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Solid-state Fermentation of Xylanase from *Penicillium canescens 10–10c* in a Multi-layer-packed Bed Reactor

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Abstract Xylanase is produced by *Penicillium canescens 10–10c* from soya oil cake in static conditions using solid-state fermentation. The impact of several parameters such as the nature and the size of inoculum, bed-loading, and aeration is evaluated during the fermentation process. Mycelial inoculum gives more production than conidial inoculum. Increasing the quantity of inoculum enhances slightly xylanase production. Forced aeration induces more sporulation of strain and reduces xylanase production. However, forced moistened air improves the production compared to production obtained with forced dry air. In addition, increasing bed-loading reduces the specific xylanase production likely due to the incapacity of the *Penicillium* strain to grow deeply in the fermented soya oil cake mass. Thus, the best cultivation conditions involve mycelial inoculum form, a bed loading of 1-cm height and passive aeration. The maximum xylanase activity is obtained after 7 days of fermentation and attains 10,200 U/g of soya oil cake. These levels are higher than those presented in the literature and, therefore, show all the potentialities of this stock and this technique for the production of xylanase.

Keywords Multi-layer-packed bed reactor · *Penicillium canescens* · Solid-state fermentation · Soya oil cake · Xylanase

Introduction

Microbial xylanases are enzymes with biotechnological potential in many industrial processes, such as pre-bleaching of kraft pulp, improvement of the digestibility of animal

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feed, juice clarification, degumming of vegetal fibers such as jute, ramie, and hemp [1–8], and bioethanol production from lignocellulosic compounds [9, 10]. A variety of microorganisms, including bacteria, yeasts, and filamentous fungi are reported to produce xylanolytic enzymes [1–8] using solid-state cultivation systems and submerged liquid cultivation processes. Most of the researches are focused on submerged culture, which allows control of the pH and the temperature of the medium and several environmental factors (homogenization of the medium, aeration, and shearing) required to optimize microbial growth [3, 7]. Thus, the availability of informations about xylanase production in solid-state bioreactors is limited [11].

However, solid-state fermentation has gained renewed interest from researchers in recent years and is often employed for the production of xylanases because of a number of economical and practical advantages, such as its simplicity, low capital costs for equipment and operating, high volumetric productivity, lower space requirements, and easier downstream processing [1, 11–13]. The tray reactor is currently the most popular technology, particularly in oriental countries, for koji or food fermentation. However, this technology suffers from its limited capacity and poor aeration control [14]. Thus, other types of reactors are investigated, including the packed-bed reactor, which has the potential to overcome the disadvantages of the tray reactor [14]. The packed-bed reactor allows using several trays (beds) of the medium simultaneously, whereas the tray technology uses only one bed of the medium in the reactor. Some applications of the packed-bed reactor in solid state fermentation are recently summarized, and the important operating parameters are identified as the air flow rate, the particle size of the substrate, and the bed loading [14]. However, the main parameters to be measured and controlled generally in solid-state fermentation processes are the temperature, the aeration homogeneity, the pH, and the water bed content [12].

This paper investigates the potential of xylanase production in solid-state fermentation from soya oil cake by *Penicillium canescens 10–10c* using a multi-layer-packed bed reactor. Previous studies reported xylanase production by this strain in solid-state fermentation from wheat straw in flasks [1].

Materials and Methods

Strain

P. canescens 10–10c is supplied by G.I Kvesidatse, Institute of Plant Biochemistry, Academy of Sciences, Tbilisi Georgia.

Culture Conditions

The enzymatic production medium is composed by 20% of soya oil cake (5-mm particles size) and 80% of a nutritive solution composed by casein peptone at 0.75% (*w*/*v*) and $Na_2HPO_4.2H_2O$ at 1% (*w*/*v*) in distilled water. The 5-mm particle size soya oil cake was obtained after crushing the whole soya oil cake with a hammer mill (Gladiator, Ets J. Mondelaers S.P.R.L, Bruxelles, Belgium) equipped with a 5-mm diameter grid. Enzymatic production medium is autoclaved in the enamel metallic trays ($17\times11\times5$ cm^3) and then placed horizontally in four distinct layers in the multi-layer-packed bed reactor as shown in Fig. 1. Enzymatic production medium is inoculated by conidia (5.10^5 or 10^7 spores/gram of soya oil cake) or mycelia issued from conidia. Bed loading varied between 50 to 250 g of soya oil cake/layer (respectively, 0.5 to 2.5 cm). In the first case, enzymatic production medium contained in the reactor is directly

