

Development of microbial Alginate and oil Productions for Bio-based Binders

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Introduction The PERFECOAT project focuses on developing sustainable bio-based binders through the production and utilization of biopolymers as key ingredients for binder formulation. Vegetable oil-based polymers to replace fossil-based plasticisers and binders in coatings and other materials are gaining increased attention. However, vegetable oil production depends on the climate and competes with food and feed production in terms of the use of arable land. Microbial oil produced by oleaginous yeast have a very similar composition to vegetable oil and can therefore be easily replaced with added value of climate-independent and more stable composition. The work presented here emphasizes on microbial alginate and oil and their potential applications as bio-based coating ingredients. The aim of this activity is to establish a scalable process for microbial alginate and oil production of defined characteristics to meet the demands of industrial-scale applications.

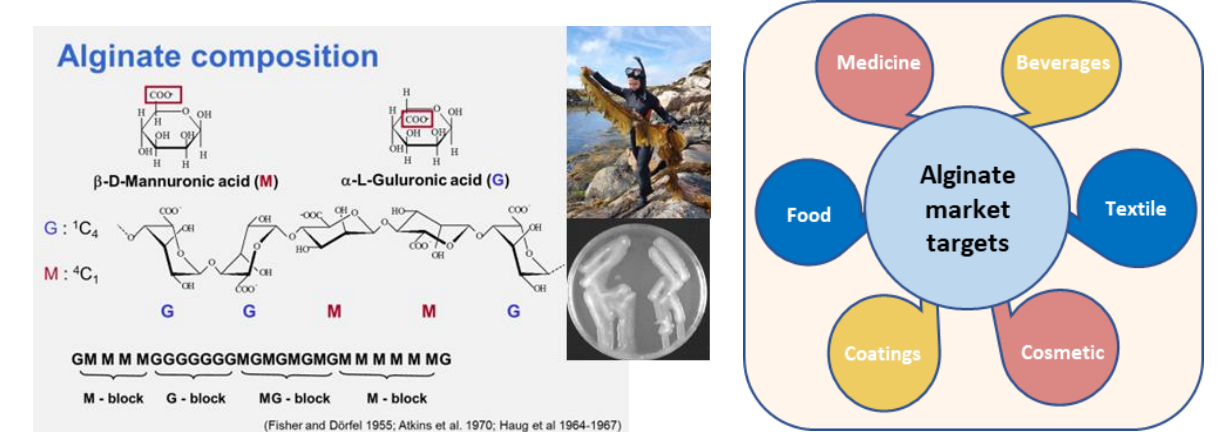


Figure 1. Microbial alginate is typically composed of repeating units of β -D-mannuronic acid (M) and α -L-guluronic acid (G), similar to algal alginate. However, the ratio of M to G residues and the overall molecular weight of microbial alginate can differ based on the specific bacterial strain and growth conditions. The market for alginate is relatively broad, encompassing various industries and applications due to its versatile properties.

