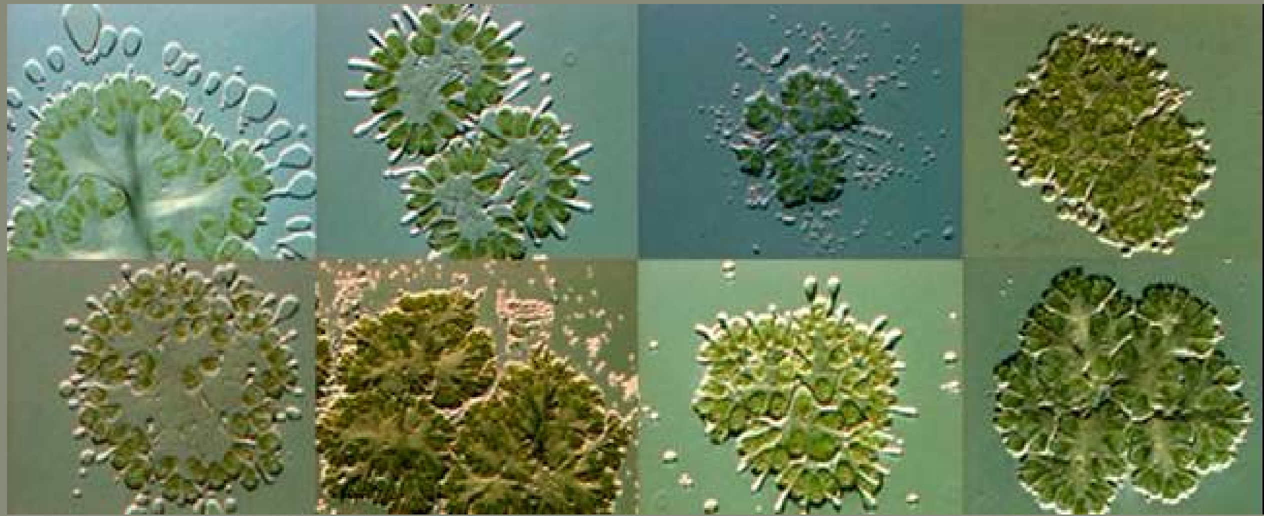
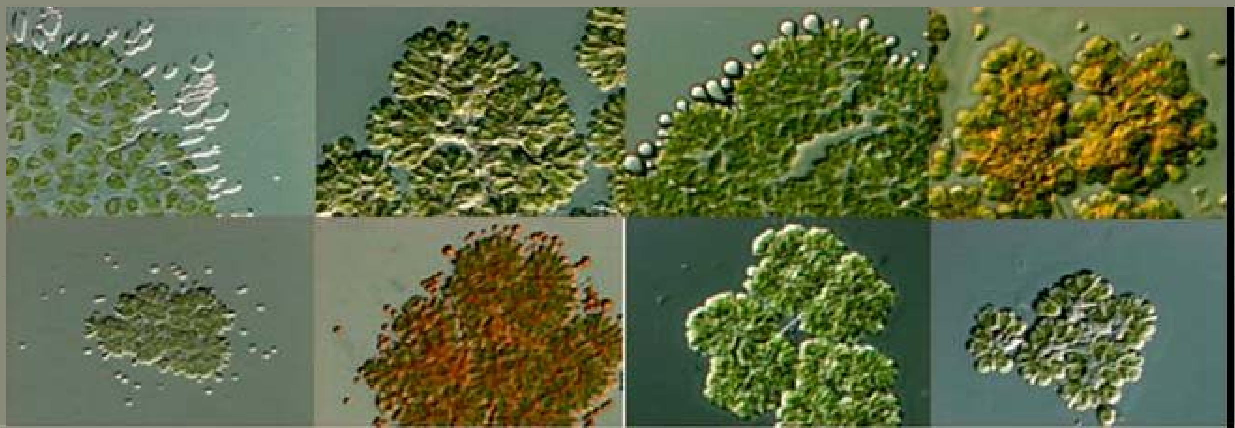
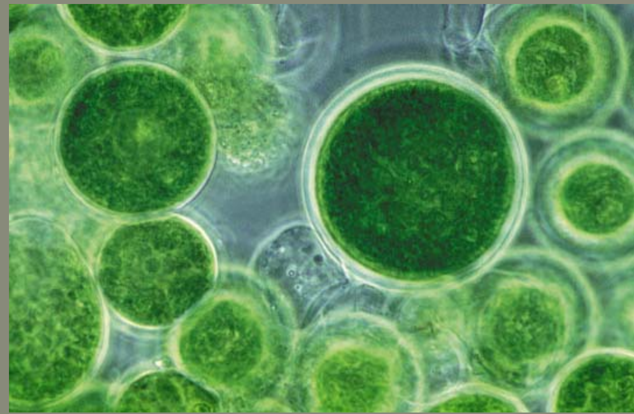


