Heterotrophic cultivation of oleaginous microbes for the production of metabolites of commercial interest using forest biomass

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BIO4ENERGY

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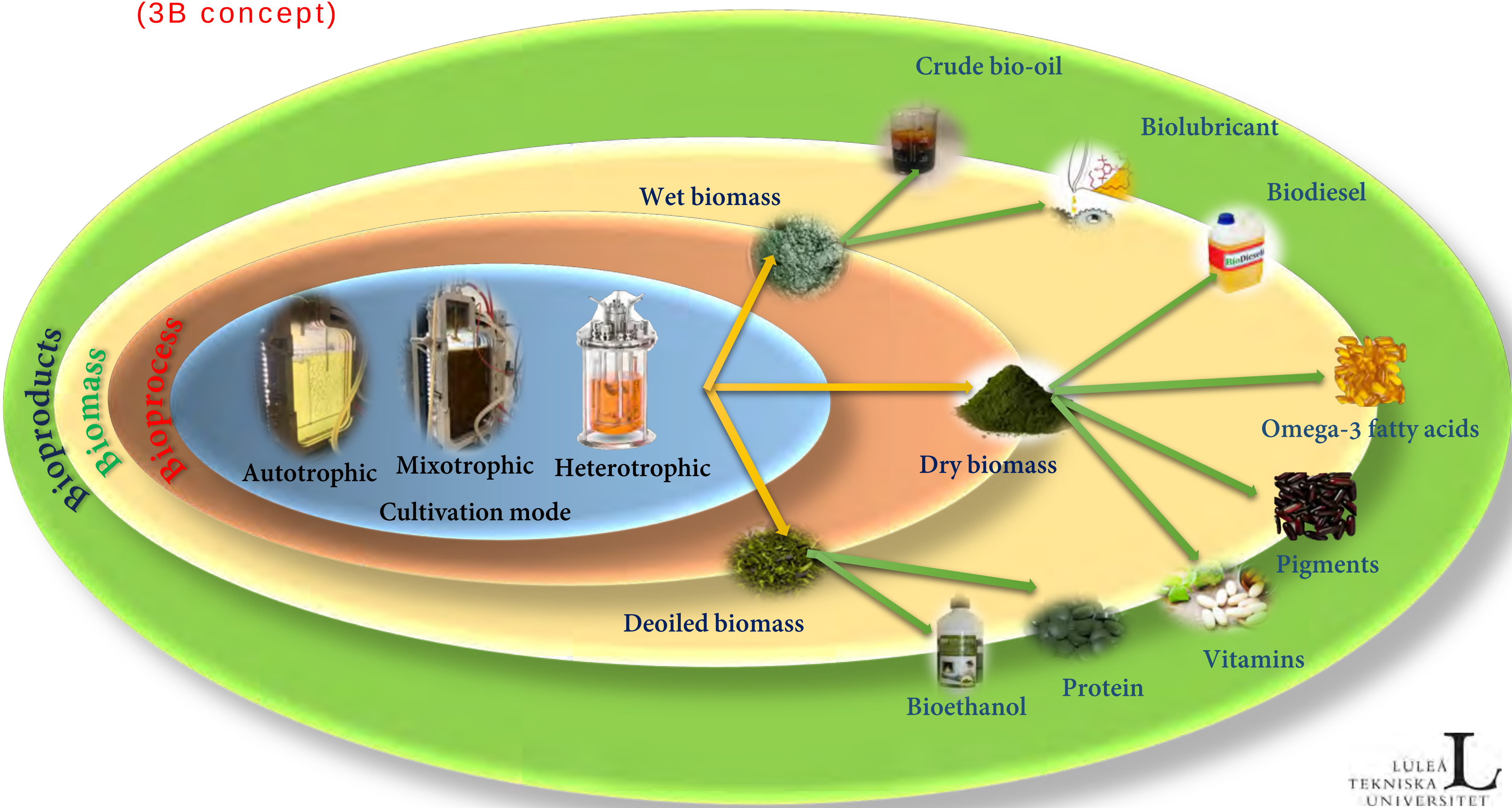
Microalgal Biorefinery Concept
(3B concept)



