Renewable Bioenergy and High-value Products from Microalgae

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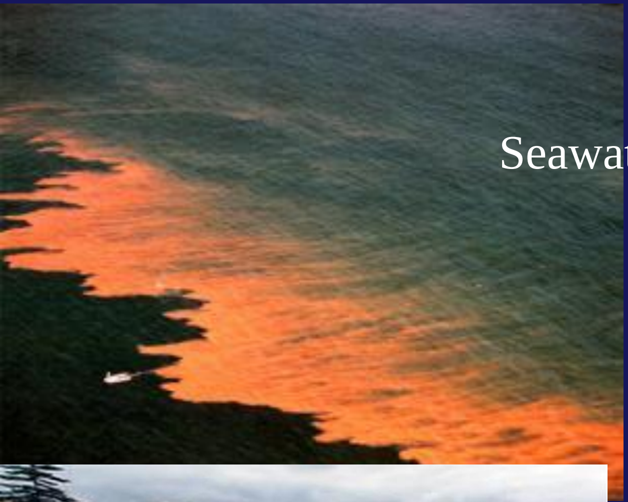
Microalgae are ubiquitous !

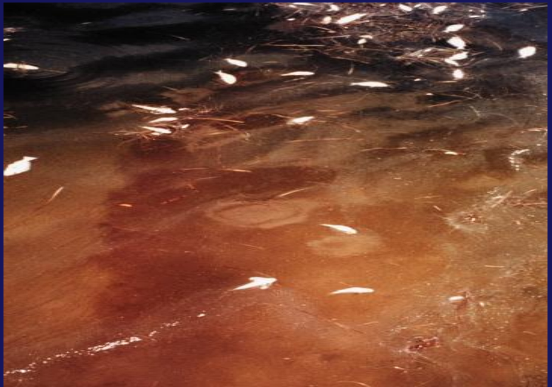
微藻无处不在

Harmful algal blooms 赤潮和水华

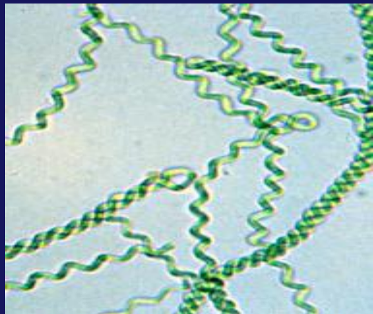


Seawater

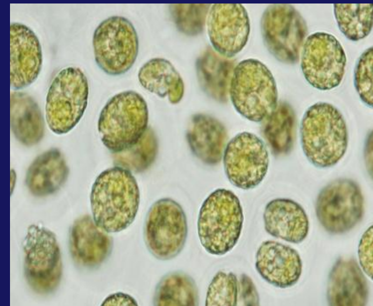
Freshwater



Microalgae (> 200,000 species; possibly up to 800,000 species)

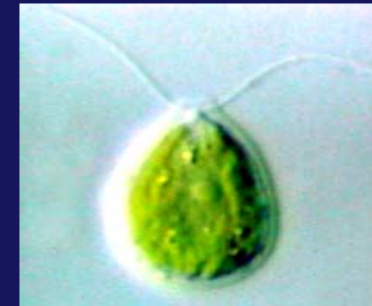


Spirulina 螺旋藻



Chlorella 小球藻

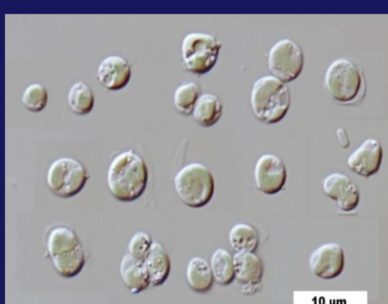
Dunaliella 杜氏藻



Chlamydomonas 衣藻

Scenedesmus 栅藻
(green algae)

Isochrysis 等鞭金藻
(golden/brown dinoflagellate
high DHA)

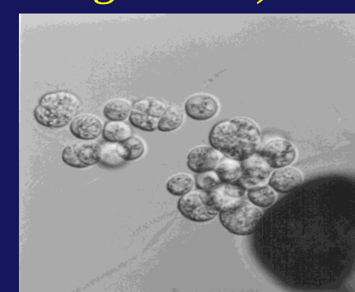


Nannochloropsis 微拟球藻
(green algae, 1-2 µm)

Tetraselmis 融合微藻
(green dinoflagellate
high lipids)

Haematococcus

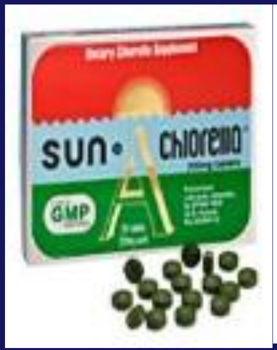
雨生红球藻



Schizochytrium

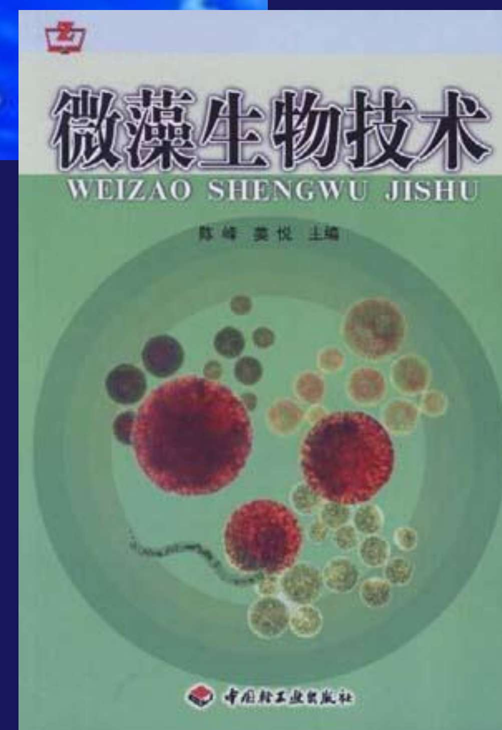
裂殖壶藻

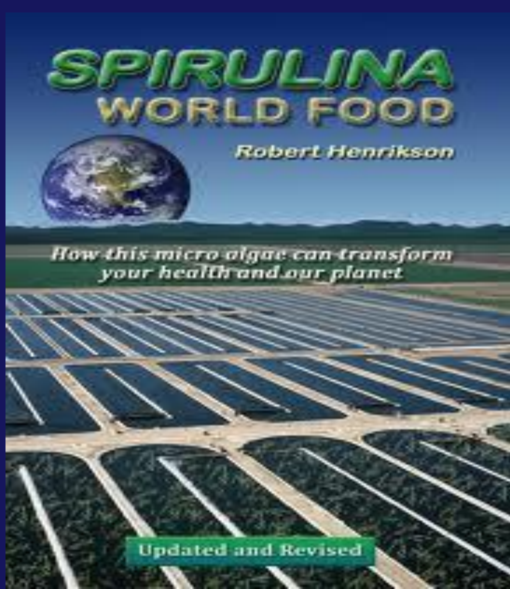
Microalgae and microalgal products in foods
微藻或含微藻成分产品

