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SUBMERGED CULTIVATION OF MYCELIUM OF TRUFFLE *TUBER MAGNATUM*

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The possibility of submerged cultivation of mycelium of *T. magnatum* has been studied in different substrates (glucose, cheese whey and partly deproteinized cheese whey). The yield of mycelium dry substance cultivated in a medium containing 4% of glucose, and 3% of corn steep liquor, was 16.0 g/l (cultivation time — 55 hours). The maximum yield of *T. magnatum* mycelium dry substance, when cultivated in cheese whey, was achieved after 20 hours of cultivation and amounted to 23.7 g/l. The cultivation parameters were as follows: optimal initial pH value 4.4; specific growth rate μ 0.137 l/h and substrate yield coefficient 0.44 g/g.

Introduction

The majority of data available in the literature about the conditions of submerged cultivation of mushrooms relate to *Morchella, Agaricus* and *Pleurotus* species (Zeltaki — Horvat 1981, Kosarić and Miyata 1981), whereas no data can be found on submerged cultivation of *Tuber magnatum.* The cultivation in »vitro« of parasitically or simbiotically living fungi, in the absence of their hosts, seems to be very difficult (Zadražil and Grabbe 1983, Bels and Pataragetvit 1982, Delmas 1978a).

Effluents from various industrial and agricultural operations contain carbohydrates that can be metabolized for the production of single cells proteins. Various species of morel mushrooms were studied for their growth in waste sulphite liquors, and in whey produced from cheese manufacturing. Of particular interest is the process utilizing cheese whey for human consumption (Kosaric and Duvnjak 1983).

The mycelium biomass of edible higher fungi produced by submerged cultivation may be utilized as a source of single-cell proteins for human sustenance, or as spawn for commercial cultivation of mushrom fruit bodies.

Recently, *Tuber melanosporum* (Delmas 1987b) in France and *Tricholoma matsutake* (Tominga 1978) in Japan have been cultivated by using the method of inoculation of trees for afforestation with mycorrhizal fungi.

Material and Methods

Microorganism. Tuber magnatum (white truffle) from the Collection of the Faculty of Food Technology and Biotechnology in Zagreb.
Media (g/l). Inoculum: (1) glucose 20, potato infusion 300, distilled water, pH 5.5. (2) wort, pH 5.5.

Cultivation: (1) glucose 40, KH_2PO_4 0.5, nitrogen sources (urea, corn steep liqour, $(NH_4)_2SO_4$ as shown in Figs. 2,3 and 4, distilled water, pH 5.5 (2) Cheese whey: lactose 45, total nitrogen 1.2. (3) Partly deproteinized whey: lactose 55, total nitrogen 0.4. Prepared by heating at 100°C/10 min. Proteins were removed by filtration.
Media were sterilized 30 min/110°C, and pH value adjusted with 10% NaOH solution.

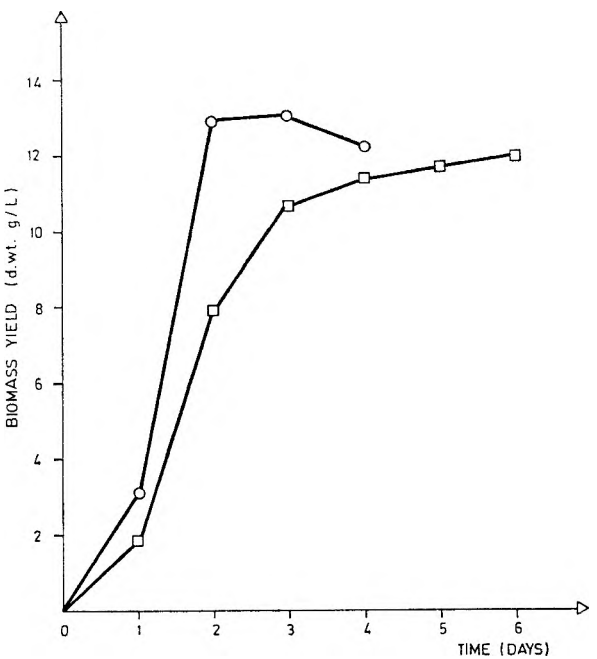

Fig. 1. Cultivation of Inoculum of *T. magnatum* in Different Media: ○ potato-glucose, □ wort.

