FEBS Workshop
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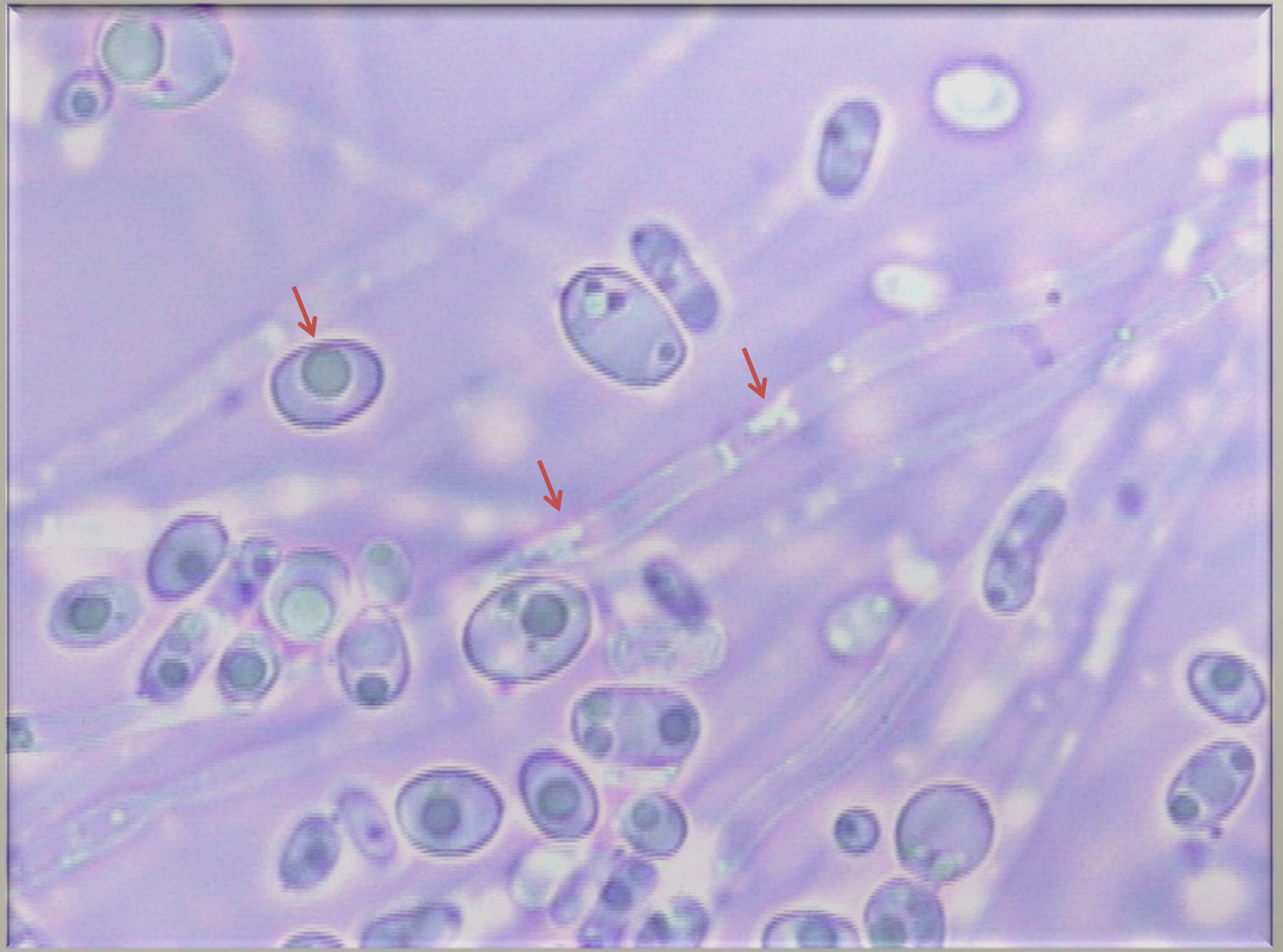
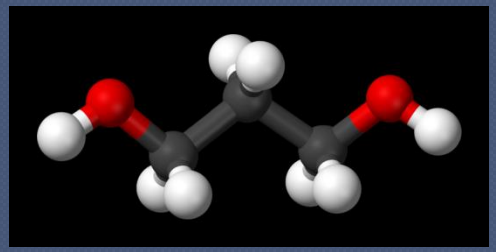
"Metabolic activities in *Yarrowia lipolytica* grown on glycerol"

