Journal of the University of Chemical Technology and Metallurgy, 46, 4, 2011, 375-380

LYOPHILIZATION OF LIQUID CULTURE, CONTAINING LICHENASE AND LAMINARINASE, PRODUCED FROM TRICHODERMA SP. 405 STRAIN M_7

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Received 08 June 2011
Accepted 10 October 2011

ABSTRACT

The lichenase and laminarinase are β-glucanases. They take part in the degradation of β-glucans, which are present in cereals- wheat, barley, oats, rye. Both enzymes find application in the production of wine and beer, in the food industry and also as a feed component in animal food. The conservation of lichenase and laminarinase by lyophilization of liquid culture, produced from Trichoderma sp. 405 Strain M_7 was investigated. This strain was bought from the National bank for industrial microorganisms and cell cultures- NBIMCC. The lyophilization was done in a freeze-drying installation „HOCHVAKUUM" TG-16. The lichenase and laminarinase activities of the liquid culture were determined during cultivation of Trichoderma sp. 405 Strain M_7 and one month after its lyophilization. It was established that both enzyme activities remain high after the process of conservation.

Keywords: lyophilization, lichenase, laminarinase, β-glucans.

INTRODUCTION

The lichenase and laminarinase pertain to the group of hemicellulases. The lichenase [E.P. 3.2.1.73] attacks lichenin and β-D-glucans in the cereals, containing 1,3- and 1,4-bonds. The laminarinase [E.P. 3.2.1.39] hydrolyses 1,3-β-D-glycosidic bonds in the 1,3-β-D-glucans. Its substrate is the polysaccharide laminarin [1]. It is found in a large quantity in some sea algae [2]. The contents of 1,3- and 1,4-β-glucans in the endosperm cell walls of Bulgarian barley varieties ranges from 3,15 % to 6,08 % [3]. For Bulgarian oats varieties it is in the range of 2,71 - 4,26 % [4]. Among the producers of enzymes that catalyse the degradation of β-D-glucans are Trichoderma reesei [5], Trichoderma harzianum [6], Trichoderma viride [7], Trichoderma koningii T 199 [8], Trichoderma sp. GXC [9], Trichoderma asperellum [10].

The β-glucanases find wide application in the production of wine and beer. More effective degradation of glucans, better filtration velocity and viscosity decreasing of the brewing wort were established when enzyme preparations with β-glucanase activity were added in the production process of beer and brewing wort [11-12]. The β-glucanases also find application as a feed component in the animal food. Adding those enzymes leads to cleaving of not fully digested food components, helps for a better absorption and conversion of the food and for weight increase of the animals, respectively [13-15].

Different methods exist for preservation of the bio-objects and conservation of their vitality. One of them is the lyophilization which successfully conserves the bio-objects for tens of years. The freeze-drying (lyophilization) is a process of taking away the water substance of the solid matrix of moisture-containing materials by sublimation in vacuum. Advantages of this method are: vast decrease of the product's mass, low transportation costs, no need for using refrigeratory installations to preserve the lyophilized bio-objects, which decreases the conservation outlay [16].

The purpose of this research is to investigate the preservation of lichenase and laminarinase activities by lyophilization.

EXPERIMENTAL

The Trichoderma sp. 405 Strain M_7 was used. It was bought from the National bank for industrial microorganisms and cell cultures - NBIMCC. The cultivation of the Trichoderma strain was carried out by the method of deep fermentation in 500 ml flasks on a shaker "Inkubations - Schüttelschrank BS-4 B.Braun".

A spore inoculation product was received from the strain producer cultivated on potato-dextrose agar (PDA) slants. Mandel's sowing media was inoculated with the spore sowing material. Mandel's fermentation media was inoculated with the sowing material [17]. Wheat bran was added to the fermentation media. Deep cultivation of the strain was done in 500 ml flasks for 168 hours. The lichenase and laminarinase activities were analysed every 24 hours.

