Řasy jako znovuobjevené zdroje

Kurz genetiky a molekulární biologie
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Řasy v historii lidstva

- Archeologické nálezy dokazují konzumaci řas u pravěkých lidí
- 500 př.n.l. sběr makrořas v Číně
- Sběr sinice *Arthrospira* Aztéky datován do 14. století, příprava pokrmu *tecuitlatl*
- Čad – využití *Arthrospira* sahá až do období Kanemské říše (8-9. století)

Table 1 Algal biotechnology historical data

Alga	Year of first record		
	Collected	Cultivated	Processed
Macroalgae			
Porphyra	530	1640	–
Chondrus/Gelidium/ Gracilaria	∞	1950	1658
Laminaria/Macrocystis/ Fucus	∞	1731	1925
Eucheuma	∞	1971	1965
Microalgae			
Diatoms	∞	1863 (selective use)	1914
Spirulina	∞	1965	1985
Chlorella	–	1975	1994
Dunaliella	–	1982	1985
Odontella	–	2002	2003

Tradiční pokrmy z řas

- Řasy bývaly konzumovány po celém světě, v průběhu let ale jejich zastoupení klesalo
- Silná tradice v asijských zemích (Japonsko, Čína, Korea, Filipíny,…), dále v Čadu a Mexiku
- V Irsku bývaly řasy tradičním pokrmem, který prakticky vymizel, dnes se znovu vrací

Světový trh s řasami

- 42 zemí se aktivně podílí na produkci makroskopických řas
- Top producentem je Čína (*Laminaria* jako produkt č. 1)
- Severní Korea, Jižní Korea, Japonsko, Filipíny, Čile, Norsko, Indonésie, USA, Indie – 10 zemí pokrývá 95 % světového trhu s řasami
- Cca. 90 % produkce je založeno na kultivacích
- Asie pokrývá 99 % produkce (Čína 75 %)

Světový trh s řasami

- *Porphyra*, *Kappaphycus*, *Undaria*, *Euchema*, *Gracilaria* and *Laminaria* představují 99 % produkovaných makrořas
- Prudký nárůst sklizně řas z 3 mil. tun v roce 1981 na 13 mil. tun v roce 2002

Total Macroalgae Harvest in All Fishing Areas of the World

All Fishing Areas of the World	2000	2001	2002
Red Macroalgae	2,275,141	2,472,253	2,791,006
Brown Macroalgae	5,608,074	5,453,534	5,782,535
Green Macroalgae	96,235	93,688	76,265

- *Porphyra* je vůbec nejcennější řasou

Summary of Edible Algae and the Corresponding Food Item

Scientific Name	Common Name	Class
Nostoc flagelliforme	Facai	Cyanophyceae
Arthrospira sp.	Dihé/Tecuitlatl	Cyanophyceae
Chondrus crispus	Pioca/Irish moss	Floridophyceae
Porphyra spp.	Nori/Laber/Zicai	Bangiophyceae
Palmaria (Rodimenia) palmata	Dulse	Floridophyceae
Callophyllis variegata	Carola	Floridophyceae
Asparagopsis taxiformis	Limu kohu	Floridophyceae
Gigartina spp.	Botelhas	Floridophyceae
Gracilaria coronopifolia	Limu manauea	Floridophyceae
Gracilaria parvisipora	Ogo	Floridophyceae
Gracilaria verucosa	Ogo-nori/Sea moss	Floridophyceae
Sargassum echinocarpum	Limu kala	Pheophyceae
Dictyopteris plagiogramma	Limu lipoa	Pheophyceae
Undaria pinnatifida	Wakame	Pheophyceae
Laminaria spp.	Kombu	Pheophyceae
Nereocystis spp.	Black kelp	Pheophyceae
Hizikia fusiforme	Hiziki/Hijiki	Pheophyceae
Alaria esculenta	Oni-wakame	Pheophyceae
Cladosiphon okamuranus	Mozuku	Pheophyceae
Codium edule	Limu wawale'iole	Bryopsidophyceae
Enteromorpha prolifera	Limu 'ele'ele/green laver	Ulvophyceae
Ulva fasciata	Limu palahalaha	Ulvophyceae
Caulerpa lentillifera	Limu Eka	Charophyceae
Monostroma nitidum	Aonori	Ulvophyceae

Major microalgae commercialized for human nutrition

Alga	Annual production (t/year)	Producer country	Applications and products
Spirulina (Arthrospira)	3000	China, India, USA, Myanmar, Japan	Human and animal nutrition, cosmetics (phycobiliproteins, powders, extracts, tablets, beverages, chips, pasta, liquid extract)
Chlorella sp.	2000	Taiwan, Germany, Japan	Human nutrition, aquaculture, cosmetics (tablets, powders, nectar, noodles)
Dunaliella salina	1200	Australia, Israel, USA, China	Human nutrition, cosmetics (ß-carotene, powders)
Aphanizomenon flos-aquae	500	USA	Human nutrition (capsules, crystals, powder)
Haematococcus pluvialis	300	USA, India, Israel	Aquaculture, astaxanthin
Crypthecodinium cohnii	240t DHA oil	USA	DHA oil
Shizochytrium sp.	10t DHA oil	USA	DHA oil

Source: Adapted from Spolaore et al. (2006) and Gouveia et al. (2008b)

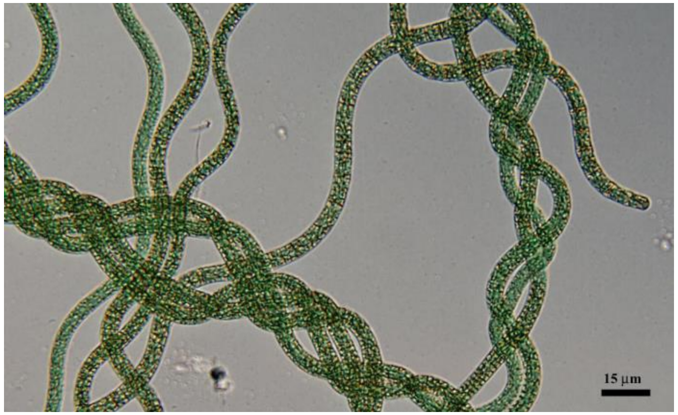
Ge-Xian-Mi

- *Nostoc sphaeroides*
- regionální využití jako potrava nebo přísada do jídel
- v Číně roste v rýžových polích
- slizové kolonie mají v průměru až 2,5 cm
- vysušený *Nostoc* se podává s ústřicemi, je možné ho použít do polévek nebo jako zahušťovadlo

Nostoc flagelliforme

- Známý jako faat choy, fa cai, black moss, hair moss nebo hair weed
- V Číně je považován za delikatesu po více jak 200 let
- Oceňován pro své vlastnosti a spirituální význam

Arthrospira



- Vláknitá sinice
- Nazývaná též Spirulina
- Oblíbená v Mexiku a Čadu
- V roce 1940 Dangeard popsal pokrm dihé z Čadu s tím, že by mělo jít o sinici Arthrospira
- Dihé je výborným zdrojem vitamínu A

Arthrospira

- Aztékové ji prokazatelně sbírali a konzumovali

- *Tecuitlatl* (kamenný exkrement) se jedl pro rychlé dodání energie, většinou ve formě suchého koláče

- Sběr v Čadu
- Vhodné s kukuřicí, rýží, …

Diet	Protein efficiency ratio
Spirulina	1.90
Maize	1.23
Rice	2.20
Wheat	1.15
Rice + *spirulina* (3:1)	2.35
Rice + *spirulina* (1:1)	2.40
Wheat + *spirulina* (3:1)	1.42
Wheat + *spirulina* (1:1)	1.90
Maize + *spirulina* (3:1)	1.80
Maize + *spirulina* (1:1)	1.72
Maize + oats + *spirulina* (3:2:5)	1.90
Maize + rice + *spirulina* (2:2:1)	1.95

Porphyra sp.

- Známa jako Nori (Japonsko), Gim (Korea) nebo Zacai (Čína)
- Makrořasa s nejlepšími nutričními vlastnostmi
- 25-50 % proteinů (75 % stravitelných)
- Zdroj vitamínů a stopových prvků
- Bohatá na jód
- Nízký obsah cukru (pouze 0,1 %)
- Za typickou chuť Nori zodpovídá obsah alaninu, glycinu a kys. glutamové

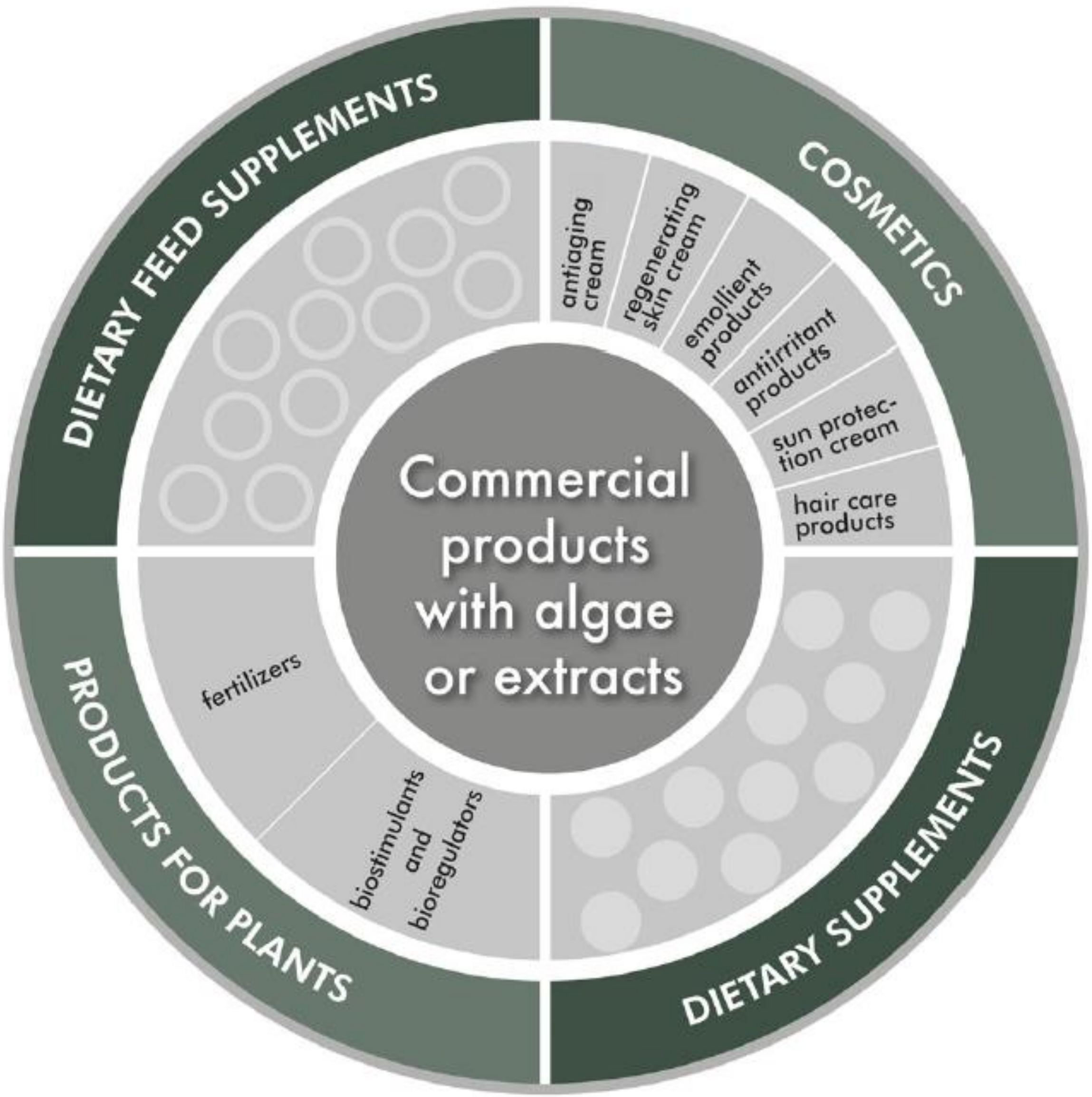
Chlorella

- Jednobuněčná nepohyblivá zelená řasa
- Taxon s největším potenciálem ve výživě a medicíně, produkce však nedosahuje úrovní arthrospiry a mkarořas
- Široké využití ve výrobě potravinových doplňků, kosmetiky a krmiv

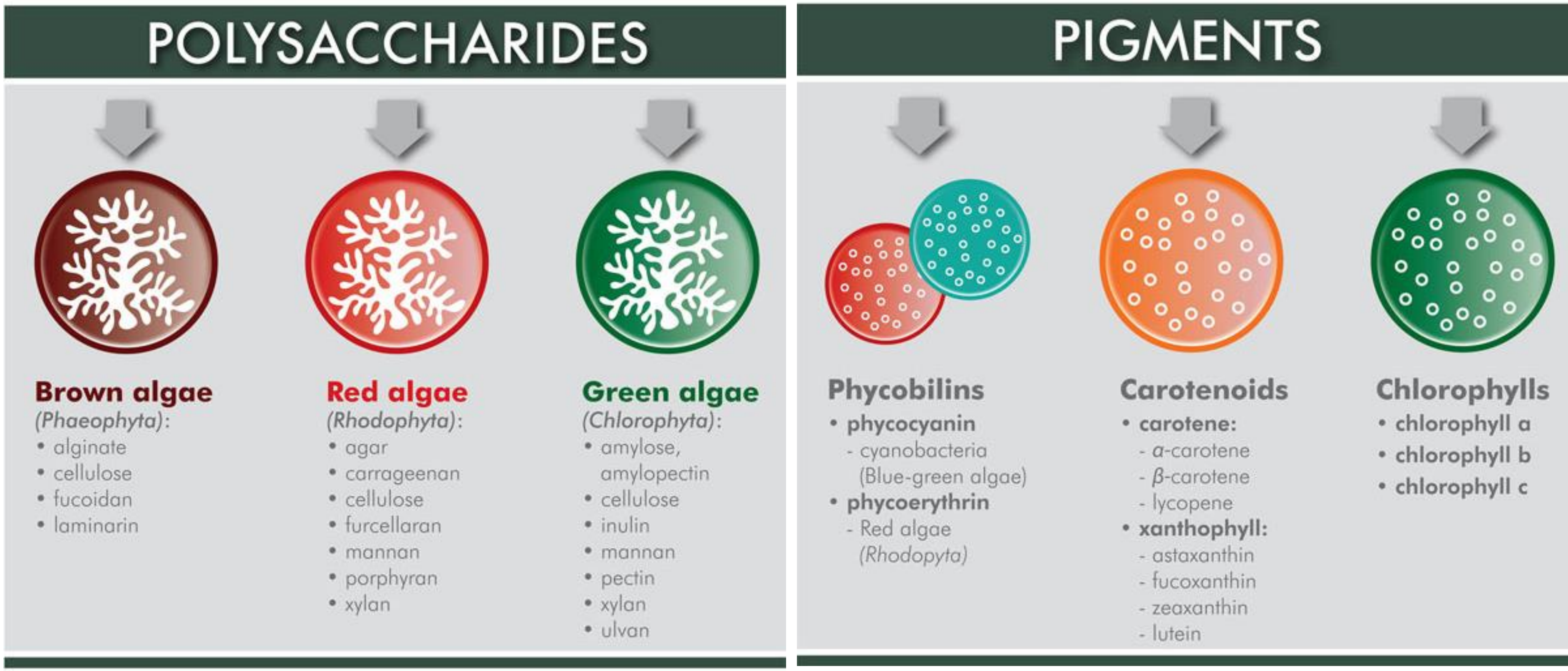
- Bohatá na proteiny, PUFA a minerální látky
- Studie z roku 2002 ukazující na přítomnost endotoxinů se nikdy neprokázala
- Oblíbená zelená potravina pro detoxikaci organismu

Interkosmos program

- Součástí sovětského vesmírného programu byla kultivace chlorelly v beztížných podmínkách


DIETARY FEED SUPPLEMENTS
COSMETICS
antiaging cream
regenerating skin cream
emollient products
antiirritant products
sun protection cream
hair care products
Commercial products with algae or extracts
PRODUCTS FOR PLANTS
fertilizers
biostimulants and bioregulators
DIETARY SUPPLEMENTS

Algal extracts



COMPOUNDS WITH ANTI-OXIDANT ACTIVITY

glutathione (GSH)		
vitamins	ascorbate (vitamin C)	
	tocopherol (vitamin E)	• α-, γ-, δ- tocopherol
carotenoids	α-carotene and β-carotene	
	fucoxanthin and astaxanthin	
polyphenols	phlorotannin – brown algal polyphenol	• fucol • phlorethol • fucophlorethol • fuhalol • isofuhalol • eckol
	catechin	• catechin (3-hydroxyflavan)/ catechin gallate • epicatechin/epicatechin gallate • epigallocatechin/epigallocatechin gallate
	phenolic acid	
	flavonoids	• anthocyanins • flavonols • flavanols • flavanones • flavones • isoflavones
	tannins	
	lignans	
mycosporine-like amino acids	mycosporine-glycine	

PLANT GROWTH-PROMOTING SUBSTANCES/HORMONES

plant hormones (phytohormones)	cytokinins	
	auxins	
	gibberellins	
	abscisic acid (ABA)	
	ethylene	
betaines		
polyamines		
sterols	fucosterol	Brown algae (*Phaeophyta*)
	cholesterol	
	cholesterol	Red algae (*Rhodophyta*)
	ergosterol	Green algae (*Chlorophyta*)
	24-methylenecholesterol	
	cholesterol	

OTHER COMPOUNDS

vitamins	B_{12}, K, C, E, A, D		
minerals	K, Ca, Mg, Na, Zn, Cu, Co, I, B		
peptides and proteins			
lectins			
lipids	fatty acids	polyunsaturated fatty acids (PUFAs)	• γ-linolenic acid (GLA) • arachidonic acid (AA) • eicosapentaenoic acid (EPA) • docosahexaenoic acid (DHA)
	sterols		
diterpenes	dolabellanes		
	hydroazulenoids		
	xenicanes		
	extended sesquiterpenoids		

Polysacharidy

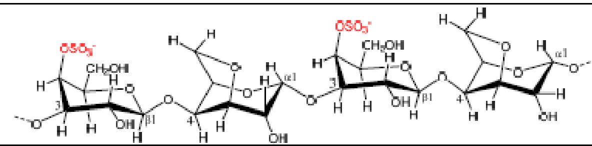
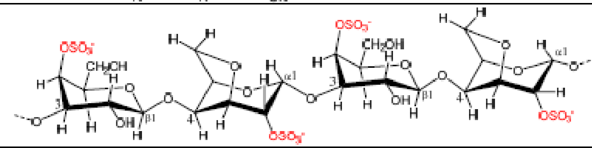
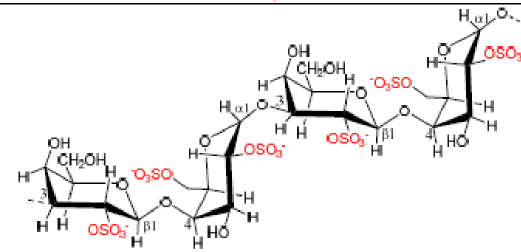
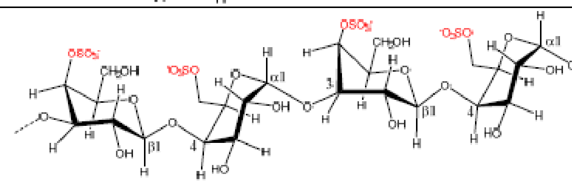
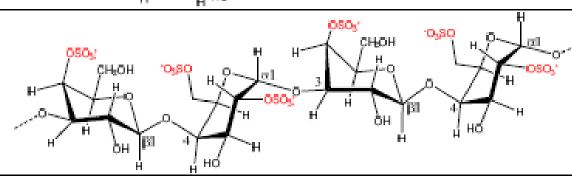
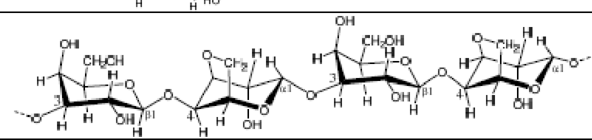
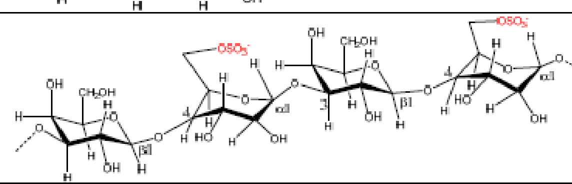
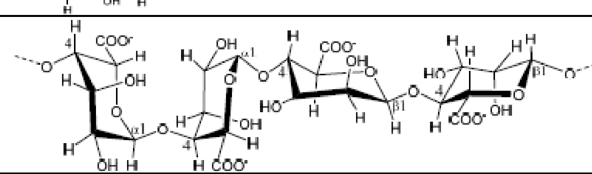
- Agar, alginát, karagenan

The market for seaweed-derived hydrocolloids, agars, alginates, and carrageenans [1].