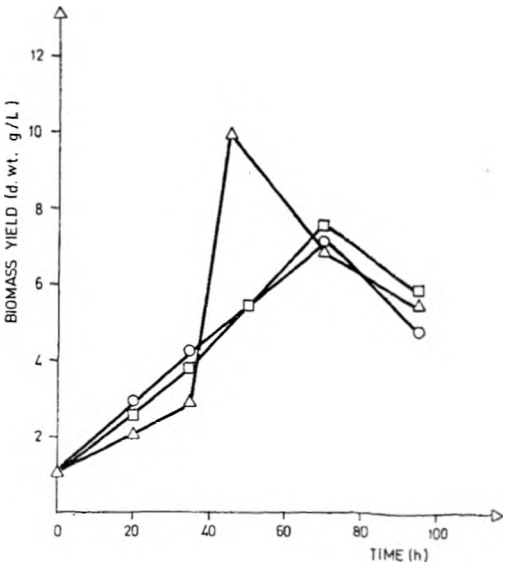

Fig. 2. Cultivation of the Mycelium of *T. magnatum* in Glucose Medium at Different Concentrations of $(NH_4)_2$ SO_4 (g/L): ○ 0.7, □ 1.4, △ 7.0.

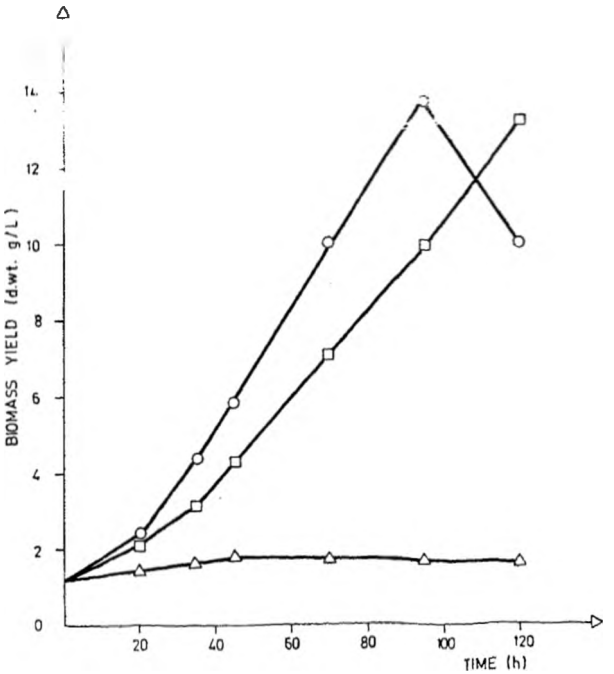

Fig. 3. Cultivation of the Mycelium of *T. magnatum* in Glucose Medium at Different Concentrations of Urea (g/L): ○ 0.7, □ 1.4, △ 7.0.