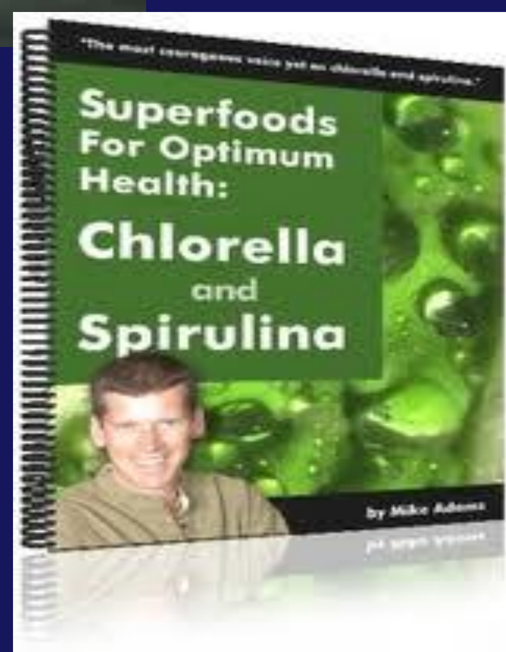


DHA 牛奶　　DHA 酸奶　　DHA 乳酸菌饮料

Microalgae as superfoods
微藻被誉为超级食品







Microalgal oils as biofuels – a world trend!
微藻油可成为最佳的生物柴油

Biodiesel production by microalgal biotechnology

GH Huang, F Chen, D Wei, XW Zhang… - *Applied Energy*, 2010 - Elsevier

被引用次数：>180

Microalgal Cultivation Strategies

Photoautotrophic growth (photosynthesis) means light (e.g., sun-light, artificial light, etc.) is required. Usually carbon dioxide (e.g., gas, bicarbonate, etc.) is used as carbon source.

Heterotrophic growth means at least one organic carbon source (e.g., sugars, organic acids, etc.) is used as energy source other than carbon source. Light is not required for growth.

Mixotrophic growth: organic carbon and light are used.



Microalgae as Bioenergy

Why microalgae are an excellent source of bioenergy?

1. Sustainable: renewable energy.

2. Economical: free sunlight and free carbon dioxide.

3. Environmentally friendly： consuming environmental and industrial (e.g., power station) carbon dioxide, flue gases, etc. Regarded as clean energy.

4. Other applications： in addition to algal bioenergy, other valuable compounds/substances can be obtained from microalgae (e.g., proteins, polysaccharides, carotenoids, etc.), and biomass after extraction of lipids can be fermented to produce methane.

More detailed reasons :

- A rich resource：grow almost everywhere, up to 800,000 species on the earth.
- A highly untapped resource: only some 20 species are being used commercially.
- High photosynthetic efficiency：more than 100 times higher than higher plant.
- Don’t waste food resources：compared to economic crops (e.g., corn, beans etc.).
- Can use lands that are not suitable for farming: salty, sandy, alkaline, etc.
- Can use seawater：don’t waste fresh water.
- High lipid content：the highest lipid containing organism (up to 77% dry weight).
- Easy industrialization: can be readily cultivated in bioreactors on a large scale.
- High-quality biodiesel：fatty acid composition can be manipulated leading to production of high-quality biodiesel (including fuels for aviation).
- Making mutants easily: using genetic/metabolic engineering or chemical/physical.
- Huge experience in large scale production: e.g., *Spirulina*, *Chlorella*, etc.
- Hot spot in research：sufficient funding from governments and industries
- More recent publications：*Journal of Applied Phycology* (> 50 % manuscripts)

《科学》杂志报道：

The world's second largest company (Exxon Mobil) announced that it will spend $600 million over 5 to 6 years to produce biofuels from algae

世界第二大公司Exxon Mobil （艾克森美孚）将在今后5-6年内花6亿美元投入生产微藻燃料。

BIOFUELS

ExxonMobil Fuels Venter's Efforts To Run Vehicles on Algae-Based Oil

The floodgates are officially open. After years of skepticism about the promise of biofuels, ExxonMobil has decided to make one of the biggest biofuel bets so far.

Last week, the world's second largest company announced that it will spend up to $600 million over 5 to 6 years to produce biofuels from algae. Half the money will go to Synthetic Genomics Inc. (SGI), a San Diego, California based start-up run by genomics pioneer J. Craig Venter. Exxon will spend another $300 million on in-house research including attempts to scale up biofuels production from algae and refine the resulting oils into finished fuels. "This is not going to be easy, and there are no guarantees of success," says Emil Jacobs, vice president of research and development for Exxon's research division.

Venter says SGI has already reengineered some strains of algae to secrete hydrocarbons from their cells. That achievement allows them to produce the compounds continuously in bioreactors. That's a big advantage over traditional "farming" methods, in which algae are grown in shallow ponds from which they are harvested and split open for oil that is then refined into fuel. The company plans to build a large facility in San Diego to test thousands of algal species in hopes of finding or reengineering strains to turn out oils at a high rate. Jacobs says Exxon's investment hinges on SGI's meeting a series of research milestones.

"The deal is tremendous for biofuels," says Bill Haywood, CEO of LS9 Inc., a biofuels start-up based in South San Francisco, California. "A move like that tells me they are serious about the future of biofuels." Exxon is the last major oil company to move into biofuels, notes Divya Reddy, an energy and natural resources analyst in Washington, D.C., with the Eurasia Group, an international policy risk assessment firm. The size of its investment "solidifies that trend," she says.

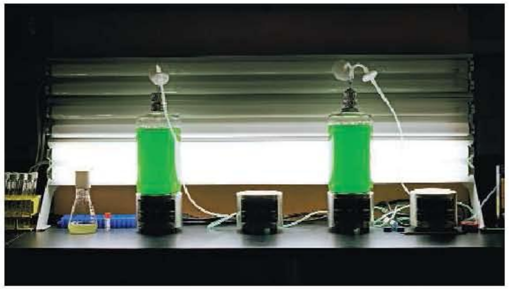
The technology's backers say algae have inherent advantages over other biofuels. They can be grown on nonagricultural land, absorb carbon dioxide, and the oils they produce can be refined into conventional transportation fuels that can be distributed using existing infrastructure. The market has begun to recognize those strengths: In 2006, Algenol Biofuels, a Florida-based company, announced an $850 million project with Mexico's Sonora Fields S.A.P.I. de C.V. to produce ethanol from algae. More recently, two algae fuel makers, Sapphire Energy in San Diego and Solazyme in South San Francisco, have raised $100 million and $76 million, respectively, from venture capitalists.

But although the area is hot, many obstacles remain. Among the biggest are reengineering algae to produce more hydrocarbons and to make molecules that more closely resemble refined gasoline. But Stephen Mayfield, a cell biologist at the Scripps Research Institute in San Diego, California, and a founder of Sapphire Energy, thinks the tallest hurdle is scaling up the process. "That's a key component Exxon will bring to this deal," Mayfield says.

