UCL Unité de génie biologique

UCL/AGRO/CABI/**GEBI**

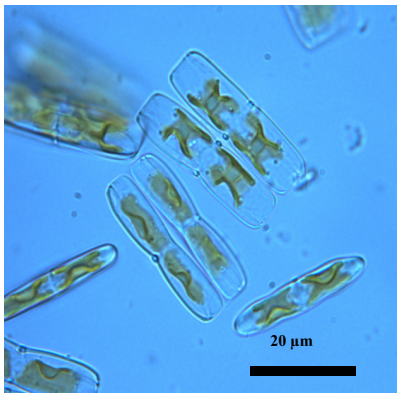
Unit of Bioengineering

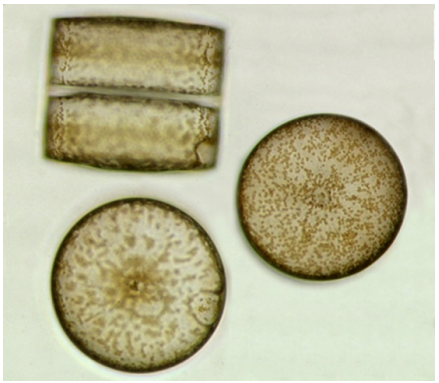
Algae as a Frontier in Bioprocessing: Technical and Economic Challenges

Clayton S. Jeffryes and Spiros N. Agathos

Unit of Bioengineering
Faculty of Bioengineering, Agronomy & Environment
Université Catholique de Louvain
Louvain-la-Neuve, Belgium

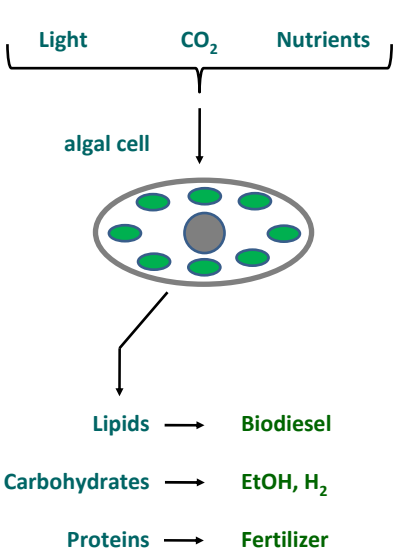
Microalgae





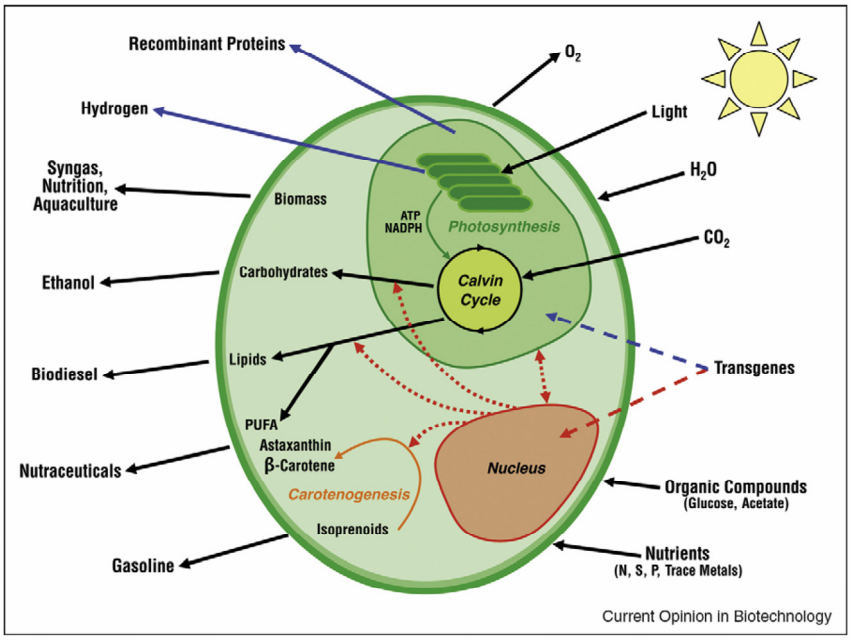
- Single-celled photosynthetic organisms
- Consume inorganic carbon and absorb photons
- Use solar energy to produce ATP which is used to synthesize lipids, carbohydrates and proteins

Microalgae: Ideal Organisms for Biofuels

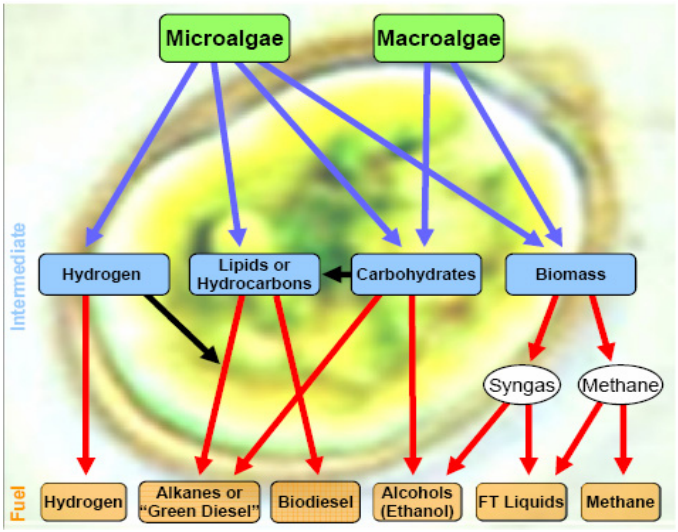


- Can be cultivated on non-arable land
  - Grown in ponds or photobioreactors
- Algae can tolerate broad ranges of pH, salinity, temperature
  - Easy to cultivate
- Continually processed, not seasonally harvested
- Algae are not a finite resource, i.e. sustainability
- Complete usage of biomass (lipids, proteins, carbohydrates)
- Algal cultivation can mitigate CO_2 from industrial sources (waste gas streams)
- Inexpensive substrates, high productivity

Commercially important metabolic pathways in microalgae



Algae can lead to several bioenergy sources



Source: Pienkos, 2007

Long-Term Studies of Microalgal Energy Potential

- **Research carried out in the framework of the Aquatic Species Program at NREL (Golden, CO) from 1978 to 1996**
- **3000 strains of microalgae collected, screened; some genetic manipulation**
- **Outdoor test facility of 1,000 m^2 (Roswell, NM), average proven productivity 10 g m^{-2} d^{-1}, peak 50 g m^{-2} d^{-1}**
- **Close-out report:**
  http://govdocs.aquake.org/cgi/reprint/2004/915/9150010.pdf
- **Current renewed interest because of**
  - → **Higher fuel prices, interest in CO_2 capture, energy security**
  - → **Tremendous progress in systems biology, "omics"**
  - → **Novel photobioreactor designs, advances in materials**

How do algae stack up?