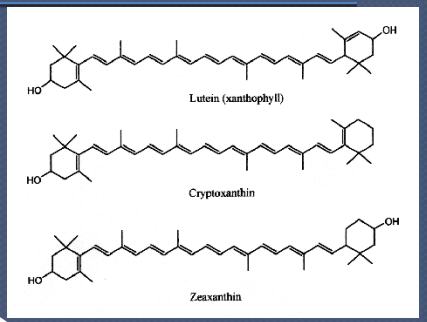
Anna Makri, Stylianos Fakas & George Aggelis

Unit of Microbiology, Division of Genetics, Cell & Development Biology, Department of Biology, University of Patras



Why Glycerol?





SCO

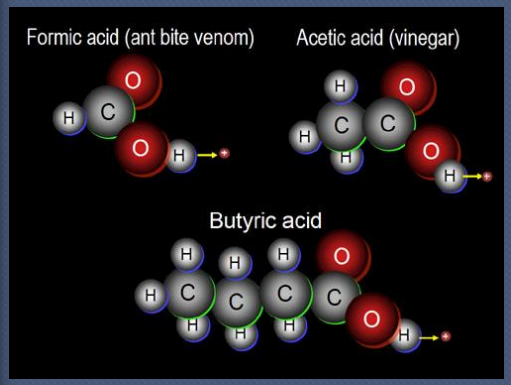
1,3-Propane diol