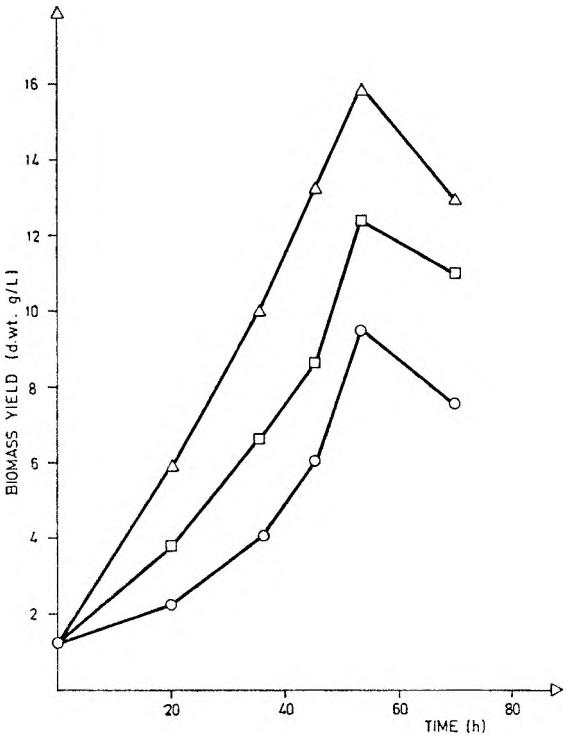

Fig. 4. Cultivation of the Mycelium of *T. magnatum* in Glucose Medium at Different Concentrations of Corn Steep Liquor (g/L): ○ 10, □ 20, △ 30.

Inoculum preparation. The organism was first grown on glucose-potato agar (5 days/25°C), and then transferred to shaken flask culture (2 days/ 25°C).
Inoculum was prepared by homogenzation of mycelium in a Waring blendor (12000 r.p.m./20 sec.)

Cultivation. Shaken flasks culture on rotary shaker (200 r.p.m.) at 25°C. Flasks volume was 500 ml with 100 ml of medium, and the quantity of inoculum was 5% vol./vol.

Analytical determ. Biomass yield was determined by drying (105°C). Lactose (conc. of reducing sugars) was determined by means of the Schoorl-Luff method and total nitrogen by Kjeldal digestion.

Result and Discussion

The possibility of submerged cultivation of mycelium of *T. magnatum* was studied in different substrates (glucose, cheese whey, and partly deproteinized cheese whey).

The inoculum cultivation was performed in a glucose-potato medium, because such cultivation can achieve a maximum mycelia dry substance yield (12.8 g/l) in only two days of cultivation. (Fig. 1)

T. magnatum cultivated in a glucose containing medium gave yields ranging from 10 to 16 g/l, depending on the nitrogen source used (Figs. 2, 3, and 4) in a cultivation time from 48 to 92 hours. The maximal yield of mycelium dry substance was achieved in the medium containing 4% of glucose, and 3% of corn steep liquor in 55 hours of cultivation, at a specific growth rate of 0.049 1/h.

The maximum yield of *T. magnatum* mycelium dry substance, when cultivated in cheese whey, was achieved after 20 hours of cultivation and amounted to 23.7 g/l (Fig. 5.). The cultivation parameters were as follows: optimal initial pH value 4.4; specific growth rate μ 0.137 1/h, and substrate yield coeficient 0.44 g/g, as shown in Fig. 6. and Table 1. Very often sporulation process started immediately after the growth stoppage.

T. magnatum, in submerged cultivation in chesse whey, achieves a relatively high value of specific growth rate which, for higher fungi, usually ranges from 0.03 to 0.06 1/h (Kosaric and Miyata 1981, Matošić, et al. 1981. Šuškovićet al. 1982).

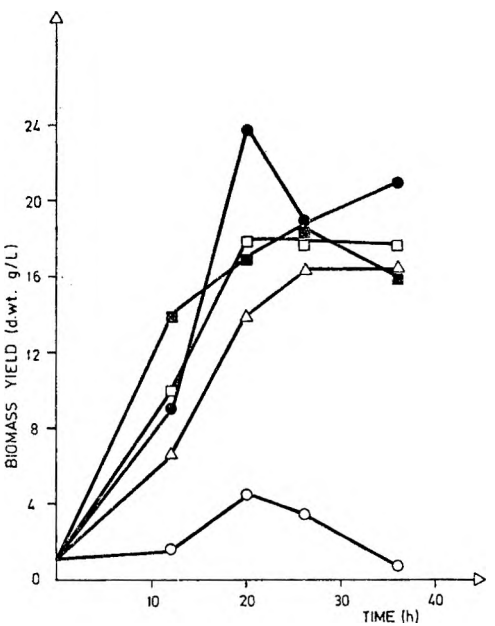

Fig. 5. Biomass Yield of *T. magnatum* in Relation to pH Value of the Medium for Cultivation (cheesy whey). pH: ○ 3.3, ● 4.4, □4.8, ■ 5.6, △ 6.5.