Comparing Potential Oil Yields

Crop	Oil Yield Gallons/acre
Corn	18
Cotton	35
Soybean	48
Mustard seed	61
Sunflower	102
Rapeseed/Canola	127
Jatropha	202
Oil palm	635
Algae (10 g/m^2/day at 15% TAG)	1,200
Algae (50 g/m^2/day at 50% TAG)	10,000

Fatty acid composition of algal oils suitable for preparation of biodiesel

Source: Pienkos, 2007

Advantages of Biodiesel from Microalgae

Crop	Oil yield L/ha/year
Corn	172
Soybean	446
Canola	1190
Coconut	2689
Oil Palm	5950
Algae (High Estimate)	136,900
Algae (Low Estimate)	58,700

Chisti, 2007

- **High productivity**
- **Up to 77 wt% lipid content**
- **Negligible lignocellulosic biomass component**
- **High fuel purity, e.g. no SO_x**
- **Diesel more versatile and energy dense than bioethanol**
- **Terrestrial oil crops are unable to meet future bio-oil demand**

Lipid Content of Microalgae (*adapted from Franck, 2008*)

Species	*Proteins*	*Sugars*	*Lipids*	*Nucleic Acids*
Soya	37	30	20	—
Scenedesmus obliquus	50–56	10–17	12–14	3–6
Scenedesmus quadricauda	47	—	1.9	—
Scenedesmus dimorphus	8–18	21–52	16–40	—
Chlamydomonas rheinhardii	48	17	21	—
Chlorella vulgaris	51–58	12–17	14–22	4–5
Chlorella pyrenoidosa	57	26	2	—
Spirogyra sp.	6–20	33–64	11–21	—
Dunaliella bioculata	49	4	8	—
Dunaliella salina	57	32	6	—
Euglena gracilis	39–61	14–18	14–20	—
Prymnesium parvum	28–45	25–33	22–38	1–2
Tetraselmis maculata	52	15	3	—
Porphyridium cruentum	28–39	40–57	9–14	—
Spirulina platensis	46–63	8–14	4–9	2–5
Spirulina maxima	60–71	13–16	6–7	3–4.5
Synechococcus sp.	63	15	11	5
Anabaena cylindrica	43–56	25–30	4–7	—

Species	*+N*	*-N*
Chlorophyceae		
Ankistrodesmus sp.	18.3	40.3
Botryococcus braunii	44.5	54.2
Chlamydomonas applanata	18.2	32.8
Chlorella pyrenoidosa	13.4	29.2
	10.0	70.0
	14.4	35.8
	20.0	86.0
Chlorella vulgaris (NH_4)[b]	11.8	52.8
C. vulgaris (NO_3)[b]	21.8	57.9
C. luteoviridis	17.5	28.8
C. capsulata	11.7	11.4
Dunaliella primolecta	23.1	16.6
D. salina (UTEX 200)	25.3	9.2
Nannochloris sp.	20.8	35.5
	20.2	47.8
Oocystis polymorpha	12.6	34.7
Ourococcus sp.	27.0	49.5
Scenedesmus obliquus (NH_4)[b]	22.4	34.6
Tetraselmis suecica	23.4	14.6

(Above: Results of different studies on the effect of nitrogen limitation on the lipid content (percentage of dry biomass) in different species of green microalgae)

Widely variable as a function of nutritional & environmental conditions:

- N limitation can cause a massive accumulation (70-85 %) of lipids

- Among diatoms, Si limitation can lead to lipid accumulation

- In some microalgae (e.g., *Euglena gracilis*), lipid content increases to 70% during senescence

- The composition in fatty acids (saturated *versus* unsaturated) varies with luminosity, T°, C-soursce

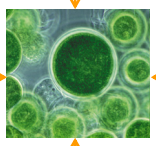
Growth Conditions of Microalgae

Energy Source for Phototrophs

- Photoinhibition
- Photolimitation

Nutritional Factors

- C-source
  - CO_2 (autotrophic mode)
  - $CO_2 + C_{org}$ (mixotrophic mode)
  - C_{org} (heterotrophic mode)
- N-source
- Minerals, trace elements and vitamins



Processing Parameters

- Gas transfer
  - C-source and supply process
  - CO_2 transfer systems and O_2 removal
- Mixing
- Light requirement

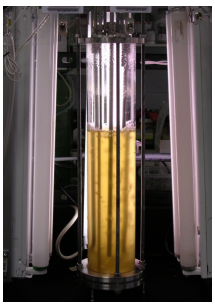
Environmental Factors

- pH
- Salinity
- Temperature

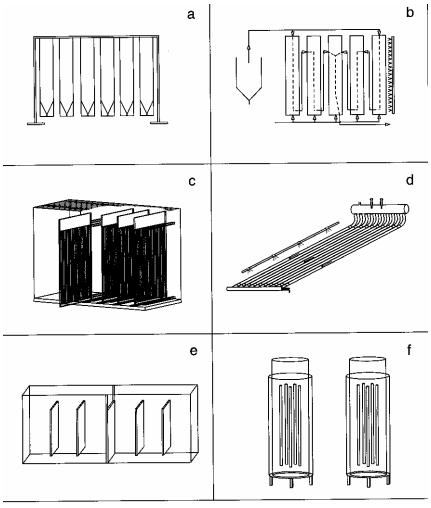
Types of Algal Cultivation Facilities

- Selected closed photobioreactor systems
  - •Tubular photobioreactors: horizontal, vertical or helical array
  - •Bubble or airlift aerated reactors, planar or cylindrical
  - •Stirred tank
- •Open systems
  - •Raceway ponds

Paddle-mixed raceway pond



Bench-scale bubble column

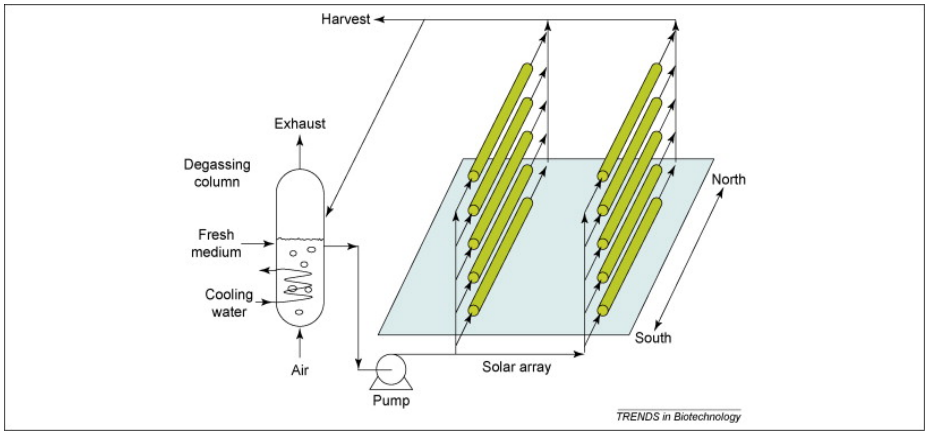


Adapted from Franck (2008)

Different types of photobioréacteurs used for the culture of *Nannochloropsis*:

a. **Polyethylene bags (indoor)**
b. **Glass-fiber cylinders (indoor)**
c. **Flat modular photobioreactor (indoor)**
d. **Tubular inclined (quasi-horizontal) photobioreactor (outdoor)**
e. **Segmented glass plate photobioreactor (outdoor)**
f. **Annular photobioreactor made of plexiglas (indoor)**