The race to market has already begun. LS9, which uses engineered *Escherichia coli* instead of algae to make fuel, hopes to open a large-scale production facility in Brazil by 2013. Aurora Biofuels in Alameda, California, expects to have a commercial algae biodiesel facility online in 2012, and Algenol plans to begin selling fuel from its facility in Mexico later this year.

Mayfield and others aren't worried about the competition. Given the global $1-trillion-a-year market for energy, Mayfield says the more biofuels players, the better. "We will use every molecule of renewable energy we can get," he says.

–ROBERT F. SERVICE



Chemical plant. ExxonMobil will pay SGI to make biofuels from algae.

CREDIT: MARK MAHANEY

*Science*Insider

From the *Science* Policy Blog

India's new environment minister, **Jairam Ramesh**, says **India will not agree to mandatory reductions** in carbon emissions. Ramesh spoke at a news conference with visiting U.S. Secretary of State Hillary Clinton. "We are simply not in a position to take over legally binding emission reduction targets," Ramesh said. Clinton said the United States would not impose targets on India or "do anything that would limit India's economic progress."

The planned restart of the **Large Hadron Collider** will be pushed back from September to at least November because of newly discovered "vacuum leaks" in two sections of the accelerator. CERN disclosed the bad news in the latest issue of its online newsletter.

Thirty-four U.S. Nobel laureates last week called on President Barack Obama to push for **a steady funding mechanism** in upcoming climate legislation **to support clean energy research**. Many billions of dollars are already flowing from stimulus funding, they note in a letter to Obama, but the money will run out in October 2010. The Nobelists want the president to follow through on an earlier pledge to spend **$15 billion a year for 10 years** for research on clean energy.

Norman Augustine, who is chairing a blue-ribbon panel examining alternative futures for the U.S. human space-flight effort, hinted last week that **the panel might shy away from advocating an expensive human mission to the moon, Mars, or an asteroid**. Recommendations to the White House are expected by the end of August.

The **Chinese government has banned** the controversial application of **electro-convulsive therapy** for so-called Internet addiction after a clinic gained notoriety for applying electric shocks to unanesthetized teenagers being treated against their will (*Science*, 26 June, p. 1630).

For other science policy news, go to **blogs.sciencemag.org/scienceinsider.**

Published by AAAS

News from US Department of Energy (www.energy.gov)

Secretary Chu Announces Nearly $80 Million Investment for Advanced Biofuels Research and Fueling Infrastructure. January 13, 2010

Washington, DC – U.S. Department of Energy Secretary Steven Chu today announced the investment of nearly $80 million under the American Recovery and Reinvestment Act for advanced biofuels research and fueling infrastructure that will help support the development of a clean sustainable transportation sector. The selections announced today – two biofuels consortia for up to **$78 million to research algae-based and other advanced biofuels** – are part of the Department's continued effort to spur the creation of the domestic bio-industry while creating jobs.

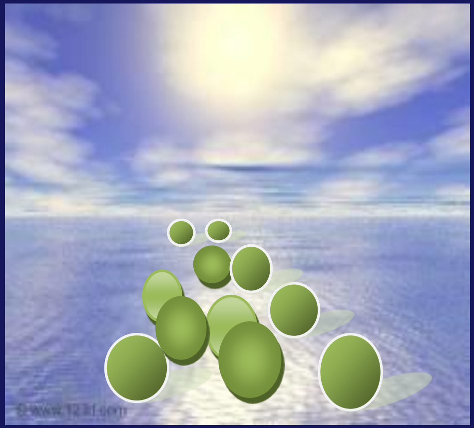
"Advanced biofuels are crucial to building a clean energy economy," said Secretary Chu "By harnessing the power of science and technology, we can bring new biofuels to the market and develop a cleaner and more sustainable transportation sector. This investment will help spur the creation of the domestic bio-industry, while creating jobs and reducing our dependence on foreign oil."

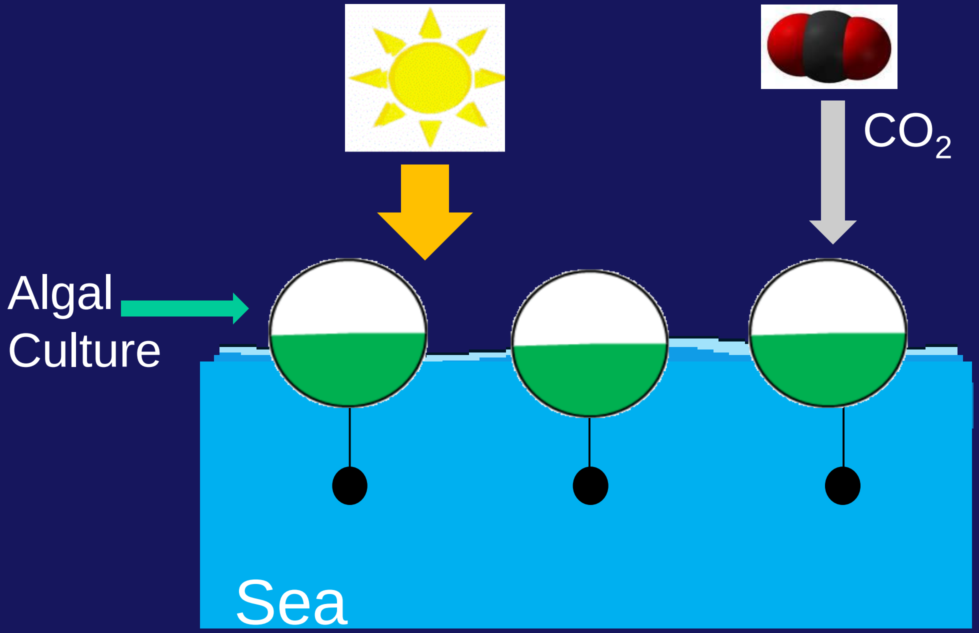
美国政府能源部2010年初宣布再拨款7.8千万美元（相当于5.4亿人民币）支持建立两个已微藻为生物能源的研究项目，以作为他们继续支持生物能源研究开发的一部分

Singapore-PKU Research Center for a Sustainable Low Carbon Future

- Biological Route:

  Set up a demonstration of floating photo-bioreactor with a target of 10-fold increase in photosynthetic productivity for CO_2 removal by genetically modified microalgae, & production of algal oil and high value products



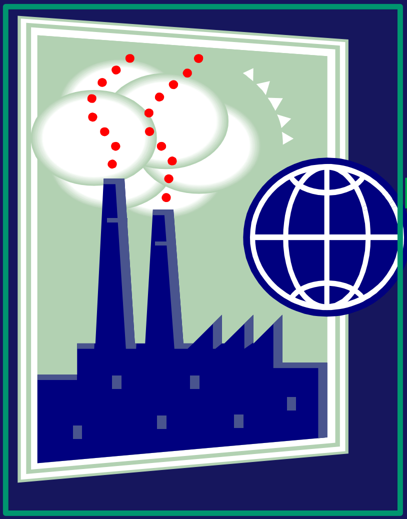


Industry Participation & interest

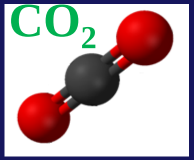
Van Der Horst

- Biodiesel feedstock (algal oil) and high value biomolecules

Technology for biodiesel production from microalgae using flue gases（Flow Chart)