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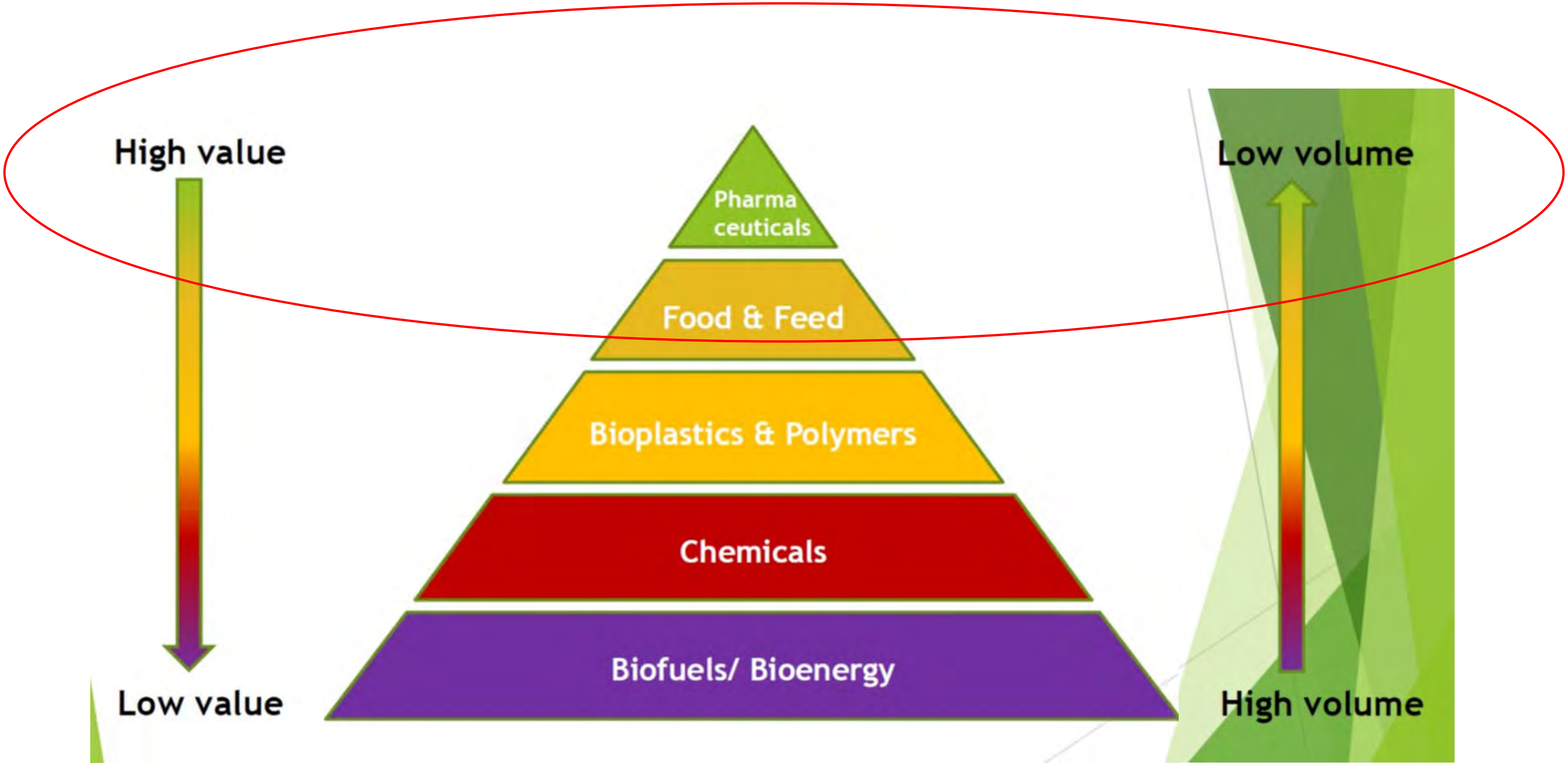
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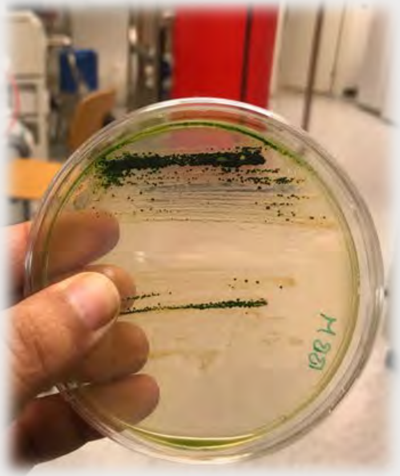
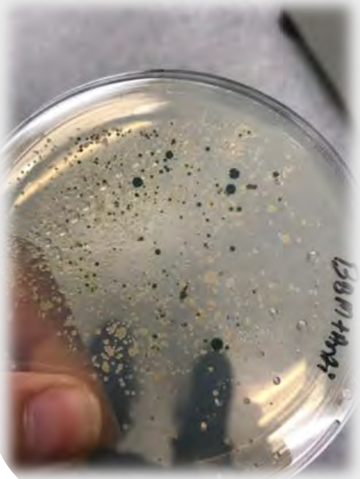
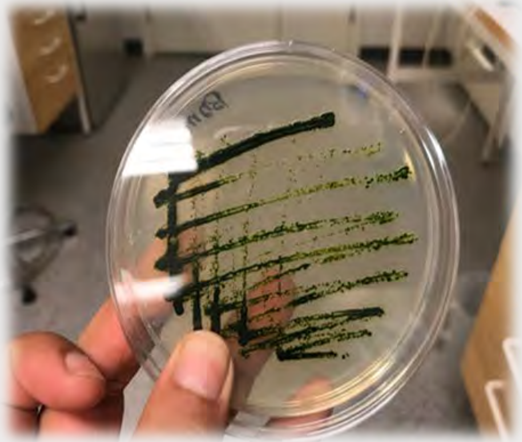
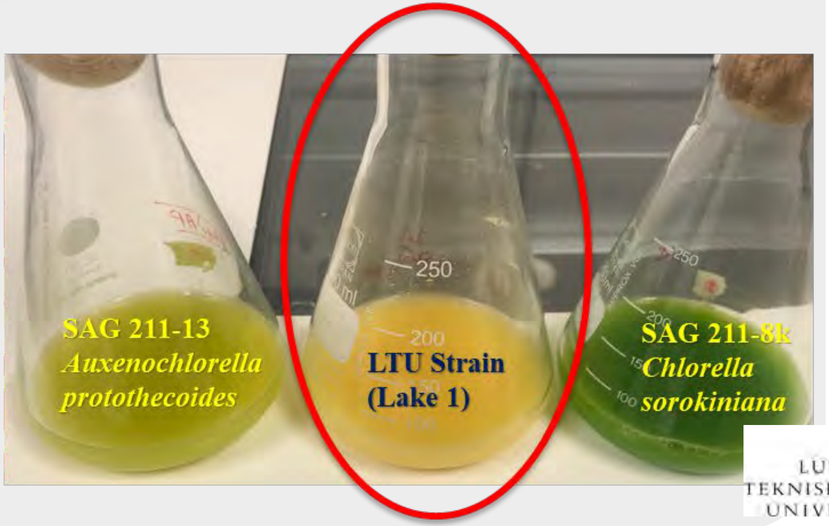
Low value
Pharma ceuticals
Food & Feed
Bioplastics & Polymers
Chemicals
Biofuels/ Bioenergy
Low volume
High volume









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- Sweden has a well-developed forestry industry that contributes substantially to its economy.
- Norway spruce (*Picea abies*), Scots pine (*Pinus sylvestris*), and birch (*Betula pendula* and *B. pubescens*) are the foremost tree species representing 40.8, 39.2, and 12.4% of the total standing volume of the forest, respectively [17].

It has a huge forest area, which covers approximately 53.1% of the total landarea, involving 3490 million m3skof total standing volume of forest [17].

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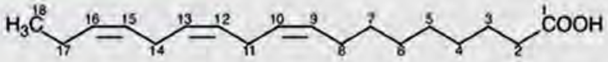
Dietary supplements/Nutraceutical Fatty Acids/ Polyunsaturated fatty acids

Figure 1a. Structures of Fatty Acids

CH_3	$CHCH_{2(n)}$	COOH
methyl or omega (ω) end	hydrocarbon chain	carboxyl end

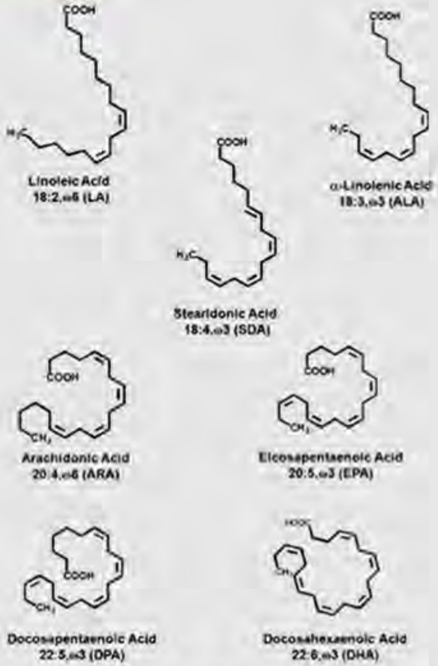
The general structure of a fatty acid (20).

Figure 1b. Structures of Fatty Acids



The chemical structure of α-linolenic acid (ALA), 18:3n-3. ALA has 18 carbon atoms (C) and 3 double bonds, the first of which is located 3 carbon atoms from the terminal methyl group (omega [ω] end).