Product	Global Production (ton/year)	Retail Price (US$/kg)	Approximate Gross Market Value (US$ million/year)
Agars	10,600	18	191
Alginates	30,000	12	339
Carrageenans	60,000	10.4	626

Physico-chemical properties for agar and carrageenans. The numbers are estimates. Viscosity values are given as (centipoise, cP) that is equivalent to $N \cdot s \cdot m^{-2}$ [56].

Properties	Agar	Carrageenan
Solubility	Boiling water	Boiling water
Gel Strength (1.5% at 20 °C)	700–1000 g/cm^3	100–350 g/cm^3
Viscosity (1.5% at 60 °C)	10–100 centipoise	30–300 centipoise
Melting point	85–95 °C	50–70 °C
Gelling point	32–45 °C	30–50 °C

Seaweed Source	Products	Main Chemical Structures	Applications
Kappaphycus alvarezii	κ-Carrageenan		Gelling agent (stiff and brittle gel)
Eucheuma spinosum	ι-Carrageenan		Gelling agent (flexible soft gel)
Gigartina spp. *Chondrus* spp.	λ-Carrageenan		Thickener
Kappaphycus alvarezii	μ-Carrageenan		κ-Carrageenan precursor
Eucheuma spinosum	ν-Carrageenan		ι-Carrageenan precursor
Gelidiella spp. *Gelidium* spp.	Agar/Agarose		Microbiology Gelling agent (strong and rigid)
Porphyra umbilicalis	Porphyran		Agar precursor
Laminaria spp. *Sargassum* spp.	Alginate		Gelling agent

Summary of Commercially Exploited Algae and the Corresponding Extracts

Scientific Name	Class	Extracts
Gracilaria chilensis	Floridophyceae	Agar
Ahnfeltia plicata	Floridophyceae	Agar/Carrageenan
Gelidium lingulatum	Floridophyceae	Agar
Pterocladia spp.	Floridophyceae	Agar
Hypnea spp.	Floridophyceae	Agar
Chondrus crispus	Floridophyceae	Carrageenan
Gigartina skottsbergii	Floridophyceae	Carrageenan
Gigartina canaliculata	Floridophyceae	Carrageenan
Mazzaella laminaroides	Floridophyceae	Carrageenan
Sarcothalia crispata	Floridophyceae	Carrageenan
Kappaphycus alvarezii	Floridophyceae	Carrageenan
Eucheuma denticulatum	Floridophyceae	Carrageenan
Iridaea spp.	Floridophyceae	Carrageenan
Laminaria hyperborea	Phaeophyceae	Alginate
Laminaria digitata	Phaeophyceae	Alginate
Laminaria japonica	Phaeophyceae	Alginate
Laminaria saccarina	Phaeophyceae	Alginate
Macrocystis pyrifera	Phaeophyceae	Alginate
Ascophyllum nodosum	Phaeophyceae	Alginate
Durvillea potatorum	Phaeophyceae	Alginate
Ecklonia spp.	Phaeophyceae	Alginate
Lessonia nigrescens	Phaeophyceae	Alginate
Lessonia trabiculata	Phaeophyceae	Alginate

Agar

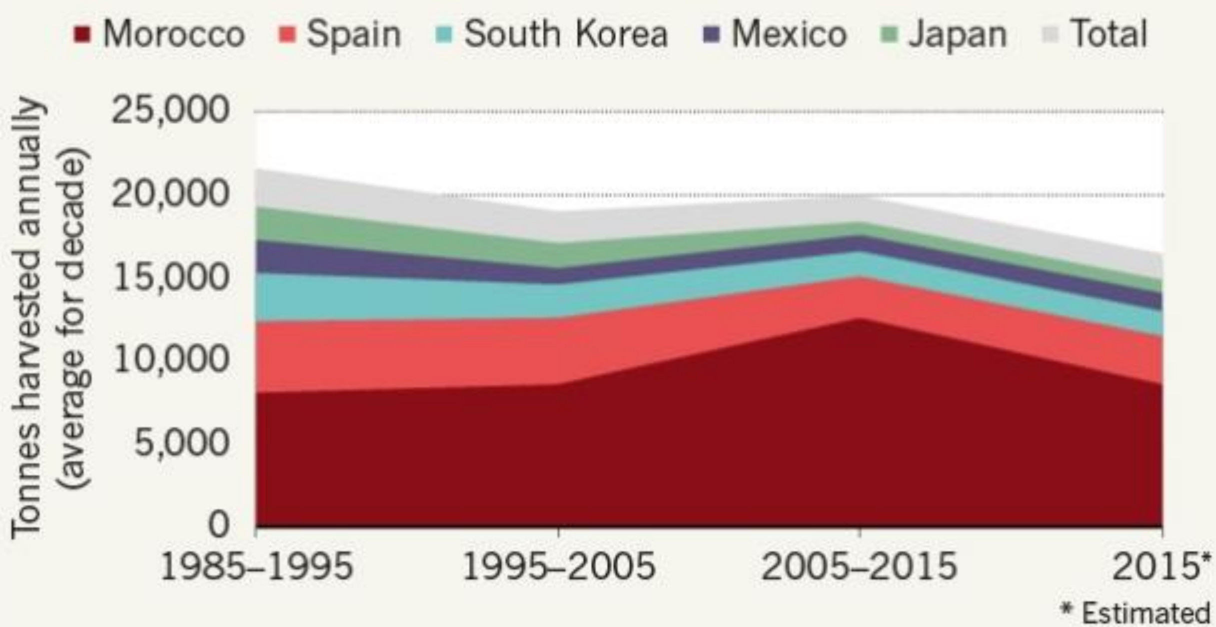
- D- and L-galactopyranosa
- Jméno dostal z malajského slova ‘agar-agar,’ což doslova znamená „makrořasa“.
- Jako želírovací přípravek katen je v Japonsku znám od 17. století
- Nejdůležitější použití agaru jako ztužovadla je při přípravě mikrobiologických půd
- Agarosa je frakce agaru zbavená iontů

Agar

- High-grade (bakretiologický) agar se získává z rodů *Pterocladia* and *Gelidium*
  - Španělsko, Francie, Portugalsko Maroko, USA, Mexiko, Nový Zéland, Jižní Korea, Indie, Čile, Japonsko, …
- Potravinářský agar z rodu Pterocladia
- Low grade agary se izolují z ruduch Gracilaria and Hypnea
- Celosvětová produkce agaru se odhaduje na cca 200 mil. USD

SEAWEED SHORTAGE

Harvests of *Gelidium* seaweed, from which the agar used in labs is made, are shrinking — particularly in Morocco, which is the world's major supplier.



Alginát

- Složka buněčné stěny u Phaeophyceae
- Mannuronová a guluronová kyselina
- Složení heteropolysacharidů závisí na druhu, kultivačních podmínkách a způsobu extrakce
- Nejvýhodnější pro získání alginátu jsou hnědé řasy (Laminariales a Fucales) rostlé ve studené vodě(<20°C)
- Roční produkce cca 50 tis tun
  - Skotsko, Norsko, Čína, USA
  - Roční obrat cca 215 mil USD

Alginát

- Algináty mají různé průmyslové využití
  - Zahušťovadlo (E401) – sodium alginate
  - Stabilizátor ve zmrzlinách
  - Látky, obvazový materiál
  - Zahušťovadlo tiskařských barev

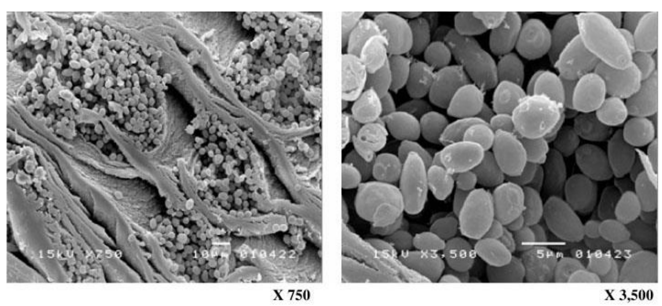


Figure 3. Yeast cells in the alginate gel layer of the ALM after 72 h of ethanol fermentation

Karagenan

- D-galactopyranosové bloky
- *Carrageenan* je irský výraz pro makrořasu
- V Irsku znám a používán od roku 1810
- Chondrus crispus býval jediným zdrojem, dnes se využívají také rody Euchema, Ahnfeltia a Gigartina
- Roční produkce 30 000 tun po celém světě
- Světový obchod s karagenanem se odhaduje na více než 640 mil USD

Karagenan

- Většina surového karagenanu je z Filipín
- Na trhu je možné najít tři třídy karagenanu
  - **Kappa** vytváří silné, tuhé gely v přítomnosti draselných iontů; dobře reaguje s mléčnými výrobky. Hlavním zdrojem je *Kappaphycus alvarezii*
  - **Iota** dělá jemnější a měkčí gely v přítomnosti vápenatých iontů. Nejvíce se získává z *Eucheuma denticulatum*
  - **Lambda** netvoří gel, použití má při zahuštění mléčných výrobků.

Výživové vlastnosti řas

Commodity	Protein	Carbohydrates	Lipids
Egg	47	4	41
Meat	43	1	34
Milk	26	38	28
Rice	8	77	2
Soybean	37	30	20
Anabaena cylindrica	43 – 56	25 – 30	4-7
Chlorella vulgaris	51 – 58	12 – 17	14 – 22
Dunaliella bioculata	49	4	8
Haematococcus	48	27	15
Spirulina platensis	64	25	7

Soy vs. Algae	Soy	Algae*
Protein	44%	55%
Lipids	2%	18%
Carbohydrates	39%	15%
Ash	15%	12%

Chemical composition of different algae (w/w)

Alga	Proteins	Carbohydrates	Lipids
Aphanizomenon flos-aquae	62	23	3
Chlorella pyrenoidosa	57	26	2
Chlorella vulgaris	51–58	12–17	14–22
Porphyridium cruentum	28–39	40–57	9–14
Schizochytrium sp.	-	-	50–77
Arthrospira maxima	60–71	13–16	6–7

Source: Adapted from Becker (2007) and Chisti (2007)

Kombu a Wakame – výživové hodnoty

Vitamin Contents of Marine Algae *Wakame* (*U. pinnatifida*) and *Kombu* (*L. japonica*) (in mg $[100\ g\ d.w.]^{-1}$)

Vitamins	Kombu	Wakame
β-carotene	2.99 ± 0.09	1.30 ± 0.12
Retinol equivalent	0.481 ± 0.015	0.217 ± 0.006
Vitamin B_1	0.24 ± 0.02	0.30 ± 0.04
Vitamin B_2	0.85 ± 0.08	1.35 ± 0.09
Vitamin B_6	0.09 ± 0.01	0.18 ± 0.02
Niacin	1.58 ± 0.14	2.56 ± 0.11

Dietary Fiber Content of *Wakame* (*U. pinnatifida*) and *Kombu* (*L. japonica*) (% d.w.)

	Soluble	Insoluble	Total
Kombu	32.6	4.7	37.3
Wakame	30	5.3	35.3

Mineral Composition of *Wakame* (*U. pinnatifida*) and *Kombu* (*L. japonica*) (in mg $[100\ g\ d.w.]^{-1}$)

Minerals	Kombu	Wakame
Ca	880 ± 20	950 ± 30
Mg	550 ± 15	405 ± 10
P	300 ± 10	450 ± 12
I	170 ± 5.5	26 ± 2.4
Na	2532 ± 120	6494 ± 254
K	5951 ± 305	5691 ± 215
Ni	0.325 ± 0.020	0.265 ± 0.015
Cr	0.227 ± 0.073	0.072 ± 0.026
Se	<0.05	<0.05
Fe	1.19 ± 0.03	1.54 ± 0.07
Zn	0.886 ± 0.330	0.944 ± 0.038
Mg	0.294 ± 0.017	0.332 ± 0.039
Cu	0.247 ± 0.076	0.185 ± 0.016
Pb	0.087 ± 0.021	0.079 ± 0.015
Cd	0.017 ± 0.007	0.028 ± 0.006
Hg	0.054 ± 0.005	0.022 ± 0.003
As	0.087 ± 0.006	0.055 ± 0.008

Table 1 Amino acid composition in mg g^{-1} protein of wakame (*U. pinnatifida*) and nori (*P. purpurea*)

Amino acid	*U. pinnatifida*	*P. purpurea*
Aspartic acid	75.60±12.12	66.58±3.63
Serine	33.96±3.04	46.25±2.47
Glutamic acid	120.85±20.26	83.04±6.13
Glycine	65.75±7.8	75.39±6.26
Histidine	17.11±1.17	22.04±1.00
Arginine	88.19±8.49	89.98±8.14
Threonine	29.22±1.24	50.10±3.98
Alanine	97.57±8.20	80.54±7.29
Proline	44.26±3.89	37.97±0.19
Cystine	3.26±0.30	4.58±0.55
Tyrosine	20.99±0.64	29.38±0.92
Valine	58.48±4.77	47.98±3.61
Methionine	1.41±0.21	13.73±0.43
Lysine	39.96±3.40	29.91±1.01
Isoleucine	50.82±4.36	34.44±1.03
Leucine	86.14±7.39	53.23±1.45
Phenylalanine	48.46±3.93	78.15±2.50

Table 2 Mineral content (mg100 g^{-1} dry weight) of wakame (*U. pinnatifida*) and nori (*P. purpurea*)

Mineral	*U. pinnatifida*	*P. purpurea*
Calcium	693.2±7.6	359.2±4.1
Phosphorus	1070.0±7.0	720.2±6.1
Iron	7.94±0.80	10.5±0.11
Magnesium	630.2±8.2	233.9±7.2
Zinc	3.86±0.27	3.29±0.24
Iodine	9.6±0.73	0.54±0.05
Sodium	3,511.0±26.0	728.2±4.04
Potassium	5,679.0±22.3	1,602.0±4.03
Manganese	0.69±0.02	2.53±0.05
Copper	0.19±0.01	0.57±0.02

Table 3 Vitamin content of wakame (*U. pinnatifida*) and nori (*P. purpurea*). Results are expressed in dry weight of sample

Vitamin	*U. pinnatifida*	*P. purpurea*
Vitamin A (UI kg^{-1})	4,729±23.3	23,830±17.2
Vitamin B_1 (mg kg^{-1})	0.30±0.03	0.40±0.02
Vitamin B_2 (mg kg^{-1})	0.68±0.03	1.89±0.09
Vitamin B_5 (mg kg^{-1})	2.0±0.11	2.7±0.12
Vitamin B_8 (μg g^{-1})	0.22±0.01	0.10±0.01
Vitamin B_{12} (μg100 g^{-1})	0.16±0.01	2.90±2.7
Vitamin B_6 (mg kg^{-1})	1.5±0.02	0.9±0.08
Vitamin B_3 (mg kg^{-1})	<5	<5
Folic acid (μg g^{-1})	0.79±0.08	<0.02
Vitamin C (mg100 g^{-1})	3.10±0.11	9.73±0.31
Vitamin E (mg kg^{-1})	6.3±0.12	9.3±0.27

Table 2. Chemical composition (g 100 g^{-1} dry weight) of *Gracilaria salicornia* and *Ulva lactuca*

Seaweed	Crude lipid	Crude protein	Crude fibre	Dry weight	Ash content
G. salicornia	2.00 ± 0.92^a	9.58 ± 0.15^a	10.4 ± 0.89^a	9.98 ± 0.15^a	38.91 ± 1.62^a
U. lactuca	0.99 ± 0.00^a	10.69 ± 0.67^a	5.6 ± 1.69^b	5.96 ± 0.33^b	18.03 ± 2.37^b

Results are the means of triplicate determinations ± SD.
Values in columns with different superscripts are significantly different ($P < 0.05$).
[a,b] Means in columns with different letters are significantly different ($P < 0.05$).

Table 3. Amino acid concentrations in *Gracilaria salicornia* and *Ulva lactuca*

Amino acid	*G. salicornia* (mg g^{-1} protein)†	*U. lactuca* (mg g^{-1} protein)†	Cereal (Pellett[22])	Legume (Pellett[22])	FAO/WHO/UNU[11] requirement pattern
Aspartic acid	53.9 ± 8.4^a	49.7 ± 5.6^b	–	–	–
Glutamic acid	75.9 ± 6.3^a	70.7 ± 6.6^a	–	–	–
Serine	34.6 ± 2.1^a	28.7 ± 2.4^b	–	–	–
Glycine	75.6 ± 3.8^a	39.7 ± 2.1^b	–	–	–
Histidine	14.3 ± 2.7^a	15.2 ± 1.5^a	–	–	–
Arginine*	75.78 ± 3.5^a	37.9 ± 1.4^b	–	–	–
Threonine*	32.9 ± 2.2^a (0.96)	31.1 ± 2.5^b (0.91)	33.6	40	34
Alanine	75.5 ± 7.3^a	43.3 ± 2.4^b	–	–	–
Proline	39.8 ± 5.6^a	37.9 ± 2.1^b	–	–	–
Tyrosine*	75.9 ± 5.6^a	23.4 ± 4.2^b	–	–	–
Valine*	41.4 ± 4.9^a (1.18)	39.2 ± 3.5^b (1.12)	51.1	50.5	35
Methionine*	77.5 ± 2.8^a (3.1)	5.9 ± 1.4^b (0.23)	41.1	25.3	25§
Isoleucine*	30.3 ± 3.4^a (1.08)	21.7 ± 2.6^b (0.77)	39.8	45.3	28
Leucine*	76.6 ± 6.1^a (1.16)	45.1 ± 4.8^b (0.68)	86.5	78.9	66
Phenylalanine*	32.7 ± 5.7^a (1.72)	28.4 ± 3.6^b (0.82)	83.0‡	84.9‡	63
Lysine*	77.1 ± 5.8^a (1.32)	25.4 ± 0.5^b (0.43)	30.5	67.1	58
Tryptophan	ND	ND	–	–	–
Essential amino acids	520.18 ± 22.47^a	258.1 ± 11.6^b	–	–	328
Non-essential amino acids[A]	369.62 ± 33.0^a	285.2 ± 17.27^b	–	–	661
Total amino acids	889.78 ± 22.64^a	543.3 ± 15.14^b	–	–	–

Table 4. Relative fatty acid content of *Gracilaria salicornia* and *Ulva lactuca* (% of total fatty acid content)

Fatty acids	*G. salicornia*	*U. lactuca*
C 12 : 0	6.98 ± 0.50^{a}	6.03 ± 0.85^{a}
C 14 : 0	5.5 ± 0.86^{a}	5.53 ± 0.13^{a}
C16 : 0	33.39 ± 8.86^{a}	34.33 ± 2.65^{a}
C 16 : 1	2.46 ± 0.12^{a}	2.48 ± 0.08^{a}
C 18 : 0	3.04 ± 0.66^{a}	2.44 ± 0.29^{a}
C 18 : 1	11.72 ± 2.01^{a}	2.63 ± 0.41^{b}
C 18:2 ω6	1.45 ± 0.38^{b}	4.89 ± 0.78^{a}
C 18:3 ω3	1.65 ± 0.04^{b}	2.77 ± 0.06^{a}
C 20:4 ω6	8.05 ± 1.98^{a}	8.53 ± 0.27^{a}
C 20:5 ω3	1.53 ± 0.27^{b}	3.65 ± 0.31^{a}
C 22:5 ω3	4.7 ± 0.19^{a}	4.98 ± 0.89^{a}
Saturated fatty acids	48.92 ± 6.83^{a}	48.34 ± 3.67^{a}
Monounsaturated	16.36 ± 1.54^{a}	5.11 ± 0.5^{b}
PUFAs	17.30 ± 1.18^{b}	24.84 ± 1.03^{a}
PUFAs ω6	10.14 ± 0.7^{b}	13.43 ± 0.5^{a}
PUFAs ω3	7.89 ± 0.43^{b}	11.41 ± 0.52^{a}
Ratio ω6/ω3	1.2	1.17