Cost of artificial illumination: from 45 to 65 USD per kg of dry algal mass



A tubular photobioreactor with fence-like solar collectors. Algal broth from the degassing column is continuously pumped through the solar array, where sunlight is absorbed, and back to the degassing column. Fresh culture medium is fed continuously to the degassing column during daylight and an equal quantity of the broth is harvested from the stream that returns to the degassing column. Cooling water pumped through a heat exchanger coil in the degassing column is used for temperature control. The degassing column is continuously aerated to remove the oxygen accumulated during photosynthesis and oxygen-rich exhaust gas is expelled from the degassing column (Chisti, 2008)

System concept and design

Table 2 Advantages and disadvantages of open and closed algal cultivation plants

Parameter	Open ponds (raceway ponds)	Closed systems (PBR systems)
Contamination risk	Extremely high	Low
Space required	High	Low
Water losses	Extremely high	Almost none
CO_2-losses	High	Almost none
Biomass quality	Not susceptible	Susceptible
Variability as to cultivatable species	Not given, cultivation possibilities are restricted to a few algal varieties	High, nearly all microalgal varieties may be cultivated
Flexibility of production	Change of production between the possible varieties nearly impossible	Change of production without any problems
Reproducibility of production parameters	Not given, dependent on exterior conditions	Possible within certain tolerances
Process control	Not given	Given
Standardization	Not possible	Possible
Weather dependence	Absolute, production impossible during rain	Insignificant, because closed configurations allow production also during bad weather
Period until net production is reached after start or interruptions	Long, approx. 6–8 weeks	Relatively short, approx. 2–4 weeks
Biomass concentration during production	Low, approx. 0.1–0.2 g/l	High, approx. 2–8 g/l
Efficiency of treatment processes	Low, time-consuming, large volume flows due to low concentrations	High, short-time, relatively small volume flows

Pulz, 2001

A review of enclosed system designs

Table 3. Main Design Features of Closed Photobioreactors (Carvalho et al., 2006)

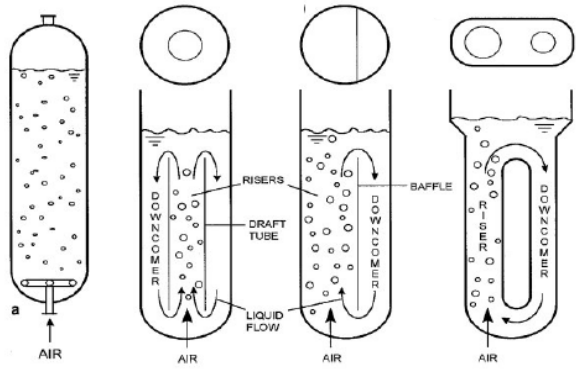
reactor type	light harvesting efficiency	degree of control	land area required	scale-up
vertical tubular	medium	medium	medium	possible
horizontal tubular	good	medium	poor	possible
helical	medium	good	excellent	easy
α-shaped	excellent	good	poor	very difficult
flat-plate	excellent	medium	good	possible
fermenter type	poor	excellent	excellent	difficult

BIOVAMAT: Conception and design of optimized PBRs taking advantage of improved materials

Table 3. Main Design Features of Closed Photobioreactors (Carvalho et al., 2006)

reactor type	light harvesting efficiency	degree of control	land area required	scale-up
vertical tubular	medium	medium	medium	possible
horizontal tubular	good	medium	poor	possible
helical	medium	good	excellent	easy

→ **Column PBRs**



BIOVAMAT: Conception and design of optimized PBRs taking advantage of improved materials

Table 3. Main Design Features of Closed Photobioreactors (Carvalho et al., 2006)

reactor type	light harvesting efficiency	degree of control	land area required	scale-up
vertical tubular	medium	medium	medium	possible
horizontal tubular	good	medium	poor	possible
helical	medium	good	excellent	easy

The greatest drawback of horizontal or helical tubular PBRs:

SPACIAL SEPARATION of photosynthesis and mass-gas exchanger

→ SPACIAL HETEROGENEITY in tubular section

→ **The length of the tubes is limited by O_2 accumulation, CO_2 depletion, and pH variations.**

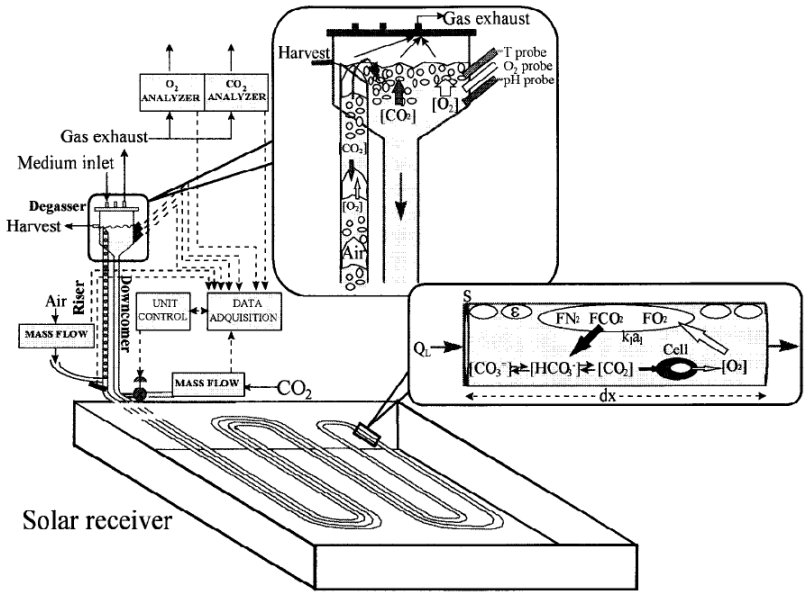
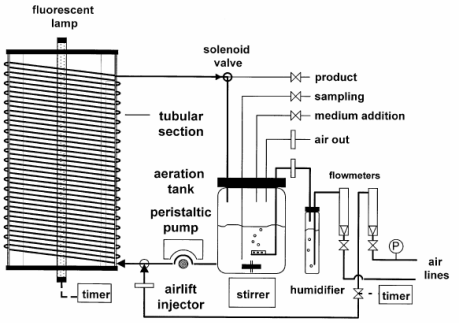
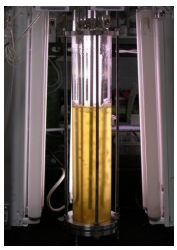


Figure 1. Schematic drawing of the tubular photobioreactor used, showing the nomenclature and phenomena taking place in the system (solar receiver 98.8 m).