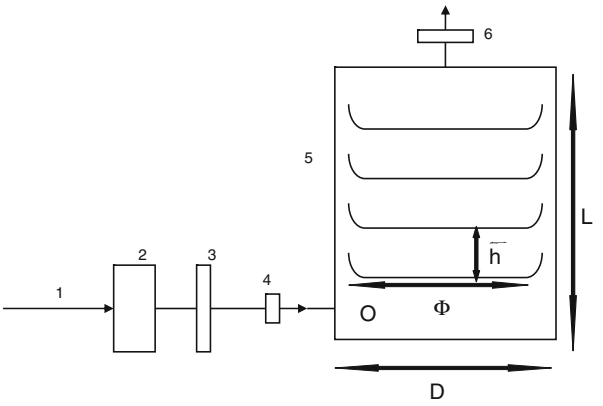

Fig. 1 Schematic diagram of the multi-layer-packed bed reactor system. *1* Circuit of air; *2* rotameter; *3* inlet filter; *4* humidifier system; *5* reactor (*L* length; *D* diameter); *6* outlet filter; *Φ* diameter of a circular layer; *h* distance between two layers; *O* air distributor

inoculated by the whole conidial suspension, whereas in the second case, a mycelial inoculum (one day old spores preculture) on 1/10 of enzymatic production medium is used as inoculum. The mycelial preculture is used to inoculate the 9/10 of the enzymatic production medium in the reactor. Production is led under static conditions at 30 °C. Passive aeration is compared to forced aeration (dry or moistened air). After production during, respectively, 3, 7, and 12 days, the fermented soya oil cake from each layer is extracted and assayed for xylanase activity, moisture content, and ergosterol content (after lyophylization). The experiments are conducted in triplicate, and the mean value is selected.

Multi-layer-packed Bed Reactor

The multi-layer-packed bed reactor (Fig. 1) contains four distinct circular layers (Φ=28.5 cm) separated each one by a distance h of 6 cm. The circular layers are composed of meshes (diameter<2 mm) facilitating the circulation of the air in the fermented medium. The dimensions of the reactor are 42 cm length L×33 cm diameter D. The distribution of air is carried out by an opening O on the reactor. In the case of forced not-moistened air, compressed air goes directly through a rotameter and then in the reactor. In the case of forced moistened air, compressed air issued from the rotameter goes through a bottle containing distilled water before going in the reactor. Filters ($\varnothing$=0. 20 μm, PTFE Midisart 2000, Sartorius Technologies, Vilvoorde, Belgium) are positioned at the inlet and at the outlet of the reactor to filter the air.

Enzyme Extraction

The fermented soya oil cake of each layer from the multi-layer-packed bed reactor is complemented with distilled water at 75% (*v*/*w*). The solution obtained is centrifuged at 10,000 RPM for 15 min at 4 °C (Avanti™ J-25 I, Beckman, Palo Alto, USA) to remove the fermented soya oil cake. This extract solution is filtrated through a folded filter ($\varnothing$= 150 mm, 595 1/2, ref. no. 10311645; Schleilcher and Schuell, Dassel, Germany). The clear supernatant filtrate is used as the enzyme source.

Enzyme Assay

Xylanase activity is measured according to Bailey et al. [15] using 1% birchwood xylan as substrate (X0502, EC 232-760-6, Sigma-Aldrich Chemie GmbH, Steinheim, Germany). Reducing sugars are assayed by dinitrosaycylic acid method with xylose as the standard

[16]. One unit of xylanase activity is defined as the amount of enzyme releasing 1 μmol of reducing sugar equivalent to xylose per minute. Xylanase activity is evaluated per layer. Each test is done in triplicate. To determine the total enzymatic activity obtained during each experiment, enzymatic activities of the four layers are added, and the average is evaluated. The average value is validated when the variation between tests values are less than 8.0%.

