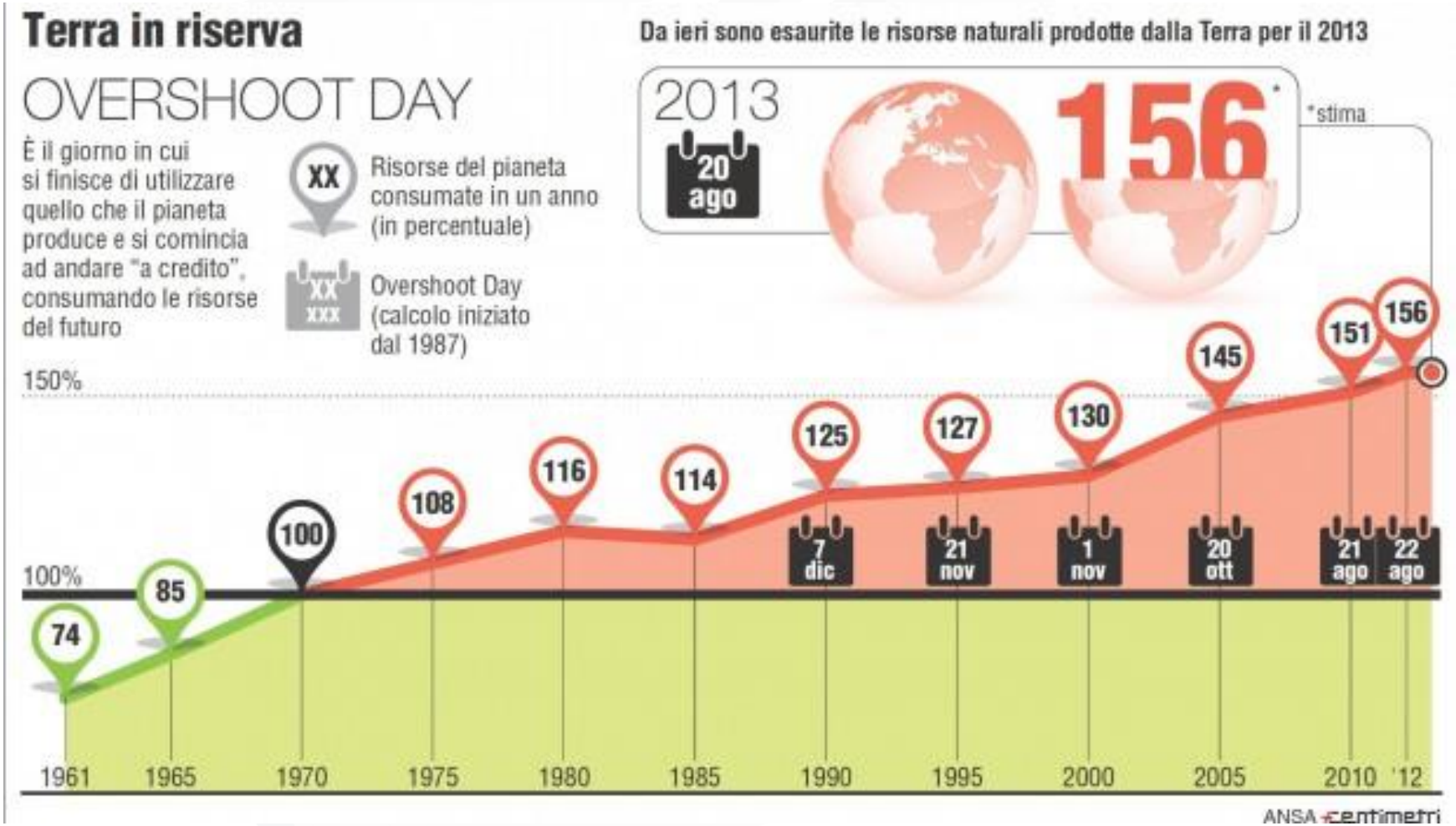
Conversione microbica di glicerolo grezzo in ingredienti per mangimi per pesci

Paola Branduardi

Department of Biotechnology and Biosciences

15-16 Febbraio 2018, Pordenone

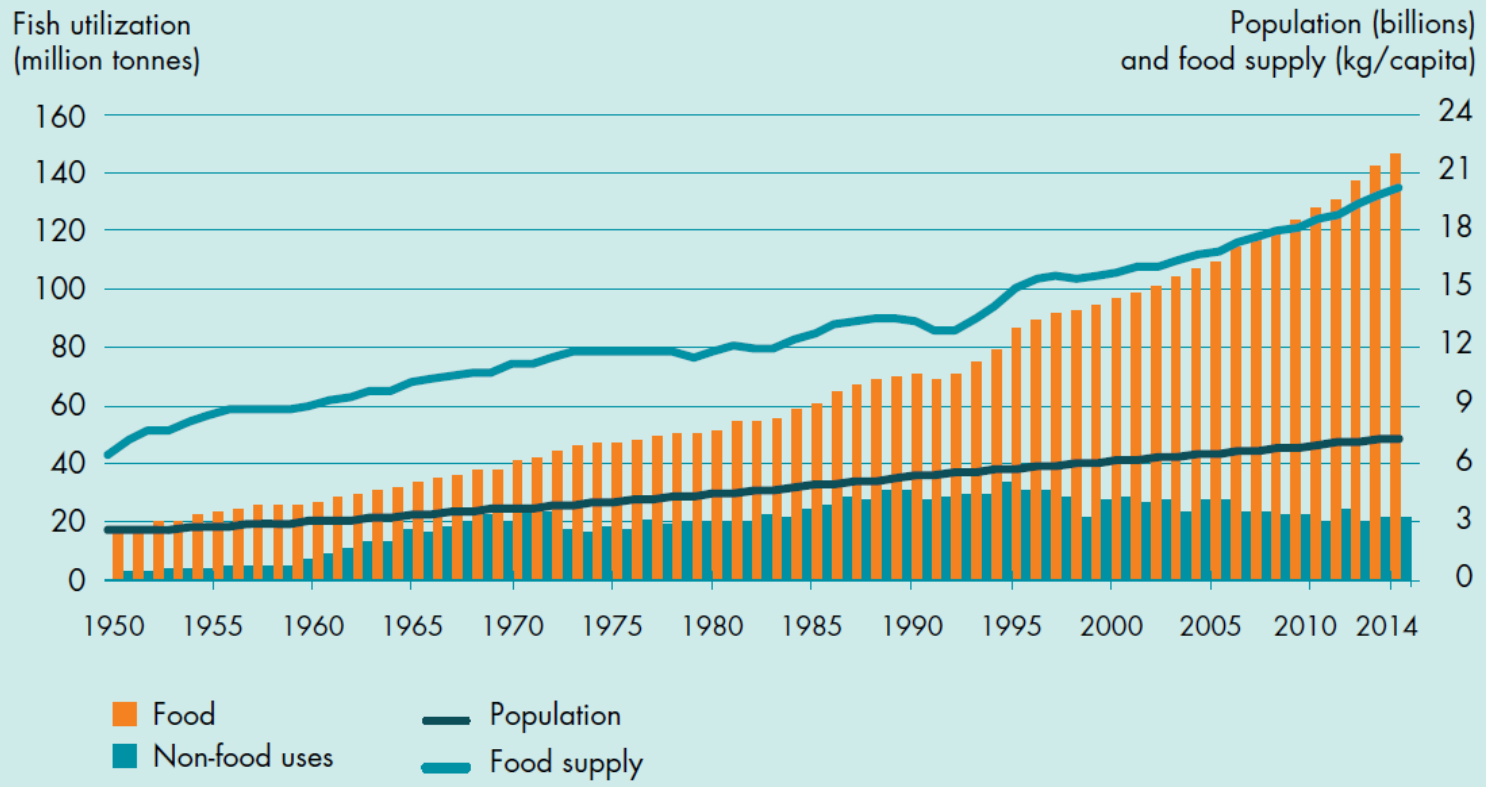
Lo scenario in cui operiamo: un pianeta in riserva



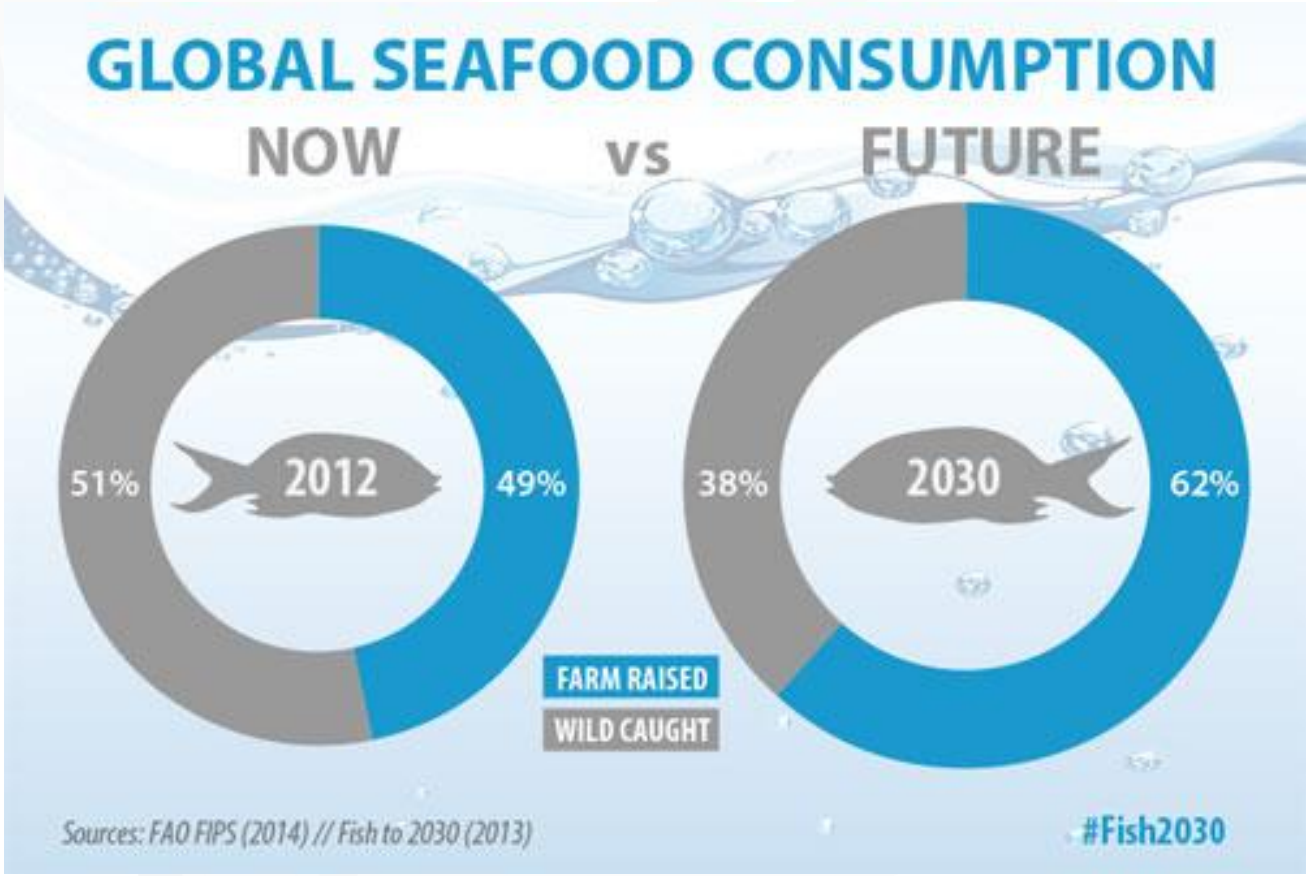
Disponibilità e consumo annuale di pescato