FIG. 1 OMEGA-3 AND OMEGA-6 FATTY ACIDS

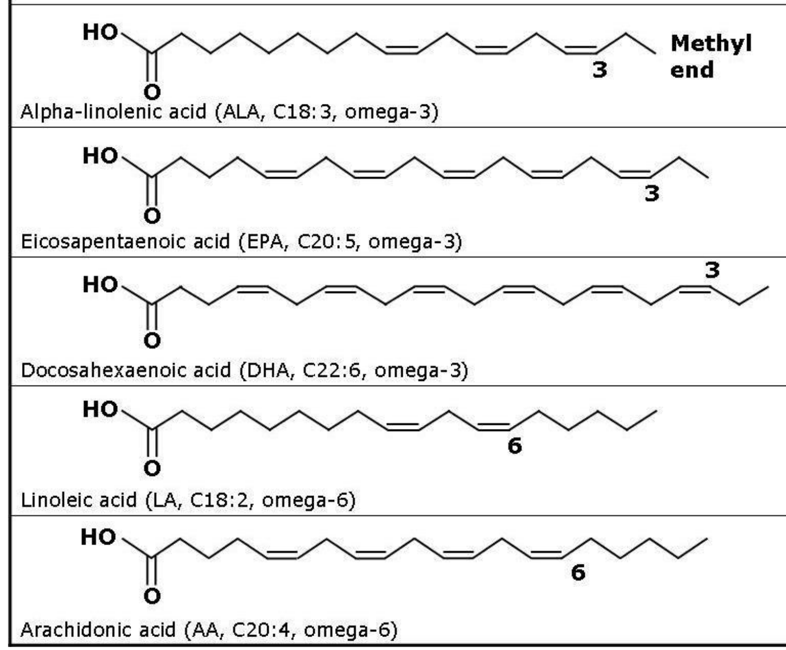
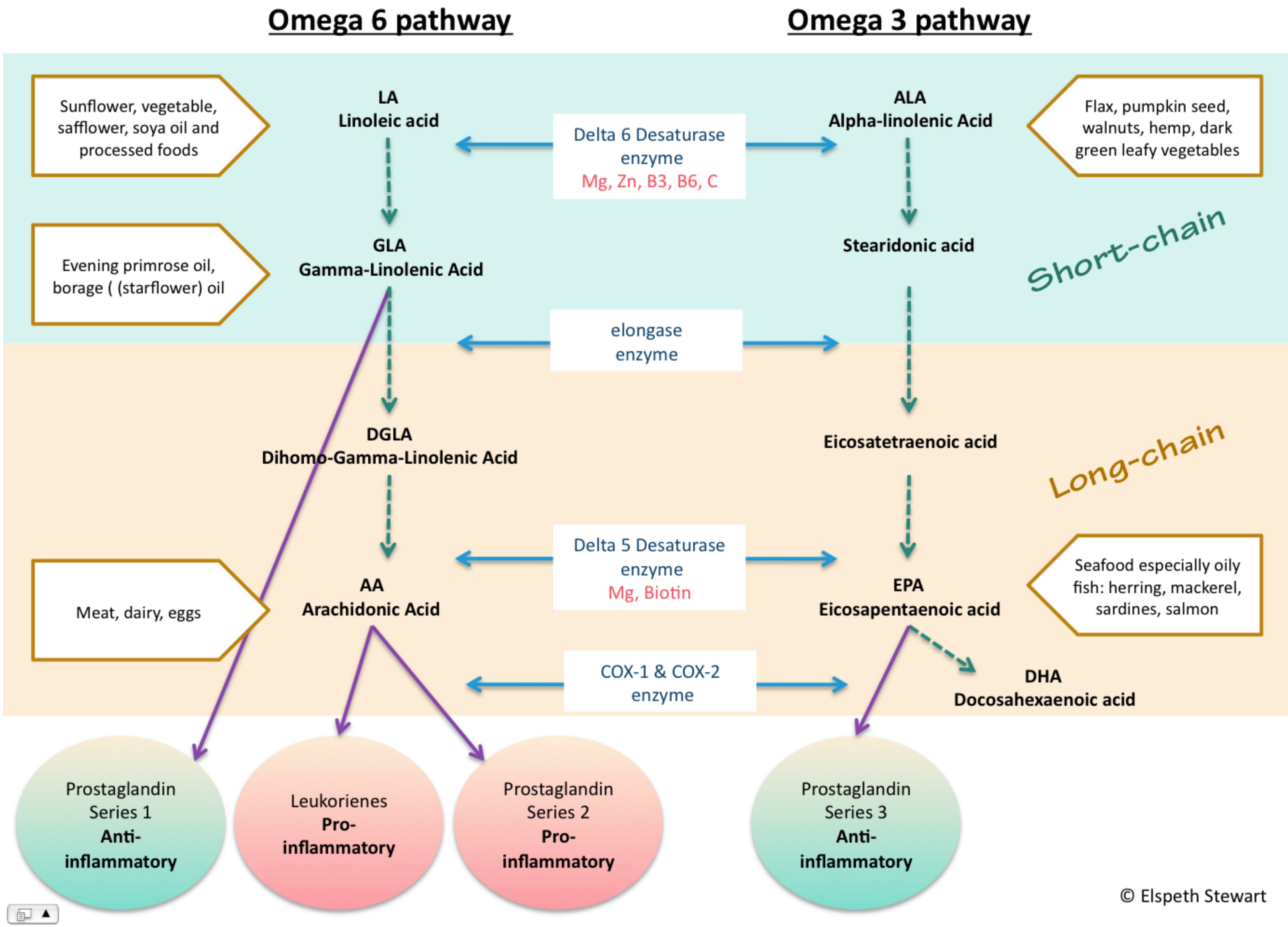


Table 5. Elemental composition in *Gracilaria salicornia* and *Ulva lactuca* (mg 100 g^{-1} dry weight)

			Foodstuff (USDA[29])				
Mineral	*G. salicornia*	*U. lactuca*	Lettuce	Cabbage	Carrots	Broccoli	Spinach
Potassium	$11\,380.06 \pm 73.45^{a}$	2414.02 ± 26.89^{b}	956.42	1931.1	3747.2	3381.2	4798.8
Calcium	948.45 ± 7.7^{b}	2782.13 ± 11.12^{a}	177.48	368.95	386.43	502.9	851.4
Sodium	1035.92 ± 61.48^{b}	1805.44 ± 58.6^{a}	138.04	141.3	807.99	353.1	679.4
Iron	67.35 ± 7.77^{b}	199.45 ± 5.86^{a}	4.23	4.63	3.51	7.81	23.3
Manganese	4.16 ± 0.05^{a}	2.11 ± 0.22^{b}	1.23	1.24	1.64	2.184	7.71
Nickel	0.92 ± 0.03^{a}	0.76 ± 0.01^{a}	–	–	–	–	–
Copper	0.57 ± 0.07^{b}	1.45 ± 0.21^{a}	0.14	0.18	052	0.52	1.11
Cobalt	0.24 ± 0.05^{a}	0.15 ± 0.03^{b}	–	–	–	–	–
Total cations	$13\,438 \pm 143^{a}$	7205.51 ± 102^{b}	–	–	–	–	–



Omega 6 pathway
Omega 3 pathway
Sunflower, vegetable, safflower, soya oil and processed foods
LA
Linoleic acid
Delta 6 Desaturase enzyme
Mg, Zn, B3, B6, C
ALA
Alpha-linolenic Acid
Flax, pumpkin seed, walnuts, hemp, dark green leafy vegetables
Evening primrose oil, borage ((starflower) oil
GLA
Gamma-Linolenic Acid
Stearidonic acid
Short-chain
elongase enzyme
DGLA
Dihomo-Gamma-Linolenic Acid
Eicosatetraenoic acid
Long-chain
Meat, dairy, eggs
AA
Arachidonic Acid
Delta 5 Desaturase enzyme
Mg, Biotin
EPA
Eicosapentaenoic acid
Seafood especially oily fish: herring, mackerel, sardines, salmon
COX-1 & COX-2 enzyme
DHA
Docosahexaenoic acid
Prostaglandin Series 1 Anti-inflammatory
Leukorienes Pro-inflammatory
Prostaglandin Series 2 Pro-inflammatory
Prostaglandin Series 3 Anti-inflammatory

Doplňky stravy, farmacie

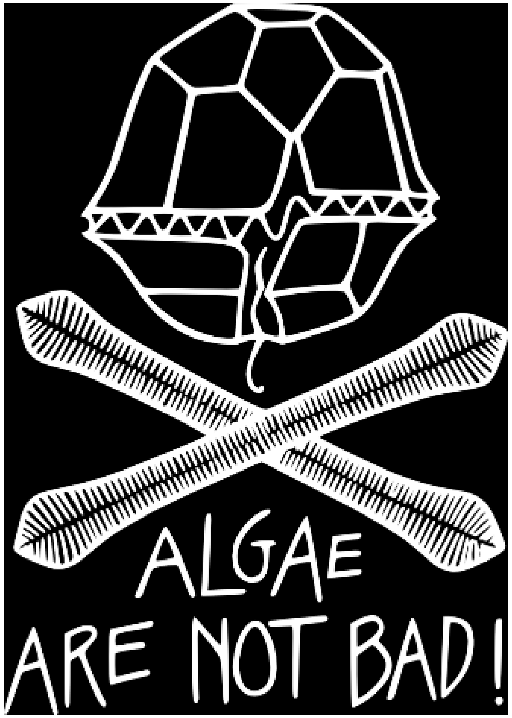
Table 2 Microalgal species with high relevance for biotechnological applications

Species/group	Product	Application areas
Spirulina platensis/Cyanobacteria	Phycocyanin, biomass	Health food, cosmetics
Chlorella vulgaris/Chlorophyta	Biomass	Health food, food supplement, feed surrogates
Dunaliella salina/Chlorophyta	Carotenoids, β-carotene	Health food, food supplement, feed
Haematococcus pluvialis/Chlorophyta	Carotenoids, astaxanthin	Health food, pharmaceuticals, feed additives
Odontella aurita/Bacillariophyta	Fatty acids	Pharmaceuticals, cosmetics, baby food
Porphyridium cruentum/Rhodophyta	Polysaccharides	Pharmaceuticals, cosmetics, nutrition
Isochrysis galbana/Chlorophyta	Fatty acids	Animal nutrition
Phaedactylum tricornutum/Bacillariohyta	Lipids, fatty acids	Nutrition, fuel production
Lyngbya majuscule/Cyanobacteria	Immune modulators	Pharmaceuticals, nutrition

- Doplňky stravy bohaté na aktivní látky
- Zdravá výživa, imunomodulace, atd.
- Studie systematicky prokazují pozitivní vliv na lidské zdraví

Biologicky aktivní látky z řas

- Mikrořasy, ale i některé makrořasy je možné využít jako zdroje medicínsky zajímavých látek s širokou škálou účinku
  - Antibabakteriální
  - Protivirové
  - Proti houbám
  - Antioxidanty
  - Protizánětlivé
  - Protirakovinové
  - …



PROPERTIES OF COMPOUNDS IN ALGAL EXTRACTS

antibacterial	• proteins • polyphenols • polysaccharides • pigments: chlorophyll and carotenoids • PUFAs
antifungal	• PUFAs • pigments: chlorophyll and carotenoids • terpens • phenols
antioxidative	• proteins • mycosporine-like amino acids • glutathione • polyphenols • polysaccharides • PUFAs • carotenoids • tocopherol • ascorbate
antiinflammatory	• proteins • carotenoids • polysaccharides • sterols - fucosterol • polyphenols - phlorotannins • porphyrin derivatives: pheophorbide a and pheophytin a
antitumor	• polyphenols • carotenoids • polysaccharides
antiviral	• proteins • diterpens • polyphenols • polysaccharides • carotenoids

Extracted compound	Algal species	Extraction method	Target
(a) **Antibacterial activity**			
PUFAs	*Gracilaria corticata* *Ulva fasciata* *Enteromorpha compressa*	Solvent extraction with hexane, chloroform, ethyl acetate, chloroform:alcohol (1:1), methanol by soaking the material in the solvents thrice overnight at room temperature (1:3v/v)	Bacteria pathogenic to fish: *Edwardsiella tarda, Vibrio alginolyticus, Pseudomonas fluorescens, P. aeruginosa, Aeromonas hydrophila*
Fats (palmitic acid) Proteins (amino acids) Bioflavonoids (rutin, quercetin, and kaempherol)	*Ulva reticulata* *Caulerpa occidentalis* *Cladophora socialis* *Dictyota ciliolate* *Gracilaria dendroides*	Extraction of powdered algal samples with ethanol, chloroform, petroleum ether, water. Samples were soaked in the solvents for 24 h and homogenized in a blender with the solvents at room temperature	*Escherichia coli, P. aeruginosa, Stapylococcus aureus, Enterococcus faecalis*
Sulfated polysaccharide	*Sargassum swartzii*	Dried seaweed powder was extracted with water at 90–95°C for 16 h. The syrup was filtered through filter paper, cooled, and precipitated with ethanol	*S. aureus, Proteus vulgaris, E. coli, Bacillus subtilis, Pseudomonas aeroginosa, Salmonella typhi, Shigella flexineri, Klebsiella pneumoniae, E. faecalis, Aeromonas hydrophilla*
Carotenoids, alkaloids, favanoids, fatty acids, saponins, amino acids, carbohydrates	*Chlorococcum humicola*	The algae were centrifuged to remove the water content. Fresh biomass was extracted for 15 min with organic solvents: acetone, benzene, chloroform, diethyl ether, ethyl acetate, ethanol, hexane, methanol	Effect of pigments: β-carotene and chlorophyll on: *E. coli, P. aeruginosa, B. subtilis, Salmonella typhimurium, K. pneumoniae, Vibreo cholerae, S. aureus*
Fatty acids, phytol, fucosterol, neophytadiene, palmitic, palmitoleic, and oleic acids	*Himanthalia elongate*, *Synechocystis* sp.	PLE in accelerated solvent extractor equipped with a solvent controller. Three different solvents hexane, ethanol, water were used	*S. aureus, E. coli*
PUFAs, indolic derivative, β-ionone, neophytadiene	*Dunaliella salina*	Sub- and supercritical CO_2 extraction	*E. coli, S. aureus*
Short-chain fatty acids	*Haematococcus pluvialis*	PLEs were performed with hexane and ethanol at different temperatures: 50, 100, 150, 200°C for 20 min	*E. coli, S. aureus*

(b) Antiviral activity			
Polysaccharide	*Constantinea simplex* *Farlowia mollis*	Frozen samples were combined with citrate-phosphate buffer at pH 7.0, homogenized in a blender, and incubated at 4°C overnight	Mice: Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), vaccinia virus, vesicular stomatitis virus, encephalomyocarditis virus, Semliki Forest virus, murine cytomegalovirus
Sulfated polysaccharide	*Sargassum patens*	Seaweed was washed and extracted with boiling water for 2 h. After centrifugation, the supernatant was concentrated and precipitated with ethanol	Vero cells (African green monkey kidney cell line): HSV-2
Sulfated galactofucan, fucan, galactan; depolymerized galactofucan sulfate, galactofucan sulfate	*Undaria pinnatifida* *Splachnidium rugosum* *Gigartina atropurpurea* *Plocamium cartilagineum*	For *U. pinnatifida* and *S. rugosum*: dry, ground samples were extracted for 6 h with 1% (w/v) H_2SO_4 at 20°C, 0.2 M HCl at 20°C, or 2% $CaCl_2$ at 75°C. For *G. atropurpurea* and *P. cartilagineum*: dry, ground algae were placed in $NaHCO_3$ solution (0.05 M), left to swell (20min) then heated (90°C, 2 h)	Human foreskin fibroblast: HSV-1 and 2
Sulfated polysaccharide	*Padina pavonia*	Algal biomass was extracted with water at 80°C for 2 h (twice)	Vero cell culture: HSV, Hepatitis A (HAV, Hep A)
Bromophenols	*Polysiphonia morrowii*	Freeze-dried alga was extracted with 80% (v/v) methanol (in water, 80% MeOH) at 80°C eight times, each of which took 1 h for a total of 10 h	Fish pathogenic viruses: infectious hematopoietic necrosis virus and infectious pancreatic necrosis virus
Sulfated polysaccharide	*Sphaerococcus coronopifolius* *Boergeseniella thuyoides*	Polysaccharides from seaweed powder were extracted in hot distilled water at 80°C for 4 h with magnetic stirring	Vero cells culture: human immunodeficiency virus (HIV) and HSV-1
Polysaccharide	*Acrosiphonia orientalis*	Polysaccharides were extracted from the dried fronds using 0.1 N HCl at 95°C for 12 h and the extract was precipitated by adding ethanol	Shrimp pathogen—white spot syndrome virus
Diterpenes	*Dictyota pfaffii* *D. menstrualis*	Air-dried specimens were extracted with CH_2Cl_2/MeOH (7:3) and MeOH	HSV-1
Diterpenes	*D. menstrualis*	Extraction with CH_2Cl_2/MeOH	HIV type 1 (HIV-1)

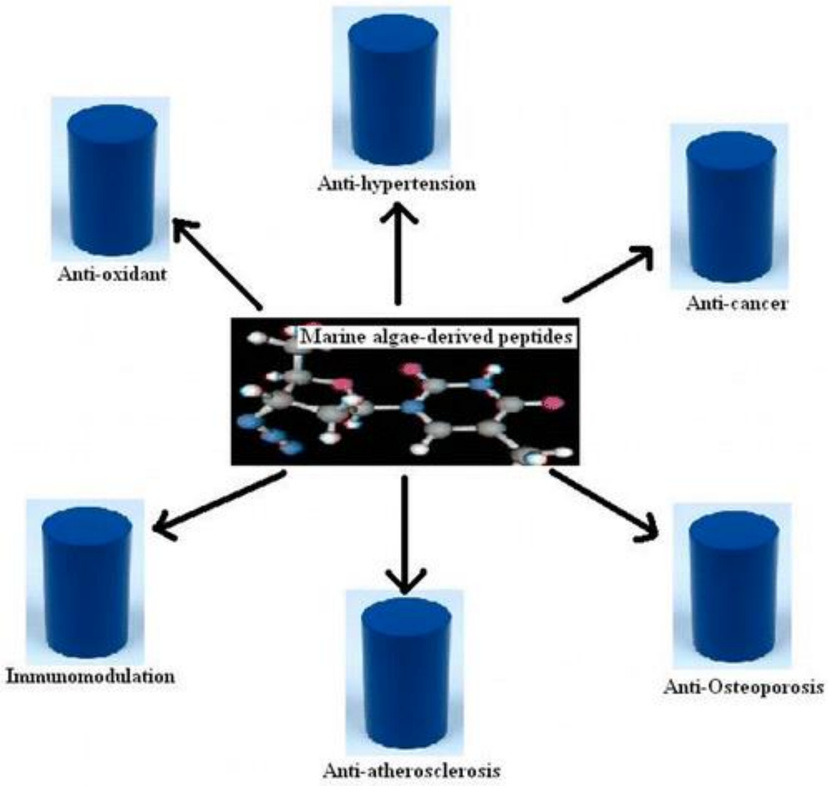
(c) Antifungal activity			
Carotenoids, alkaloids, flavonoids, fatty acids, saponins, amino acids, carbohydrates	*C. humicola*	The algal samples were centrifuged to remove the water content. Fresh algae were extracted for 15 min with organic solvents: acetone, benzene, chloroform, diethyl ether, ethyl acetate, ethanol, hexane, methanol	Effect of pigments: β-carotene and chlorophyllon: *Candida albicans*, *Aspergillus niger*, *A. flavus*
Terpenes and phenols (terpenes were present in all algal extracts, phenols for ∗)	*Stypopodium zonale* *Laurencia dendroidea*, *Ascophyllum nodosum* (∗) *Sargassum muticum* (∗) *S. filipendula*, *S. stenophyllum*, *Pelvetia canaliculata* (∗) *Fucus spiralis* *Laminaria hyperborea* *Gracilaria edulis*	The algae were washed, air-dried, powdered, and extracted with ethanol (95%)	*Colletotrichum lagenarium*, *A. flavus*
Phenols	*Padina pavonica* *Sargassum vulgare*	First method—marine alga was macerated for 3 days in methanol at room temperature in an orbital shaker; the second extraction was in methanol using a Soxhlet extractor for 6 h	*Candida*
Fatty acids, phytol, fucosterol, neophytadiene, palmitic, palmitoleic and oleic acids	*H. elongate*, *Synechocystis* sp.	PLE in accelerated solvent extractor equipped with a solvent controller. Three solvents hexane, ethanol, water were used	*C. albicans*, *A. niger*
PUFAs, indolic derivative, β-ionone, neophytadiene	*D. salina*	Sub- and supercritical CO_2 extraction	*C. albicans*, *A. niger*
Short-chain fatty acids	*H. pluvialis*	PLEs were performed with hexane and ethanol at different temperatures: 50, 100, 150, 200°C for 20 min	*C. albicans*, *A. niger*