Carotenoids

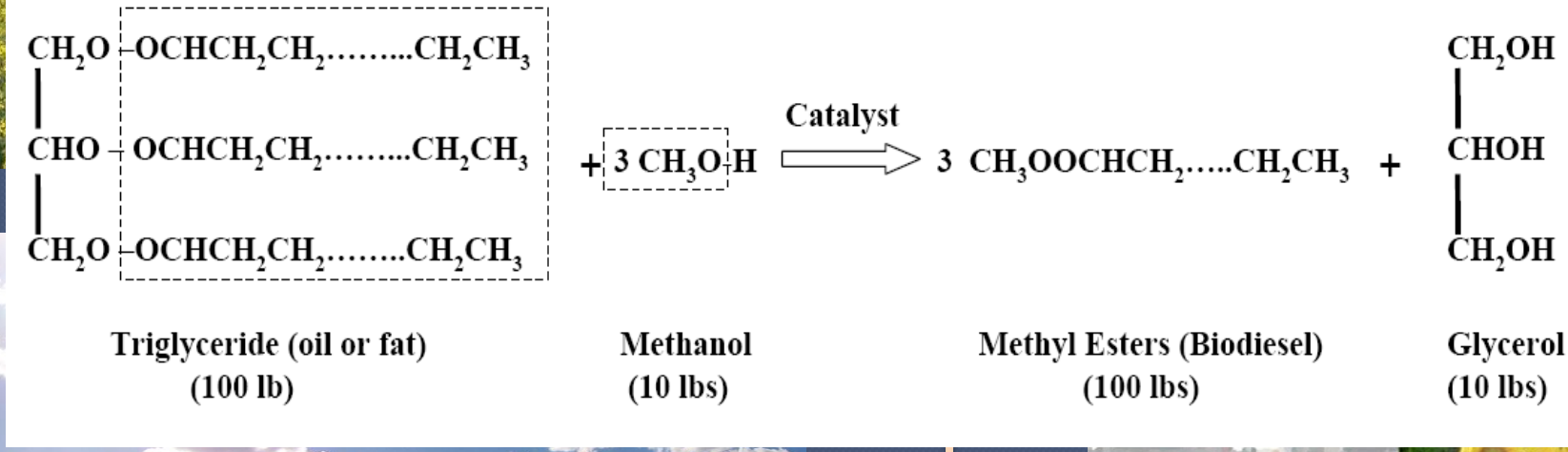
Glycerol

Organic acids

Bioplastics



Glycerol



Industrial Biodiesel System 40.000 ton/year – Client: Brasil Ecodiesel

Biotechnological interest of SCO

Oleaginous species

nutrient limitation

oil contents >20% biomass weight

