Available online at www.sciencedirect.com
ScienceDirect
Bioresource Technology 99 (2008) 76–82
BIORESOURCE TECHNOLOGY

Alternative medium for production of *Pleurotus ostreatus* biomass and potential antitumor polysaccharides

Regina Maria Miranda Gern [a,*], Elisabeth Wisbeck [b], Jamile Rosa Rampinelli [c], Jorge Luiz Ninow [d], Sandra Aparecida Furlan [b]

[a] *Department of Biology Sciences, University of the Region of Joinville, UNIVILLE, M Box 246, CEP 89.201-972 Joinville, Santa Catarina, Brazil*
[b] *Department of Environmental Engineering, University of the Region of Joinville, UNIVILLE, M Box 246, CEP 89.201-972, Joinville, Santa Catarina, Brazil*
[c] *Department of Industrial Chemistry, University of the Region of Joinville, UNIVILLE, M Box 246, CEP 89.201-972 Joinville, Santa Catarina, Brazil*
[d] *Department of Food and Chemical Engineering, Federal University of Santa Catarina, UFSC/CTC/EQA, M Box 476, Campus Universitário Trindade, 88040-900 Florianópolis, Santa Catarina, Brazil*

Received 18 September 2006; received in revised form 27 November 2006; accepted 28 November 2006
Available online 15 February 2007

Abstract

Pleurotus species are recognized for producing β-glucans with important medicinal properties as a constituent of the cellular wall of the fruiting body or of the mycelium. The aims of this work were to select a culture medium that maximized the production of biomass and polysaccharides produced by *Pleurotus ostreatus* DSM 1833 and to evaluate the selected medium in two values of initial oxygen transfer rate – K_La (10.2 and 19.3 h^{-1}). A 2**4 factorial design was constructed to evaluate the supplementation of wheat extract with corn steep liquor – CSL (10 or 20 g L^{-1}), yeast extract – YE (2 or 5 g L^{-1}), ammonium sulfate – AS (0 or 5 g L^{-1}) and glucose (20 or 40 g L^{-1}). In terms of maximum productivity in biomass and global productivity in polysaccharides, the best values were obtained when 5 g L^{-1} of YE and 40 g L^{-1} of glucose were used. In terms of maximum concentration of biomass, the best results were obtained when 20 g L^{-1} of CSL and 40 g L^{-1} of glucose were used. The best results in terms of production of biomass and polysaccharides were achieved when lower initial K_La (10.2 h^{-1}) was used.

Keywords: *Pleurotus ostreatus*; Antitumor polysaccharides; Mushrooms; Alternative culture medium

1. Introduction

Fungi of the *Pleurotus* genus have an important place among the commercially employed basidiomycetes because they have gastronomic, nutritional and medicinal properties and can be easily cultivated on a large range of substrates. Besides the studies in solid culture aiming for the production of fruit bodies, the submerged culture of the genus *Pleurotus* has also been studied by several authors with the most varied objectives including the production of liquid inoculum (Rosado et al., 2002), extracellular enzymes (Garzillo et al., 1994), flavoring agents (Martin, 1992), β-glucosidases (Morais et al., 2002), antimicrobials (Beltran et al., 1997; Wisbeck et al., 2002; Benkortbi et al., in press) and vitamins (Solomko and Eliseeva, 1988). Biomass and intra and extracellular polysaccharides are also the aim of several studies. Jung et al. (2003) investigated the effect of thinned fruits, apple, pear and peach on the mycelial growth of *Pleurotus* finding superior yields of biomass than those found in control medium. Wang et al. (2005) used a response surface methodology to determine the optimal conditions for production of water soluble polysaccharides of the culture broth of *Pleurotus citrinopileatus*. Sarangi et al. (2006) purified proteoglycan

* Corresponding author. Tel.: +55 47 3461 9017; fax: +55 47 3461 9077.
E-mail address: rgern@univille.edu.br (R.M.M. Gern).

0960-8524/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2006.11.059

fractions from *P. ostreatus* mycelia that could be used as immnomodulators and anticancer agents.

The polysaccharides represent the major constituent that determines the rigidity and morphological properties of the fungal cell wall and, depending on the culture conditions they can also be excreted to the culture medium. Among the polysaccharides produced by *Pleurotus* spp., β-1,3 glucans play an important role as biological response modifiers (BRMs) (Bohn and BeMiller, 1995) which stimulate the immune system of the host and exert an extensive range of immunopharmacological activities, in particular an antitumor effect and the inhibition of metastasis, as well as the stimulation of hematopoiesis (Mizuno and Zhuang, 1995; Gunde-Cimerman, 1999; Wang et al., 2005).

The possibility of application of these biopolymers in human health led to intensive research on the characterization and evaluation of these polysaccharides (Karácsonyi and Kuniak, 1994; Gutiérrez et al., 1996; Rout et al., 2005). The present work was designed to evaluate alternative culture media in order to maximize the production of biomass and polysaccharides by *P. ostreatus* DSM 1833. Aiming for a clean process and to reduce the medium costs, two kinds of residues were used in the composition of the culture medium: corn steep liquor, generated as a residue of the corn industry and already tested with good results as a nutrient for bacteria and fungi, and wheat extract, generated as a residue of the spawn industry.