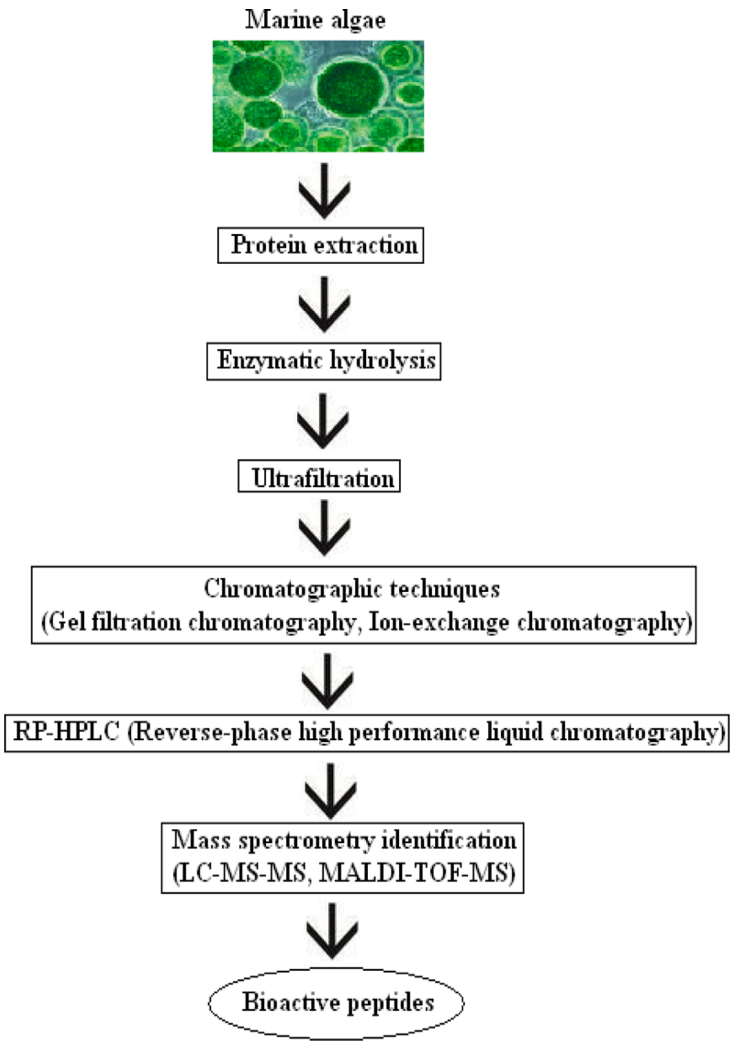
(d) Antioxidative activity			
Sulfated polysaccharide	*S. swartzii*	Dried seaweed powder was extracted with water at 90–95°C for 16 h. The syrup was then filtered through filter paper, cooled, and precipitated with ethanol	Total antioxidant activity of the extract Reducing power of the extract 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay Hydrogen peroxide scavenging assay 2,2′-Azinobis-(3-ethylbenzo-thiazoline-6-sulfonic acid) inhibition assay
Sulfated polysaccharides: (1) iota, kappa, and lambda carrageenans, fucoidan, (2) fucans	*(1) Fucus vesiculosus* *(2) Padina gymnospora*	Powdered algae were suspended with NaCl. pH was adjusted to 8.0. For proteolytic digestion protease from *Esporobacillus* was added. Incubation at 60°C under shaking lasted for 24 h	Superoxide anion scavenging activity Hydroxyl radical scavenging activity Liver microsomal (from Wistar rats) lipid peroxidation
Phenols	*Bifurcaria bifurcata* *Cystoseira tamariscifolia* *Fucus ceranoides* *Halidrys siliquosa*	Accelerated solvent extraction system. The biomass was extracted with a mixture of dichloromethane methanol (1:1, v:v) at 75°C and 1500 psi	DPPH Reducing activity *β*-carotene–linoleic acid system
Usujilene—kind of mycosporine-glycine like amino acid	*Porphyra yezoensis*	Ground freeze-dried material was extracted with *n*-hexane, ethyl acetate, acetone, chloroform/methanol (2:1), methanol, and hot water (90°C) under stirring	Ferric thiocyanate method Thiobarbituric acid method
Phenols	*P. pavonica* *S. vulgare*	First method—marine alga was macerated for 3 days in methanol at room temperature in an orbital shaker; the second extraction was in methanol using a Soxhlet extractor for 6 h	DPPH
Phenols	*Caulerpa racemosa*	Seaweed powder was placed into an extraction vessel and was extracted with solvent under different MAE conditions	Hydroxyl radical scavenging assay DPPH determination of reducing power
Phenols	*A. nodosum* *P. canaliculata* *F. spiralis* *Ulva intestinalis*	SLE and PLE was employed to extract algae with 100% water, ethanol/water (80:20, v:v), and acetone/water (80:20, v:v)	DPPH Ferric reducing antioxidant power Ferrous ion chelating capability assay
Fatty acids, phytol, fucosterol, neophytadiene, palmitic, palmitoleic, and oleic acids	*H. elongate* *Synechocystis sp.*	PLE in accelerated solvent extractor equipped with a solvent controller. Three solvents hexane, ethanol, water were used	Trolox equivalent antioxidant capacity assay
Carotenoid	*D. salina*	PLEs were performed using an accelerated solvent equipped with a solvent controller. Three solvents hexane, ethanol, water were used	Trolox equivalent antioxidant capacity assay
Antioxidants—carotenoids	*Spirulina platensis*	Extractions were performed in accelerated solvent extractor equipped with a solvent controller. Three solvents hexane, petroleum ether, ethanol were used. Extractions were performed at temperatures (60, 115, 170°C) and extraction times (3, 9, and 15 min)	DPPH
Polyphenol, flavonoid	*Chlorella vulgaris C-C*	Supercritical fluid equipment and ultrasonic extraction	DPPH Ferric reducing antioxidant power Metal chelating activity Superoxide anion radical scavenging capacity

(e) Anti-inflammatory activity			
Fucosterol, phlorotannins (phloroglucinol, eckol, dieckol, 7-phloroeckol, phlorofucofuroeckol A, dioxinodehydroeckol)	*Eisenia bicyclis*	The powdered leafy thallus of alga was refluxed with methanol for 3 h	Inhibition against production of lipopolysaccharide (LPS) induced nitric oxide (NO) and tert-butylhydroperoxide induced reactive oxygen species, suppression against expression of inducible NO synthase, and cyclooxygenase-2 in LPS-stimulated RAW 264.7 macrophages
Porphyrin derivatives: pheophorbide a, pheophytin a	*Saccharina japonica*	The powder of the whole plant of alga was refluxed with methanol for 3 h	Inhibitory activities against LPS-induced NO production, inducible NO synthase, and cyclooxygenase-2 expression in RAW 264.7 murine macrophage cells
Lactones, phenols, triterpenes, steroids, reduced carbohydrates	*Dichotomaria obtusata*	Distilled water was added to algal powder and vortexed in a shaker for 24 h at room temperature	Tests in mice: ear edema induced by 12-O-tetradecanoylphorbol acetate and writhing induced by acetic acid
(f) Antitumor activity			
Fucoidan	*Fucus evanescens*	Hot extraction	Mice with transplanted Lewis lung adenocarcinoma
Polyphenol: phlorotannin—dioxinodehydroeckol	*Ecklonia cava*	The lyophilized powder of alga was percolated in hot EtOH. The crude extract was partitioned with organic solvents to yield *n*-hexane, CH_2Cl_2, EtOAc, and *n*-BuOH fractions, as well as an H_2O residue	Inhibition of the proliferation of human breast cancer cells
Phenols	*B. bifurcata* *C. tamariscifolia* *F. ceranoides* *H. siliquosa*	Accelerated solvent extraction system. The biomass was extracted with a mixture of dichloromethane methanol (1:1, v:v) at 75°C and 1500 psi	Cytotoxic assay with three different tumoral cells lines (Daudi, Jurkat, and K562)
Crude polysaccharide	*Sargassum coreanum*	Biomass was pulverized into powder with a grinder. Buffer solution was added to the dried sample and then Neutrase. The reaction was performed for 12 h	HL-60 (human promyelocytic leukemia cell line); >30-kDa fraction of crude polysaccharides exhibited a marked anticancer activity in HL-60 cells
Polyphenol, flavonoid	*C. vulgaris C-C*	Supercritical fluid equipment and ultrasonic extraction	Extract of *C. vulgaris C-C* inhibits human lung cancer H1299, A549, and H1437 cells in a dose-dependent manner

Bioaktivní peptidy z řas

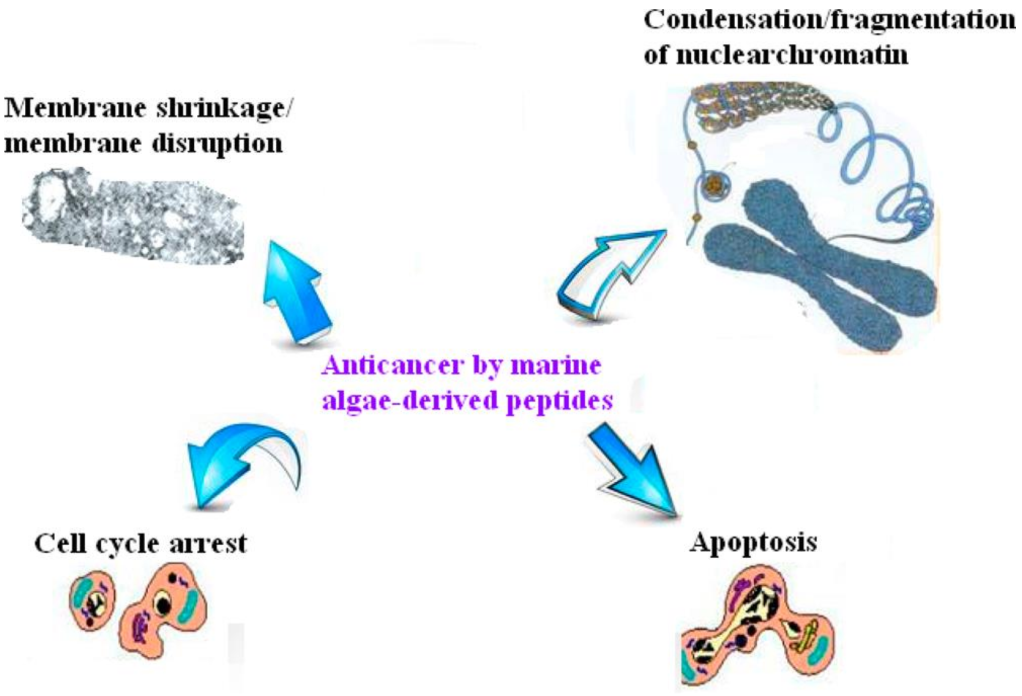
- Krátké řetězce (2-20 AA)
- Různé účinky na živé organizmy





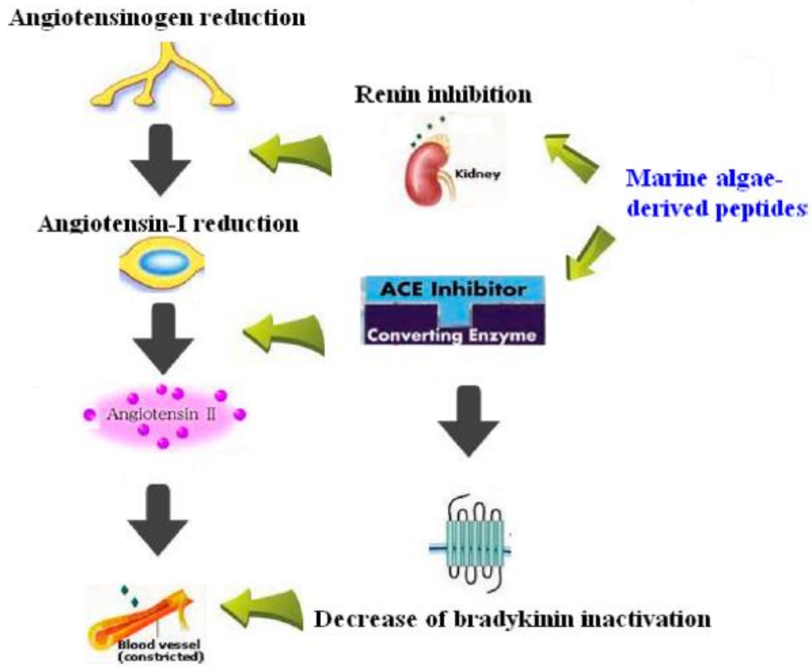
Protirakovinové peptidy

peptide name or sequence	source	enzyme	IC_{50}	in vitro/in vivo	mechanism of action
VECYGPNRPQF	*Chlorella vulgaris*	pepsin	70 μg/mL	in vitro (gastric cancer AGS cells)	antiproliferation and post-G1 cell cycle arrest
polypeptide CPAP	*Chlorella pyrenoidosa*	papain, trypsin, and alcalase	426 μg/mL	in vitro (HepG2 cells)	apoptosis
polypeptide Y2	*Spirulina platensis*	trypsin, alcalase, pepsin, and papain	61 μg/mL	in vitro (MCF-7 and HepG-2 cells)	



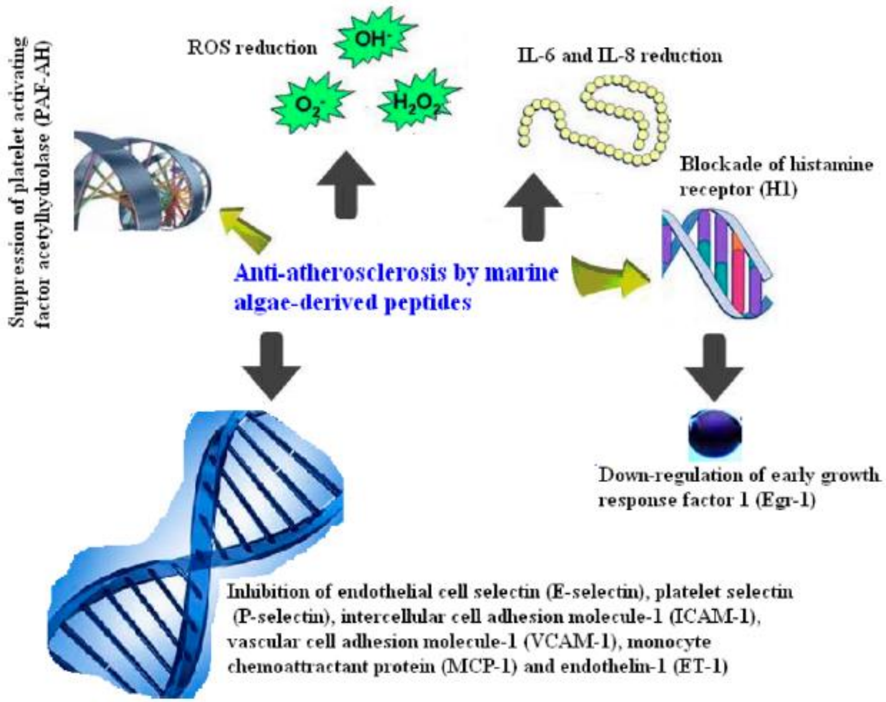
Biopeptidy proti vysokému tlaku

peptide name or sequence	source	enzyme	IC_{50}	in vitro/in vivo	mechanism of action
YH, KY, FY, IY	*Undaria pinnatifida*	no enzyme used	2.7−43.7 μmol/L	in vitro and in vivo (rats)	ACE inhibition
enzymatic digests	*Ecklonia cava*	Kojizyme, Flavourzyme, Neutrase, Alcalase, and Protamex	2.33−3.56 μg/mL	in vitro	ACE inhibition
VECYGPNRPQF	*Chlorella vulgaris*	pepsin	29.6 μM	in vitro	ACE inhibition
VEGY	*Chlorella ellipsoidea*	Protamex, Kojizyme, Neutrase, Flavourzyme, Alcalase, trypsin, α-chymotrypsin, pepsin, and papain	128.4 μM	in vitro and in vivo (rats)	ACE inhibition
GMNNLTP, LEQ	*Nannochloropsis oculata*	pepsin, trypsin, α-chymotrypsin, and papain	123−173 μM	in vitro	ACE inhibition
IRLIIVLMPILMA	*Palmaria palmata*	papain	3.3 mM	in vitro	renin inhibition



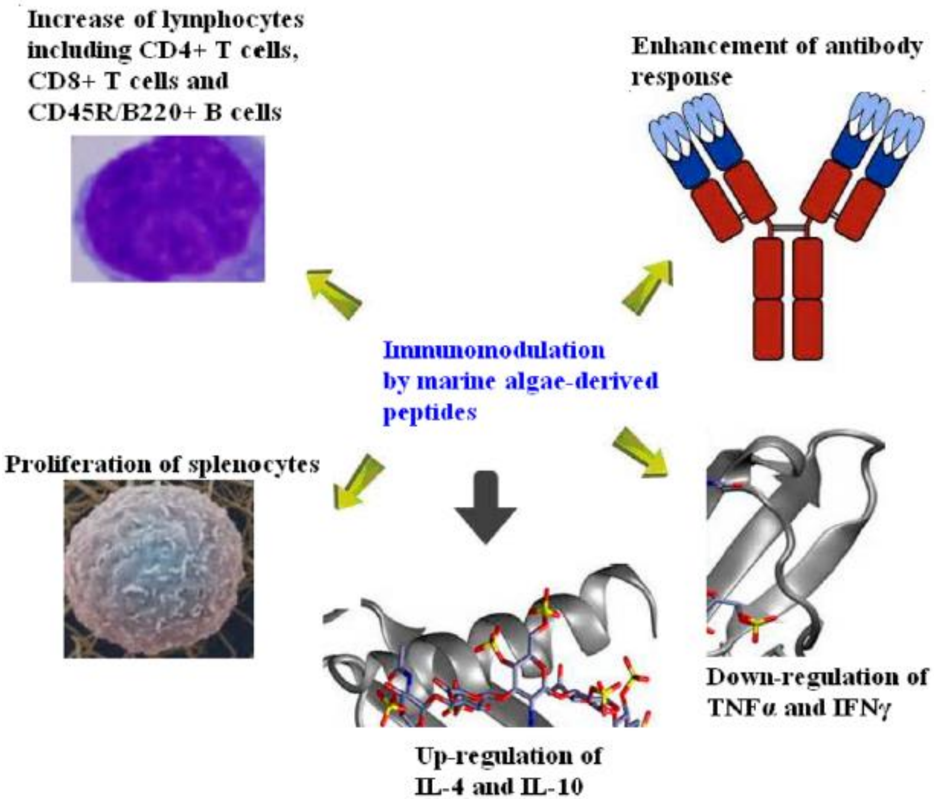
Anti-ateriosklerotické peptidy

peptide name or sequence	source	enzyme	IC_{50}	in vitro/in vivo	mechanism of action
VECYGPNRPQF	*Chlorella* sp.	pepsin, Flavourzyme, Alcalase, and Papain		in vitro (endothelial cells Svec4-10 and macrophage RAW 264.7 cells)	inhibition of vascular adhesion molecules (E-selectin, ICAM, VCAM, MCP-1 and ET-1) gene expression
NIGK	*Palmaria palmata*	papain	2.32 mM	in vitro	platelet activating factor acetylhydrolase (PAG-AH) inhibition
LDAVNR, MMLDF	*Spirulina maxima*	trypsin, α-chymotrypsin, and pepsin		in vitro (EA hy926 cells and U937 cells)	adhesion inhibition/anti-inflammatory (inhibition of IL-6, IL-8, MCP-1, P-selectin, ROS, and Egr-1)



Imunomodulační biopeptidy

peptide name or sequence	source	enzyme	IC_{50}	in vitro/ in vivo	mechanism of action
protein hydrolysates	*Chlorella vulgaris*	pancreatin		in vivo (mice)	stimulation of both humoral and cell-mediated immune functions (T-dependent antibody response and the reconstitution of delayed-type hypersensitivity response)
protein hydrolysates	*Ecklonia cava*	Kojizyme		in vivo (mice)	increases in lymphocytes, monocytes, and granulocytes; down-regulation of TNF-α and IFN-γ, up-regulation of IL-4 and IL-10
protein hydrolysates	*Porphyra columbina*	trypsin, alcalase	2.1−5.6 g/L	in vivo (rats)	cytokine modulations (inhibition of TNF-α and IFN-γ, increase of IL-10)



Další oblasti biotechnologického využití řas

Summary of Commercially Exploited Algae and the Corresponding Products or Applications

Scientific Name	Class	Products/Applications
Lyngbya lagerheimii	Cyanophyceae	Sulpholipids/spirulan
Nostoc spp.	Cyanophyceae	Cryptophycin 1
Arthrospira spp.	Cyanophyceae	Health food
Palmaria mollis	Floridophyceae	Abalone feed
Phymatolithon calcareum	Floridophyceae	Fertilizers
Lithothamnion coralloides	Floridophyceae	Fertilizers
Nannochloropsis spp.	Eustigmatophyceae	EPA/fish fry feed
Monodus subterraneus	Eustigmatophyceae	EPA
Skeletonema spp.	Bacillariophyceae	Fish fry feed
Chaetoceros spp.	Bacillariophyceae	Fish fry feed
Nitzschia alba	Bacillariophyceae	EPA
Nitzschia laevis	Bacillariophyceae	EPA
Petalonia binghamiae	Phaeophyceae	fucoxanthin
Scytosiphon lomentaria	Phaeophyceae	fucoxanthin
Ascophyllum nodosum	Phaeophyceae	Fertilizers
Sargassum spp.	Phaeophyceae	Fertilizers
Laminaria digitata	Phaeophyceae	Animal feed
Macrocystis pyrifera	Phaeophyceae	Abalone feed
Isochrysis spp.	Haptophyceae	DHA/fish fry feed
Tetraselmis spp.	Haptophyceae	Fish fry feed
Pavlova spp.	Haptophyceae	Fish fry feed
Crypthecodinium cohni	Dinophyceae	DHA
Euglena gracilis	Euglenophyceae	β-1,3-glucan
Haematococcus pluvialis	Chlorophyceae	astaxanthin
Dunaliella salina	Chlorophyceae	β-carotene
Chlorella spp.	Chlorophyceae	Health food/fish fry feed