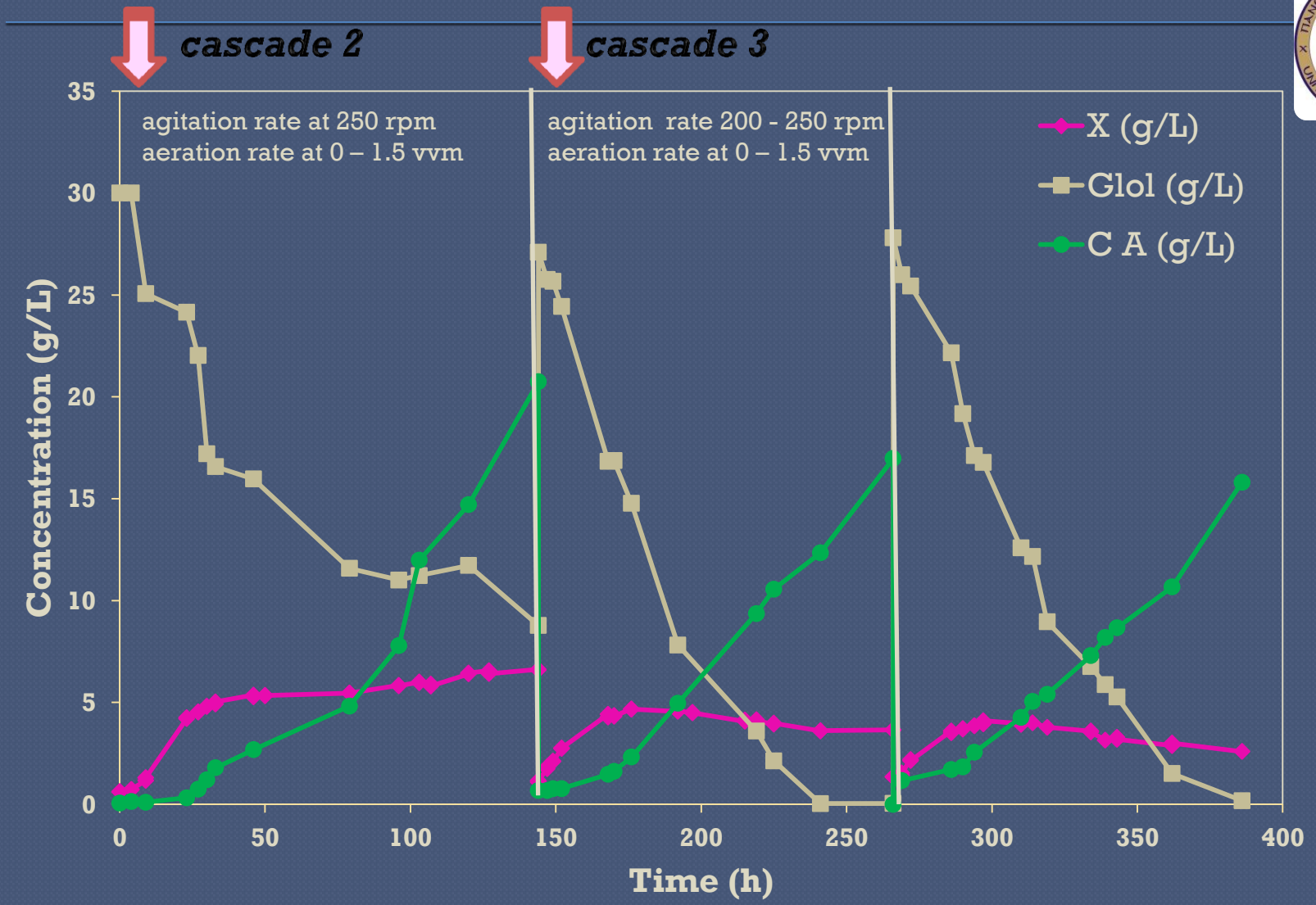
Dietary and pharmaceutical importance

Conversion into bio-diesel


BIOENGINEERING
AIR
DELL

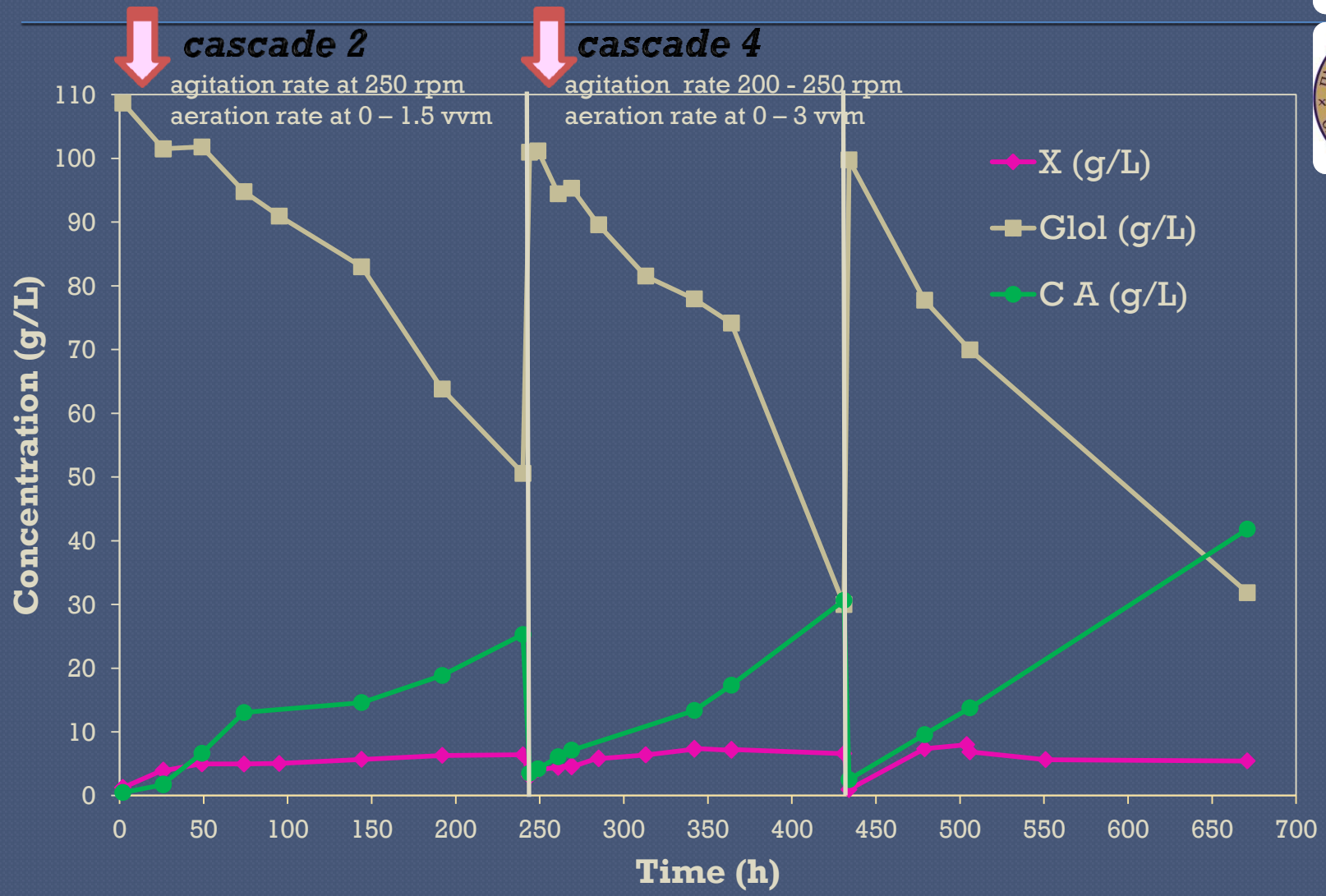
Kinetics of biomass (x), substrate (Glol) and citric acid (CA) in repeated batch cultures of *Y. lipoltytica*.