FIGURE 2

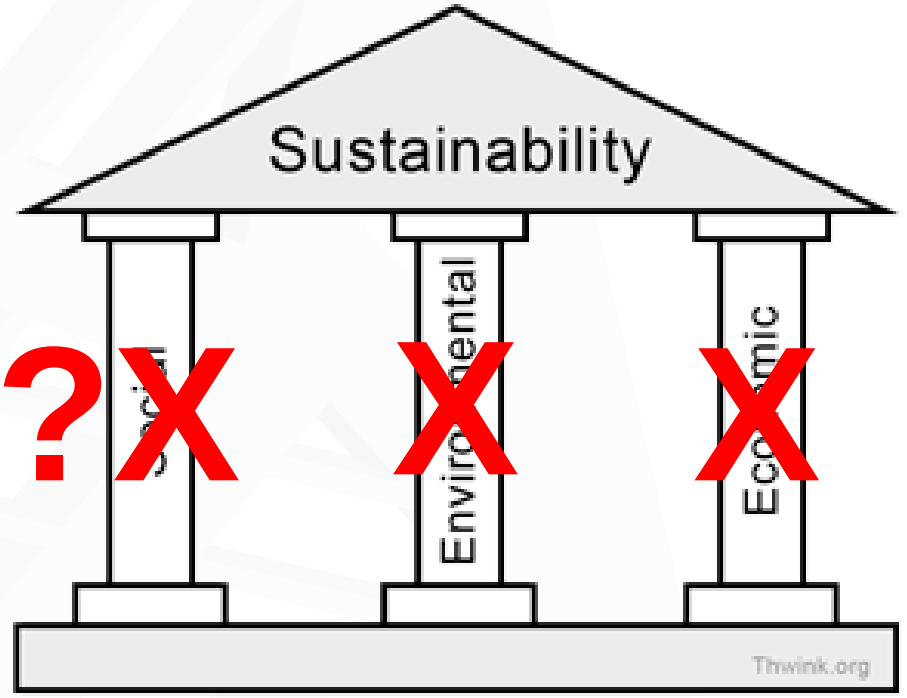
WORLD FISH UTILIZATION AND SUPPLY



Disponibilità e consumo annuale di pescato: Acquacoltura come soluzione?



Acquacoltura e sostenibilità: il mangime



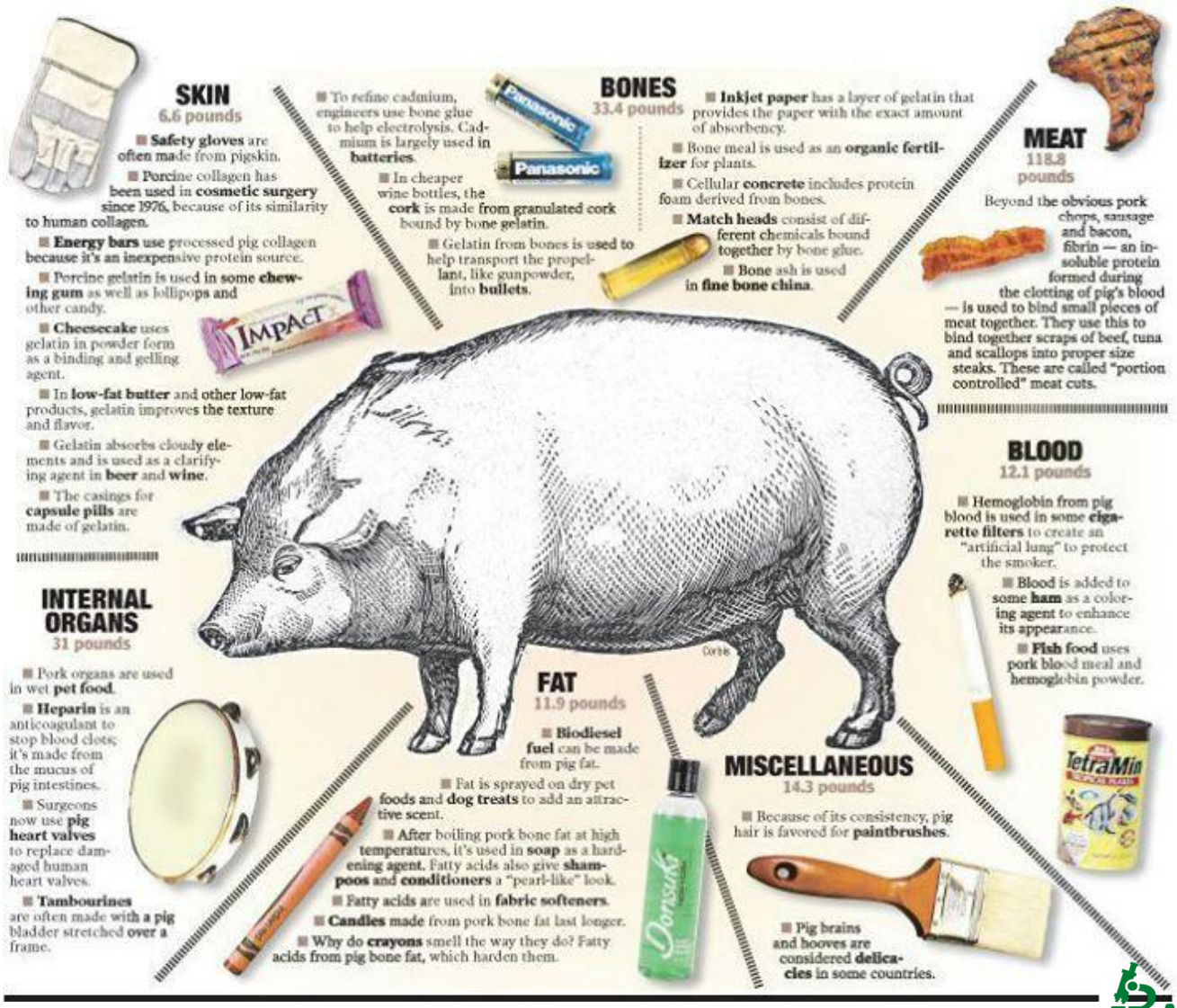
Possiamo usare meglio i prodotti del Pianeta? Sostenibilità e disponibilità

Prodotti e residui

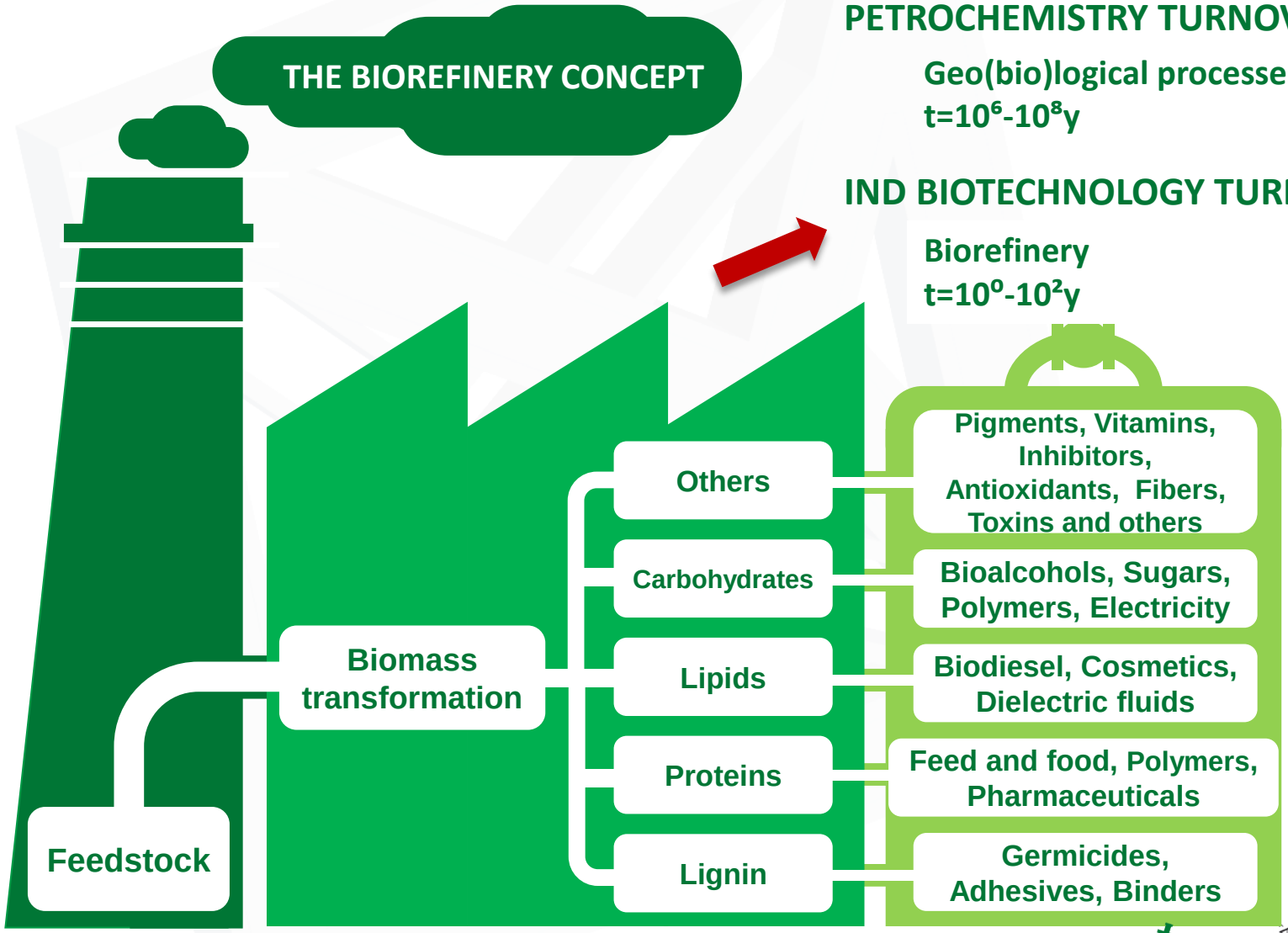





La logica del maiale: everything but the Oink…



… diventa bioraffineria



PETROCHEMISTRY TURNOVER:

Geo(bio)logical processes

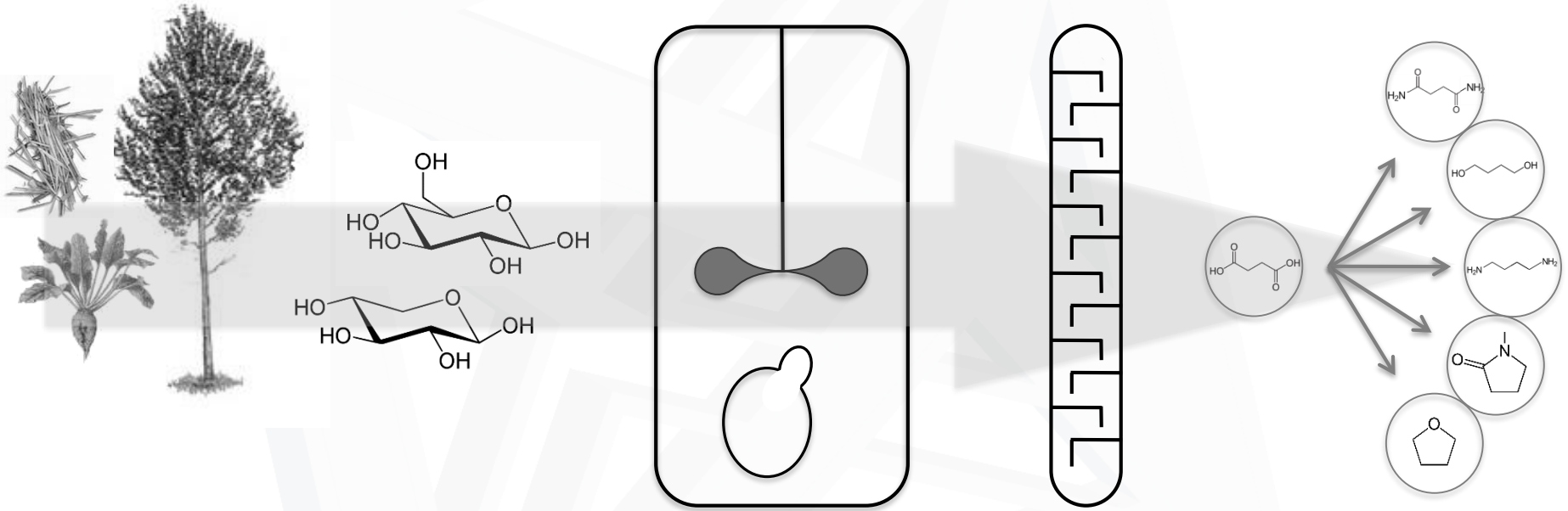
$t=10^6$-10^8y

IND BIOTECHNOLOGY TURNOVER:

Biorefinery

$t=10^0$-10^2y

Possiamo usare meglio i prodotti del Pianeta? Fermentazioni microbiche e bioprocessi

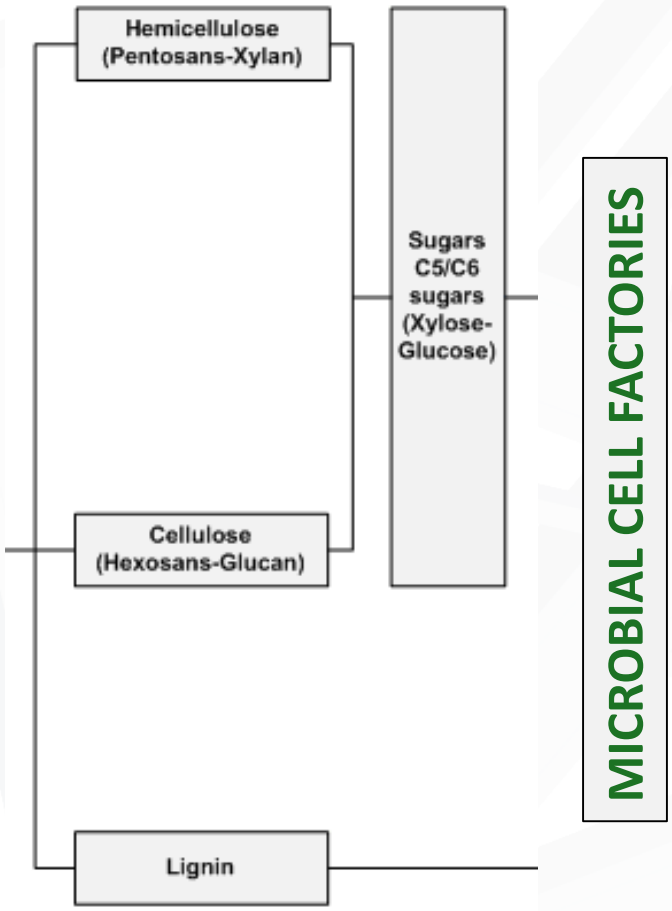


Adapted from: Sauer 2106 FEMS Microbiol Lett

Fermentazioni microbiche e bioprocessi a IndBioTech

BIOMASS:

- MOLASSES
- SUGAR BEET PULP
- CRUDE GLYCEROL
- SPRUCE
- ARUNDO DONAX
- WHEY
- RESIDUAL STARCH



Recombinant Proteins (since 1990)

Lactic Acid and PLA (since 1996)

Ascorbic Acid (since 2002)

Glucosinolates (since 2007)

Butanol and Biodiesel (since 2008)

II Generation Ethanol (since 2009)

Microbial oil (since 2016)

Substrate for enzymes (since 2014)

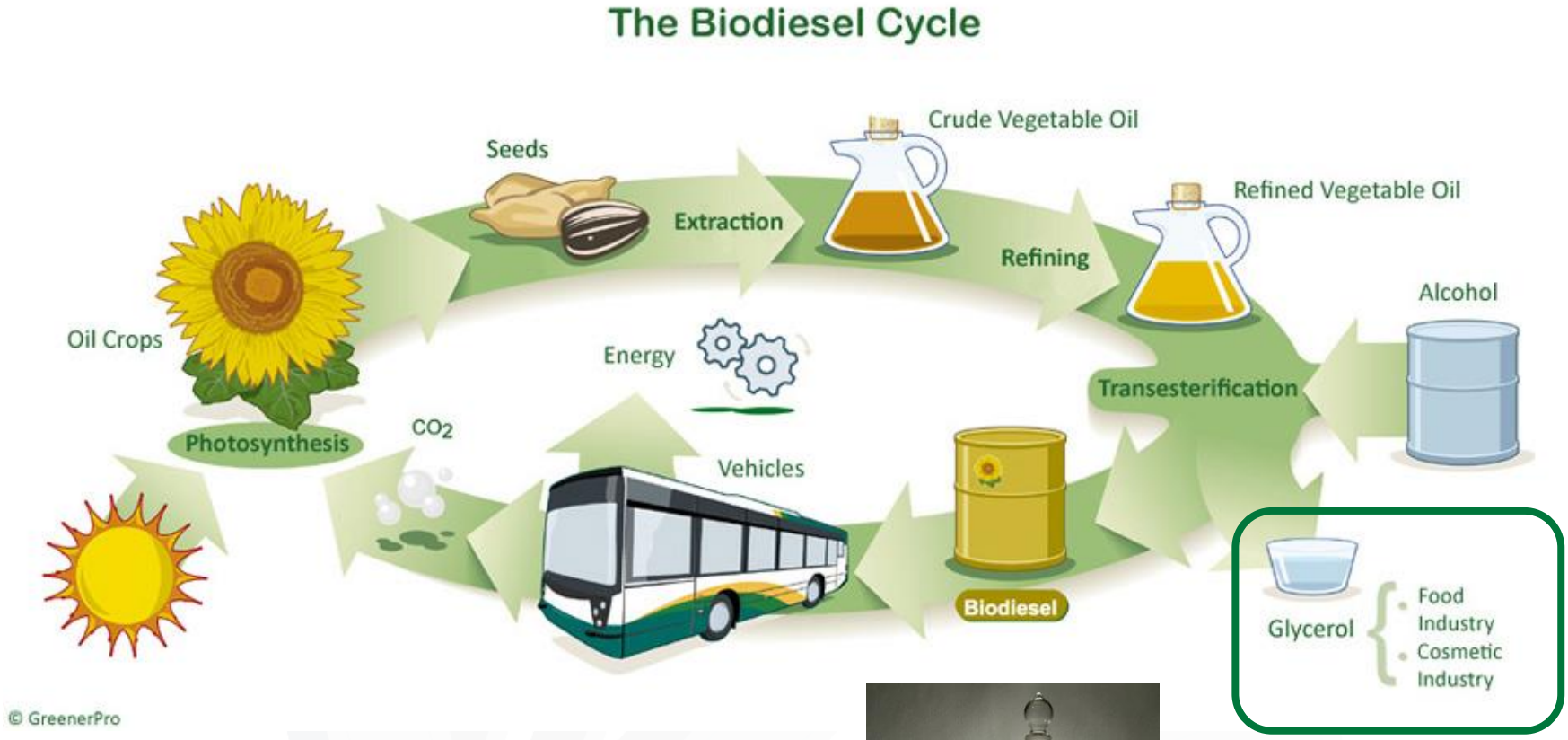
Adapted from: Villegas et al., BPE(LASEN)-ENAC-EPFL, 2008

