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Evaluating the lipid and carotenoid production capability of novel *Rhodotorula* strains cultivated in crude biodiesel-derived glycerol.

Summer Camp | July 7 – 11, 2025 | Marseille, France



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Agri-food Waste Management for Sustainable bio-economy through Higher Education curricula and upskilling

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PORTUGAL EDUCAÇÃO E FORMAÇÃO

Goal

AGRIMA aims to foster universities' **capacity building** for the **green transition** through **innovative practices** and **higher education curricula updating** in **agri-food waste**

AGRIMA addresses the: **circular bioeconomy**.

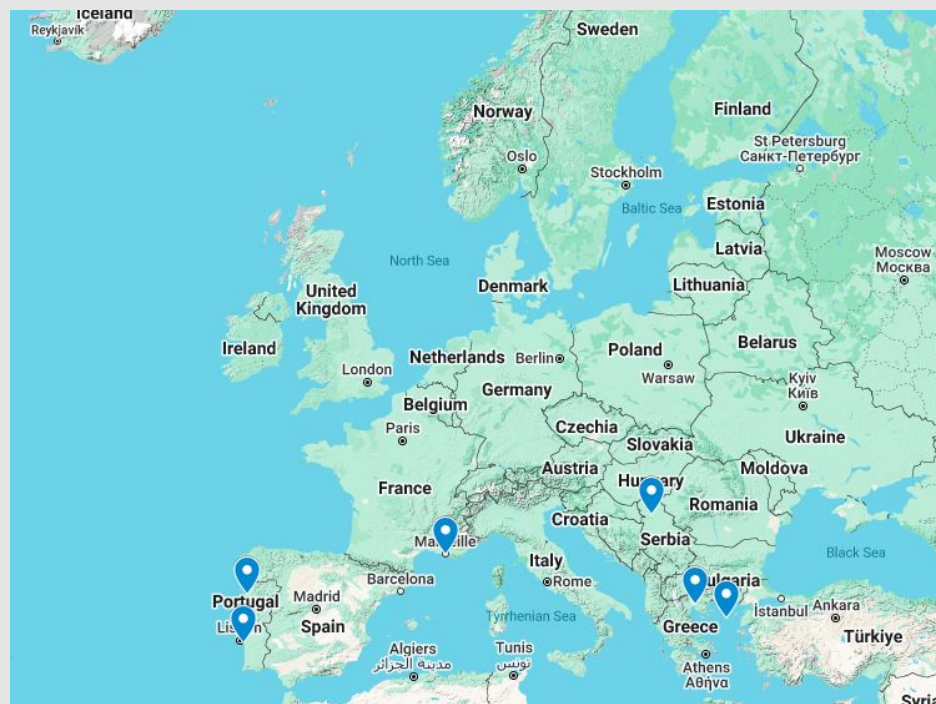
1. **Advancing pedagogical methods** for industrial agri-food waste valorisation **based on business-academia synergies**.
2. **Integrating citizen science** in bio-economy-enhanced waste valorisation as a means of **civic engagement and environmental advocacy**.



Partners



AGRIMA



P. PORTO



Web2Learn
Open, social learning



What is crude glycerol?



Biodiesel Production



Soap Manufacturing Industry



Fatty Acid Industry



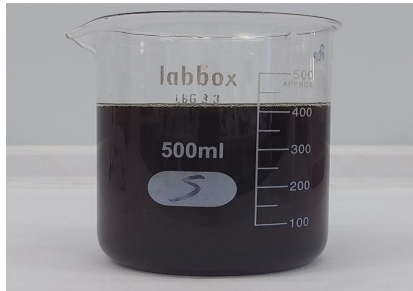
Fatty Ester Industry

Biodiesel Production

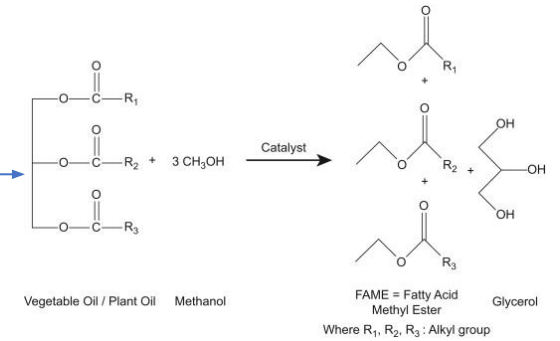
Feedstock (vegetable oils, animal fats, fatty acid material)



Crude Glycerol



Transesterification



Biodiesel



Purification

Biodiesel Production Feedstock

Food-Feed vs Biofuel

01

1st
Generation

Edible feedstock (vegetable oils–animal fats)

02

2nd
Generation

Non-edible feedstock (raw materials– non-edible seeds)

