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Improvement of *Aspergillus niger* invertase production in stationary fermentative system

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GABRIELA BAHRIM, MARIELA GHETEU
[*]University "Dunărea de Jos" Faculty of Food Science and Engineering, Bioengineering Department, Str.Domnească No. 111, Galaţi, Romania
e-mail: Gabriela.Bahrim@ugal.ro

Abstract

The highly active Aspergillus niger MIUG 1.15 strain can produce extracellular invertase on media containing sucrose and peptone with higher biosynthesis yields when is cultivated without agitation as surface culture. For a concentration of the nitrogen source of 0.5 % and a constant mineral composition, the best biosynthesis yield for exogenous invertase is obtained at a concentration of 2 % sucrose, used as unique carbon source. For peptone concentration varying from 0.2 - 0.6 % it is noticed that, in rapport of total enzyme quantity, the level of exogenous invertase increases, the maximum percent value being attained at 0.6 % peptone.

Keywords: Fungal invertase, β-fructofuranosidase, *Aspergillus niger*, stationary fermentation systems

Introduction

The main invertase producers are yeasts *Saccharomyces cerevisiae*, *Saccharomyces carlsbergensis*, *Candida utilis* and some species of *Aspergillus*, that produce extracellular invertase. Because the yeast *Saccharomyces cerevisiae* is important in bread-making industry, establishing the biotechnologies for producing invertase using moulds is a valuable perspective for enzyme preparation industry [2].

In any biotechnological process the strain selected as catalysis agent plays an important role, but it is also important to optimise the fermentation medium and biosynthesis condition in order to increase the yield of enzymes and the economic efficiency [1,3]

The present study aimed to establish the quantitative effect of carbon and nitrogen sources upon the yield of invertase biosynthesis and its location when *Aspergillus niger* MIUG 1.15 grown in surface culture on liquid media containing sucrose and peptone.

Material and Methods

The strain *Aspergillus niger* MIUG 1.15 which is preserved in the Laboratory of Industrial Microbiology Collection under mineral oil, was reactivated and used to obtain the spores inoculum.

Cultivation took place in Roux bottles on liquid media (pH=4.2) containing Czapek salts (1g % KH_2PO_4; 0.5 g % $MgSO_4 \cdot 7H_2O$; 0.01g % $FeSO_4$; 0.5 g% KCl) and different concentrations of sucrose and peptone as carbon and, respectively nitrogen sources.

Six days after cultivation at 28°C, the extra and intracellular invertase accumulation was determinated. The endogenous enzyme content was assayed after mycelium lysis by mechanical grinding with quartz sand, extraction in 0.05 M acetate buffer (pH=4.6), at a ration of 1:10 and filtration [4].

One unit of invertase activity represents the number of inverted sugar micromoles released by hydrolytic action of a one cm^3 crude enzyme preparation (or 1g d.m. mycelium), during one minute in the following conditions: 20% sucrose as substrate, 0.02 M acetate buffer pH=4.6, at 45°C.

Inverted sugar produced by hydrolysis or present in filtrate at the moment of analysis was determined by means of Schaffer Shomogy (Methods of Analysis-AOAC) [5].

Results and Discussions

Influence of sucrose concentration upon invertase biosynthesis

In order to study the influence of sucrose as unique carbon source upon invertase biosynthesis by strain *Aspergillus niger* MIUG 1.15, there were conceived four variants of the culture medium. While the nitrogen source (0.5% peptone) and mineral salts concentration was maintained at the optimum level established in previous studies, the sucrose concentration varied from 2 to 8 %.

Six days after stationary cultivation at 28°C, the mould displayed a differentiated growth concerning the sporulation level and the vegetative mycelium abundance, correlated to the carbon source concentration (**Table 1**).

Table 1. The variation of the morphological characters of the mould at stationary cultivation on liquid media

Sucrose, g %	Morpho-cultural characters, after 6 days at 28°C	
	Derma aspect	**Sporulation level***
2	Thin wrinkled derma extended on the entire surface of the culture medium	+ + +
4	Thin wrinkled derma extended on the entire surface of the culture medium	+ + -
6	Thin, weak wrinkled derma extended on the entire surface of the culture medium	+ - -
8	Thin, weak wrinkled derma extended on the entire surface of the culture medium	- - -

* Sporulation level quantification: high sporulation (+ + +); moderate sporulation (+ + -); low sporulation; (+ - -); absence of sporulation (- - -)

For better analysing the mould growth in correlation to the carbon source, the characteristics of the fermented medium were appreciated and the biomass yield was calculated (g dry biomass × 100 cm^{-3} fermented medium) (**Table 2**).

