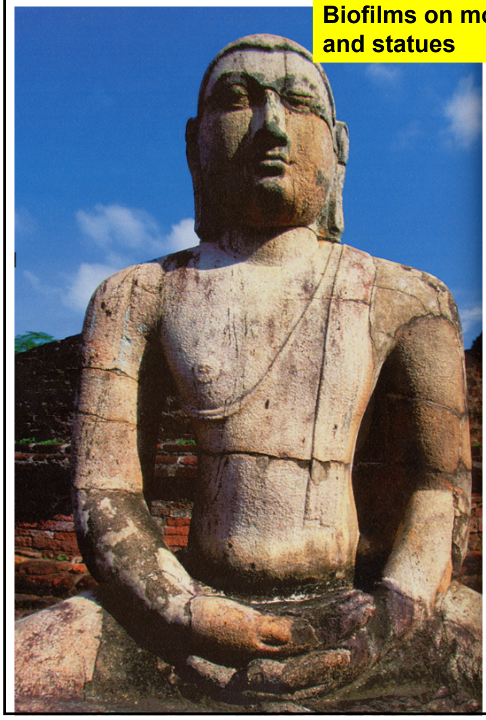
Department of Agricultural
Biotechnology
University of Florence

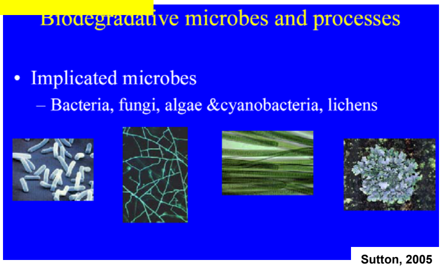
Microalgae: tools for the next Green Revolution?

When microalgae are a problem!

Microalgae biofilms on walls and roofs

Biofilms on monuments and statues

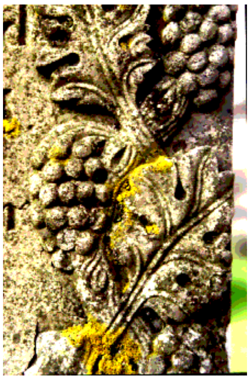




Biodegradative microbes and processes

- Implicated microbes
  - Bacteria, fungi, algae &cyanobacteria, lichens

Sutton, 2005



Algae growth in a swimming pool

Eutrophication and algal blooms

Eutrophication in lakes caused by massive use of fertilizers is characterized by an abundant growth of algae, the decay of which depletes the waters of oxygen. (T.D Brock).

Toxic blooms

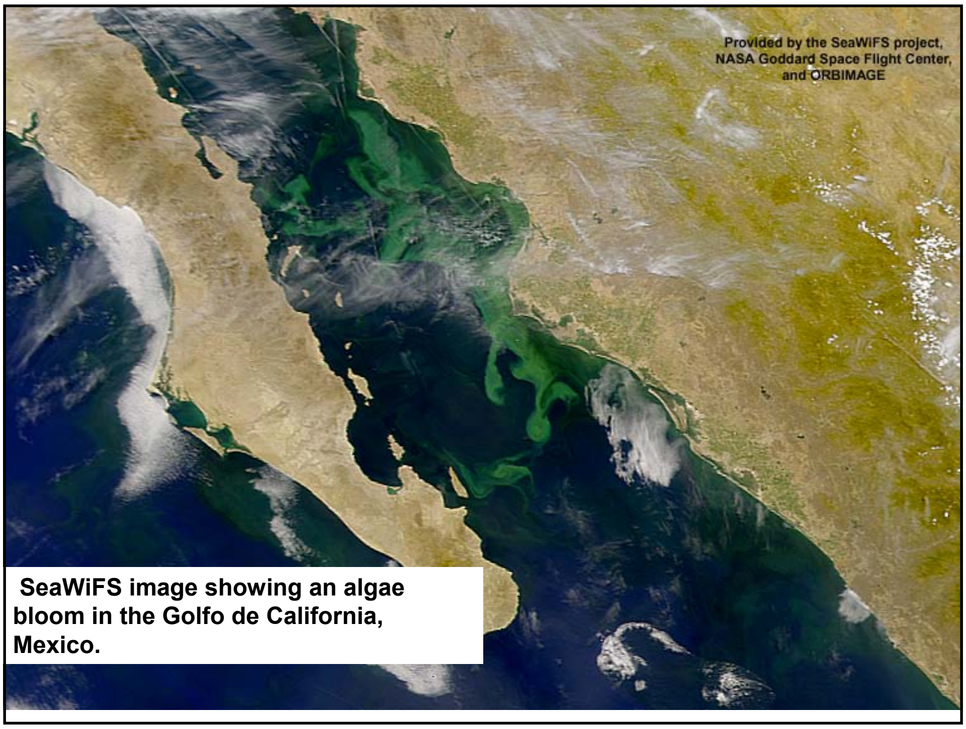

Nodularia


Mycrocistis

FIORITURE DI ALGHE TOSSICHE NELLE ACQUE TOSCANE

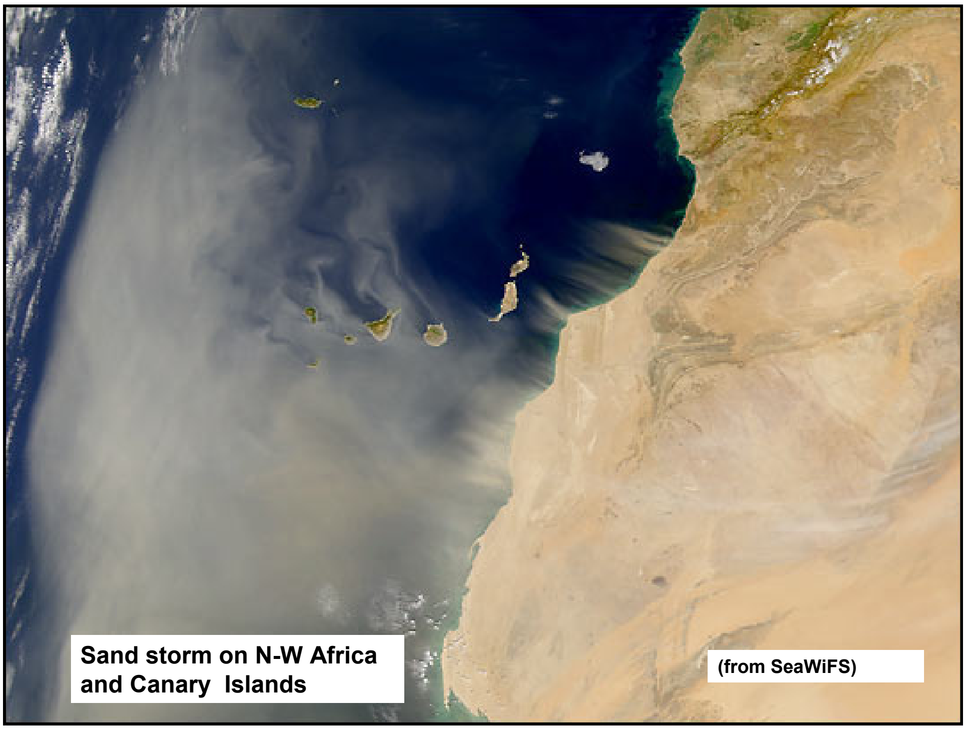
REGIONE TOSCANA AMBIENTE e TERRITORIO

L'organismo responsabile è stato identificato in *Ostreopsis ovata*, una dinoficea di origine tropicale che da alcuni decenni, si è ben adattata ai nostri climi. Produce neurotossine che danno vertigine, febbre, difficoltà respiratorie, ecc.



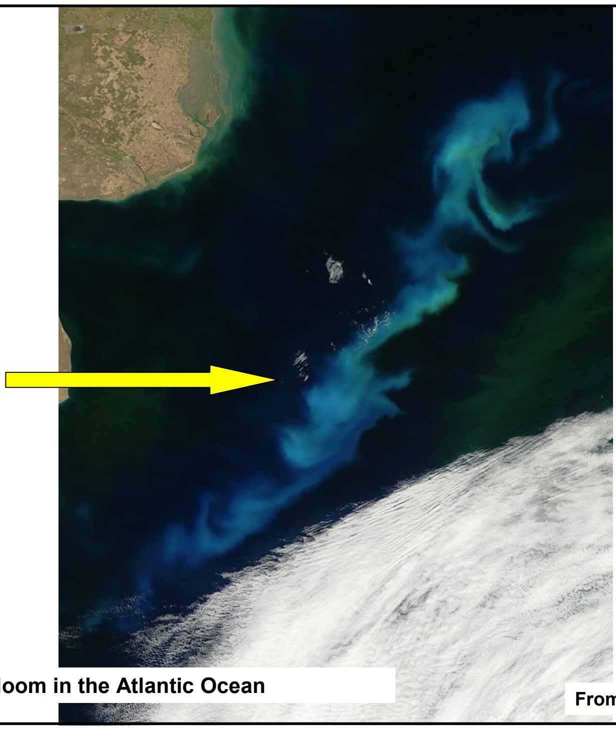
Provided by the SeaWiFS project,
NASA Goddard Space Flight Center,
and ORBIMAGE

SeaWiFS image showing an algae bloom in the Golfo de California, Mexico.



Sand storm on N-W Africa and Canary Islands

(from SeaWiFS)

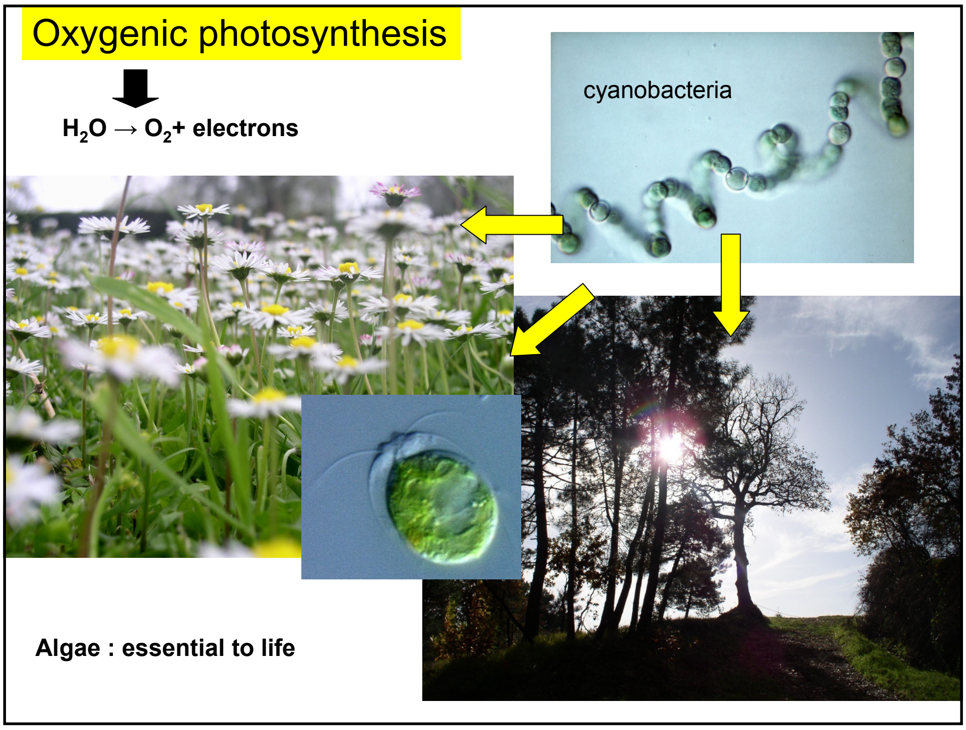


Algal bloom in the Atlantic Ocean

From SeaWiFS)

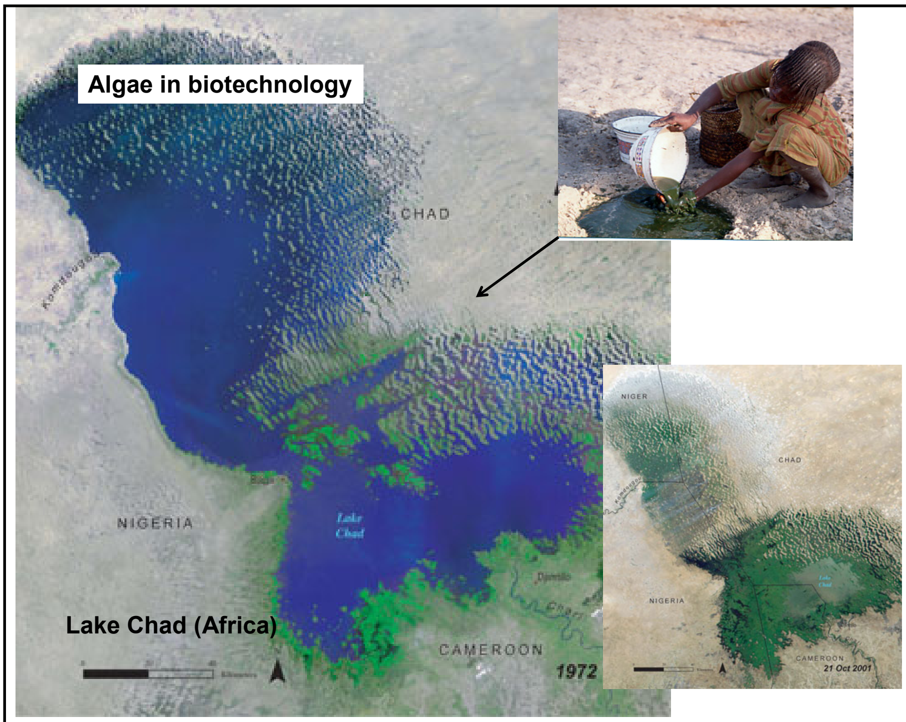
Oxygenic photosynthesis

$H_2O \rightarrow O_2$+ electrons



Algae : essential to life

Algae in biotechnology



Lake Chad (Africa)

Kanembu women harvesting *Arthrospira platensis* (Chad)