Methods

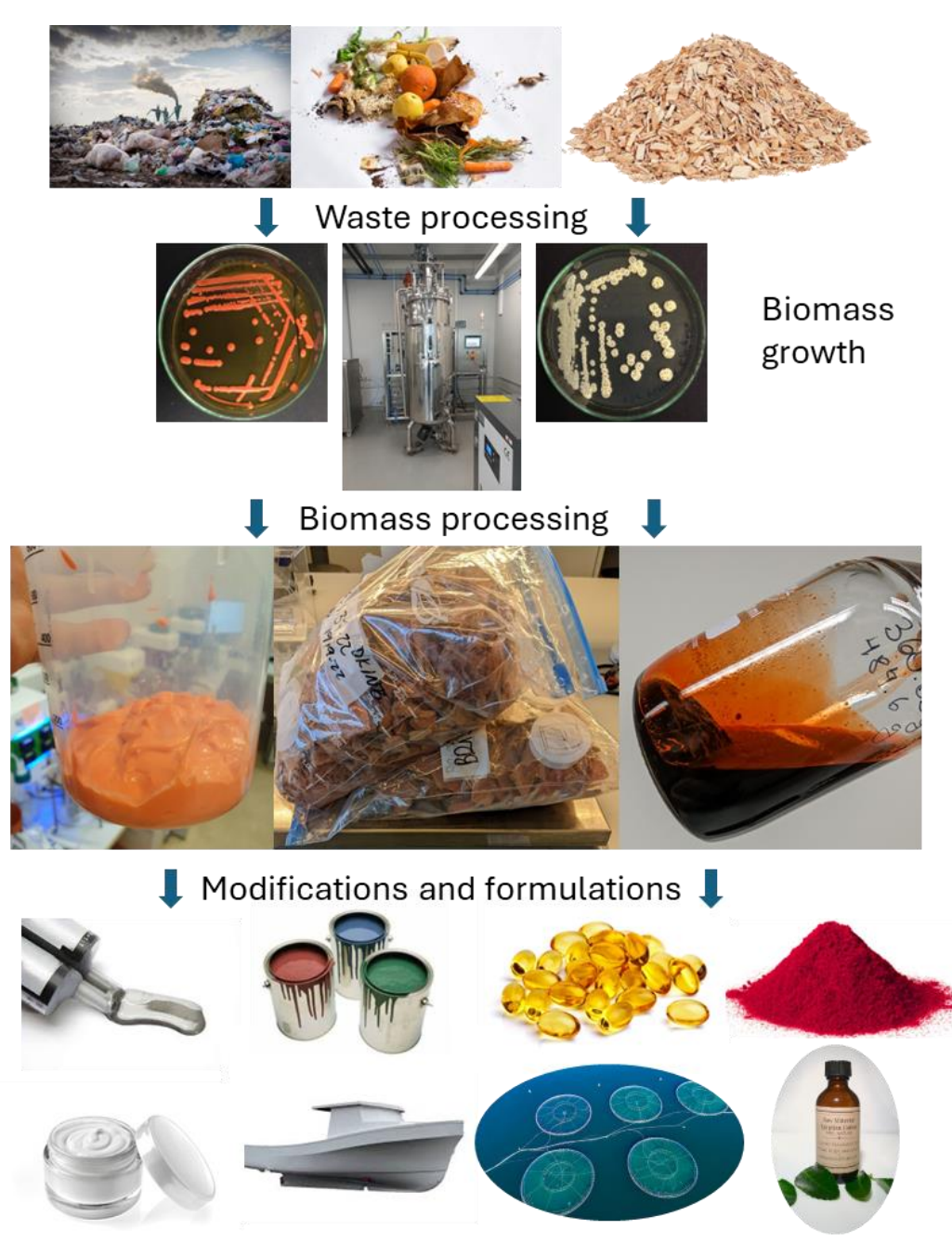


Figure 3. Successfully demonstrated process for conversion of several waste streams into greener ingredients for 2nd generation biofuels, biochemicals, nutraceuticals, novel food, and feed. The processes are currently undergoing further improvement and scale-up.