The results concerning mycelium growth were correlated to the biosynthesis potential of producing extra and intracellular invertase. Enzyme assessment was performed both in the cultural liquid and the extracted mycelium (**Fig. 1**).

Analysing the data concerning the mould growth it is noticed that the best dry biomass yields are obtained for 4 and 6 % sucrose concentrations. At a sucrose concentration of 2 % the mould presents a good sporulation level but the biomass yield is the lowest of the three

cultivation variants. A decrease in the biomass yield and a poor utilisation of the carbon source is also noticed for 8 % sucrose. The fact could be explained through a possible growth inhibition of the high substrate concentration.

Table 2. Mould growth parameters at different sucrose concentrations

Sucrose, g %	Biomass yield		
	Wet biomass, g×100 cm^{-3}	d.m., %	Dry biomass, g d.m.×100 cm^{-3}
2	6.77	15.47	1.05
4	10.78	20.04	2.16
6	10.68	21.21	2.26
8	9.43	17.38	1.64

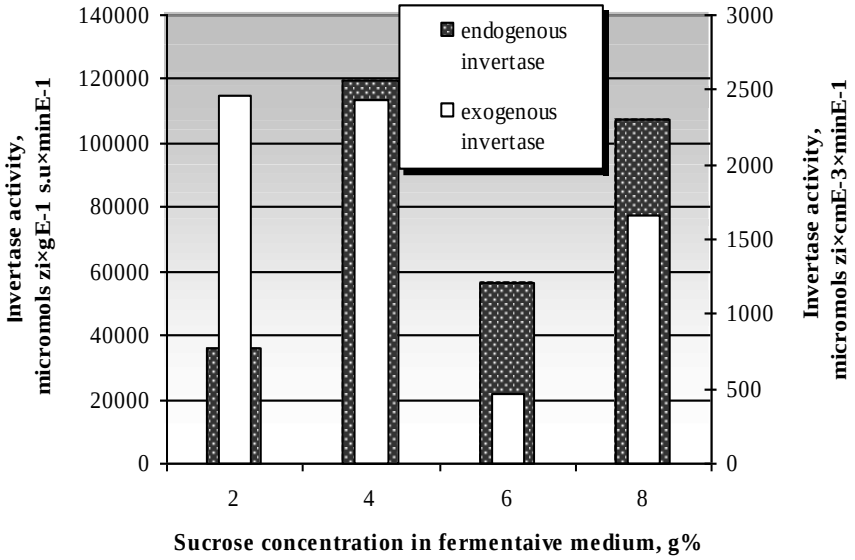

Fig. 1. The *Aspergillus niger* MIUG 1.15 potential to produce intracellular and exogenous invertase by varying the sucrose concentration of the fermentative medium

The invertase biosynthesis potential and enzyme location also varied with sucrose concentration in the fermentative medium. The best exogenous enzyme yield is obtained for a sucrose concentration of 2 % that orients the filamentous fungus metabolism to extracellular enzyme supersynthesis while the mycelium growth is weak.

Good results concerning enzyme biosynthesis were obtained for a sucrose concentration of 4 %. At a sucrose concentration of 6 % when a best biomass yield is recorded, the enzyme biosynthesis takes place at low levels, the metabolism being focused on cellular compounds biosynthesis. At a sucrose concentration of 8 % the invertase quantity increases but higher proportions of exogenous enzyme are obtained.

The variation of the extracellular enzyme percent from the entire enzyme potential that *Aspergillus niger* MIUG 1.15 develops when the carbon source varied is, presented in **Fig. 2.**

In conclusion for a nitrogen source concentration of 0.5 % and a constant mineral composition, the higher biosynthesis yield for the exogenous invertase is obtained at a sucrose concentration of 2 %, the sucrose being the unique carbon source of the fermentative medium.