Moisture Content Analysis

Fifteen grams of fresh fermented soya oil cake from each layer is dried during 48 h at 105 °C until a stable weight is obtained. Moisture content is determined by the difference between the fresh matter and the dried mater. Then, the percentage of the ratio moisture content/fresh matter of the fermented soya oil cake during the process is determined by layer. Each test is done in triplicate. To determine the total moisture content of the fermented soya oil cake during each experiment, moisture contents of the fours layers are added, and the average value is evaluated. The variation between tests values are less than 8.0%.

Ergosterol Analysis

To extract ergosterol, the fermented soya oil cake of each layer is lyophilized (Lyophylizator Liogamma, Koeltechniek Louw, Rotselaar, Belgium). About 100 mg of the lyophilized sample in 2.5 ml of 4% (*w*/*v*) methanolic solution of NaOH is saponified for 30 min at 80 °C. After cooling to room temperature, 3 ml of hexane is added. The hexane phase (upper phase) is transferred to new test tubes. Three hexane extractions are performed. The three hexane fractions are combined and evaporated under vacuum at 30 °C. The dried residue is dissolved in 1.0 ml of methanol. Ergosterol is measured by high performance liquid chromatography (Hewlett-Packard 1100 Agilent Technologies, Diegem, Belgium) after filtration of the samples through on a 0.2-μm pore-size filter (Chromofil PET-20/15 MS-Macherey-Nagel, Duren, Germany). Ten microliters of the sample is injected on a C18 column (length, 150 mm; ID, 3.0 mm; Alltech, Lokeren, Belgium). Ergosterol is eluted with a mixture of methanol acetonitrile 90:10 (*v*/*v*) at a flow rate of 0.40 ml/min and at 30 °C. Peaks are detected at a wavelength of 282 nm. A standard solution of ergosterol (Acros Organics; Geel, Belgium) in methanol is used for quantifications purposes. Standards and blanks are treated in the same manner. Each test is done in triplicate. To determine the total ergosterol content obtained during each experiment, ergosterol content of the four layers are added, and the average is evaluated. The variation between tests values are less than 8.0%.

Results and Discussion

Xylanase Production in Passive Aeration Conditions

In preliminary studies at small scale in flask and in enamel metallic trays (submitted for publication), several cheap substrates (soya oil cake, soya meal, wheat bran, complete bran, and pulp sugar beet) have been tested. The best substrates were issued from soya. However, soya oil cake gave better production than soya meal. The average of best activities were obtained after 7 days, 14,485±1,090 U/g in flasks and 8,133±540 U/g in enamel metallic trays. To improve the scale up of the production, we have decided to test xylanase production in a multi-layer-packed bed reactor. The fermentations conditions are similar.

Firstly, impacts of bed loading, type of inoculum, and cultivation duration on the enzyme production in the multi-layer-packed bed reactor have been studied. Bed loading varied between 50 to 250 g of soya oil cake/layer.

Figure 2 shows that the mycelial inoculum gives higher xylanase activity than conidial inoculum. The highest specific production (7,900 U/g) and the highest total production (1,020,000 U) are observed after 7 days incubation with 100 g (2X, 1-cm bed thickness) and 150 g (3X, 1.5-cm bed thickness) of soya oil cake, respectively. Xylanase first increases and then decreases with increasing the bed loading of soya oil cake. The *Penicillium* strain sporulated and was unable to colonize the entire culture medium. The microorganism growths primarily at the surface of the culture medium and colonizes the entire medium when depth is comprised between 0.5 cm to 1 cm (visual observation). Similar observations were reported in the cases of penicillin production by *Penicillium chrysogenum* and in alkaline protease production by *Teredinobacter turnirae* using agricultural wastes under solid state fermentation [17, 18]. With mycelial inoculum, the lag phase during the strain growth is reduced. The strain grows quickly and, therefore, produces more xylanase. Production increases with the cultivation duration until the seventh day and then decreases. Many works describe this phenomenon as a sudden increase and subsequent decrease in enzymes activities during the cultivation period [5]. The sporulation of the strain reduces the enzymatic activity [19, 20]. Maybe, the xylanase produced during days 1–7 is consumed or denaturated after onset of sporulation during days 7–12. These assumptions had to be verified.