Possibility of Algal Lipids as Industrial Ingredients



Kunimitsu Kaya
University of Tsukuba

Lipid accumulating microorganisms



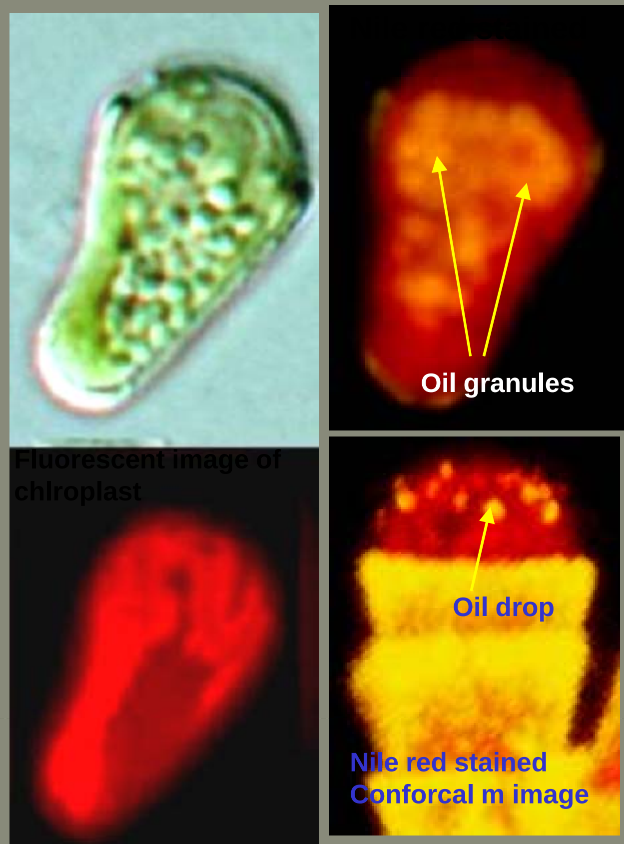
Green Algae

Pseudochoricystis ellipsoidea

Botryococcus braunii

Euglenoid

Euglena gracilis



Cost of Algae Production

algae	cost (JPYen/kg DW)	market price (JPYen/kg DW)
Botryococcus	80	?
Chlorella	159	2,000 – 10,000
Spirulina	160	2,000 – 7,000
Euglena	?	50,000 – 100,000

Lipid Composition of Algae

Botryococcus *		
Hydrocarbons	--	5-75%/DW
Triglyceride	--	1-3%/DW
Glycolipids	--	3-5%/DW
Phosphlipids	--	1-2%/DW

Euglena	(light condition)	(dark condition)
Triglyceride	8.2%	20.5%
Glycolipids	70.4%	5.9%
Phospholipids	20.7%	74.3%

*The lipid content and composition are dependent on media nutrients and culture conditions.

Hydrocarbons of *Botryococcus braunii*

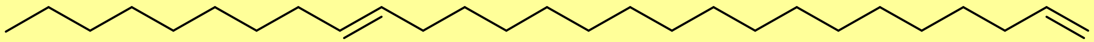
Strain No	H.C. content (% in algae DW)	Growth rate (μ/day)	Mol.Formula of the most abundant H.C.	Purity (%) of the H.C.
001	40.8	0.078	$C_{34}H_{58}$ (T) mw=466	92
002	21.1	0.187	$C_{33}H_{64}$ (A) mw=460	54
003	42.3	0.066	$C_{34}H_{58}$ (T) mw=466	97
004	45.3	0.158	$C_{31}H_{58}$ (A) mw=430	56
005	25.1	0.33	$C_{34}H_{58}$ (T) mw=466	94
006	45.7	0.2	$C_{34}H_{58}$ (T) mw=466	95
007	25.3	0.088	$C_{19}H_{38}O$ (E) mw=282	51

T: terpene; A: alkene; E: alkane epoxide

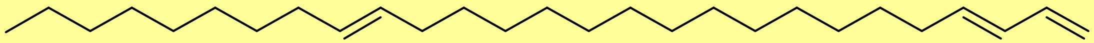
Structures of hydrocarbons produced from *Botryococcus*

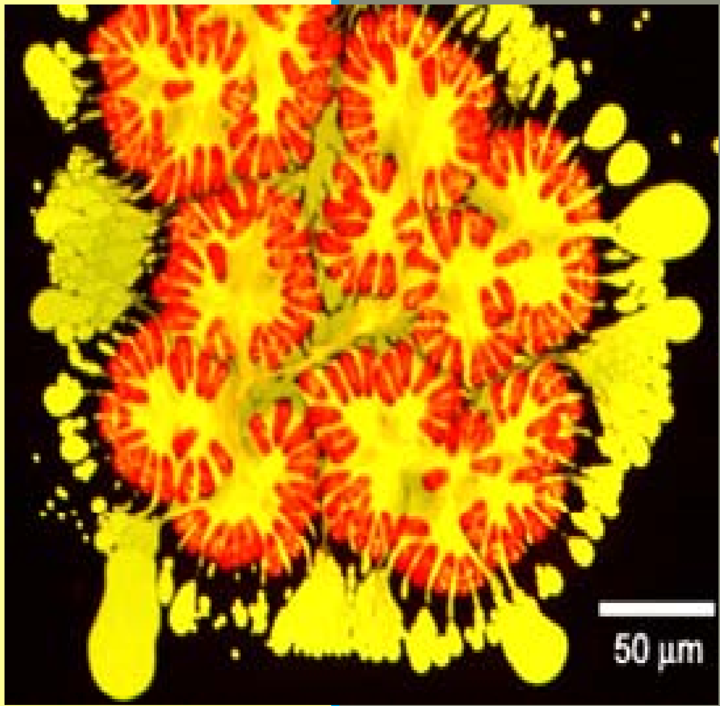
Race A

$C_{27}H_{52}$ E/Z



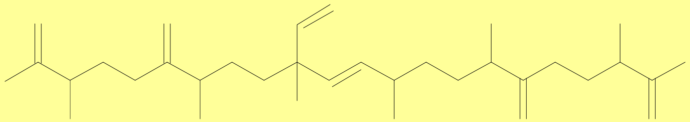
$C_{27}H_{50}$ E/Z





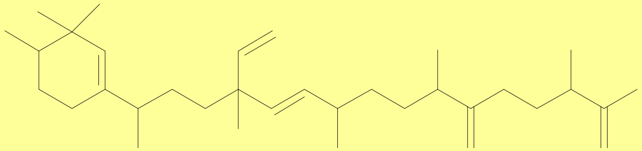
Race B

Botryococcene ($C_{34}H_{58}$, *MW* 466)