Culture conditions: initial glycerol concentration 27.8 g/L; dissolved oxygen concentration 30 %of saturation; pH 6 ± 0.03; T=28 °C



Kinetics of biomass (x), substrate (Glol) and citric acid (CA) in repeated batch cultures of *Y. lipoltytica*.

Culture conditions: initial glycerol concentration 104.9 g/L; dissolved oxygen concentration 30 % of saturation; pH 6 ± 0.03; T=28 °C



Growth of *Y. lipolytica* in repeated batch culture and conversion yields in different initial glycerol concentration.

Culture conditions: dissolved oxygen concentration 30 % of saturation; pH 6 ± 0.03; T=28 ºC; agitation rate 200 – 250 rpm; aeration rate 0 – 1.5 and 0 – 3.0 vvm for initial glycerol concentration 27.8 and 104.9 g/L, respectively. *Abbreviations*: initial substrate (So); remaining substrate (S); biomass produced (x); lipid content (L/x, %); citric acid produced (CA); and conversion yields (*Y*) in the various growth phases.

S_o (g/L)	Growth phase	Time (h)	S (g/L)	x (g/L)	L/x (%)	CA (g/L)	$Y_{x/S}$	$Y_{L/S}$	$Y_{CA/S}$
27.8	Biomass Production	30	23.09 ±1.06	4.37 ±0.17	13.90 ±0.10	0.53 ±0.21	0.97 ±0.18	0.13 ±0.03	0.11 ± 0.02
	Lipogenic	50	14.07 ±1.90	4.68 ±0.68	22.30 ±0.39	3.87 ±1.19	0.36 ±0.10	0.08 ±0.02	0.28 ± 0.05
	CA Production	130	0.44 ±0.36	3.18 ±0.27	11.83 ±0.66	13.95 ±1.47	0.17 ±0.10	0.01 ±0.01	0.51 ± 0.05
104.9	Biomass Production	30	94.86 ±0.43	4.57 ±0.17	12.13 ±0.03	6.63 ±0.51	0.46 ±0.04	0.06 ±0.01	0.66 ±0.08
	Lipogenic	50	78.29 ±2.56	6.71 ±0.52	20.41 ±1.31	13.83 ±2.27	0.25 ±0.01	0.05 ±0.01	0.53 ±0.09
	CA Production	240	41.22 ±9.37	5.92 ±0.49	13.40 ±0.33	33.55 ±8.26	0.10 ±0.02	0.01 ±0.01	0.52 ±0.05

Enzymatic activities in *Y. lipolytica* grown on glycerol in repeated batch culture.

Culture conditions: initial glycerol concentration 27.8 g/L; dissolved oxygen concentration 30 % of saturation; pH 6 ± 0.03; T=28 °C; agitation rate 200 – 250 rpm; aeration rate 0 – 1.5 vvm.

Abbreviations: **$NADP^+$-ICDH** – $NADP^+$ dependent isocitric dehydrogenase; **NAD^+-ICDH** – NAD^+ dependent isocitric dehydrogenase; **GK** – glycerol kinase; **NAD^+-G-3-P DH** – glycerol -3- phosphate dehydrogenase; **Mit.** – mitochondial; **Cyt.** – cytoplasmic; Units/mg dry weight – U/mg DW.