Krmivo hospodářských zvířat

- Zařazení řas (především Chlorella) do krmiva zlepšuje zdraví zvířat a zvyšuje kvalitu masa
- Efekt je viditelný už při malých dávkách

Table 5 Results of *Chlorella* feeding trials with sows and piglets during farrowing at the Regional Research Center (LVA; Iden, Germany; Weber and Grimmer 2001)

Parameter	Trial 1		Trial 2		Trial 3		Total	
	Control	Alga	Control	Alga	Control	Alga	Control	Alga
Sow daily weight gain								
Lactating time (g/day)	290	305	319	318	303	300	304	308
Weight after lactating (kg)	7.5	7.9	8.5	8.5	7.2	7.18	7.8	7.8
End weight (kg)	23.8	24.9	26.9[a]	29.8[b]	24.5	25.7	25.1[a]	26.8[b]
Husbandry (days)	42	42	46.2	45.8	47	46.1	45	44.6
Piglet daily weight gain								
Growth (g/day)	388	404	396[a]	466[b]	369	403	386[a]	424[b]
Feed conversion (kg/kg)	1.67	1.66	1.74	1.66	1.73	1.57	1.71	1.63
Dead animals	0	0	1	0	3	0	4	0

[a,b]Level of significance $P>0.05$

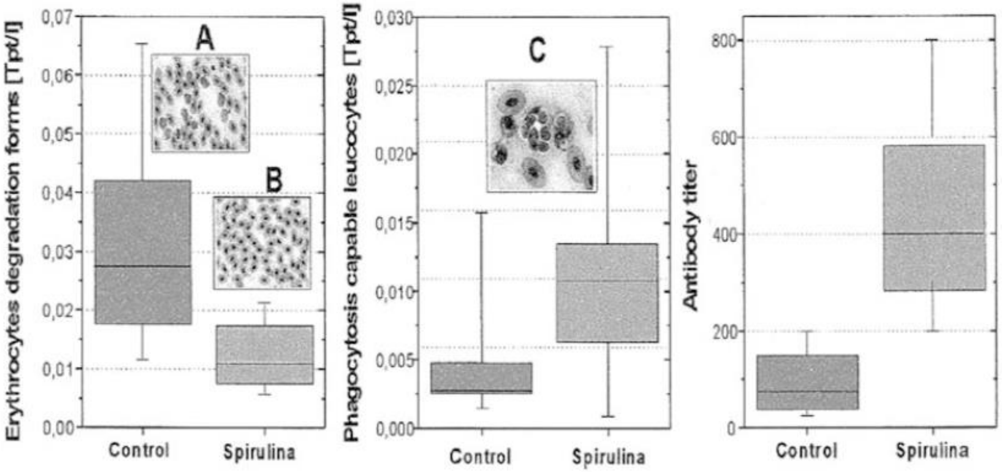
Rybí akvakultury

- Posílení a vylepšení produkce ryb v akvakulturách

Table 6 Important genera of microalgae used in aquaculture

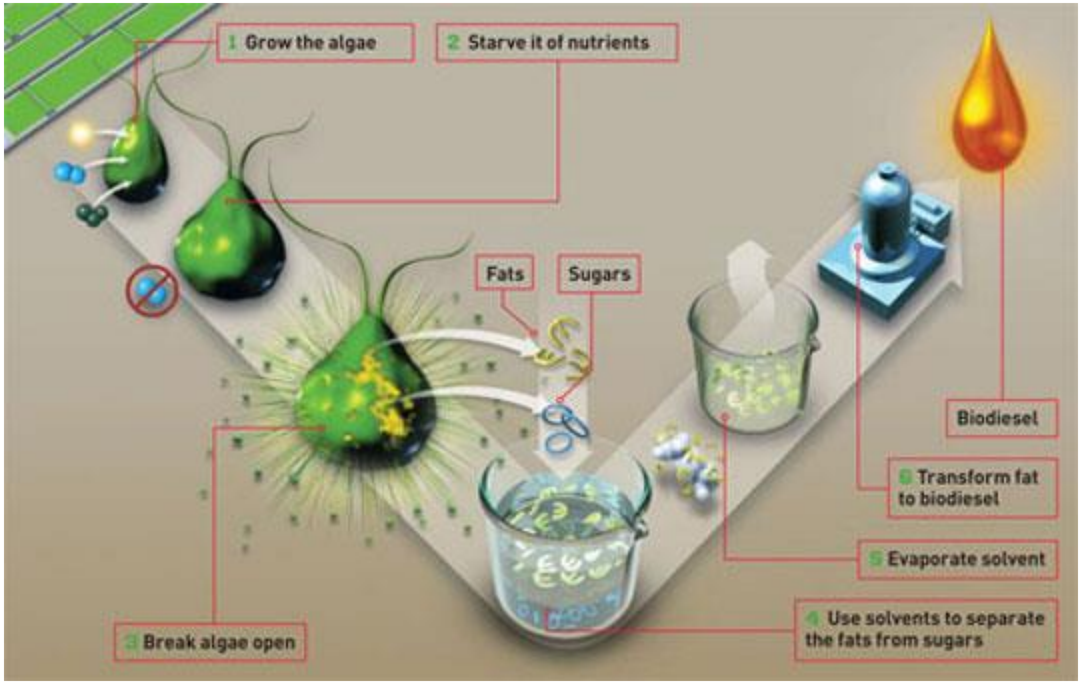
Taxon	Genera
Bacillariophyta	*Skeletonema*, *Chaetoceros*, *Phaeodactylum*, *Nitzschia*, *Thalassiosira*
Prymnesiophyta	*Isochrysis*, *Pavlova*
Prasinophyceae	*Tetraselmis*
Chlorophyceae	*Chlorella*, *Scenedesmus*, *Dunaliella*
Cyanobacteria	*Spirulina*

Fig. 4 Influence of adding microalgae to feed for carp. *A–C* Views of cells mentioned in *y*-axis (Schreckenbach et al. 2001)



	Products currently on market	Producers of current products	Micro-algae / product from micro-algae
Cosmetics	Personal care skin products	Solazyme (US) + Unilever Fuji Chemicals [77]	(NA)
		Soliance (France) [78]	*Spirulina*
		LVMH (France) [87]	*Chlorella*
		Daniel Jouvance (France) [87]	(NA)
		Algenist /Solazyme (USA, California) [79]	'Alguronic acid' (trade name for a undetermined mix of polysaccharides produced by micro-algae clogging filters in algae cultures)
	Anti aging skin product (lipid)	Soliance (FR) [78]	*Skeletonema costatum*
		Exsymol S.A.M. (Monaco) [87]	Arthropira (*Spirulina*)
		Pentapharm (Switzerland) [87]	*Nannochloropsis* *Dunaliella Salina*
	Hydrating skin product	Soliance [78]	*Porphyridium cruentum*
		Codif (France) [87	*Chlorella*
	Anti – inflammation (peptide)	Soliance [78]	*Phaeodactylum tricornutum*
	Slimming products	Soliance [78]	*Dysmorphococcus globosus*
Other products	Fluorescent protein markers	Martek/DSM	(NA)
	Stable isotope biochemicals	Spectra Gases/Martek/DSM [87]	(NA)

Řasy jako zdroje biopaliv



Advantages and disadvantages of biofuel production using microalgae.

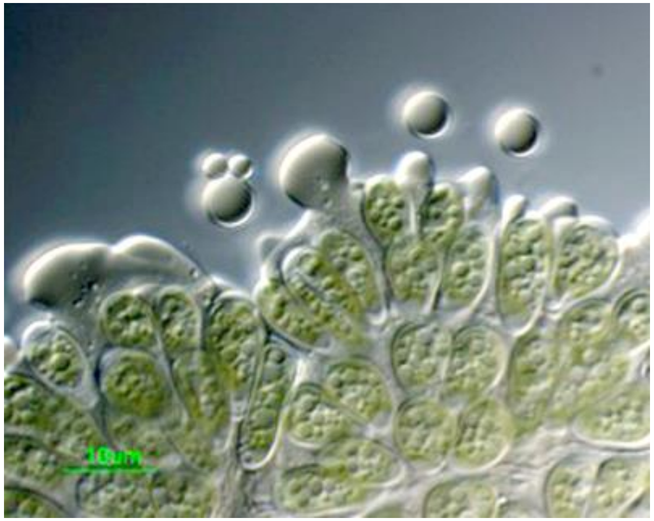
Advantages	Disadvantages
High growth rate	Low biomass concentration
Less water demand than land crops	Higher capital costs
High-efficiency CO_2 mitigation	
More cost effective farming	

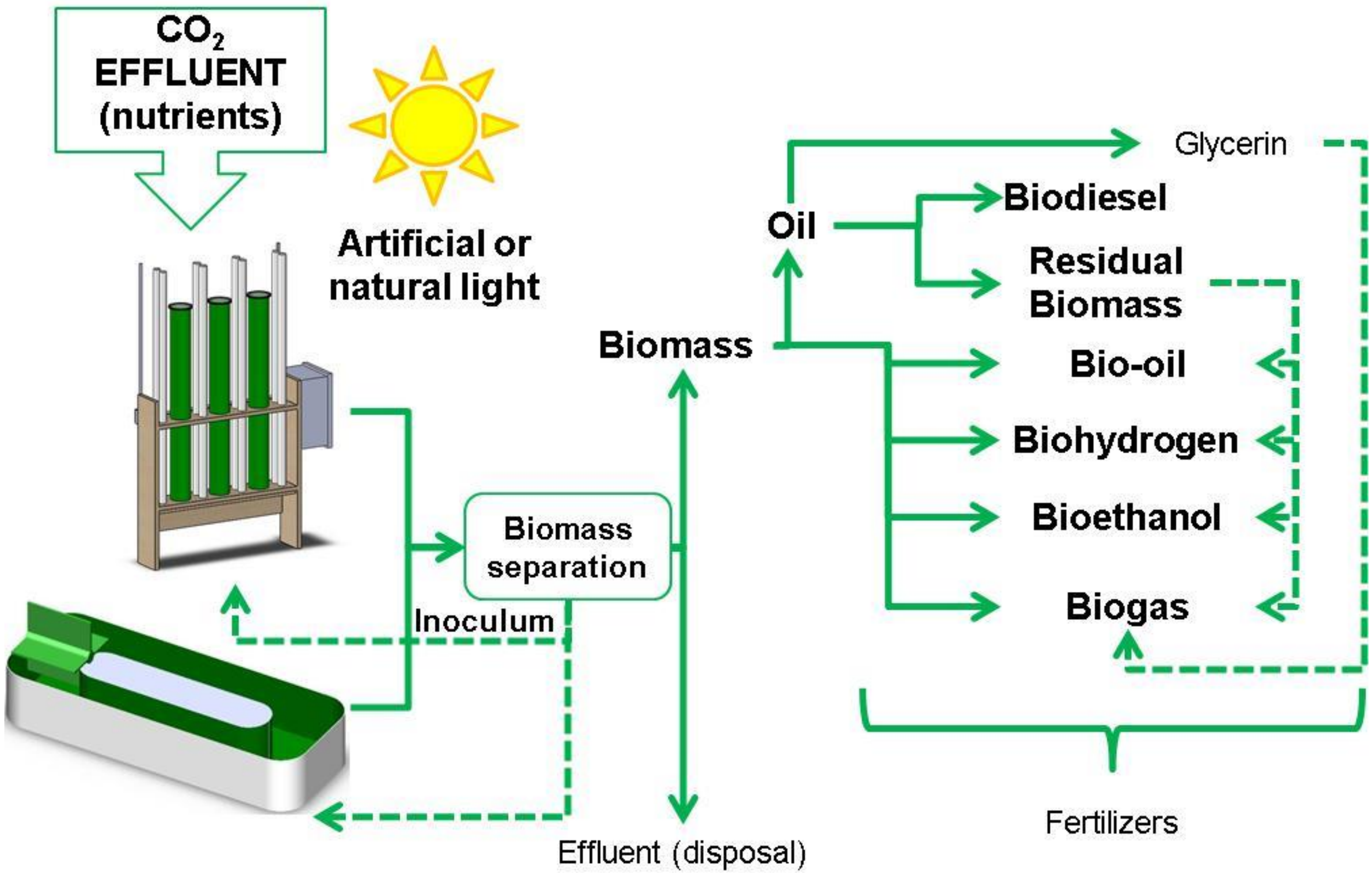
Botryococcus braunii

- Zelená řasa produkuje olejovité látky
- Oleje ale nejsou vhodné pro transesterifikaci
- Triterpeny je možné rafinovat
  - Octane
  - Kerosene
  - Diesel

Hydrocarbon Oil Constituents of Botryococcus braunii [5]

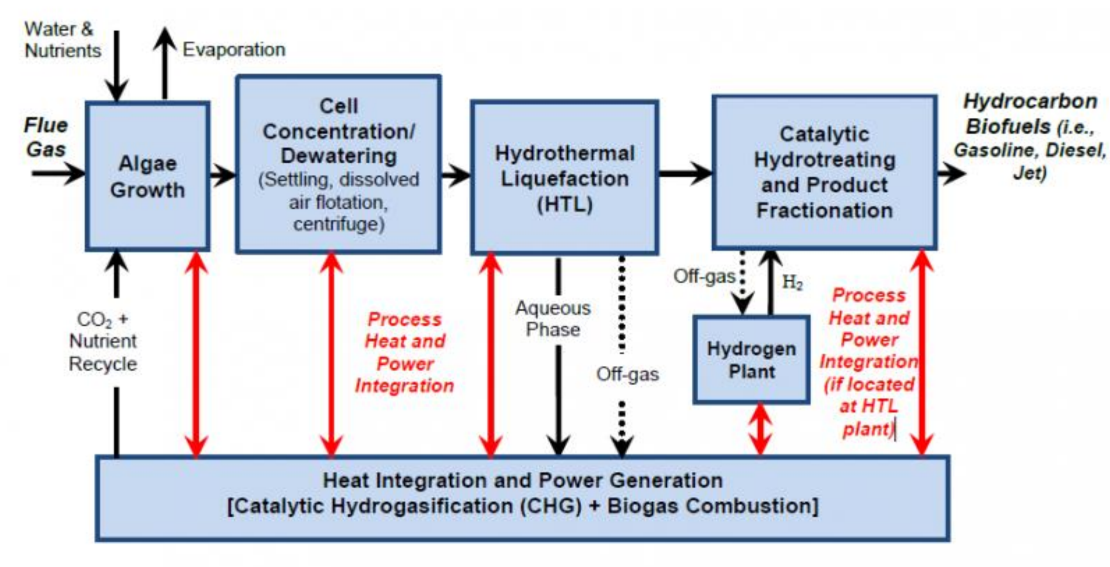
Compound	% mass
Isobotryococcene	4%
Botryococcene	9%
$C_{34}H_{58}$	11%
$C_{36}H_{62}$	34%
$C_{36}H_{62}$	4%
$C_{37}H_{64}$	20%
Other hydrocarbons	18%





CO_2
EFFLUENT
(nutrients)
Artificial or
natural light
Biomass
separation
Inoculum
Biomass
Effluent (disposal)
Oil
Glycerin
Biodiesel
Residual
Biomass
Bio-oil
Biohydrogen
Bioethanol
Biogas
Fertilizers

Předpříprava řasové biomasy



- Nejdůležitější je odstranění vody
- Po odvodnění zůstává 20 %

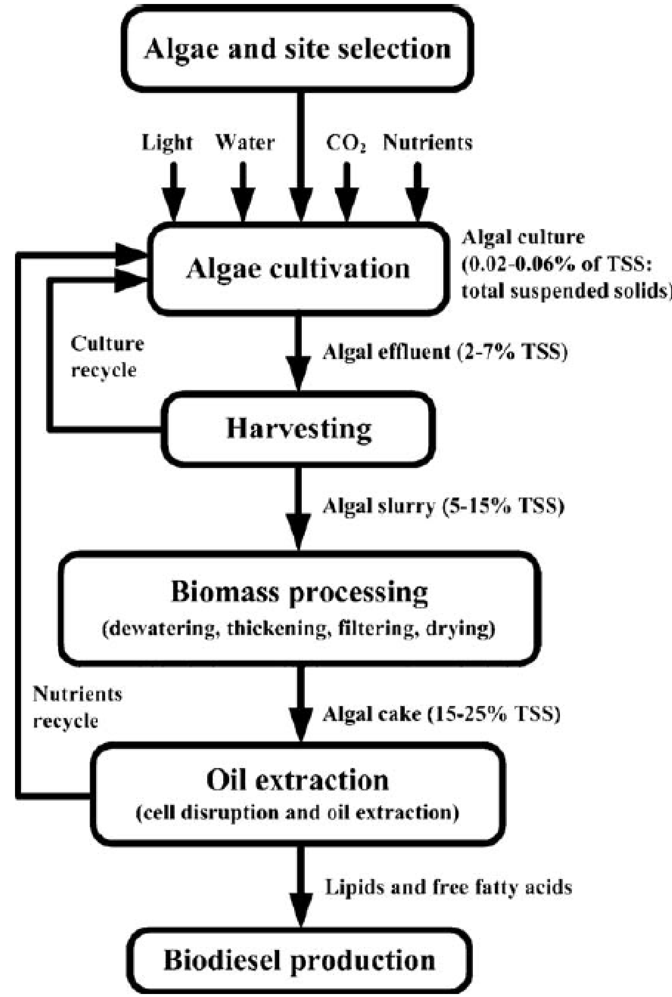
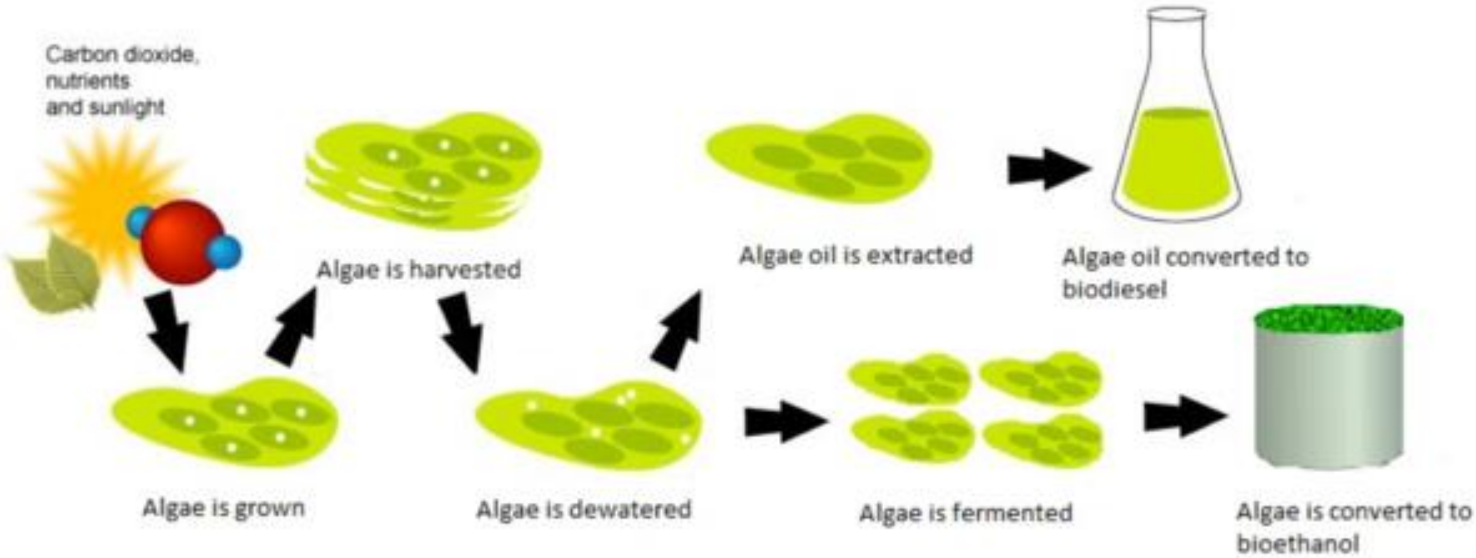


Fig. 1. Microalgae biodiesel value chain stages.