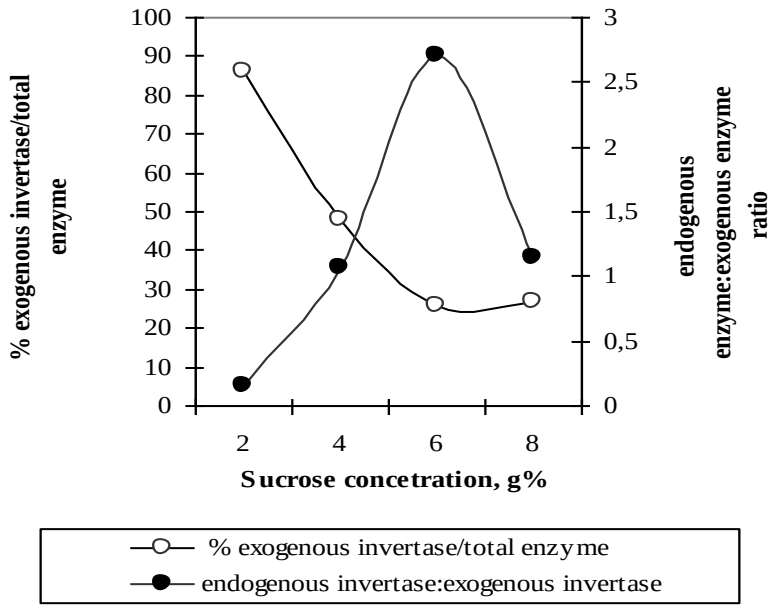

Fig. 2. Influence of sucrose concentration upon exogenous enzyme production

The effect of nitrogen source concentration upon invertase biosynthesis

The effect that peptone concentration has upon biosynthesis yield and enzyme location has been put into evidence by cultivating the mould similarly to the previous experiment conditions. Thus 5 variants of the culture medium having different peptone concentrations were tested. The peptone concentration varied from 0.2 to 1 %, the variation levels being 0.2 %. The sucrose concentration was maintained at the same value during these experiments (6 %) as well as mineral salt composition.

The results concerning the mould growth and invertase biosynthesis are presented in **Table 3**, **Table 4** and **Fig. 3**.

Table 3. The variation of morphological characteristics with peptone concentration

Peptone, g%	Morpho-cultural characteristic	
	Derma aspect	**Sporulation level***
0.2	Thin wrinkled derma, extended to the entire culture medium surface	+ + +
0.4	Well developed derma, extended to the entire culture medium surface	+ + -
0.6	Thick derma, weak wrinkled, extended to the entire culture medium surface	+ ± -
0.8	Thick derma, weak wrinkled, extended to the entire culture medium surface	- - -
1.0	Thick derma, weak wrinkled, extended to the entire culture medium surface	- - -

* Sporulation level quantification: high sporulation (+ + +); moderate sporulation (+ + -); low sporulation; (+ - -); absence of sporulation (- - -)

Table 4. The variation of biomass yield with the nitrogen source concentration

Peptone, g%	Biomass yield		
	Wet biomass, g×100 cm^{-3}	d.m. %	Dry biomass, g d.m.×100 cm^{-3}
0.2	12.19	12.15	1.48
0.4	12.90	13.60	1.75
0.6	12.16	18.06	2.20
0.8	12.10	21.94	2.65
1.0	12.02	19.88	2.39

Similarity to the variation on sucrose concentration, when peptone concentration varies, morphological and physiological changes take place.

At peptone concentrations of 0.2-0.4 % the mycelium growth is weak, fact demonstrated by the reduced biomass yield. After 6 days of stationary cultivation, the mycelium sporulates best at low nitrogen source concentrations.