03

3rd
Generation

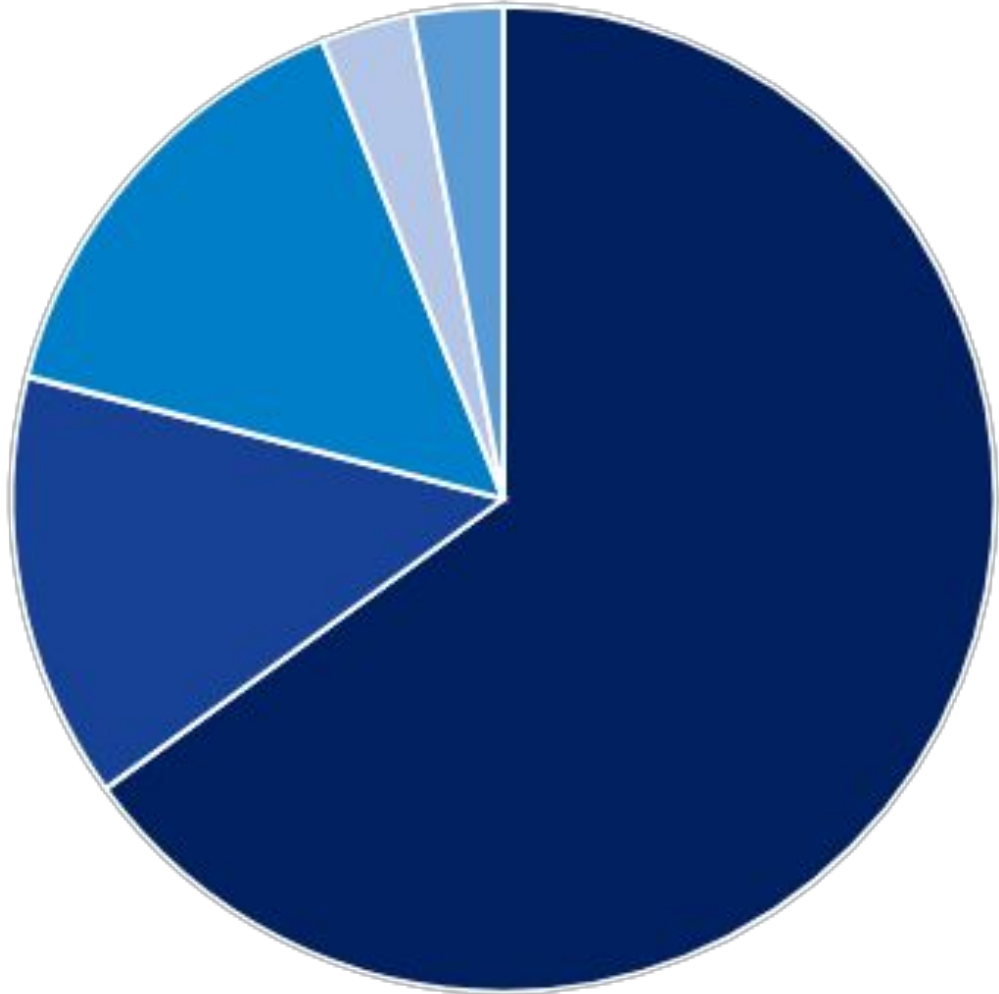
Waste cooking oil, macro–microalgae

04

4th
Generation

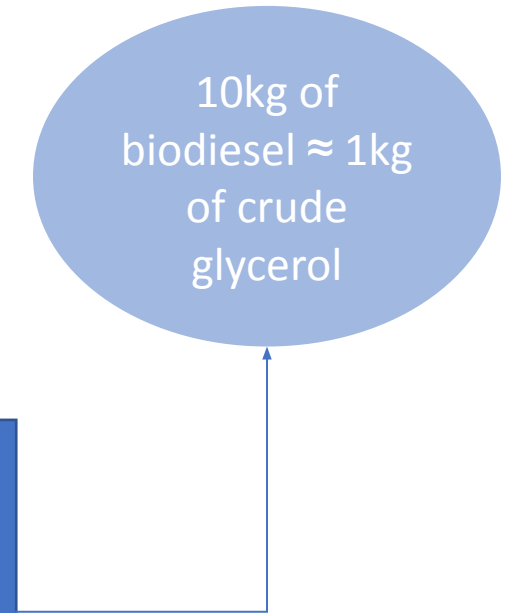
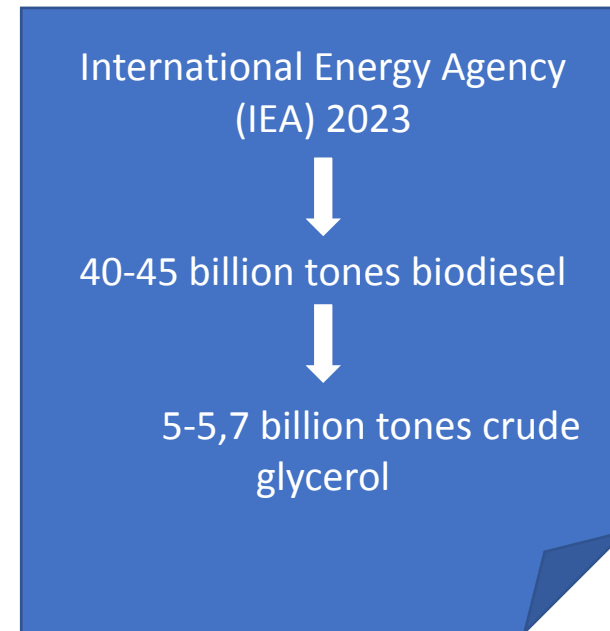
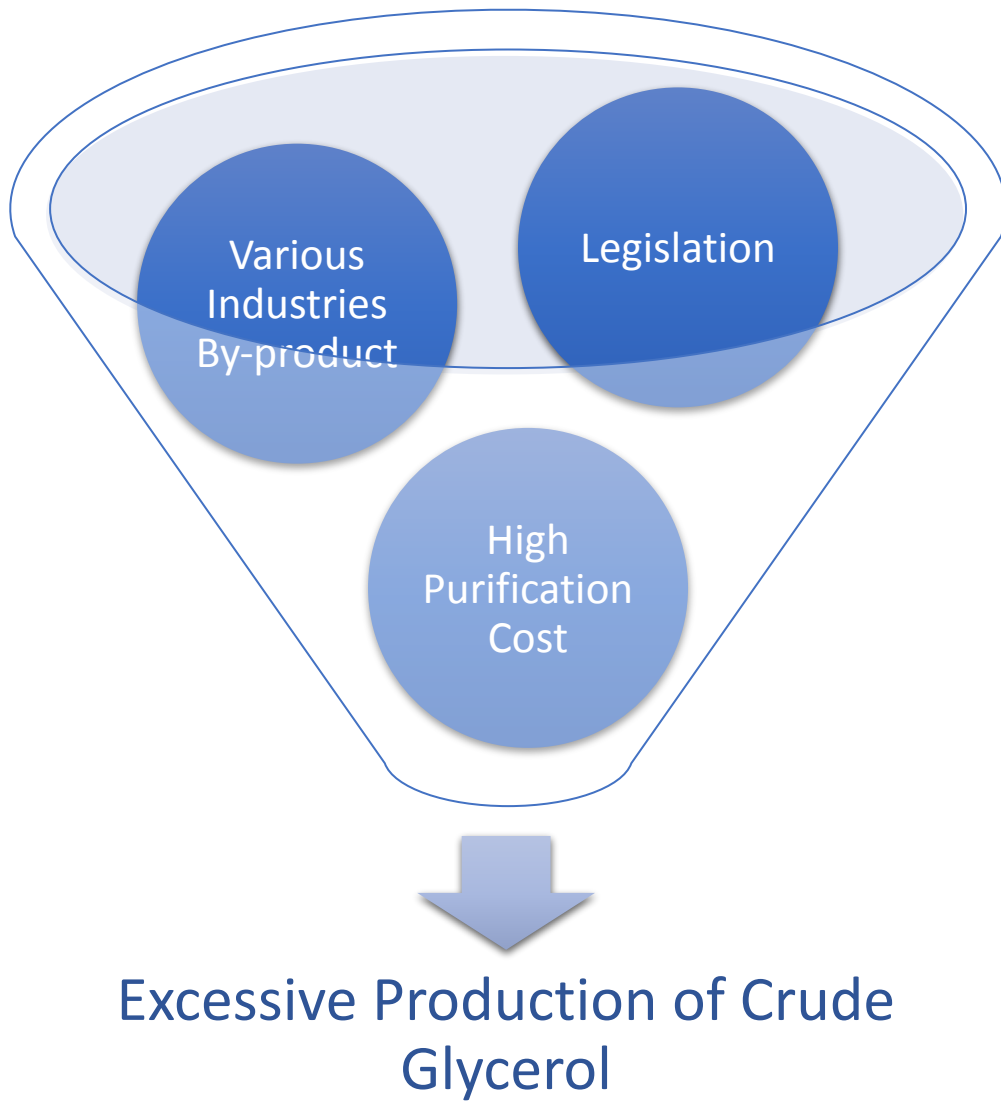
Microbial oil, greener technologies, biotechnology