Bioethanol



- Produkce závisí na obsahu kvasitelných cukrů
- Aby byla výroba udržitelná, je nutné dosáhnout výtěžku nad 4 % (40 g/L)

Table 1. Comparison of the productivities of lignocellulosic biomass and seaweeds

Biomass	Productivity [dry g/(m^2·year)]	Reference
Lignocellulosic biomass		
Switchgrass	560–2,240	65
Corn stover	180–790	65
Eucalyptus	1,000–2,000	65
Poplar	300–612.5[a]	66
Willow	46–2,700	67
Seaweeds		
Green seaweeds	7,100[b]	19, 20
Brown seaweeds	3,300–11,300	21
Red seaweeds	3,300–11,300	21

[a]Mean value calculated from the amount of biomass produced for 8 y; [b]calculated value.

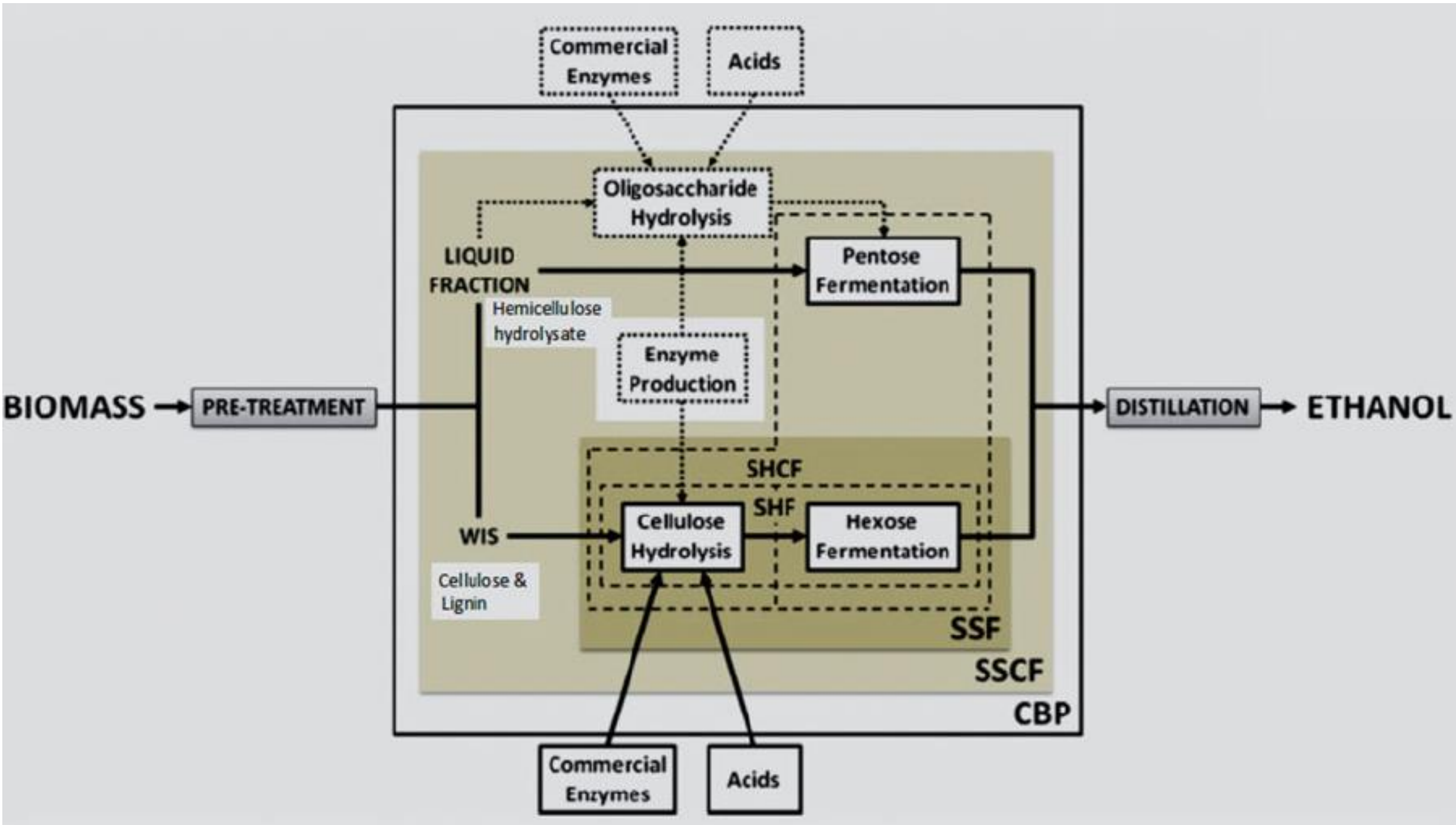

Commercial Enzymes
Acids
Oligosaccharide Hydrolysis
LIQUID FRACTION
Pentose Fermentation
Hemicellulose hydrolysate
Enzyme Production
BIOMASS
PRE-TREATMENT
DISTILLATION
ETHANOL
SHCF
SHF
WIS
Cellulose Hydrolysis
Hexose Fermentation
Cellulose & Lignin
SSF
SSCF
CBP
Commercial Enzymes
Acids

Table 2. Various hydrolysis treatments methods and their bioethanol yields

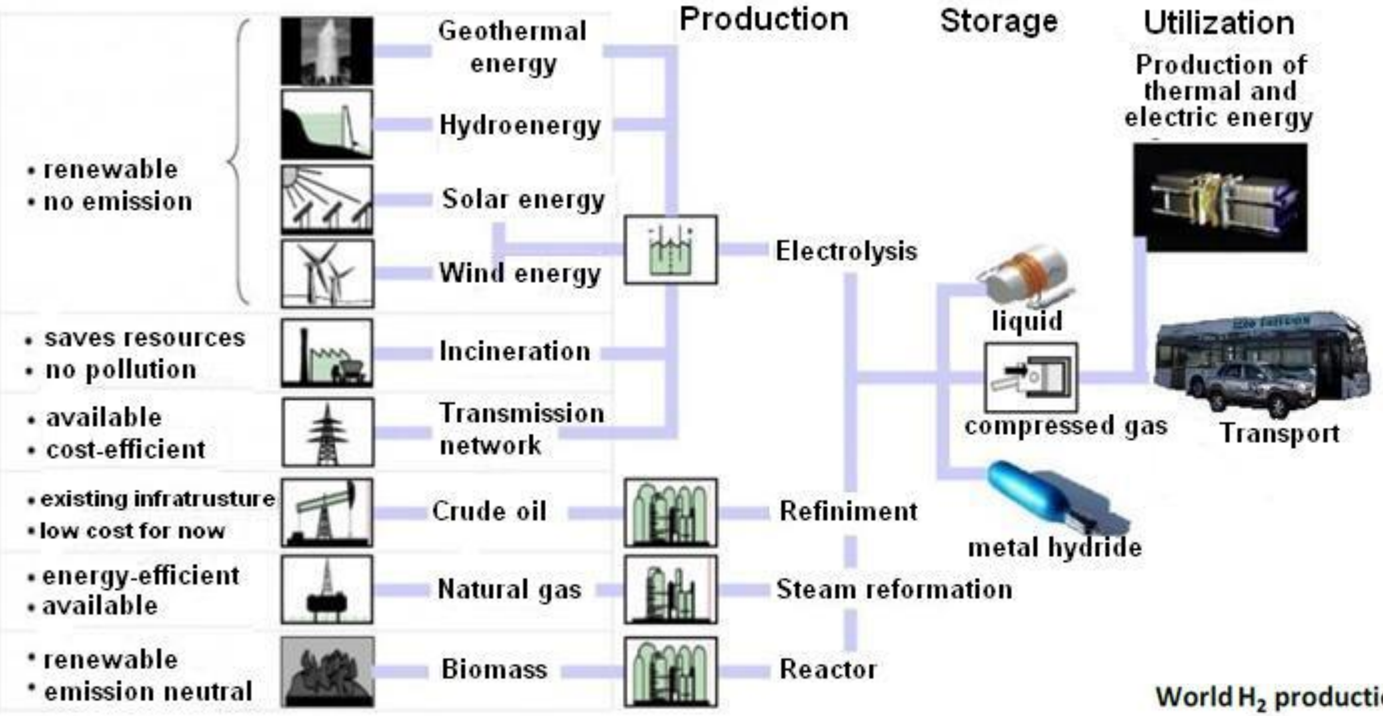
Hydrolysis type	Hydrolysis source	Fermentation Mode[a)]	Algae species	Algae type	Yield (g ethanol/g algae)	Reference
Acid	HCl/ $MgCl_2$	SHF	*Chlorella* sp.	Micro	0.47	[36]
Alkaline	NaOH	SHF	*Chlorococcum infusionum*	Micro	0.261	[10]
Chemical	H_2SO_4	SHF	*Chlorococcum humicola*	Micro	0.48	[9]
Chemical[b)]	H_2SO_4	SHF	*Chlorella vulgaris*	Micro	0.233	[61]
Chemo-enzymatic[c)]	HCl/ H_2SO_4 + amyloglucosidase + endocellulase + β-glucosidase	SHF	*Dunaliella tertiolecta*	Micro	0.14	[46]
Enzymatic	α-amylase + amyloglucosidase	SHF	*Chlamydomonas reinhardtii*	Micro	0.235	[18]
Enzymatic	endoglucanase + β-glucanase + amyloglucosidase	SSF	*Laminaria japonica*	Macro	0.196	[38]
Enzymatic[b)]	cellulase + amylase	SHF	*C. vulgaris*	Micro	0.178	[61]
Enzymatic[d)]	cellulase + β-glucosidase	SHF	*Gracilaria verrucosa*	Macro	0.43	[14]
Enzymatic[e)]	cellulase + β-glucosidase	SSF	*Saccharina japonica*	Macro	0.111	[31]
Enzymatic[b)]	cellulase + Amylase	SSF	*C. vulgaris*	Micro	0.214	[61]
Physical[c)]	supercritical CO_2	SHF	*Chlorococum* sp.	Micro	0.383	[45]

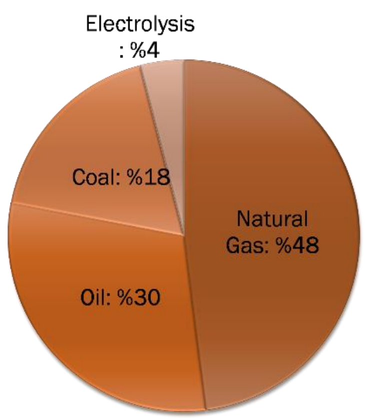
a) SHF: separate hydrolysis and fermentation; SSF: simultaneous saccharification and fermentation
b) Sonicated algal biomass was utilized
c) Lipid-extracted algal biomass was utilized
d) Agar pulp was extracted after alkali treatment and hydrolyzed
e) Algal biomass received extremely low acid pretreatment.

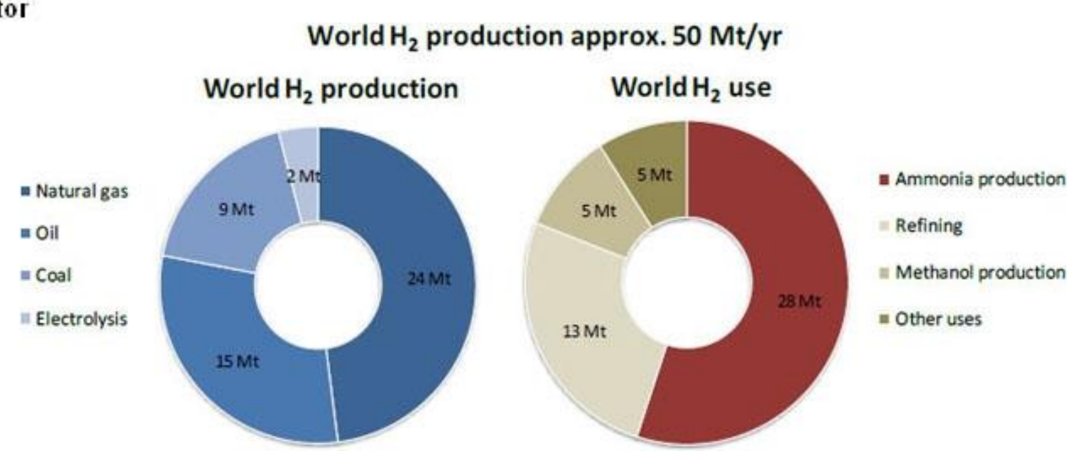
Table 3 | Comparative study between algal biomass and terrestrial plants for bioethanol production.

Feedstock	Conditions	Bioethanol	Reference
ALGAE			
Chlorococcum infusionum	Alkaline pre-treatment, temp. 120°C, *S. cerevisiae*	260 g ethanol/kg algae	Harun et al. (2011)
Spirogyra	Alkaline pre-treatment, synthetic media growth, saccharification of biomass by *Aspergillus niger*, fermentation by *S. cerevisiae*	80 g ethanol/kg algae	Eshaq et al. (2010)
Chlorococcum humicola	Acid pre-treatment, temp. 160°C, *S. cerevisiae*	520 g ethanol/kg microalgae	Harun and Danquah (2011a)
TERRESTRIAL PLANTS			
Madhuca latifolia	Strain *Zymomonas mobilis* MTCC 92, immobilized in *Luffa cylindrical* sponge disks, temp. 30°C	251.1 ± 0.012 g ethanol/kg flowers	Behera et al. (2011)
Manihot esculenta	Enzyme termamyl and amyloglucosidase, 1 N HCl, *Saccharomyces cerevisiae*, ca-alginate immobilization	189 ± 3.1 g ethanol/kg flour cassava	Behera et al. (2014)
Sugarcane bagasse	Acid (H_2SO_4) hydrolysis, *Kluyveromyces* sp. IIPE453, Fermentation at 50°C	165 g ethanol/kg bagasse	Kumar et al., 2014
Rice straw	Cellulase, β-glucosidase, solid state fermentation, strain *Trichoderma reesei* RUT C30, and *Aspergillus niger* MTCC 7956	93 g ethanol/kg pretreated rice straw	Sukumaran et al. (2008)

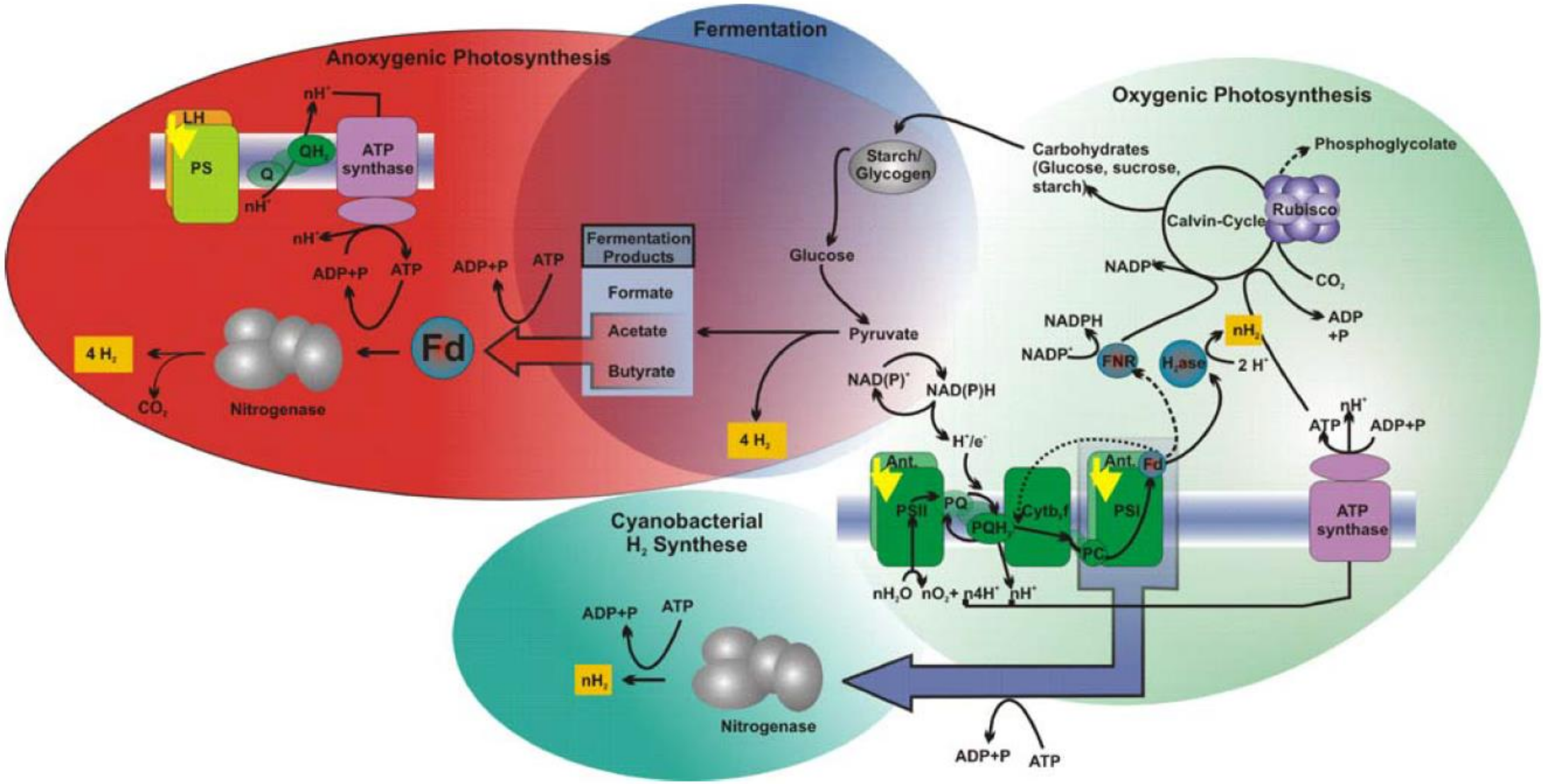
Výroba vodíku







Biovodík z řas



Nitrogenasa jako klíčový enzym

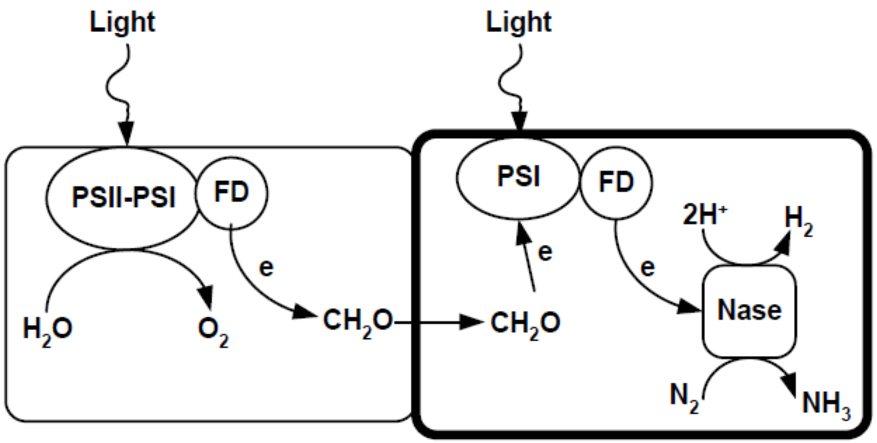
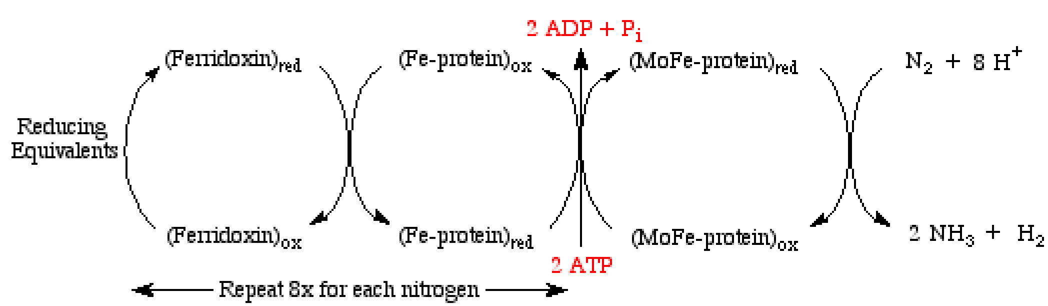


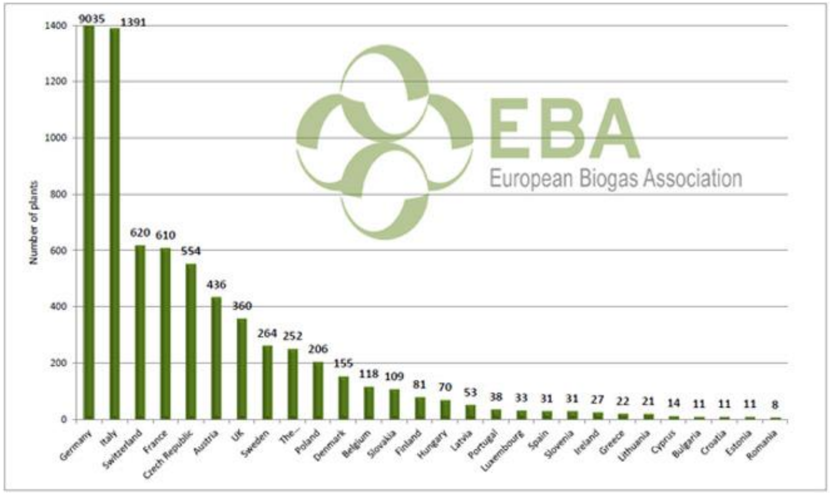
Fig. 2. Nitrogenase(Nase)-mediated hydrogen evolution in a heterocyst of nitrogen-fixing heterocystous cyanobacteria [10, 30, 32]. The oxygen and hydrogen evolution are carried out separately and the energy-rich carbohydrate (CH_2O) is used as the electron source in the oxygen-free heterocyst.