Types of Algal Cultivation Facilities

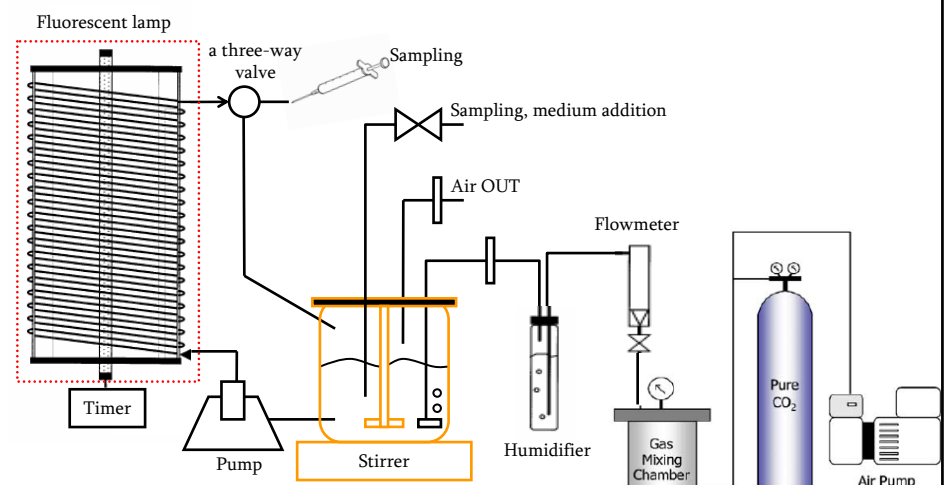




Bench scale helical photobioreactor,
Rorrer and Mullikin, 1999

19

BIOVAMAT: Conception and design of optimized PBRs taking advantage of improved materials



TUBULAR SECTION - Light harvesting unit

AERATION TANK - Gas-mass exchanger

BIOVAMAT: Conception and design of optimized PBRs taking advantage of improved pumping

Critical Design Elements for Algal Cultivation

- Delivery of photons to the cell culture
- CO_2 transport, oxygen removal and gas exchange
- Mixing
- Temperature and pH control
- Light/dark cycles
- Nutrient supply (N, P, S, Si trace elements)
- Water loss
- Contamination
- Scale-up

Comparison of Algal Cultivation Platforms

Parameter	Open pond	Closed Photobioreactor
Productivity		X
Cost	X	
Photon utilization		X
Process control		X
Mixing and gas exchange		X
Contamination		X
Water loss		X

Open systems are prone to:

- **Low lipid content**
- **Microbial and native algae contamination**
- **Temperature variation**
- **Water loss**
- **Oxygen inhibition due to poor mixing**

Closed systems are prone to:

- **High construction costs**
- **High maintenance requirements**

Comparison of Algal Cultivation Platforms

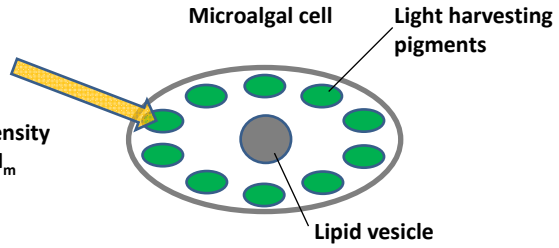
Parameter	Open pond	Closed Photobioreactor
Productivity		X
Cost	X	
Photon utilization		X
Process control		X
Mixing and gas exchange		X
Contamination		X
Water loss		X

Closed photobioreactor systems outperform open pond systems, but currently cost more to operate

Photobioreactor Design and Modeling

- **Components of Design**
  - **Light delivery**
  - **Carbon delivery**
- **Flue gas mitigation and biofuel production**
- **Case study**
  - **Lab scale bubble column configuration**
  - **Photobioreactor modeling**

Light Delivery and Photosynthesis



- **Photons, hv**
- **Mean light intensity within reactor I_m**

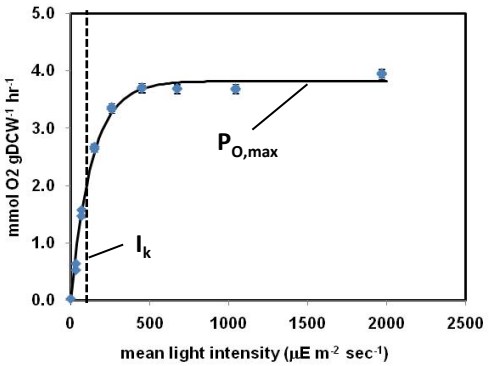
Simplified photosynthetic reaction

$$2H_2O + h\lambda \rightarrow O_2 + 4H^+ + 4e^-$$

CO_2 **, nutrients** →

- **Lipids**
- **Carbohydrates**
- **Proteins**

Photosynthetic Rate vs. Light Intensity



$$P_O = P_{O,\max}\left[1 - \exp\left(-\frac{I}{I_k}\right)\right]$$

$P_{O,max}$ = 3.81 ± 0.05 mmol O_2 / (g dry cell mass hr)

I_k = 134 ± 7 µE m^{-2} s^{-1}

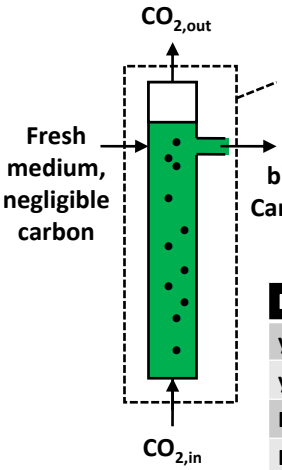
± 1 standard error

- $P_{O,max}$ is the maximum relative photosynthetic rate of a microalgal cell culture
- I_k is the mean light intensity at 50% of the maximum photosynthetic rate
- Photobioreactor design and optimization:
  - Adequate light to maintain a high photosynthetic rate
  - Avoid saturation, "wasted photons" dissipated as heat instead of biomass

Photobioreactor Design and Modeling

- Components of Design
  - Light delivery
  - Carbon delivery
- Flue gas mitigation and biofuel production
- Case study
  - Lab scale bubble column configuration
  - Photobioreactor modeling

Microalgal Carbon Mass Balance



Analyzing the extent of carbon capture & biomass productivity

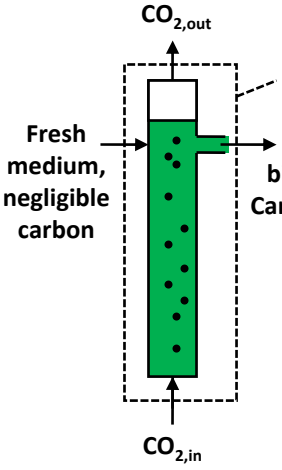
Steady State Overall Mass Balance

$CO_{2,in} = CO_{2,out}$ + algal biomass carbon out

$$y_{CO2,in}F_{in} = y_{CO2,out}F_{out} + Y_{X/C}R_XV$$

Parameter	Definition	units
$y_{CO2,in}$	Mole fraction CO_2 inlet	mol CO_2 /mol total
$y_{CO2,out}$	Mole fractionCO_2 outlet	mol CO_2 /mol total
F_{in}	Molar flow rate, inlet gas	mol / hr
F_{out}	Molar flow rate, outlet gas	mol / hr
$Y_{X/C}$	Carbon content of biomass	mol C / kg biomass
R_xV	Rate of algal biomass production	Kg biomass / hr