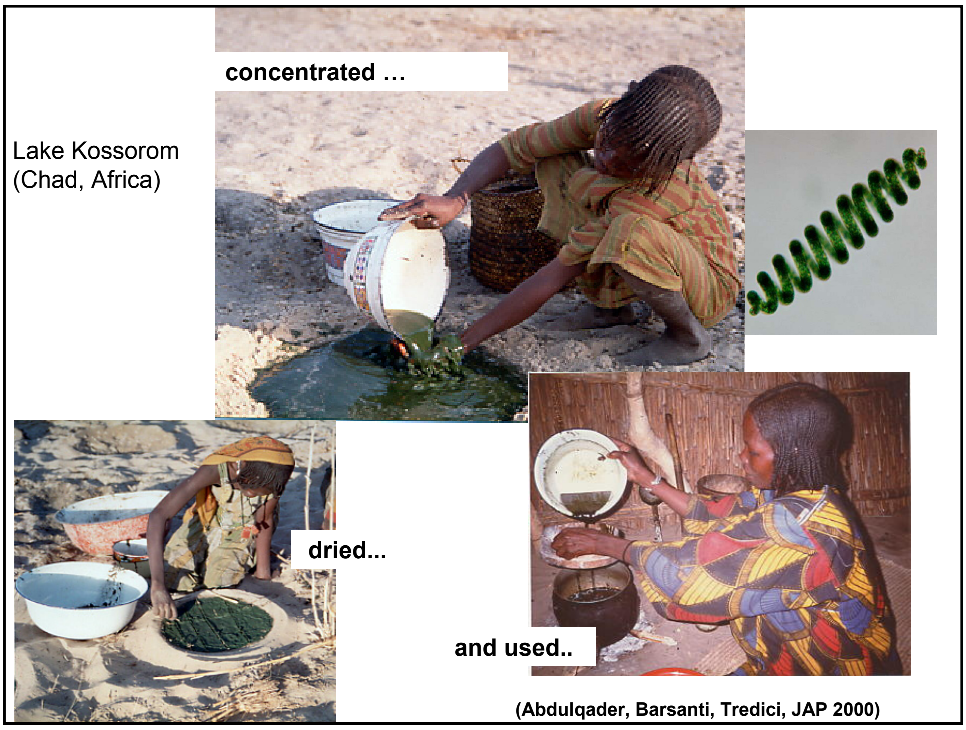
Lake Kossorom (Chad, Africa)

(Abdulqader, Barsanti, Tredici, 2000)

Lake Kossorom
(Chad, Africa)

Algae are collected…

(Abdulqader, Barsanti, Tredici, 2000)



concentrated …

Lake Kossorom
(Chad, Africa)

dried...

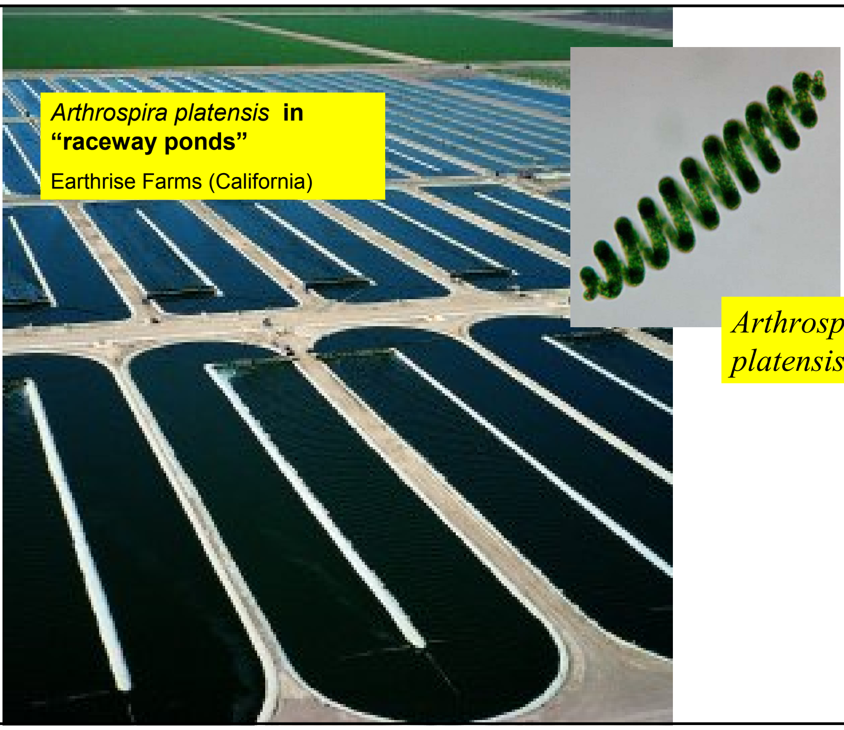
and used..

(Abdulqader, Barsanti, Tredici, JAP 2000)

Klamath Lake (Oregon, USA)

Aphanizomenon flos-aquae

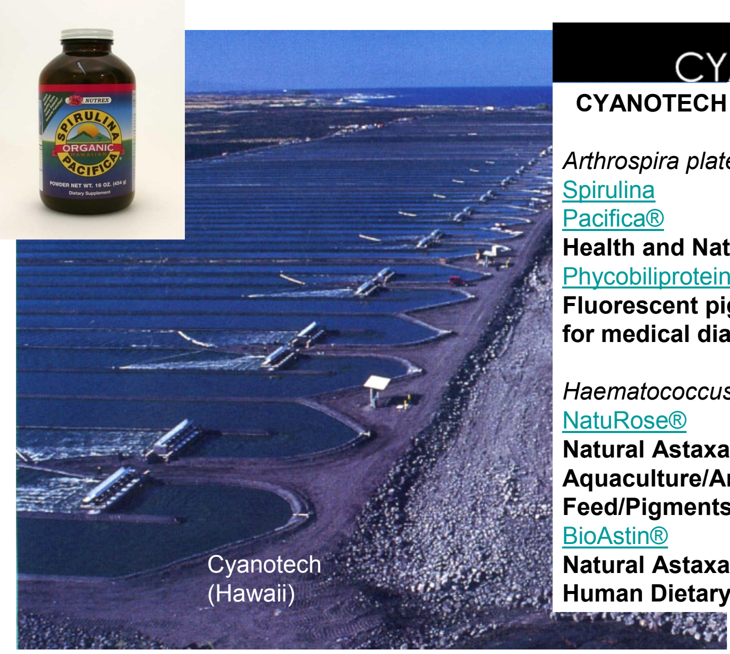
Microalgae mass cultures



Arthrospira platensis **in “raceway ponds”**

Earthrise Farms (California)

Arthrospira platensis



Cyanotech (Hawaii)

CYANOTECH

CYANOTECH PRODUCTS

Arthrospira platensis
Spirulina
Pacifica®
Health and Natural Foods
Phycobiliproteins
Fluorescent pigments used for medical diagnostics.

Haematococcus pluvialis
NatuRose®
Natural Astaxanthin
Aquaculture/Animal Feed/Pigments
BioAstin®
Natural Astaxanthin
Human Dietary Supplement

Raceway ponds: materials

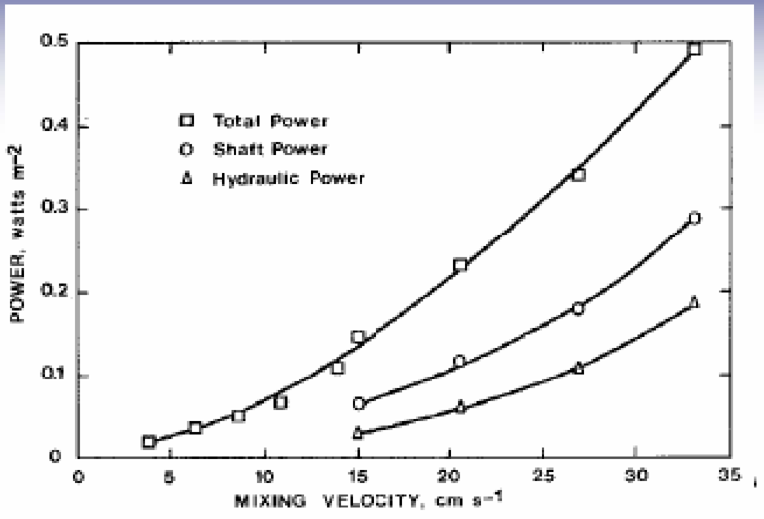
The most simple example of raceway ponds consists of a shallow ditch dug into the ground and covered with plastic sheets draped up the sloping earth embankments. This construction is relatively inexpensive, but its cost is strongly influenced by the characteristics of the ground. The lining must be fixed very carefully to the ground to avoid displacement by winds.

In a different design, used in several commercial plants in Asia, the walls of the pond are erected on the ground with concrete blocks, bricks or even adobe (sun-dried clay) blocks which are covered with a plastic membrane that covers also the pond bottom.

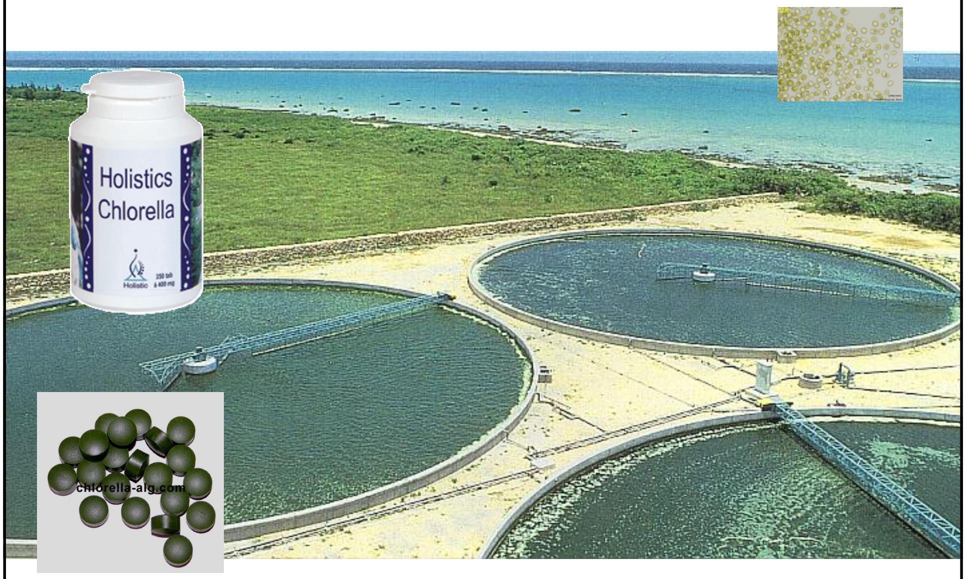
Paddle wheels for mixing high rate ponds.

(Mixing at or below 30 cm/sec minimizes energy use)

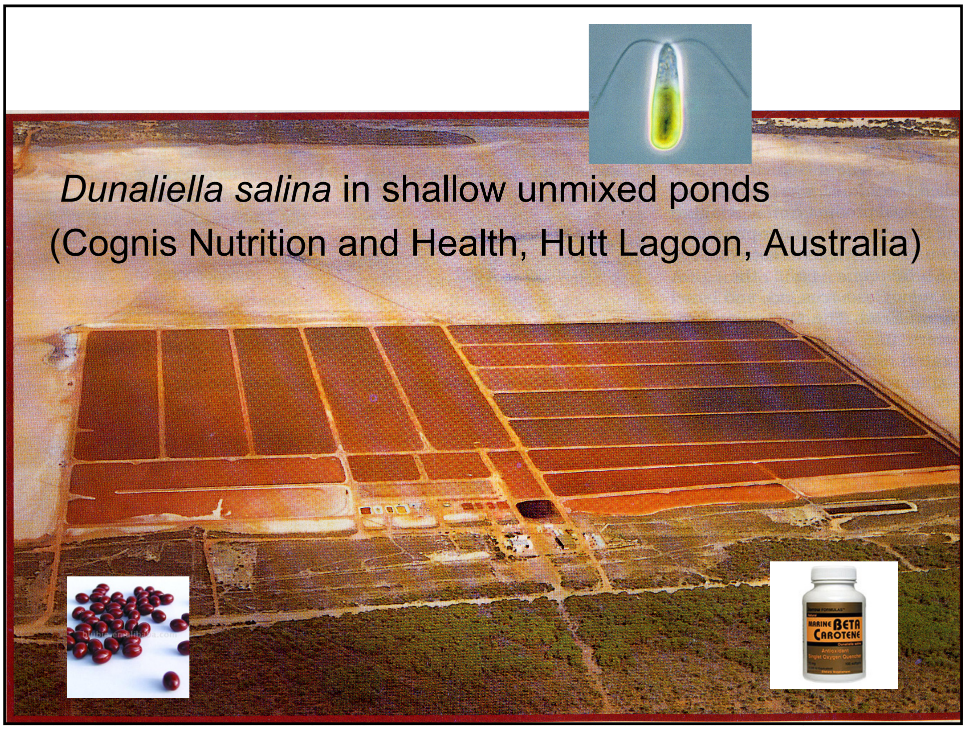
Power required for mixing ponds



Chlorella (Japan)



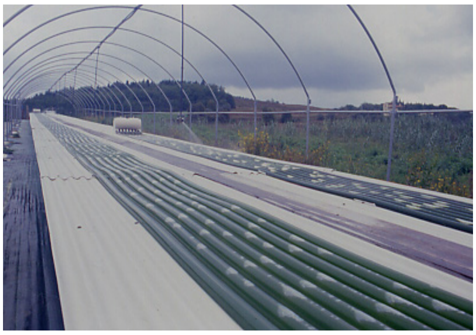
Dunaliella salina in shallow unmixed ponds (Cognis Nutrition and Health, Hutt Lagoon, Australia)



Algae biomass production

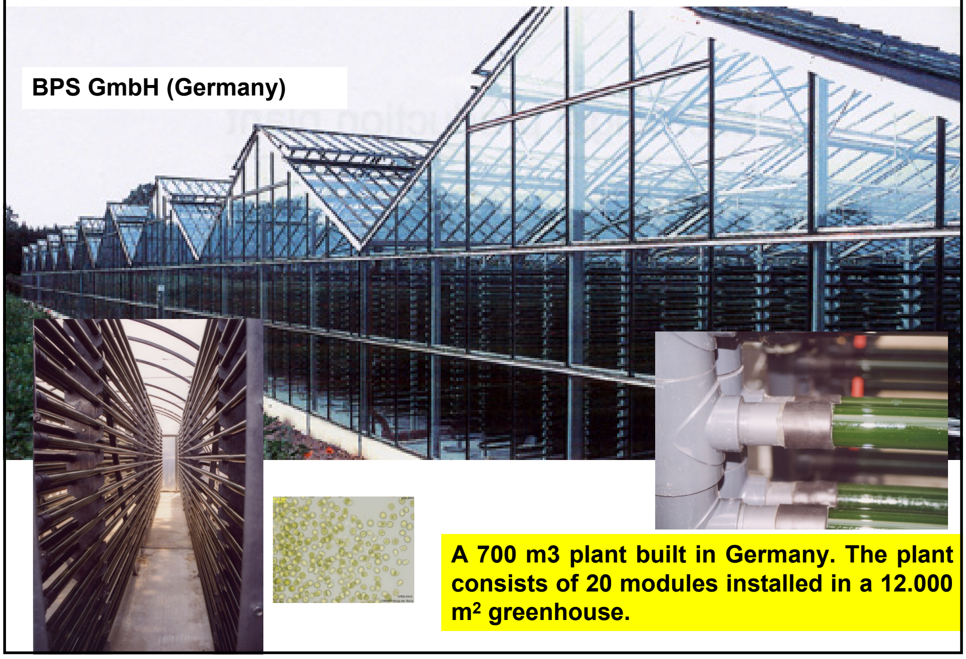
Many microalgae cannot be maintained long enough in outdoor open systems because of contamination by fungi, bacteria and protozoa, and competition by other microalgae that tend to dominate regardless of the original species used as inoculum (Richmond, 1999).