Power station



Collection of flue gases and treatment

Photobioreactors for microalgal cultivation

Electric flocculation

Wet biomass

Wet extraction and transesterification

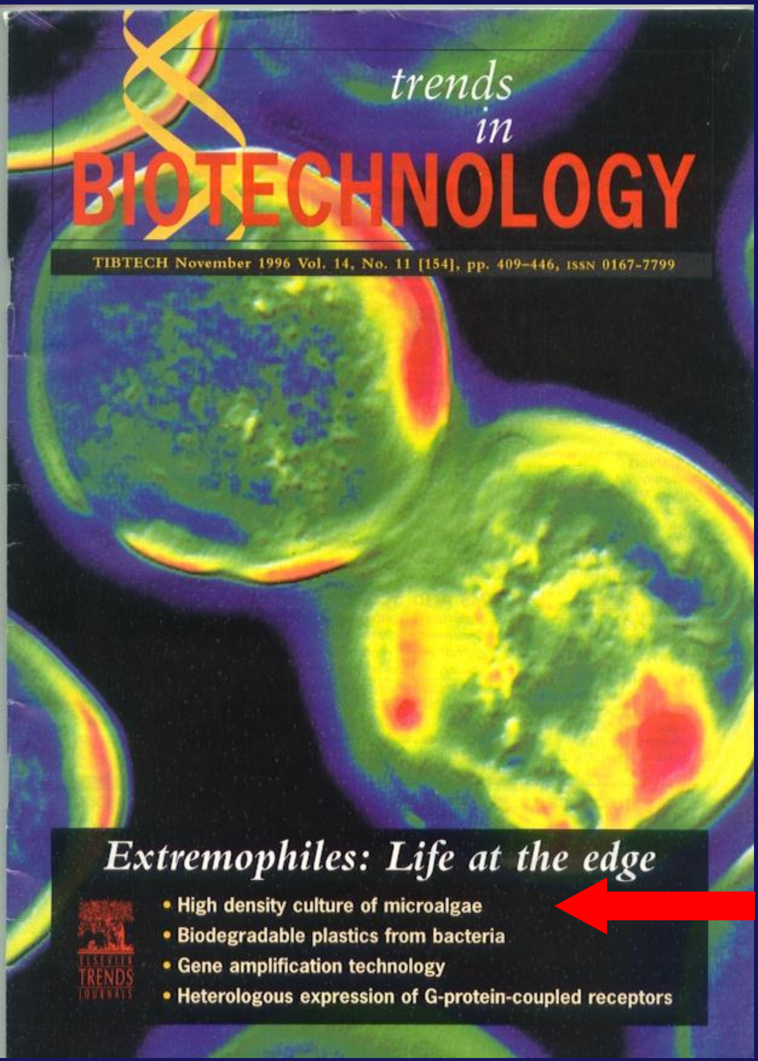
Biodiesel

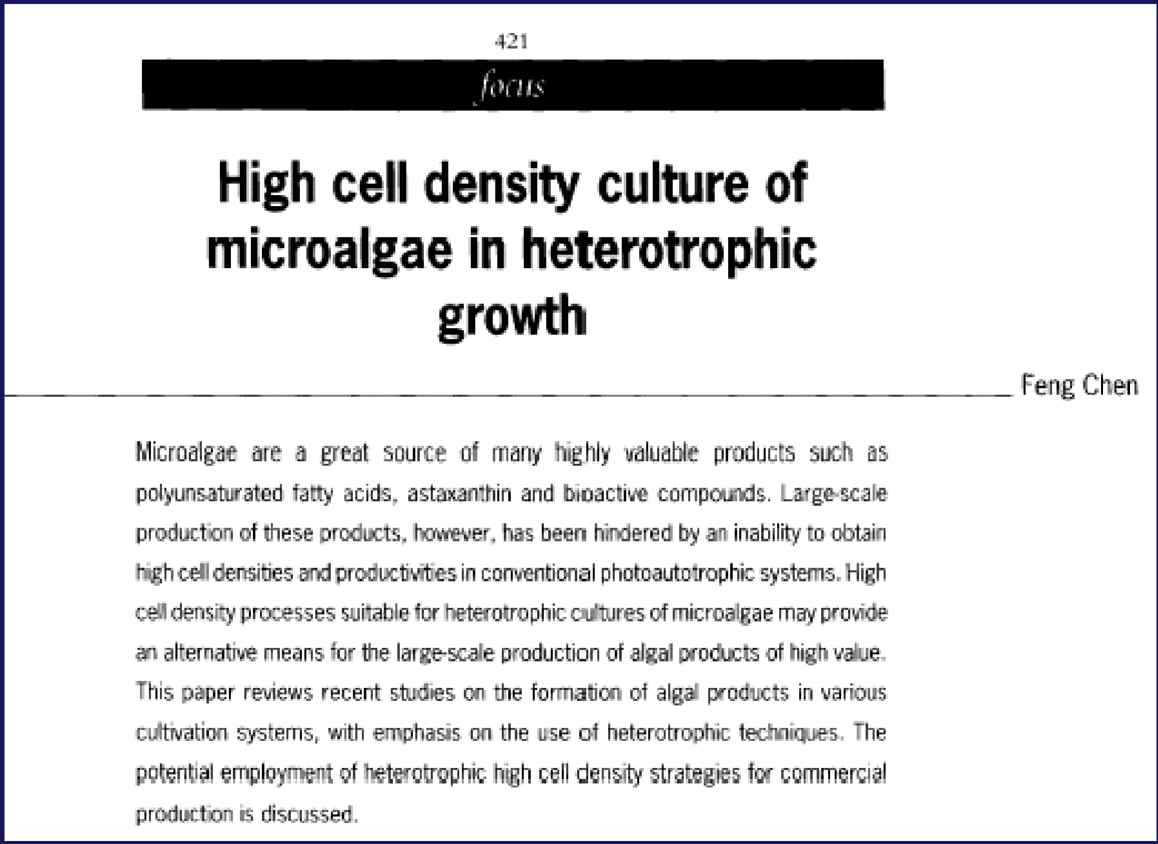
New functional materials

Functional foods & drugs

Heterotrophic Culture: Alternative Means

- Mono-culture
- High cell density
- Better control of environment
- May grow on cheap substrate
- Easy scale up