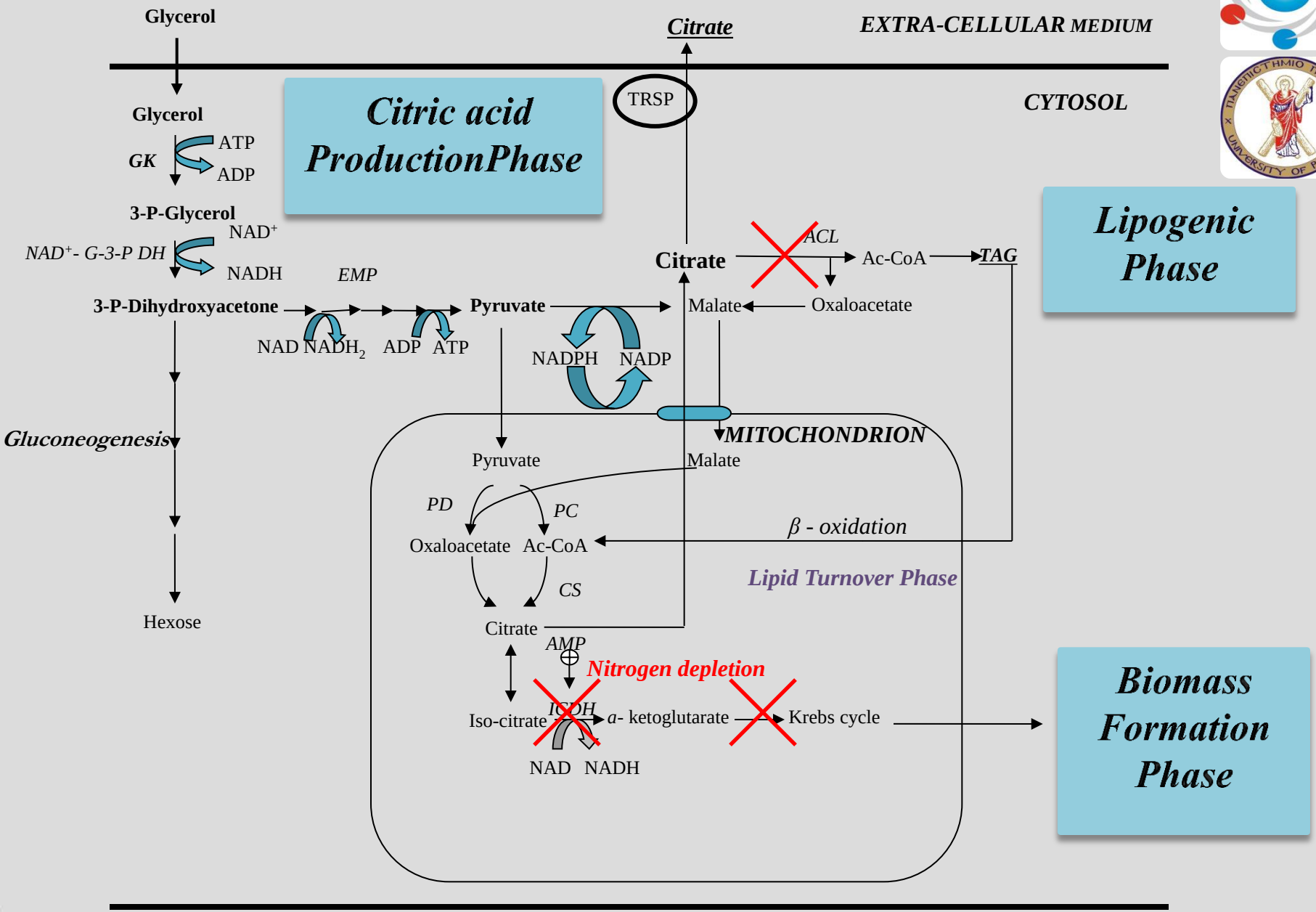
Growth phase	Fermentation Period (h)	$NADP^+$-ICDH (U/mg DW)	NAD^+-ICDH (U/mg DW)	GK (U/mg DW)	NAD^+-G-3-P DH (U/mg DW)
Biomass Production	0 - 24	192.55 ± 4.61 Mit./Cyt. 10 %/ 90 %	45.53±0.89	39.40±6.23	14.93±8.68
Lipogenic	30 - 52	187.82±13.68 Mit./Cyt. 0 %/ 100 %	13.66±0.94	49.48±6.99	17.67±6.48
CA Production	55-130	208.05±0.15 Mit./Cyt. 0 %/ 100 %	0.39±0.01	18.00±0.10	18.00±0.07