Microalgal Carbon Mass Balance



Analyzing the extent of carbon capture and biomass productivity

Solving

Mole fraction of CO_2 in outlet

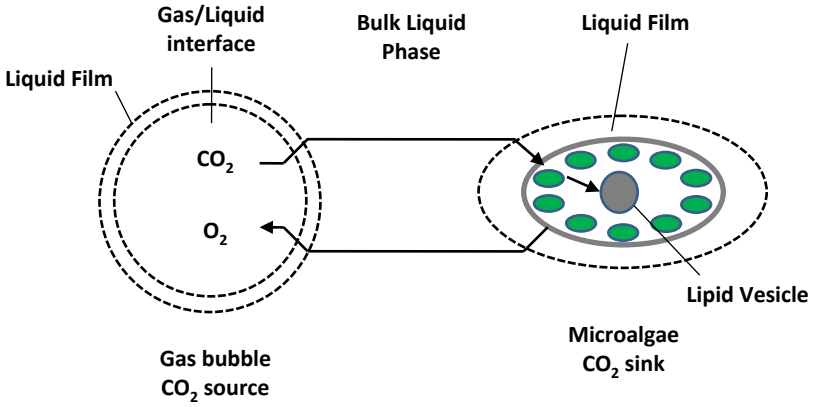
$$y_{CO2,out} = \frac{y_{CO2,in}F_{in} - Y_{X/C}R_XV}{F_{out}}$$

Rate of CO_2 capture (biomass productivity)

$$R_{CO\ 2} = Y_{X/C}R_XV$$

- If carbon capture is desired $y_{CO2,out}$ is minimized
- If biomass productivity is desired, R_{CO2} is maximized

CO_2 Transfer to Microalgae

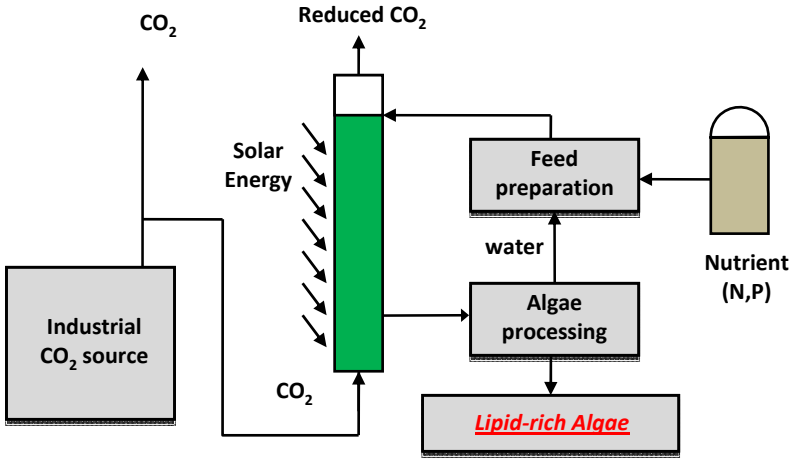


- Interphase mass transfer limitations are decreased with adequate mixing
- Higher k_La
- Increased CO_2 concentration in the gas phase increases molar transfer to liquid phase, but leads to incomplete carbon scrubbing

Photobioreactor Design and Modeling

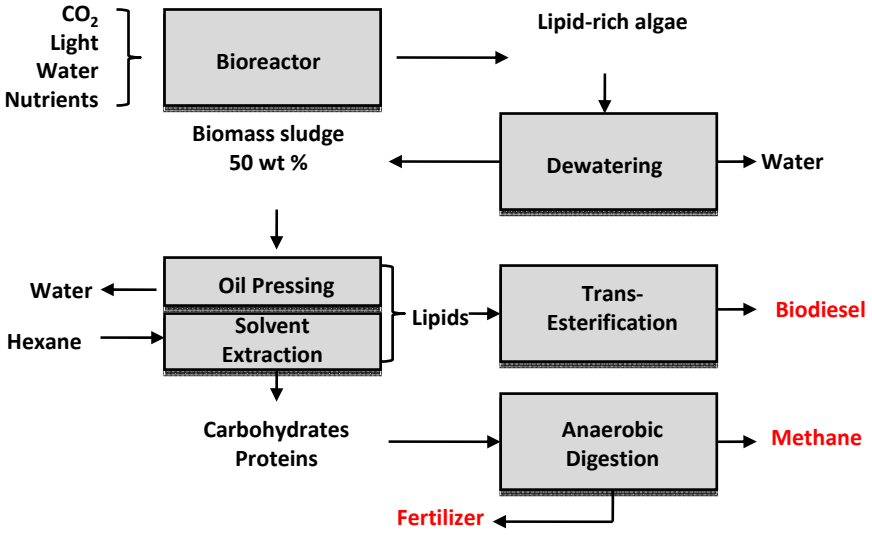
- Components of Design
  - Light delivery
  - Carbon delivery
- Flue gas mitigation with biofuel production
- Case study
  - Lab scale bubble column configuration
  - Photobioreactor modeling

Microalgal Mitigation of CO_2



Integration of microalgae cultivation with an industrial CO_2 source for carbon capture and biomass production

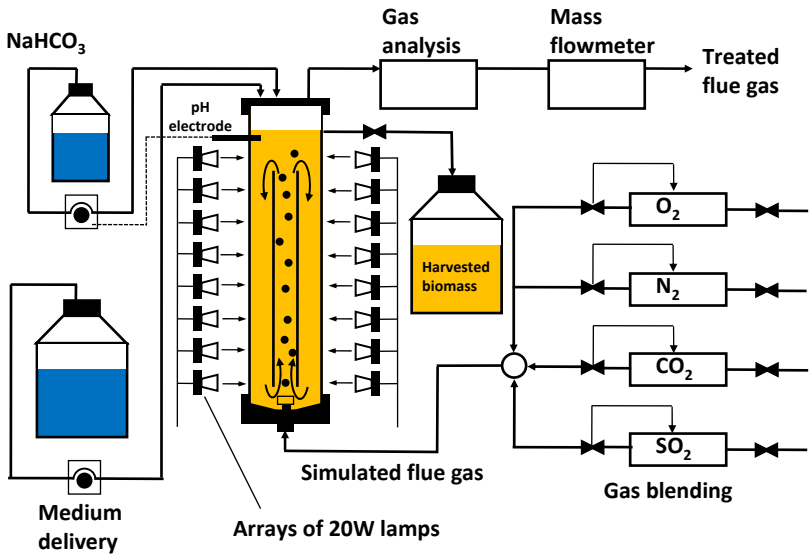
Biomass Processing



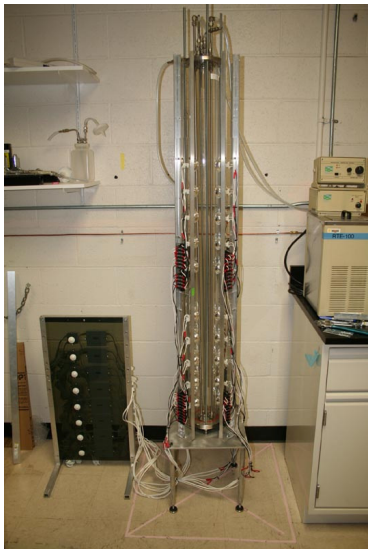
Photobioreactor Design and Modeling

- Components of Design
  - Light delivery
  - Carbon delivery
- Flue gas mitigation with biofuel production
- Case study
  - Lab scale bubble column configuration
  - Photobioreactor modeling