The activity of both enzymes was obtained by Somogyi-Nelson's method [18-19]. One unit (IU) of lichenase activity is the amount of enzyme which catalyses the transformation of 1μmol of reducing sugars in 1 cm^3 per minute at 40°C in an acetate buffer of pH 4.00. One unit (IU) of laminarinase activity is the amount of enzyme which catalyses the transformation of 1μmol of reducing sugars in 1 cm^3 per minute at 40 °C in a citric phosphate buffer of pH 5.00. The protein contents in the liquid culture in mg/ml was obtained by spectrophotometric method (OD_{280}/OD_{260}) [20].

The lyophilization was carried out in a freeze-drying installation „HOCHVAKUUM" TG-16. The freezing process of the liquid culture was done at minus 34°C, without addition of cryoprotectants. Lyophilization's duration was 28 hours. The cooling temperature of desublimator was minus 75°C and the temperature of the plates was minus 35°C.

The enzyme activities were recovered by adding 10ml distilled water to the lyophilized liquid culture and then they was analysed by the mentioned above Somogyi-Nelson's method [18-19].

RESULTS AND DISCUSSION

The conservation by lyophilization of the liquid culture produced from Trichoderma sp. 405 Strain M_7 was investigated. The strains of genus Trichoderma are mainly producers of cellulase enzymes. They also produce hemicellulase enzymes. It was determined that the enzyme activity of the β-glucanase enzymes produced from Trichoderma sp. 405 Strain M_7 is lower than the β-1,3-1,4-glucanase activity of a recombinant yeast, for example, the activity of which reaches up to 45,1 IU/ml [21]. The lichenase and laminarinase activities of liquid culture were determined before and one month after the lyophilization. Table 1 shows the protein contents in the liquid culture during the fermentation. Table 2 summarizes the results from analyses of the enzymatic activities at the time of fermentation and one month after lyophilization. The percentage of both enzyme activities preservation after lyophilization was calculated. In Table 3 is presented the preservation percentage of the liquid culture's enzymatic activities after lyophilization. Table 4 presents the specific lichenase and laminarinase activities during the fermentation.

During the fermentation both enzymes show highest activity levels at the 144th hour of cultivation. The obtained results show that one month after lyophilization both enzymes preserve their activities at 97 - 98 % compared to the results received before lyophilization.

Table 1. Protein contents in the liquid culture during the fermentation with Trichoderma sp. 405 Strain M_7.

Hour of liquid culture analysis		24 th	48 th	72 nd	96 th	120 th	144 th	168 th
Protein contents in the liquid culture mg ml^{-1}	mean $\overline{X}$	4,553	5,618	5,768	6,375	6,915	8,445	8,445
	standard deviation, SD	0,394	0,164	0,184	0,454	0,611	0,252	0,252
	standard error, Sx	0,176	0,073	0,083	0,203	0,273	0,113	0,113

Total number of trials (n) = 5.

Table 2. Lichenase and laminarinase activities before and one month after lyophilisation of the liquid culture recieved during fermentation with Trichoderma sp. 405 Strain $M_{7.}$

Hour of liquid culture analysis	Lichenase activity, IU/ml liquid culture before lyophilisation			Lichenase activity, IU/ml liquid culture one month after lyophilisation			Laminarinase activity, IU/ml liquid culture before lyophilisation			Laminarinase activity, IU/ml liquid culture one month after lyophilisation		
	mean $\overline{X}$	standard deviation SD	standard error Sx	mean $\overline{X}$	standard deviation SD	standard error Sx	mean $\overline{X}$	standard deviation SD	standard error Sx	mean $\overline{X}$	standard deviation SD	standard error Sx
24 th	0,043	0,003	0,001	0,042	0,002	0,001	0,127	0,039	0,017	0,125	0,038	0,017
48 th	0,061	0,005	0,002	0,059	0,005	0,002	0,248	0,036	0,016	0,244	0,036	0,016
72 nd	0,070	0,004	0,002	0,068	0,005	0,002	0,300	0,006	0,003	0,294	0,006	0,003
96 th	0,085	0,004	0,002	0,084	0,005	0,002	0,343	0,047	0,021	0,335	0,048	0,021
120 th	0,096	0,005	0,002	0,094	0,005	0,002	0,456	0,028	0,013	0,449	0,029	0,013
144 th	0,107	0,006	0,003	0,106	0,006	0,003	0,520	0,061	0,027	0,508	0,061	0,027
168 th	0,095	0,002	0,001	0,093	0,002	0,001	0,458	0,021	0,009	0,447	0,023	0,010

Total number of trials (n) = 5.