2. Methods

2.1. Microorganism

P. ostreatus DSM 1833, obtained from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen) was maintained at 4 °C on WDA (Wheat Dextrose Agar) (Furlan et al., 1997) Petri dishes.

2.2. Conduction of the experiments

Seventeen culture media were generated by a 2^4 factorial design, with a central point, to evaluate the effect of medium composition on the production of biomass and polysaccharides by *P. ostreatus*. The effects of wheat extract (WE: infusion obtained by boiling 1 kg of wheat grains in 2 L of water, for 10 min) supplementation with different sources and concentrations of organic nitrogen (2 or 5 g/L of yeast extract – YE or 10 or 20 g/L of corn steep liquor – CSL), different glucose concentrations (20 or 40 g/L) and the presence of ammonium sulfate were evaluated. The experimental design is presented in Table 1.

The experiments were carried out in duplicate, in 500 mL Erlenmeyer flasks containing 100 mL of the evaluated medium, autoclaved at 121 °C, for 15 min, inoculated with three agar disks colonized with the mycelia of *P. ostreatus*. The initial pH was not adjusted. Media containing yeast extract showed an average pH of 6.0 and media containing corn steep liquor showed average pH of 4.5. The flasks were incubated at 30 °C under agitation at 120 rpm, for a period of 8–14 days. Periodically, the whole volume of a flask was removed for biomass and polysaccharides assay. The medium in which *P. ostreatus* achieved the best results in terms of biomass and polysaccharides was evaluated in a 4 L bioreactor using two values of initial K_La (10.2 h^{-1}, at 330 rpm and 1 L of air/min, and 19.3 h^{-1}, at 400 rpm and 2 L of air/min). The pH and the temperature were kept at 4.0 and 30 °C, respectively. The medium was inoculated with the resultant biomass of *P. ostreatus* cultured in 400 mL of WE with 20 g/L of glucose (Furlan et al., 1997).

Table 1
Evaluated factors in the factorial design used for definition of a culture medium for the production of biomass and polysaccharides by *P. ostreatus*

Factors	Level		
	–	0	+
Organic nitrogen source	CSL	–	YE
Organic nitrogen source concentration (g/L)	2.0 (YE) or 10.0 (CSL)	0 (YE or CSL)	5.0 (YE) or 20.0 (CSL)
Ammonium sulfate concentration (g/L)	0	2.5	5
Initial glucose concentration (g/L)	20.0	30.0	40.0

CSL – corn steep liquor.
YE – yeast extract.
(−), (0) and (+) indicate the factors level as inferior, central and superior, respectively.

2.3. Biomass concentration

The culture broth was filtered through Whatman no. 1 filter paper, washed with distilled water and the retained biomass were dried at 60 °C for 48 h for the determination of the biomass dry weight.

2.4. Intracellular polysaccharides and exopolysaccharides concentration

For the extraction of the intracellular polysaccharides, the mycelia were initially washed with ethanol (96%), at a volume ratio of 1:2 (biomass:alcohol) to eliminate the low molecular weight components (Berovic et al., 2003). The biomass was washed and filtered to eliminate the residual ethanol, before adding 5 vol of distilled water and boiling for 4 h to extract the crude polysaccharides. The suspension was filtered and the filtrate was treated with cooled ethanol (8 °C) at a volume ratio of 4:1 (ethanol:sample). After 48 h under refrigeration (4 °C) the sample was centrifuged at 5000*g* for 10 min (Xu et al., 2003; Lee et al., 2003). The exopolysaccharides were extracted from the culture broth after the separation of the biomass, and the broth was treated with ethanol, following the same method described for intracellular polysaccharides. Finally, the concentration of the polysaccharides and exopolysaccharides obtained was determined by the phenol–sulfuric method (Dubois et al., 1956).

3. Results and discussion

3.1. Evaluation of different culture media for production of biomass and polysaccharides by P. ostreatus

The results obtained with *P. ostreatus* grown in the different culture media and the effects of the evaluated factors on the maximum biomass concentration (X_{max}) expressing the highest value of biomass in g/L, maximum biomass productivity ($P_{X_{max}}$), expressing the highest value of productivity in grams of biomass/L day^{-1}, maximum specific growth rate ($\mu_{X_{max}}$), intracellular polysaccharides concentration (PSX – grams of intracellular polysaccharides/gram of dried biomass), and global polysaccharides productivity (P_{PS}-concentration of intracellular polysaccharides at the end of the process) are shown in Tables 2 and 3, respectively.

