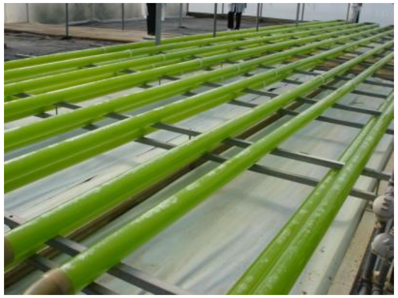


CELL CULTURE

- MICROALGAE PLATFORM

METABOLIC ENGINEERING OF LIPID PRODUCTION IN MICROALGAE

CONTENT TABLE:

Microalgae introduction

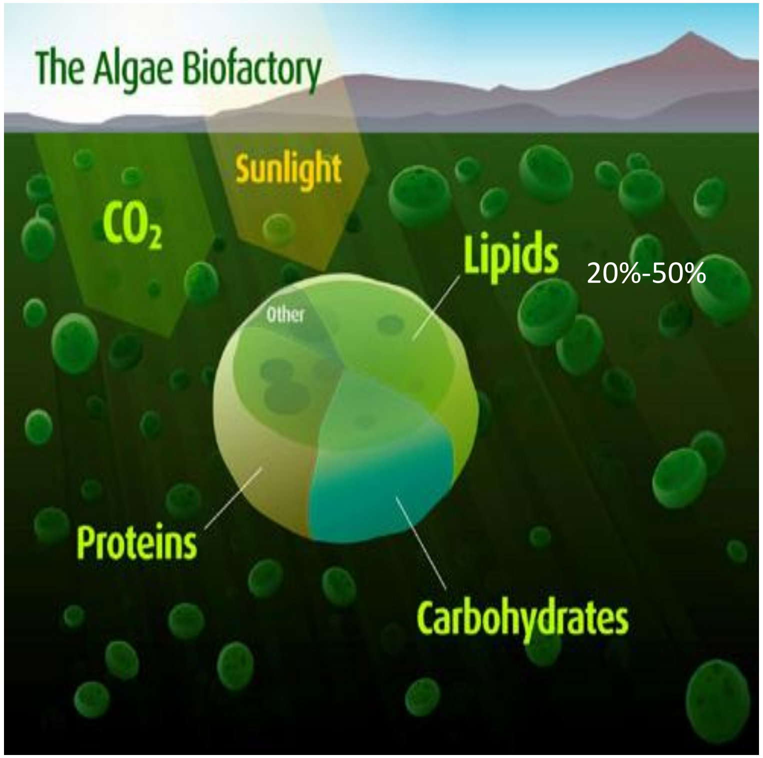
How do you know about microalgae?

Microalgae are unicellular species of differing sizes, shapes, colors and structures.

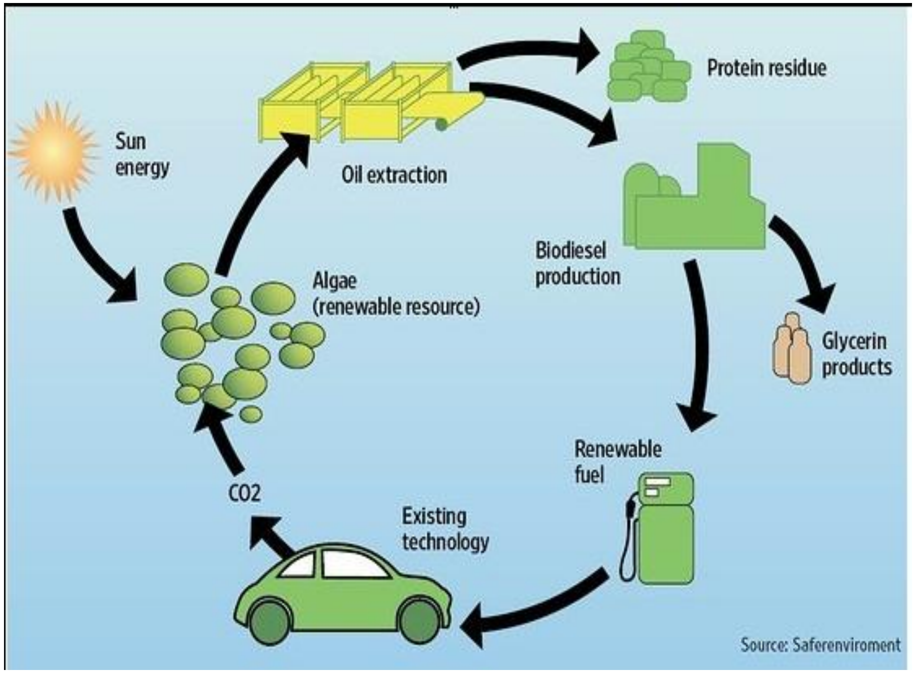
For more sources: http://www.rbgsyd.nsw.gov.au/science/Plant_Diversity_Research/australian_freshwater_algae/algpic/nonmotile_microalgae

Characters

They have the common property of photosynthesis that capture carbon dioxide and assimilate it as a part of their central metabolism.



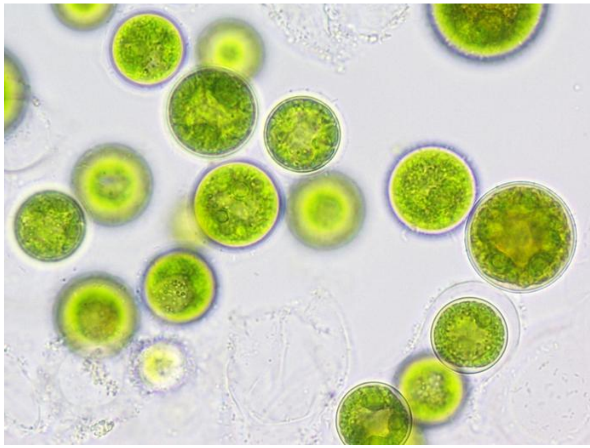
http://www2.hci.edu.sg/y11hci0149/website/algae.html