PBR offer a closed culture environment, which is protected from direct fall-out, relatively safe from invasion by competing microorganisms, and where conditions are better controlled ensuring dominance of the desired species (Tredici, 2003).



Microalgae mass cultures: photobioreactors

Commercial scale photobioreactors



BPS GmbH (Germany)

A 700 m3 plant built in Germany. The plant consists of 20 modules installed in a 12.000 m^2 greenhouse.

Photo Bioreactors Ltd (Murcia, Spain)

Photo Bioreactors Ltd, set up in southern Spain in the late 1980s, is one of the bigges disasters in the field of microalgal biotechnology. The quality of the basic work done a Queen Elizabeth College by John Pirt and the high projected productivities (more than 20 ton ha-1 year-1) attracted investments and led to the creation of Photo Bioreactors Ltd (PB UK) in 1986. Three years later, in 1989, PBL Spain (PBL SA) was founded with investment from private industries and public Spanish sources and a commercial plant for the productio of *Dunaliella* was established in Santa Ana near Cartagena (Murcia, Spain).

Hidrobiologica SA (La Rioja, Argentina)

In 1996 Hidrobiologica SA built the largest photobioreactor known at that time (Tredici 1999). The system consisted of 96 polyethylene tubes 120 m long and 25.5 cm in diameter. The tubes were laid parallel on the ground and arranged like a manifold with feeding, connecting and collecting channels made of concrete. The surface occupied by the whole plant was about 5,000 m2; the total culture volume was 600 m3. Main problems were the limited capacity to control temperature, a major drawback in summer, and oxygen build up to dangerous levels.

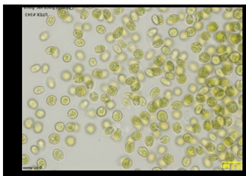
Microalgae biomass production (2006) (from J Benemann and others, modified)

Product	Species	Status
Health food and feed supplements	***Arthrospira (3000 t)*** ***Chlorella (2000 t)*** ***Dunaliella (1200 t)*** ***Aphanizomenon (500 t)*** ***Haematococcus (300 t)***	**Commercial** (Raceway ponds, circular ponds, lagoons, PBR)
Pigments (carotenoids, phycobiliproteins)	***Dunaliella*** ***Arthrospira*** ***Haematococcus***	**Commercial** (as above)
ω 3 PUFA (DHA)	***Schyzochitrium (10 t oil)*** ***Crypthecodiniun (240 t oil)***	**Commercial** (10-100 m^3 fermenters)
Fluorescent diagnostics **Labeled compounds (stable isotopes)** **Restriction enzymes**	***Arthrospira*** ***Anabaena*** ***Anacystis***	**Commercial** (small PBR)
Aquaculture feeds	Various spp. (1000 t)	**Commercial** (cylinders, bags, tanks)
Polysaccharides		**Research**
Biofertilizers		**Research**
Bioactive molecules (biopesticides, probiotics, pharmaceuticals, biosensors,cosmetics)		**Research**
Bioremediation (xenobiotics, heavy metals)		
CO_2 biofixation		
Energy (biodiesel, H_2)		

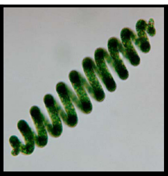
Algae commercial applications (2006)

< 10,000 tons

A *niche* technology

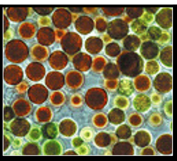


Chlorella



Arthrospira

Dunaliella



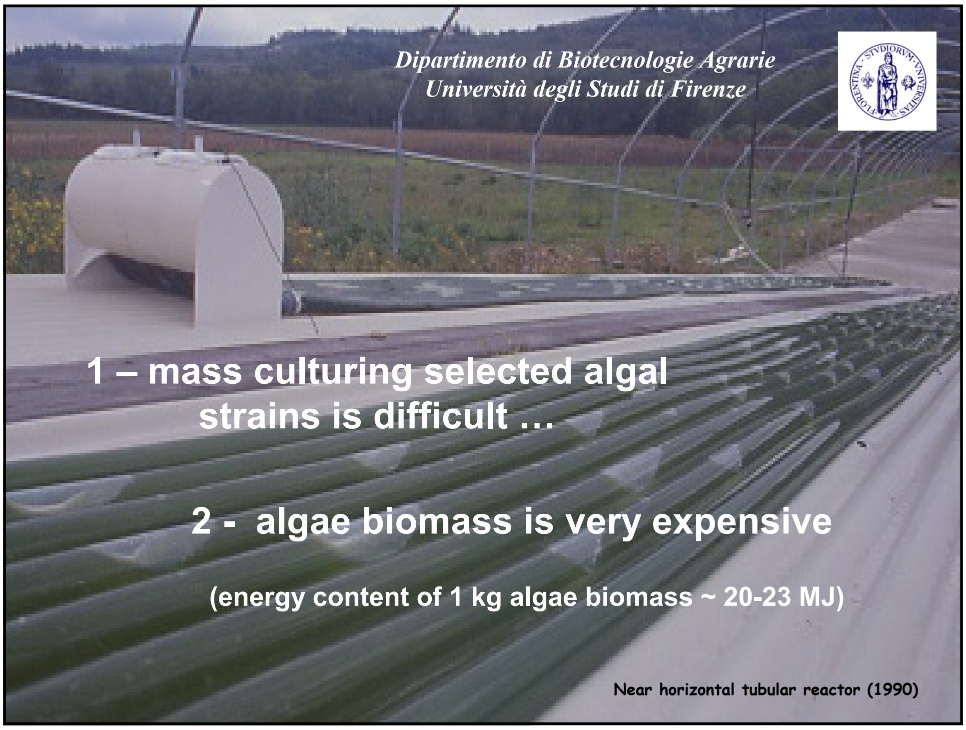
Haematococcus

INTRODUCTION

- Four microalgae are at present produced at commercial level:
- *Chlorella*
- *Arthrospira*
- *Dunaliella*
- *Haematococcus*

- Their commercial cultivation is carried out, in open systems (with the exclusion of the green stage of *Haematococcus*).

- The main reason for this is that large (commercial) open ponds are easier and less expensive to build and operate, and more durable than large closed reactors.



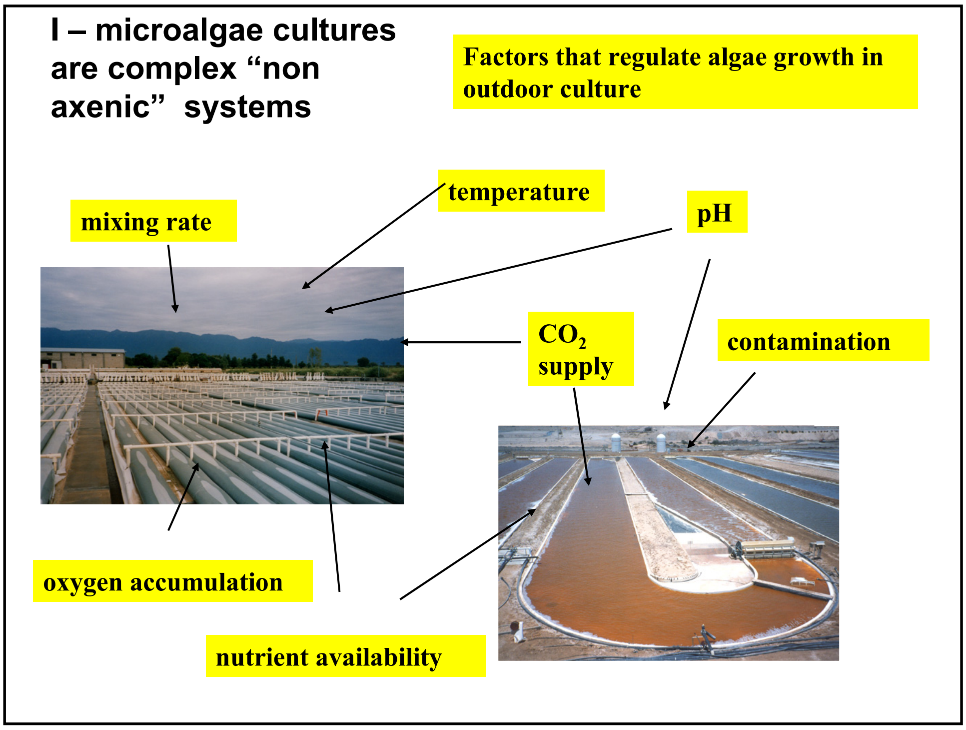
Dipartimento di Biotecnologie Agrarie
Università degli Studi di Firenze

1 – mass culturing selected algal strains is difficult …

2 - algae biomass is very expensive

(energy content of 1 kg algae biomass ~ 20-23 MJ)

Near horizontal tubular reactor (1990)



I – microalgae cultures are complex “non axenic” systems

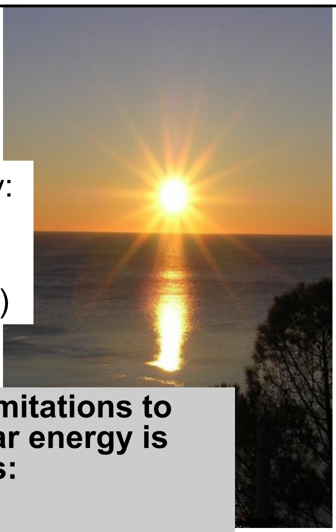
Factors that regulate algae growth in outdoor culture

Photons as energy source

1. **must be continuously provided**
2. **can not be stored in the culture medium**
3. **even when there is plenty of supply, there is competition for photons**
4. **high μ does not necessarily leads to high VP (μ X)**

scale up of PBR is difficult

II - Low productivity!



The main drivers of productivity:

1- sunlight

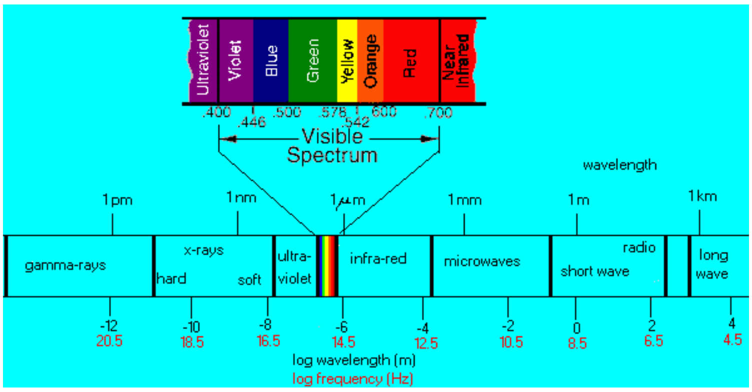
2-PE (photosynthetic efficiency)

There are two fundamental limitations to the efficiency with which solar energy is converted into algae biomass:

- **A - PAR (useful component of sunlight)**
- **B - mechanisms of photosynthesis**

A – PAR, the useful component of solar light

Only 45% of sunlight has the suitable wavelength (400 to 700 nm) (PAR) to drive (oxygenic) photosynthesis

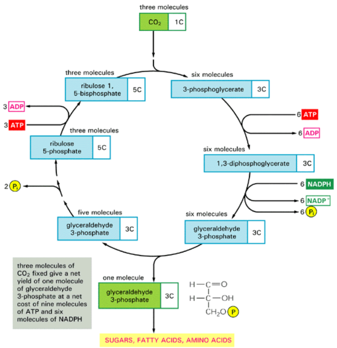


B - mechanisms of photosynthesis

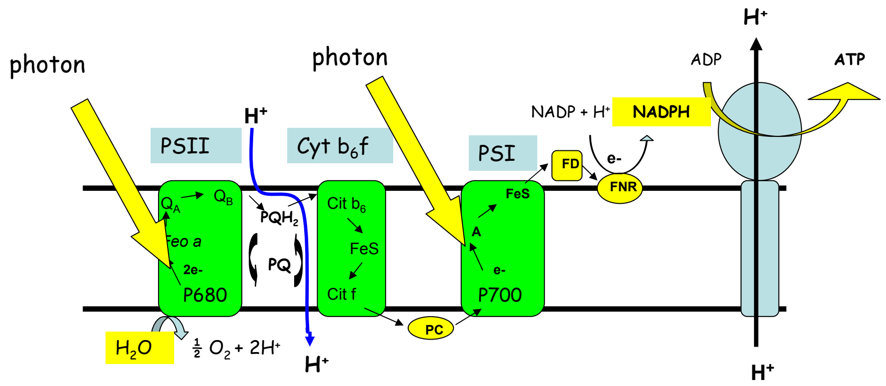
To fix one molecule of CO_2 (Calvin cycle)

2 NADPH + 3 ATP + CO2 → Carbohydrate (CH2O)

4e- + 4H$^+$ + 2 NADP → 2 NADPH



B - mechanisms of photosynthesis



Photosynthetic membrane

2 photons are required to transfer 1 electron from water to NADP

A total of **8 photons** is required to fix on molecule of CO_2. Their transport also moves 12 protons (→3 ATP)