Crude Glycerol Composition



■ Glycerol
 ■ Methanol/Ethanol residues
 ■ Free fatty Acids
 ■ Water
 ■ Inorganic soaps-micronutrients

Component	Percentage
Glycerol	40-75%
Methanol or Ethanol residues	10-14%
Free fatty acids	10-15%
Water	2-3%
Inorganic matter	2-3%



Oleaginous Microorganisms

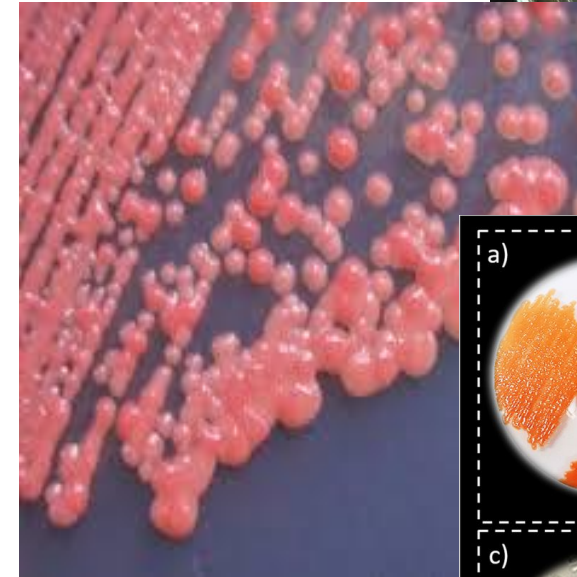


Oleaginous Yeasts

- Consume a wide range of carbon and nitrogen source
- Characteristic orange/reddish/rose color of colonies
- Ability to accumulate up to 20% (DCW) intracellular lipids (triacylglycerols) and other secondary metabolites
- Produce aromatic compounds
- Rapid growth rates
- Available knowledge about their genetic modifications

Rhodotorula spp

- ❑ More than 165 species
- ❑ Isolated from diverse sources, including extreme environments
- ❑ Typically spherical, ovoid or elongated shape
- ❑ Grow as individual cells or in clusters
- ❑ Sexual or asexual reproduction depends on the strain
- ❑ Classified as mesophilic, optimum temp. 25-28°C
- ❑ Non-GRAS, some species = opportunistic pathogens
- ❑ Consume a wide range of carbon and nitrogen source including agro-industrial and food industries waste
- ❑ Ability to synthesize simultaneously multiple products:
 - ✓ Intracellular microbial lipid (SCO)
 - ✓ Carotenoids (β -carotene, torulene, torularhodin)
 - ✓ Enzymes
 - ✓ Exopolysaccharides
 - ✓ Organic acids