Considering the values of the individual effects presented in Table 3 it can be concluded that the organic nitrogen source (CSL or YE) has a significant effect on the maximum concentration of biomass. In this case, the substitution of YE by CSL provides better results. The fact that this substitution did not significantly affect the maximum productivity in biomass can be explained by the longer

Table 2
Maximum cell concentration (X_{max}), maximum cell productivity ($P_{X_{max}}$), maximum specific growth rate ($\propto X_{max}$), intracellular polysaccharides concentration (PSX), and total polysaccharides productivity (P_{PS}) for *P. ostreatus* grown on the 17 different culture media

Medium	ONS[a]	[AS][b]	[ON][c]	[GLU][d]	X_{max} (g/L)	$P_{X_{max}}$ (g/L day)	$\mu_{X_{max}}$ (day^{-1})	PSX (mg/g)	P_{PS} (mg/L day)
1	YE	5	5	40	11.15 ± 0.14	1.16 ± 0.00	0.87 ± 0.08	19.46 ± 1.12	17.12 ± 0.97
2	YE	5	5	20	10.86 ± 0.51	0.90 ± 0.07	0.40 ± 0.07	21.51 ± 0.83	18.55 ± 1.44
3	CSL	5	20	40	22.45 ± 0.37	1.57 ± 0.03	0.38 ± 0.03	4.72 ± 0.00	7.95 ± 0.08
4	CSL	5	20	20	17.12 ± 1.82	1.16 ± 0.11	0.34 ± 0.02	10.57 ± 0.00	12.32 ± 1.48
5	YE	5	2	40	6.38 ± 0.62	0.50 ± 0.00	0.27 ± 0.05	6.41 ± 0.80	3.49 ± 0.74
6	YE	5	2	20	4.99 ± 0.02	0.44 ± 0.04	0.35 ± 0.03	26.25 ± 1.55	10.16 ± 0.43
7	CSL	5	10	40	9.45 ± 0.81	0.66 ± 0.04	0.33 ± 0.02	8.06 ± 0.07	4.97 ± 0.56
8	CSL	5	10	20	8.05 ± 0.16	0.54 ± 0.00	0.26 ± 0.00	0.96 ± 0.24	0.53 ± 0.15
9	YE	0	5	40	20.49 ± 1.83	2.78 ± 0.23	0.85 ± 0.00	7.25 ± 0.55	20.05 ± 3.37
10	YE	0	5	20	14.15 ± 0.58	1.55 ± 0.15	0.69 ± 0.10	14.84 ± 1.96	19.99 ± 3.41
11	CSL	0	20	40	29.64 ± 1.54	2.00 ± 0.14	0.41 ± 0.03	8.44 ± 1.61	17.01 ± 4.15
12	CSL	0	20	20	16.33 ± 0.07	1.30 ± 0.04	0.50 ± 0.03	9.80 ± 0.93	11.30 ± 1.01
13	YE	0	2	40	17.60 ± 0.17	2.09 ± 0.10	0.58 ± 0.02	4.98 ± 1.35	11.00 ± 2.93
14	YE	0	2	20	13.63 ± 0.80	1.65 ± 0.11	0.68 ± 0.02	7.93 ± 0.19	11.48 ± 0.94
15	CSL	0	10	40	17.80 ± 0.37	2.07 ± 0.30	0.70 ± 0.06	7.06 ± 0.55	9.84 ± 0.86
16	CSL	0	10	20	13.09 ± 0.61	1.34 ± 0.06	0.44 ± 0.01	12.14 ± 4.33	12.99 ± 5.17
17	–	2.5	0	30	1.69 ± 0.27	0.25 ± 0.04	0.34 ± 0.03	9.95 ± 0.00	1.61 ± 0.23

CSL – Corn steep liquor & YE – yeast extract.
[a] Organic nitrogen source.
[b] Ammonium sulfate concentration (g/L).
[c] Organic nitrogen concentration (g/L).
[d] Initial glucose concentration (g/L).

Table 3
Effect of the evaluated factors on the maximum cell concentration (X_{max}), maximum cell productivity ($P_{X_{max}}$), maximum specific growth rate ($\propto X_{max}$), intracellular polysaccharides concentration (PSX), and total polysaccharides productivity (P_{PS})