Cox, RE et al. J. Chem Soc.- Chem. Commun. 12, 284-285 (1973)

$C_{34}H_{56}$, mw 466

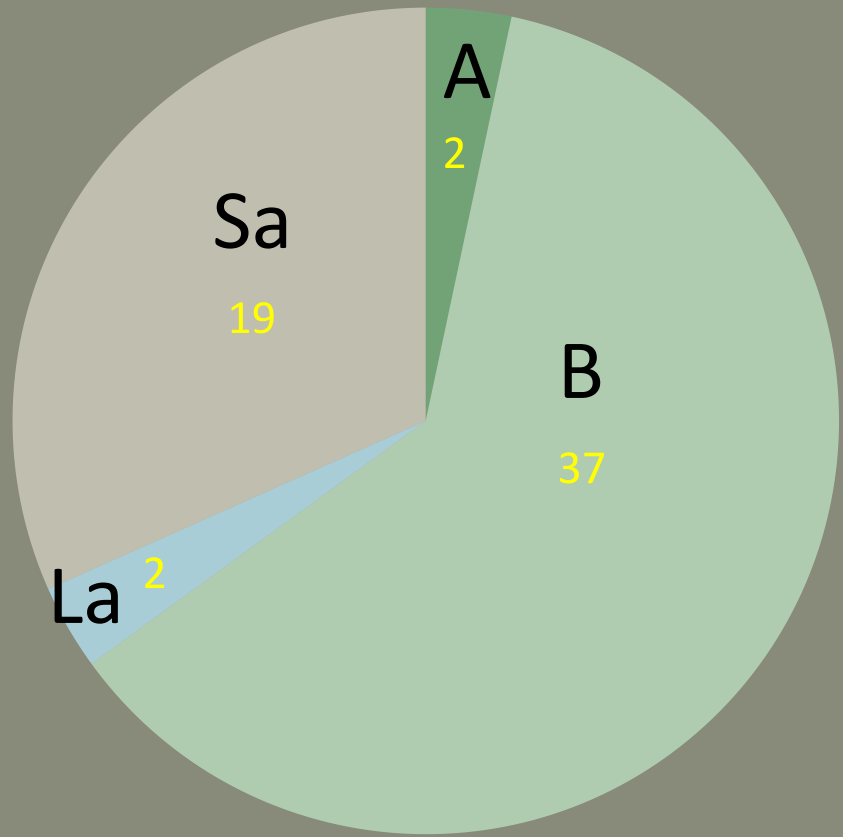


Hydrocarbon compositions of *B. braunii Races* Isolate from Japan

	Rt	Mol.%	mw	Molecular formula	Unsatu.
Race-A:	40.32	40	432	$C_{31}H_{60}$	2
	40.78	4	374	$C_{27}H_{50}$	3
	41.34	56	430	$C_{31}H_{58}$	3
Race-B:	41.29	39	466	$C_{34}H_{58}$	6
	43.18	50	466	$C_{34}H_{58}$	6
	43.52	11	466	$C_{34}H_{58}$	6
Race-Sa:	25.94	73	268	$C_{18}H_{36}O$	1
	27.61	27	278	$C_{19}H_{34}O$	3
Race-La:	25.92	10	268	$C_{18}H_{36}O$	1
	31.93	5	310	$C_{21}H_{42}O$	1
	44.48	42	557	$C_{39}H_{72}O$	4
	49.04	44	575	$C_{40}H_{78}O$	2

GC/MS Column: methylsilicon; Temp: 60ºC(2min)- 5ºC/min -280ºC

Major Hydrocarbons of Races of *B. buraunii* collected from Japanese freshwaters



A: $C_{27}H_{50}$, $C_{27}H_{52}$,$C_{29}H_{56}$

B: $C_{32}H_{54}$, $C_{34}H_{58}$

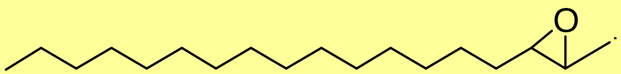
La: $C_{39}H_{72}O$, $C_{40}H_{78}O$

Sa: $C_{18}H_{36}$,O,$C_{19}H_{34}O$

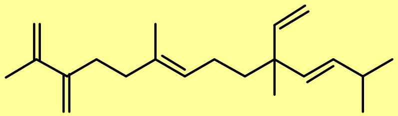
Hydrocarbon composition of *Botryococcus* isolated from a dam

	Rt		composition	unsaturation	compound
1.	18.01(min)	$C_{18}H_{36}O$, mw 268	6.4% (weight %)	1	epoxide
2.	20.32	$C_{21}H_{34}$, mw 286	19.1	5	diterpene
3.	27.19	$C_{33}H_{56}$, mw 452	20.2	6	triterpens
4.	27.30	$C_{33}H_{56}$, mw 452	26.0	6	triterpene
5.	27.57	$C_{32}H_{54}$, mw 438	28.3	6	triterpene

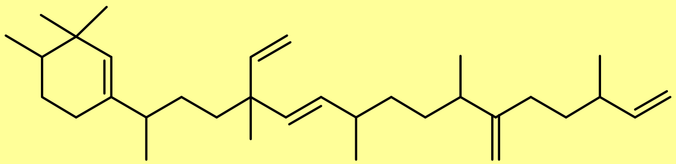
1.