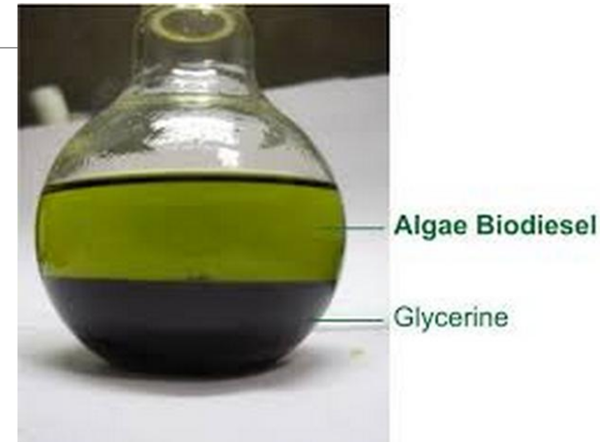


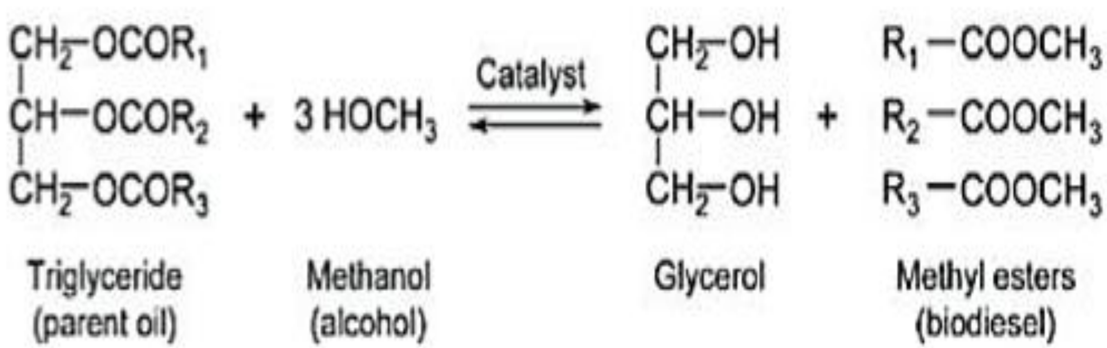
Recycling cell factory

http://theharborandthehudson.wordpress.com/2010/12/05/new-algae-biofuel-pilot-project-at-the-rockaway-wastewater-treatment-plant/

Microalgae products: biodiesel-from lipids to biodiesel







Comparison of lipid production

300L/hectare/year | **30000L/hectare/year**

Soybeans

http://northcountrypublicradio.net/news/npr/167558840/peak-farmland-some-researchers-say-it-s-here

Microalgae

http://greenglobalmalaysia.blogspot.ca/

100-folds

"Foster" destroyer

The now-decommissioned destroyer Paul F. Foster approaches the starboard side of a Military Sealift Command ship to take on fuel in October 2002. Foster will be part of the Navy's largest demonstration in alternative fuels when it takes to sea next week with a mixture of algal oil and diesel fuel. (Navy)

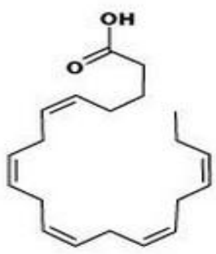
The injection of biofuels is a "algae fuel", a mixture of 50 percent of traditional petroleum products and 50 percent biofuel from algae oil

Cited from Navy times: http://www.navytimes.com/article/20111110/NEWS/111100338/Experimental-Navy-ship-set-alt-fuel-demo

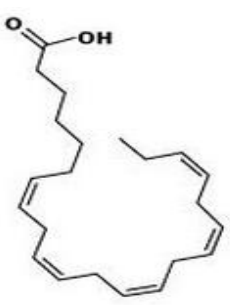
Microalgae products: nutritional lipids-long chain multi-unsaturated fatty acids: EPA DHA

Previously: fish oil was famous for its omega-3 fatty acid content, however fish don't actually produce omega-3s, instead accumulating their omega-3 reserves by consuming microalgae.

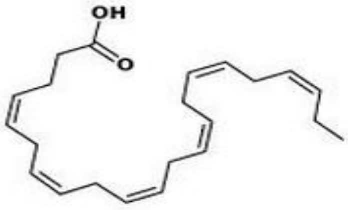
DHA and EPA: improving human cardiovascular health;
disease prevention;
cancer prevention and treatment;
physical and cognitive development of infants;
anti-aging;
Treatment of mental health disorders; lessening the impact of rheumatoid arthritis, and prevention of liver disease.



Eicosapentaenoic acid
EPA (20:5n-3)



Docosapentaenoic acid
DPA (22:5n-3)



Docosahexaenoic acid
DHA (22:6n-3)

Microalgae products: irons, proteins and other carbohydrates products

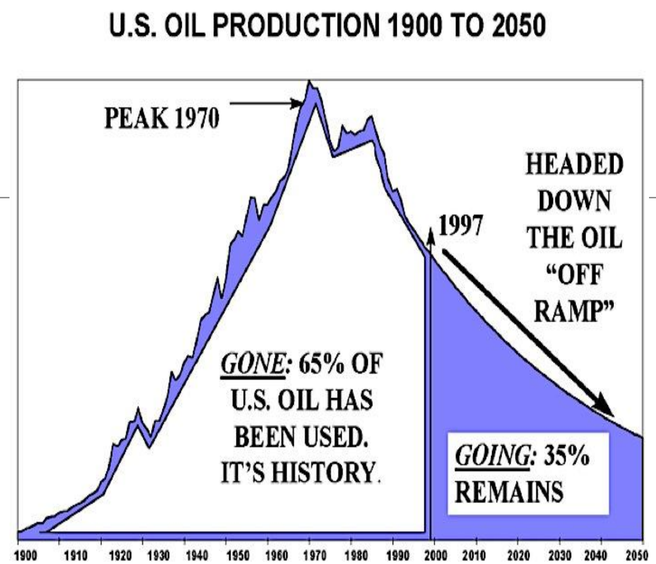
Look how packed Spirulina is with Nutrition:

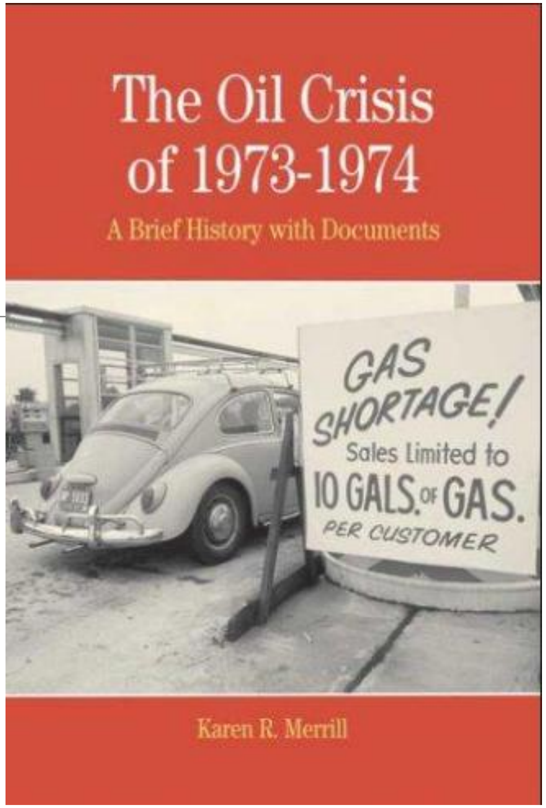
- *Spirulina has 2300% more iron than spinach*
- *Spirulina has 3900% more beta carotene than carrots*
- *Spirulina has 300% more calcium than whole milk*
- *Spirulina has 375% more protein than tofu*
- *Comparing phytonutrient levels, Spirulina is 31 times more potent than blueberries and 60 times more potent than spinach!*

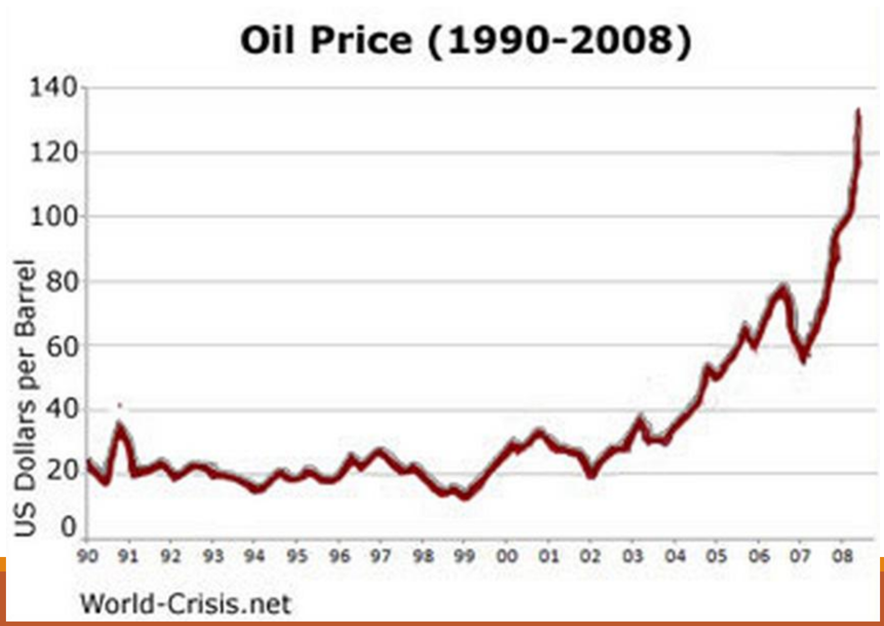
Research history

Oil crisis

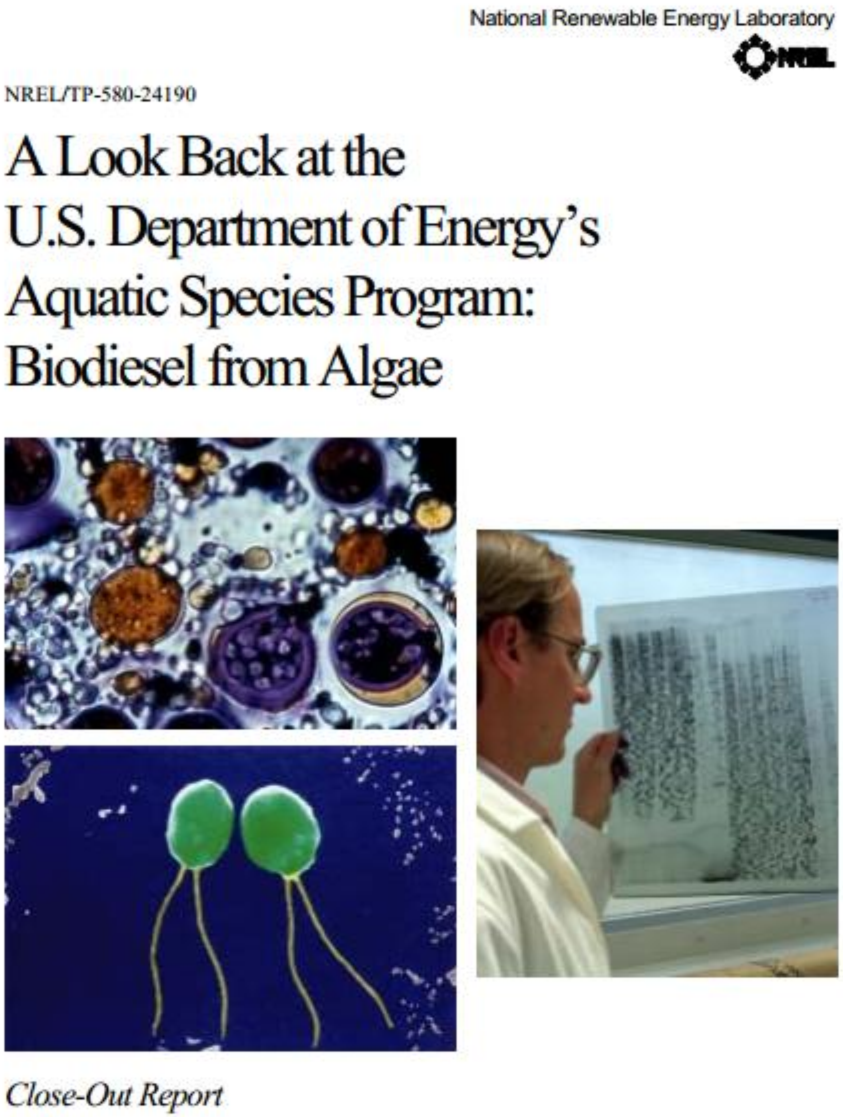
overexploitation







Algae biodiesel


National Renewable Energy Laboratory

NREL/TP-580-24190

A Look Back at the U.S. Department of Energy's Aquatic Species Program: Biodiesel from Algae