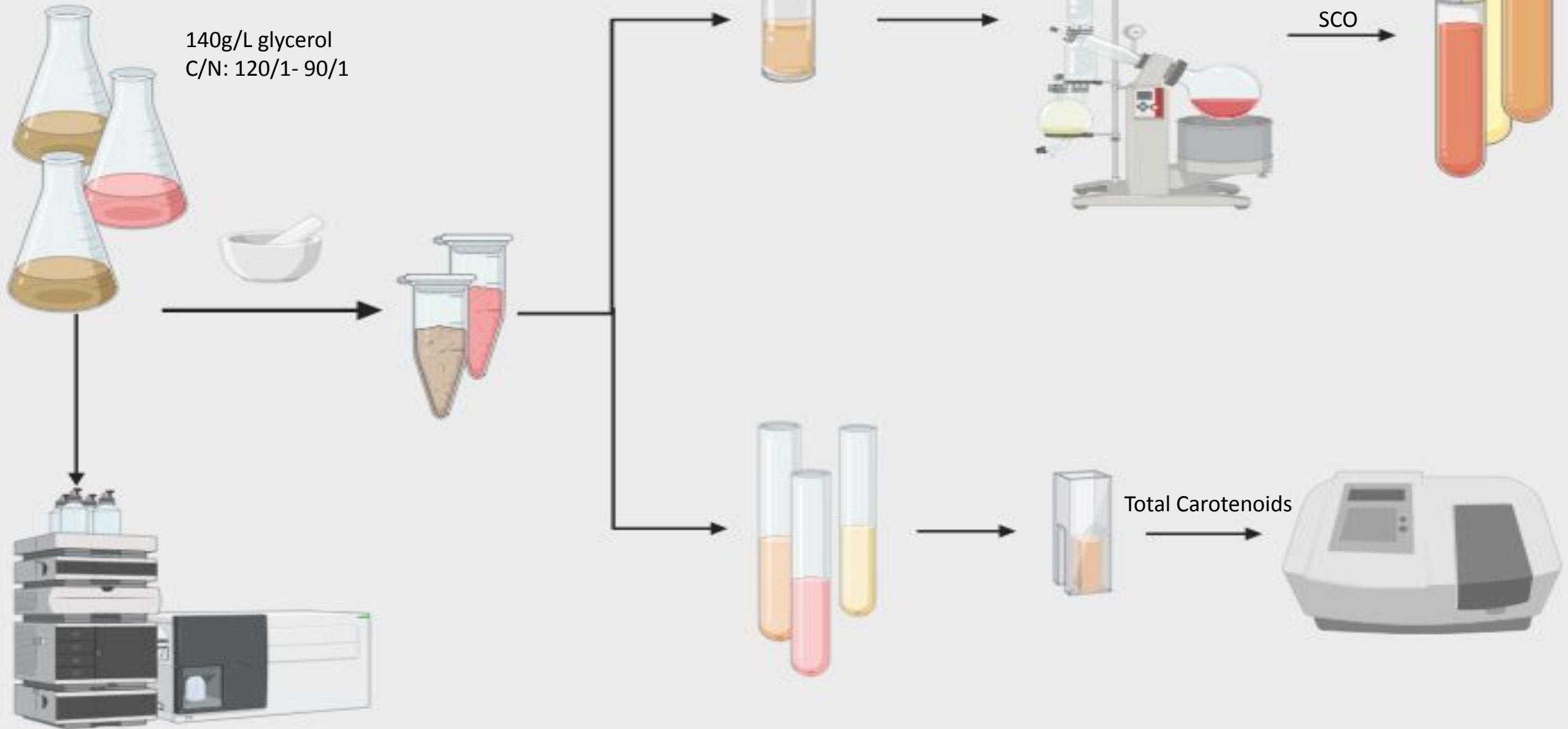


Study Objective

Investigate the potential of four novel *Rhodotorula* yeast (*R.diobovata*, *R.kratochvilovae*, and two strains of *R.mucilaginosa*) to grow under extreme cultivation conditions (high glycerol concentration, low nitrogen, high C/N ratio, low pH (without adjustment), raw crude glycerol) for the biosynthesis of intracellular lipids and carotenoids, aiming at a more sustainable and industry-compatible approach for the valorization of crude biodiesel-derived glycerol.



METHODOLOGY

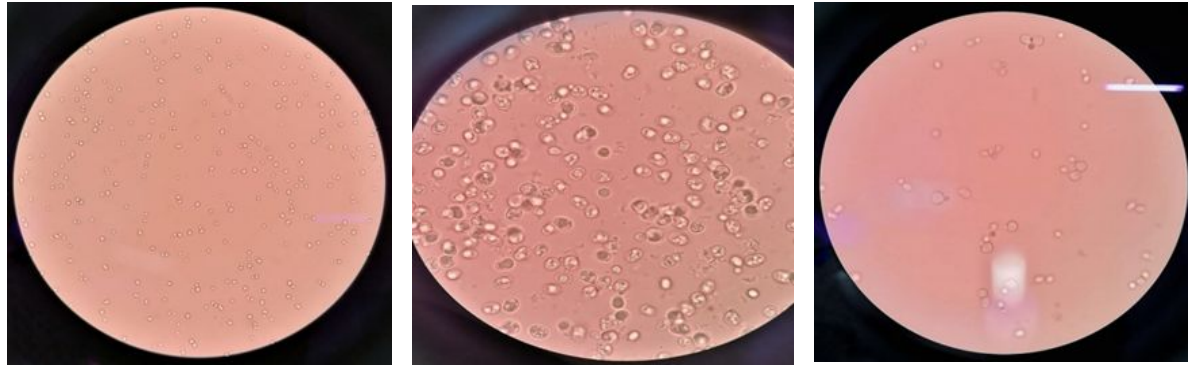


Maximum prices of Biomass, Lipids and Carotenoids per strain and substrate

Strain	Time culture (h)	X(g/L)	L (g/L)	Y _{L/X} (% wt/wt)	Total Carotenoids (µg/g)
<i>R. diobovata</i> EXF-6843	456 ^{a,b}	29.740	13.097	44.105	N.A.
	288 ^c	25.614	12.146	51.998	63.778
	384 ^d	28.334	13.038	45.422	468.933
<i>R.kratochvilovae</i> EXF-3471	240 ^a	19.196	N.A.	N.A.	473.325
	384 ^b	17.214	5.460	31.718	N.A.
	120 ^{c,d}	9.636	4.383	45.486	531.246
<i>R.mucilaginosa</i> EXF-8984	384 ^a	15.046	3.577	23.777	98.533
	408 ^{b,c}	14.130	3.746	26.512	149.366
	24 ^d	1.189	0.227	18.576	931.988
<i>R.mucilaginosa</i> EXF-9791	336 ^a	15.698	1.881	11.979	167.975
	456 ^{b,c}	10.986	2.343	21.323	275.385
	192 ^d	9.790	1.007	10.281	711.977

Strain	Time culture (h)	Biomass (g/L)	Lipids (g/L)	Y _{L/X} (% wt/wt)	Total Carotenoids (µg/g)
Crude glycerol C/N:120/1 (S3)					
<i>R. diobovata</i> EXF-6843	336 ^a	4.444	0.721	16.210	534.420
	288 ^b	4.286	0.809	18.995	557.996
	432 ^c	3.629	0.749	20.868	485.647
	144 ^d	2.694	0.463	17.512	901.090
<i>R.kratochvilovae</i> EXF-3471	336 ^a	4.424	1.404	31.925	306.966
	144 ^{b,d}	0.300	1.936	82.130	914.553
	120 ^c	1.627	1.477	90.985	381.607
<i>R.mucilaginosa</i> EXF-8984	480 ^a	3.884	0.409	10.506	220.747
	120 ^{b,c}	1.582	0.925	58.438	201.891
	96 ^d	1.454	0.546	37.709	446.153
<i>R.mucilaginosa</i> EXF-9791	480 ^a	5.568	0.228	4.133	36.750
	120 ^{b,c}	2.905	1.275	43.498	411.701
	336 ^d	5.356	0.292	5.400	412.032
Crude glycerol C/N:90/1 (S2)					
<i>R. diobovata</i> EXF-6843	384 ^a	4.662	0.473	10.341	N.A.
	288 ^b	4.392	0.608	14.024	649.802
	96 ^{c,d}	3.121	0.550	16.512	838.512
<i>R.kratochvilovae</i> EXF-3471	288 ^a	9.876	1.469	14.887	391.201
	240 ^{b,d}	8.864	2.114	23.954	1041.161
	384 ^c	5.247	1.391	26.454	N.A.
<i>R.mucilaginosa</i> EXF-8984	480 ^a	3.458	0.326	10.100	N.A.
	336 ^{b,c}	2.790	0.560	16.364	55.076
	120 ^d	2.735	0.303	11.074	184.955
<i>R.mucilaginosa</i> EXF-9791	288 ^{a,b}	16.386	1.598	9.751	330.708
	48 ^c	3.532	0.745	21.103	555.000
	192 ^d	11.534	0.625	7.395	1214.813