Figure 1c. Structures of Fatty Acids



The molecular structures of dietary omega-6 and omega-3 fatty acids. The presence of a double bond in the hydrocarbon chain of polyunsaturated fatty acids (PUFA) introduces a "kink" in the molecule, creating different secondary structures that influence physical properties (5).

Table 1. Names and Abbreviations of the Omega-6 and Omega-3 Fatty Acids					
Omega-6 Fatty Acids			Omega-3 Fatty Acids		
Linoleic acid	LA	18:2n-6	α-Linolenic acid	ALA	18:3n-3
γ-Linolenic acid	GLA	18:3n-6	Stearadonic acid	SDA	18:4n-3
Dihomo-γ-linolenic acid	DGLA	20:3n-6	Eicosatetraienoic acid	ETA	20:4n-3
Arachidonic acid	AA	20:4n-6	**Eicosapentaenoic acid**	**EPA**	20:5n-3
Adrenic acid		22:4n-6	Docosapentaenoic acid	DPA (n-3)	22:5n-3
Tetracosatetraenoic acid		24:4n-6	Tetracosapentaenoic acid		24:5n-3
Tetracosapentaienoic acid		24:5n-6	Tetracosahexaenoic acid		24:6n-3
Docosapentaenoic acid	DPA (n-6)	22:5n-6	**Docosahexaenoic acid**	**DHA**	22:6n-3

The capacity to generate DHA from ALA is higher in women than men

Studies of ALA metabolism in healthy young men indicate that approximately 8% of dietary ALA is converted to EPA and 0-4% is converted to DHA (6). In healthy young women, approximately 21% of dietary ALA is converted to EPA and 9% is converted to DHA (7).

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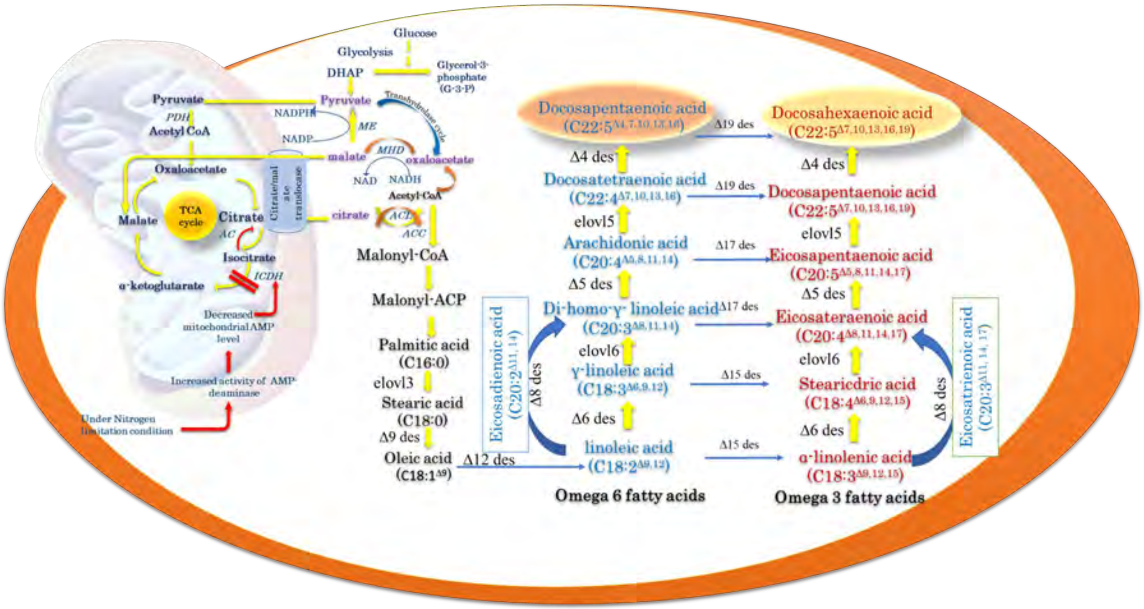

Humans can synthesize longer omega-6 and omega-3 fatty acids from the essential fatty acids LA and ALA through a series of desaturation (addition of a double bond) and elongation (addition of two carbon atoms) reactions. Delta-6 desaturase (FADS2) is considered the rate-limiting enzyme in this metabolic pathway. Retroconversion of DHA to EPA in peroxisomes occurs at low basal rates and following DHA supplementation (4, 5). *FADS2, delta-6 desaturase; FADS1, delta-5 desaturase; Elovl2, Elovl5, elongases.*

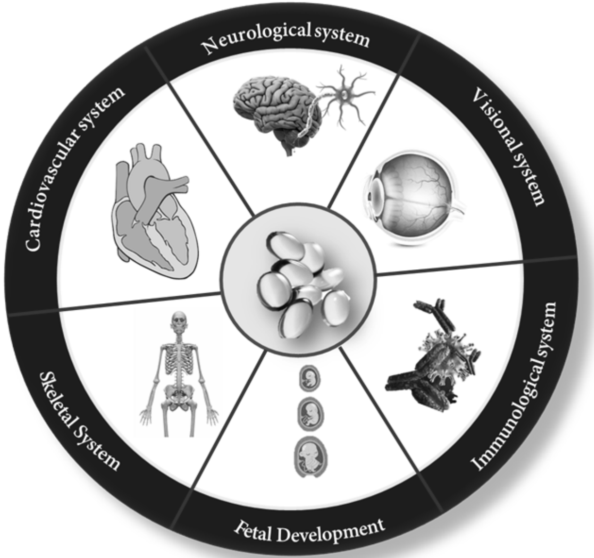

Figure 5. Bioactive Lipid Mediators Derived from Omega-6 and Omega-3 Fatty Acids

Dietary intake can alter the fatty acid composition of cell membranes and influence the local production of bioactive lipid mediators. Each PUFA precursor gives rise to a variety of molecul with a range of immune modulating activities: inflammatory (red and pink), anti-inflammator (blue), and pro-resolving (green); isoprostanes (yellow) are markers of oxidative stress (16, 22