Close-Out Report

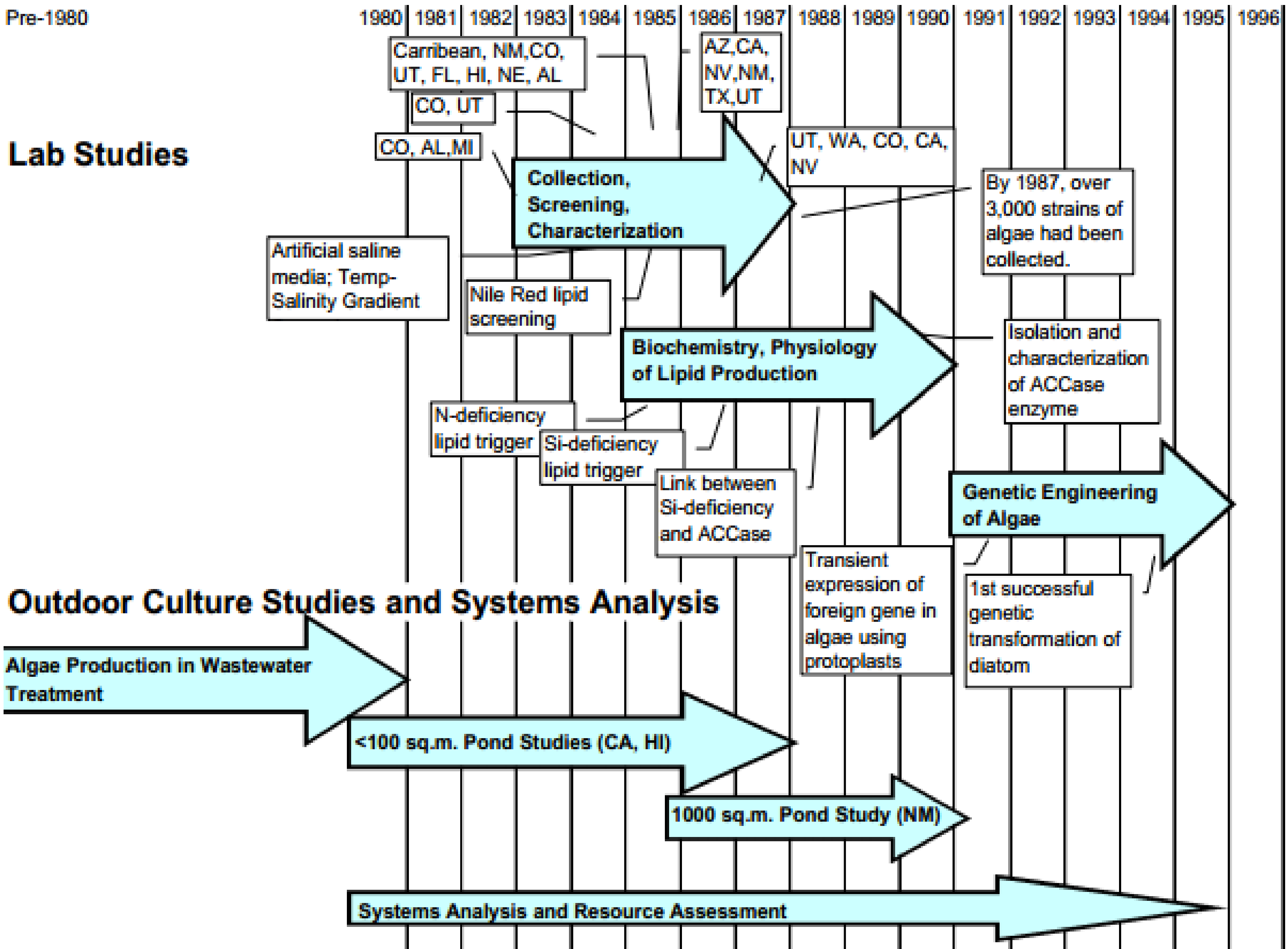
Global research wave

United States: "Aquatic Species Program (ASP)", "Mini-Manhattan Project"

Japan: "Microalgae diesel research program"

Companies: UK "Carbon Trust", U.S. "Solix Biofule" "Green Fuels technology", Canada "International energy" etc.

In 2007, **Canada International Energy, Inc.** announced that it has launched its "algae to oil" research and development initiatives.

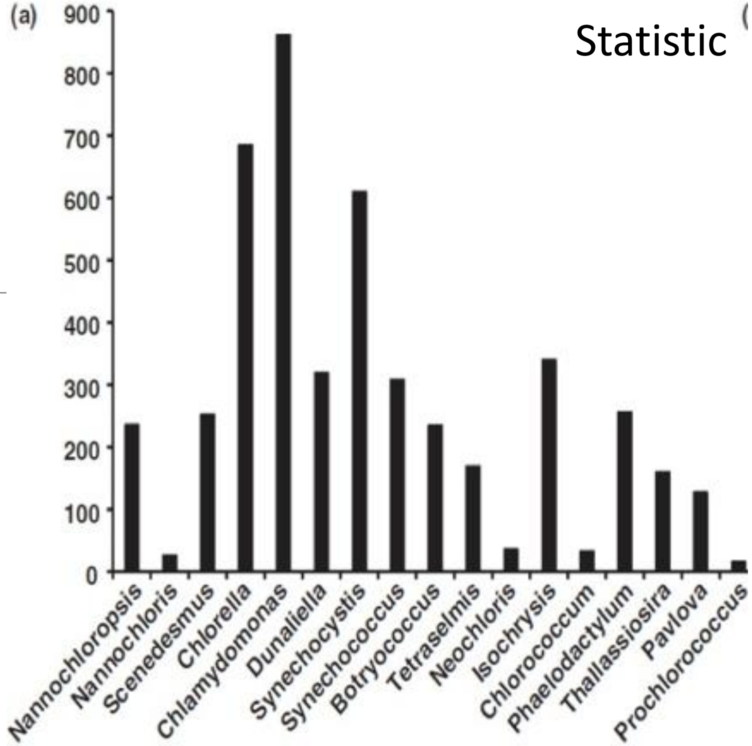


ASP final report, 1998

Oleaginous algae strains:

Lipid content of some microalgae species

Microalgae	lipid content (% dry wt)
Botryoccus braunii	25-75
Chlorella sp.	28-32
Chlorella protothecoides *(autotrophic/heterotrophic)*	15-55
Dunaliella. Tertiolecta	36-42
Nannochloropsis sp.	31-68
Nannochlosis sp.	15-32
Crypthecodinium cohnii	20
cylindrotheca sp.	16-37
Dunaliella primolecta	23
Neochloris oleoabundans	35-54
Nitzschia sp.	45-47
phaeodactylum tricornutum	20-30
Schizochytrium sp.	50-77
Tetraselmis sueica	15-23



Statistic (

Chlamydomonas reinhardtii has arisen as the hallmark, model organism. Although the lipid content is not high, it's the first algae species that finished genome sequencing and annotation (Merchant, Prochnik et al. 2007). *C. reinhardtii* has been widely used to study photosynthesis, cell motility and phototaxis, cell wall biogenesis, and other fundamental cellular processes (Harris 2001). These studies laid a foundation for researches in other species.