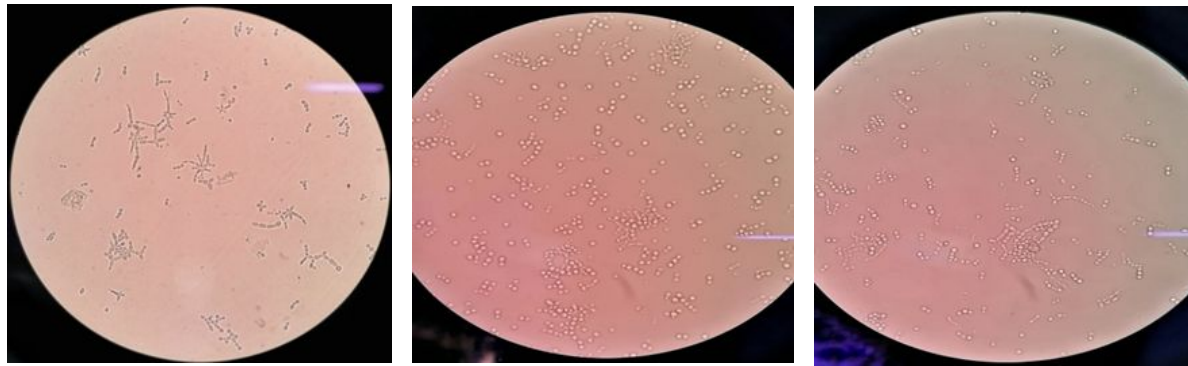
Microscopic Observation



R. diobovata: 96h and 432h of growth (pure-crude)



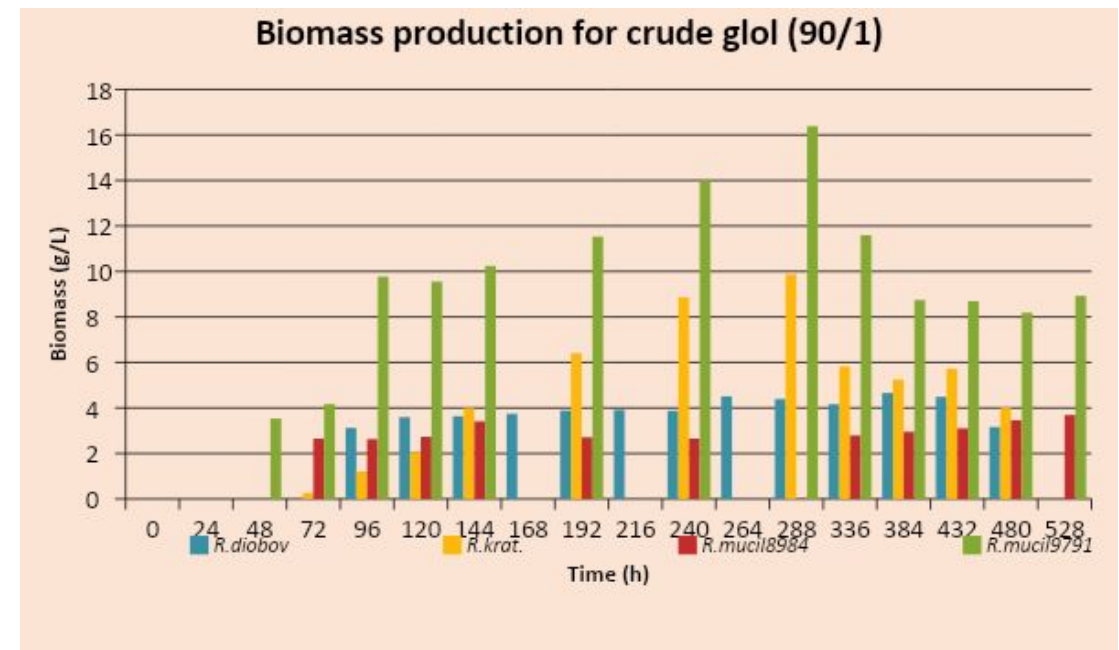
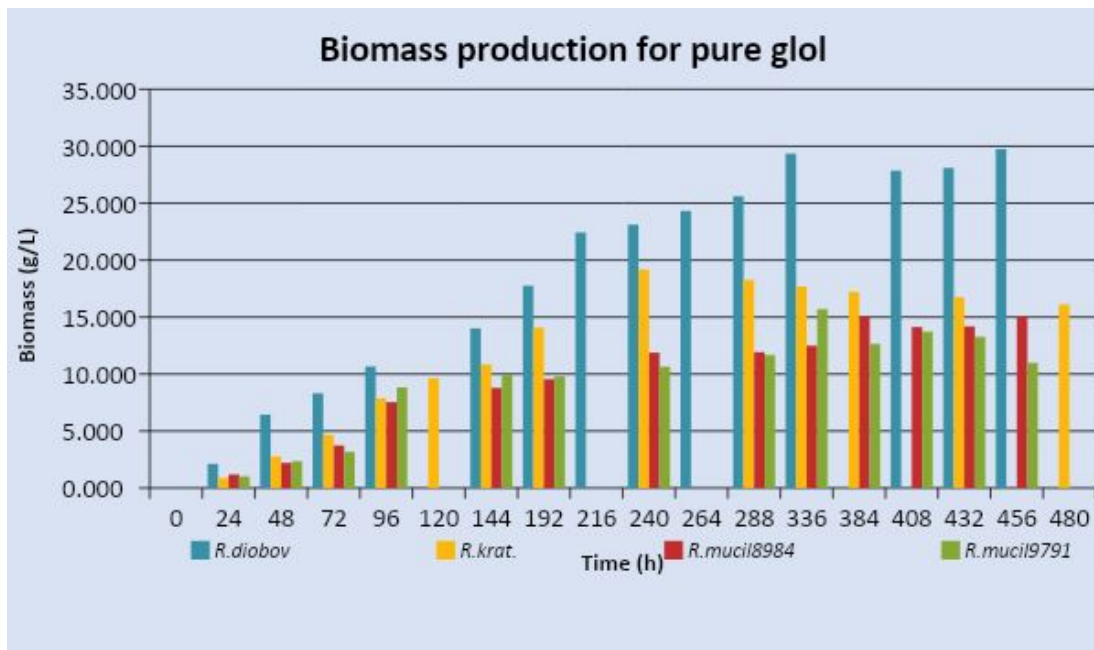
R. kratochvilovae: 96h and 288 (pure-crude)