Acquacoltura: dai problemi alle soluzioni

Microalgae and Yeasts SUStainable fermentation for HIgh quality fish feed formulation - MYSUSHI

http://www.mysushibiotech.com/it/

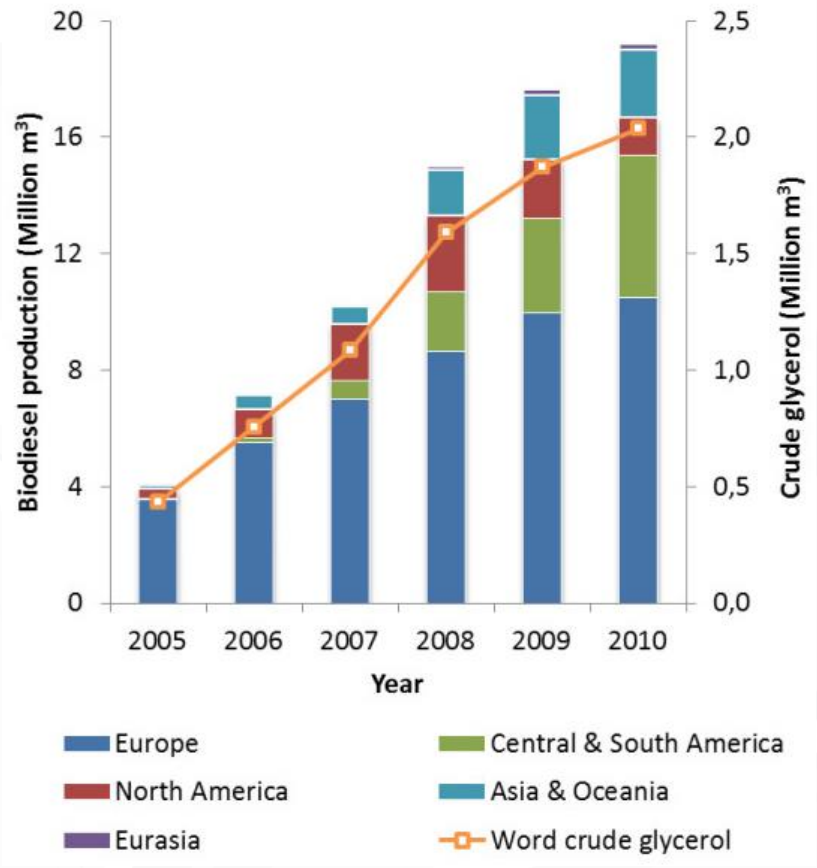
La produzione di biodiesel



BioDiesel →

Glicerolo grezzo →

La produzione di biodiesel



Produzione mondiale di biodiesel (barre, divisa per continenti) e glicerolo grezzo (linea, totale) tra il 2005 ed il 2010

Biodiesel biorefinery: Opportunities and challenges for microbial production of fuels and chemicals from glycerol waste *2012 Biotechnology for Biofuels*

Microalgae and Yeasts SUStainable fermentation for HIgh quality fish feed formulation - MYSUSHI

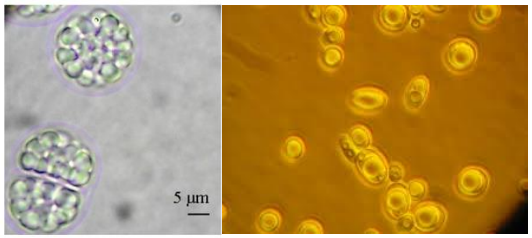
Bio Diesel

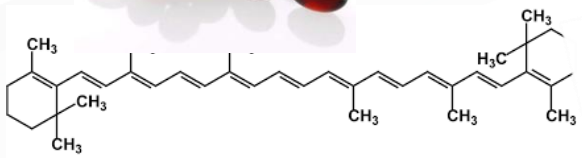
Crude glycerol

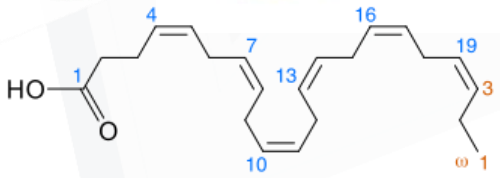
Microrganismi oleaginosi

Carotenoidi e LC-omega 3

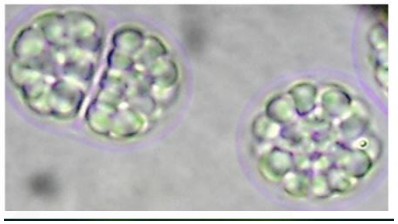
Mangime

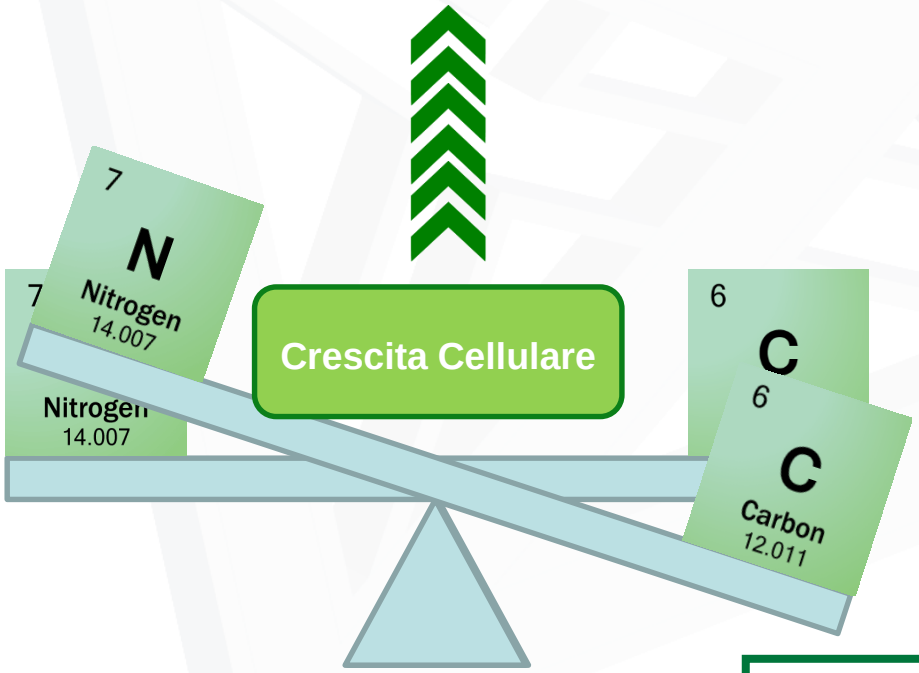






Lieviti oleaginosi e microalghe

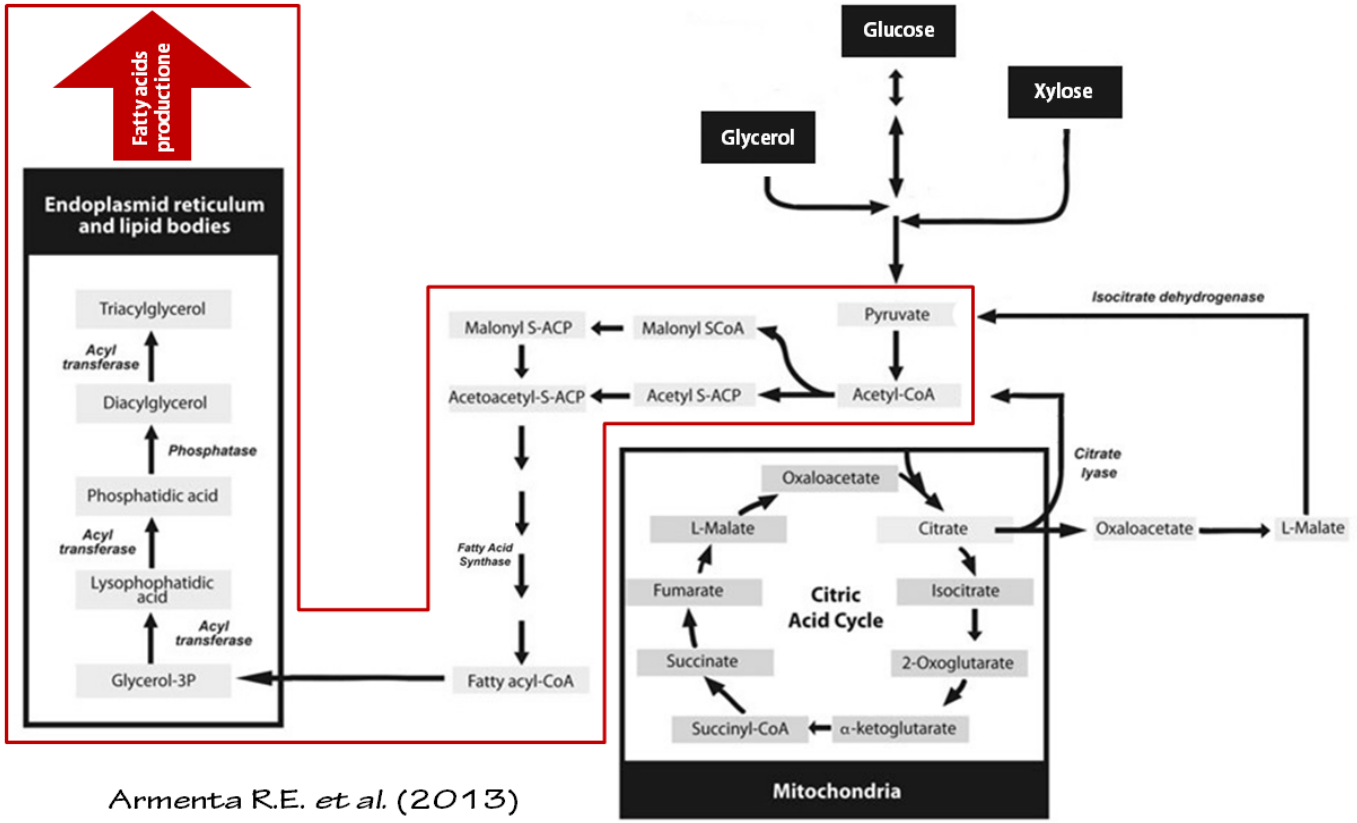




Accumulo di Olio Microbico

GLICEROLO GREZZO

Lieviti oleaginosi, microalghe e olio microbico



Armenta R.E. *et al.* (2013)

In condizioni di crescita sbilanciata i microrganismi oleaginosi possono accumulare fino al 70% del loro peso in forma di oli microbici