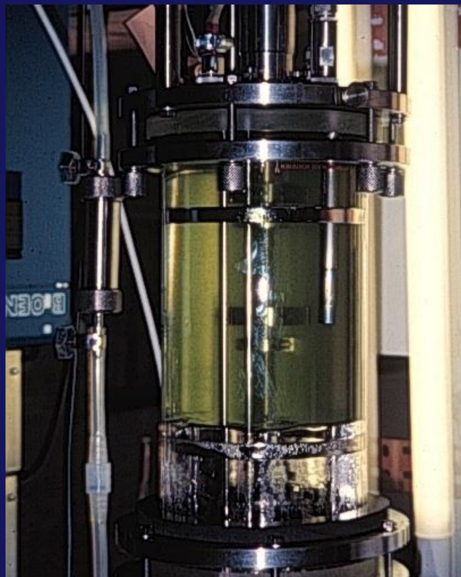


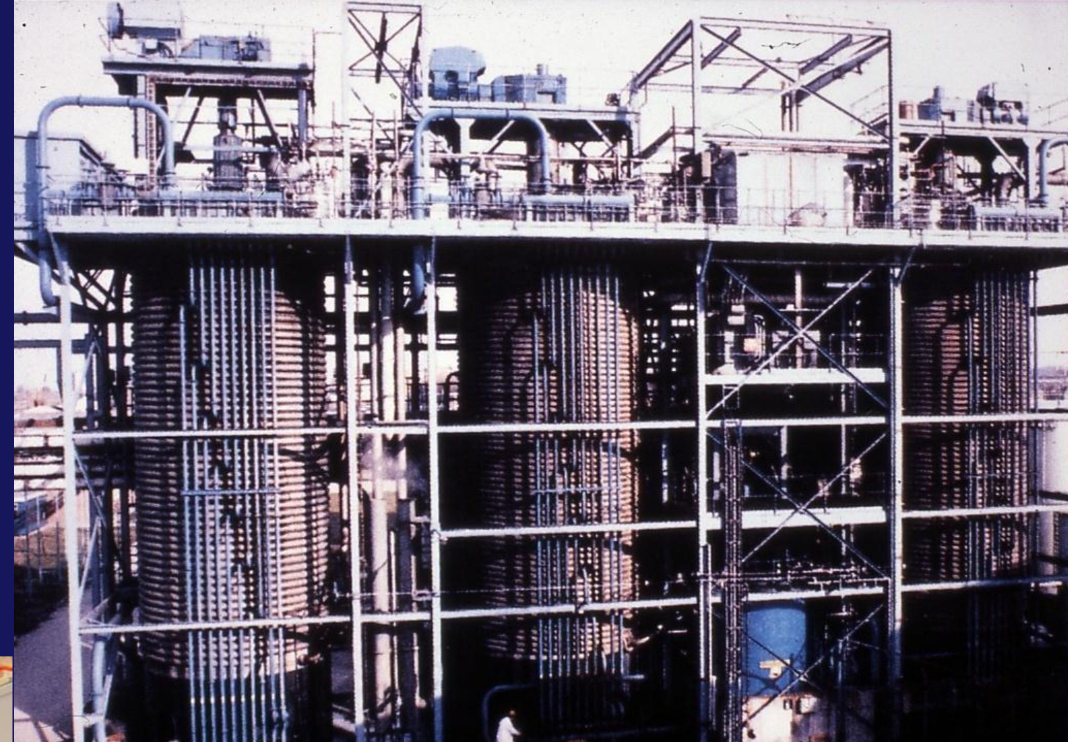
focus

High cell density culture of microalgae in heterotrophic growth

Feng Chen

Microalgae are a great source of many highly valuable products such as polyunsaturated fatty acids, astaxanthin and bioactive compounds. Large-scale production of these products, however, has been hindered by an inability to obtain high cell densities and productivities in conventional photoautotrophic systems. High cell density processes suitable for heterotrophic cultures of microalgae may provide an alternative means for the large-scale production of algal products of high value. This paper reviews recent studies on the formation of algal products in various cultivation systems, with emphasis on the use of heterotrophic techniques. The potential employment of heterotrophic high cell density strategies for commercial production is discussed.





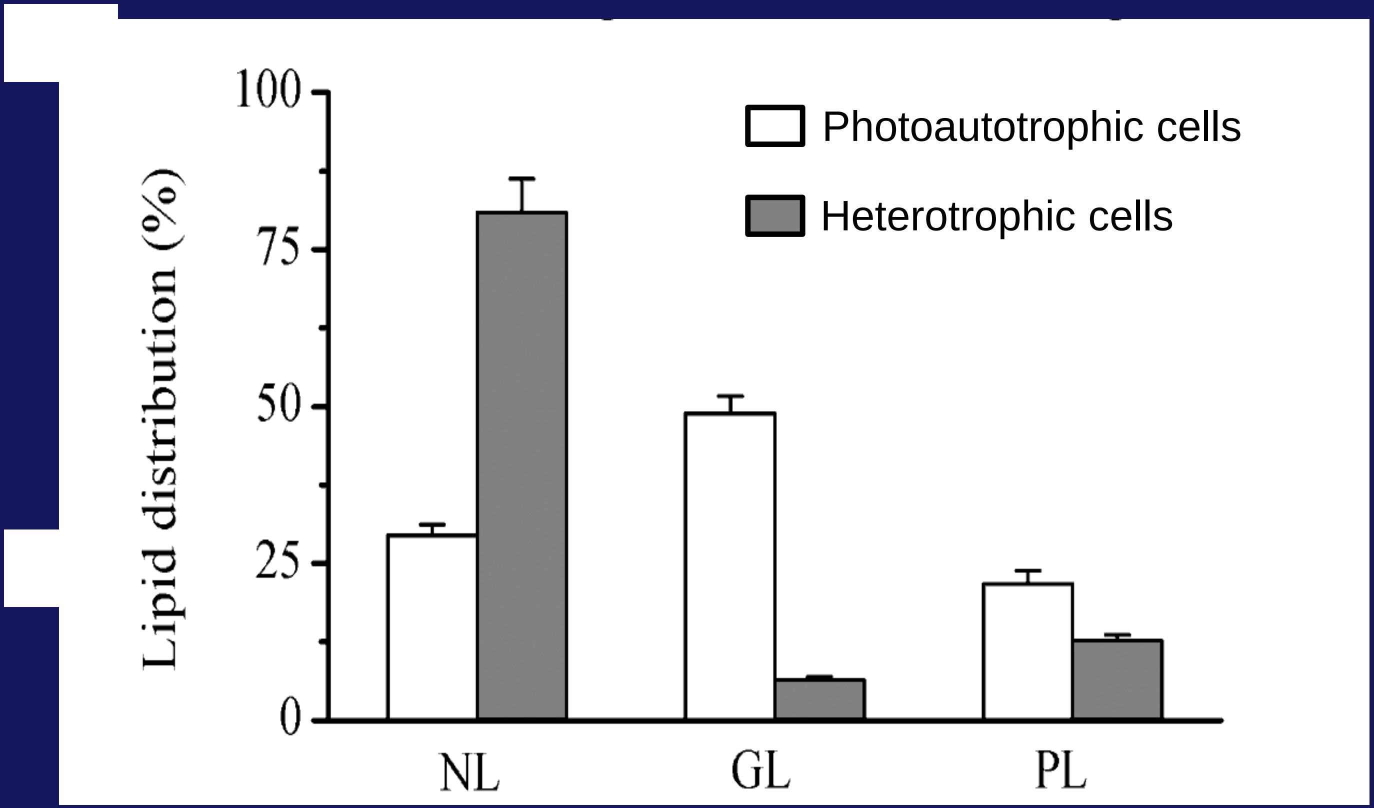
Software tool for real-time fermentation process control

Assessment of *Chlorella zofingiensis* as a potential biodiesel feedstock – *AN EXAMPLE*