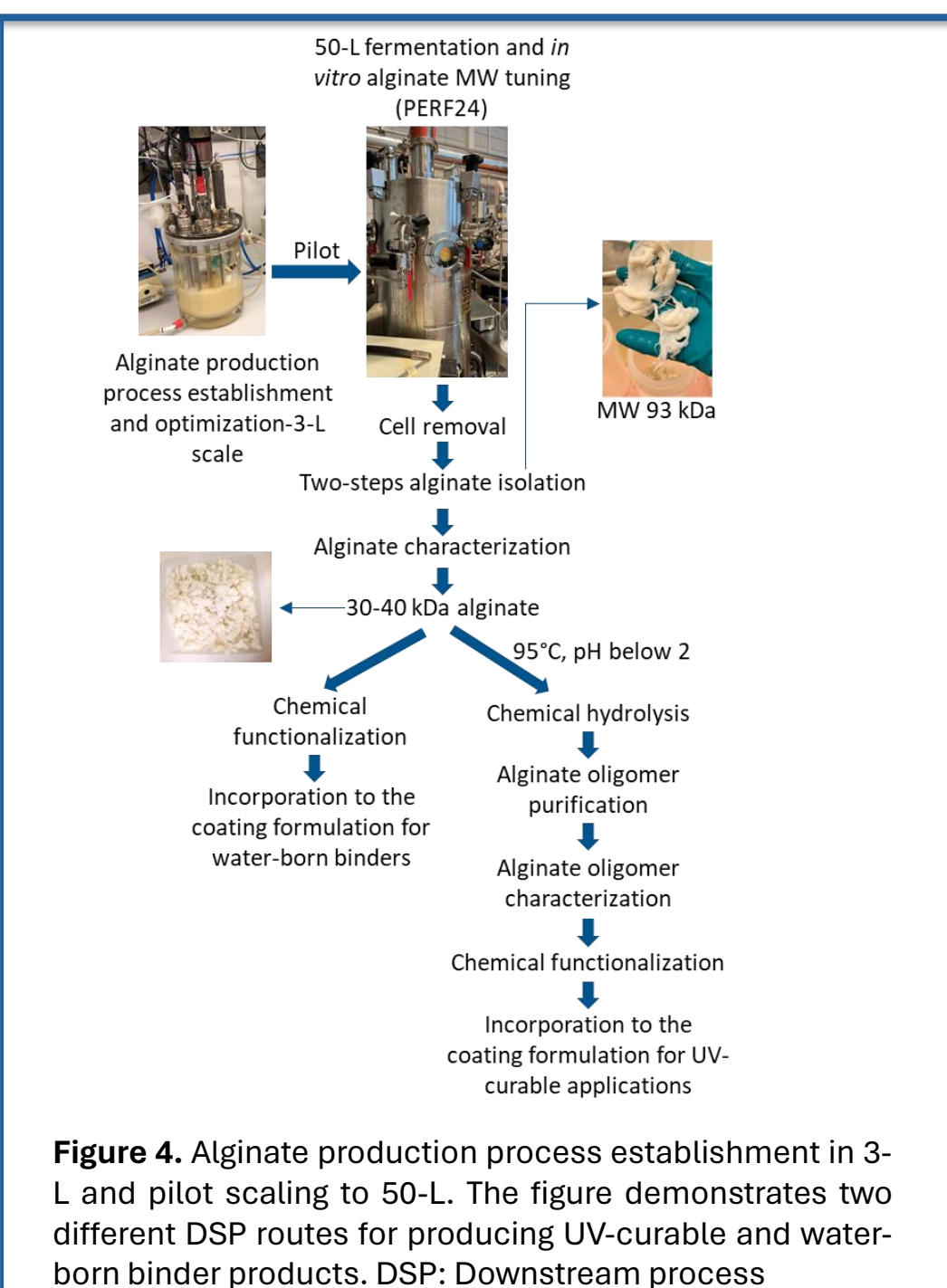
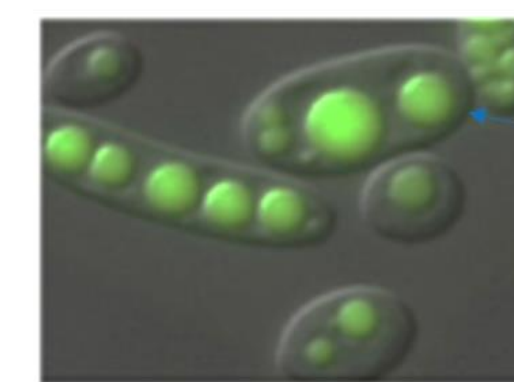


Figure 4. Alginate production process establishment in 3-L and pilot scaling to 50-L. The figure demonstrates two different DSP routes for producing UV-curable and water-born binder products. DSP: Downstream process



Lipid droplet

Figure 2. Oleaginous yeasts are highly efficient in converting numerous waste streams into lipids as a value-added raw material for several industries. The ability to engineer the yeasts, and change their properties makes the production platform more suitable for providing tailor-made industrially relevant greener ingredients.