$$N_2 + 8H^+ + 8e + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi \quad \ldots 2$$

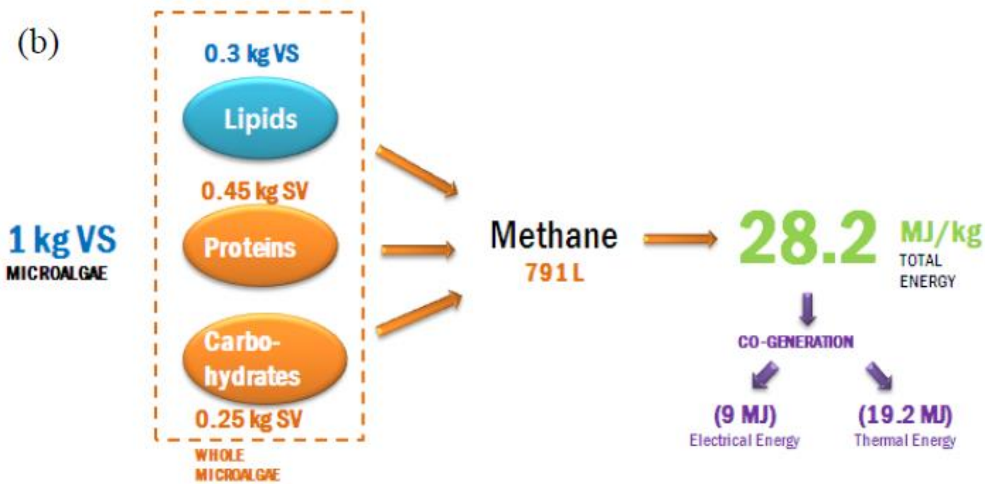
$$8H^+ + 8e + 16ATP \rightarrow 4H_2 + 16ADP + 16Pi \quad \ldots 3$$

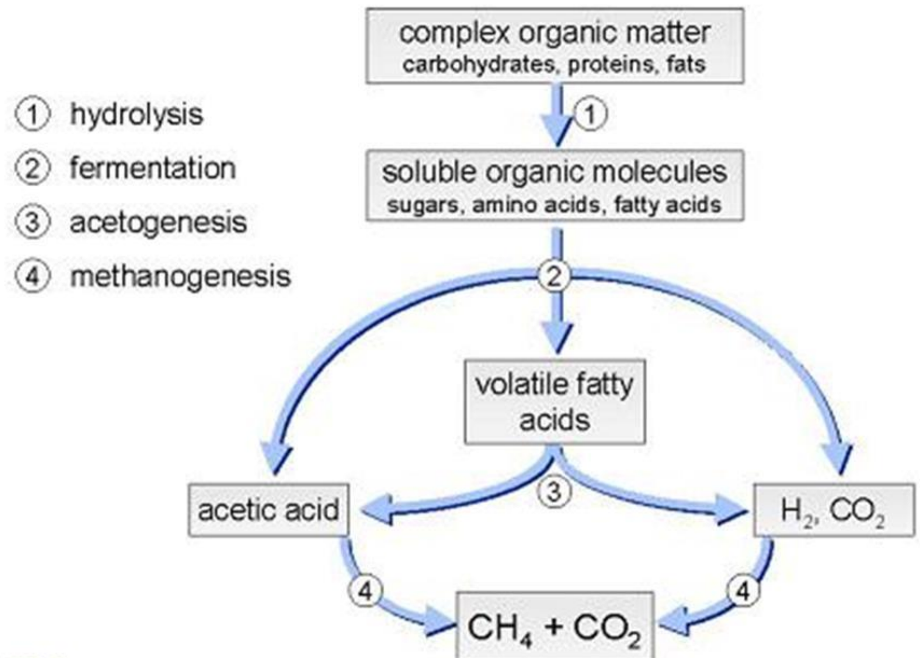


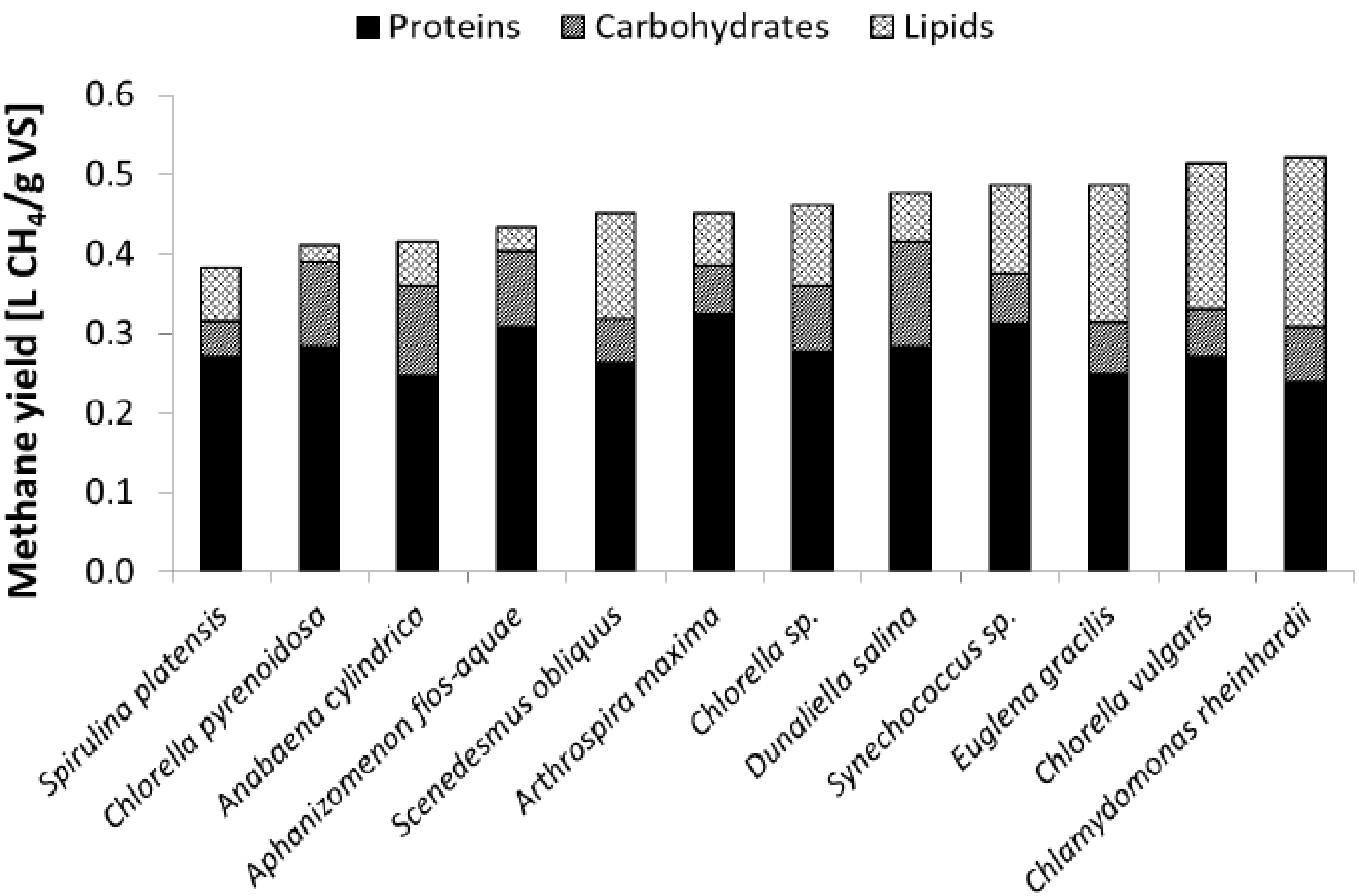
Bioplyn



14 563 biogas plants in Europe with total installed capacity of 7 857 MWel (2013)






Proteins
Carbohydrates
Lipids
Methane yield [L CH4/g VS]
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Spirulina platensis
Chlorella pyrenoidosa
Anabaena cylindrica
Aphanizomenon flos-aquae
Scenedesmus obliquus
Arthrospira maxima
Chlorella sp.
Dunaliella salina
Synechococcus sp.
Euglena gracilis
Chlorella vulgaris
Chlamydomonas rheinhardii

Inženýrství sinic a řas

- Modifikace fotoautotrofních organizmů je rozvíjející se oblast moderních biotechnologií
- Na výběr je jaderná nebo chloroplastová transformace
- Nejdůležitějšími cíli jsou produkce biopaliv, především vodíku, a příprava čistých chemikálií
- Mezi nejvíce studované a vhodné organizmy patří Chlamydomonas a Synechocystis

Box 1. Nuclear versus chloroplastic transformation

Integration of transgenes into the chloroplast has important advantages. It enables controlled site-directed recombination of constructs and results in high expression levels with no silencing drawbacks (Table I). However, nuclear transformation might enable a wider range of possibilities both for transgenic protein expression (e.g. excretion, different cell-compartment expression, and glycosylation) and for manipulation of algal metabolism (gene inactivation or overexpression, and gain of additional pathways) (Table I).

Table I. Main characteristics of nuclear and chloroplastic transformations

	Nuclear	Chloroplastic
Cell compartment of expression	Extracellular, cytosol and chloroplast, among others	Chloroplast
Recombination machinery for integration of exogenous DNA	Mostly non-homologous	Homologous
Gene silencing	Probable	Not probable
Inheritance of integrated gene	Mendelian	Maternal
Level of expression (gene copy number)	Low to intermediate	High
Co-transformation of different markers	High	High
Versatility to express genes from different organisms	Intermediate to low	High
Glycosylation pattern of proteins	Similar to plants and animals	None

Box 2. Main problems associated with foreign gene expression in microalgae

- Inadequate method of DNA delivery
- No integration into the chromosome
- Inadequate recognition of the promoter region
- Biased codon usage
- Lack of adequate regulatory sequences
- Incorrect polyadenylation
- Inappropriate nuclear transport
- Instability of mRNA
- Positional effects
- Silencing by methylation
  Epigenetic silencing mechanisms

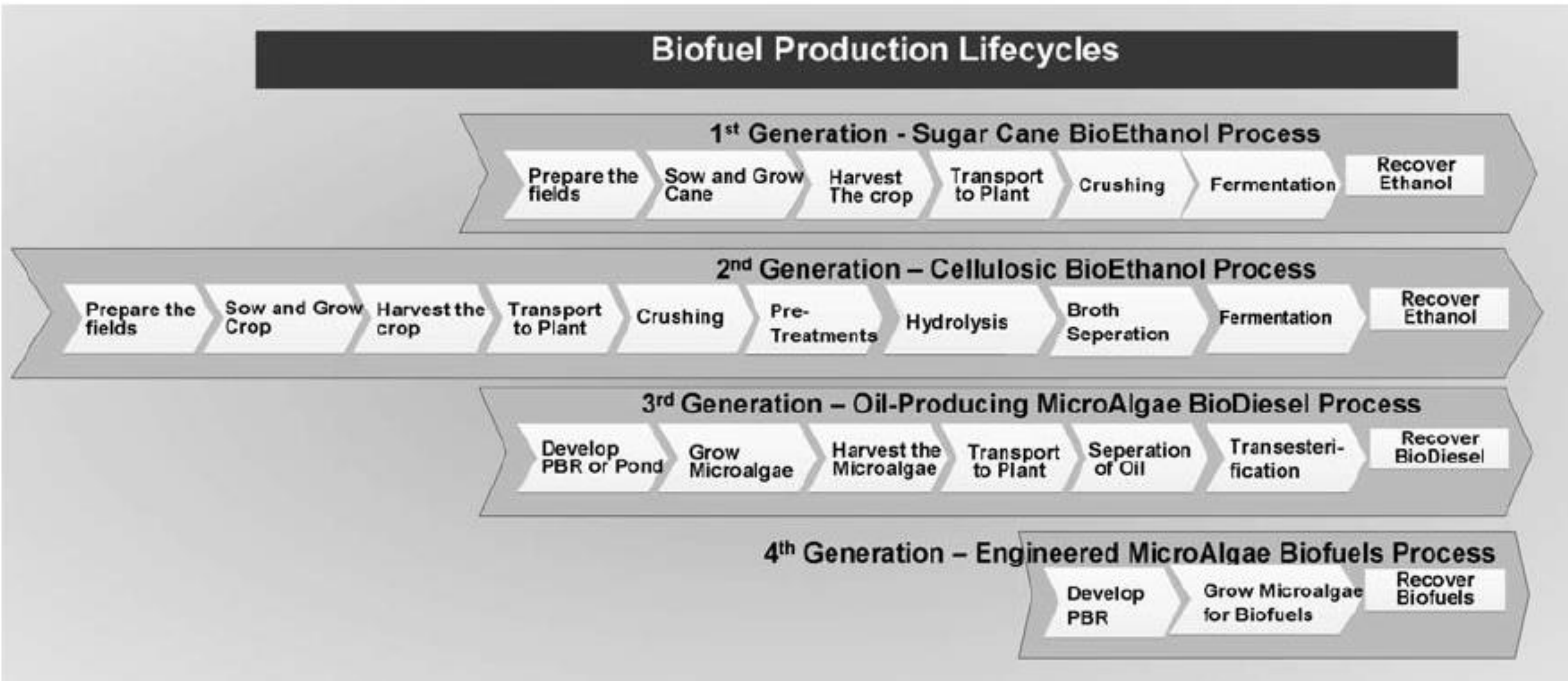


Fig. 5 Comparison of the typical bioprocess steps required for four generations of biofuels production.

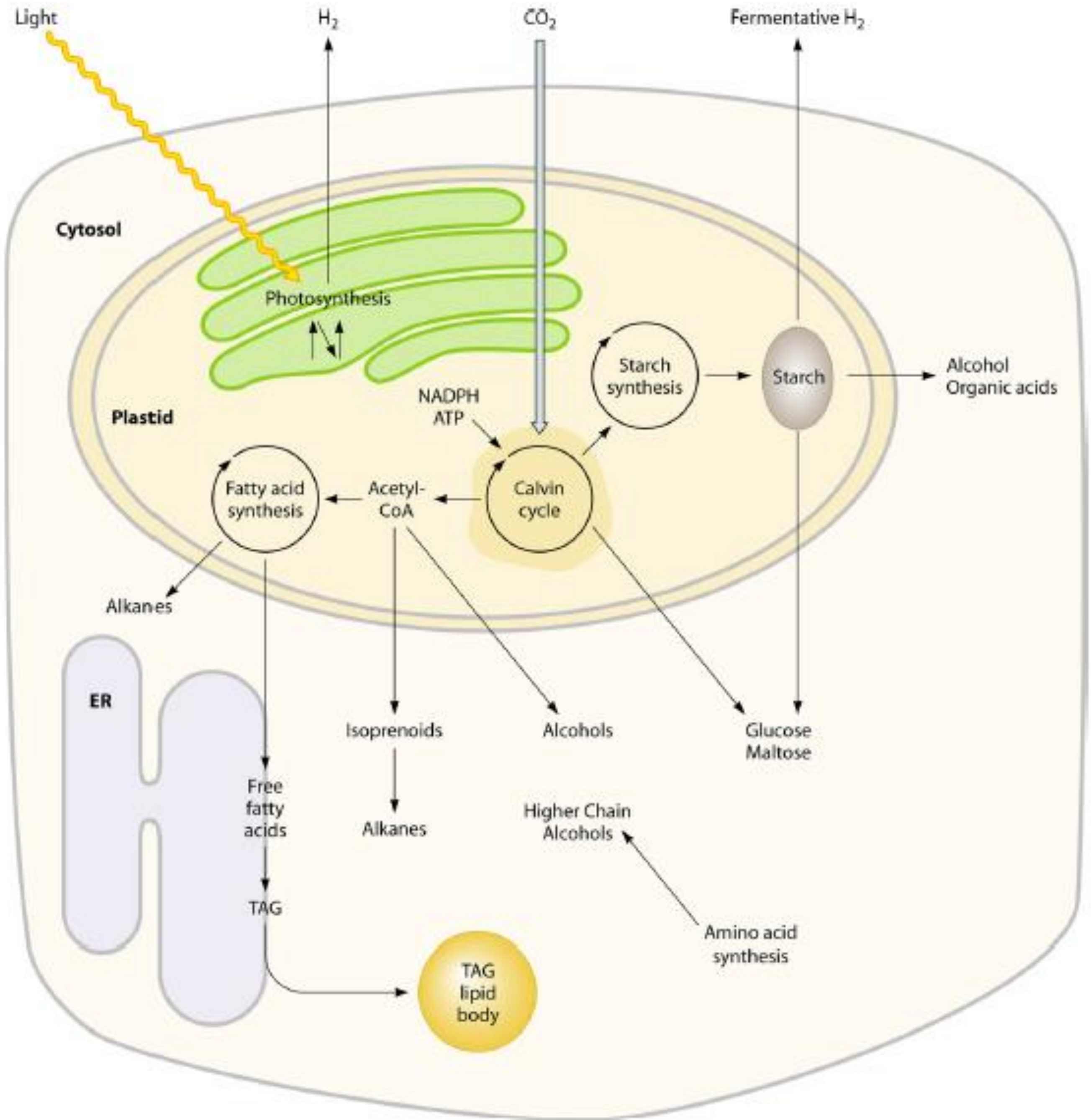


FIG. 1. Microalgal metabolic pathways that can be leveraged for biofuel production. ER, endoplasmic reticulum.

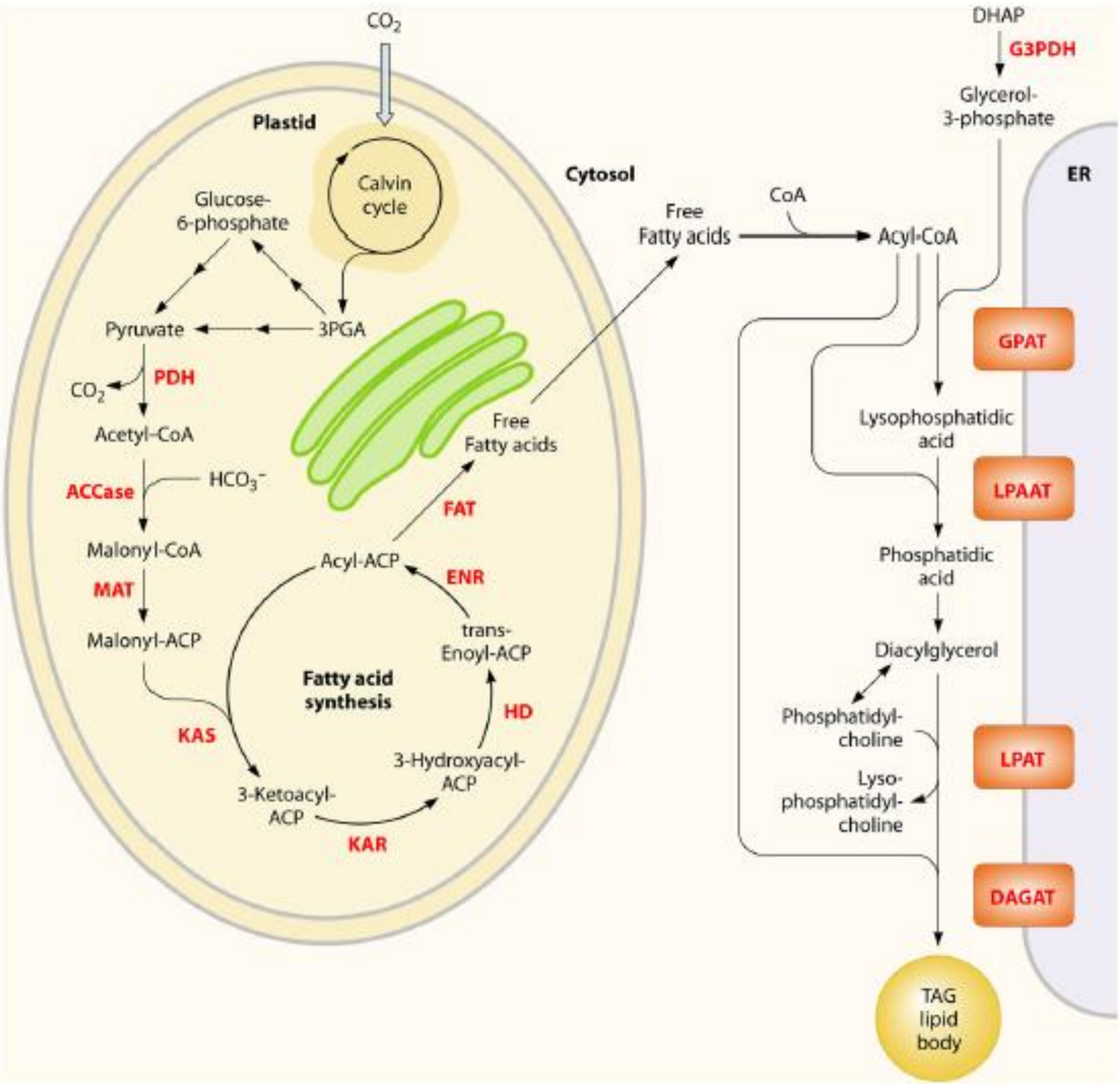


FIG. 2. Simplified overview of the metabolites and representative pathways in microalgal lipid biosynthesis shown in black and enzymes shown in red. Free fatty acids are synthesized in the chloroplast, while TAGs may be assembled at the ER. ACCase, acetyl-CoA carboxylase; ACP, acyl carrier protein; CoA, coenzyme A; DAGAT, diacylglycerol acyltransferase; DHAP, dihydroxyacetone phosphate; ENR, enoyl-ACP reductase; FAT, fatty acyl-ACP thioesterase; G3PDH, gycerol-3-phosphate dehydrogenase; GPAT, glycerol-3-phosphate acyltransferase; HD, 3-hydroxyacyl-ACP dehydratase; KAR, 3-ketoacyl-ACP reductase; KAS, 3-ketoacyl-ACP synthase; LPAAT, lyso-phosphatidic acid acyltransferase; LPAT, lyso-phosphatidylcholine acyltransferase; MAT, malonyl-CoA:ACP transacylase; PDH, pyruvate dehydrogenase complex; TAG, triacylglycerols.