Ergosterol level is an indicator of the strain growth. Figure 3 indicates that it decreases slightly when the bed loading increases. About 100 g of fermented soya oil cake allows obtaining higher values of ergosterol. Ergosterol content is affected by the inoculum form and the time of cultivation. Mycelial inoculum gives more ergosterol content than conidial inoculum. Twelve days cultivation duration gives the highest ergosterol content.

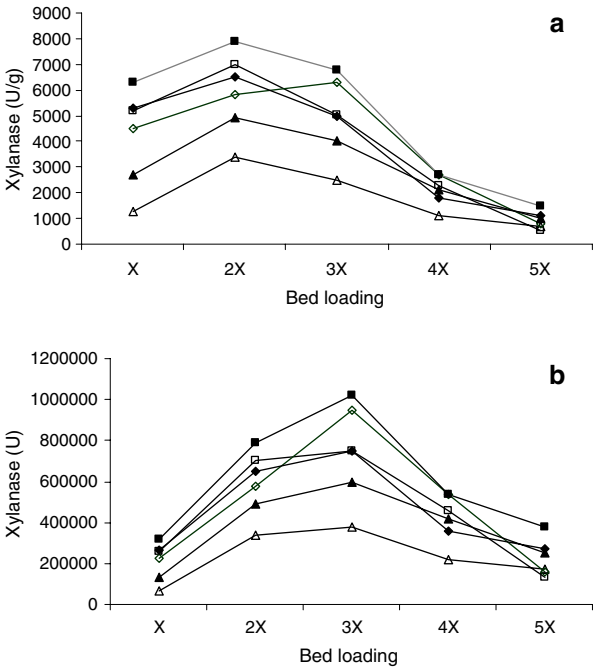

Fig. 2 Xylanase production in the multi-layer-packed bed reactor with passive aeration conditions; **a** specific activity, **b** total activity. *X* represents 50 g of soya oil cake supplemented with 200 ml of the nutritive solution [casein peptone at 0.75% (*w*/*v*) and $Na_2HPO_4.2H_2O$ at 1% (*w*/*v*) in distilled water]. Inoculum is 5.10^5 spores/gram of soya oil cake or the results of the development of these spores in 1 day (mycelia). The ratio values are averages based on addition of the enzymatic activity from the four layers, and the experiment was performed in triplicate. The deviation standard was below 8% in all cases. Conidial inoculum: 3 days (*open triangle*), 7 days (*open square*), 12 days (*open rhombus*). Mycelial inoculum: 3 days (*closed triangle*), 7 days (*closed square*), 12 days (*closed rhombus*)

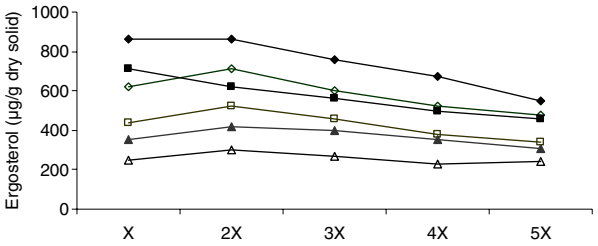

Fig. 3 Evolution of ergosterol content during xylanase production in the multi-layer-packed bed reactor in passive aeration conditions. The ratio values are averages based on addition of the ergosterol content from the four layers, and the experiment was performed in triplicate. The deviation standard was below 8% in all cases. *Symbols* are identical to those of the Fig. 2

Impact of Forced Air on Xylanase Production

To enhance oxygen transfer during the process in the multi-layer-packed bed reactor, xylanase production is realized with forced air (moistened or dry) at 0.1, 0.5, and 1 l/min. The other parameters of the culture remain identical to those used before.