2.

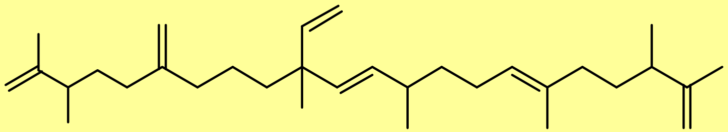


3.



4 is a geometric isomer of 3

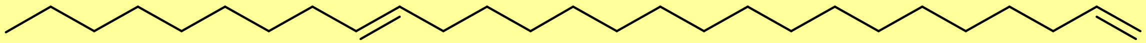
5.



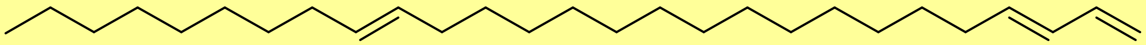
Application to fuel

Race A

$C_{27}H_{52}$ E/Z



$C_{27}H_{50}$ E/Z

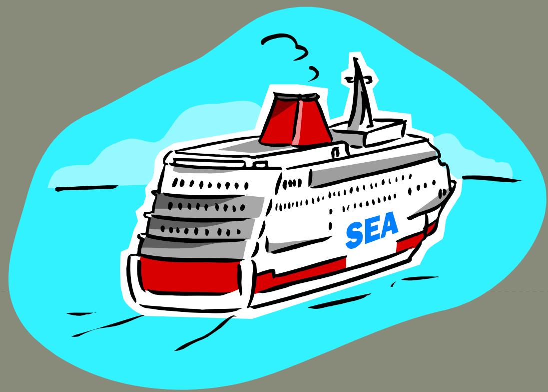


Direct Use

Catalytic Cracking

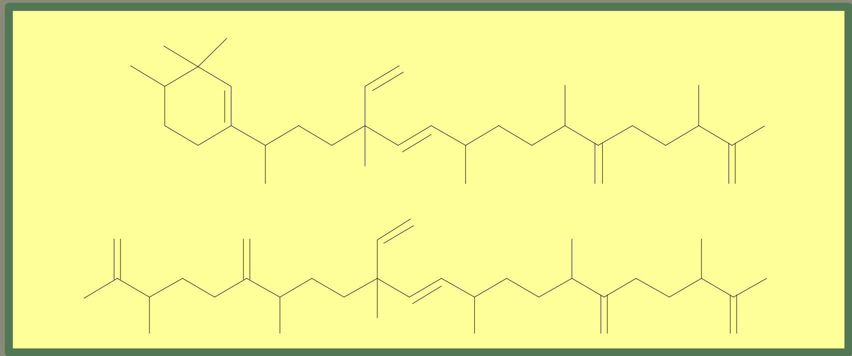
Diesel oil

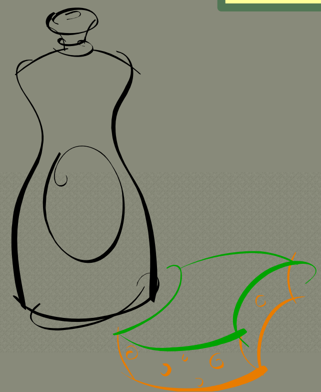
Gasoline (C5 –C8)



