS), 0.2 (CC) and 0.0 (OB) (Table 1). These points correspond to the left lower and right lower points indicated in the triangle of experimental shaded region (Fig. 1). ANOVA analysis indicated significant differences ($P < 0.05$) between maximum and minimum responses for LDM (Table 1). The sample coefficient of variation ([standard deviation/mean] × 100) of experimental results were less than 10.1%, for all the cases.

As indicated previously, LDM data were fitted to the Scheffé model [18] and coefficients estimated by multiple regression given:

$$\begin{aligned}\text{LDM} = &-14.65\,X_1 - 41.33\,X_2 + 109.23\,X_3 \\ &+177.87\,X_1\,X_2 - 21.12\,X_1\,X_3 + 234.36\,X_2\,X_3 \\ &-788.75\,X_1\,X_2\,X_3 \qquad (5)\end{aligned}$$

where the correlation coefficient (R^2) obtained was 0.976.

Response surface and contours of LDM were plotted using Eq. (5) and results are shown in Fig. 5. At the bottom right side of the triangle (dry matter fractions of 0.8, 0.2 for OS and CC, respectively) the lower degradation level (LDM = 8.5%) of the mixture by *P. ostreatus* was observed. The best results of substrate utilisation, expressed as loss of dry matter, were found at low mass fractions of OS (≈ 0.55) and higher fractions of CC (≈ 0.45) and OB (≈ 0.25). This corresponds to the upper and left lower points of the experimental region (Fig. 5), where high proportions of easily assimilable nutrients are provided by the CC or OB. Similar results of LDM have been reported for *Pleurotus* grown on wheat straw where lignocellulosic substrates are less utilised than starchy materials [26]. These results are also supported by experimental evi-

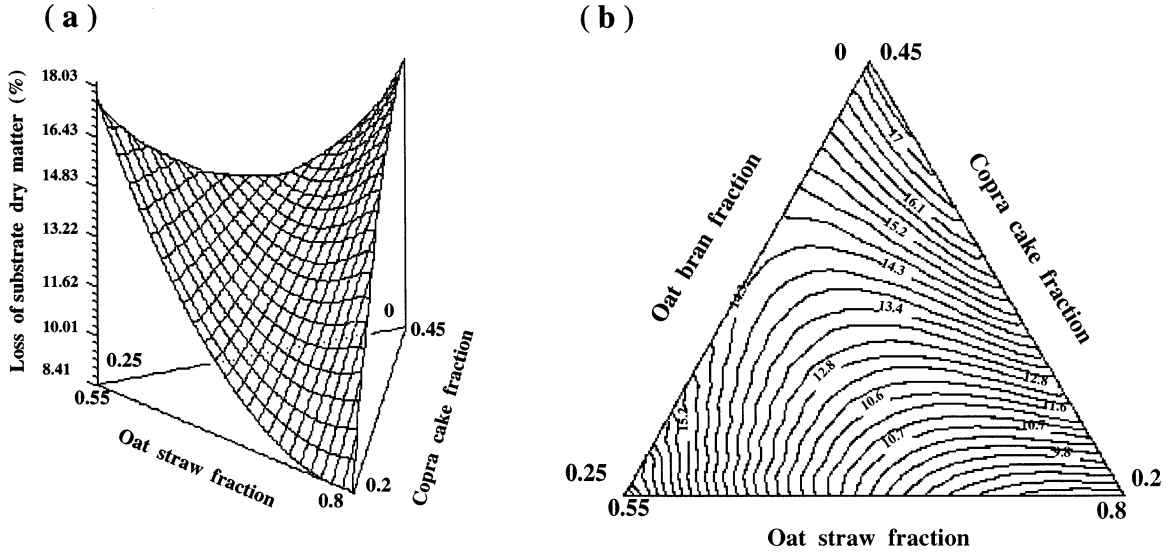

Fig. 5. Effect of oat straw, copra cake and oat bran mass fractions (g g^{-1} mixture, dry basis) on the loss of dry matter (LDM, g per 100 g) of the mixture used as substrate during solid cultivation of *Pleurotus ostreatus*. Response surface (a) and contours (b).