	X_{max}	$P_{X_{max}}$	$\mu_{X_{max}}$	PSX	P_{PS}
1 ONS	−3.62 ± 1.45*	0.06 ± 0.14	0.17 ± 0.03*	5.86 ± 1.31*	4.29 ± 1.68*
2 [ON]	6.16 ± 1.45*	0.39 ± 0.14*	0.10 ± 0.03*	2.85 ± 1.31*	7.40 ± 1.68*
3 [AS]	−6.78 ± 1.45*	−0.99 ± 0.14*	−0.21 ± 0.03*	3.18 ± 1.31*	−4.75 ± 1.68*
4 [GLU]	4.94 ± 1.45*	0.50 ± 0.14*	0.09 ± 0.03*	−4.70 ± 1.31*	−0.81 ± 1.68
1 and 2	−2.17 ± 1.45	0.04 ± 0.14	0.13 ± 0.03*	1.53 ± 1.31	2.35 ± 1.68
1 and 3	−1.19 ± 1.45	−0.28 ± 0.14	−0.02 ± 0.03	6.47 ± 1.31*	1.59 ± 1.68
1 and 4	−1.02 ± 1.45	−0.003 ± 0.14	0.02 ± 0.03	−3.41 ± 1.31*	−1.47 ± 1.68
2 and 3	1.72 ± 1.45	0.27 ± 0.14	0.09 ± 0.03*	0.80 ± 1.31	1.79 ± 1.68
2 and 4	1.89 ± 1.45	0.16 ± 0.14	0.06 ± 0.03	0.49 ± 1.31	0.66 ± 1.68
3 and 4	−2.57 ± 1.45	−0.28 ± 0.14	0.03 ± 0.03	−0.46 ± 1.31	−1.20 ± 1.68
1, 2 and 3	−0.37 ± 1.45	−0.13 ± 0.14	0.00 ± 0.03	−1.01 ± 1.31	−0.53 ± 1.68
1, 2 and 4	−1.46 ± 1.45	0.09 ± 0.14	0.15 ± 0.03*	2.80 ± 1.31*	0.64 ± 1.68
1, 3 and 4	−0.37 ± 1.45	−0.06 ± 0.14	0.05 ± 0.03	−2.38 ± 1.31	−0.57 ± 1.68
2, 3 and 4	−0.91 ± 1.45	−0.03 ± 0.14	0.08 ± 0.03*	0.72 ± 1.31	−1.55 ± 1.68
ASSV**	|3.03|	|0.29|	|0.06|	|2.74|	|3.51|

* Statistically significant effects (95% confidence limits).
** Absolute statistically significant value.

lag phase presented when *P. ostreatus* is cultivated in the medium containing CSL, probably due to the necessity for adaptation to this new medium, since the inoculum was prepared in a medium composed only of wheat extract and glucose and the pH of the medium containing CSL (≅4.5) was lower than that of the inoculum medium (≅6.0). Another factor to be considered when the organic nitrogen source is evaluated is the initial pH of the culture medium. The media that contained CSL presented an initial pH about 25% lower than those containing YE. This fact may also have been responsible for the best results found for biomass concentration when CSL was used. In contrast to the results obtained for X_{max}, the maximum specific growth rate, the polysaccharides concentration and the productivity in polysaccharides were negatively affected by CSL. In this case, the use of YE provides better results.

An increase in the organic nitrogen concentration had a significant positive effect on all the variables related to the biomass, notably the biomass concentration. The organic nitrogen concentration at the higher level also favored the increase in the concentration and the productivity of polysaccharides.

The addition of inorganic nitrogen (ammonium sulfate) led to a decrease of all evaluated parameters related to the biomass. The low values obtained for X_{max} and $P_{X_{max}}$ (1.69 g/L and 0.25 g/L day, respectively) when ammonium sulfate was employed in the absence of an organic nitrogen source (central point of the factorial design) suggests that *P. ostreatus* does not use this nitrogen source. Moreover, when these results are compared with those obtained with a medium containing only wheat extract and 20 g/L of glucose (10.58 g/L and 1.48 g/L day, data not shown), it can be seen that the maximum concentration of biomass was approximately sixfold higher, showing a clear inhibition of *P. ostreatus* growth. The polysaccharide productivity also decreases when ammonium sulfate is used. However, the concentration of polysaccharides is positively affected by the presence of this salt.

The increase in the glucose concentration from 20 to 40 g/L had a positive significant effect on the concentration of biomass, the maximum biomass productivity and the maximum specific growth rate. However, using 40 g/L of glucose, only about 67% of the initial concentration of glucose was consumed (with the exception of medium 15), even when the organic nitrogen concentration was increased. This fact can indicate that the inhibition of the glucose consumption is associated with a factor other than the nitrogen concentration. It is presumed that the increase in the final concentration of biomass was provided by the increase of the initial glucose concentration. Furthermore, the increase in the viscosity of the culture medium due to the release of exopolysaccharides made the transfer of oxygen and glucose to the cells difficult, leading to a phase of limitation of nutrients. Burns et al. (1994) also observed that after 20 days cultivation of *P. ostreatus* var *florida*, the exopolysaccharides and biomass production were limited, even with the presence of glucose in the medium, probably due to a polymer layer surrounding the cell. A higher glucose concentration also presents a negative effect on the polysaccharides concentration. Rosado et al. (2002) and Wisbeck et al. (2005) reported that a high glucose concentration favors the production of exopolysaccharides. This may suggest that glucan-synthases produced by *Pleurotus* are directed to the synthesis of extracellular polysaccharides instead of cell wall polysaccharides.

Data presented in Table 3 show the effects of interaction between the evaluated factors. The maximum specific growth rate is affected by the interaction between the factors, returning better results when organic nitrogen and ammonium sulfate concentrations are lower and the glucose concentration is at the highest level. The interaction between the organic nitrogen source and the ammonium sulfate concentration also is significant for the polysaccharides concentration, with better results being achieved when YE is used in the presence of ammonium sulfate. It was also found that when the nitrogen source is CSL, the ammonium sulfate always has a negative effect on the biomass and polysaccharides productivities, leading us to presume that the interaction between this salt and some constituent of the CSL has an inhibitory effect on the production of polysaccharides.