Application to chemical ingredients

The purities are over 90%

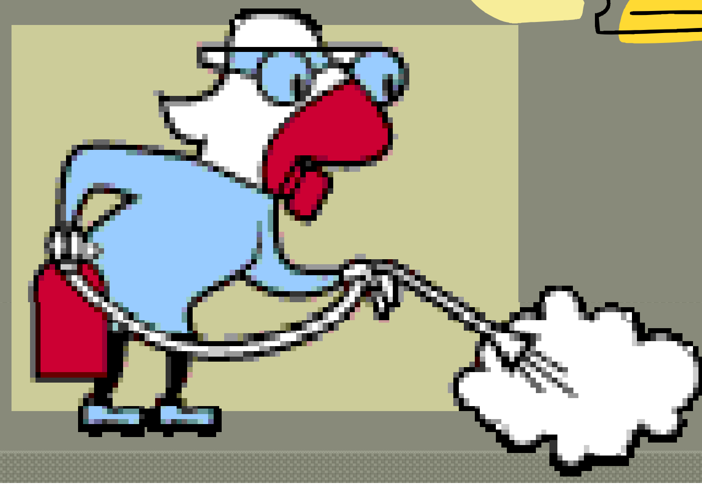




Polymer

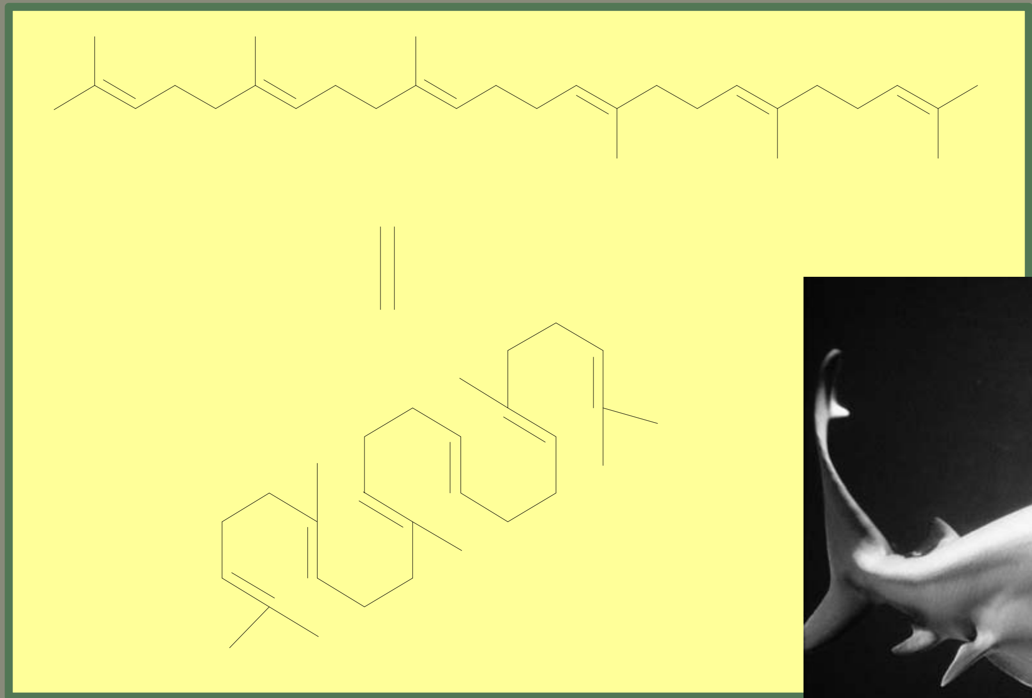
Chemicals

Fuel



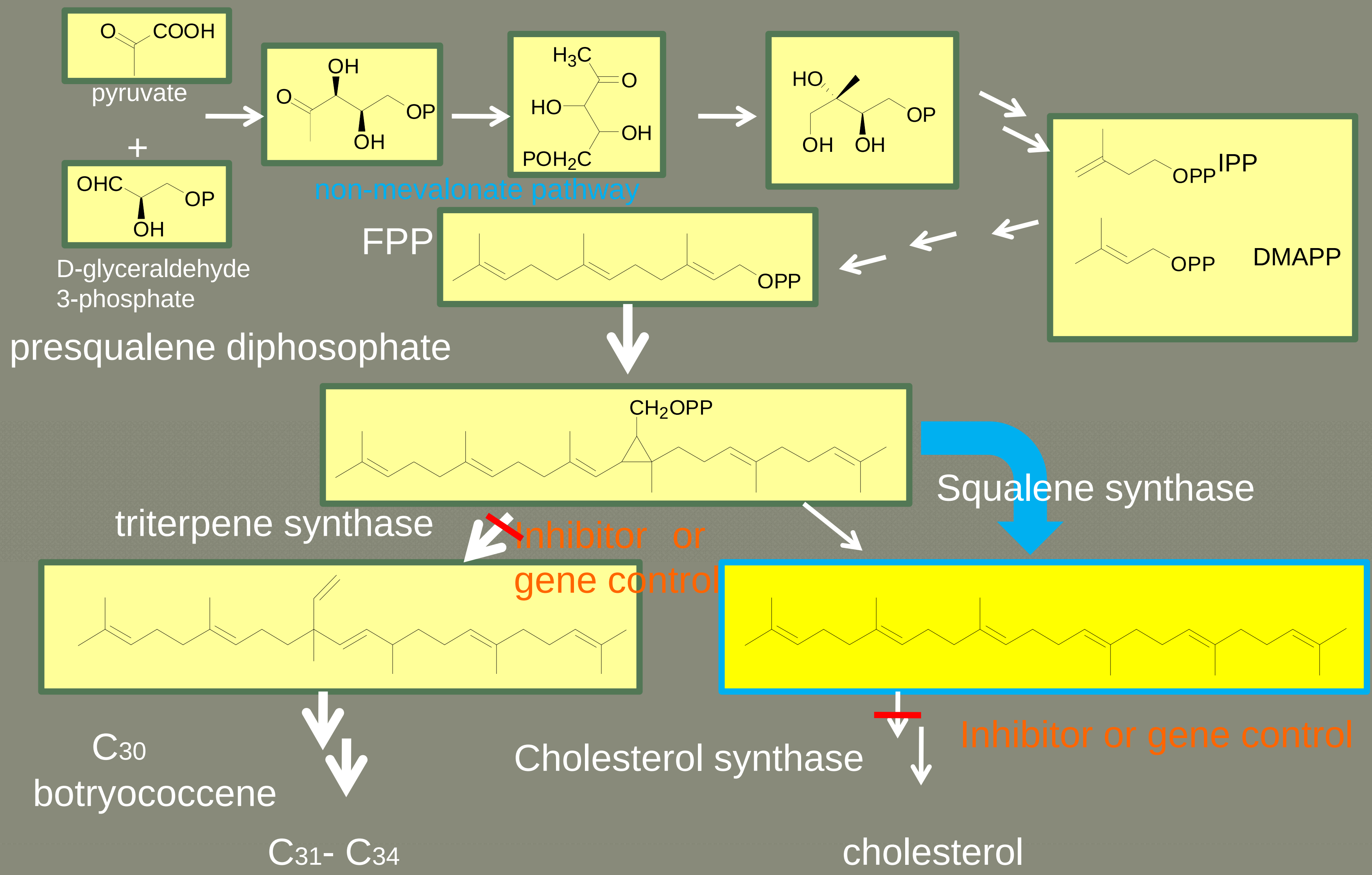
Squalene is a minor component of hydrocarbon fraction of microalgae

Squalene is isolated from shark liver oil. The market price of squalene for cosmetics is JPYen16,000/100g.

