Bioconversion of raw glycerol into triacylglycerols rich in γ- linolenic acid

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Introduction

Raw glycerol, a byproduct from the bio-diesel production process, is produced at a percentage of 10% of the produced biodiesel [1]. The management of this byproduct is a major problem for the bio-diesel industry. Therefore, the use of raw glycerol as a carbon substrate in several biotechnological applications could be considered as an alternative approach. I.e., oleaginous fungi, such as *Thamnidium elegans, Mortierella ramanniana* etc., having the ability to accumulate large amounts of microbial lipids (Single Cell Oils, SCO) by metabolizing raw glycerol, are among the potential sources for the production of polyunsaturated fatty acids (PUFAs) [2]. PUFAs are of great dietary, pharmaceutical and cosmetic importance. Among them γ-linolenic acid (GLA) is known for its anticancer activities [3, 4].

Materials and Methods

Microorganisms: *Th. elegans* and *M. ramanniana* were cultivated in batch cultures at T=28°C.

Medium: Raw glycerol at 25 g/l, minerals and yeast extract.

Lipid extraction: According to Folch protocol [5].

Lipid fractionation: By using a column of silicic acid activated by heating overnight at T=80 °C. Successive applications of dichloromethane, acetone and methanol produced fractions containing neutral lipids (NL), glycolipids plus sphingolipids (G+S) and phospholipids (P), respectively [6].

GC analysis: Fatty acid analysis of the various lipid fractions was performed after trans-methylation according to the AFNOR method [7], in an Agilent Technologies 7890 A GC system (conditions: column 60 m x 0,25 mm HP- 88 112- 8867; oven T=200 °C; injector T=250 °C, detector (FID) T=280 °C.

Results and Discussion

Growth of oleaginous fungi was studied on nitrogen – limited media.

Th. elegans and *M. ramanniana* cultivated on raw glycerol (used as sole carbon substrate) presented remarkable growth (up to 7 g/l) while significant fat quantities were accumulated inside the fungal mycelia (34- 44 %, wt/wt oil/dry biomass) (e.g. in Fig. 1). These lipids comprised mainly of NL (80%), the concentration of which increased during growth. On the contrary the proportion of polar lipids (G+S and P), decreased with time, since some quantities of NL were accumulated into the mycelium after the growth cessation (Table 1).

Although there were variations in the amounts and composition of lipid fractions, the dominant fatty acid was oleic acid (C18:1, n-9, 38-49% of total fatty acids). The concentration of γ- linolenic acid (GLA) was also remarkable (up to 12,6 %). GLA concentration was decreased in the various fractions, especially in phospholipids during lipid accumulation process (Fig. 2).

It is concluded that SCO accumulation is related to secondary microbial metabolism, while GLA biosynthesis seems to be related to the primary metabolic growth. Cited papers: [6-8].

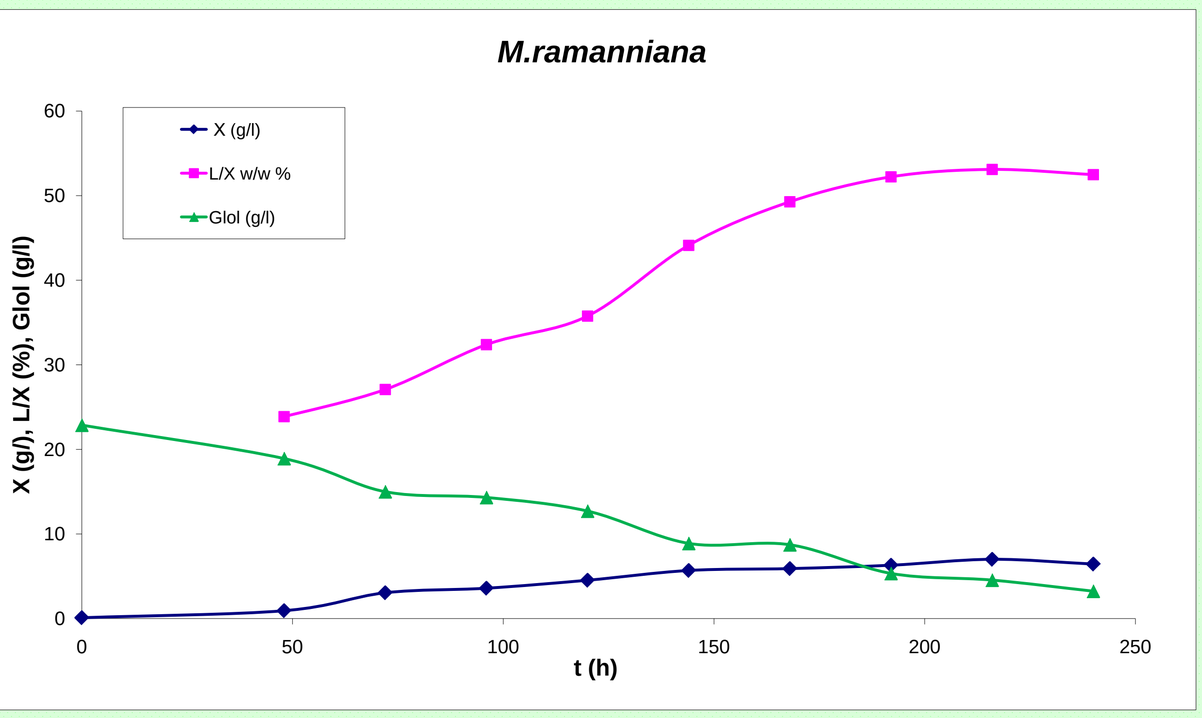
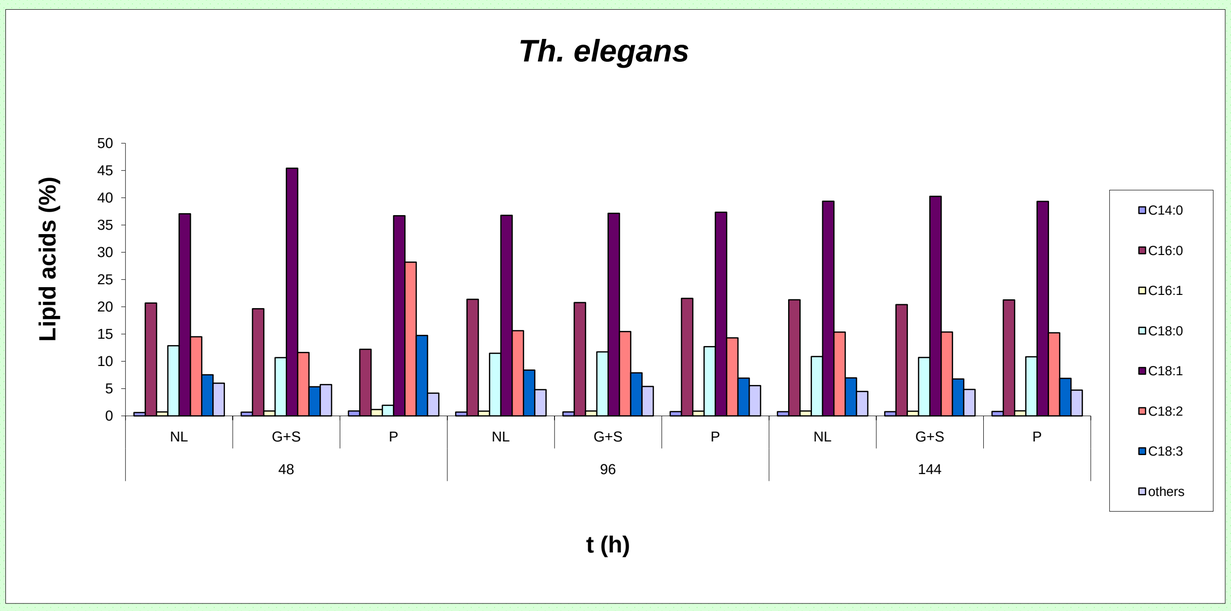