Laboratory Scale Photobioreactor



Laboratory Scale Photobioreactor



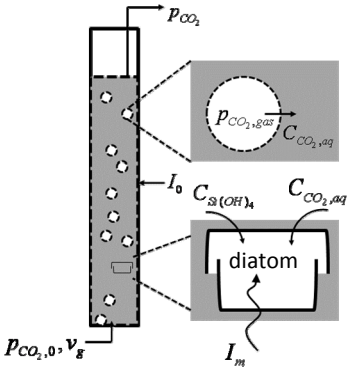
- Photobioreactor vessel
  - 150 cm h
  - 12 L working volume
- Variable incident light intensity
  - 0-4000 µE m^{-2} s^{-1}
  - 0-800W total power
- Air and CO_2 mass flow controllers with rotometer redundancy
- Online CO_2 analyzer

Photobioreactor Design and Modeling

- Components of Design
  - Light delivery
  - Carbon delivery
- Flue gas mitigation with biofuel production
- Case study
  - Lab scale bubble column configuration
  - Photobioreactor modeling

Photobioreactor Modeling of Diatom Microalgae

- **Key Assumptions**
  - **Only $CO_{2,aq}$ and $Si(OH)_4$ are consumed**
  - **Monod substrate uptake and growth**
  - **Well-mixed liquid and gas phases**
  - **Uniform external illumination**
  - **$SiO(OH)_3^-$ and $Si(OH)_4$ are always in equilibrium**



Key Photobioreactor Material Balances

Biomass

$$\frac{dX}{dt} = \mu X$$

Silicon

$$\frac{dC_{Si,total}}{dt} = -\frac{\mu X}{Y_{X/Si}}$$

Dissolved CO_2

$$\frac{dC_{CO_2}}{dt} = -k_L a\left(\frac{p_{CO_2}}{H_{CO_2}} - C_{CO_2}\right) + k_{12}C_{HCO_3^-} - \frac{\mu X}{Y_{X/CO_2}} - k_{11}C_{CO_2} - Xm_{CO_2}$$

Dissolved H^+

$$\frac{dC_{H^+}}{dt} = -k_{11}C_{CO_2} + (k_{21} - k_{12})C_{HCO_3^-} - k_{22}C_{CO_3^{2-}}C_{H^+} - \frac{\mu X}{\left(1+10^{pH-pKa,Si}\right)Y_{X/Si}}$$

Complete Material Balance

$$\frac{dX}{dt} = \left(\mu - \frac{F}{X} - k_d\right)X$$

$$\frac{dC_{Si(OH)4}}{dt} = \frac{dC_{Si,T}}{dt} = \frac{-\mu X}{Y_{X/Si}} + \frac{F}{V}\left(C_{Si,T,f} - C_{Si,T}\right)$$

$$\frac{dC_{CO2}}{dt} = k_L a\left(\frac{P_{CO2}}{H_{CO2}} - C_{CO2}\right) + k_{12}C_{HCO3} - \frac{\mu X}{Y_{X/CO2}} - k_{11}C_{CO2} - Xm_{CO2} + \frac{F}{V}\left(C_{CO2,0} - C_{CO2}\right)$$

$$\frac{dC_{HCO3}}{dt} = k_{11}C_{CO2} + k_{22}C_{CO3}C_{H+} - (k_{12} + k_{21})C_{HCO3} + \frac{F}{V}\left(C_{HCO3,0} - C_{HCO3}\right)$$

$$\frac{dC_{CO3}}{dt} = k_{21}C_{HCO3} - k_{22}C_{CO3}C_{H+} + \frac{F}{V}\left(C_{CO3,0} - C_{CO3}\right)$$

$$\frac{dC_{H+}}{dt} = k_{11}C_{CO2} + (k_{21} - k_{12})C_{HCO3} - k_{22}C_{CO3}C_{H+} + \frac{F}{V}\left(C_{H+,0} - C_{H+}\right) - \frac{\mu X}{(1 + 10^{pH-pKa,Si})Y_{X/Si}}$$

Photobioreactor Modeling of Diatom Microalgae

Silicon speciation

$$C_{Si(OH)_4} = \frac{C_{Si,total}}{1+10^{pH-pKa,Si}}$$

Gas phase CO_2 mass balance

$$p_{CO_2} = p_{CO_2,0} - \frac{VRT\mu X}{v_g Y_{X/CO_2}}$$

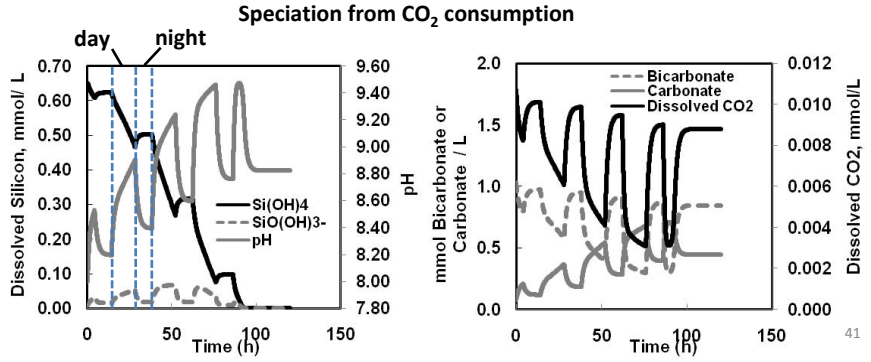
Mean light intensity

$$I_m = \frac{4I_0 \exp^{-L(k_c X + k_0)} \sinh(L(k_c X + k_0))}{L(k_c X + k_0)}$$

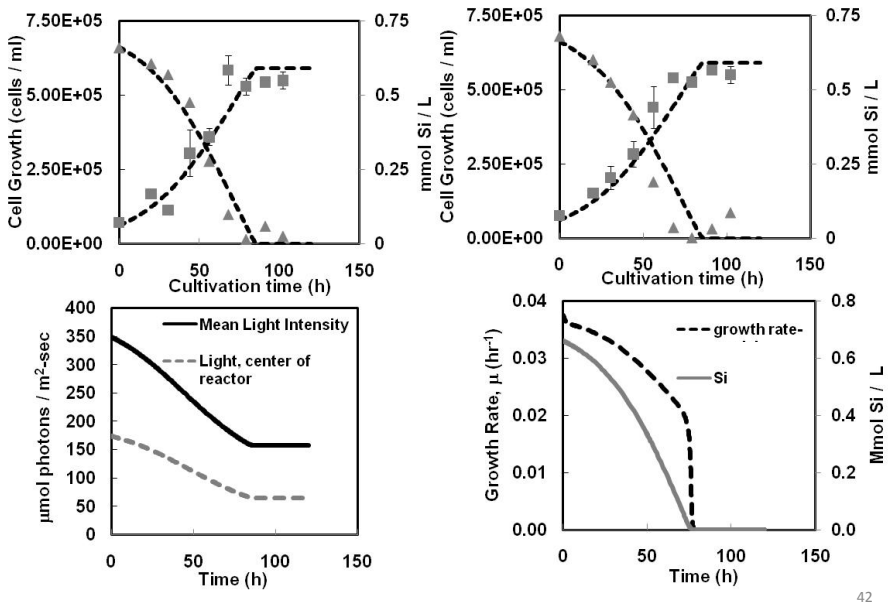
Growth rate

$$\mu = \mu_{max} \underbrace{\frac{C_{CO_2}}{K_{CO_2} + C_{CO_2}}}_{\text{Dissolved } CO_2} \underbrace{\frac{C_{Si(OH)_4}}{K_{Si(OH)_4} + C_{Si(OH)_4}}}_{\text{Dissolved silicon}} \underbrace{\left(1 - \exp^{-I_m/I_k}\right)}_{\text{Light intensity}} \underbrace{\frac{1}{1 + \left(\frac{C_{H^+}}{K_1}\right) + \left(\frac{K_2}{C_{H^+}}\right)}}_{\text{pH}}$$