Burns et al. (1994) studied the effect of organic and inorganic nitrogen on the biomass and exopolysaccharides production by *Pleurotus* sp. *florida*. These authors achieved better results in terms of biomass when higher organic nitrogen concentrations were employed. The substitution of organic nitrogen (asparagine and phenylalanine) by inorganic nitrogen (ammonium tartrate) led to a lower performance. On the other hand, conflicting results have been found for the production of exopolysaccharides. In this case, media containing a low nitrogen concentration were the most appropriate for the production of polysaccharides. Rosado et al. (2002), studying the production of exopolysaccharides by *P. ostreatoroseus* and *P. ostreatus* "*florida*" tested the ammonium sulfate concentration at 5.0 and 2.5 g/L, obtaining better results with the latter concentration. Wang et al. (2005) studied the effect of various nitrogen sources on the production of biomass and exopolysaccharides by *P. citrinopileatus*. Values that were 62.5% and 100% lower were found for the biomass concentration and the concentration of polysaccharides, respectively, when ammonium sulfate was used in place of peptone. The analysis of the results shown in Table 2 allows to conclude that the best condition for the increase in the maximum concentration of biomass was reached with 40 g/L of glucose and 20 g/L of CSL, in the absence of ammonium sulfate (medium 11), providing a concentration of 29.64 g/L of biomass. The best condition for the increase in the maximum productivity in biomass was found when 40 g/L of glucose and 5 g/L of YE were used, in the absence of ammonium sulfate (medium 9), providing a maximum productivity in biomass of 2.78 g/L day. The best maximum specific growth rate ($\mu_{X_{max}} = 0.85\ day^{-1}$)

was also achieved in this condition. Similar values were found by Chahal (1989) with the cultivation of *P. sajor-caju*.

Fasidi and Olorunmaiye (1994) evaluated five different inorganic nitrogen sources in the mycelial growth of *P. tuber-regium*: $NaNO_3$, KNO_3, NH_4NO_3, $Ca(NO_3)_2$ and $(NH_4)_2SO_4$. Except for the media containing $Ca(NO_3)$ and NH_4NO_3, in which 2.0 and 1.67 mg/mL of biomass were produced, respectively, all the other inorganic nitrogen sources presented lower biomass concentrations than that obtained with the basal control medium, which did not contain a nitrogen source. Amongst the tested organic nitrogen sources (peptone, urea, yeast extract and casein), yeast extract presented the best results (103 mg/30 mL), suggesting a strong preference of this fungus for organic nitrogen. Manu-Tawiah and Martin (1987) evaluated the effect of the supplementation of organic and inorganic nitrogen in a medium containing turf extract. The authors observed a low production of *P. ostreatus* biomass when ammonium sulfate was used (1.20 g/L), and the best production of biomass was reached with yeast extract (4.98 g/L).

Based on the total productivity, the best condition for the production of polysaccharides (20.05 mg/L day) was found with 5 g/L of YE and 40 g/L of glucose, in the absence of ammonium sulfate (medium 9). Although the results indicate the use of YE in the composition of the medium to obtain more polysaccharides, the high cost of this extract, allied to the small difference found between the productivities in polysaccharides (~18%) obtained with medium 11 (17.01 mg/L day), containing CSL in place of YE, led to the selection of this medium as the most appropriate for the production of biomass and polysaccharides by *P. ostreatus* in submerged culture.

3.2. Evaluation of initial K_La influence

Medium 11 was evaluated in a 4 L bioreactor using an initial K_La of 19.3 h^{-1} and 10.2 h^{-1}. Figs. 1 and 2 show the biomass concentration (X), the substrate consumption (S), the partial pressure of dissolved oxygen – measurement range of 0 to 100% of saturation (pO_2) and the concentration of exopolysaccharides (E) for both conditions tested.

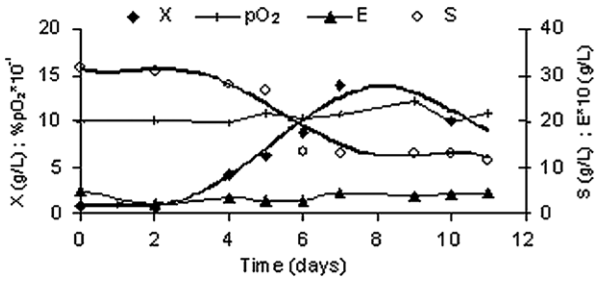

Fig. 1. Kinetics of biomass production (X), substrate consumption (S), dissolved oxygen partial pressure (pO_2) and production of exopolysaccharides (E) for *P. ostreatus* cultivated with an initial K_La of 19.3 h^{-1}.