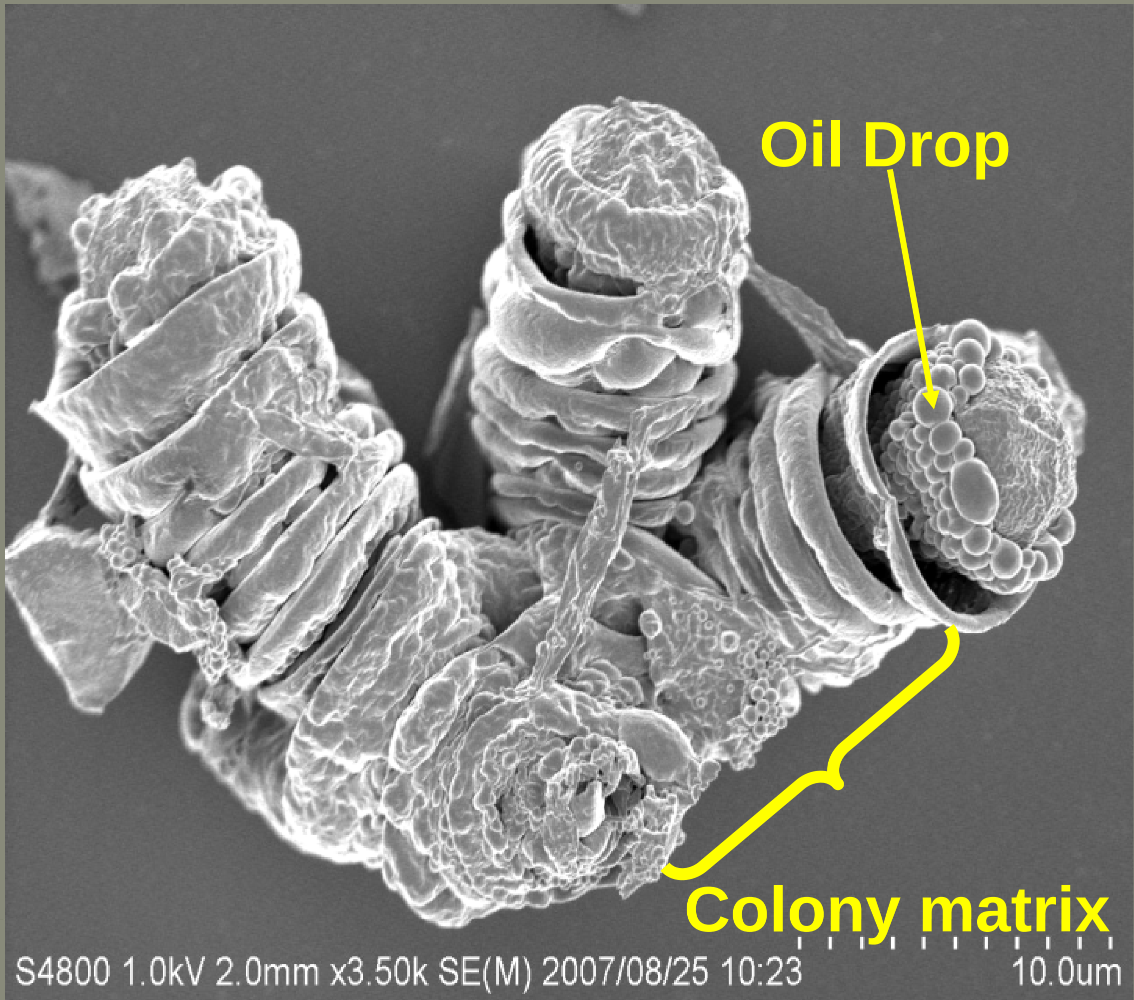


Squalene is used to keep skin moisture, and protect skin surface against oxidants.

How to control squalene production in microalgae



Algaenans (insoluble biopolymers as the main component of colony matrix)



Algaenans :

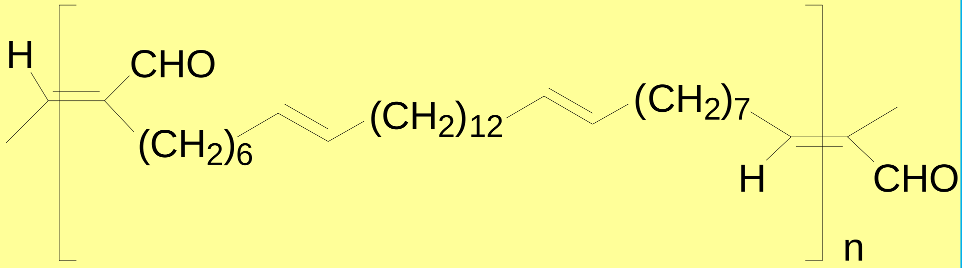
◎ the major component of colony matrix

◎ insoluble with organic solvents (chloroform/methanol, ethanol/diethy lether)-----Stubborn biopolymers