Table 3. Preservation of lichenase and laminarinase activities in % one month after lyophilisation of liquid culture recieved during fermentation with Trichoderma sp. 405 Strain $M_{7.}$

Hour of liquid culture analysis	% of lichenase activity preservation one month after lyophilisation	% of laminarinase activity preservation one month after lyophilisation
24 th	97,02	97,87
48 th	97,78	98,54
72 nd	97,52	98,17
96 th	98,19	97,47
120 th	98,28	98,43
144 th	98,08	97,64
168 th	98,09	97,75

Protein contents in the liquid culture

The protein contents in the liquid culture was determined during the fermentation (Table 1). During the cultivation the protein contents was increased. At the 24th hour it was an average 4,553 mg ml^{-1}. At the 120th hour the protein content reached 6,915 mg ml^{-1}. The results show that the protein contents is highest at the 144th hour of cultivation.

Lichenase activity preservation

The lichenase activity at 24th hour before lyophilization is at the rate of 0,043 IU/ml liquid culture (Table 2). One month after the process of drying this activity was preserved at 97,02 % compared with the activity before the conservation process (Table 3). At the 48th hour of Trichoderma sp. 405 Strain M_7 cultivation the enzyme's activity reached 0,061 IU/ml liquid culture. One month after its lyophilization the lichenase activity dropped to 0,059 IU/ml liquid culture. Therefore the lichenase activity was preserved at 97,87 % after freeze-drying. At the 72nd hour this activity was preserved at 97,52 % compared with the activity before lyophilization. It was established that at the 96th, 120th and 144th hour the enzyme preserved its activity at 98,19 %, 98,28 % and 98,08 %, after freeze-drying. At the 168th hour of the strain producer's cultivation the lichenase activity was 0,095 IU/ml liquid culture and it was 0,093 IU/ml liquid culture one month after the process of lyophilization, which means that 98,09 % of its activity was preserved. The specific lichenase activity during the fermentation of Trichoderma sp. 405 Strain M_7 is presented in Table 4.

Laminarinase activity preservation

The laminarinase activity at the 24th and the 48th hour of the strain producer's cultivation was 0,127 IU/ml liquid culture and 0,248 IU/ml liquid culture, respectively (Table 2). One month after the freeze-drying process this enzyme preserved its activity at the rate of 97,87 % and 98,54 %, respectively (Table 3). The laminarinase activity at the 72th, 96th and 120th hour remained at 98,17 %, 97,47 % and 98,43 % one month after lyophilization of the liquid culture. At the 144th hour this activity determined one month after the process of freeze-drying was preserved at 97,64 %, at 168th hour – at 97,75 %, respectively. In Table 4 is presented the specific laminarinase activity during the fermentation of Trichoderma sp. 405 Strain M_7.

Table 4. Specific lichenase and laminarinase activities during the fermentation with Trichoderma sp. 405 Strain $M_{7.}$

Hour of liquid culture analysis	24 th	48 th	72 nd	96 th	120 th	144 th	168 th
specific lichenase activity IU/mg	0,009	0,011	0,012	0,013	0,014	0,013	0,011
specific laminarinase activity IU/mg	0,028	0,044	0,052	0,054	0,066	0,062	0,054

CONCLUSIONS

The results received from this research on lichenase and laminarinase activities preservation through lyophilization showed that both enzymes preserved their activity determined one month after the process of freeze-drying at more than 97 % compared with their activities before conservation. It was established that the lichenase activity remained between 97,02 % and 98,28 % compared to the lichenase activity received during the cultivation of the strain producer. The laminarinase activity one month after lyophilization remained between 97,47 % and 98,54 % compared to its activity established during the cultivation process of Trichoderma sp. 405 Strain $M_{7.}$ Cryoprotectants were not used in the freeze-drying process which made it less expensive.

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