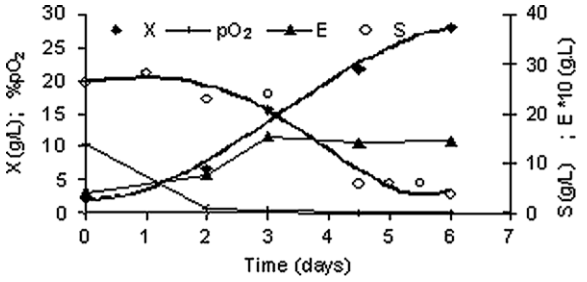

Fig. 2. Kinetics of biomass production (X), substrate consumption (S), dissolved oxygen partial pressure (pO_2) and production of exopolysaccharides (E) for *P. ostreatus* cultivated with an initial K_La of 10.2 h^{-1}.

The curves of X and S for the K_La of 10.2 h^{-1} were adjusted with polynomials of 3rd and 4th order, respectively, and for the K_La of 19.3 h^{-1}, with polynomials of 5th order. Table 4 shows the kinetic parameters – maximum biomass concentration (X_{max}), $\mu_{X_{max}}$ (maximum specific growth rate), $P_{X_{max}}$ (maximum biomass productivity), $Y_{X/S}$ (yield of substrate on biomass), PSX (intracellular polysaccharides concentration – grams of intracellular polysaccharides/gram of dried biomass), P_{PS} (global polysaccharides productivity – concentration of intracellular polysaccharides at the end of the process), P_E (global exopolysaccharides productivity – concentration of extracellular polysaccharides at the end of the process), $Y_{PS/S}$ (yield of substrate on intracellular polysaccharides) and $Y_{E/S}$ (yield of substrate on extracellular polysaccharides) obtained for the experiments carried out at 19.3 and 10.2 h^{-1}, respectively.

The large mass of cells generated when *P. ostreatus* was cultivated with an initial K_La of 10.2 h^{-1} in the bioreactor, together with the production of exopolysaccharides, made the conditions of submerged culture not usual. With the

Table 4
Kinetic parameters for *P. ostreatus* cultivated with initial K_La of 19.3 and 10.2 h^{-1}

	K_La (h^{-1})	
	19.3	10.2
X_{max} (g/L)	12.45	27.72
$\mu_{X_{max}}$ (day^{-1})	0.92	0.84
$P_{X_{max}}$ (g/L day)	1.60	4.56
$Y_{X/S}$ (g/g)	0.64	1.18
PSX (mg/g)	7.35	7.62
P_{PS} (mg/L day)	4.96	35.11
P_E (mg/L day)	0.00	168.33
$Y_{PS/S}$ (mg/g)	3.03	11.70
$Y_{E/S}$ (mg/g)	0.00	47.14

X_{max} – maximum biomass concentration.
$P_{X_{max}}$ – maximum biomass productivity.
PSX – intracellular polysaccharides concentration.
P_E – global exopolysaccharides productivity.
$Y_{E/S}$ – yield of substrate on extracellular polysaccharides.
$\mu_{X_{max}}$ – maximum specific growth rate.
$Y_{X/S}$ – yield of substrate on biomass.
P_{PS} – global polysaccharides productivity.
$Y_{PS/S}$ – yield of substrate on intracellular polysaccharides.

rheology of the medium totally modified, the transmitted agitation frequency was not the same at all points of the bioreactor, leaving cells at the surface practically stationary. During the process, the difficulty in transferring oxygen to the cells was increased. However, these adverse conditions appear to favor the production of exopolysaccharides. It must be pointed that the distinction between biomass, intracellular polysaccharides and exopolysaccharides is difficult. The gelatinous appearance of the pellets due to the presence of exopolysaccharides adhered to the mycelia led to an overestimation of the biomass and the underestimation of exopolysaccharides concentration. When the initial K_La of 19.3 h^{-1} is used, the air flow (2 L/min) is enough to maintain oxygen saturation in the culture broth. However, when an initial K_La of 10.2 h^{-1} is used, the oxygen is quickly consumed (2 days) and *P. ostreatus* grows under oxygen limitation, a condition that appears to favor cell growth and the production of intracellular polysaccharides. The production of exopolysaccharides rapidly increases when the concentration of dissolved oxygen is stabilized at zero. Although some authors report the consumption of this polymer during the process (Rosado, 2002; Wisbeck et al., 2005), this phenomenon was not observed in our study, what could be attributed to the existence of approximately 5 g/L of residual glucose in the culture medium at the end of the process.

Smits et al. (2001), in a review on biogenesis of the cell wall in yeasts, reported that changes in the metabolism and the composition of polysaccharides of the cell wall occur under stress conditions. In response to some signals of stress, such as the limitation of some nutrients and osmotic or thermal stress, the cells activate a regulatory glucan-synthase subunit, increasing the production of cell wall glucans. The influence of agitation and aeration is clear on all the kinetic parameters obtained in the culture of *P. ostreatus*. Increases of 123%, 185% and 608% in X_{max}, $P_{X_{max}}$ and P_{PS}, respectively, were observed when the initial K_La of 10.2 h^{-1} was used. The production of exopolysaccharides was not detected when the initial K_La was 19.3 h^{-1}, while 168.33 mg/L day were produced when an initial K_La of 10.2 h^{-1} was used. Zadrazil (1978) also observed a decrease in the *Pleurotus* biomass yield with the increase in the concentration of oxygen in the solid culture. However, the complete removal of the oxygen causes the inhibition of cell growth. Márquez-Rocha et al. (1999), cultivating *P. ostreatus* observed a 15% and 28% reduction in the maximum specific growth rate when the aeration was increased from 1 to 1.5 vvm and the agitation was increased from 200 to 400 rpm, respectively. The authors also observed a decrease in the size of the pellets when the agitation and the aeration were increased, caused probably by damage to the stability of the pellets. Shu and Wen (2003) studied the production of exopolysaccharides by *Agaricus blazei*, known for its medicinal properties associated with the production of β-glucans, and observed that an increase in the agitation speed negatively affected the production of biomass and polysaccharides.