Valorizzazione di glicerolo grezzo in carotenoidi con lieviti oleaginosi

Riccardo Posteri

Lorenzo Signori

Chiara Pesciaroli

Olio microbico da glicerolo grezzo: ottimizzazione del processo fermentativo

a

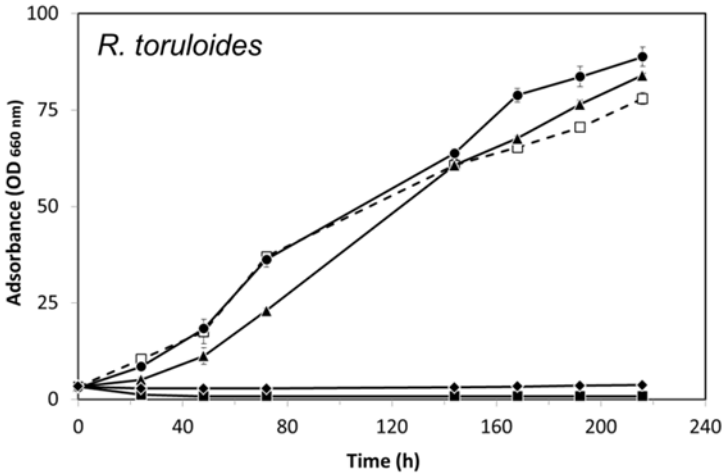
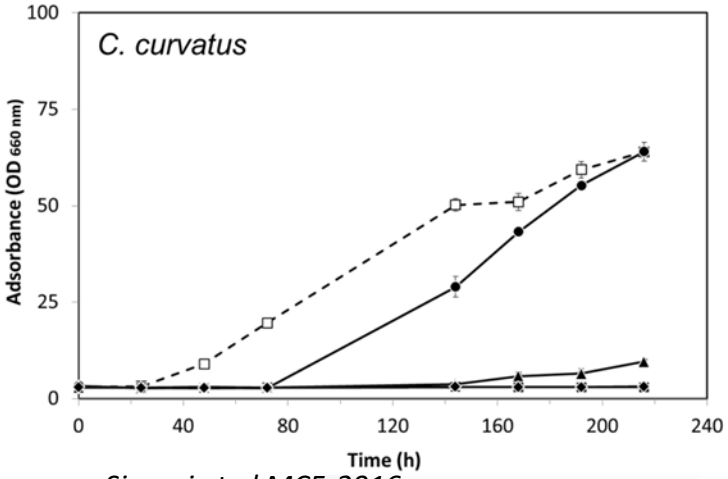


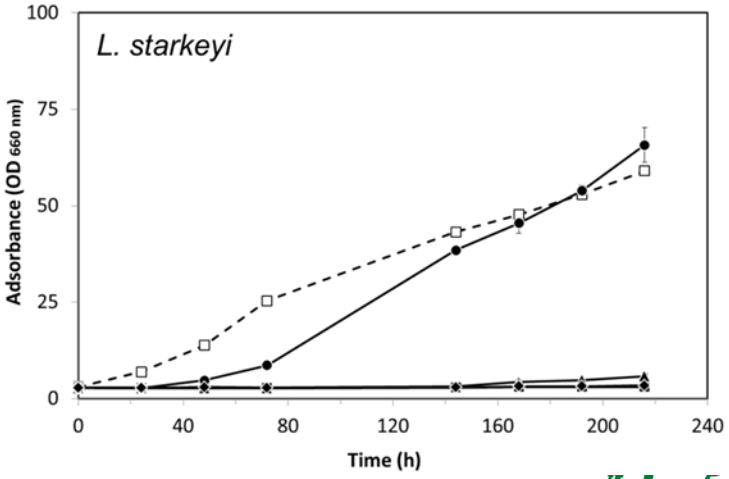
Figure ...h curves (OD660 nm) of *R. toruloides* (a), *C. curvatus* (b) and *L. starkeyi* (c) cells. Five different mix of pure and crude glycerol were evaluated:

- 100 % pure glycerol
- 80 % pure and 20% crude glycerol
- 70 % pure and 30% crude glycerol
- 50 % pure and 50% crude glycerol
- 100 % crude glycerol

b



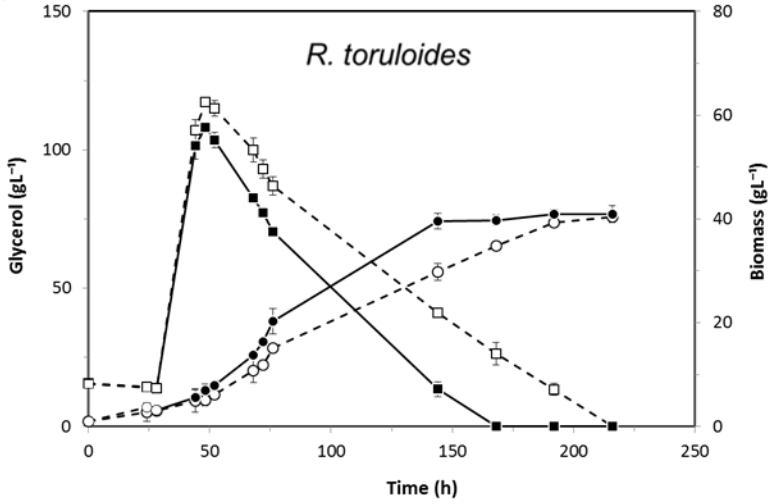
c



Signori et al MCF, 2016

Olio microbico da glicerolo grezzo: ottimizzazione del processo fermentativo

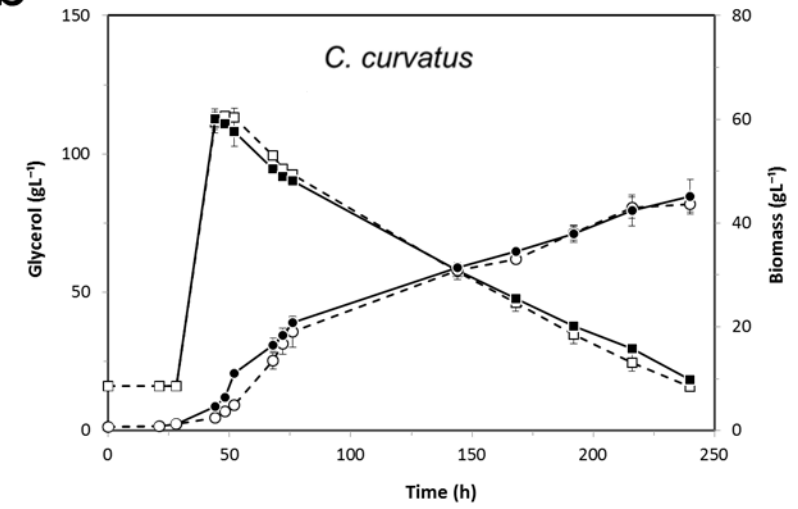
a



– Growth profiles (CDW; g L^{-1}) and glycerol consumption profiles (g L^{-1}) of *R. toruloides* (a), *C. curvatus* (b) and *L. starkeyi* (c) grown on pure (dashed line) and crude (continuous line) glycerol.

Pure glycerol Crude glycerol

b



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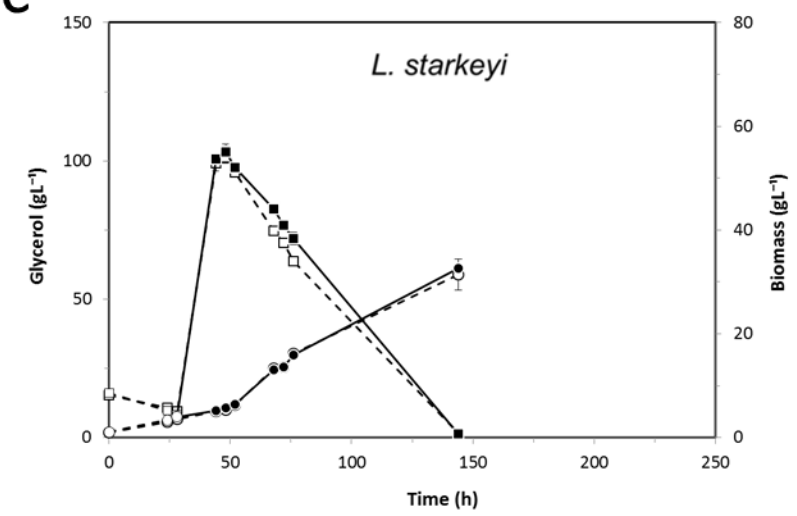

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Olio microbico da glicerolo grezzo: ottimizzazione del processo fermentativo

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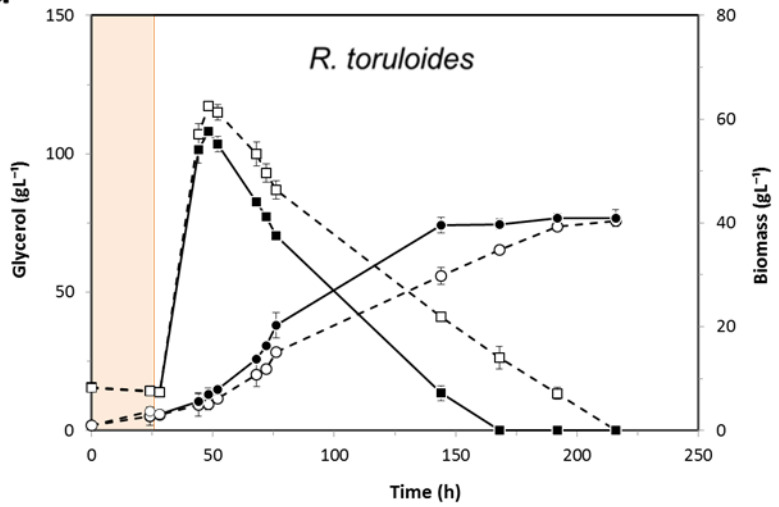