Results-Alginate

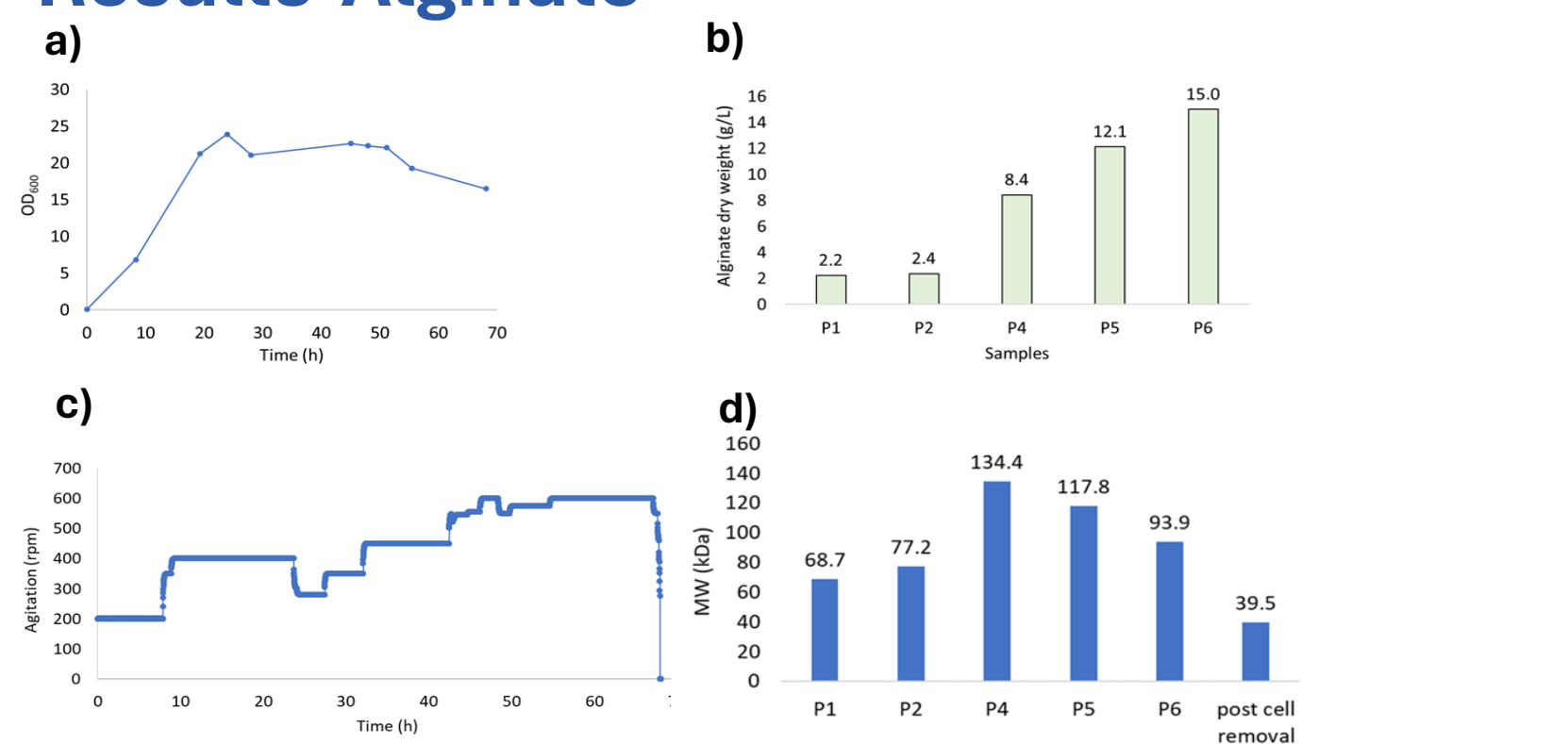


Figure 8. a) Growth profile, b) Alginate production yield, c) Agitation development, and d) Molecular weight analyses obtained during pilot scale alginate fermentation.