Heterotrophic cells can grow faster and accumulate more lipids than photoautotrophic ones

Parameters[b]	*C. zofingiensis*	
	Photoautotrophic	Heterotrophic
Specific growth rate (d^{-1})	0.235 ± 0.014	0.769 ± 0.046
Biomass ($g\ L^{-1}$)	1.9 ± 0.11	9.7 ± 0.33
Lipid content ($g\ g^{-1}$)	0.258 ± 0.016	0.511 ± 0.027

Lipids from heterotrophic cells consist mainly of neutral lipids



NL = Neutral lipids; GL = Glycolipids; PL = Phospholipids

Heterotrophic cells contain higher proportion of oleic acid (C18:1)

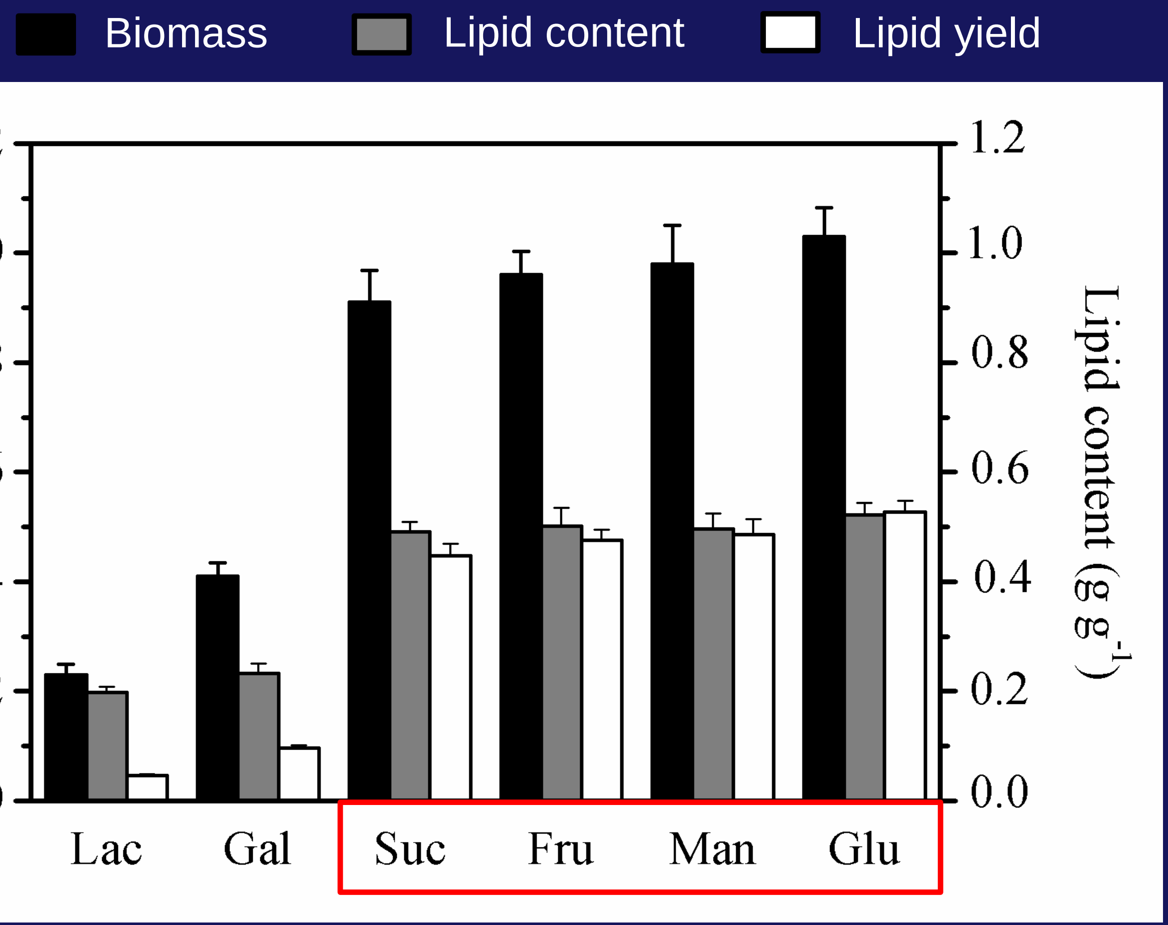
Fatty acids	16:0	16:1	16:2	16:3	16:4	18:0	18:1	18:2	18:3 n-6	18:3 n-3	18:4
Photo-autotrophic	26.6	2.2	7.7	6.6	1.1	3.7	17.9	20.8	1.4	10.8	1.1
Hetero-trophic	22.2	1.7	8.3	2.1	0.2	1.2	35.2	20.2	0.5	7.8	0.4

Data are expressed as percentage of total fatty acids (TFA)

High proportion of oleic acid is preferred for biodiesel

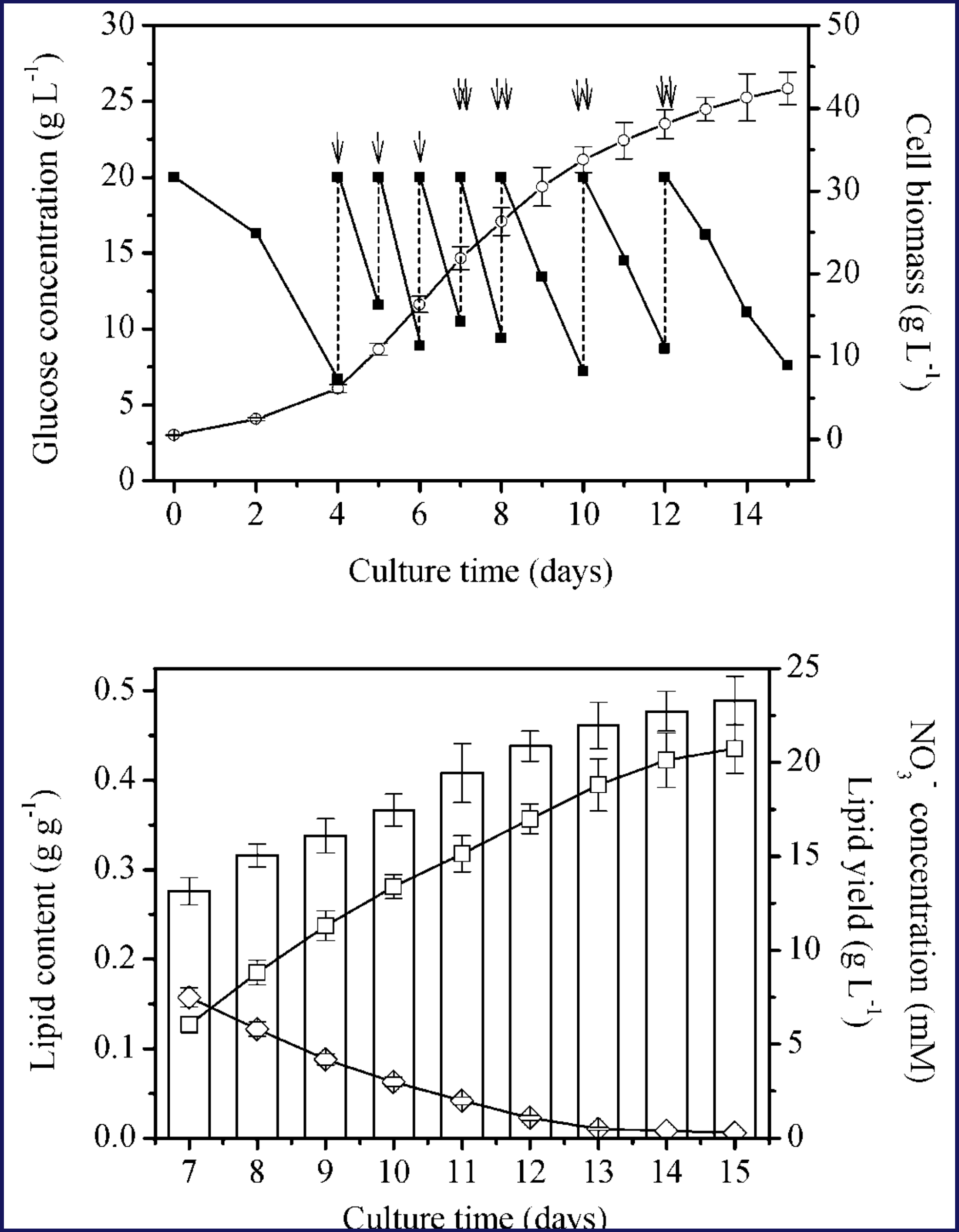
Oils from heterotrophic cells appear to be more feasible for biodiesel production