Mechanisms of photosynthesis

- **8 photons** are required to fix one molecule of CO_2
- PAR photons have average energy content of 217 KJ per mole
- one mole of fixed CO_2 is equivalent to 475 KJ (1/6 mole glucose)

Thus:

the maximum theoretical efficiency of conversion of PAR into the chemical energy of biomass is

475 KJ / (217 x 8 KJ) = **27%**

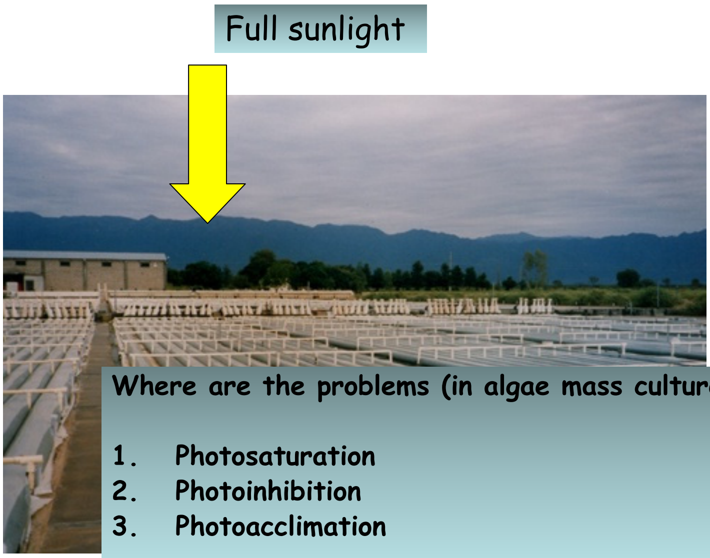
PAR (useful component of light) is 45% of total sunlight

The maximum conversion efficiency of total solar light by photosynthesis is:

→ 27% x 45% = 12%

Most scientists consider 10% the maximum PE attainable

These efficiencies and productivities can not be attained under natural sunlight



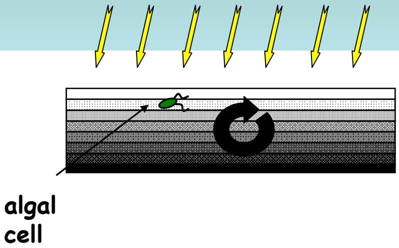
Where are the problems (in algae mass cultures):

1. **Photosaturation**
2. **Photoinhibition**
3. **Photoacclimation**

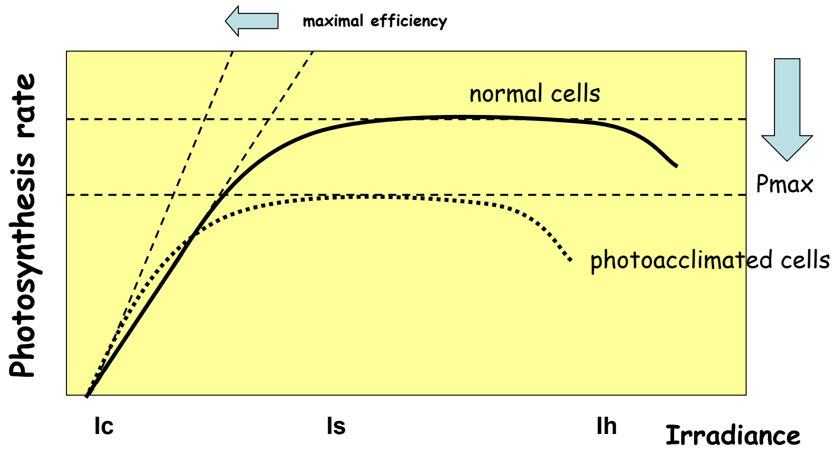
Photoacclimation under fluctuating irradiance

Under fluctuating irradiance (medium high-frequency light-gradient/dark cycles that prevail in mixed dense algae cultures), the cells acclimate to irradiances approximately 3 times lower than the average irradiance of the fluctuating regime.

Havelkova-Dousova et al. (2004)

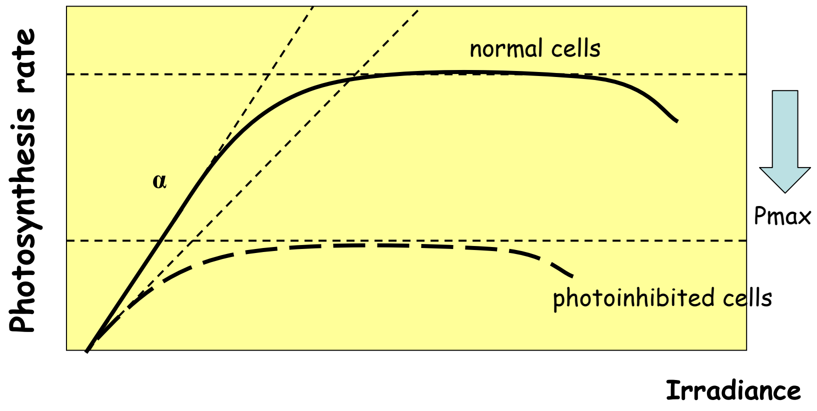


Consequences of photoacclimation to low irradiance



PI curve that relates photosynthesis to light intensity

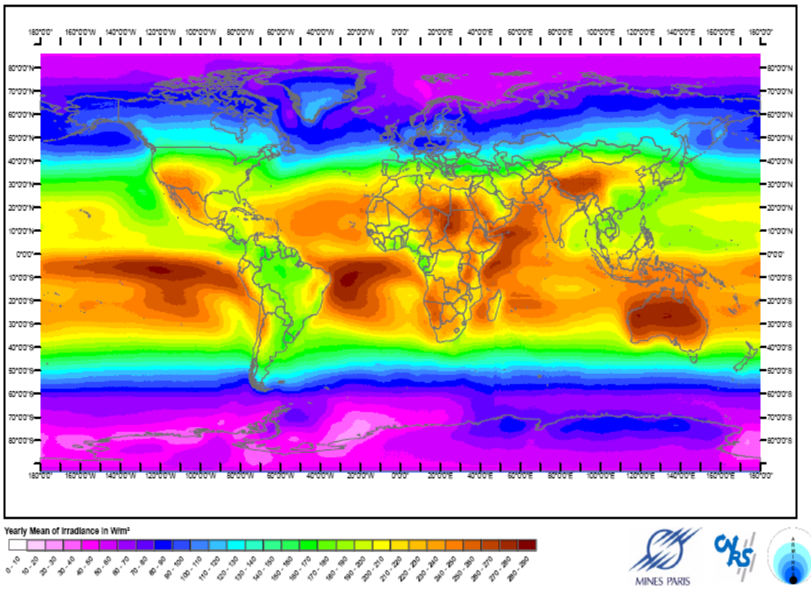
Consequences of photoinhibition



PI curve that relates photosynthesis to light intensity

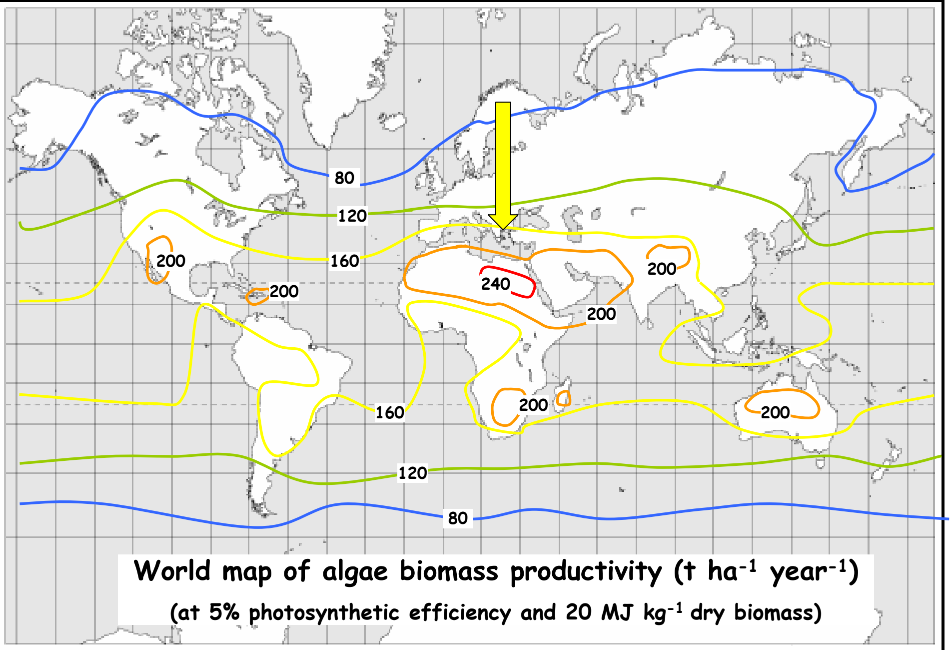
Minimum energy losses	**Maximum energy remaining**		
	C3 plants	**C4 plants**	**Microalgae**
Total incident solar radiation	100%	100%	100%
Radiation outside PAR (55%)	45%	45%	45%
Degradation of absorbed PAR photons to excitation energy at 700 nm (21 %)	35.6%	35.6%	35.6%
Conversion of excitation energy at 700 nm to the chemical energy of glucose (65%)	12.4% (MPE)	8.2% (MPE)	12.4% (MPE)
Reflection (10%)	11.2%	7.4%	11.2%
Photorespiration (40 % in C3 plants, none in C4 plants and microalgae)	6.7%	7.4%	11.2%
Respiration (20%)	5.4%	5.9%	9.0%
Photosaturation & photoinhibition (20% in C3 plants; 10% in C4 plants ; 40 % in microalgae)	**4.3 %**	**5.3%**	**5.4%**

Averaged Solar Radiation 1990-2004



MINES PARIS

Realized by Michel Albuisson, Mireille Lefèvre, Lucien Wald.
Edited and produced by Thierry Ranchin. Date of production: 23 November 2006.
Centre for Energy and Processes, Ecole des Mines de Paris / Armines / CNRS.

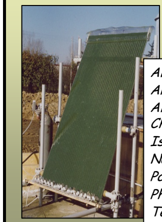


World map of algae biomass productivity (t ha^{-1} $year^{-1}$)

(at 5% photosynthetic efficiency and 20 MJ kg^{-1} dry biomass)

Photobioreactors developed at the Department of Agricultural Biotechnology University of Florence

1980 – The Vertical Alveolar Panel (20 L)

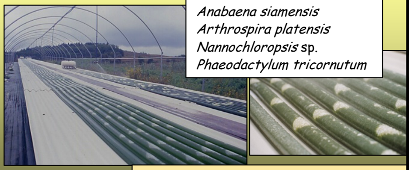


Anabaena azzollae
Arthrospira platensis
Alexandrium minutum
Chlorella sp.
Isochrysis galbana
Nannochloropsis sp.
Pavlova lutheri
Phaeodactylum tricormutum
Tetraselmis suecica

1986 – The Coil Reactor (120 L)

Anabaena siamensis
Arthrospira platensis

1990 - The Near Horizontal Tubular Reactor (10 - 1800 L)



Anabaena siamensis
Arthrospira platensis
Nannochloropsis sp.
Phaeodactylum tricornutum

1997 - The Annular Column (115 L)

Chlorella sp.
Isochrysis sp. T-ISO
Monodus subterraneus
Nannochloropsis sp.
Pavlova lutheri
Phaeodactylum tricornutum
Tetraselmis suecica
Skeletonema sp.
Nostoc spp.

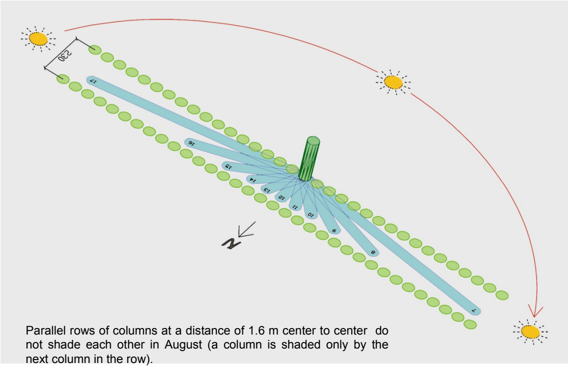
2004 – Patent WO2004/074423A2

The Annular Column (120 L)

Isochrysis sp. T-ISO
Nannochloropsis sp.
Tetraselmis suecica

The Green-Wall Reactor (500 L)

Fotosintetica & Microbiologica s.r.l.
Spin-off dell'Università degli Studi di Firenze

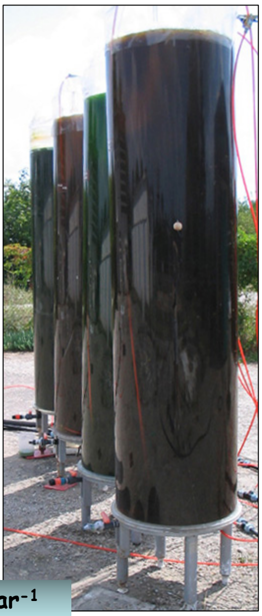


Parallel rows of columns at a distance of 1.6 m center to center do not shade each other in August (a column is shaded only by the next column in the row).