Speciation from CO_2 consumption



Photobioreactor Modeling of Diatom Microalgae



Photobioreactor Modeling Results

- **Cell culture biomass and substrate concentrations were predicted from a first principles photobioreactor model**
- **Light had greatest effect upon growth the rate until the rate limiting substrate depleted**
- **Growth related CO_2 consumption changes pH which drives speciation and effects substrate uptake**

Perspectives

The future of algae as an energy supplier is bright, but substantial displacement of fossil fuels (25-50%) is not going to occur overnight

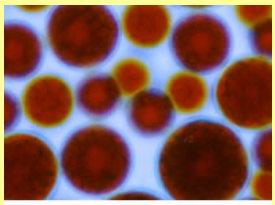
Algal biofuels cost one at least 10 times the cost of 'traditional' biofuels (bioethanol, biodiesel)

Major breakthroughs are needed in obtaining robust algae species with higher oil content, enhancing productivity through novel potoboioreactor cultivation and developing improved harvesting

Commercial applications - Real

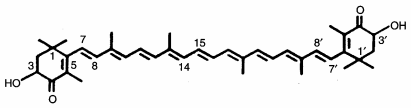
ALGA Technologies	Israel, www.algatech.com	Closed tubular PBR
BIOREAL SWEDEN	Sweden, www.bioreal.se	Closed cylindrical PBR
Cyanotech	Hawai, www.cyanotech.com	Closed tubular PBR + Open ponds
Mera Pharmaceuticals	Hawai, www.merapharma.com	Closed tubular PBR
FUJI healthscience	Hawai, www.fujihealthscience.com	Enclosed hemispherical PBR
Parry Nutraceuticals	India, www.parrynutraceuticals.com	Open ponds

Photoreactor-based production of astaxanthin by *Haematococcus pluvialis*



'Red phase', rich in carotenoids

'Green phase', rich in chlorophyl



Astaxanthin:

- **A powerful antioxidant**
- **Nutritional additive for salmon in aquaculture**
- **Current commercial value : 2000 $ / kg of pure pigment**
- **Already commercial**
- **Market expanding**

Mass culture of *Haematococcus* using tubular photobioreactors in the Negev desert (Israel, Alga Technologies)

UCL Unité de génie biologique

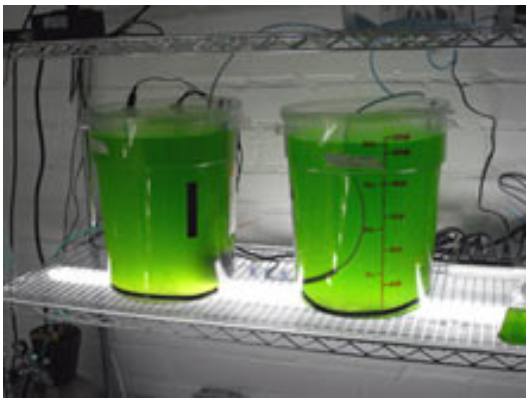
UCL/AGRO/CABI/**GEBI**

Biodiesel production from microalgae

Solazyme and **NIST** collaboration to commercialize algal biodiesel

UCL Unité de génie biologique
UCL/AGRO/CABI/GEBI

Oil extraction from microalgae

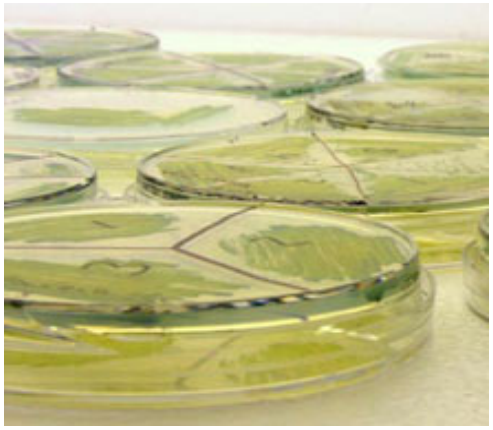


OriginOil is testing a more efficient extraction route for algal lipids

UCL Unité de génie biologique
UCL/AGRO/CABI/GEBI

Synthetic biology in microalgae

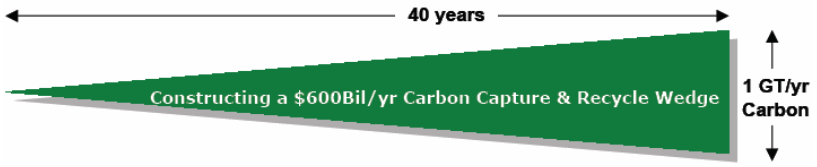
July 2009: **ExxonMobil** announced a commitment to invest $300 million over 5 to 6 years in **Synthetic Genomics**, which J.Craig Venter founded and now leads as CEO, and to spend an additional $300 million on a complementary internal algae program



Ethanol production from microalgae

Algae-to-ethanol bioreactors at an **Algenol** test facility. The company has teamed up with **Dow Chemical** to build a demonstration plant that could end up producing 100,000 gallons of ethanol annually.

Carbon Capture Sequester & Recycle (CCS&R)
National Pipeline Grid proposed by A2BE



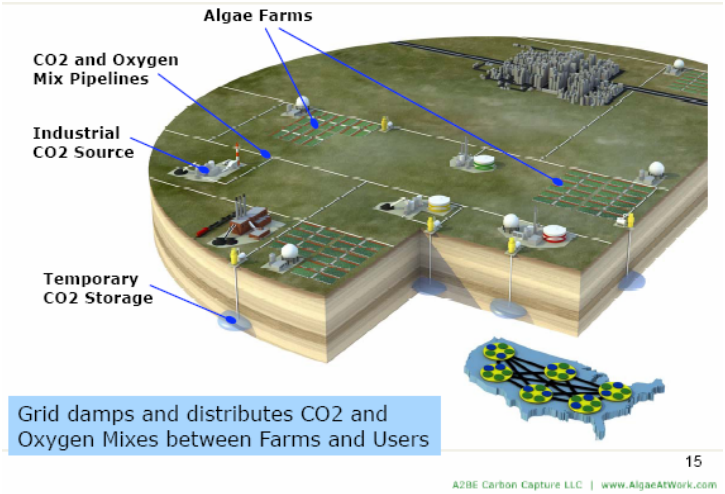
Yearly Global investment	Total for 1 Carbon Wedge
• 800,000 acres/year	• 33 Million CC&R acres
• $66 Billion infrastructure/yr	• $2.6 Trillion build-out
• 57,000 new direct jobs/year	• 2.3 Million direct jobs
• $15 Billion revenue growth	• $600 Billion revenue/year