Figures 4 and 5 show that aeration affects the parameters of the culture. For the three air flows tested, moistened air gives best activities than dry air. The higher xylanase productions appear at 0.1 l/min moistened air after 7 days (4,000 U/g) with the mycelial inoculum (Fig. 4) and after 12 days (2,300 U/g) with the conidial inoculum (Fig. 5). The xylanase production increases during days 3–7 in all the cases. However, during days 7–12, the xylanase production increases slowly or decreases. Maybe, this weak increase of xylanase activity after the seventh day is caused by the effects of forced aeration. Indeed, this situation did not appear in passive aeration. It has clearly established in submerged fermentation that *P. canescens* is very sensitive to hydrodynamic stress generated by aeration and mixing of the medium [21–23]. A lowering stress due to aeration would be beneficial as well for the synthesis of several enzymes by filamentous microorganisms in general as for the synthesis of xylanase by *P. canescens* in particular [21–23].

Figures 6 and 7 show ergosterol content during *P. canescens* growth. Ergosterol content is related to the *Penicillium* strain growth. As we can see, ergosterol content increases with

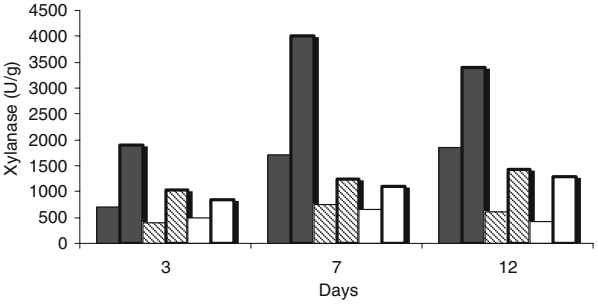

Fig. 4 Xylanase production in the multi-layer-packed bed reactor with active aeration conditions. Mycelial inoculum, bed loading of 50 g of soya oil cake. The ratio values are averages based on addition of the enzymatic activity from the four layers, and the experiment was performed in triplicate. The deviation standard was below 8% in all cases. Ambient air 0.1 l/min (*black*); Moistened air 0.1 l/min (*shaded black*); ambient air 0.5 l/min (*stripes*); moistened air 0.5 l/min (*shaded stripes*); ambient air 0.1 l/min (*white*); moistened air 0.1 l/min (*shaded white*)

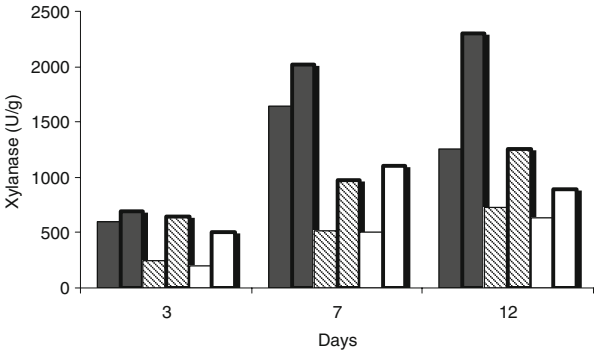

Fig. 5 Xylanase production in the multi-layer-packed bed reactor with active aeration conditions conidial inoculum, bed loading of 50 g soya oil cake. The ratio values are averages based on addition of the enzymatic activity from the four layers, and the experiment was performed in triplicate. The deviation standard was below 8% in all cases. *Symbols* are identical to those of the Fig. 4

time. Forced moistened air at 1 l/min gives the higher ergosterol content, and the lowest content is obtained at 0.1 l/min forced dry air. Visual observations of culture show also that aeration does appear to have a pronounced effect on spore formation. Indeed, with forced dry air, conidia formation takes place rapidly, and it is visible at the second day. With forced moistened air, spore formation is less important and appeared at the fifth day. With passive aeration, spore formation appears at the seventh day of fermentation. The temperature at the inner of the reactor is not controlled during the process. Maybe, formation of spores is favored by a fall of the temperature in the reactor and/or by a stress of the strain caused by forced air injection [21]. In addition, literature indicates many other examples where xylanase production decreases with forced aeration level [11, 24–26]. Moreover, forced aeration could cause a cellular mortality and enzymatic damage [27, 28]. Oxygen could be toxic for aerobic strains at critical values [19]. It could also inhibit or inactivate the enzymes [25]. Forced dry air decreases also the moisture content during the fermentation until the microorganisms cannot grow well (Fig. 8). With passive aeration, moisture content remains constant. Our results suggest that the optimum operating conditions includes 100 g bed loading (1-cm thickness), mycelial inoculum, and passive aeration. However, when using lower bed loading, the advantage of the process for scaling-up is lost. Increasing bed loading reduces production (Fig. 9). To improve xylanase production, we decide to increase