Results

Areal productivity → **37-41 g m^{-2} d^{-1}**

Photosynthetic efficiency → **4.5%**



On an annual basis→ **20 g m^{-2} d^{-1}** and **70 t ha^{-1} year^{-1}**

Examples of High Biomass Productivity

(Slesser & Lewis, 1979; Leight el al. 1987; Klass, 2004) (Higher heating value: 15.6-20.0 Mj kg^{-1})

Biomass community	**Location**	**Yield (t dry w. ha^{-1} year^{-1})**	**Photosynthetic efficiency (%)**
Hybrid poplar (Populus spp.) (C3)	Minnesota	8 -11	0.3- 0.4
Water hyacinth (Eichornia crassipes)	Missisipi	11 – 33 (>150)	0.3- 0.9
Switchgrass (Panicum virgatum) (C4)	Texas	8-20	0.2- 0.6
Sweet sorghum (Sorghum bicolor) (C4)	Texas-California	22 - 47	0.6-1.0
Coniferous forest	England	34	1.8
Maize (Zea mays) (C4)	Israel	34	0.8
Tree plantation	Congo	36	1.0
Tropical forest	West Indies	60	1.6
Algae	***Different locations***	***70-80***	***2-2.5***
Sugar cane (Saccharum officinarum)	Hawaii-Java	64-87	1.8-2.6
Napier grass (Pennisetum purpureum)	Hawaii, Puerto Rico	85-106	2.2-2.8

III - energy balance

70 tons ha^{-1} $year^{-1}$

~

1500 GJ ha^{-1} $year^{-1}$

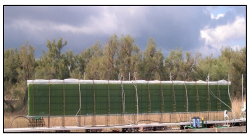
Open ponds vs. PBR: Productivity and energy output

(with the marine microalga *Tetraselmis* [1])

	Pond (Calabria, Italy, 1983) 	Tubular reactors (Tuscany and Lazio, Italy, 1998-2005) 	GW reactor (Tuscany, Italy, 2006) 
Productivity ($ton\ ha^{-1}\ y^{-1}$)	~50	57- 60	~ 70
Energy output ($GJ\ ha^{-1}\ y^{-1}$)	1150	1350	1600 ~ 40%

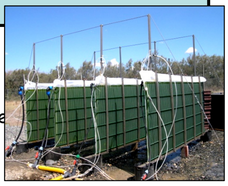
(1) - biomass energy content: 23 kJ g^{-1}

Energy cost of bioreactor materials

	Total embodied energy	
	(GJ ha^{-1} y^{-1})	% of the productivity
	**1200** (10 year lifespan)	**80%**
	**120** HDPE membrane + dividers + paddle wheel (12 year lifespan)	**10%**

Open ponds vs PBR: energy consumption for mixing

	Raceway pond(1)	Tubular reactor(2)	GW reactor
GJ ha^{-1} y^{-1}	60	180	600
% of the energy in biomass	5	14	40



(1) From Weissman et al. 1988

(2) from Burgess and Fernandez-Velasco, 2007 (0.05 m diameter tube

Energy cost for harvesting and biomass concentration

Even with efficient systems, 10% of the energy content of the biomass is required to concentrate it

Picture: Evodos Algae Paste

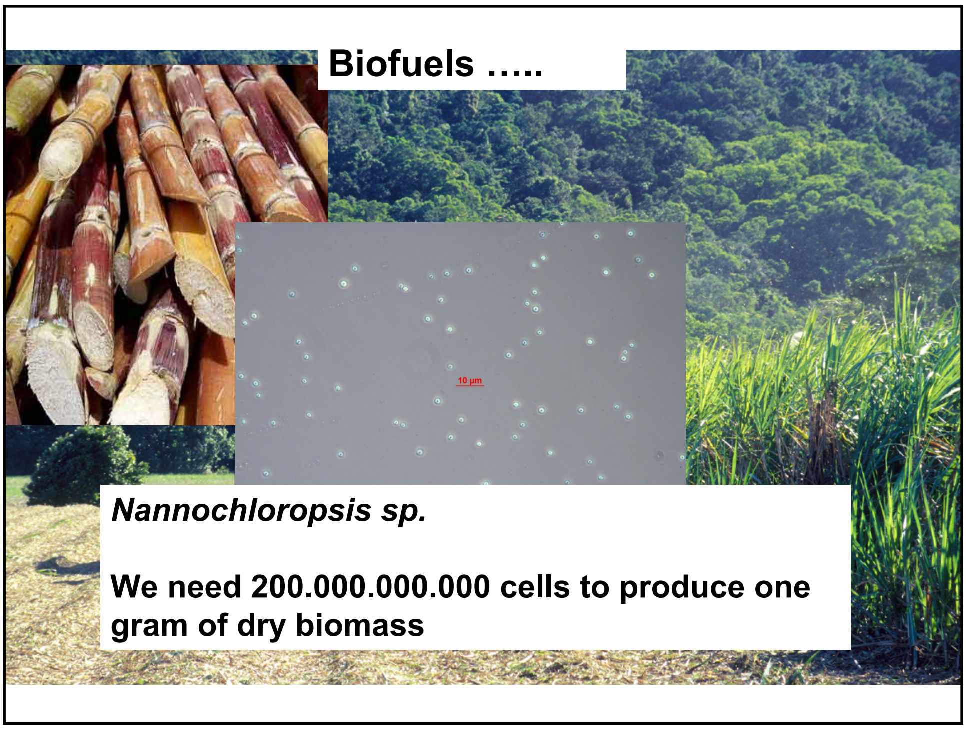
Main limitations and barriers to large-scale algae farming

- Low actual PE (photosynthetic efficiency) and productivities
- A negative Net Energy Ratio *(due to high energy consumption for water pumping, CO_2 distribution, mixing and harvesting, etc.)*
- Instability of the culture (difficulty in maintaining the selected species) *(and we do need selected species)*
- No experience on large scale (thousands of hectares) cultures
- Large variability in perfomances among culture systems and difficulty to standardize techniques
- Limited data on large scale algae biomass processing (e.g. extraction)

Dipartimento di Biotecnologie Agrarie
Università degli Studi di Firenze

Algae: fuel of the future ?

Near horizontal tubular reactor (1990)



Biofuels …..

Nannochloropsis sp.

We need 200.000.000.000 cells to produce one gram of dry biomass

Iniziative commerciali su biodiesel da microalghe (2006-2007)

Company	Location	Web	Links
Algatech	Israel		
Algoil	Bamgalore, India		
AlgaeFuels (Owned by Bioking)	Netherlands	www.algaefuels.org	
Aquaflow Bionomic Corporation	New Zealand	www.bio-diesel.co.nz	
Biofuel Systems (BSF)	Spain.		
De Beer's Fuels BPK	South Africa		GFT Technology
Ecogenics Research Center	Tennessee, USA	www.ecogenicsresearchcenter.org	
Energetix (Victor Morgan Group)	Victoria,Australia		GFT technology.
Enhanced Biofuels & Technologies			Ponds and PBR through GreenFuel
Energy Farms	Texas, USA	www.nanoforcetechnologies.com	owned by Nanoforce Inc.
GreenFuel Technologies Corp.	Massachusetts, USA	www.greenfuelonline.com	MIT
GreenShift Industrial Design Corporation	New York, USA	www.greenshift.com	through Veridiun and Ohio University
Green Star Products and De Beers Fuel Limited	South Africa		GFT Technology
GS Clean Tech	New York, USA	www.gs-cleantech.com	GreenShift Corp. & Veridium Corp. &Ohio State University
Kwikpower Int. Advanced Biofuels Technologies	Gibraltar, UK	www.kwikpower.com	
Infinifuel Corporation	Nevada, USA	www.infinifuel.com	
Needful Provision, Inc	Oklaoma USA	www.needfulprovision.com	
PetroAlgae. LLC (XL Tech Group, Inc.)	Florida, USA	www.xltg.com/html/activity/PetroAlgae.asp	Arizona State University
PetroSun Drilling Inc. (with Algae Biofuels)	Arizona, USA	www.petrosun.us www.petrosuninc.com	
Solazyme, Inc.	California, USA	www.solazyme.com	
Solix Biofuels, Inc. (Sun Source Industries)	Colorado, USA	www.solixbiofuels.com	Colorado State University
Sun Source Industries	Colorado, USA	www.cobioscience.com	Solix Biofuels, Inc.
Valcent Products, Inc	Vancouver, Canada	www.valcent.net	
Veridium Corp.	New York, USA	www.veridium.com	
XL Tech Group Inc.	Florida, USA	www.xltechgroup.com	www.xltechgroup.com

---

Photobioreactors and algae biofuels

> 900 M $

Why?

Oil Yield

Cultivating Algae for Liquid Fuel Production
(*http://oakhavenpc.org/cultivating_algae.htm*)

Gallons of Oil per Acre per Year

Corn	→	20 - 30
Soybeans	→	50
Sunflower	→	110
Rapeseed	→	130
Oil Palm	→	600
Microalgae	→	**5.000-25.000**

↓

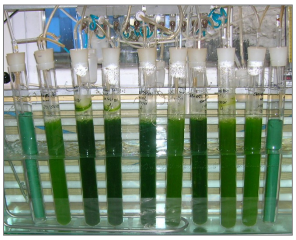
50.000-250.000 L oil per ha and year

Possible?

NO

Why is algae productivity overestimated?

I - Growth rate (μ) is confused with productivity (μX)



μ high=
few hours

μ low, X high

Microalgae can double their biomass in one day!

Let’s start with 1 g of algae and let them grow for 1 month

days	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	
Biom (g)	2	4	8	16	32	64	128	256	512	1024	**1 kg**
days	***11***	***12***	***13***	***14***	***15***	***16***	***17***	***18***	***19***	***20***	
Biom (Kg)	2	4	8	16	32	64	128	256	512	1024	**1 t**
days	***21***	***22***	***23***	***24***	***25***	***26***	***27***	***28***	***29***	***30***	
Biom (tons)	2	4	8	16	32	64	128	256	512	1024	**1000t**

➡ **From 1 g to 1000 t in 30 days!!!**

---

days	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	
Biom. (g)	2	4	8	16	32	64	128	256	512	1024	**1 kg**
Surf. (m2)	**0.04**	0.08	0.16	0.32	0.64	1.28	2.56	5.12	10.24	20.5	
days	***11***	***12***	***13***	***14***	***15***	***16***	***17***	***18***	***19***	***20***	
Biom (kg)	2	4	8	16	32	64	128	256	512	1024	**1 t**
Surf. (m2)	41	82	164	328	656	1312	2624	5248	10496	21.000	
days	***21***	***22***	***23***	***24***	***25***	***26***	***27***	***28***	***29***	***30***	
Biom (t)	2	4	8	16	32	64	128	256	512	1024	**1000t**
S (ha)	4.2	8.4	16.8	33.6	67.2	134.4	268.8	537.6	1.075	**2.150 ha**	

From 0.04 m^2 to 2.150 ha in 30 days!!!

1 Ton Dry Biomass per day Algae production plant

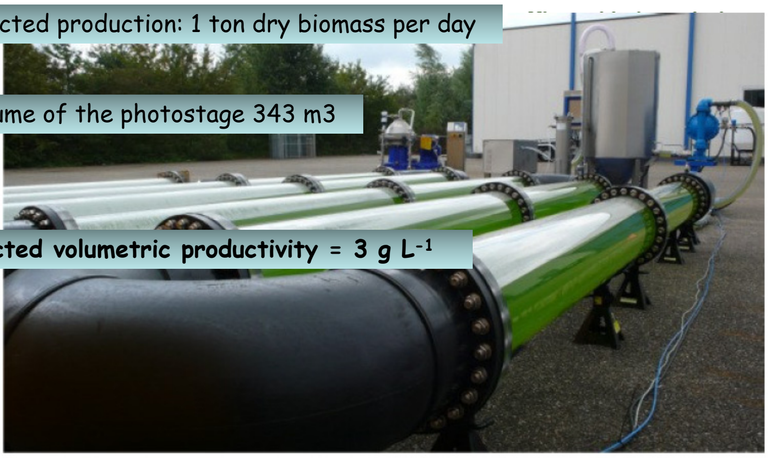
Complete automated turnkey photo-bioreactor with production capacity of 1 ton dry biomass per day:

- 1,068 meters of transparent synthetic tubes with a diameter of 640 mm Ø and a volume of 667 m³
- Pumps and valves
- Selected algae
- Electricity for pumps involved in the process is 55 kW

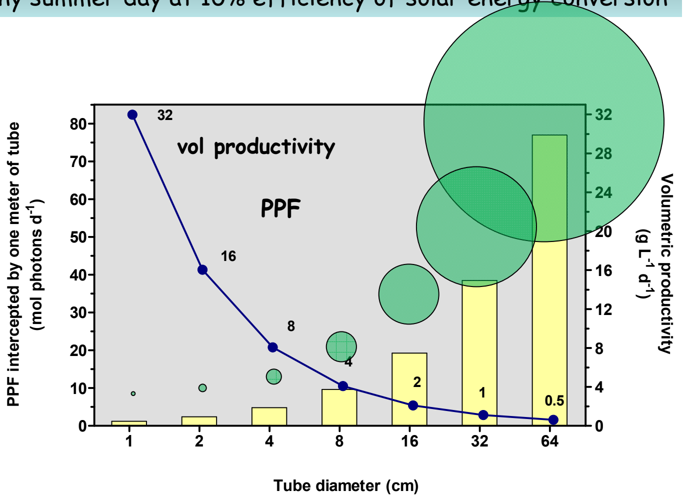
expected production: 1 ton dry biomass per day

volume of the photostage 343 m3

expected volumetric productivity = 3 g L^{-1}



Expected volumetric productivity of tubular bioreactors in a sunny summer day at 10% efficiency of solar energy conversion



40 MW from 10 ha of PBR

IL MESSAGGERO
MARTEDÌ
31 MARZO 2009

SPECIALE
IL MESSAGGERO 23

Un doppio, innovativo brevetto in esclusiva per l'Italia sarà sfruttato da una società tra Autorità portuale ed Enalg

ENERGIA&AMBIENTE

La CO2 prodotta "nutrirà" i microrganismi acquatici
Il presidente Costa e l'a.u. Bordon: «Due anni per il via»

"Rivoluzione" a Venezia: il Porto s'illuminerà con l'alga

Parte il progetto per la centrale a plancton: 40 Mw a emissioni zero

di ANTONIO PAOLINI

ALGHE. Meglio, microalghe. Per illuminare il porto di una città unica al mondo: Venezia. Per rifornire di energia le navi alla fonda. A costo sostenibile (180 milioni d'investimento ammortizzabili in 7 anni, secondo stime definite «prudenti»). E a emissioni zero. Non un grammo di CO2 nell'aria. Residui: solo silicio "solido", riciclabile in vari settori industriali.

Gli ingredienti del "prodigio" promesso? Da 10 a 12 ettari di superficie, assai meno - rimarcano gli interessati - di quanti ne servono ad altri tipi d'impianto capaci di produrre i 50-60 Megawatt (35-40 al netto dell'energia usata nel ciclo produttivo) attesi qui. Poi, due brevetti «rivoluzionari», targati Solena Group e acquisiti in esclusiva per l'Italia (5 anni) da una società nata ad hoc, l'Enalg, guidata dall'ex ministro dell'Ambiente Wil-

raddoppia in 8-12 ore, i silos in plastica trasparente lavorano 8.000 ore all'anno, 11 mesi, ma per il "24 ore su 24" basta l'ausilio di lampade alimentate a energia solare), quindi essiccate e "trattate" con torcia al plasmaa 10.000° (che non "brucia" ma induce dissociazioni a livello molecolare), diverranno biomassa gassosa: biogas per le turbine che produrranno energia elettrica.