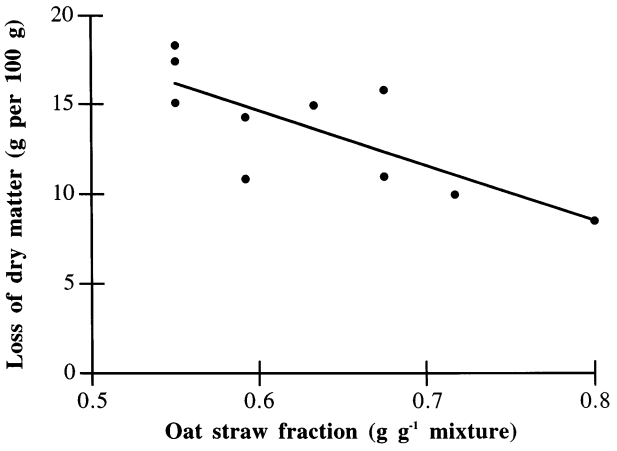

Fig. 6. Effect of the oat straw mass fraction (g g^{-1} mixture, dry basis) on the loss of dry matter (LDM, g per 100 g) of the mixture used as substrate during solid cultivation of *Pleurotus ostreatus*.

dence. Fig. 6 shows a linear decrease of LDM with increasing amounts of oat straw. *Pleurotus* has a particular ability to degrade lignocellulosic substrates which have a poor content of nitrogen as well as other nutrients [27]. This deficiency can be overcome if lignocellulosic residues are mixed with other substrates such as copra cake and oat bran. A better mycelial development could be achieved by addition of easily assimilable nutrients from OB or CC. Nevertheless, the dry matter fraction of lignocellulosic residues must be kept high enough in order to profit from agricultural residues. In this sense, C/N ratio as well as carbon source origin (lignin or starch) should be considered in mixture design.

4. Conclusions

The use of mixture methodology allowed the development of an experimental design to study the effects of main characteristics (water retention capacity, C/N ratio and cost) of the mixtures upon the mycelial growth of *P. ostreatus*.

Once the upper and lower limits of each substrate (OB, OS and CC) were established, the use of the response surface methodology lead to a mixture composition where the maximum apical growth rate was observed. The maximum value of AGR (0.50 ± 0.02 cm day^{-1}) was found by using 0.633, 0.284 and 0.083 (g g^{-1} mixture, dry basis) for OS, CC and OB fractions, respectively. AGR was inversely related to the incubation time required to complete mycelial invasion of the fermenting bulk.

Loss of dry matter decreased from 16.9 to 8.5% as the OS fraction (lignin and cellulose source) was increased from 0.55 to 0.80. This is related to the content of the starchy substrate (OB). Its maximum value was not observed within the experimental studied region. Higher OB concentration will increase LDM with an undesirable reduction on AGR and the increase of mixture cost.

Mixture and response surface methodologies, in complex biological systems, are useful approaches when different process variables (LDM, AGR) are evaluated while selecting the appropriate mixtures in cultivation of edible mushrooms.