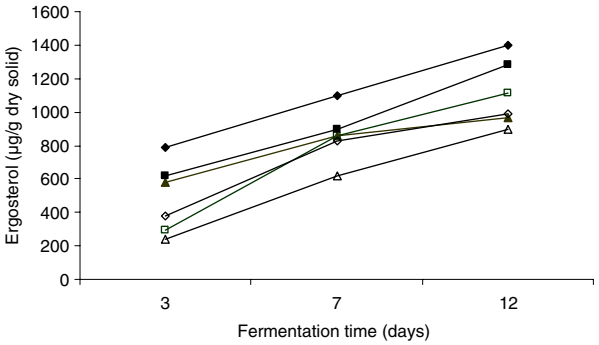

Fig. 6 Evolution of ergosterol content during xylanase production in the multi-layer-packed bed reactor with active aeration conditions, mycelial inoculum, bed loading of 50 g soya oil cake. The ratio values are averages based on addition of the ergosterol content from the four layers, and the experiment was performed in triplicate. The deviation standard was below 8% in all cases. Ambient air 0.1 l/min (*open triangle*); moistened air 0.1 l/min (*closed triangle*); ambient air 0.5 l/min (*open square*); moistened air 0.5 l/min (*closed square*); ambient air 1 l/min (*open rhombus*); moistened air 1 l/min (*closed rhombus*)

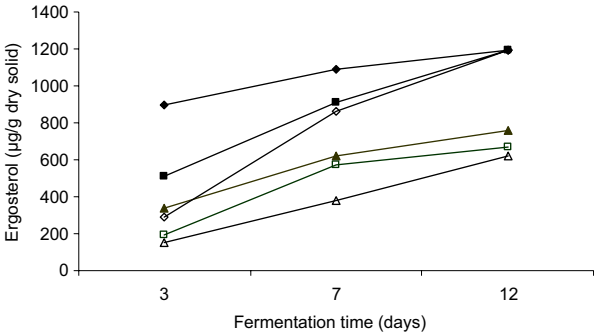

Fig. 7 Evolution of ergosterol content during xylanase production in the multi-layer-packed bed reactor with active aeration conditions, conidial inoculum, bed loading of 50 g of soya oil cake. The ratio values are averages based on addition of the ergosterol content from the four layers, and the experiment was performed in triplicate. The deviation standard was below 8% in all cases. *Symbols* are identical to those of the Fig. 6

quantity of initial inoculum to 10^7 spores/gram of soya oil cake. Better production (10,200 U/g of soya oil cake) is obtained with mycelial inoculum and about 100 g of soya oil cake in passive aeration condition (Table 1). Therefore, during solid-state fermentation xylanase production by *P. canescens*, it is desirable not to use active aeration if temperature is not controlled.

Comparison of Fermentations in Flasks, Enamel Metallic Trays and in the Multi-layer-packed Bed Reactor

Table 2 presents a comparison of the fermentation parameters used in the framework of this study by the multi-layer-packed bed reactor and data from experiments conducted in flasks and in enamel metallic trays using the same substrate and the same strain in passive aeration. It is evident that in flasks, xylanase production is higher than in the multi-layer-packed bed reactor and in the enamel metallic trays. A major difference in these three systems tested is the depth (bed height) of the fermented soya oil cake. Indeed, the production requires an appropriate thickness of medium for the microbial growth, the complete colonization of the medium, and not sporulation of the strain. A great quantity of inoculum enhances slightly the production. However, we think that it is not the best solution to improve the production. Indeed, with a low quantity of inoculum and an appropriate depth of medium, the entire