Figure 1: Kinetics of *Thamnidium elegans* cultivated in media of raw glycerol (25 g/l) as sole carbon source. Biomass (X, g/l), cellular lipids (L, g/l), glycerol (Glol, g/l).

Fungus	Cultivation Time (h)	Total lipids in mycelial mass (%)	NL (%)	G+S (%)	P (%)
Th. elegans	48	19	66,5	20,9	12,6
	96	34	76,6	16,1	7,3
	144	37	82,2	16,2	1,6
M. ramanniana	48	23	64,3	24,9	10,8
	96	34	85,6	10,1	4,3
	144	44	85,8	11,4	2,8

Table 1: Lipid fractions (NL, G+S, P) in the lipid produced by *Th. elegans* and *M. ramanniana* cultivated on raw glycerol.



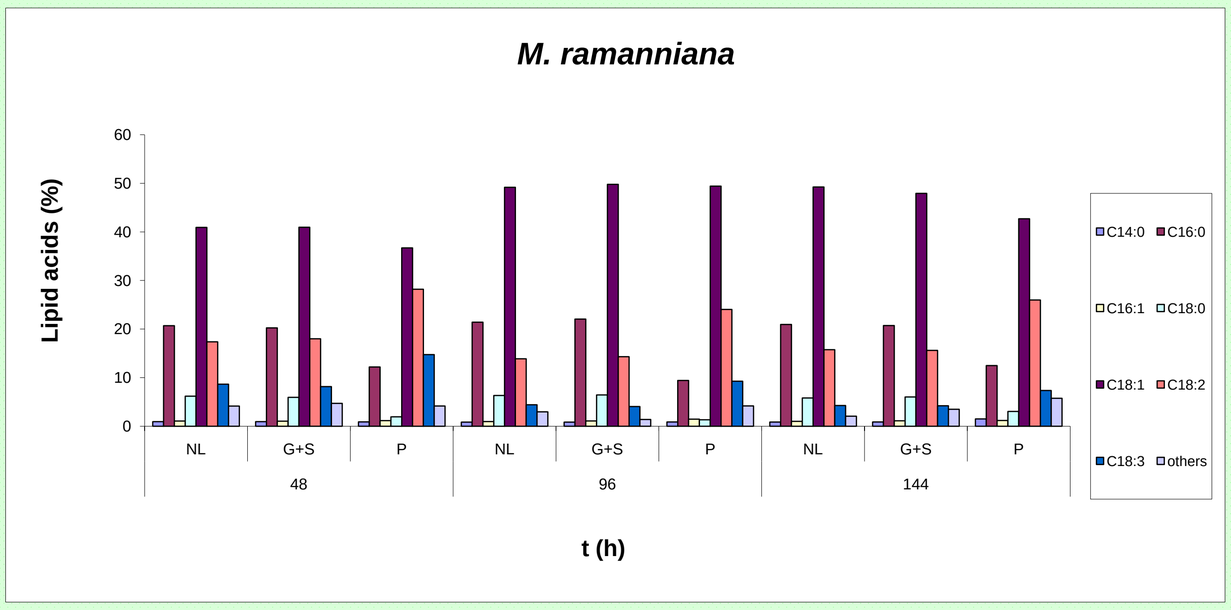


Figure 2: Fatty acid composition of NL, G+S and P during growth of *Th. elegans* and *M. ramanniana* on raw glycerol.

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