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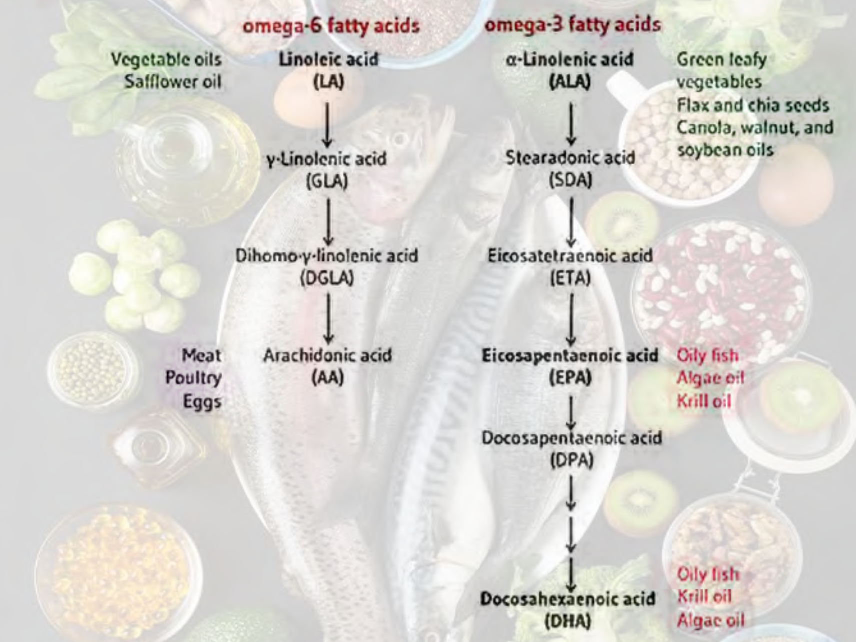
Figure 2. Classes of Essential Fatty Acids



Omega-6 (n-6) and omega-3 (n-3) fatty acids comprise the two classes of essential fatty acids (EFA). The parent compounds of each class, linoleic acid (LA) and α-linolenic acid (ALA) (bold font), give rise to longer chain derivatives inside the body. Due to low efficiency of conversion of ALA to the long-chain omega-3 PUFA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), it is recommended to obtain EPA and DHA from additional sources. Dietary sources of the different LC-PUFA are listed in the colored boxes (23).

Krill (animal)

Krillolja , 1000 mg, 120 Gelékapslar

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Futuristic food fortification with a balanced ratio of dietary ω-3/ω-6 omega fatty acids for the prevention of lifestyle diseases

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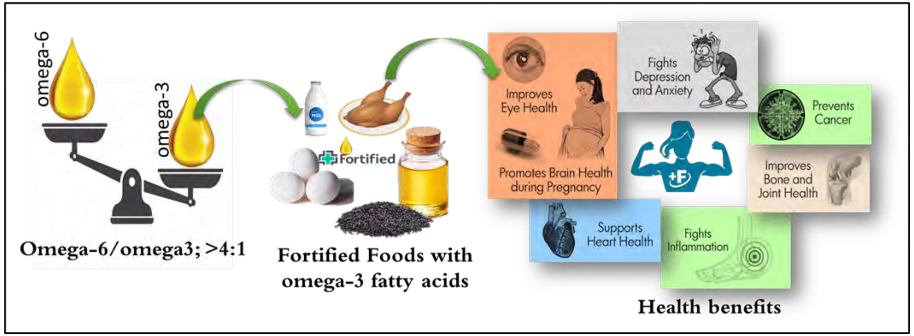

Over the last three decades, total fat and saturated fat consumption has steadily declined in Western diets as a proportion of total calories, while omega-6 fatty acid intake has risen with decreasing omega-3 fatty acids content, resulting in a substantial rise in the **omega-6/omega-3 ratio from 1:1 during evolution to 20:1** or much higher.

This changes in the ratio has significant increase in the prevalence of several vital diseases such as **coronary heart disease, hypertension, cancer, diabetes, overweight, obesity, rheumatoid arthritis, other autoimmune or probably neurodegenerative disorders**. The poor intake of omega-3 fatty acids maybe attributed due to lacking these fatty acids in diet or lack of awareness about dietary source of omega-3 fatty acids.

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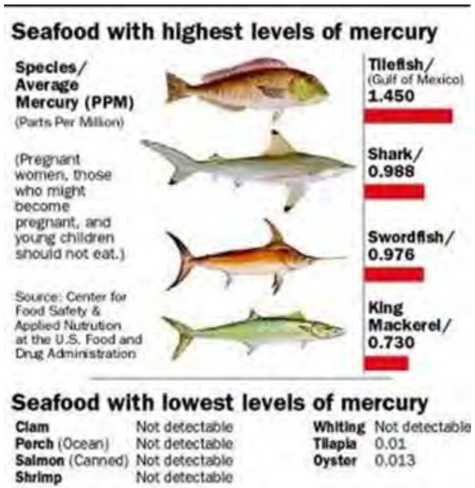

- Utilization of ω-3PUFAs from fish oils are possibly unsustainable and risky and thus have not met the ever rising worldwide demand for ω-3PUFAs because global fish stocks are diminishing, and environmental pollution of marine ecosystems has become a persistent and global problem.

Hamilton et al., 2020. **Systems approach to quantify the global omega-3 fatty acid cycle.** Nat. Food 1, 59–62.

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ACS Sustainable Chemistry & Engineering

The process aims to replace traditional non sustainable sources for squalene and DHA, such as shark liver oil and marine fish oil respectively, with sustainable sources. Furthermore, to make this strategy economically feasible, the waste generated during the suggested process is utilized as a biolubricant.

Parameters	Cultivation in flask	Cultivation in bioreactor
Cell dry weight (g/L)	26.87 ± 0.69	29.07 ± 0.84
Biomass yield ($g/g_{substrate}$)	0.44 ± 0.01	0.48 ± 0.01
Biomass productivity (g/L/d)	5.37 ± 0.23	9.69 ± 0.34
Total lipid concentration (g/L)	12.87 ± 0.95	15.21 ± 0.72
Lipid content (%, w/w)	47.90 ± 1.23	52.32 ± 1.45
Lipid yield ($g/g_{substrate}$)	0.21 ± 0.01	0.25 ± 0.01
Lipid productivity (g/L/d)	2.57 ± 0.25	5.07 ± 0.13
DHA content (%, $w/w_{total\ lipids}$)	45.54 ± 0.23	66.72 ± 0.31
DHA concentration (g/L)	5.86 ± 0.34	10.15 ± 0.64
DHA yield (mg/g_{CDW})	218.08 ± 1.16	349.15 ± 1.54
DHA productivity (g/L/d)	1.17 ± 0.12	3.38 ± 0.27
Squalene yield (mg/g_{CDW})	16.34 ± 1.81	32.12 ± 4.37
Squalene concentration (mg/L)	439.05 ± 1.34	933.72 ± 6.53