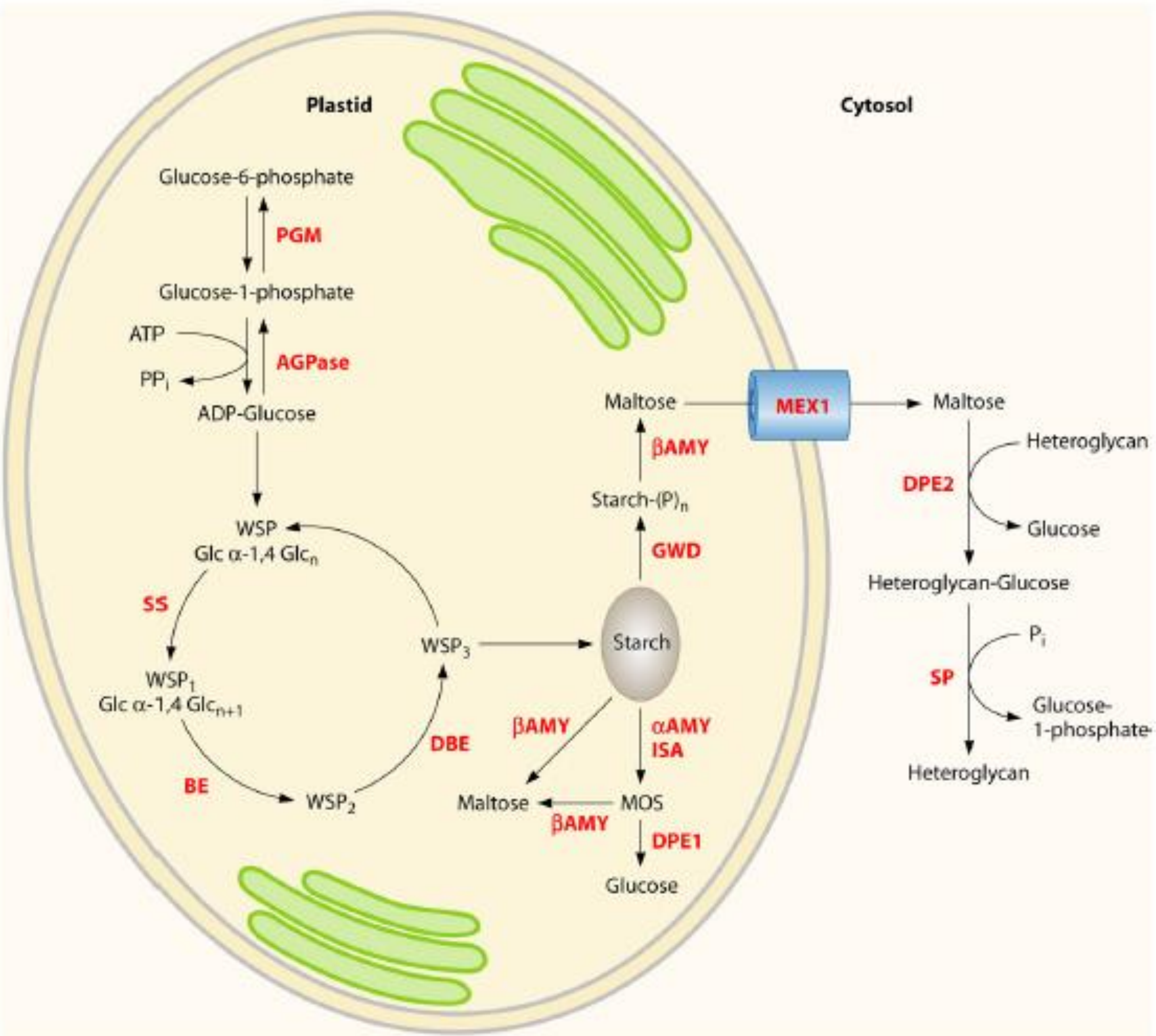


FIG. 3. Starch metabolism in green microalgae. The metabolites and simplified representative pathways in microalgal starch metabolism are shown in black, and enzymes are shown in red. Glucans are added to the water soluble polysaccharide (WSP) by α-1,4 glycosidic linkages (WSP_1) until a branching enzyme highly branches the ends (WSP_2). Some of these branches are trimmed (WSP_3), and this process is repeated until a starch granule is formed. Phosphorolytic [Starch-$(P)_n$] and hydrolytic degradation pathways are shown. αAMY, α-amylase; AGPase, ADP-glucose pyrophosphorylase; βAMY, β-amylases; BE, branching enzymes; DBE, debranching enzymes; DPE, disproportionating enzyme (1 and 2) α-1,4 glucanotransferase; Glc, glucose; GWD, glucan-water dikinases; ISA, isoamylases; MEX1, maltose transporter; MOS, malto-oligosaccharides; PGM, plastidial phosphoglucomutase; P, phosphate; P_i, inorganic phosphate; PP_i, pyrophosphate; SP, starch phosphorylases; SS, starch synthases.

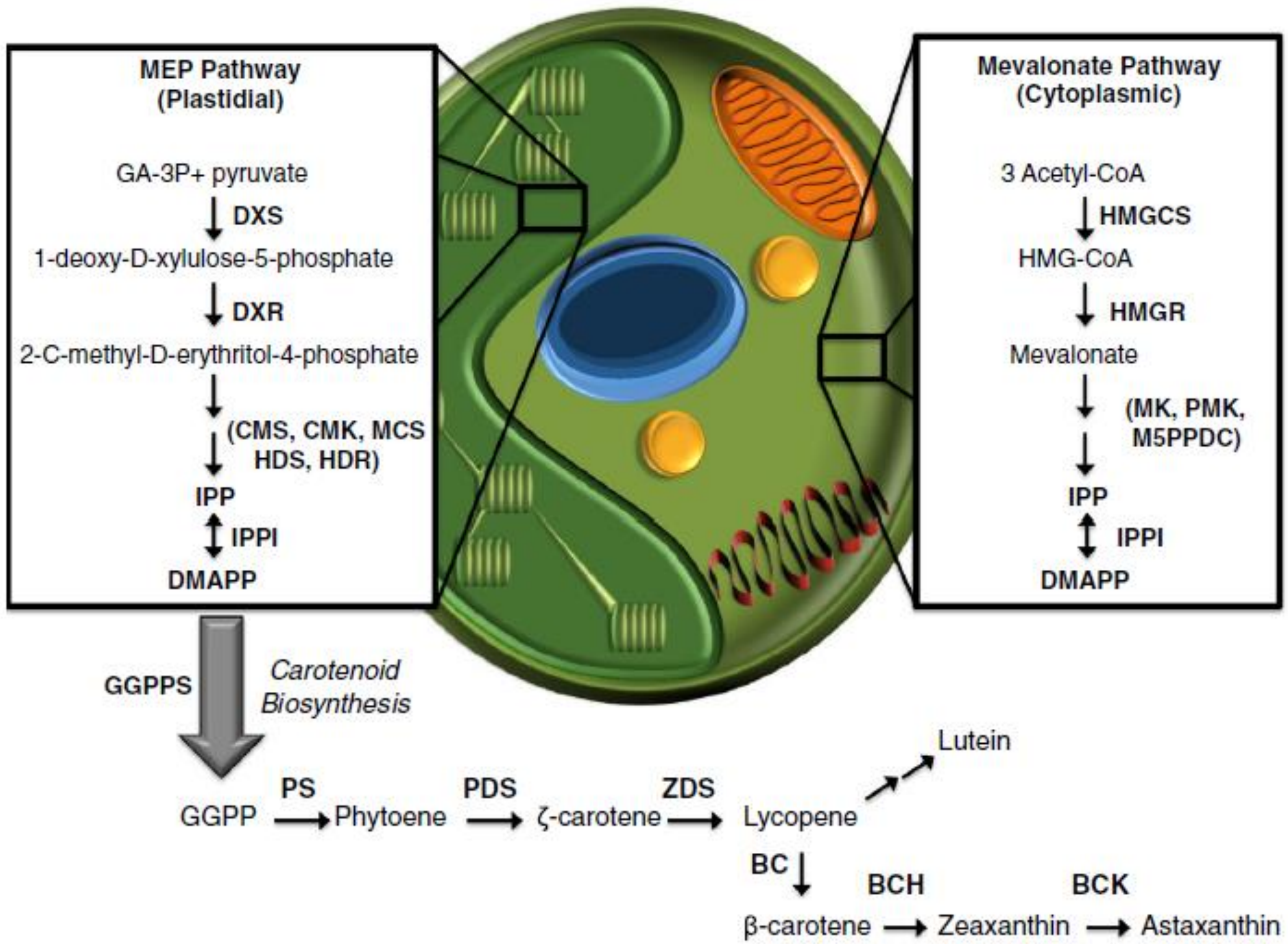


Fig. 2 Algal terpenoid biosynthesis pathways. Not all intermediates are displayed. Plastidial carotenoid biosynthesis represents one potential fate of DMAPP. Plastidial MEP components: *GA-3P* glyceraldehyde 3-phosphate; *DXS* 1-deoxy-D-xylulose 5-phosphate (DOXP) synthase; *DXR* DOXP reductase; *CMS* 2-c-methyl-D-erythritol 2-phosphate synthase; *CMK* 4-diphosphocytidyl-2C-methyl-D-erythritol kinase; *MCS* methyl-erythritol-cyclo-diphosphate-synthase; *HDS* hydroxy-methyl-butenyl-diphosphate (HMBPP) synthase; *HDR* HMBPP reductase; *IPP* isopentyl diphosphate; *DMAPP* dimethylallyl diphosphate. Cytoplasmic mevalonate components: *HMGCS* 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase; *HMGR* HMG-CoA reductase; *MK* mevalonate kinase; *PMK* phosphomevalonate kinase; *M5PPDC* mevalonate-5-diphosphate decarboxylase; *IPPI* isopentyl diphosphate isomerase. Carotenoid biosynthetic components: *GGPPS* Geranylgeranyl diphosphate (GGPP) synthase; *PS* phytoene synthase; *PDS* phytoene desaturase; *ZDS* zeta-carotene desaturase; *BC* beta-cyclase; *BCH* beta-carotene hydroxylase; *BCK* beta-carotene ketolase

Table 1 Summary of the genetically engineered strains with improved H_2 production

Barriers	Target	Strain	Genetic engineering technique	Phenotype	H_2 production vs control strain	Ref
O_2 sensitivity	D1 protein	*L1591-N230Y*	Site-directed mutagenesis	↑quantum yield of photosynthesis ↑ respiration rate	~20 fold↑ compared to WT 11/32b	Torzillo 2009
	PSII subunit O	*antiPSBO*	RNA interference	↑hydrogenase ↓ Fv/Fm	~10 fold↑ compared to WT *Chlorella sp. DT*	Hsin Di Lin 2013
	PSII	*cyc6nac2.49*	Random mutagenesis	↓ O_2	~3 fold↑ compared to *nac2-26*	Surzycki 2007
	P/R ratio	*apr1+* glycoaldehyde	DNA insertional mutagenesis	↑respiration rate ↓photosynthesis rate	~2-3 fold↑ compared to CC-425	Ruhle 2008
	Leg hemoglobin	CC-849 *+codon optimized hemH-lbA*	Heterologous expression from Bradyrhizobium and Glycine max in *Chlamydomonas*	↓growth ↑O_2 consumption	~4-fold ↑ compared to CC-849 *+non codon optimized hemH-lbA*	Wu 2010 Wu 2011
	Pyruvate oxidase	*ccHPC*	Heterologous expression from *E.coli* in *Chlamydomonas*	↓O_2 evolution	~3 fold ↑ compared to CC-503	Xu 2011
	Sulfate permase	*antisulp*	RNA Antisense	↓uptake sulfate ↓O_2 evolution ↓steady state levels of PSII	~4 fold ↑ compared to CW-15	Chen 2005

Proton gradient	Cyclic Electron Flow PGRL1 protein	*pgrl1*	DNA insertional mutagenesis	↑ETR ↓NPQ	~3 fold ↑ compared to 137C	Tolleter 2011
State transition	State transitions	*stm6*	DNA insertional mutagenesis	↑ starch reserves ↑ respiration No state transitions ↓ PSII activity	~5-13 fold↑ compared to CC-1618	Kruse 2005, Volgusheva 2013
Photosynthetic efficiency	Antenna size	*tla1*	DNA insertional mutagenesis	↓ Chl/reaction center ↑O_2 evolution/Chl ↑ H_2 rate/Chl	~4 fold ↑ compared to CC-4169	Kosourov 2011, Polle 2003
	Light harvesting complex LHCBM1,2,3	*stm6GLC4lo1*	RNA interference	↓Chl ↑ H_2 rate/Chl	~2 fold ↑ compared to *stm6GLC4*	Oey 2013
Competition for electron	Rubisco large subunit	CC-2803	DNA insertional Mutagenesis	Light sensitive Reduced	~2 fold ↑ compared	Heimscheier 2008
				RuBisCo Activity ↓O_2 evolution	to 137C	
	Rubisco small subunit	*Y67A*	Site-directed mutagenesis	Reduced RuBisCo Activity ↓O_2 evolution	~10 fold↑ compared to *RBCS-T60-3*	Pinto 2013
Low reductant flux	Sugar reserve	*stm6GLC4*	Heterologous expression of HUP1 from Chlorella in *Chlamydomonas*	Import glucose ↑ growth rate	~1.5 fold↑ compared to *stm6*	Doebbe et al. 2007, 2010
	Starch enzyme	*std3 sda6*	DNA insertional mutagenesis	↑residual starch amounts	~1.5 and 1.2 fold↑ compared to 137C	Chochois 2010
Low level of hydrogenase	Hydrogenase from *Chlorella sp.DT*	*C.s DT hydA*	Homologous overexpression in Chlorella	↑Fv/Fm	~7 fold↑ compared to WT *Chlorella sp. DT*	Chien 2012

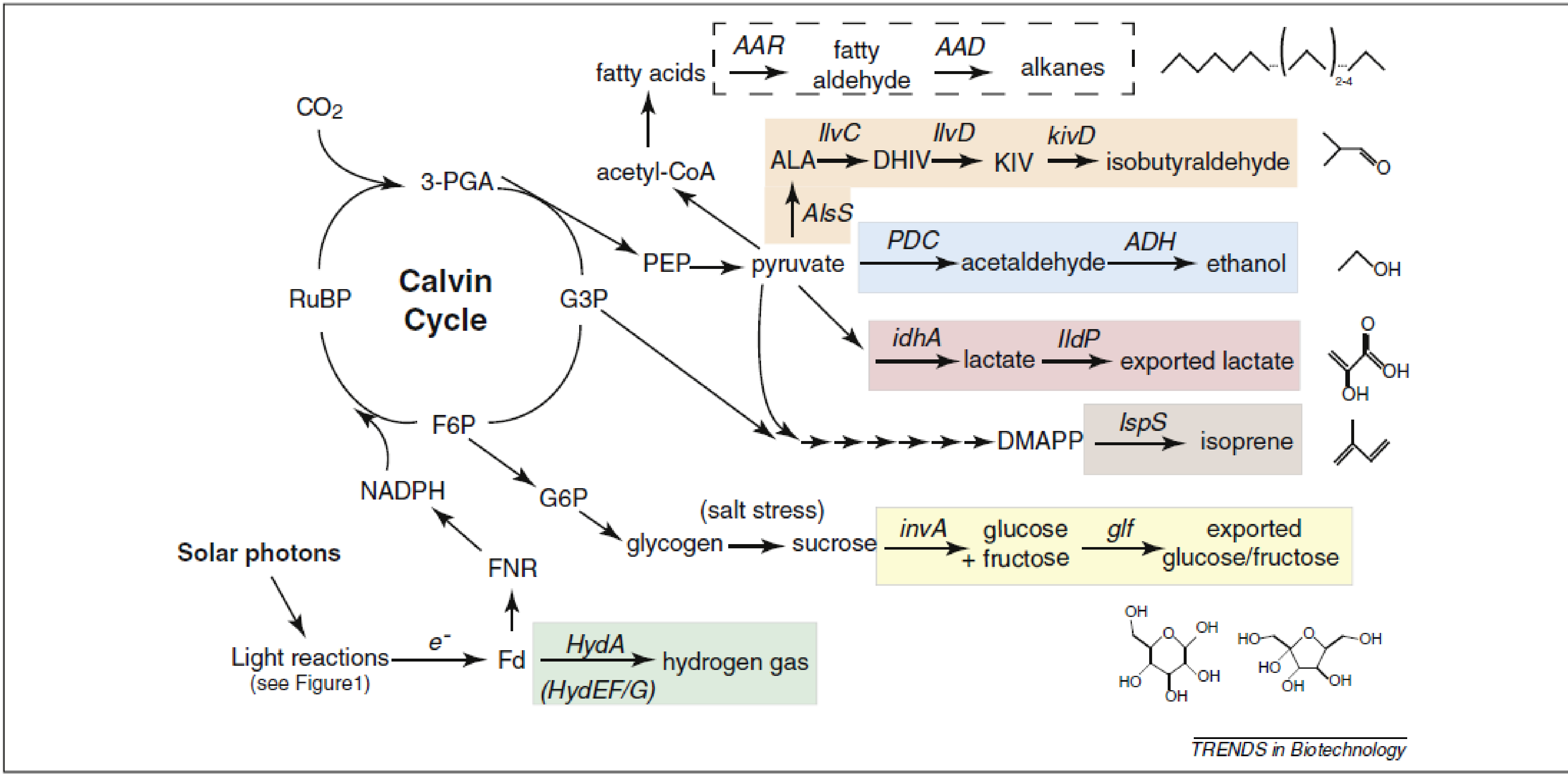


Figure 2. Schematic representation of engineered biochemical pathways in cyanobacteria. Core metabolism of photosynthetic processes is shown in black text. Branch points utilized for the production of various compounds discussed in this review are indicated (highlighted pathways) with relevant enzymes catalyzing specific reactions indicated in italics. Abbreviations: 3-PGA, 3-phosphoglycerate; AAD, aldehyde decarbonylase; ADH, alcohol dehydrogenase II; ALA, 2-acetolactate; AlsS, acetolactate synthase; DHIV, 2,3-dihydroxy-isovalerate; F6P, fructose 6-phosphate; FNR, ferredoxin NADP+ reductase; G6P, glucose 6-phosphate; HydA, [FeFe] hydrogenase; HydEF/G, hydrogenase maturation factors; ldhA, lactate dehydrogenase; IlvD, dihydroxy-acid dehydratase; IlvC, acetohydroxy acid isomeroreductase; PDC, pyruvate decarboxylase; PEP, phosphoenolpyruvate.

Oblasti využití řas - shrnutí

- Potraviny (celé řasy)
- Doplňky stravy
- Přísady v potravinářství
  - barviva
  - antioxidanty
  - zahušťovadla
- Farmacie
- Kosmetika
- Krmiva
- Mikrobiologie
- Látky, barvy, …
- Biopaliva
  - biodiesel
  - bioplyn
  - bioetanol
  - vodík