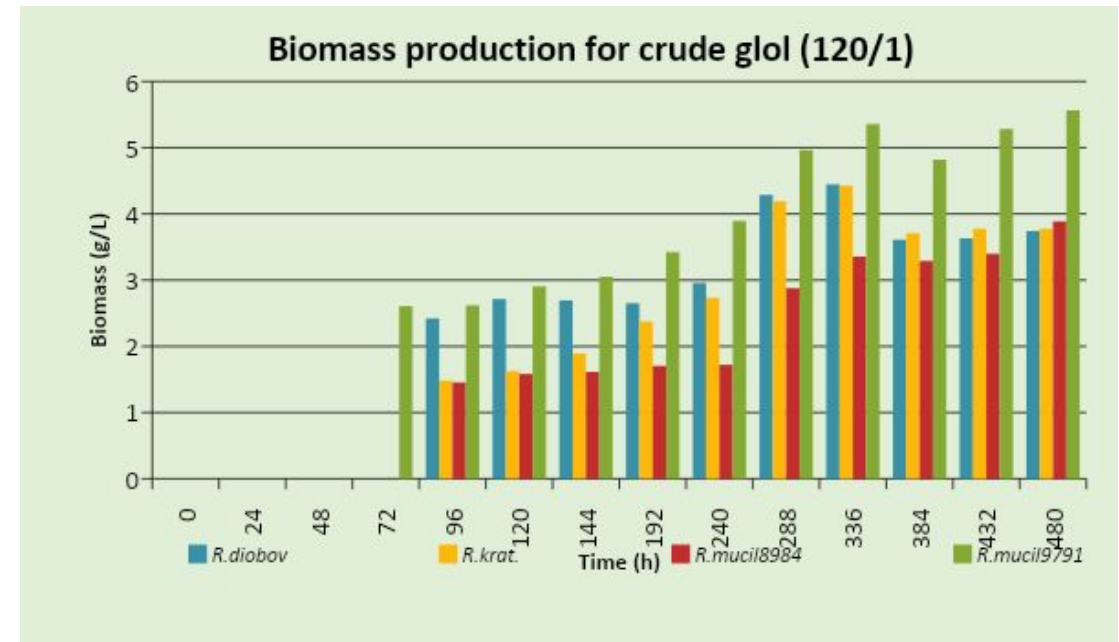
R. mucilaginosa 8984: 72h and 336h growth (pure-crude)



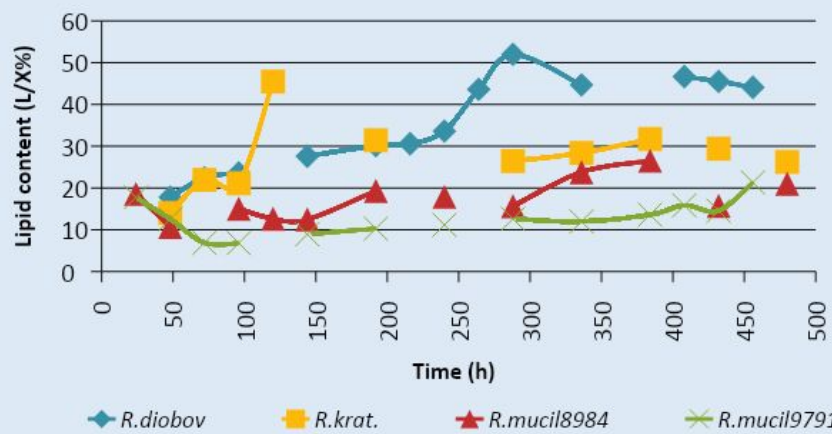
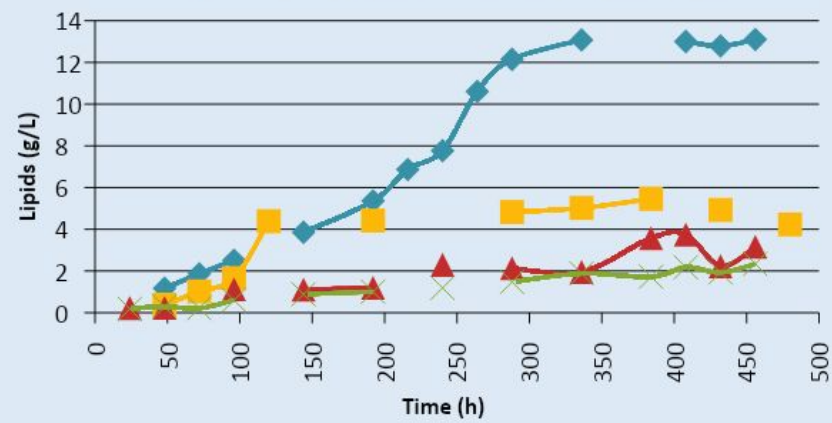
R. mucilaginosa 9791: 72h and 336h growth (pure-crude)



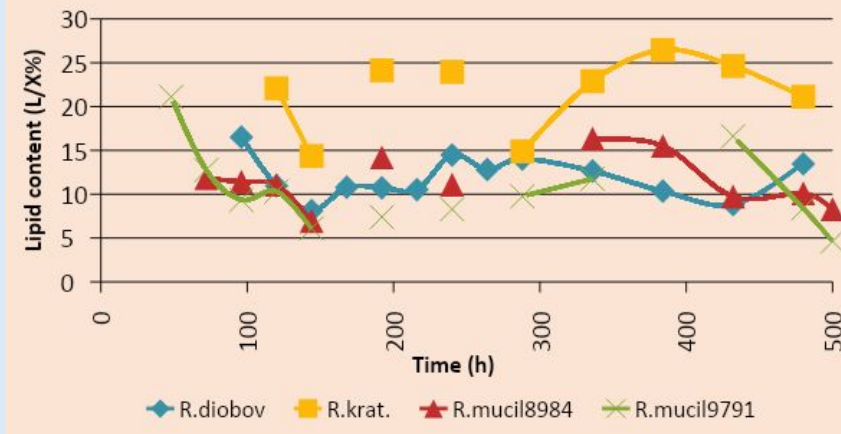
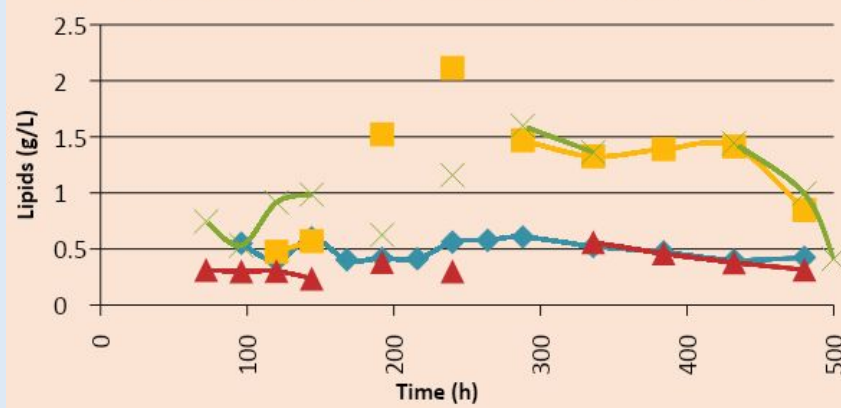
- *R. diobovata* achieved the higher biomass production in pure glycerol up to 29,740g/L.
- All strains achieve higher yield at pure glycerol substrate
- In pure glol the growth begins within the first 24h, while in crude it is delayed (possible due to the adaptation)
- *R. kratochvilovae* and *R. mucilaginoso* 9791 are more resistant in extreme environments, while *R. diobovata* is more predictable