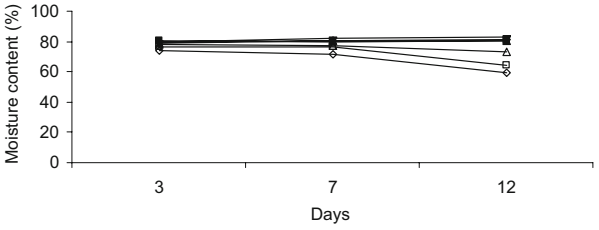

Fig. 8 Evolution of moisture content during xylanase production in the multi-layer-packed bed reactor in active aeration conditions, bed loading of 50 g of soya oil cake. The ratio values are averages based on addition of the moisture content from the four layers, and the experiment was performed in triplicate. The deviation standard was below 8% in all cases. Passive aeration (*feature*); ambient air 0.1 l/min (*open triangle*); moistened air 0.1 l/min (*closed triangle*); ambient air 0.5 l/min (*open square*); moistened air 0.5 l/min (*closed square*); ambient air 1 l/min (*open rhombus*); moistened air 1 (*closed rhombus*)

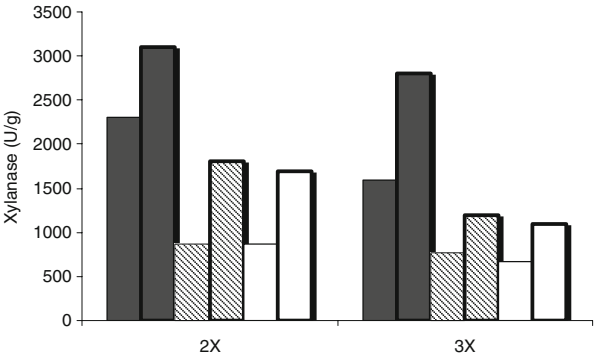

Fig. 9 Xylanase production in the multi-layer-packed bed reactor with active aeration conditions, mycelial inoculum (X=50 g of soya oil cake + 5.10^5 spores/gram). The ratio values are averages based on addition of the enzymatic activity from the four layers, and the experiment was performed in triplicate. The deviation standard was below 8% in all cases. *Symbols* are identical to those of the Fig. 4

medium will be colonized but tardily, and the level of the production would be equivalent to production obtained with a great quantity of inoculum in the same cultural conditions. However, a rigorous control of the sporulation of *P. canescens* is strongly recommended. One of the possible solutions would consist to increase the cultural surface by increasing the dimensions of the layers in the reactor.

Comparison of This Work and Others in the Literature

The number of publications on solid-state fermentation in a large scale in general and on xylanase production in particular is very small. The majority of the works are limited to studies in flasks. Indeed, many problems appear that limit production during the scaling-up. The aerobic microbes grow at the surface of the culture medium and cannot grow in-depth in the solid cultural mass. Thus, with *Thermoascus aurantiacus* in a double-jacketed glass column, better xylanase production was 1,597 U/g [11]. This activity is obtained after 10 days cultivation with only 8 g of bagasse at an airflow rate of 6 l/(h g). A static environment gave 71 U/g. The authors noted that the best conditions of the process required better oxygen transfer, which depends on the porosity of the substrate and interparticular spaces. This loading is very weak compared to bed loading used in our studies with the multi-layer-packed bed reactor. *T. aurantiacus* strain in a 2-l rotating drum bioreactor gave optimal xylanase activity (4,490 U/g) from 100 g of dry wheat straw after 7 days cultivation duration [29]. This activity is obtained at a humidified air flow rate of 10 l/min/kg dry wheat straw. Initial moisture content was 80%. This activity is two times inferior to the optimal activity (10,200 U/g of soya oil cake) obtained in the present study. This confirms the hyperproductor character of xylanase by the strain *P. canescens.* Khamgkhang and Wisutharom [30] used a packed bed fermenter, with a diameter and height ratio of 1 to produce xylanase. They obtained only 497 U/g dry solid, and overheating problems occurred that limit production. Prasertsri [31] obtained with a tray fermenter a maximum of 1,764 U/g dry solid of xylanase. Although the xylanase concentration in tray is high, the larger area requirement in tray fermenters is the limitation of large scale production. With *Aspergillus sulphurous*, the xylanase activity of the dry koji was more than 1,000 U/g when the medium temperature and water activity are balanced [32]. However, the xylanase activity of the dry koji was only 650 U/h if the solid state fermentation is carried out naturally. In our cultivation conditions, 10,200 U/g of xylanase is obtained in passive aerations after 7 days incubation. Bed loading of 100 g of soya oil cake (1-cm bed height) is required to obtain this activity. This production is better obtained compared to other works reported in literature. However, this comparison must be considered with care because of

Table 1 Xylanase production in the multi-layer-packed bed reactor with a great quantity of inoculum (10^7 spores/gram of soya oil cake).