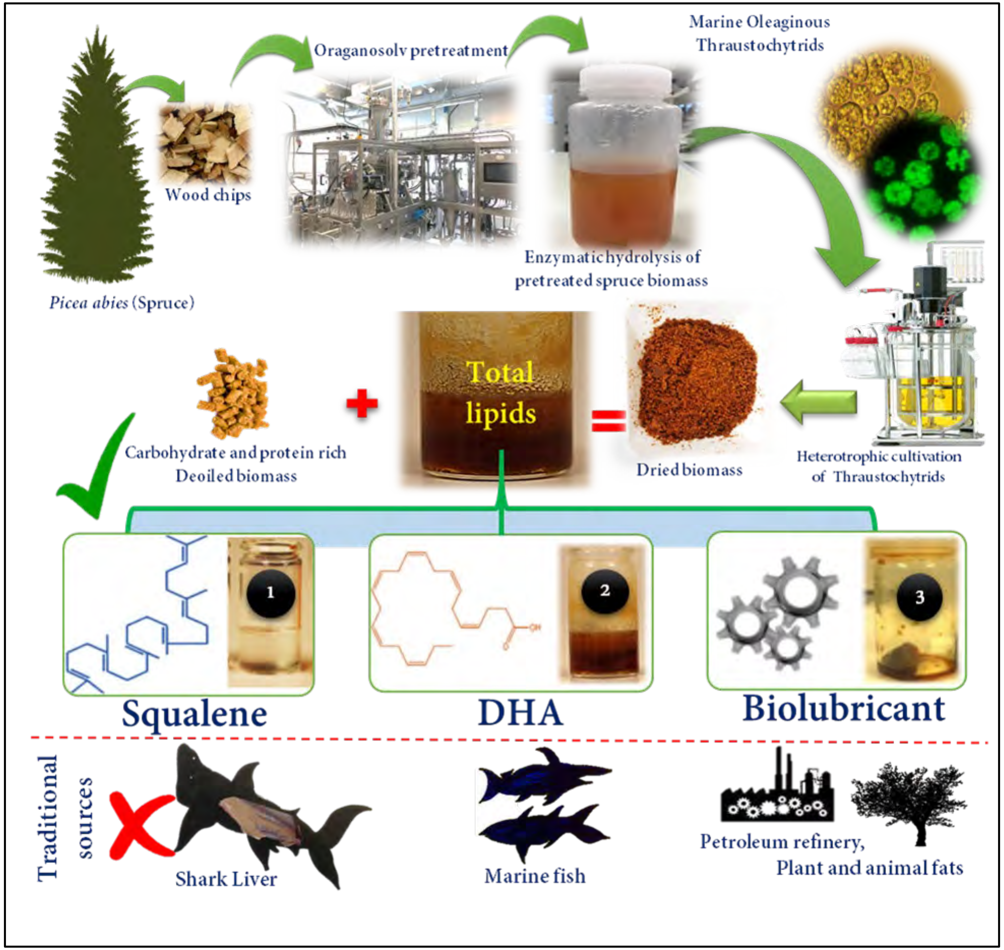
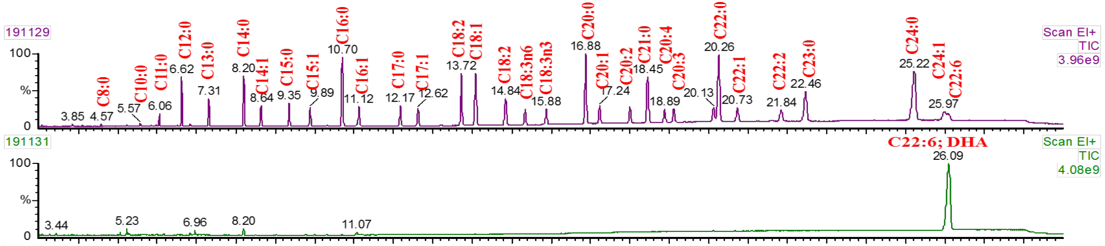


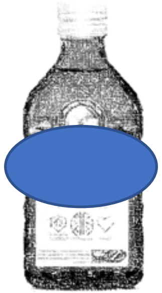
Table 2 Nutritional profile of microalgae and fish species per kg DM

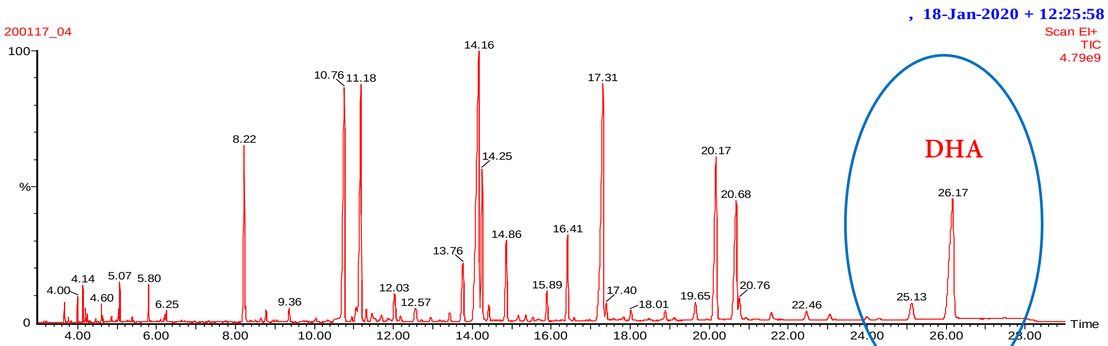
Product	Calories in kcal kg^{-1} DM	Fat in g kg^{-1} DM	EPA in g kg^{-1} DM	DHA in g kg^{-1} DM	Protein in g kg^{-1} DM	EPA+DHA in g kg^{-1} DM
Thraustochytrids						
Schizochytrium limacinum SR21		**775.00**		**271.25**		**271.25**
Thraustochytriidae PKU#Mn16		**522.00**		**225.21**		**225.21**
Microalgae						
Nannochloropsis sp.	4218.00	206.00	42.00	-	300.00	42.00
Phaeodactylum tricornutum	4120.00	180.00	31.10	1.40	364.00	32.50
Fish: capture production						
Salmon	4839.77	238.39	8.76	20.59	783.85	29.35
Alaska pollack	3577.11	38.39	4.17	9.54	818.92	13.71
Herring	4985.97	312.73	24.60	26.70	566.76	51.30
Tuna	4406.66	235.48	16.40	56.07	605.00	72.47
Codfish	3763.39	30.75	2.94	5.51	817.39	8.44
Mackerel	5787.33	392.13	25.35	39.55	525.09	64.90
Fish: aquaculture production						
Salmon	5113.64	363.51	23.53	35.44	596.69	58.97
Trout	4968.15	214.01	13.46	28.95	758.47	42.41
Carp	4768.46	115.57	5.44	5.41	667.96	10.85
Pangasius	6165.58	227.34	0.81	3.10	842.44	3.91
Tilapia	3915.50	122.88	0.65	3.04	670.52	3.69