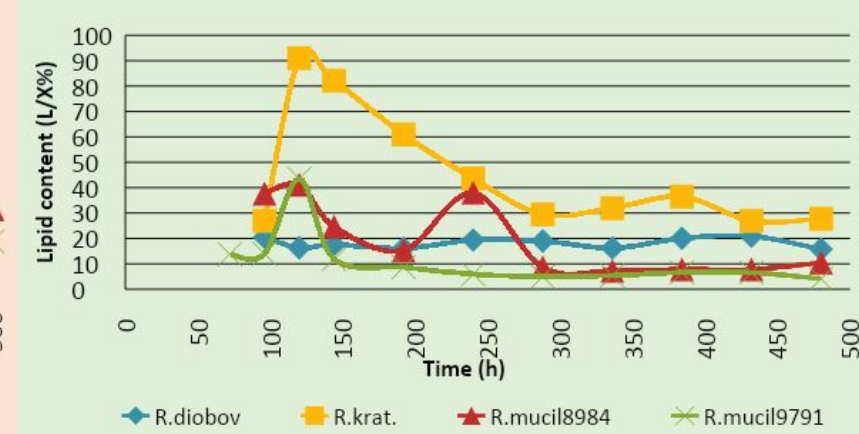
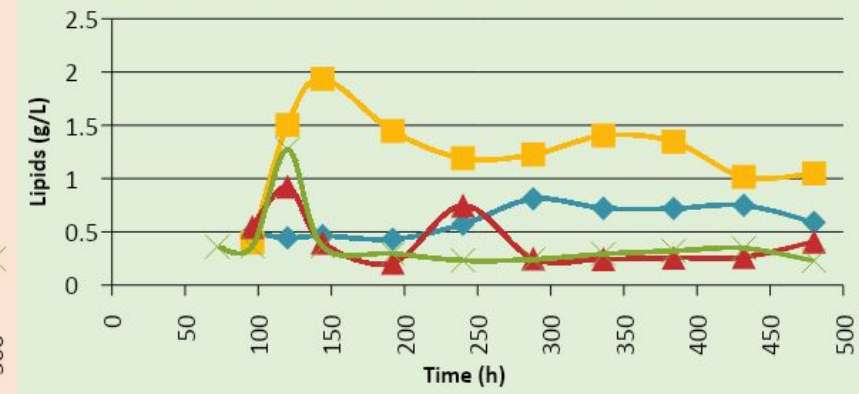
Lipids and Lipid content for pure glol



Lipids and Lipid content for crude glol (90/1)

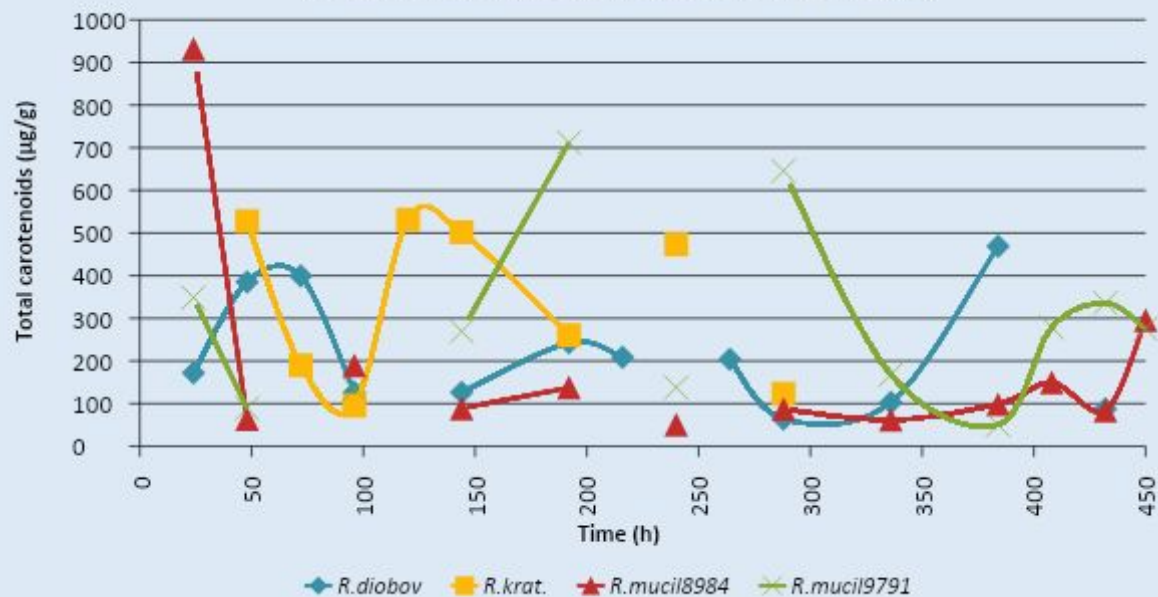


Lipids and Lipid content for crude glol (120/1)