References

[1] Ghosh N, Chakravarty DK. Predictive analysis of the protein quality of *Pleurotus citrinopileatus*. J Food Sci Technol 1990;27:236–8.
[2] Martínez-Carrera D, Sobal M, Morales P, Martínez-Sánchez W, Aguilar A, Larqué-Saavedra A. Edible mushroom cultivation and sustainable agriculture in Mexico. Afr J Mycol Biotechnol 1995;3:13–8.
[3] Flegg PB. Future strategies for mushroom production. Mushroom Res 1992;1:25–32.
[4] Martínez-Carrera D. Producción de *Pleurotus* en México. Paper presented at the VI Congreso Nacional de Micología, Tapachula, Chiapas, México, 15–17 October 1997.
[5] Ragunathan R, Gurusamy R, Palaniswamy M, Swaminathan K. Cultivation of *Pleurotus* spp. on various agro-residues. Food Chem 1996;55:139–44.
[6] Chang ST, Quimio TH. Tropical mushrooms, biological nature and cultivation methods. Hong Kong: The Chinese University Press, 1982.
[7] Zadrazil F, Ostermann D, Dal Compare G. Production of edible mushrooms. In: Doelle HW, Mitchell DA, Rolz CA, editors. Solid substrate cultivation. London and New York: Elsevier Applied Science, 1992:283–320.
[8] Zadrazil F, Dube HC. The oyster mushroom—importance and prospects. Mushroom Res 1992;1:25–32.
[9] Stölzer S, Grabbe K. Mechanisms in substrate selectivity in the cultivation of edible fungi. In: Maher MJ, editor. Science and cultivation of edible fungi, Conference, Dublin, 1–6 Sept. 1991. p. 141–146.
[10] Houdeau G, Olivier JM, Libmond S, Bawadikji H. Improvement of *Pleurotus* cultivation. In: Maher MJ, editor. Science and cultivation of edible fungi, Conference, Dublin, 1–6 Sept. 1991. p. 549–554.
[11] Cornell JA. Experiments with mixtures. New York: John Wiley and Sons, 1981.
[12] Myers H, Khuri AY, Carter WH. Response surface methodology: 1966–1988. Technometrics 1989;31:137–57.
[13] Roka F, Hoag D, Vickner S. Response surface methodology for modeling complex-systems. Am J Agric |Econ 1996;78:1406–6.
[14] Diniz FM, Martin AM. Use of response surface methodology to describe the combined effects of temperature and E/S ratio on the hydrolysis of dogfish (*Squalus acanthias*) muscle. Int J Food Sci Technol 1996;31:419–26.
[15] Guinard JX, Zoumas-Morse C, Mori L, Panyam D, Kilara A. Effect of sugar and fat on the acceptability of vanilla ice cream. J Dairy SciTechnol 1996;79:1922–7.
[16] Hwang S, Hancen CL. Modeling and optimization in anaerobic bioconversion of complex substrates to acetic and butyric acids. Biotechnol Bioeng 1997;54:451–60.
[17] Rastogi NK, Rajesh G, Shamala TR. Optimization of enzymatic degradation of coconut residue. J Sci Food Agric 1998;76:129–34.

[18] APHA, AWWA and WPCF. Standard methods for the analysis of water and wastewater. American Public Health Association, Washington, 1989.
[19] Scheffé H. The simplex centroid design for experiments with mixtures. J Roy Stat Soc (Series B) 1963;25:235–63.
[20] Sato K, Miyazaki SI, Matsumoto N, Yoshizawa K, Nakamura KI. Pilot-scale solid state ethanol fermentation by inert gas recirculation using moderately thermophilic yeast. J Ferment Technol 1988;66:173–80.
[21] Martínez-Carrera D, Morales P, Sobal M. Cultivo de diversas cepas mexicanas de *Pleurotus ostreatus* sobre pulpa de café y paja de cebada. Rev Mex Mic 1988;4:153–60.
[22] von Boguslawsky E, Debruck J. La paja y la fertilidad de los suelos. México D.F.: Compañia Editorial Continental, 1983.
[23] Kent NL. Technology of cereals. An introduction for students of food science and agriculture. Oxford: Pergamon Press Ltd, 1986.
[24] Sanchez C, Viniegra-Gonzalez G. Detection of highly productive strains of *Pleurotus ostreatus* by their tolerance to 2-deoxy-D-glucose in starch-based media. Mycol Res 1996;100:455–61.
[25] Jwanny EW, Rashad MM, Abdu HM. Solid state fermentation of agricultural wastes into food through *Pleurotus* cultivation. Appl Biochem Biotechnol 1995;50:71–8.
[26] Royse DJ, Zaki SA. Yield stimulation of *Pleurotus flavelatus* by dual nutrient suplementation of pasteurised wheat straw. In: Maher MJ, editor. Science and cultivation of edible fungi, Conference, Dublin, 1–6 Sept. 1991. p. 545–547.
[27] Galli E, Tomati U, Grappelli A, Di Lena G, Pietrosanti W. Solid state degradation of agricultural wastes by *Pleurotus* species. In: Maher MJ, editor. Science and cultivation of edible fungi, Conference, Dublin, 1–6 Sept. 1991. p. 705–708.