Cell culture

What kind of special culture nutrition and conditions do a “green cell” need?

Culture nutrition:

1. Macro elements : C, H, O, N, Si---Cell synthesis, energy source, cell wall structure

2. Minor elements: P, K, S, Mg----ATP , phosphorylation of proteins, nucleic acids ; cofactor of several enzymes , signal transfer; composed of several amino acids , nucleic acids and protein.

3. Vitamins and hormones, etc.

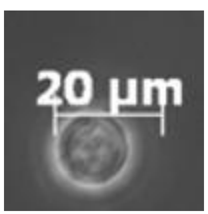
•Growth factors: organic nutrients incorporated into the cellular structure.

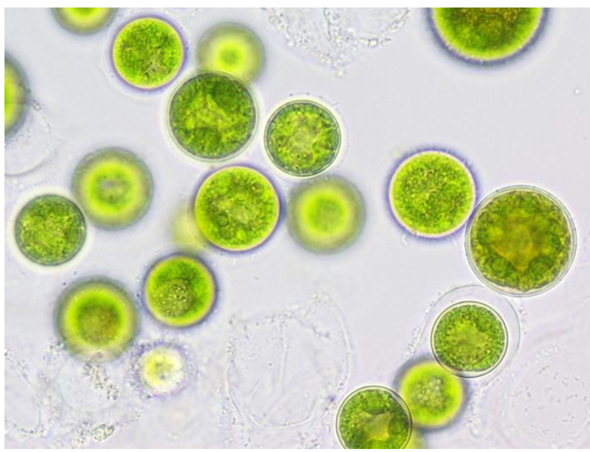
4. Trace elements: Fe, Zn, Cu, Mn, Co, Mo, B----enzyme cofactors

Culture medium

Chlorophyta:

Clorella protothecoides belongs to genus *Clorella*, it is a *chlorophyta*.
Cell size:
Culture medium: Modified basal Medium (MBM)





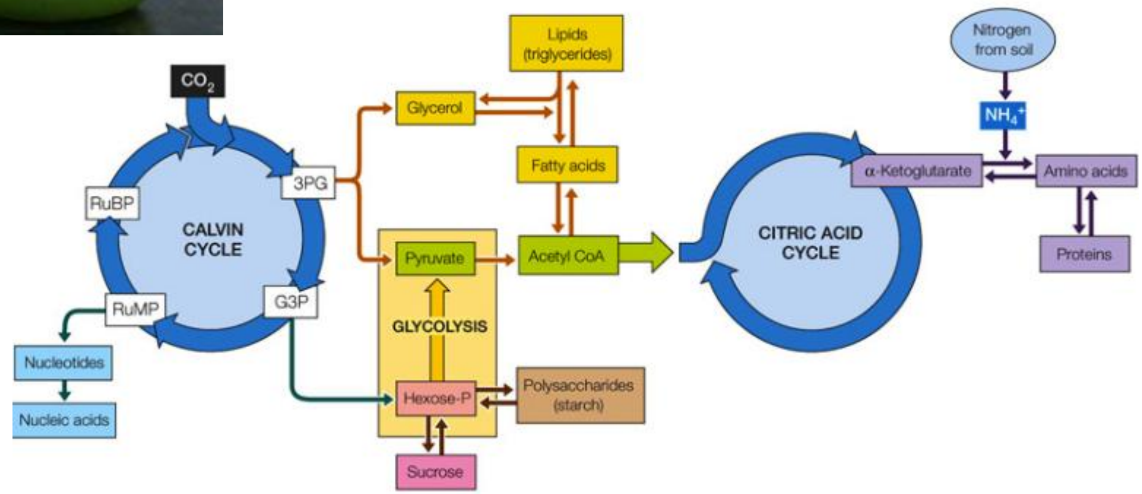
Components	Concentration	Utility
KH2PO4	0.7g/L	Maintain PH, provide PO4 for nucleotide synthesis.
K2HPO4	0.3g/L	
MgSO4.7H2O	0.3g/L	
FeSO4.7H2O	0.3mg/L	
Glycine	0.1g/L	Nitrogen source
Vitamine B1	0.01mg/L	Coenzyme and cofactors, regulate metabolism or energy transformation

A5 trace mineral solution components	Add to 100ml of distilled water
H3BO3	286mg
MnCl2.4H2O	181mg
ZnSO4.5H2O	22mg
CuSO4.5H2O	7.9mg
(NH4)6MoO24.4H2O	3.9mg

Carbon utilization

Chlorella protothecoides can use organic carbon source such as glucose, glycerol, starch *etc*. to live a heterotrophic life, the cell culture density and lipid content are all greatly increased.

Meanwhile, it can also use inorganic and organic carbon source simultaneously to live mixtrophy life.