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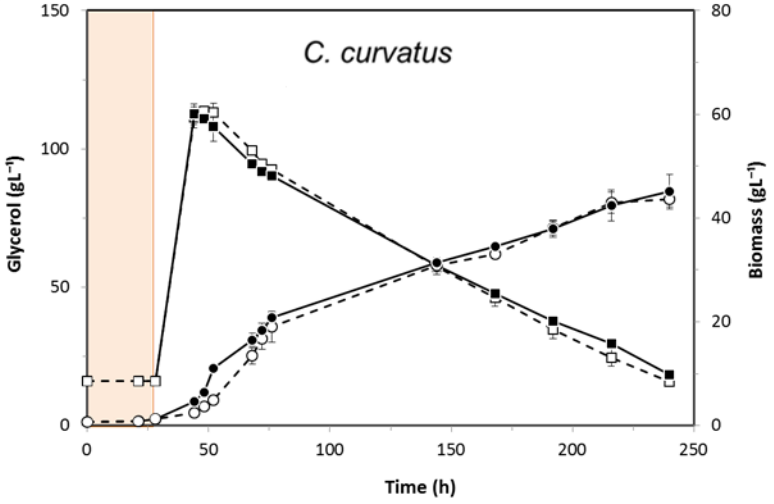
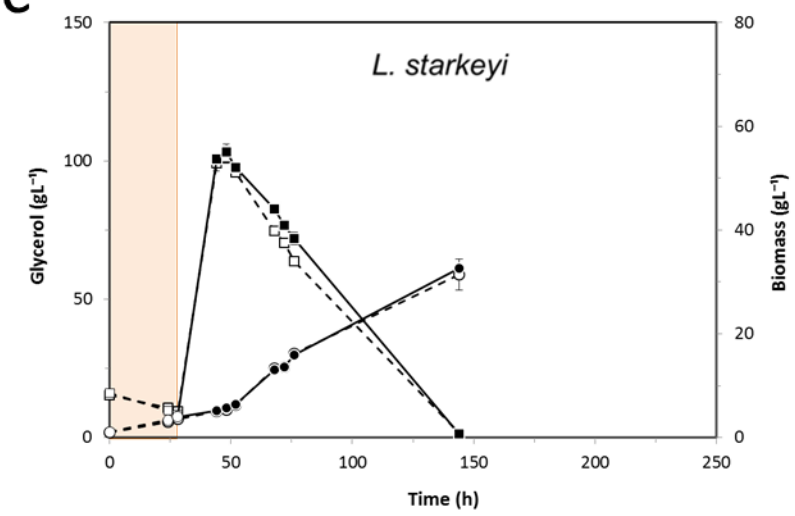


c

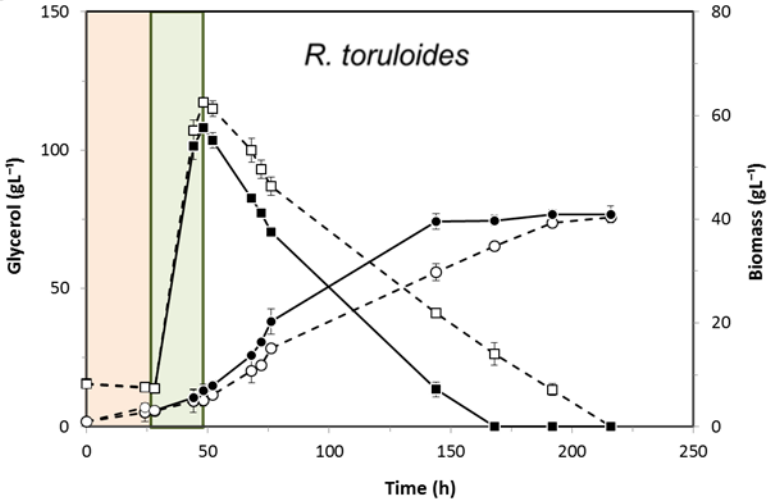


Signori et al MCF, 2016

IndTech UNIVERSITÀ DEGLI STUDI DI MILANO BICOCCA

Olio microbico da glicerolo grezzo: ottimizzazione del processo fermentativo

a



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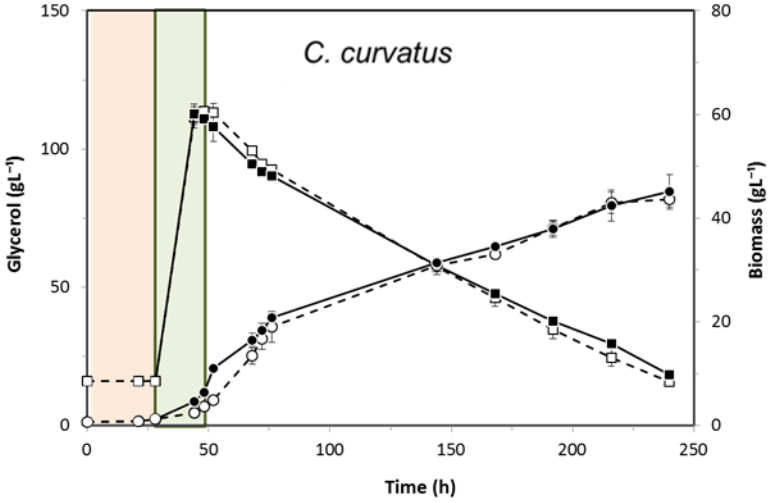

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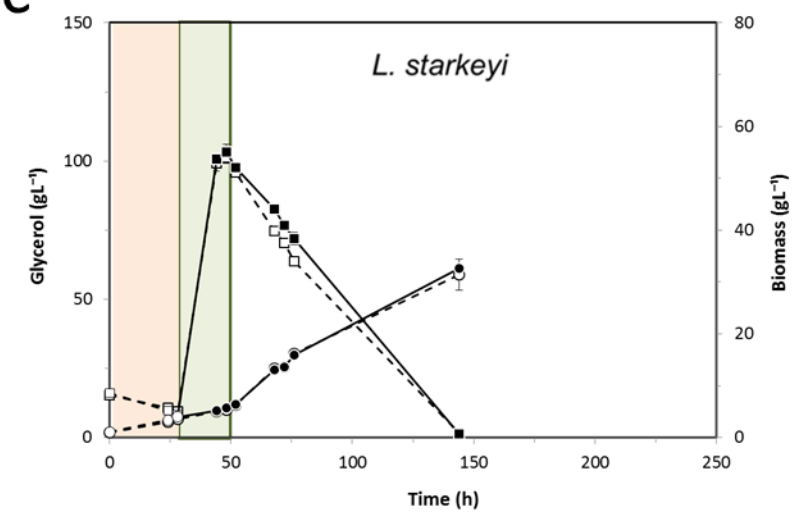
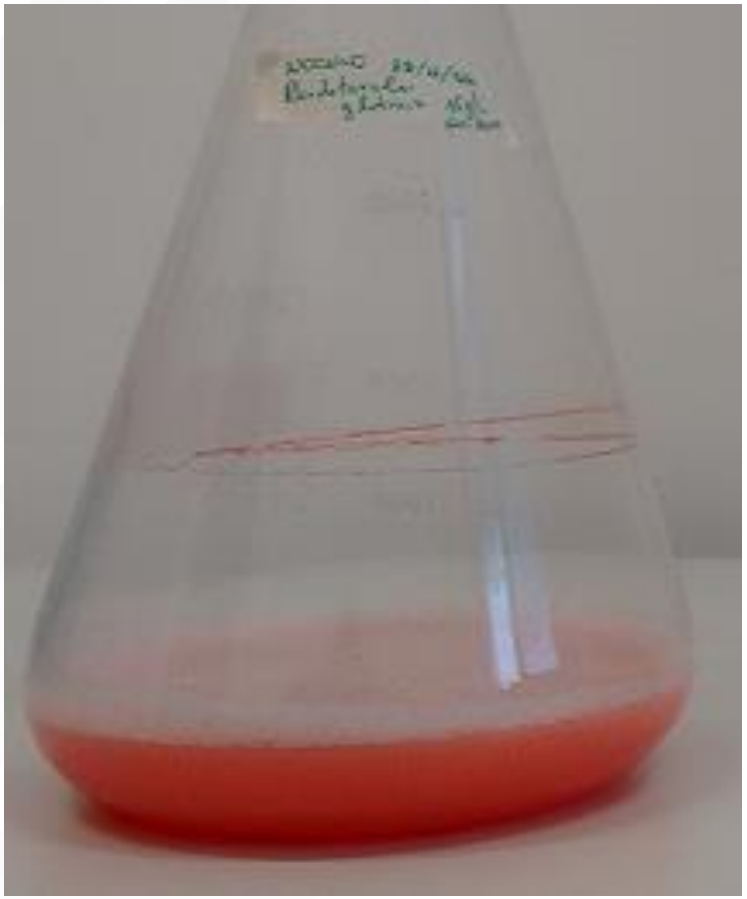


Signori et al MCF, 2016

Carotenoidi microbici da glicerolo grezzo

***R. toruloides* and *R. glutinis* dopo fermentazione fed-batch**



In valutazione:
composizione e quantità di carotenoidi, aminogramma

Valorizzazione di glicerolo grezzo in DHA con microalghe (*Schizochitrium limacinum*)