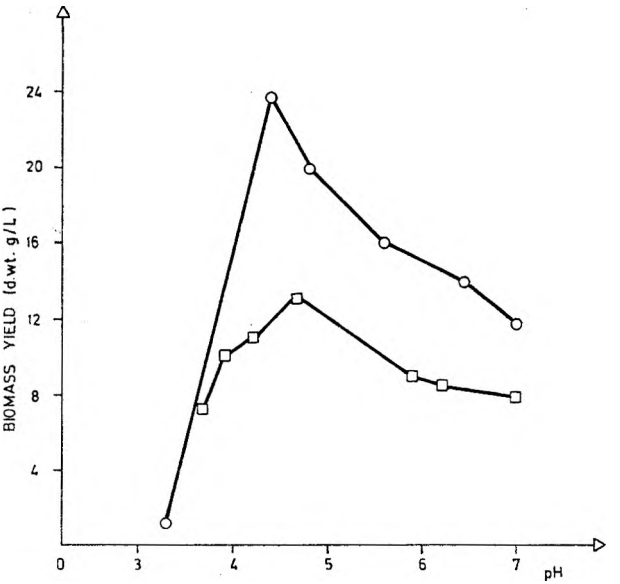

Fig. 6. Yield of the Mycelium of *T. magnatum* in Relation to pH Value of the Medium for Cultivation: ○ cheesy whey (20 hours of cultivation), □ deproteinized whey (35 hours of cultivation).

In partly deproteinized cheese whey the maximal *T. magnatum* mycelium dry matter yields amounted to 12.7 g/l after 35 hours of cultivation (probably due to a diminished content of nitrogen source in the medium), lactose yield coefficient into mycelium biomass amounted to 0.281 g/g (Table 1).

Table 1. Parameters of Cultivation of *T. magnatum* in Cheese Whey and Partly Deproteinized Whey

Parameters			Medium	
			Whey	Deproteinized Whey
Cultivation time	t	(h)	20	35
Sugar consumed		(%)	88.8	68.2
Nitrogen consumed		(%)	91.9	76.2
Biomass yield d. wt.	Y_x	(g/l)	23.7	12.7
Spec. growth rate	μ	(1/h)	0.137	0.067
Productivity	P	(g/lh)	0.83	0.36
Substrate yield coefficient	$Y_{x/s}$	(g/g)	0.44	0.281

Conclusion

Submerged cultivation of *T. magnatum* may be performed in chemically defined and complex media (cheese whey and partly deproteinized cheese whey). However, owing to a higher biomass yield and shorter cultivation time, i. e. a higher specific growth rate, cheese whey is more suitable for the production of *T. magnatum* mycelia than other substrates investigated.

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SAŽETAK

SUBMERZNI UZGOJ MICELIJA TARTUFA *TUBER MAGNATUM*

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Proučavana je mogućnost submerznog uzgoja micelija *T. magnatum* u različitim supstratima (glukoza, sirutka i djelomično deproteinizirana sirutka). Prinos suhe tvari micelija pri kultivaciji u podlozi koja sadrži 4% glukoze i 3% kukuruznog ekstrakta iznosi 16,0 g/l (vrijeme uzgoja — 53 sata).

Maksimalni prinos suhe tvari biomase *T. magnatum* pri kultivaciji u sirutki iznosi 23,7 g/l, a postignut je za 20 sati uzgoja. Pokazatelji uspješnosti procesa bili su: optimalna početna pH vrijednost podloge 4.4, specifična brzina rasta μ 0.137 l/h, a stupanj prijetvorbe supstrata 0.44 g/g. U djelomično deproteiniziranoj sirutki maksimalno postignuti prinos suhe tvari biomase iznosio je 12.7 g/l nakon 35 sati uzgoja, a stupanj prijetvorbe laktoze u micelijsku biomasu bio je 0.281 g/g.

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