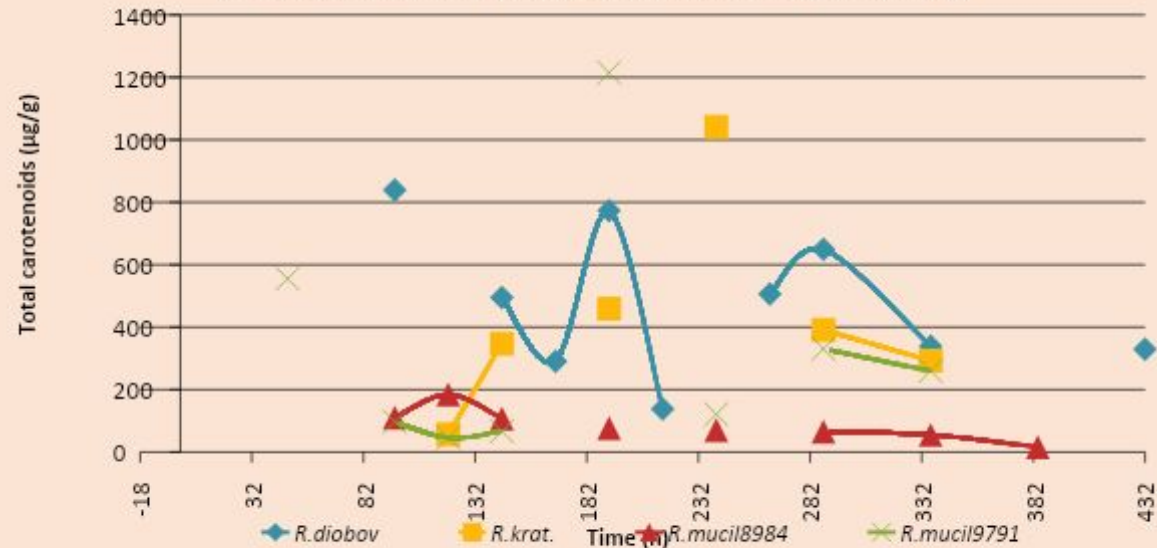


- Regarding pure glycerol, *R.diobovata* achieved a maximum lipid biosynthesis of 19g/L, with a highest lipid content of 52%. The other strains showed not only minimal lipid accumulation, but also low lipid content, except for *R.kratochvilovae*, which reached a lipid content of 45,5%.
- In the case of crude glycerol, all strains demonstrated low levels of lipid accumulation and lipid content, with *R.kratochvilovae* attaining the highest values under both C/N ratios.
- A remarkable lipid content of 90% was observed by *R.kratochvilovae* in crude glycerol under C/N ratio 120/1.

Total carotenoids production in pure glol



Total carotenoids production in crude glol (90/1)

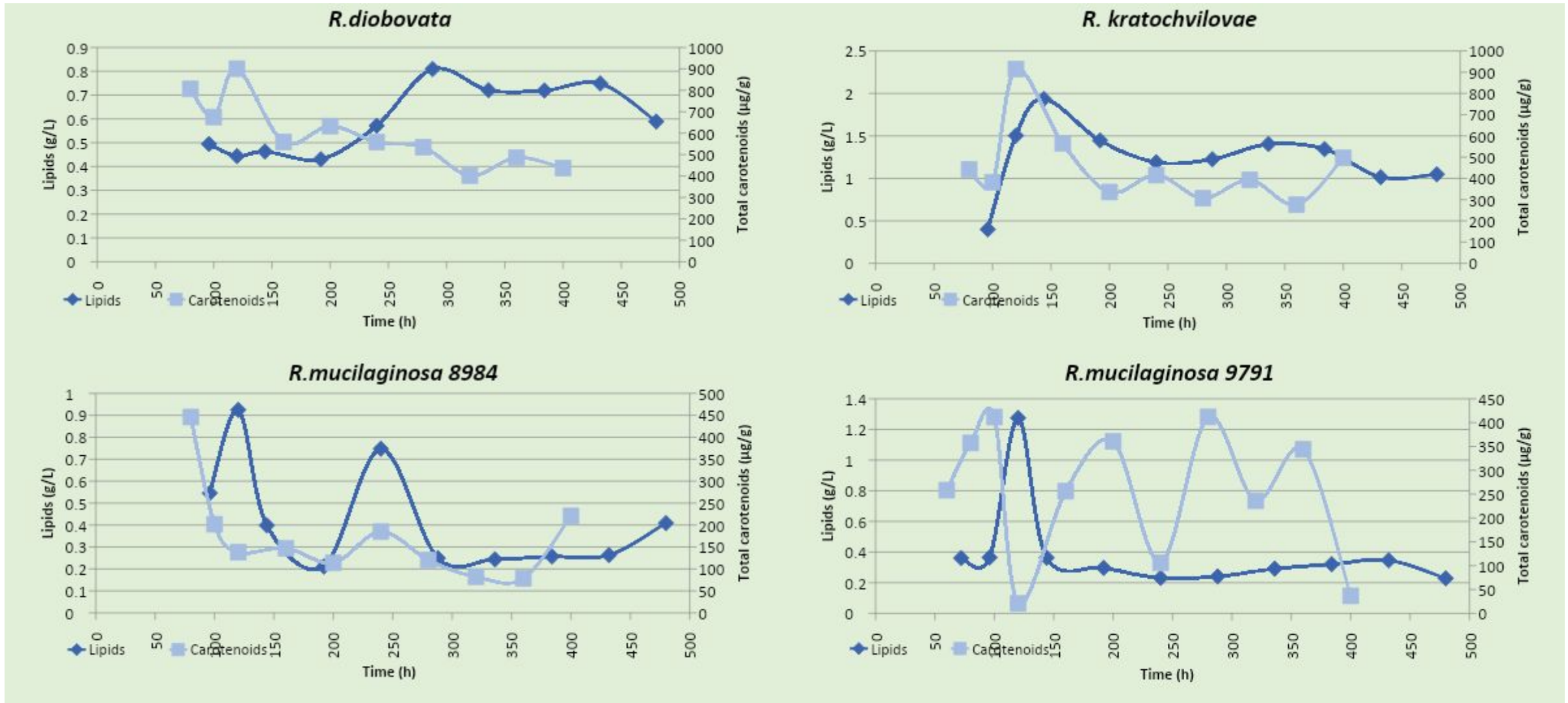


- Initially elevated carotenoid synthesis was observed in nearly all strains and across the three substrates, potentially reflecting a stress response mechanism activated by the strain to cope with multiple environmental stressors.
- With the exception of *R.mucilaginoso 8984*, all strains achieve their maximum carotenoid production when cultivated on crude glycerol.

Total carotenoid production in crude glol (120/1)



Correlation of lipids and carotenoids pathways ?



Conclusions

- ✓ Strains belonging to the same genus may exhibit significant phenotypic and genotypic variability.
- ✓ Strain efficiency is influenced by environmental factors, such as nutrient availability and pH.
- ✓ All strains are highly resilient as they grow under extreme nutrient limitations and pH stress conditions.
- ✓ Each strain exhibits a distinct carotenoid biosynthesis profile, which is likely influenced by its requirements.
- ✓ It is appeared to be no consistent correlation between lipids and carotenoids biosynthesis, but the metabolic pathways activated may be influenced by the microorganism's demands and the environmental conditions it encounters.
- ✓ This study presents a prospective biorefinery model, drive by the combination of high waste utilization, low processing costs and high product yields.
- ✓ Further investigation is necessary to optimize the cultivation conditions and to evaluate the process at large scale, such as in a bioreactor system.





THANK YOU!