4. Conclusion

The maximum concentration of biomass was achieved in the media where corn steep liquor (CSL) was used. However, this variable did not present a significant effect on the maximum biomass productivity. Better results in terms of global productivity of polysaccharides were found when yeast extract (YE) was used. The increased concentration of the organic nitrogen source promoted an increase in all the evaluated variables. The ammonium sulfate used as inorganic nitrogen source clearly presented a negative effect on all the evaluated parameters, except on the global productivity of polysaccharides. The higher glucose concentration (40 g/L) provided the best results in terms of maximum productivity in biomass and maximum concentration of biomass. However, the increase in the glucose concentration presented a negative effect on the concentration of polysaccharides. The best conditions in terms of maximum biomass concentration were achieved when wheat extract was supplemented with 20 g/L of CSL and 40 g/L of glucose, in the absence of ammonium sulfate. The best condition for maximum biomass productivity was found when wheat extract was supplemented with 5 g/L of YE and 40 g/L of glucose, in the absence of ammonium sulfate. Even so, the results pointed to the use of YE to obtain more polysaccharides, the low cost of CSL allied to the small difference found between the productivities in polysaccharides (~18%) obtained with the medium containing CSL (17.01 mg/L day), in place of YE (20.05 mg/L day), led to the choice of medium 11 as the most attractive for the production of biomass and polysaccharides by *P. ostreatus* in submerged culture. The comparison of the results obtained for the two different values of initial K_La (10.2 and 19.3 h^{-1}) showed that the performance of *P. ostreatus* in terms of biomass and polysaccharides production is extremely favored by an initial K_La of 10.2 h^{-1}. The comparison of the results obtained with wheat extract supplemented in the conditions defined in this work, with the results reported in the literature led us to confirm the viability of using of this medium in the process of biomass and polysaccharides production by *P. ostreatus*.

References

Beltran, G.M.J., Estarron, E.M., Ogura, T., 1997. Volatile compounds secreted by the oyster mushroom (*Pleurotus ostreatus*) and their antibacterial activity. J. Agric. Food Chem. 45 (10), 4049–4052.

Benkortbi, O., Hanini, S., Bentahar, F., in press. Batch kinetics and modeling of Pleuromutilin production by *Pleurotus mutilis*. Biochem. Eng. J.

Berovic, M., Habijanic, J., Zore, I., Wraber, B., Hodzar, D., Boh, B., Pohleven, F., 2003. Submerged cultivation of *Ganoderma lucidum* biomass and immunostimulatory effects of fungal polysaccharides. J. Biotechnol. 103, 77–86.

Bohn, J.A., BeMiller, J.N., 1995. (1,3)-β-D-Glucans as biological response modifiers: a review on structure–functional activity relationships. Carbohydr. Polym. 28, 3–14.

Burns, P.J., Yeo, P., Keshavarz, T., Roller, S., Evans, C.S., 1994. Physiological studies of exopolysaccharide production from

basidiomycetes *Pleurotus* sp. *Florida*; effect of C and N source on polysaccharide production for potential as a hypocholesterolemic, antitumor and a fat mimetic. Enzyme Microbial. Technol. 34, 566–572.

Chahal, D.S., 1989. Production of protein-rich mycelial biomass of a mushroom, *Pleurotus sajor-caju*, on corn stover. J. Ferment. Bioeng. 68, 334–338.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.

Fasidi, I.O., Olorunmaiye, K.S., 1994. Studies on the requirement for vegetative growth of *Pleurotus tuber-regium* (Fr.) Singer, a Nigerian mushroom. Food Chem. 50, 397–401.

Furlan, S.A., Virmond, L.J., Miers, D.A., Bonatti, M., Gern, R.M.M., Jonas, R., 1997. Mushroom strains able to grow at high temperatures and low pH values. W. J. Microbiol. Biotechnol. 13, 689–692.

Garzillo, A.M.V., Di Paolo, S., Ruzzi, M., Buonocore, V., 1994. Hydrolytic properties of extracellular cellulases from *Pleurotus ostreatus*. Appl. Microbiol. Biotechnol. 42, 476–481.

Gunde-Cimerman, N., 1999. Medicinal value of the genus *Pleurotus* (Fr.) P. Karst. (Agaricales s.I., Basidiomycetes). Int. J. Med. Mushrooms 1, 69–80.