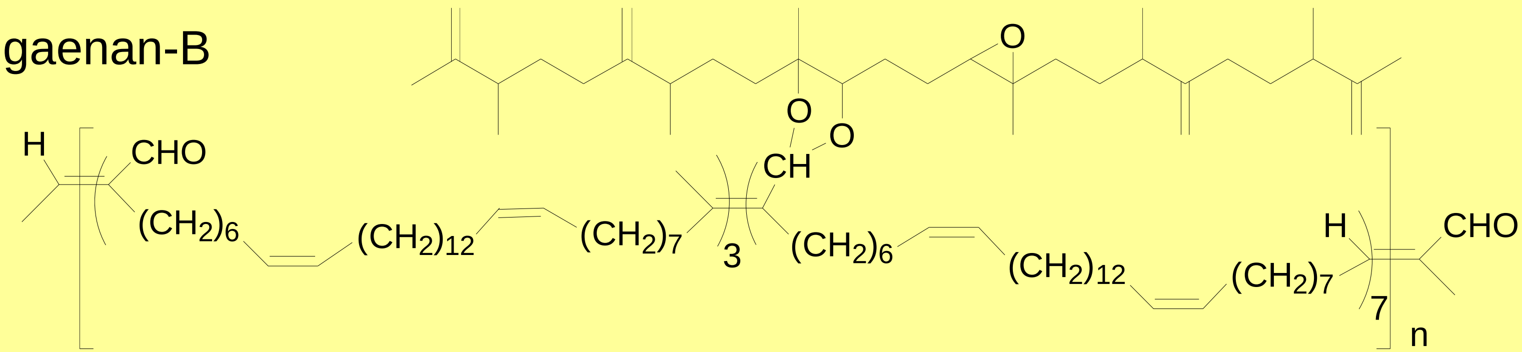
◎ partially soluble by the treatment with KOH/Methanol or trifluoroacetic acid and HCl/methanol),but not all

◎ consist of hydrocarbon units, mainly methylenic chains, $–(CH_2)_n-$

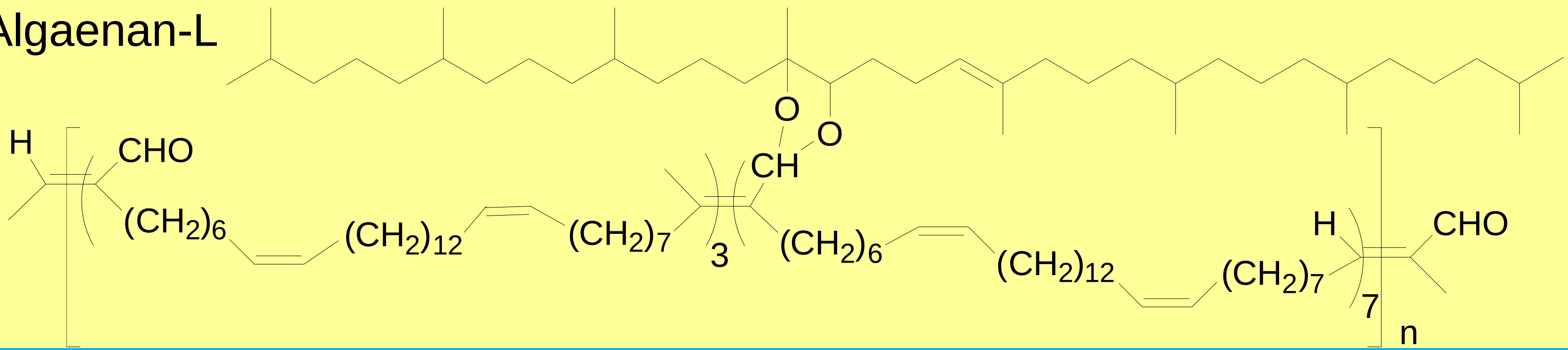
Algaenan-A



Algaenan-B



Algaenan-L

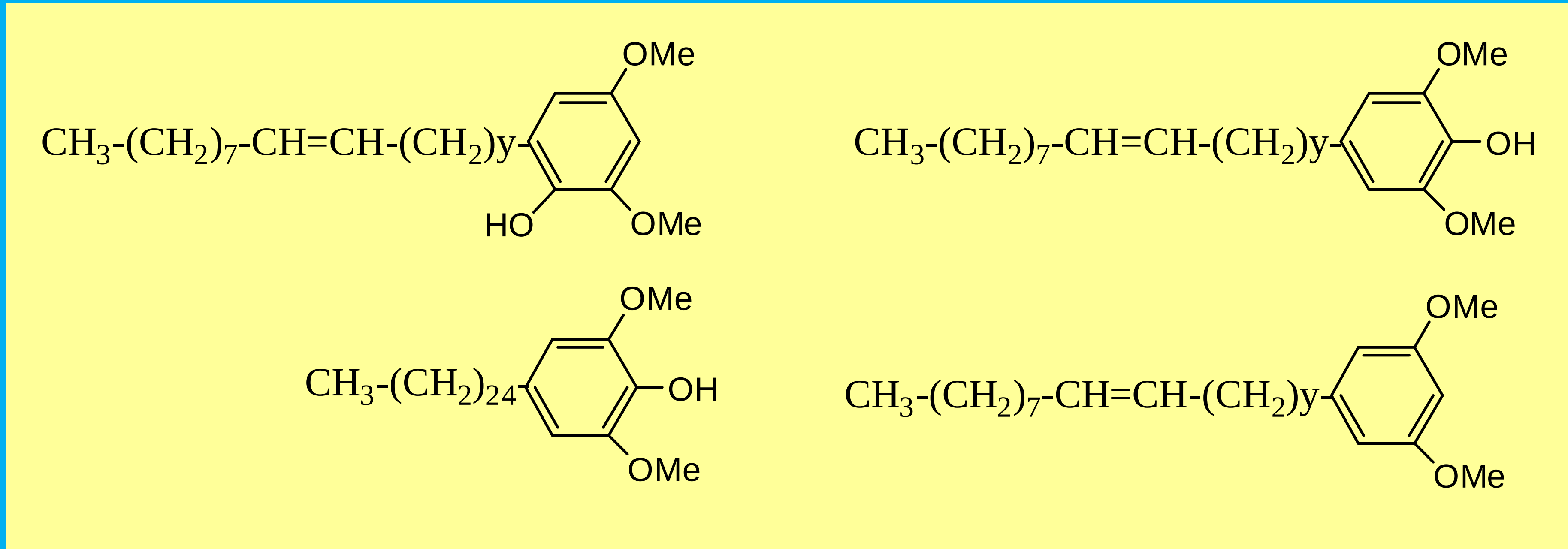


Algaenan forms sponge-like shape (reticulum structure), and hold a lot of hydrocarbon secreted to out-side of cells.