Inoculum form	Air flow rate (l/min)	Bed loading (g)	Xylanase (U/g)	Final moisture content (%)	Ergosterol (mg/g dry solid)
Spore	Passive	50	8,100	82	6.7
Spore	Passive	100	7,100	82	8.6
Spore	Passive	150	5,700	82	7.6
Spore	Passive	200	3,800	82	6.7
Spore	Passive	250	1,380	82	5.7
Mycelia	Passive	50	9,200	82	10.9
Mycelia	Passive	100	10,200	82	11.9
Mycelia	Passive	150	8,500	82	10
Mycelia	Passive	200	5,100	82	9
Mycelia	Passive	250	3,800	82	7.1
Spore	0.1	50	2,980	77	6.2
Spore	0.1	100	3,100	77	9
Spore	0.1	150	1,800	77	7.6
Spore	0.1	200	1,600	77	6.7
Spore	0.1	250	1,100	77	5.7
Spore	0.1[a]	50	4,800	80	7.1
Spore	0.1[a]	100	5,100	80	8.6
Spore	0.1[a]	150	2,450	80	7.6
Spore	0.1[a]	200	1,800	80	6.7
Spore	0.1[a]	250	1,300	80	5.7
Mycelia	0.1	50	4,700	77	8.1
Mycelia	0.1	100	3,600	77	9.5
Mycelia	0.1	150	2,550	77	10.5
Mycelia	0.1	200	1,200	77	8.6
Mycelia	0.1	250	870	77	7.6
Mycelia	0.1[a]	50	7,380	80	9
Mycelia	0.1[a]	100	6,150	80	10
Mycelia	0.1[a]	150	4,000	80	9.5
Mycelia	0.1[a]	200	2,900	80	9
Mycelia	0.1[a]	250	3,000	80	8.1

All the values presented are mean value of triplicates. Standard deviations were essentially within 2–8%.

[a] Cultures were conducted with moistened air.

Table 2 Comparison of fermentations in flasks, enamel metallic trays, and in the multi-layer-packed bed reactor.

Parameters	Flask	Enamel metallic trays	Multi-layer packed bed reactor
Maximum xylanase production (U/g)	14,485	8,133	10,200
Productivity (U/g/day)	2,069	1,162	1,457
Initial inoculum (spores/gram of soya oil cake)	1×10^6	5×10^5	1×10^7
Form of inoculum	Spore	Spore	Mycelia
Bed loading (g of soya oil cake)	5	50	100/layer
Bed height of fermented soya oil cake (cm)	0.5	1	1

nonstandardization of the different reactors used during all the works. Indeed, in function of the desired scale production, the objectives to be reached vary according to the reactor. In the studies, problems occurred concerning bed loading, aeration conditions, and heat accumulation in the medium. In our case, one of the main problems is the sporulation of the strain when production is done with forced aeration, although no control of cultivation temperature is realized during our studies. Maybe, this production would be enhanced with association of a low moistened air flow rate and rigorous control of cultivation temperature at 30 °C. Therefore, it is probable that forced aeration in the form of steam vapor at 30 °C would be advantageous for the strain growth and so the xylanase production. This system has been used in other work [29, 32].

Conclusion

The present work has established the potential of a laboratory multi-layer-packed bed reactor for xylanase production in solid-state fermentation by *P. canescens*. Optimal production obtained is largely superior to those related in literature. Two problems are identified. Initially, the forced aeration causes sporulation of the *Penicillium* strain and so decreases xylanase production. In addition, increasing the cultural surface more than 100 g/layer of soya oil cake (1-cm bed height) decreases significantly xylanase production and constitutes a disadvantage for this process. To increase efficiency of the multi-layer-packed bed reactor, we can increase the dimensions of the layers. Why not associate mixing the culture medium, moistened air, and a rigorous control of the temperature at the inner the reactor? Another approach would be to develop a reactor in this direction to improve the production.

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