Gutiérrez, A., Prieto, A., Martínez, A.T., 1996. Structural characterization of extracellular polysaccharides produced by fungi from the genus *Pleurotus*. Carbohydr. Res. 281, 143–154.

Jung, G.T., Ju, I.O., Ryu, J., Choi, J.S., Choi, Y.G., 2003. Mycelial yield of *Pleurotus ostreatus* using thinned apple, pear, and peach on submerged culture. Biotechnol. Bioproc. Eng. 8 (5), 286–290.

Karácsonyi, S., Kuniak, L., 1994. Polysaccharides of *Pleurotus ostreatus*: isolation and structure of pleuran, an alkali-insoluble β-D-glucan. Carbohydr. Polym. 24, 107–111.

Lee, B.C., Bae, J.T., Pyo, H.B., Choe, T.B., Kim, S.W., Hwang, H.J., Yun, J.W., 2003. Biological activities of the polysaccharides produced from submerged culture of the edible Basidiomycete Grifola frondosa. Enzyme. Microbial Technol. 32, 574–581.

Manu-Tawiah, W., Martin, A.M., 1987. *Pleurotus ostreatus* requirements for P, K, Mg and Mn in submerged culture. Can. J. Microbiol. 34, 620–624.

Márquez-Rocha, F., Guillén, N.G.K., Sánchez, V.J.E., Vázquez-Duhalt, R., 1999. Growth characteristics of *Pleurotus ostreatus* in bioreactor. Biotechnol. Tech. 132, 29–32.

Martin, A.M., 1992. Study of the growth and biomass composition of the edible mushroom *Pleurotus ostreatus*. Food Sci. Human Nutr., 239–248.

Mizuno, T., Zhuang, C., 1995. Houbitake, *Pleurotus sajor-caju*: antitumor activity and utilization. Food Rev. Int. 11, 185–187.

Morais, H., Ramos, C., Matos, N., Forgács, E., Cserháti, T., Almeida, V., Oliveira, J., Darwish, Y., Illés, Z., 2002. Liquid chromatographic and electrophoretic characterization of extracellular β-glucosidase of *Pleurotus ostreatus* grow in organic waste. J. Chromatogr. B 770, 111–119.

Rosado, F.R., Carbonero, E.R., Kemmelmeier, C., Tischer, C.A., Gorin, P.A.J., Iacomini, M., 2002. A partially 3-O-methylated (1→4)-linked β-D-galactan and β-D-mannan from *Pleurotus ostreatoroseus* Sing. FEMS Microbiol. Lett. 212, 261–265.

Rout, D., Mondal, S., Chakraborty, I., Pramanik, M., Islam, S.S., 2005. Chemical analysis of a new (1 → 3)-, (1 → 6)-branched glucan from an edible mushroom, *Pleurotus florida*. Carbohydr. Res. 340 (16), 2533–2539.

Sarangi, I., Ghosh, D., Bhutia, S.K., Mallick, S.K., Maiti, T.K., 2006. Anti-tumor and immunomodulating effects of *Pleurotus ostreatus* mycelia-derived proteoglycans. Int. Immunopharmacol. 6, 1287–1297.

Shu, C.H., Wen, B.J., 2003. Enhanced shear protection and increased production of an anti-tumor polysaccharide by *Agaricus blazei* in xanthan-supplemented cultures. Biotechnol. Lett. 25, 873–876.

Smits, G.J., Van Den Ende, H., Klis, F.M., 2001. Differential regulation of cell wall biogenesis during growth and development in yeast. Microbiology 147, 781–794.

Solomko, E.'F., Eliseeva, G.S., 1988. Biosynthesis of group B vitamins by the fungus *Pleurotus ostreatus* in submerged culture. Prikladnaya Biokhimiya 24 (2), 64–169.

Wang, J.C., Hu, S.H., Liang, Z.C., 2005. Optimization for the production of water-soluble polysaccharide from *Pleurotus citrinopileatus* in submerged culture and its antitumor effect. Appl. Microbiol. Biotechnol. 67, 759–766.

Wisbeck, E., Robert, A.P., Furlan, S.A., 2002. Avaliação da produção de agentes antimicrobianos por fungos do gênero *Pleurotus*. Health Environ. J. 3 (2), 07–10.

Wisbeck, E., Furlan, S.A., Ninow, J., 2005. Efeito da concentração inicial de glicose e do pH na produção de exopolissacarídeos de potencial antitumoral por *Pleurotus ostreatus* DSM 1883. Health Environ. J. 6 (2), 19–22.

Xu, Chun-Ping, Kim, Sang-Woo, Hwang, Hye-Jin, Choi, Jang-Won, Yun, Jong-Won, 2003. Optimization of submerged culture conditions for mycelial growth and *exo*-biopolymer production by *Paecilomyces tenuipes* C240. Process Biochem. 38, 1025–1030.

Zadrazil, F., 1978. Cultivation of *Pleurotus*. In: Chang, S.T., Hayes, W.A. (Eds.), The Biology and Cultivation of Edible Mushrooms. Academic Press Inc., New York, pp. 521–557.