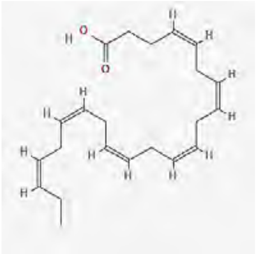
Standards

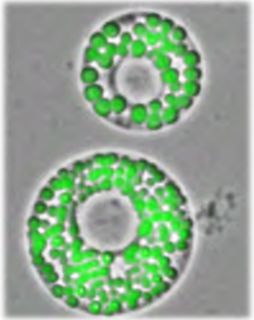


Norwegian arctic cod

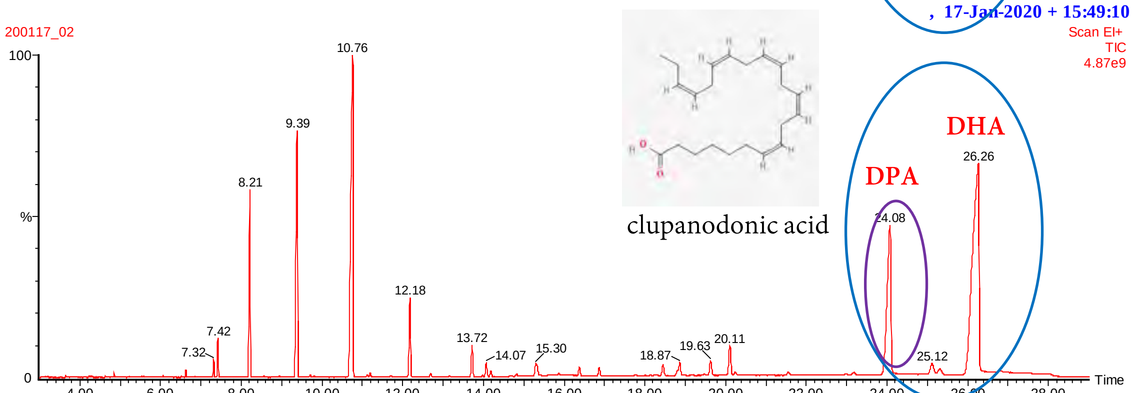








clupanodonic acid



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FORMAS (INVENTION)

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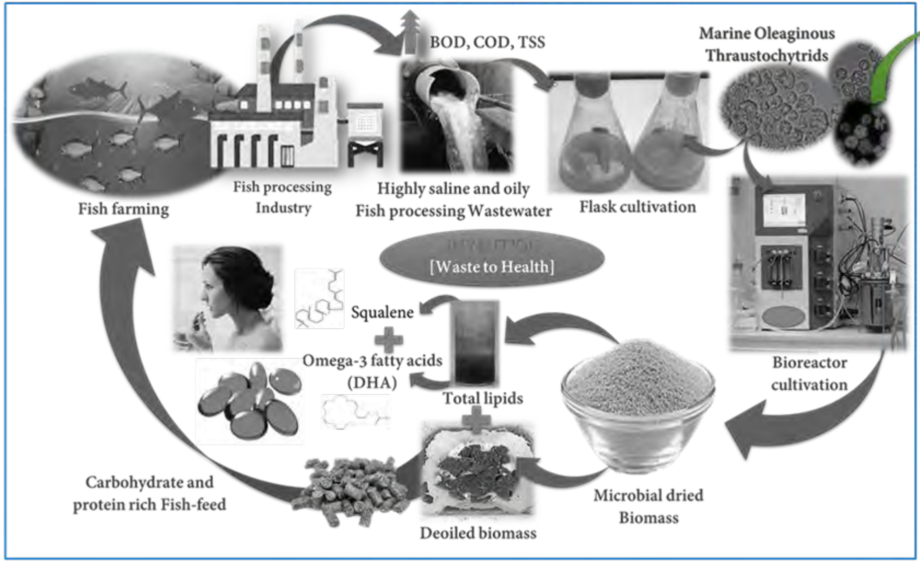
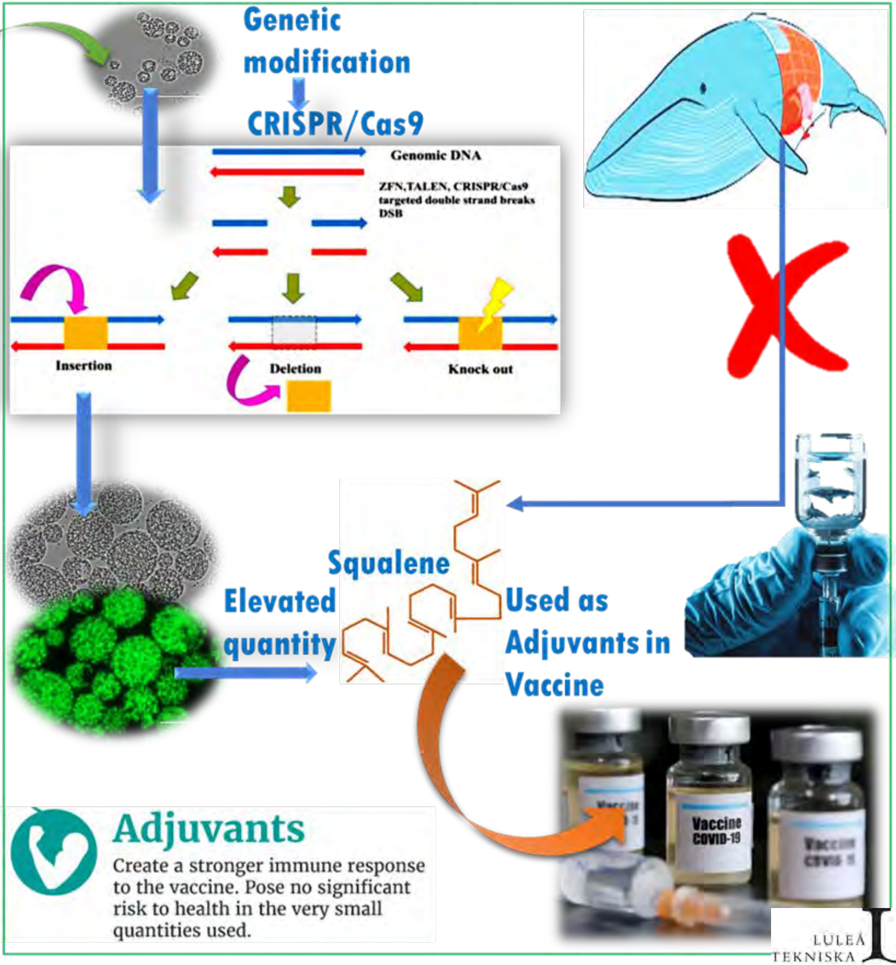
KEMPE (SQUALENE)

Formas Project (*INVENTION*) → **Kempestiftelserna Project Proposal (2021)**





- **Shark squalene has been used as the main ingredient in some adjuvants to boost efficacy in vaccines. Squalene made from shark liver oil is used most commonly because it is cheap to obtain and easy to come by, not because it is more effective than other sources.**
- **Currently, there are 34 candidate vaccines in clinical evaluation and 142 vaccines in preclinical evaluation, according to the WHO. Of these vaccines, 17 use adjuvants, and 5 of those adjuvants are shark-squalene base.**
- **This project will consist the first CRISPR-CAS 9 based genetic engineering approach to improve non-animal based squalene content in thraustochytrids to replace the adjuvant in COVID 19 vaccines that is mainly obtained from shark.**