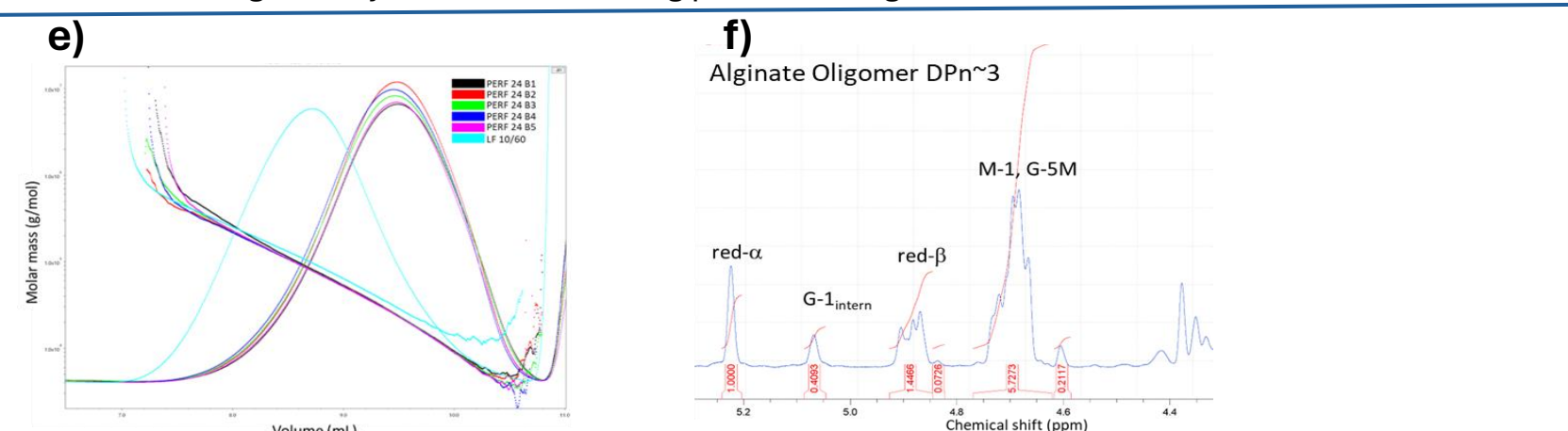


Figure 9. e) Reflective index chromatograms with overlaid molecular weight-volume lines of samples PERF24 B1-B5 taken during alginate production in pilot fermentation. f) NMR data showing anomeric region of the sample alginate oligomer with DPn 3. g) Table presenting NMR and SEC/MALLS data for alginate polymer and oligomer originating from pilot fermentation.

Sample	F _G	F _M	F _{GG}	F _{GM}	F _{MM}	F _{MGM}	F _{GGG}	N _{G>1}	DP _n	MW (kDa)
PERF24	0.14	0.86	0	0.14	0.72	0.14	0	0	40	
Alginate oligomer	0.05	0.095	0				0	0	3	0.57

