Biotechnological conversion of raw glycerol into Single Cell Oils (SCOs)

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Introduction

Raw glycerol is an important renewable feedstock as it is the principle side-product of the biodiesel production process, which is nowadays applied on a large commercial scale. Taking into consideration the huge quantities of raw glycerol produced and its low-cost, it is of urgency to find alternative ways to convert this substrate into value-added products [1]. Its use as carbon substrate in several biotechnological applications could be considered as a potential approach, i.e. oleaginous microorganisms could metabolize raw glycerol and accumulate large amounts of microbial lipids (Single Cell Oils, SCOs) reach in polyunsaturated fatty acids (PUFAs) [2]. PUFAs are of great dietary, pharmaceutical and cosmetic importance and some of them such as γ-linolenic acid (GLA) is known for its anticancer activities [3, 4].

Materials and Methods

Microorganisms: *Cunninghamella echinulata* ATHUM 4411; *Mortierella isabellina* MUCL 15102; *Mortierella ramanniana* MUCL 9235; *Mucor* sp. LGAM 365; *Zygorhynchus moelleri* MUCL 1430; *Thamnidium elegans* CCF-1465; *Candida oleophila* ATCC 20177; *Zygosaccharomyces ruxii* and *Rhodotorula* sp. **Culture conditions:** cultures were performed in 250 ml Erlenmeyer flasks containing 50 ml of a liquid medium, under nitrogen limited conditions and were incubated in a rotary shaker at T=28°C and 180 rpm. **Medium:** Raw glycerol at 30 g/l, minerals and yeast extract. **HPLC analysis:** Glycerol was determined in filtered (through 0.2 μm pore size bacteriological filter, Whatman) aliquots of the culture by an HPLC apparatus (Ultimate 3000, Dionex, Germering, Germany) equipped with an HPX-87H column and a R.I. detector. Conditions: eluant H_2SO_4 0.004 N, flow rate 0.9 ml/min, T =55 °C. **Lipid extraction:** According to Folch protocol [5]. **GC analysis:** Fatty acid analysis was performed after trans-methylation according to the AFNOR method [6], in an Agilent Technologies 7890 A device equipped with a HP-88 (J&W scientific) column (60 m x 0.25 mm). Conditions: carrier gas helium, flow rate 1 ml/min, oven T=200 °C, injector T=250 °C, detector (FID) T=280 °C.

Microorganism	Time (h)	X (g/)	L/X %	Glol (g/)	FFA (mg/l)
Mortierella isabellina	45-55	2,77	24,04	17,64	75
	96-110	4,43	22,45	14,74	61
	140-165	5,50	24,86	10,54	42
Zygorhynchus moelleri	45-55	1,80	23,33	20,12	103
	96-110	2,54	30,05	15,36	77
	140-165	3,49	33,81	16,61	54
Thamnidium elegans	48-55	1,57	27,78	22,84	70
	96-110	3,68	30,87	18,38	64
	140-165	5,16	33,89	13,19	39
***Mucor* sp.**	60	1,23	21,43	27,53	70
	96-110	1,92	24,48	22,99	-
	140-165	2,32	22,60	23,26	-
Cunninghamella echinulata	48-55	1,05	35,19	18,99	240
	96-110	2,84	40,36	19,52	180
	140-165	4,54	33,03	15,85	66
***Rhodotorula* sp.**	48-55	4,42	8,70	13,91	30
	96-110	5,74	18,00	8,21	34
	140-150	6,34	15,30	11,29	26

Table 1: Growth of *M. isabellina*, *Z. moelleri*, *Th. elegans*, *C.echinulata*, *Rhodotorula* sp., *Z. ruxii* on raw glycerol.
Biomass (X, g/l), SCOs (L/X, %), Glycerol (Glol, g/l), medium free fatty acids (FFAs, g/l).