www.algaeatwork.com 8

1 GT Carbon = Carbon in 3.66 Billion tons of CO2

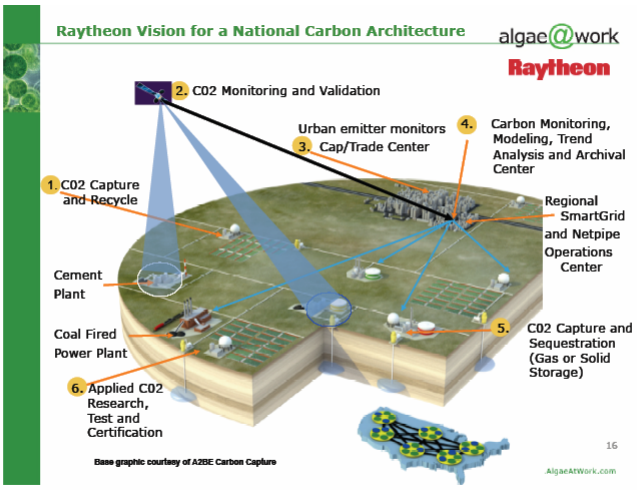
Source: Jim Sears, 2007

Carbon Capture Sequester & Recycle (CCS&R)
National Pipeline Grid Integrates Industries



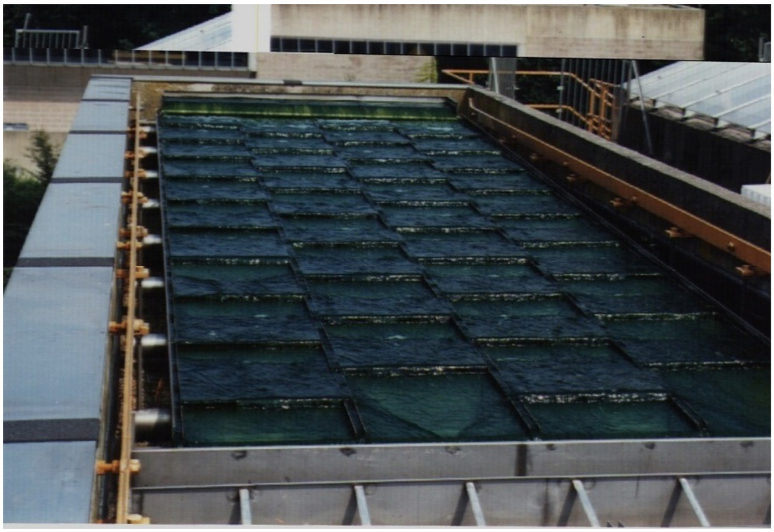
Source: Jim Sears, 2009

Carbon Capture Sequester & Recycle (CCS&R)
National Pipeline Grid Integrates Industries



Source: Jim Sears, 2009

Backup slides for Discussion

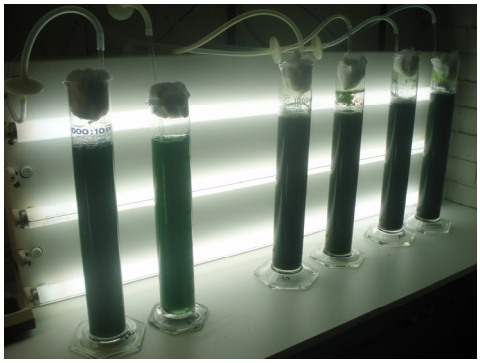


Outdoors culture of microalgae at Université de Liège (partner, BEMA project): a cascade system

Algal Cultures in Closed Photobioreactors

Cultures in polyethylene bags

Preculture



Open-air culture in Tunisia (INSTM): pilot-scale pond, A = 100 m^2 (V = 30 m^3)

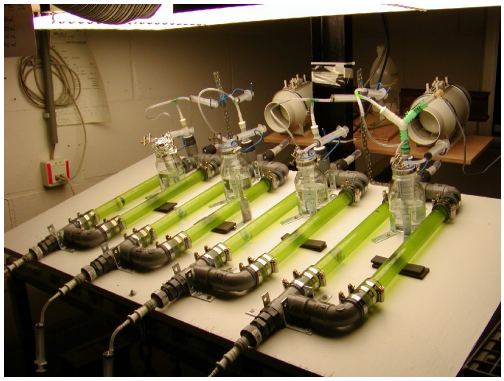
a: récolte

b: roue à aubes

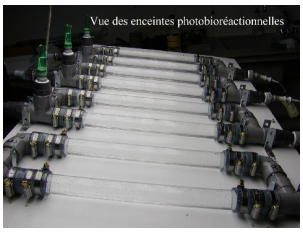
c: sèchage

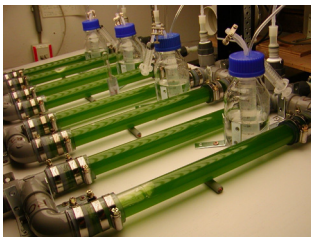
Using the photosynthetic apparatus of microalgae to produce biohydrogen

Trends in Plant Sciences
(2002) 7(6)



A new experimental photobioreactor





Adapted from Mignolet, M. and Franck, F. (2006)

Questions to ponder in the BEMA project

- **To what extent can we scale up to the industrial size industrielle the high yields obtained at laboratory scale under optimal conditions?**

- **Can we reach high enough yields (per hectare) in temperate climate regions? (energy investment, light limitation?)**

- **What is the photosynthetic efficiency of CO_2 capture by our microalgae ?**

- **How to optimize the exploitation of the algal biomass produced: lipids (extraction, processing) and residual biomass**

Production techniques of astaxanthin by *Haematococcus pluvialis*

A two-stage process

1. 'Green' stage: growing cells

Production of green biomass under optimal growth conditions (nutrient-sufficient conditions and low average irradiance)

2. 'Red' stage: non-growing cells

Haematocyst (aplanospore) formation and accumulation of astaxanthin induced by adverse environmental conditions (e.g., deprivation of nutrients, tempreature increase or salt addition, and high average irradiance)

A one-stage process

Continuous culture conditions allow substantial astaxanthin biosynthetic activity in actively growing *H. pluvialis* cells under

- careful control of nitrogen input (limiting nitrogen regime)
- a high irradiance

Prof. Emilio Molina-Grima (Almería)

Prof. Miguel G. Guerrero (Sevilla)

System conception and design

1. Sequential production system

- Green biomass growth
- Red pigment accumulation

Adjusting optimized conditions for each stage independently

2. Photoautotrophic induction is more effective for astaxanthin accumulation

3. Closed systems

- No selective environment available for *H. pluvialis*
- *H. pluvialis* cultures very sensitive to extreme environmental conditions (high and low temperature or light).