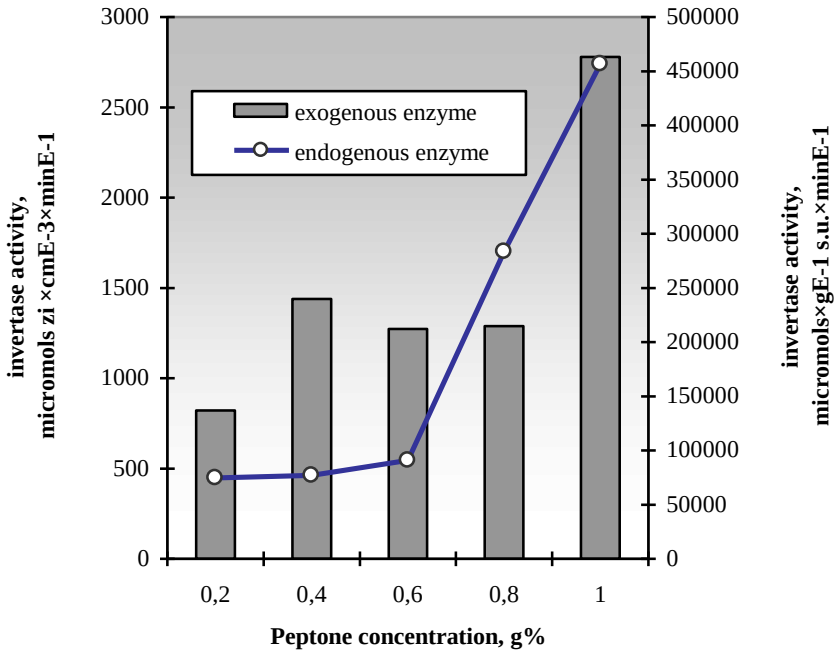

Fig. 3. The influence of peptone concentration upon fungal invertase location and biosynthesis yield

Analysing the data that have been presented in **Fig. 3** it is noticed that for peptone concentration of 0.2-0.6 % the biosynthesis potential of intracellular invertase varies at low extent. Better results are obtained at 0.6 % peptone when the superior enzyme yield correlates well to the biomass content, which is higher in this cultivation variant.

For peptone concentrations varying from 0.2 to 0.6 % it is noticed the increased of the proportion of the exogenous enzyme from total enzyme content (**Fig. 4**). The maximum extracellular per cent is obtained for peptone concentration of 0.6 %.

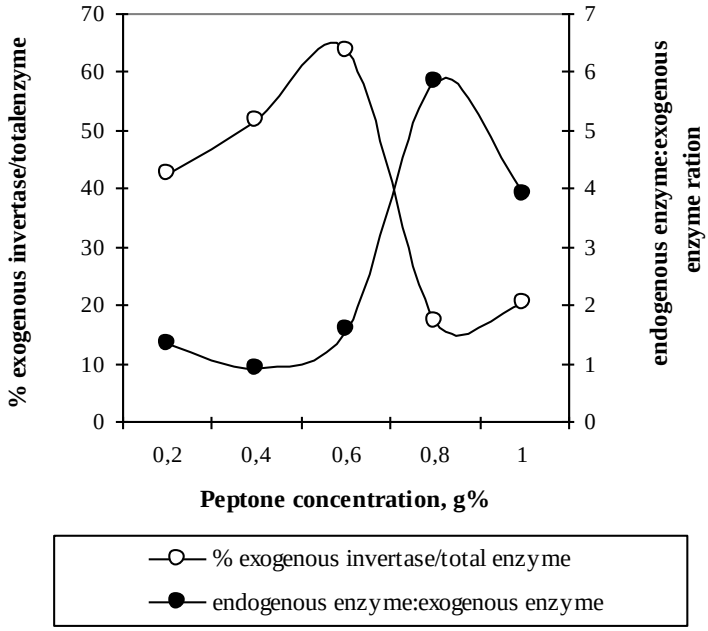

Fig. 4. The effect of peptone concentration upon exogenous enzyme accumulation

Conclusion

1. Starting from the composition of a fermentative medium (6 % sucrose, 0.5 % peptone and Czapek salts) that was proved by previous studies as being favourable for invertase production with strain *Aspergillus niger* MIUG 1.15, it was studied the way of controlling the enzyme location and biosynthesis yield by quantitative modification of carbon and nitrogen sources.
2. Maintaining constant the nitrogen sources concentration (peptone 0.5 %) and the mineral salt content but varying the sucrose concentration from 2 to 8 % it was proved that the best extracellular invertase yield is produced at a sucrose concentration of 2 %.
3. When sucrose concentration remained constant 6 % and the peptone concentration varied from 0.2 to 1 % the maximum exogenous and endogenous yield is obtain at 1% peptone, but the best proportion of exogenous enzyme of total biosynthesis potential is obtained for 0.6 % peptone.
4. This study offers preliminary information concerning the effect of two independent variables, sucrose and peptone concentration upon fungal invertase location and biosynthesis yield.

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