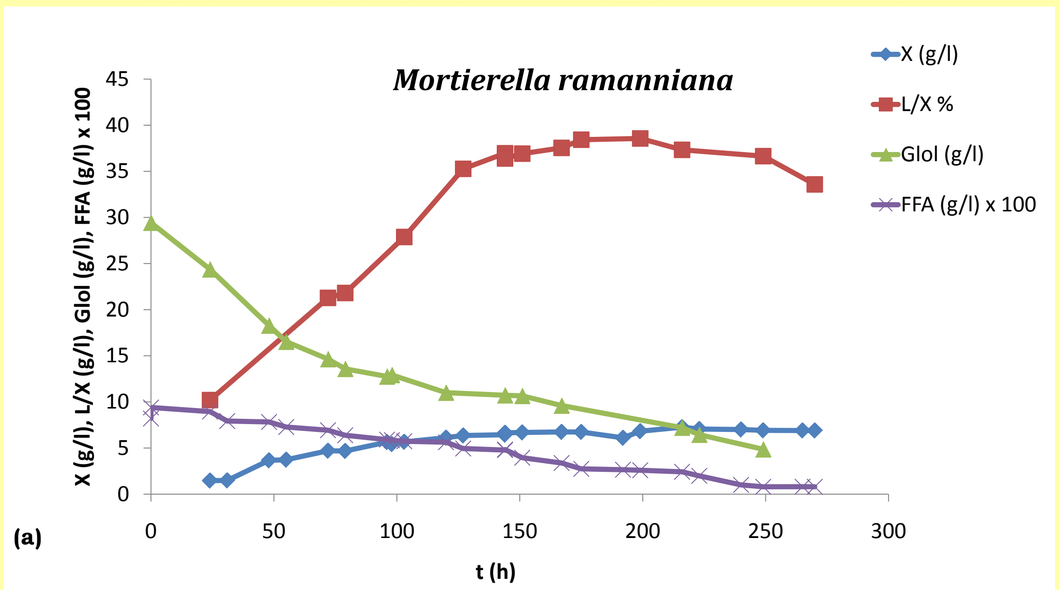

Figure 1: Kinetics of **(a)** *M. raman*niana **(b)** *C. oleophila* on raw glycerol.
-♦- Biomass (X, g/l), -■- SCOs (L/X, %), -▲- Glycerol (Glol, g/l),
-x- medium free fatty acids (FFAs, g/l x 100).

Results and Discussion

The microbial growth was studied under nitrogen-limited conditions. *M. ramanniana* (Fig. 1a), *Th. elegans* (Table 1), *Rhodotorula* sp. (Table 1) and *C. oleophila* (g/l), showed remarkable biomass synthesis cultivated on raw glycerol as sole carbon source, while significant lipid quantities (i.e. up to 42 %, for *Th. elegans* and 24% for *C. oleophila*, wt/wt oil in dry biomass) were accumulated.
Fungal lipids were reach in PUFAs such as γ-linolenic acid, an important fatty acid due to its pharmaceutical properties. GLA was produced in significant quantities reaching 7% in *M. isabellina* and 6% in *M. ramanniana* in total lipid produced. It is also remarkable that GLA excretion (up to 14,66% of total lipids) in the culture medium was noticed in some fungi strains (*Z. moelleri*), fact that may open new perspectives in the biotechnological production of GLA (Table 2a).
On the other hand, yeasts' produced SCOs could be suitable for biodiesel production (Table 2b).