«La CO2 generata sarà reimmessa nei silos: le alghe si nutrono di quella e di luce - dice Willer Bordon, che ha come partner in Enalg l'imprenditore romano Giancarlo Giglio. - La resa è alta, un Mw da solare vuole 10 metri quadri di superficie, le alghe 0,31. E il nostro costo d'investimento è di 3.000 euro a Mw contro 6.000. Con le alghe s'è già fatto

Il plastico del possibile sviluppo dell'impianto ad alghe Coprirà una superficie tra i 10 e i 12 ettari nelle aree da recupero di Marghera

L'IDEA ENAVE PER IL PORTO

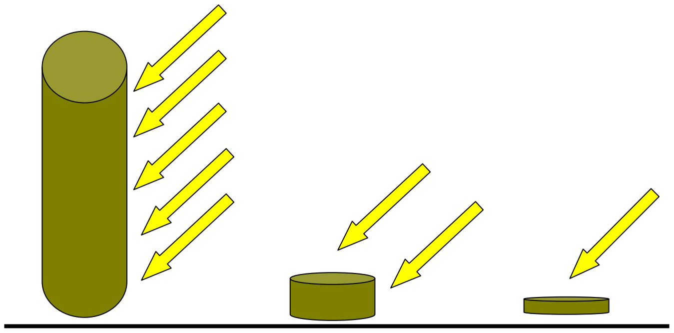
I SILOS DOVE CRESCE L'ALGA

biocarburante per aerei, la Virgin l'ha usato e molti sono interessati. Ma il nostro ambo di brevetti porta un passo oltre. Tanto che siamo già in contatto con una possibile seconda location (Trieste ndR.) e abbiamo manifestazioni d'interesse dalla Campania».

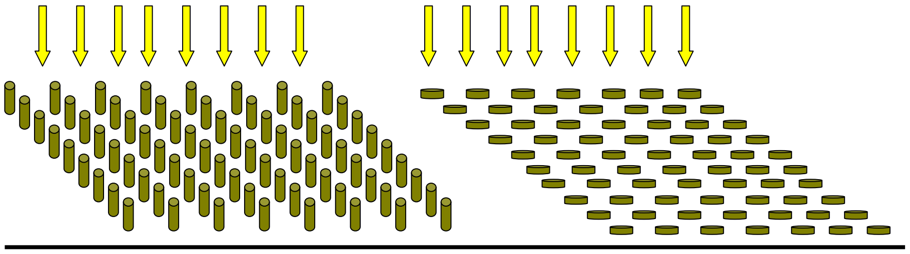
Pronto a clonare l'idea anche l'altro partner, il Porto. Spiega Paolo Costa, presidente dell'Autorità: «Qui zero problemi di aree, a Marghera c'è solo l'imbarazzo della scelta tra quelle dismesse. C'è interesse sotto il profile del business, rifornire le navi ormeggiate è un plus nodale. C'è interesse ambientale perché quella che partirebbe da Venezia sarebbe una piccola rivoluzione nel produrre energia pulita. E c'è infine interesse ad allargare il discorso, magari su un polo adriatico che coinvolga Slovenia e Croazia».

BFS Design of: BIO-ELECTRICAL power plant without any CO_2 emissions

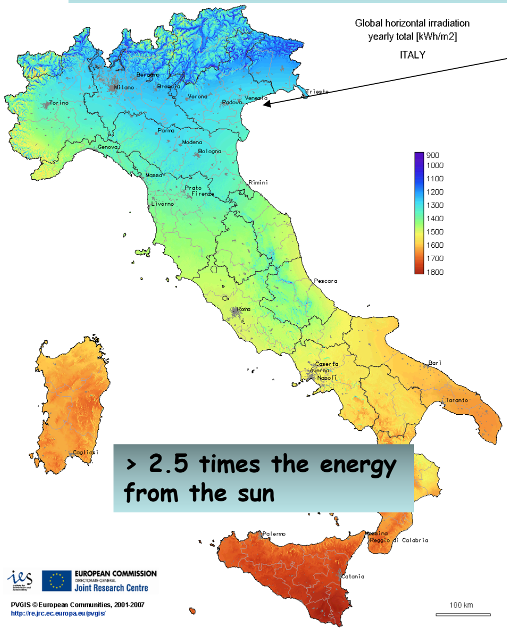
Vertical reactors are considered to be highly productive



At large scale vertical PBR do not intercept more light than horizontal ones



Is algae productivity overestimated?



> 2.5 times the energy from the sun

1300 KWh/m2 year

46.800.000 MJ/ ha year

With a PE of 10% and an energy content of algae of 20 MJ/Kg maximum productivity is ≤ 240 ton/ ha year

The project targets 10.000 ton/ ha year

> 40 times maximum PE

Last limitation

not acknowledging that there are limitations

Algae are not miracles…. they

1 - obey the laws of thermodynamics

2- convert solar energy into biomass by oxygenic photosynthesis

Biofuels from microalgae: the weak points

1. Low productivity
2. High cost of the photobioreactor/pond
3. High cost for mixing
4. High cost of harvesting/dewatering the biomass
5. Cost for water pumping, etc.

High cost of biomass production

How close the gap between

→ present cost of algal biomass production : 3 – 30 € Kg^{-1}

→ the cost for biofuel production < 0.3 € Kg^{-1}

Dipartimento di Biotecnologie Agrarie
Università degli Studi di Firenze

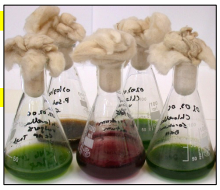
OIL FROM MICROALGAE

Liliana Rodolfi, Niccolò Bassi, Giulia Padovani, Gimena Bonini, Graziella Chini Zittelli, Natascia Biondi and Mario R. Tredici

Near horizontal tubular reactor

Biomass and lipid production by 31 microalgal strains

Microalgae	Biomass productivity (mg L^{-1} day^{-1})	Lipid content (% biomass)	Lipid productivity (mg L^{-1} day^{-1})
Porphyridium cruentum	613.3 ± 77.8	9.4 ± 0.2	57.5 ± 7.3
Tetraselmis suecica OR	448.0 ± 0.0	8.4 ± 0.3	37.5 ± 0.0
Tetraselmis sp. LW	414.0 ± 11.3	14.9 ± 0.1	61.8 ± 1.7
Tetraselmis suecica CV	383.6 ± 1.3	14.9 ± 0.1	57.3 ± 0.2
Chlorococcum sp. UMACC 112	380.0 ± 2.6	19.5 ± 0.7	74.2 ± 0.5
Scenedesmus sp. DM	348.2 ± 2.6	21.8 ± 0.6	75.8 ± 0.6
Phaeodactylum tricornutum	335.0 ± 31.1	19.2 ± 0.4	64.3 ± 6.0
Chlorella sorokiniana	315.5 ± 10.3	19.8 ± 0.7	62.3 ± 2.0
Chlorella sp. AMI2	307.3 ± 7.7	19.2 ± 0.4	59.0 ± 1.5
Scenedesmus sp. cvc3	283.6 ± 5.1	20.6 ± 0.8	58.4 ± 1.1
Nannochloropsis sp. RM	278.2 ± 0.0	31.0 ± 0.5	86.3 ± 0.0
Ellipsoidium sp. LW 277/01	275.5 ± 21.9	22.5 ± 0.8	62.1 ± 4.9
Chlorella vulgaris UTEX 1200	274.5 ± 21.9	19.4 ± 0.9	53.2 ± 4.2
Nannochloropsis sp. MRS	270.0 ± 2.6	24.9 ± 0.7	67.2 ± 0.6
Scenedesmus quadricauda	260.0 ± 1.3	19.0 ± 0.5	49.3 ± 0.2
Monodus subterraneus UTEX 151	257.3 ± 20.6	15.5 ± 0.5	39.9 ± 3.2
Isochrysis (T-ISO) CS 177	252.5 ± 1.8	22.0 ± 1,6	55.4 ± 0.4
Nannochloropsis sp. ZM	241.8 ± 7.7	33.1 ± 1.7	79.9 ± 2.6
Pavlova salina	240.0 ± 7.1	31.1 ± 1.4	74.6 ± 2.2
Nannochloropsis sp. MI	237.3 ± 1.3	22.3 ± 0.5	52.8 ± 0.3
Ellipsoidium sp. LW 70/01	235.5 ± 1.3	28.4 ± 0.4	67.0 ± 0.4
Nannochloropsis sp. RP	232.7 ± 25.7	37.0 ± 0.5	86.1 ± 9.5
Nannochloropsis sp. CS 246	231.8 ± 1.3	30.4 ± 0.3	70.4 ± 0.4
Chlorella vulgaris CCAP 211/11b	231.8 ± 1.3	19.7 ± 0.3	45.7 ± 0.3
Pavlova lutheri	212.5 ± 10.6	37.1 ± 0.5	78.9 ± 3.9
Isochrysis sp. MRS	194.0 ± 5.7	28.7 ± 0.5	55.6 ± 1.6
Thalassiosira pseudonana	135.0 ± 5.3	22.0 ± 1.7	29.7 ± 1.2
Skeletonema sp. CS 252	128.8 ± 5,0	32.9 ± 0.2	42.4 ± 1.6
Skeletonema sp. CS 181	123.8 ± 3.5	21.1 ± 0.9	26.1 ± 0.8
Chaetoceros muelleri	92.0 ± 4.2	34.7 ± 0.2	32.0 ± 1.5
Chaetoceros calcitrans	62.0 ± 1.4	40.9 ± 0.1	25.3 ± 0.6



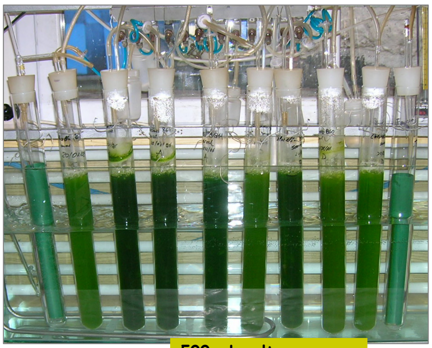
Lipid production in bubbled tubes under NITROGEN STARVATION

Freshwater species:

- *Chlorella* sp. AMI2
- *Scenedesmus* sp. DM

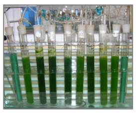
Marine species:

- *Tetraselmis suecica* OR
- *Nannochloropsis* sp. ZM

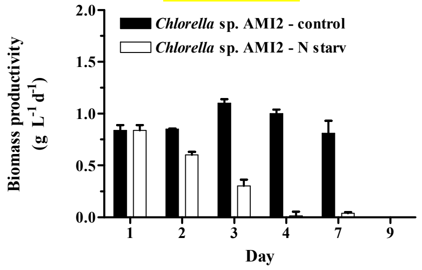


·500 mL cultures
·50% daily harvest rate
·N – sufficient medium
·N – free medium

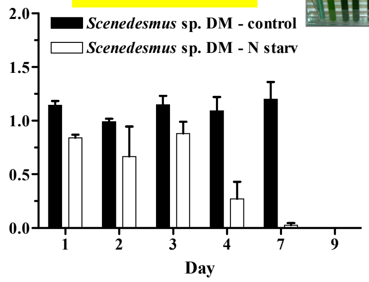
Freshwater microalgae under NITROGEN STARVATION

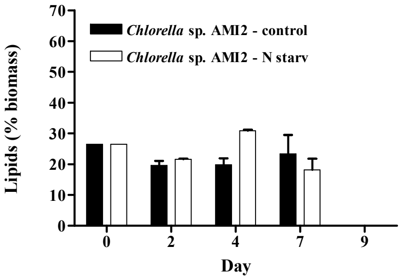


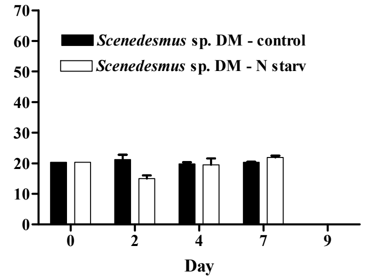
Chlorella



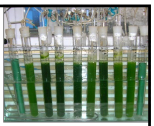
Scenedesmus



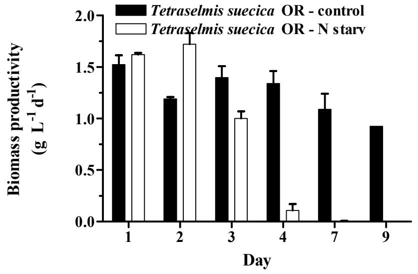




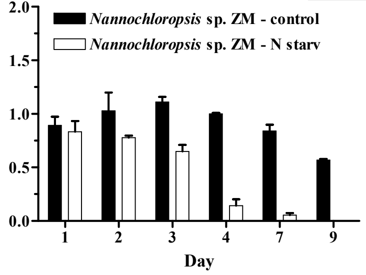
Marine microalgae under NITROGEN STARVATION