Heterotrophic growth and lipid production of *C. zofingiensis* on various sugars



Lac = Lactose; Gal = Galactose; Suc = Sucrose; Fru = Fructose; Man = Mannose; Glu = Glucose

Lipid production by *C. zofingiensis* in fed-batch fermentation



Fed-batch can extend exponential growth phase and increase biomass

■ Glucose concentration
○ Cell biomass dry weight
↓ Medium containing glucose
↓↓ Only glucose (i.e., no nitrogen)

Lipid yield: 20.7 g/L

█ (bar) lipid content
□ lipid yield
◇ NO_3^- concentration

Lipid production by *C. zofingiensis* from cane molasses

Cane molasses is a low-cost and readily available by-product of sugar industry, consisting of around 50% total sugars (sucrose, glucose and fructose)

Pretreatment of molasses	Cell biomass (g L^{-1})	Lipid content (g g^{-1})	Lipid yield (g L^{-1})	Residual total sugar (g L^{-1})	Sugar unitization (%)
No pretreatment	5.5 ± 0.29	0.32 ± 0.02	1.76 ± 0.10	15.8 ± 0.65	47.4 ± 2.5
Pretreatment with sulfuric acid	10.8 ± 0.43	0.48 ± 0.03	5.18 ± 0.22	4.6 ± 0.28	84.7 ± 3.3

Metal ions/suspended colloids inhibit growth & inactivate enzymes

↓

Pretreatment of molasses is needed

Comparison of pretreated molasses with glucose in fed-batch fermentation for lipid production

Sugar	Cell biomass ($g\ L^{-1}$)	Lipid content ($g\ g^{-1}$)	Lipid yield ($g\ L^{-1}$)	Consumed sugar ($g\ L^{-1}$)	Lipid yield coefficient
Glucose	42.5 ± 1.9	0.48 ± 0.02	20.4 ± 0.8	83.9 ± 4.5	0.243 ± 0.01
Pretreated molasses	46.9 ± 2.3	0.42 ± 0.02	19.7 ± 1.2	82.3 ± 3.1	0.239 ± 0.02

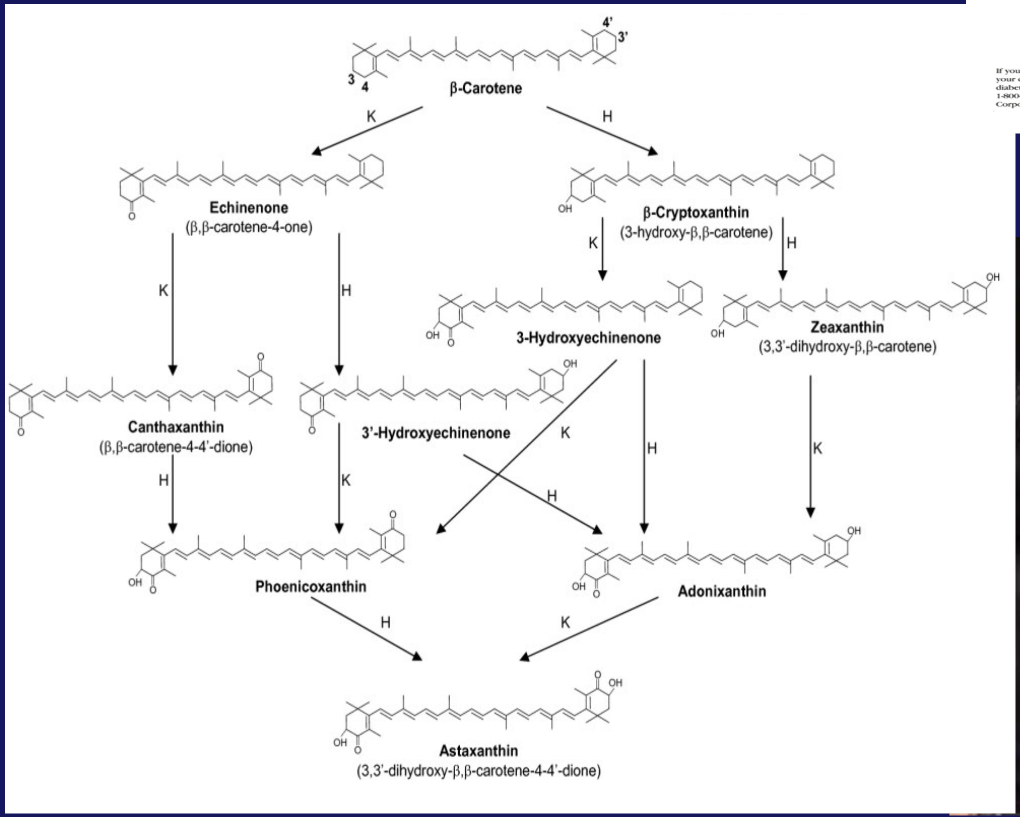
Lipid yield coefficient = lipid yield ($g\ L^{-1}$) / consumed sugar ($g\ L^{-1}$)