Fungi		t (h)	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3γ	C18:3α	Others
Mortierella isabellina		**14**	**30,74**	**2,71**	**19,13**	**31,41**	**3,47**	**6,45**	**1,15**	**4,73**
	SCOs	134	18,81	2,84	7,49	43,26	15,07	6,12	1,25	5,14
		182	20,67	3,39	6,01	44,89	14,47	4,36	0,89	5,31
		206	21,65	3,54	5,11	47,76	14,63	5,29	0,46	1,55
	FFA	24	10,59	1,05	5,89	52,89	18,83	4,29	1,38	5,08
Mortierella ramanniana	SCOs	55	29,53	1,58	6,05	46,58	10,92	1,62	0,61	3,14
		103	25,35	1,36	6,91	46,79	12,11	3,82	0,63	3,03
		151	25,56	1,43	6,37	47,63	12,24	3,85	0,57	2,79
		240	25,55	1,99	4,34	42,92	16,27	6,06	0,41	3,47
	FFA	24	15,11	2,38	17,66	37,55	19,14	2,81	2,29	3,08
		31	23,58	4,01	6,54	44,21	13,27	3,31	0,74	4,35
Zygorhynchus moelleri	SCOs	50	17,83	1,37	6,37	20,23	47,02	1,32	2,59	3,26
		128	18,37	1,28	6,78	19,32	48,07	1,28	2,25	2,66
		150	17,04	1,75	5,85	23,46	43,49	3,72	2,03	2,67
		192	13,39	1,21	4,86	19,43	42,13	2,23	2,75	2,95
	FFA	24	3,08	8,24	6,86	18,66	34,55	14,66	5,09	3,08
Thamnidium elegans	SCOs	54	23,89	2,38	14,66	42,23	11,31	3,43	0,74	1,37
		115	20,30	1,42	11,80	46,74	12,89	4,81	0,66	1,37
		154	19,51	1,22	12,57	47,26	12,67	4,99	0,43	1,35
		219	18,24	1,17	10,74	50,95	12,63	4,64	0,48	1,14
	FFA	24	21,81	6,69	19,47	23,91	15,74	6,08	2,76	3,54
Cunninghamella echinulata	SCOs	38	25,17	3,47	11,18	36,76	13,32	4,07	1,11	4,92
		88	20,91	2,10	8,21	42,01	15,27	8,66	0,58	2,25
		158	20,27	2,16	4,90	44,52	17,40	8,74	0,62	1,39
		230	19,34	2,36	3,63	47,37	16,50	8,78	0,89	1,13
	FFA	14	13,41	1,17	13,08	25,26	41,01	0,56	1,16	4,35
***Mucor* sp.**	SCOs	39	30,97	3,66	14,73	28,60	11,17	3,44	4,19	3,23
		60	25,72	3,80	8,40	29,59	21,13	6,60	0,49	4,29
		193	23,92	2,16	5,35	27,49	24,20	13,43	0,47	2,99
		237	25,04	2,13	6,49	27,07	22,93	11,43	0,44	4,47

(a)

Yeasts		t (h)	C16:0	C16:1	C18:0	C18:1	C18:2	Others
Candida oleophila	SCOs	33	14,36	2,45	11,93	56,94	12,26	2,05
		57	13,40	2,05	10,55	60,51	11,42	2,07
		74	13,48	1,70	9,52	62,71	11,38	1,20
		150	12,91	2,52	6,61	65,55	11,53	0,87
		170	11,99	1,97	9,04	65,91	9,21	1,88
	FFA	14	11,16	1,18	6,11	53,97	18,58	2,75
		24	10,59	1,05	5,89	52,89	18,83	5,08
***Rhodotorula* sp.**	SCOs	52	25,59	1,14	8,33	52,31	10,68	1,95
		91	21,90	1,01	8,25	55,37	12,14	1,33
		141	21,75	1,14	8,10	55,07	12,24	1,70
		187	21,73	1,14	7,37	55,85	12,40	1,52
	FFA	8	15,90	3,42	6,06	51,23	21,33	2,06

(b)

Table 2: Fatty acid composition of microbial lipids (SCOs) and culture medium (FFAs) during growth of **(a)** fungi, **(b)** yeasts on raw glycerol. Medium FFAs contained 13,5% C16:0, 2,04% C16:1, 7,51% C18:0, 42,67% C18:1, 23,91% C18:2, 5,58% C18:3a, 4,79% others.

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