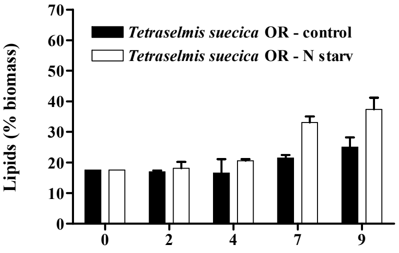


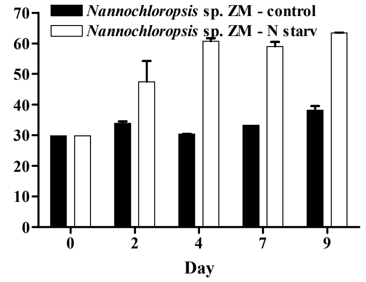
Tetraselmis



Nannochloropsis

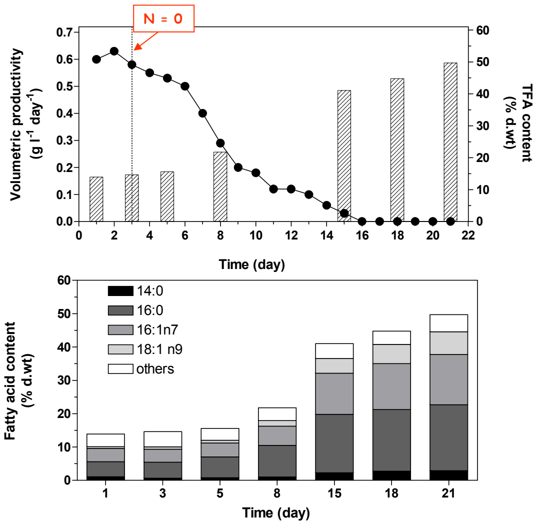




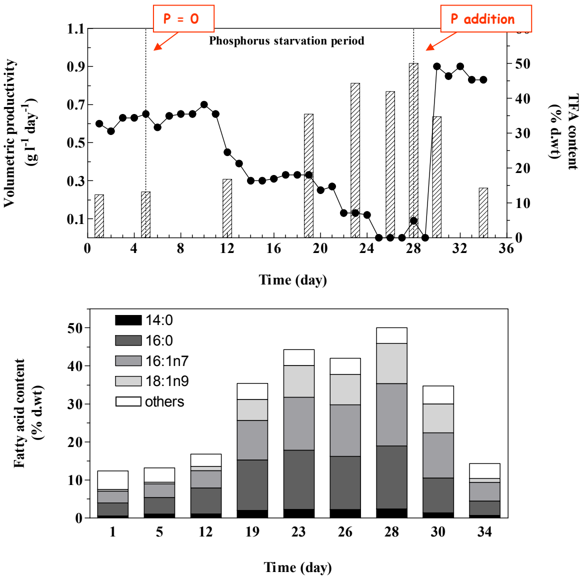


Nannochloropsis sp. ZM in alveolar panels with artificial illumination
Fatty acid accumulation under NITROGEN STARVATION

20 L
30% harvest rate



Nannochloropsis sp. ZM in alveolar panels with artificial illumination
Fatty acid accumulation under PHOSPHORUS STARVATION

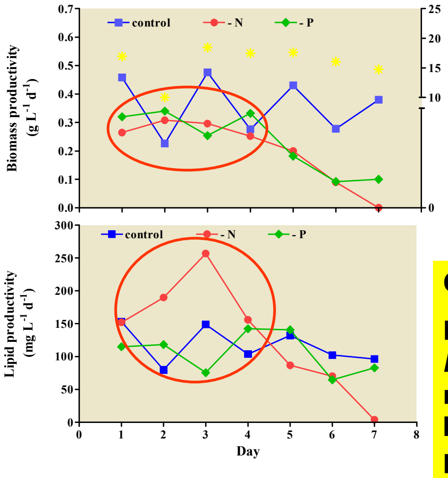


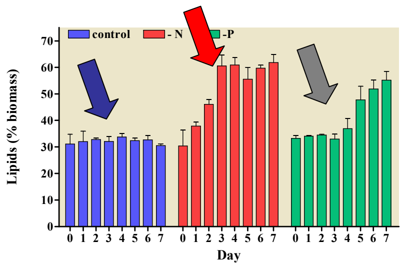
Outdoor cultivation of *Nannochloropsis* sp. ZM in green-wall reactors

Influence of **NITROGEN & PHOSPHORUS** starvation/limitation on lipid productivity

110 and 500 L GW reactors
40% daily harvest rate

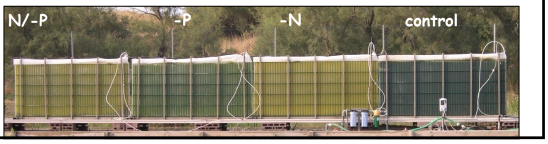
Nannochloropsis sp. ZM outdoor in green-wall reactor - **NITROGEN & PHOSPHORUS** STARVATION





Conclusion:

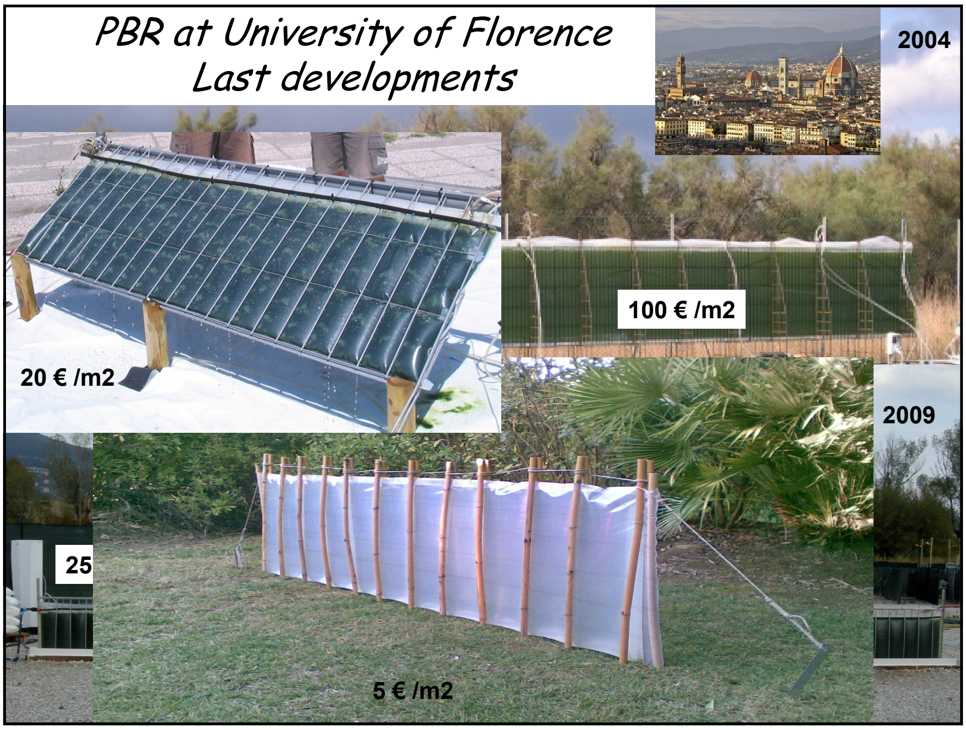
Nitrogen starvation in a growing *Nannochloropsis* culture causes a rapid increase of lipid content and leads to a significant increase of lipid productivity



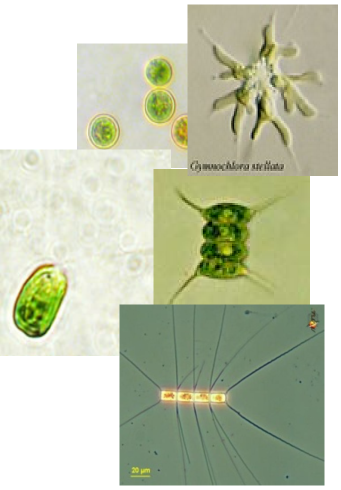
The pilot plant was arranged so as to simulate a full-scale system and the areal lipid productivity could be calculated

The experiments showed that *Nannochloropsis* has a potential for producing more than **20 tons of lipid per ha per year** in the Mediterranean basin and more than **30 tons of lipid per ha per year** in sunny tropical areas (20 MJ m^{-2} d^{-1})

PBR at University of Florence
Last developments



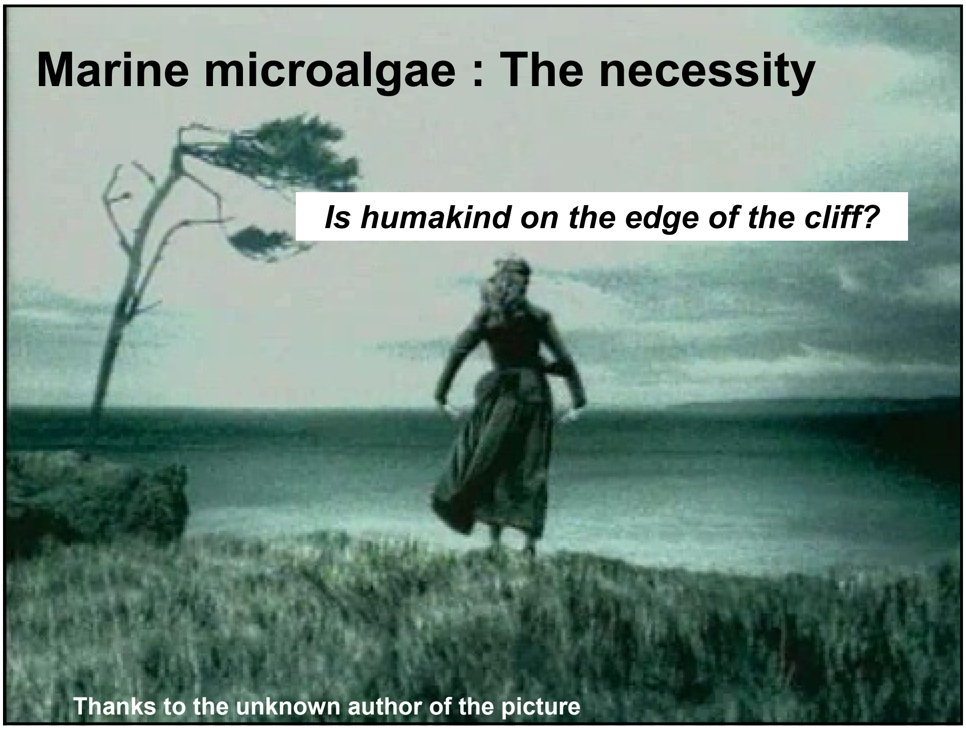
Marine Microalgae: The opportunity



Biofuels from marine microalgae: advantages

1. No need of arable/fertile soils
2. No need of freshwater
3. Biomass production may be combined with wastewater treatment
4. Carbon from flue gases
5. No need of pesticides and herbicides
6. No production of toxic substances
7. No need of GMO
8. *Higher oil production than traditional crops*
9. *Cultivation can (and must) be coupled with food production*

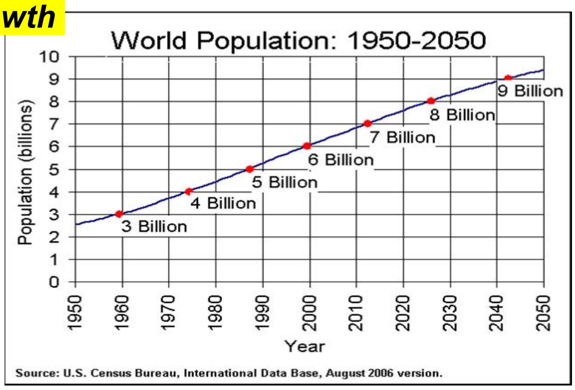
Marine microalgae : The necessity



Is humakind on the edge of the cliff?

Thanks to the unknown author of the picture

I-Population growth



Source: U.S. Census Bureau, International Data Base, August 2006 version.

There will be more than 9 billion people living in the world of 2050 and hundreds of millions of people will afford diets far richer in protein.

To meet future world's food needs, global food production must double (UN Environment Program)

(Julian Cribb, FTSE, May 2008)

II-Food shortage

Today 860 million people lack food !

Sustaining food production is the global scientific challenge of our era, more urgent even than global warming.

Source: FAO

III - the end of water

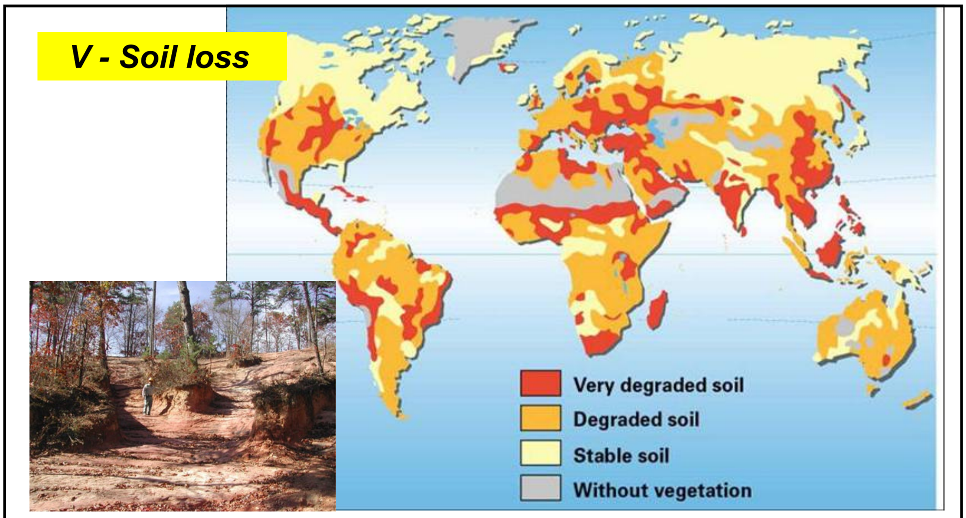
Groundwater is in decline everywhere

By 2050 cities will consume half the world's fresh water

(Julian Cribb, FTSE, May 2008)

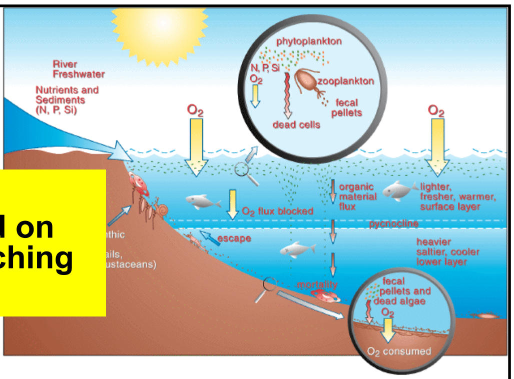
IV - The end of oil

V - Soil loss



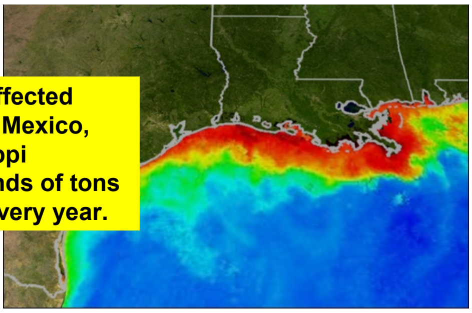
5-10 million ha arable land are lost per year

VI - Ocean dead zones

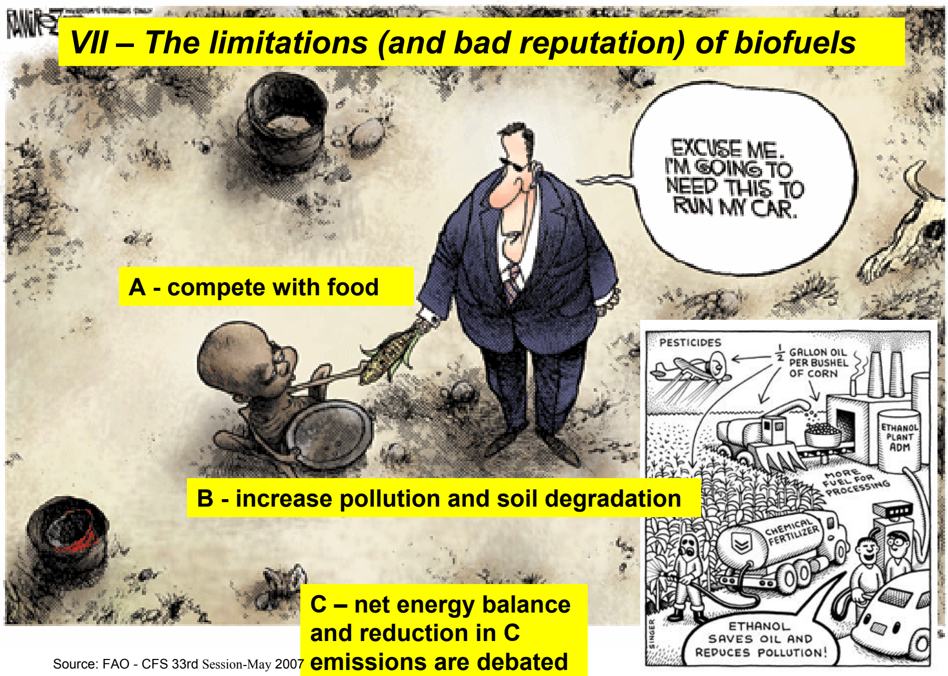


Half of all nutrients applied on farm are lost in runoff, leaching or erosion.