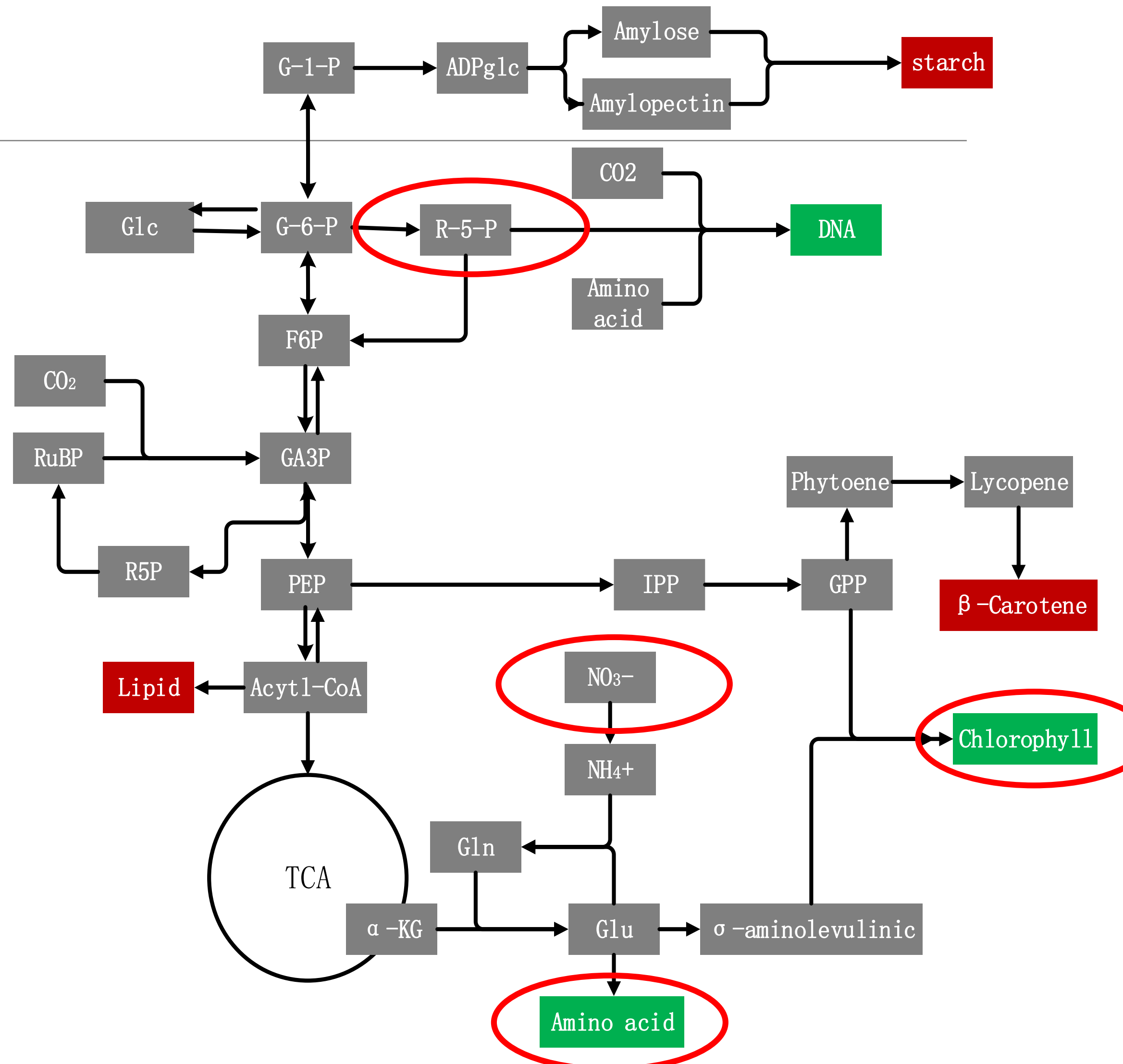


http://www.vi.cl/foro/topic/1071-apuntes-de-biologia-y-quimica/page-42

Nitrogen utilization

N is an essential nutrient factor for algae growth. It is the essential element formatting cell amino acids, purines, pyrimidines, amino sugar, and the amine compound. And especially in algae cell, it is also a essential component of chlorophyll.

In limited N condition, protein, nucleic acid synthesis slow down so cell division and growth are restricted, most assimilated carbon are led to produce storage and stress-resistant materials such as lipid, starch, beta-carotene.



Cell metabolism reprogramming in N limitation

Baeillariophyta :

Navicula pelliculosa belongs to genus *Navicula*, it is a diatom (*Baeillariophyta*). It's a freshwater alga

Size: 7.5-10um*4-5.5um

Culture Medium: CHU-10 medium

http://www.algae.md/Anketa.aspx?id=i355

CHU-10 (MODIFIED by J. Acreman, CPCC)

Reference: Stein, J (ED.) 1973. Handbook of Phycological methods. Culture methods and growth measurements. Cambridge University Press. 448 pp.

STOCK	STOCK SOLUTION	ml/Litre
1. $Na_2SiO_3.9H_2O$*	5.8 g/L	10 ml
2. $Ca(NO_3)_2.4H_2O$	57.56 g/L	1 ml
3. K_2HPO_4	10 g/L	1 ml
4. $MgSO_4.7H_2O$	25 g/L	1 ml
5. Na_2CO_3	20 g/L	1 ml
6. F/2 Vitamins	see page 2	1 ml
7. Na_2 EDTA. $2H_2O$	1.00 g/L	1ml
8. Trace metal mix	see below**	1 ml
9. Ferric citrate and	6.00 g/L	1 ml
Citric acid	6.00 g/L	
10. H_2SeO_3	0.163 g/L	0.5 ml

* Adjust pH of $Na_2SiO_3.9H_2O$ to neutral before adding to the media

If Ferric citrate and citric acid not available, substitute:

1. $FeCl_3.6H_2O$ (3.15 g/L) and $Na_2EDTA.2H_2O$ (4.36 g/L). Add 1ml/L to medium

**** Trace metal mix**

Substance	g/Litre
1. H_3BO_3	2.86 g
2. $MnCl_2.4H_2O$	1.81 g
3. $ZnSO_4.7H_2O$	0.222 g
4. Na_2 $MoO_4.2H_2O$	0.390 g
5. $CuSO_4.5H_2O$	0.079 g
6. $Co(NO_3)_2.6H_2O$	0.0494 g

Dissolve each of the above substances separately prior to adding the next.

Adjust pH of the final CHU-10 medium to 6.4 for diatoms and green algae or to 8.5 for cyanobacteria and *Cladophora* (for *Cladophora*, mix 50% of CHU-10 @ pH 8.5 with 50% filter-sterilized lake water).

Silicon utilization in diatoms

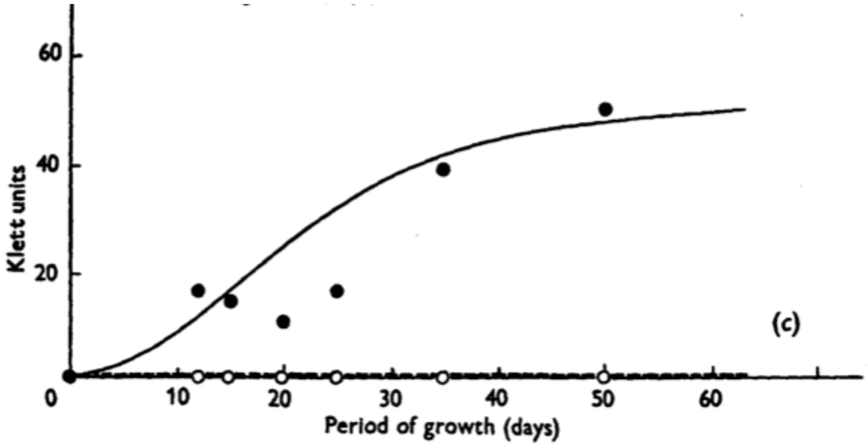
Silicon is major component of diatom cell walls. Similar to the lipid trigger effect produced by N deficiency, Si depletion also results in a decrease in cell growth and often is accompanied by an accumulation of lipid within the cells.