Results-Microbial oils

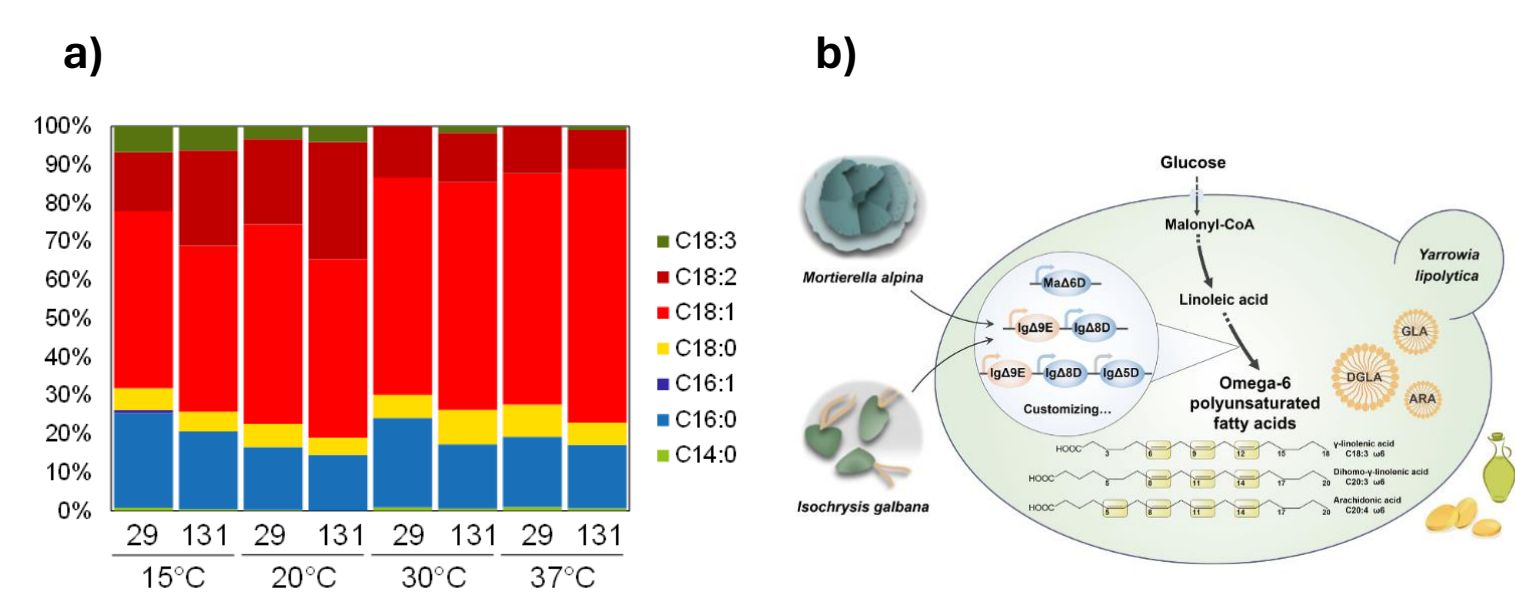


Figure 5. a) Cultivation dependent changes in Free Fatty Acid (FFA) profile in *Rhodotorula toruloides* b) *Yarrowia lipolytica* engineered for production of poly-unsaturated Fatty Acids.

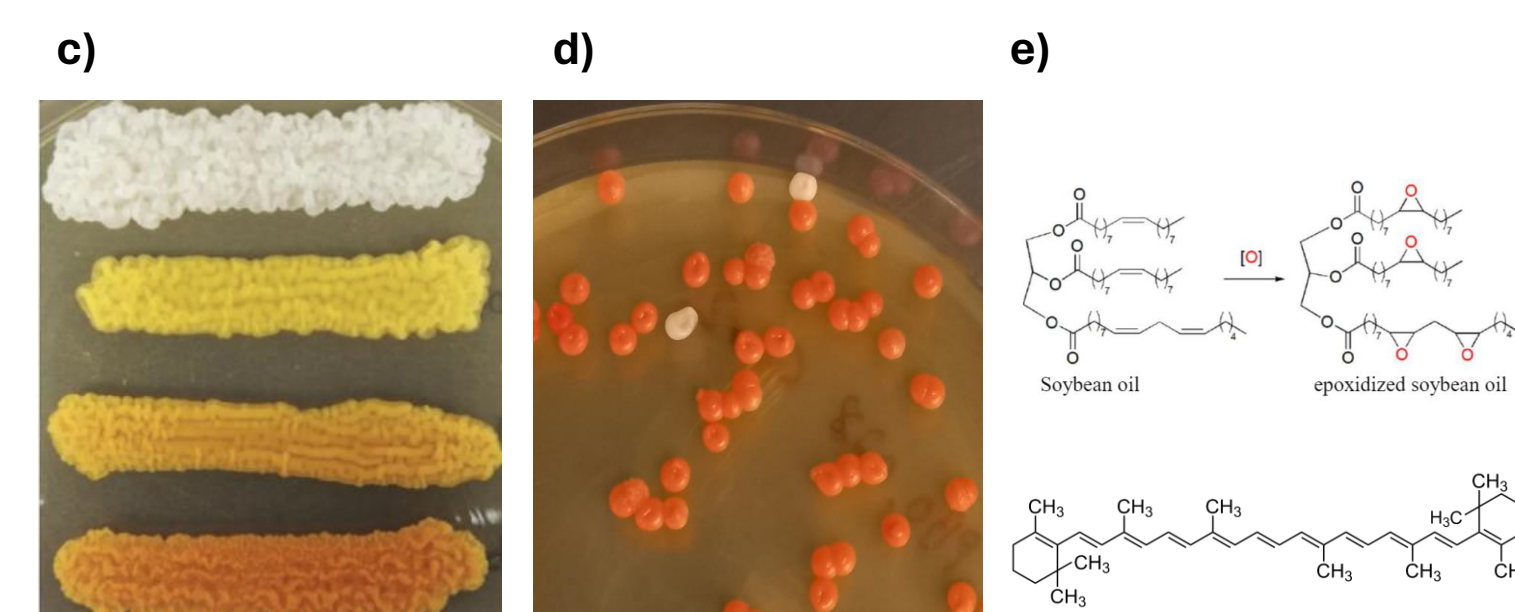


Figure 6. c) *Yarrowia lipolytica* engineered for high β -carotene production, d) *Rhodotorula toruloides* engineered for production of non-pigmented microbial oils, e) structure of oil, epoxidized oil, and β -carotene.