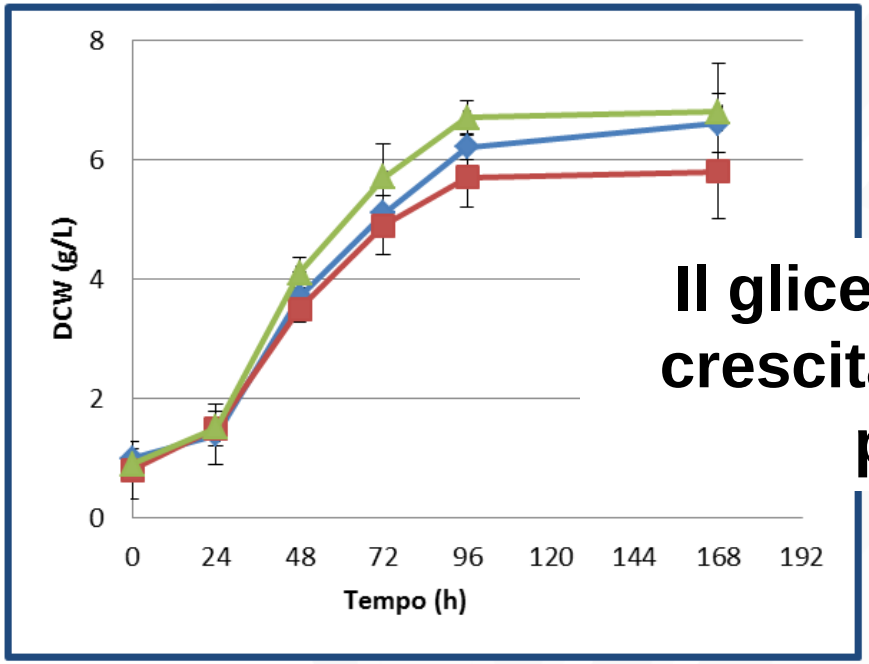
Chiara Pesciaroli

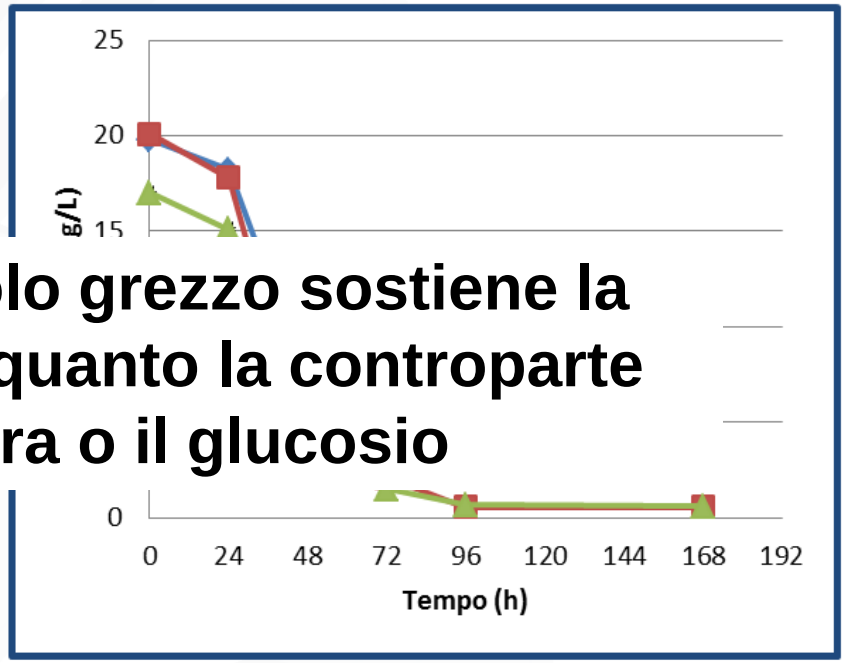
Arianna Salvetti

UNIVERSITÀ DEGLI STUDI DI MILANO BICOCCA

S. limacinum e produzione di DHA

Crescita e consumo di substrato





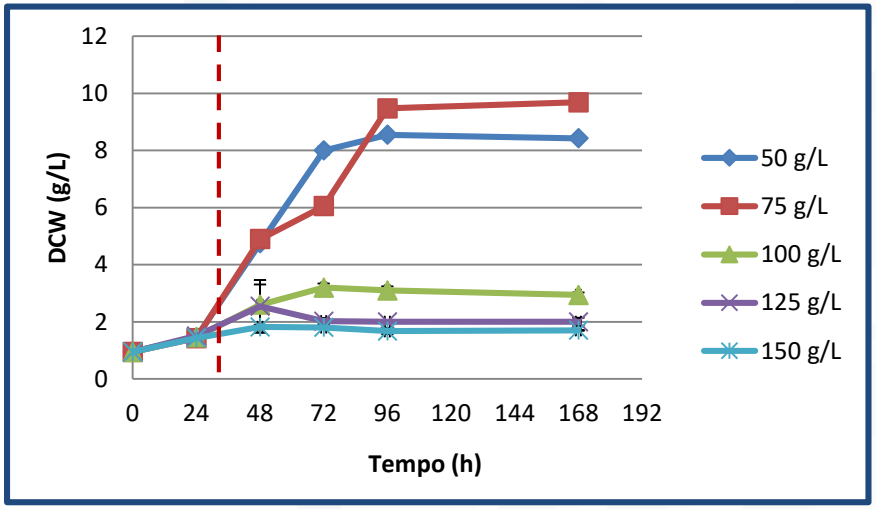
Il glicerolo grezzo sostiene la crescita quanto la controparte pura o il glucosio

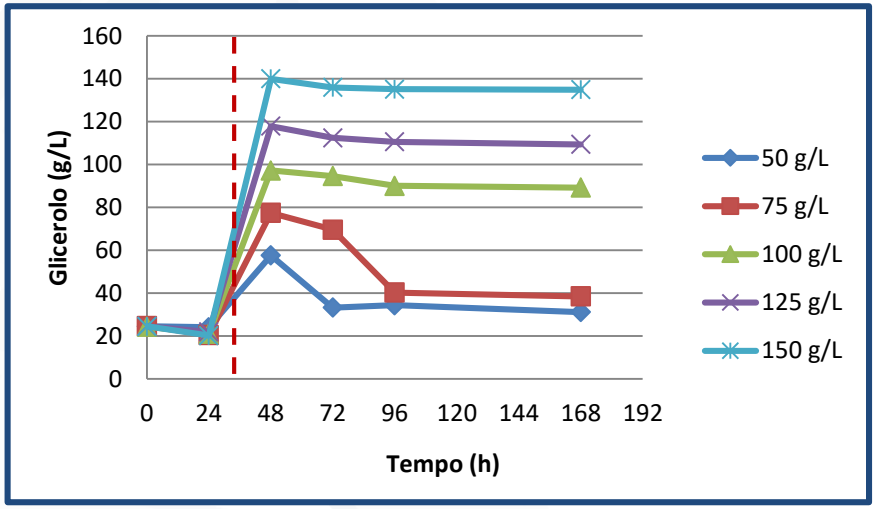
Parametri	Substrato: Glucosio	Glicerolo	Glicerolo grezzo
DCW max (g/L)	6,6 ± 0,49[a]	5,8 ± 0,78[b]	6,8 ± 0,80[a]
Biomass yield (g/g)	0,38 ± 0,02[a]	0,29 ± 0,04[b]	0,41 ± 0,02[a]

Differenze tra a e b sono significative ($p < 0,050$)