Among the worst affected areas is the Gulf of Mexico, where the Mississippi discharges thousands of tons of agrochemicals every year.



VII – The limitations (and bad reputation) of biofuels



A - compete with food

B - increase pollution and soil degradation

C – net energy balance and reduction in C emissions are debated

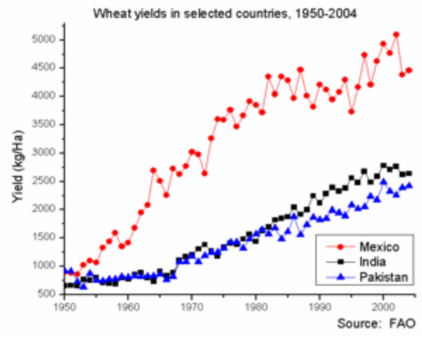
Source: FAO - CFS 33rd Session-May 2007

VIII - Climate change !

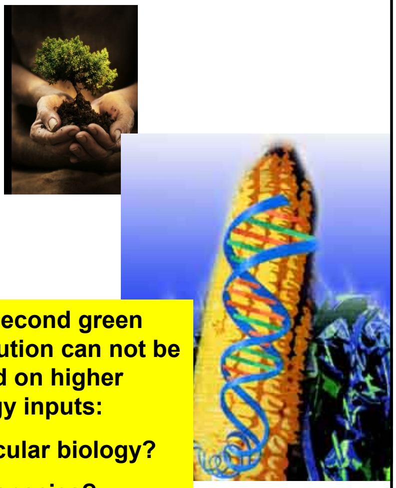
In this list of constraints, the only one somewhat uncertain is the scale of impact of climate change

(Julian Cribb, FTSE, May 2008)

The world needs a second "green revolution"



First green revolution was based on fertilizers, pesticides, productive crops (high energy inputs)



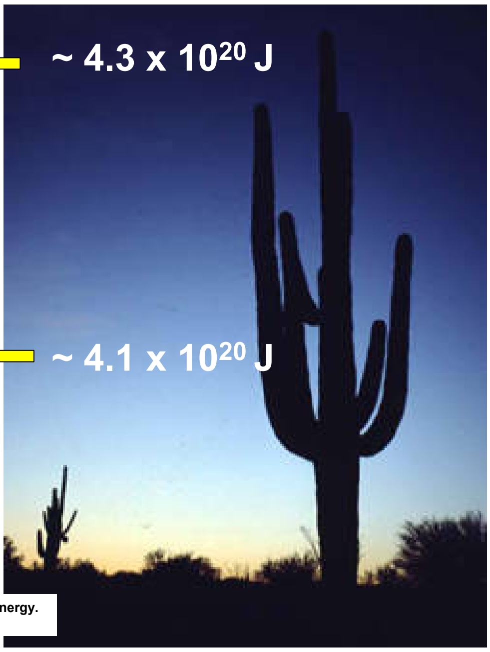
The second green revolution can not be based on higher energy inputs:

Molecular biology?

New species?

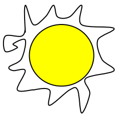
Algae?

Energy consumed in the world per year



~ 4.3 x 10^{20} J

~ 4.1 x 10^{20} J



Energy from the sun every hour

Donohue T. and Cogdell R. Microorganisms and clean energy. Nature Reviews Microbiology 4, 800 (November 2006)

Mass culture of algae for energy farming in coastal deserts

Balloni W., Florenzano G., Materassi R., Tredici M. R., Soeder C.J. and Wagener K.

In: Energy from Biomass (1982) A. Strub, P. Chartier and G. Schlesser, eds. Appl. Science Pubs, London.

Annual yield of marine algae in outdoor ponds ≥50 t ha^{-1}

Effect of nutrient concentration on the productivity of outdoor mass cultures of* Tetraselmis *sp. (Tredici et al. 1987).

Constituent (%)	High N input (2.4 g m^{-2} d^{-1})	Low N input (0.34 g m^{-2} d^{-1})
Protein	48	14
Lipid	30	27
Carbohydrate	22	58
Total N	7.7	2.3

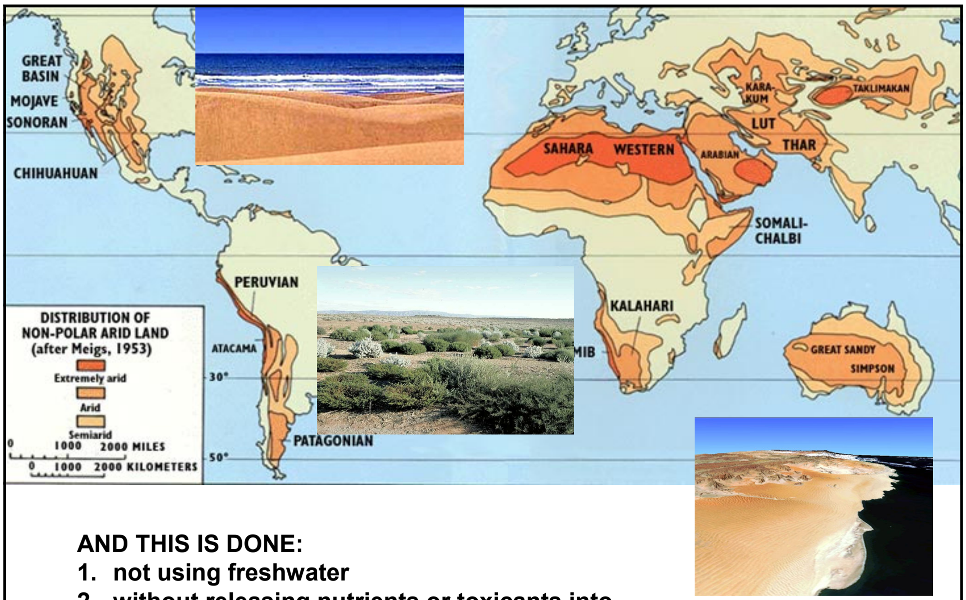
No effect on productivity

Mariculture on arid lands

Large-scale cultivation of a selected, robust, resilient marine microalga able to achieve:

1. 70-80 t ha^{-1} y^{-1} of biomass → 3-4% PE
2. 15-20 t ha^{-1} y^{-1} of oil + 30-45 t ha^{-1} y^{-1} of protein
3. unialgality (e.g. by the use of suitable strain and inoculation from PBR)
4. CO$_2$ and nutrients (N&P) use with almost 100% efficiency
5. a positive NER (net energy balance) is possible

An algae biomass industry is created, that produces food, fuel, feed and a variety of chemicals exploiting every single component (vitamins, algenans, fatty acids, carbohydrates) of the algal cell…



AND THIS IS DONE:

1. **not using freshwater**
2. **without releasing nutrients or toxicants into the environment**
3. **in the coastal desert areas of the world**

Food and fuels from marine microalgae:

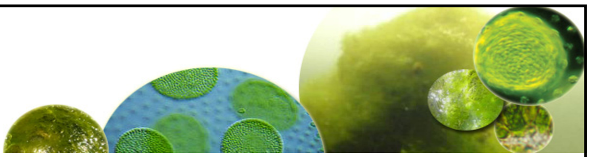
Dream?

or necessity?

1st EABA Conference and General Assembly was held

June 3-4, 2009 at the Home of the New York University in Florence, Italy

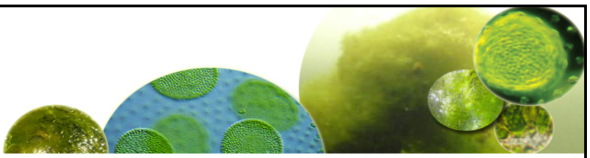
The EU algae biomass sector and the role of the EABA



Objectives of EABA:

1. Support the conditions for the development of an ecomically viable algae production chain and use
2. Represent algae industry at EU and national level
3. Defend the interests of the Algae Biomass sector
4. Promote interchange among the various heterogeneous stakeholders
5. Study solution to problems (technical, economic, environmental)
6. Make algae research and industry alive in the public debate
7. Spreading responsible and reliable knowledge and verified scientific information on algae
8. Promote investment in algae based technologies
9. Establish a permanent liaison with EU institutions
10. Define and express common positions on EU related issues and legislation

---



The EABA what for?

Algae-based biofuels need to be defined in the EU legislative framework

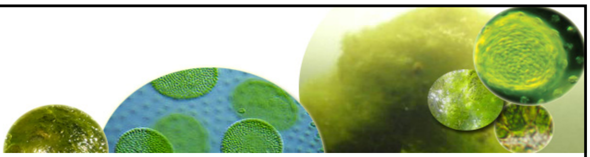
☐UNCLEAR if algae are in the group of biofuels made from wastes residues, non-food cellulosic material, ligno-cellulosic material (and algae?) counting double

☐No CO_2 emission default value for algae-based biofuels exists

☐Definition itself as algae as biofuel is at stake and needs to be defended

Algae must be in the review of the next EU animal feed legislation

If no initiative at these levels is taken algae risk to remain a theoretical promise and not become a real opportunity

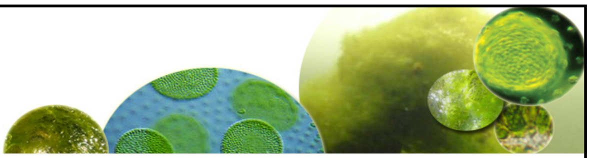


EABA Structure and functioning:

Approved during the 1st General Assembly meeting held in Florence, Italy – June '09

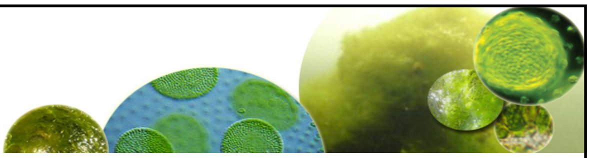
EABA GOVERNING BODIES

1. *General Assembly*
2. *Steering Board*, *President*, *Vice-Presidents*, *Executive Director*
3. *Scientific Committee* (also serving as Committee of publications)
4. *Industrial Committee*



EABA members

- There are at present:
- 58 members (and 12 new candidates)
  - 28 Industrial Members
  - 18 Scientific Members, 11 Individual, 1 Observer
  - Many medium size industries active in cultivation ofo algae and in algae technology (Necton, PetroAlgae, SBAE, Biovalue, Fotosintetica & Microbiologica Srl.)
  - Universities and research centres active in algae research (Wageningen UR, University of Florence, University of Ben-Gurion, IFREMER, CNR of Italy, University of Sevilla, etc.)
  - Industries from the fuel and renewable energy sector (Neste, Oil, Oxem, Repsol, Enel, etc.), strong support from the EU biofuel industry (EBB)
  - Air transport companies (Lufthansa)



Membership and financial support:

The Association is financially supported by its Members.

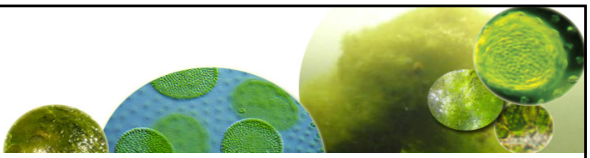
As FULL MEMBERS: located or having relevant activities in Europe, i.e. in the European Union, or in a country candidate to become a part of the European Union, including the EEE and Israel, full members are subdivided in three cathegories:

1. SCIENTIFIC MEMBERS: non-profit research institutes, universities, research and academic centres active in, with proven scientific activities, interest and/or publications in the field of algae biomass. Similar scientific legal entities coming from countries outside Europe -as defined above - can become Scientific Observers within the Association.
2. INDUSTRIAL MEMBERS: companies, pilot projects, algae-biomass technology providers, research groups and final users as the case may be, that are able to produce or contribute to produce, transform or use algae biomass in Europe - as defined above. European companies or legal entities that have already established precise plans of investment in view of producing or transforming or using algae biomass at industrial level can also become Industrial Members.
3. INDIVIDUAL MEMBERS: individuals or individual research fellows with proven interest in the development of the algae biomass research

SUPPORTING MEMBERS (Sponsors): Supporting membership status is reserved to Members which voluntarily support an extraordinary contribution whose level is fixed by the Steering Committee every year.

OBSERVERS (Scientific, Industrial or individual) all the legal entities or individuals that are active in the field of algae biomass and that because of the criteria detailed above and their geographical situation outside Europe cannot become full Members of the Association

---



For more information visit the EABA Web-portal:

www.eaba-association.eu

Or send your questions to:

eaba@eaba-association.eu

The 2nd General Assembly and EABA Conference will be held in June 2010