Enzymatic activities in *Y. lipolytica* grown on glycerol in continuous culture.

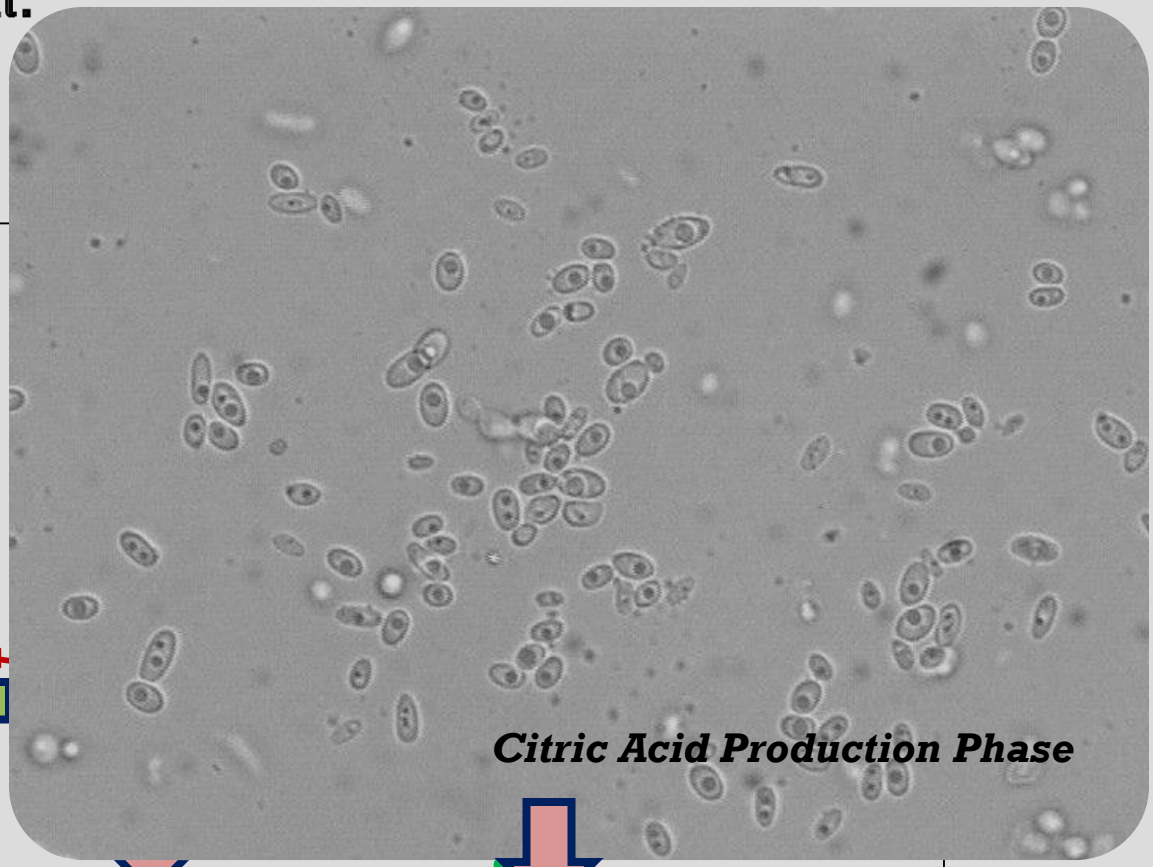
Culture conditions: initial glycerol concentration 50 g/L; dissolved oxygen concentration 20 % of saturation; pH 6 ± 0.03; T=28 oC; agitation rate 200 – 250 rpm; aeration rate 0 – 1.5 vvm.