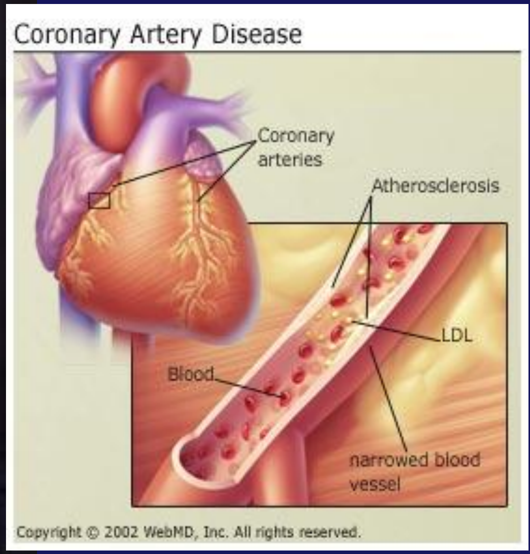
Cane molasses, when pretreated with proper methods, can substitute glucose to feed *C. zofingiensis* for economical biodiesel production

Astaxanthin from *Chlorella zofingiensis*

- Mainly carotenoids (类胡萝卜素）
- Protecting against neurological dysfunction
- Anti-cancer activities
- Improving immune system
- Interrupting glycation process
- Preventing heart/vascular diseases

Astaxanthin = $7000/kg





C. zofingiensis

mutagenesis

Mutants

characterization

PDS mutation

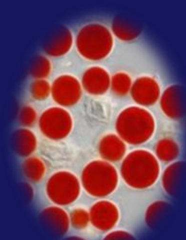
(PDS-L516F)

Transformation

selectable marker
(PDS-L516F)

Transformants

Astaxanthin



Lipid/fatty acid analyses

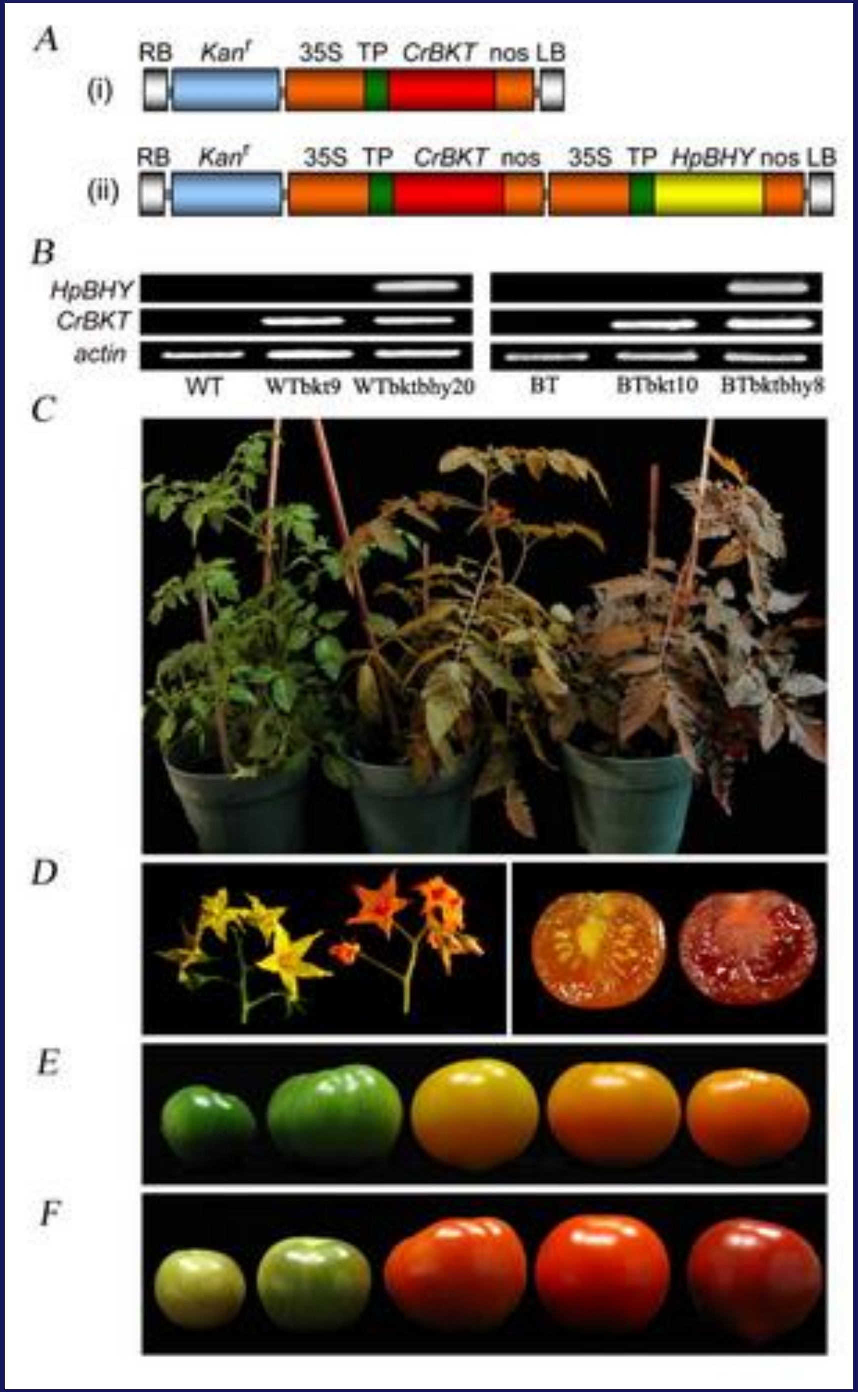
Sugar-based lipid production

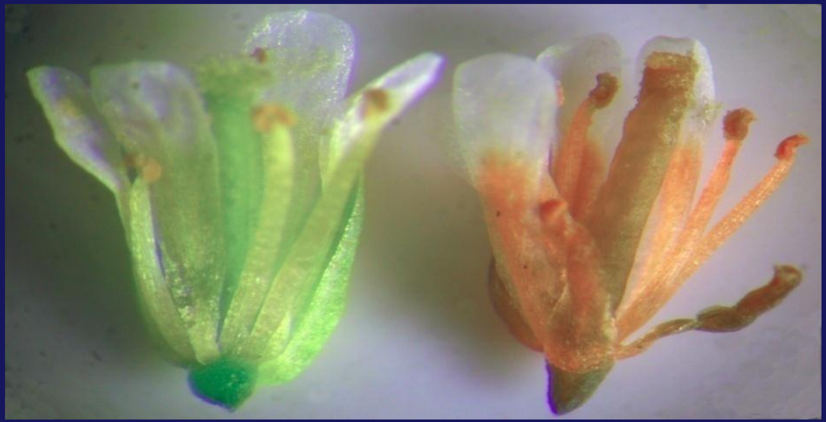
Cane molasses

Biodiesel

Expression of a *BKT* gene (from green microalgae including *Chlorella zofingiensis* enables plants to produce astaxanthin

Metabolic Engineering 17（2013）59-67





Wild-type Arabidopsis Transgenic

Wild-type Tobacco Transgenic

Conclusions

• Microalgae are considerable untapped resources of bioactive compounds and are perfectly suitable for making functional (health) foods. Some microalgae can be used for production of biofuels using industrial wastes.

• More researches are needed to help understand the benefits and action mechanisms of microalgal ingredients.

• Development of effective large-scale high-cell density cultivation processes is the key for commercialization of microalgal products.

• It is possible that genetic engineering/metabolic engineering can help overcome some of the practical problems and extend the range of microalgal products.

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Thank You!