Silicon deficiency led to an increase in the lipid content of all strains (although in some cases the increase was small and probably not statistically significant). The mean lipid content of the eight strains increased from 12.2% in nutrient-sufficient cells to 23.4% in Si-deficient cells.

Carbon utilization

Table 2. *Carbon compounds which supported heterotrophic growth of* N. pelliculosa

	Autotrophy	Heterotrophy	
	Light ($+CO_2$)	Light ($-CO_2$)	Dark ($+CO_2$)
Control	+ +	−	−
Glucose	+ +	+	+ +
Glycerol	+ +	+	−
Fructose	+ +	+	−

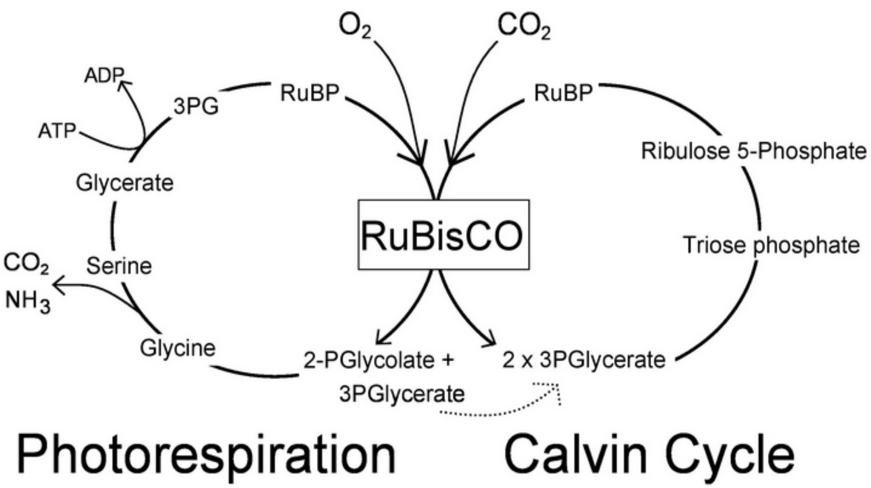


Although it can grow under heterotrophic conditions, the growth rate is quite low.

There isn't any mix trophic culture conditions for this species yet.

CO_2/O_2 ratio:

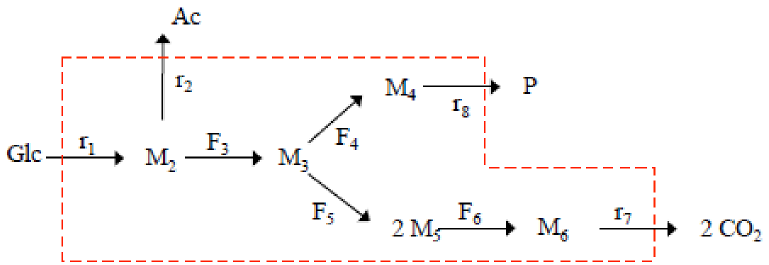
Photorespiration and Calvin cycle



http://en.wikipedia.org/wiki/Photorespiration

Using metabolic flux modeling with measured rates for each steady state we were able to quantify the ratio of the oxygenase reaction to the carboxylase reaction of Rubisco and the fluxes through the photorespiration pathway. This showed that the observed decrease in yield can be explained from an increase in oxygenase activity of Rubisco and the resulting process of photorespiration, up to 20.5% of the carboxylase activity. In conclusion, this study shows that elevated oxygen concentrations and the corresponding increase in the ratio of O2 versus CO2 common in photobioreactors leads to a reduction of the biomass yield on light of the microalgae *C. reinhardtii.*

Metabolic flux analysis



$$\frac{dM}{dt} = d\begin{bmatrix} M2 \\ M3 \\ M4 \\ M5 \\ M6 \end{bmatrix} / dt = Sv = \begin{bmatrix} 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & -1 \\ 0 & 0 & 0 & 0 & 2 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 \end{bmatrix} * \begin{bmatrix} r1 \\ r2 \\ F3 \\ F4 \\ F5 \\ F6 \\ r7 \\ r8 \end{bmatrix}$$

Flux intracellulaires versus concentrations intracellulaires

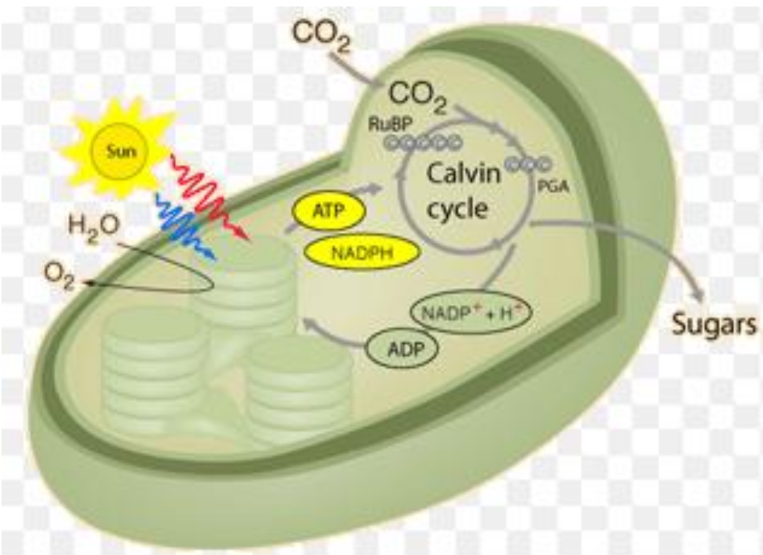
$$\frac{dM}{dt} = 0 \quad \Rightarrow \quad Sv = 0$$

Source from the course "Biochemical Engineering" given by professor Mario Jolicoeur

Light:

Light cycle: photoperiod

Light is essential for microalgae growth under autotrophic and mixtrophic conditions. Since light can active the enzyme of Rubisoco (carbon fixation) and relative enzymes to synthesis chlorophyll.



http://fhs-bio-wiki.pbworks.com/w/page/12145771/Factors%20effecting%20the%20rate%20of%20photosynthesis