Abbreviations: **NAD+-ICDH** – NAD+ dependent isocitric dehydrogenase; **GK** – glycerol kinase; **NAD+-G-3-P DH** – glycerol -3- phosphate dehydrogenase; **ATP:CL**- ATP citrate lyase; **ME**- malic enzyme Units/mg dry weight – U/mg DW.

D (h^{-1})	GK (U/mg DW)	NAD^+-G-3-P DH (U/mg DW)	NAD^+-ICDH (U/mg DW)	ATP:CL (U/mg DW)	ME (U/mg DW)
0.017±0.001	101.84	3.42	15.95	218.33	142.93
0.104±0.003	161.55	33.07	26.93	18.84	648.95



The morphology of the unconventional yeast *Y. lipolytica* grown on glycerol is growth phase-dependent.



Lipid fractions and individual phospholipid classes during growth of *Y. lipolytica* on glycerol in repeated batch culture.

Analyses were performed in duplicate.
Culture conditions: initial glycerol concetration 27.8 g/L; dissolved oxygen concetration 30 per cent of saturation; pH 6 ± 0.03; T=28 oC; agitation rate 200 – 250 rpm; aeration rate 0 – 1.5 vvm.
Abbreviations : NL – Neutral lipids; G+S – glycolipids plus sphingolipids; P – phospholipids; PC – phosphatidylcholine; PE – phosphatidylethanolamine; PI – phosphatidylinositol; PA - phosphatidic acid; others - unidentified phospholipids.

	Lipid Fractions [% in total lipids]			Lipid Fractions [% in x]			P classes [% (w/w) in P]					
Time (h)	**NL**	**G+S**	**P**	**NL**	**G+S**	**P**	**PS**	**PI**	**PA**	**PE**	**PC**	**others**
28	82.81	12.20	4.99	11.51	1.70	0.69	2.38	24.35	1.10	11.66	29.38	31.11
48	92.45	4.35	3.20	20.61	0.97	0.71	0.22	20.45	5.88	15.57	30.11	27.76
130	60.96	24.48	14.55	7.21	2.90	1.72	n.d.*	n.d.	n.d.	n.d.	n.d.	n.d.

***n.d.: not determined**

Fatty acid composition of lipids fractions of *Y. lipolytica* grown on 27.8 g/L glycerol in repeated batch culture.

Analyses were performed in duplicate.
Culture conditions: initial glycerol concetration 27.8 g/L; dissolved oxygen concetration 30 per cent of saturation; pH 6 ± 0.03; T=28 oC; agitation rate 200 – 250 rpm; aeration rate 0 – 1.5 vvm.

	Time (h)	C16:0	C16:1	C18:0	C18:1	C18:2	others
NL	28	14.71	14.05	6.96	55.65	7.96	0.67
	48	13.07	16.97	5.96	55.88	7.06	1.05
	130	17.06	14.13	7.70	43.50	16.94	0.67
G+S	28	16.52	13.30	2.95	50.80	13.29	3.14
	48	13.65	17.28	4.66	53.24	9.62	1.55
P	28	8.38	17.00	0.73	50.14	21.64	2.10
	48	7.31	23.00	1.45	48.93	15.87	3.45
	130	10.62	19.92	1.07	34.47	30.66	3.25

Conclusions

Growth

- Three distinct phases

Regulation

- GK
- NAD^+-ICDH
- ME
- ATP:CL

Lipids

- NL
- P

Unit of Microbiology

- **FEBS Workshop**

- **Project K. Karatheodori, University of Patras, Kinetics of growth of oleaginous microorganisms and biosynthesis dynamics of Polyunsatusated Fatty Acids (PUFAs), (2007-2010)**

- **DRACOIL SA, Biodiesel production from agricultural by- products, (2006-2010)**


Thank you!