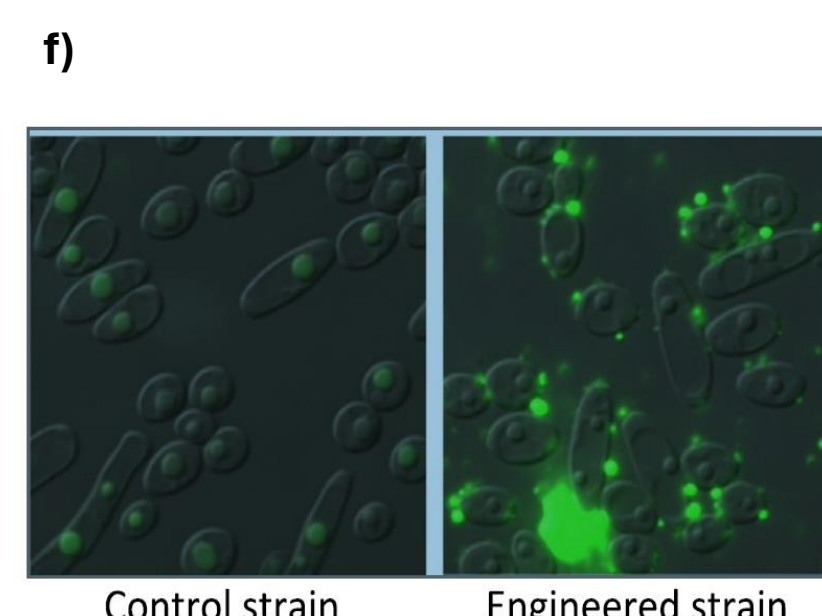


Figure 7. f) Microscopic image of *Yarrowia lipolytica* strain engineered for secretion of Free Fatty Acids (FFA), g) Emulsion of FFA in cultivation medium, h) FFA clumps in cultivation medium.

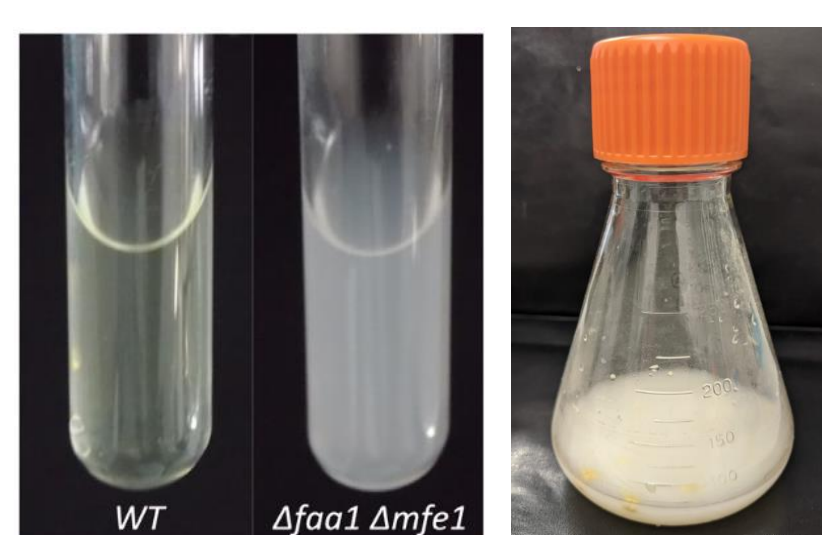


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Conclusions The technical advancements achieved in PERFECOAT demonstrate significant progress towards establishing scalable processes for microbial alginate and oil productions. The DSP work for alginate processing was successfully upscaled at Evonik for 3 kg material. Furthermore, Evonik successfully scaled-up microbial oil production to 200 L fermentation, which resulted in an estimated ~2 kg of oils. Further optimization efforts, coupled with the exploration of alternative feedstocks, hold promise for enhancing the sustainability and performance of bio-based binders in coating applications.