S. limacinum e produzione di DHA

Tolleranza al glicerolo grezzo

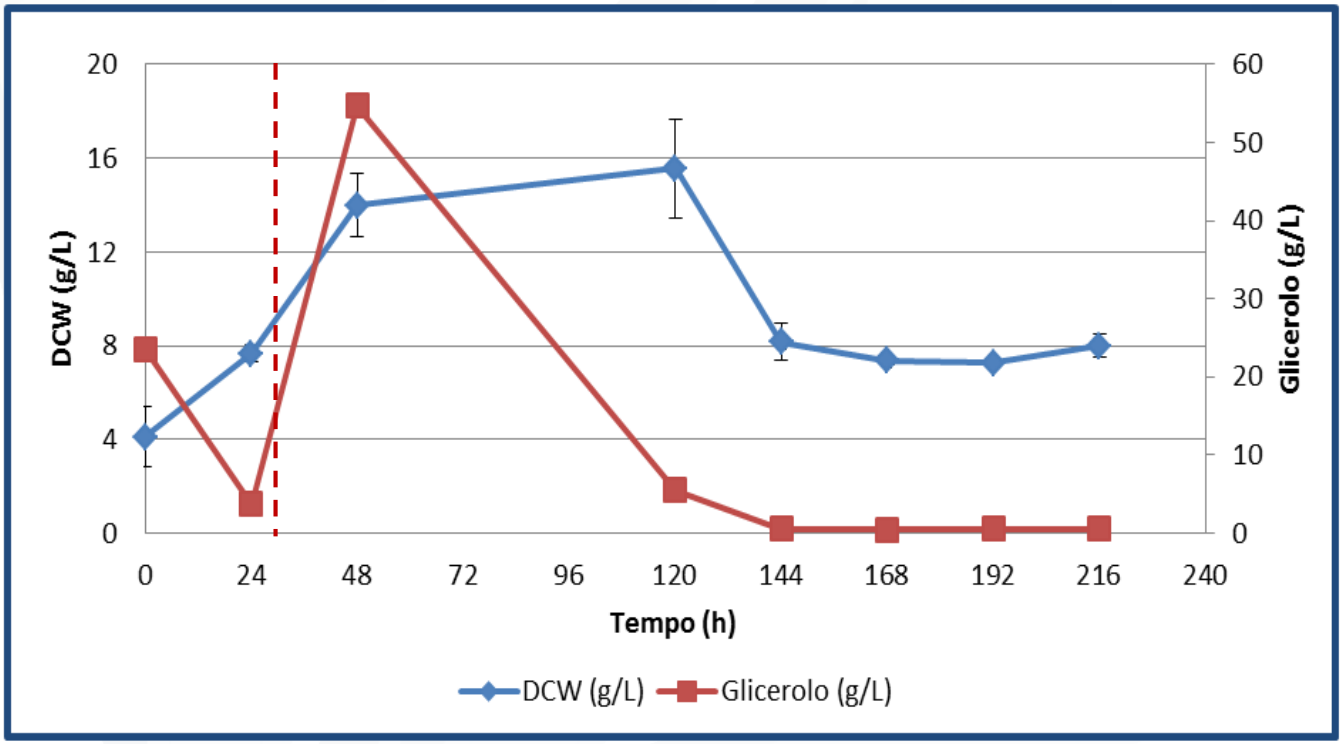




Concentrazioni superiori a 75-80 g/L di glicerolo grezzo inibiscono la crescita

S. limacinum e produzione di DHA

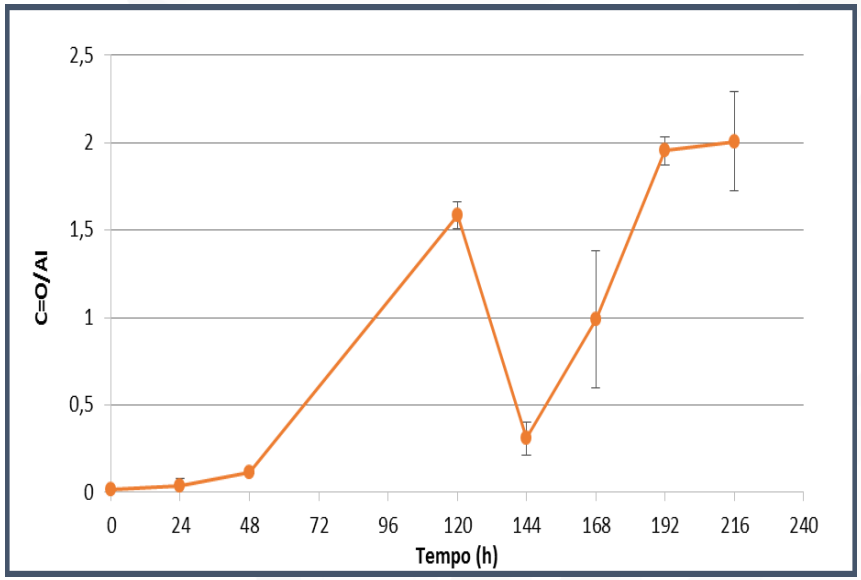
Produzione in bioreattore

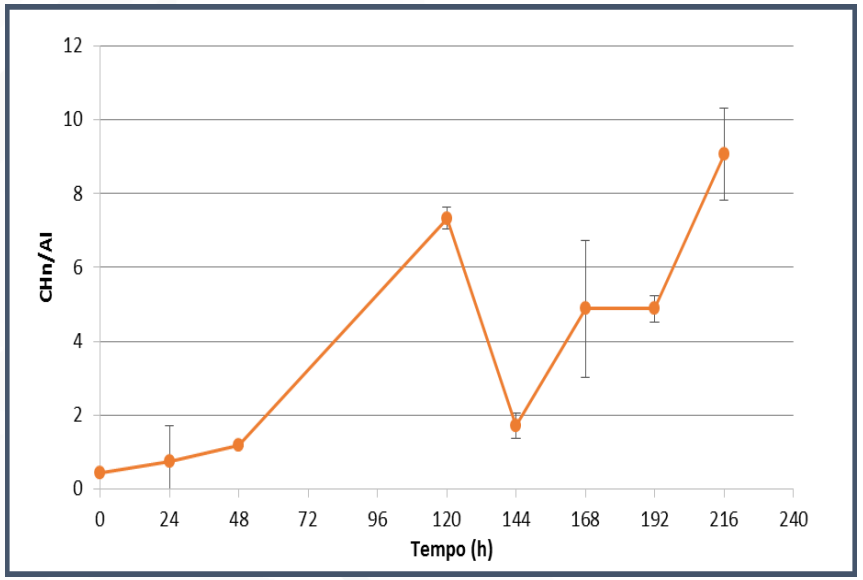


S. limacinum e produzione di DHA

Analisi quali-quantitative

FTIR





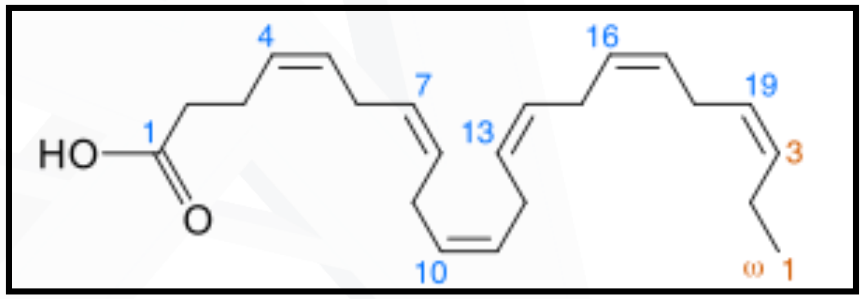
GC

DCW (g/L)	Biomass yield (g/g)	Lipid (g/L)	Lipid on biomass (g/g)	DHA (g/L)	DHA on biomass (g/g)
8 ± 1,09	0,11 ± 0,09	4,8 ± 0,51	0,6 ± 0,4	1,08 ± 0,28	0,13 ± 0,25

S. limacinum e produzione di DHA

% DHA = 22,5 %

degli oli totali



DCW (g/L)	Resa in biomassa (g/g)	Lipidi (g/L)	Frazione lipidica (g/g)	DHA (g/L)	Frazione DHA (g/g)
8 ± 1,09	0,11 ± 0,09	4,8 ± 0,51	0,6 ± 0,4	1,08 ± 0,28	0,13 ± 0,25

Microalgae and Yeasts SUStainable fermentation for HIgh quality fish feed formulation - MYSUSHI

Innovative Biotech Process

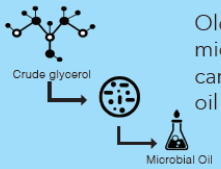
Sustainability

Biomass waste

Crude glycerol can be used as substrate for the biotransformation process.

→ Crude glycerol is a waste byproduct of biodiesel synthesis, derived from renewable vegetal biomasses.

Microorganisms



Oleaginous yeasts and heterotrophic microalgae have the ability to utilize glycerol as carbon source for the production of microbial oil (MO).

→ Microorganism as cell factories: their metabolism is naturally able to convert renewable feedstocks into bulk chemicals.

Nutraceuticals

Production of the key lipid ingredients for the innovative MO: n-3 Long Chain poli-unsaturated Fatty Acids (LCFAs) and carotenoids.

→ The microbial conversion of crude glycerol into value added compounds represents anenvironmental friendly alternative strategy.

Fish Feed

The Microbial Oil (MO) is expected to efficiently substitute Fish Oil (FO) in the novel fish feed, maintaining fish performances, health and quality

→ The environmental sustainability of aquaculture will be improved, preserving fish health and productivity

MySushi, shares part of the aims with another project named "**4F: Fine Feed For Fish**" (founded by AGER), especially concerning the definition of new feed formulations that are sustainable in terms of water and environmental impact. An innovative approach based on smart analytical detection systems is used to calculate a water quality index and the Water Footprint for the developed feeds.

THE PROJECT ALSO PRESENTS A RESPONSIBLE RESEARCH INNOVATION (RRI) AIM: TO TRANSFER THE KEY ENABLING TECHNOLOGIES TO AQUACULTURE BUSINESS AND TO SPREAD SCIENTIFIC CONTENTS OF THE PROJECT TO THE PUBLIC AUDIENCE.

The IndBioTech Lab

Paola Branduardi, Danilo Porro

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Chiara Pesciaroli*, Marco Brambilla, Stefano Bertacchi, Raffaella Di Lorenzo*
Fellowship: ***Arianna Salvetti****, Ilaria Vitali, Mattia Torchio*
Master Students: Luca Spezzati, Letizia Maestroni

Collaborazioni e Finanziamenti

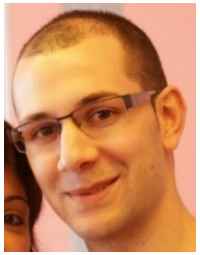
ACADEMIC COLLABORATIONS

CHALMERS · VTT · BOKU · UCD DUBLIN · UCC Coláiste na hOllscoile Corcaigh, Éire University College Cork, Ireland · GOETHE UNIVERSITÄT FRANKFURT AM MAIN · LUND UNIVERSITY · UNIVERSIDADE DO MINHO

Genciana Terova,
Marco Saroglia

UNIVERSITAS STUDIORUM INSUBRIAE

PAST MEMBERS OF THE GROUP



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Riccardo Posteri

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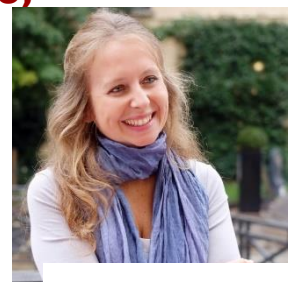
Ausilia Campanaro,
Chiara Magoni

MYSUSHI



Diletta Ami

FUNDING

HORIZON 2020 · MARIE CURIE ACTIONS · fondazione cariplo · M&G · CHEMTEX · BETA RENEWABLES · eni · stahl · NOVAMONT

PUBLIC ENGAGEMENT:
Dissemination and Outreach

ECO-ZINDER s.r.l.

Eridania

Lipidogramma *S. limacinum*

Acidi Grassi	Quantità (%)
Ac. Laurico (12:0)	3,33
Ac. Miristico (14:0)	6,78
Ac. Pentadecanoico (15:0)	4,3
Ac. Palmitico (16:0)	56,35
Ac. Eptadecanoico (17:0)	1,11
Ac. Eptadecenoico (17:1)	0,13
Ac. Stearico (18:0)	1,24
Ac. Oleico (18:1-c, n-9)	2,54
Ac. Linoleico (18:2-c n-6)	0,59
Ac. Arachico (20:0)	0,13
Ac. Eicosapentaenoico (20:5 n-3)	0,38
Ac. Docosaesaenoico (22:6 n-3)	22,82
Ac. Grassi Monoinsaturi	2,67
Ac. Grassi Polinsaturi	23,8
Ac. Grassi Saturi	73,2

Lipidogramma lieviti oleaginosi

Strain	Carbon source	Fatty acids composition (%, wt/wt)										
		C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	Others	S	M	P
R. toruloides	Pure Glycerol	1.5 ± 0.1	27.9 ± 0.1	2.1 ± 0.1	12.3 ± 0.1	35.2 ± 0.1	17.4 ± 0.2	2.8 ± 0.1	0.7 ± 0.1	42.4 ± 0.2	37.3 ± 0.1	20.2 ± 0.2
	Crude Glycerol	1.5 ± 0.1	27.5 ± 0.1	2.0 ± 0.3	12.5 ± 0.2	37.8 ± 0.1	15.8 ± 0.2	2.2 ± 0.1	0.7 ± 0.1	42.2 ± 0.1	39.8 ± 0.4	18.0 ± 0.3
C. curvatus	Pure Glycerol	1.6 ± 0.3	29.6 ± 0.1	3.7 ± 0.3	18.6 ± 0.1	27.6 ± 0.2	14.9 ± 0.1	1.9 ± 0.2	2.2 ± 0.6	51.9 ± 0.1	31.3 ± 0.1	16.8 ± 0.2
	Crude Glycerol	1.6 ± 0.3	30.6 ± 0.1	4.0 ± 0.3	15.4 ± 0.1	31.2 ± 0.1	14.5 ± 0.1	1.6 ± 0.1	1.0 ± 0.1	48.7 ± 0.4	35.2 ± 0.4	16.1 ± 0.1
L. starkeyi	Pure Glycerol	3.1 ± 0.3	31.0 ± 0.1	4.3 ± 0.1	12.9 ± 0.1	39.4 ± 0.3	7.6 ± 0.1	0.5 ± 0.1	1.2 ± 0.3	48.2 ± 0.4	43.8 ± 0.3	8.1 ± 0.1
	Crude Glycerol	3.0 ± 0.2	32.2 ± 0.1	4.2 ± 0.1	10.9 ± 0.1	40.9 ± 0.1	7.2 ± 0.1	0.6 ± 0.1	1.0 ± 0.2	47.1 ± 0.1	45.1 ± 0.1	7.8 ± 0.2

S, saturated; M, monounsaturated; P, polyunsaturated

Microalgae as cell factory for omega3 production