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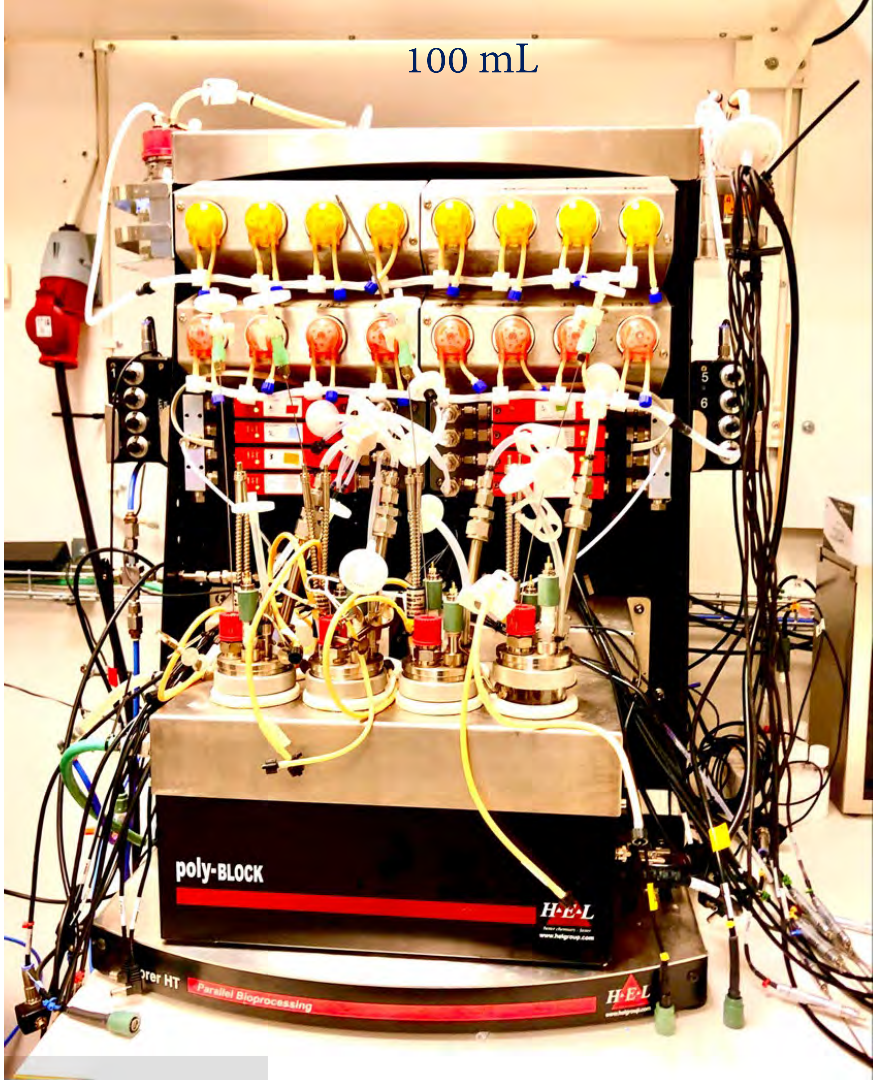
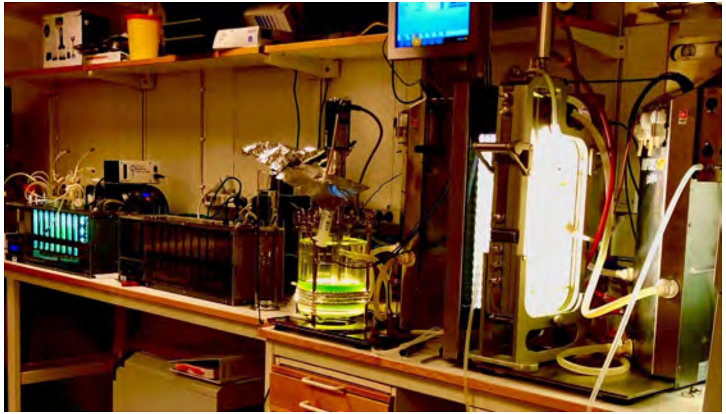
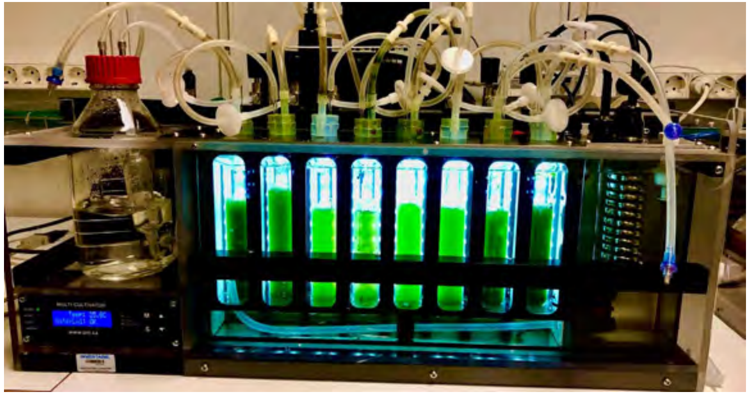
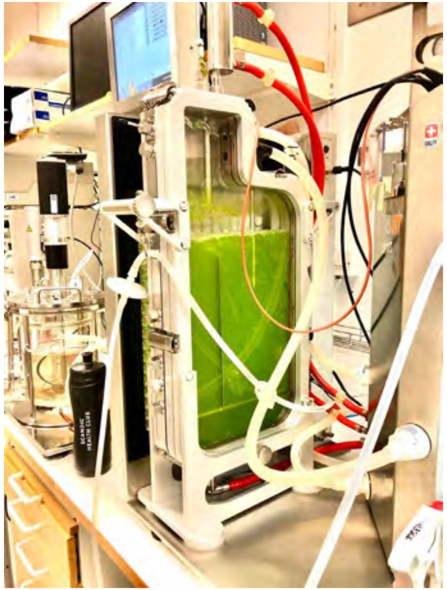