The colony is ensured by hydrophobic cohesion of algaenans .

Can we use algaenans as some ingredients?

N-Alkyl and Alkenyl phenols *of B. buraunii* Race- A



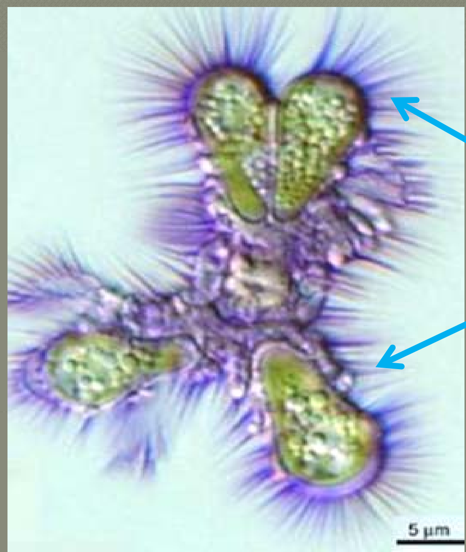
Y= 15-21, odd number

The phenolic moieties protect the aliphatic chains against degradation by Bacteria and fungi.

Productions of hydrocarbon and slime exopolysaccarides by *Botryococcus braunii*

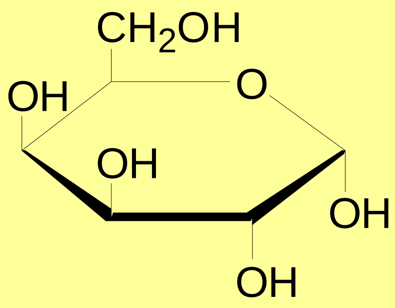
strain	Biomass (g/L)	Hydrocarbon (% of w/w)	Exopolusaccarides g/L
LB 572	2.0-3.6	20-35	1-2
SAG30.81	1.5-2.2	40-50	0.5-2
UC58			4-4.5
SI30	10	10-28	

Radial secretion of polysaccharides, stained with methyl violet

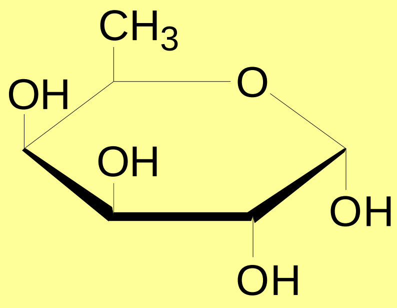


Exopolysaccarides (violet color)

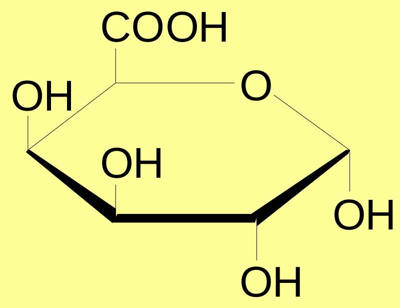
Sugar composition of the slime exopolysaccarides



α-*D*-Galactose



α-*D*-fucose



α-*D*-Galacturonic acid

In a case of strain UC58, the carbohydrate of the slime exopolysaccarides is mainly composed of α-*D*-glucose, α-*D*-fucose and α-*D*-galacturonic acid. Fucose may be related with apoptosis of cancer cells

Content and composition of slime exopolysaccarides of UT009 and 010

	Total*	Sugar composition (mol ratio) (Fuc / Gal / G-uron.A / Unknown compound)
009		
Exocarbohydrate	15mg/100mL	1/2/1/1
Cell debris (upper 5μm)	11 mg	1/2/1/1
010		
Exocarbohydrate	1.5g/L	1/3/2**

Fuc: α-*D*-fucose; Gal: α-*D*-galactose; G-uron. A: α-*D*-galcturonic acid.
* Excretion of slime exopolysaccarides is dependent on culture conditions
**not containing unknown compound.

Summary

1) Many microalgae accumulate hydrocarbons or triglycerides.
2) In culture costs, *Botryococcus* is the cheapest. The Hydrocarbon (HC) content reaches about 50% in *Botryococcus* dry cells.
3) The major HC are terpene, alkene and alkane epoxide, these chemical species are dependent on strains. Especially, many Japanese strains produce alkane epoxides. The purities of terpens in HC are over 90%.
4) Alkene and alkane epoxide can be utilized as fuel. Terpenes can be utilized as ingredients of polymer, detergents and other medicinal and industrial chemicals.
5) Squalene is an expensive ingredient of cosmetics, but a minor component of H.C. of microalgae. If we use inhibitors or gene modification of key enzymes of squalene biosynthesis, we obtain a large amount of squalene
6) Algaenans are insoluble biopolymers consisted of HC.
7) Slime exopolysaccarides is a major by-product of *Botryococcus*, and is composed of α-*D*-glucose, α-*D*-fucose and α-*D*-galacturonic acid. Also, these can be utilized as industrial ingredients.

When the lipids and by-products of microalgae will be utilized in various industries, the cost of biofuel produced by microalgae will be reduced. As the results, bioleum (bio + oleum: oil of bioresources) will become an alternative resource of petroleum.

Thank you for your attention

This study is supported by JST-CREST (Algal Oil Project)