100 mL
poly-BLOCK
H·E·L
Parallel Bioprocessing

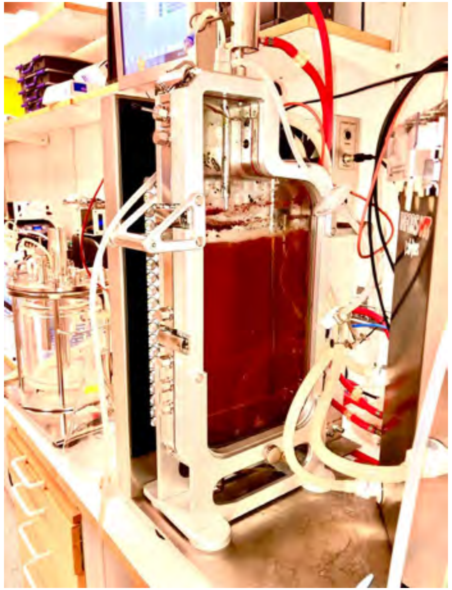

12L
DELL
WARNING

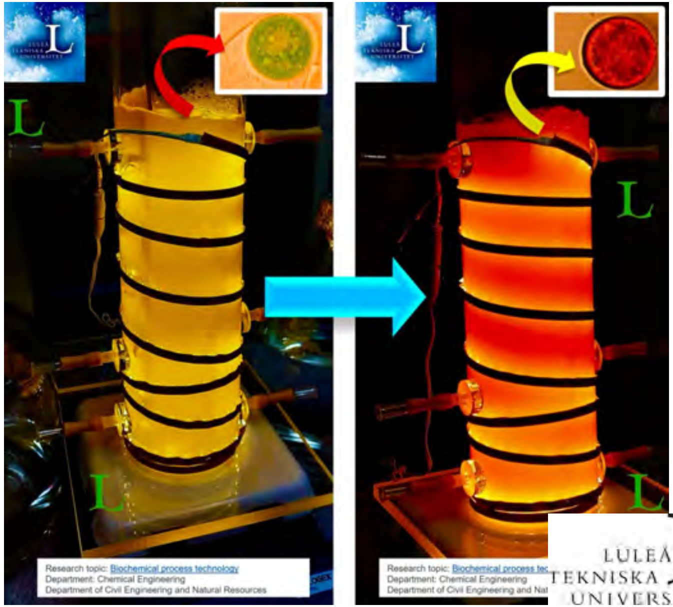
Astaxanthin Production











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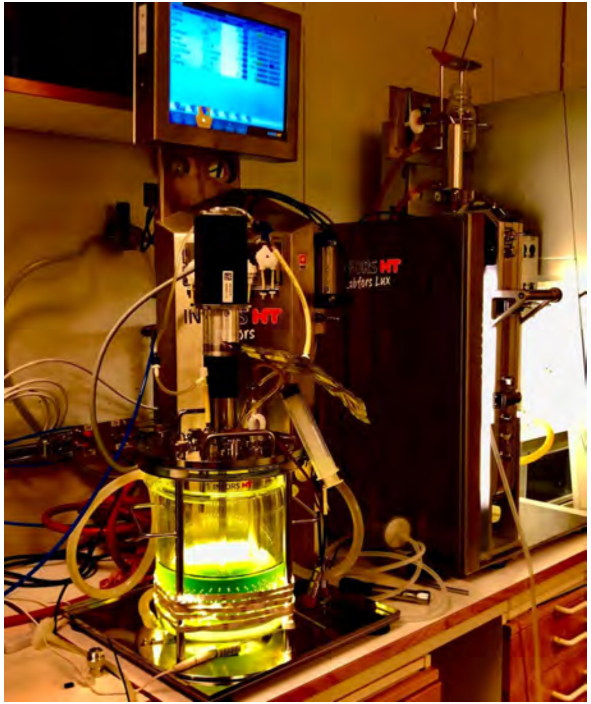
Hydrocarbon production

Thermochemical and catalytic upgrading



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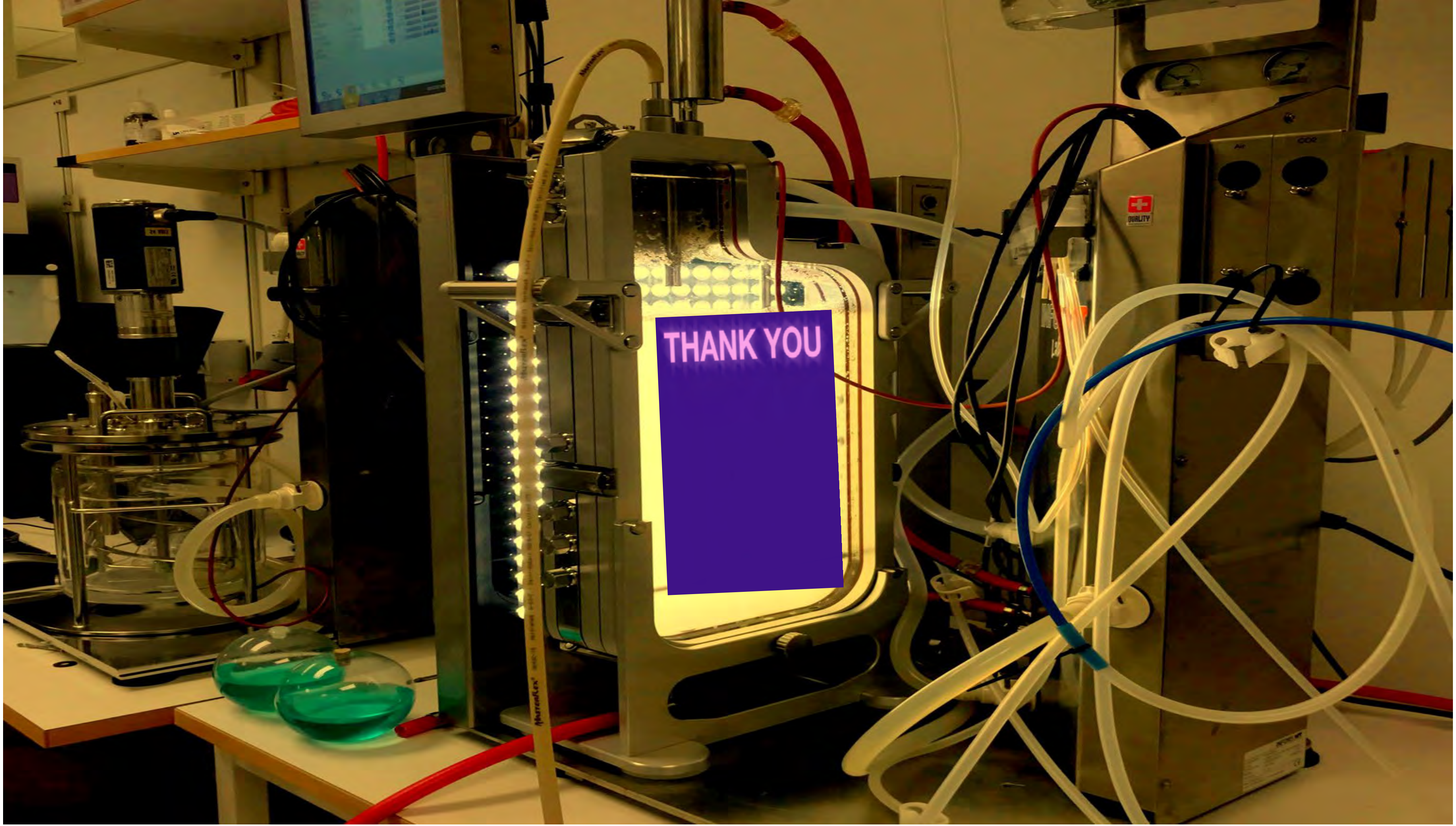
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