Cell growth:

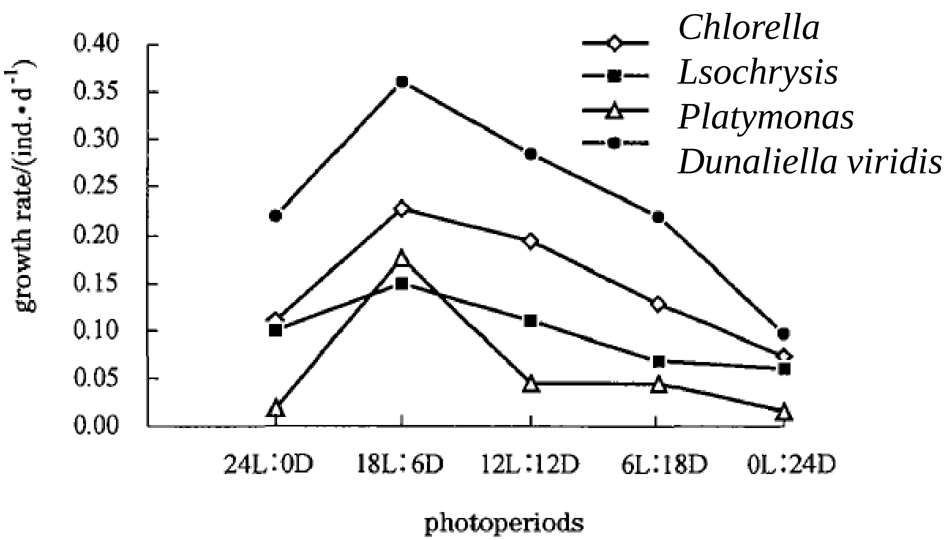


Fig. 1 The growth rate of four unicellular alga species under different photoperiods

Liu Qing, 2006

Light cycle: Chlorophyll synthesis under different photoperiod

Tab. 1 The chlorophyll content in unicellular algae under different photoperiods

Microalgae species	Photo period	Chlorophyll content in unit volume /($\mu g \cdot L^{-1}$)				Chlorophyll content per cell /($10^{-6} \mu g \cdot ind.^{-1}$)			
		Chlorophyll a	Chlorophyll b(c)	Total amount	a/b(c)	Chlorophyll a	Chlorophyll b(c)	Total amount	a/b(c)
Chlorella	24L: 0D	217. 01	92. 10	309. 11	2. 36	10. 83	4. 60	15. 43	2. 35
	18L: 6D	297. 15	114. 62	411. 77	2. 59	14. 31	5. 52	19. 83	2. 59
	12L: 12D	219. 78	101. 80	321. 58	2. 16	11. 46	5. 31	16. 77	2. 16
	6L: 18D	168. 24	84. 18	252. 42	2. 01	26. 58	13. 30	39. 88	2. 01
	0L: 24D	108. 80	54. 04	162. 84	2. 01	24. 39	12. 12	36. 51	2. 01
Isochrysis zhanjiangensis	24L: 0D	377. 35	61. 11	438. 46	6. 17	8. 62	2. 47	11. 09	3. 49
	18L: 6D	733. 70	178. 91	912. 61	4. 10	10. 70	2. 62	13. 32	4. 08
	12L: 12D	682. 26	178. 00	860. 26	3. 83	15. 16	3. 96	19. 12	3. 83
	6L: 18D	345. 06	57. 38	402. 44	6. 01	9. 86	2. 64	12. 50	3. 74
	0L: 24D	327. 86	54. 40	382. 26	6. 03	10. 40	2. 72	13. 12	3. 82
Platymonas helgolandica	24L: 0D	165. 52	76. 60	242. 12	2. 16				
	18L: 6D	346. 74	155. 20	501. 94	2. 23				
	12L: 12D	236. 30	107. 43	343. 73	2. 20				
	6L: 18D	181. 46	66. 47	247. 93	2. 73				
	0L: 24D	120. 44	43. 99	164. 98	2. 74				
Dunaliella viridis	24L: 0D	870. 01	377. 03	1247. 04	2. 31				
	18L: 6D	1008. 28	144. 26	1452. 54	2. 27				
	12L: 12D	971. 44	385. 49	1356. 93	2. 52				
	6L: 18D	874. 93	348. 58	1223. 51	2. 51				
	0L: 24D	686. 01	254. 50	940. 51	2. 70				

Liu Qing, 2006

Light intensity:

limitation of light for high cell densities is critical for growth and lipid production Wen Zhiyou et al. (Wen Zhiyou 2003), but excessive light can also cause “photoinhibition” (Jack Myers 1940).

Tab. 2 The chlorophyll content in unicellular algae under different light intensities

Microalgae species	Photo intensity (lx)	Chlorophyll content in unit volume (μg · L^{-1})				Chlorophyll content per cell /(10^{-6} μg · $ind.^{-1}$)			
		Chlorophyll a	Chlorophyll (c)	Total amount	a/b(c)	Chlorophyll a	Chlorophyll b(c)	Total amount	a/b(c)
Chlorella	500	66. 59	27. 86	94. 45	2. 39	9. 57	4. 00	13. 57	2. 39
	1 000	72. 32	32. 14	104. 46	2. 25	9. 68	4. 30	13. 98	2. 25
	3 000	161. 88	57. 61	219. 49	2. 81	14. 98	5. 33	20. 31	2. 81
	5 000	243. 15	100. 06	343. 21	2. 43				
	10 000	110. 41	43. 81	154. 22	2. 52				
Isochrysis zhanjiangensis	500	108. 62	41. 76	150. 38	2. 60				
	1 000	127. 98	50. 56	178. 54	2. 53				
	3 000	177. 47	71. 22	248. 68	2. 49				
	5 000	201. 55	81. 09	282. 64	2. 48				
	10 000	115. 25	49. 53	164. 78	2. 33				
Platymonas helgolandica	500	100. 54	44. 68	145. 22	2. 25				
	1 000	121. 12	52. 02	173. 14	2. 33				
	3 000	181. 56	89. 49	271. 05	2. 03				
	5 000	234. 12	107. 01	341. 13	2. 19				
	10 000	112. 33	46. 23	158. 56	2. 43				
Dunaliella viridis	500	122. 23	51. 36	173. 59	2. 38				
	1 000	286. 75	118. 98	405. 73	2. 41				
	3 000	442. 46	165. 81	608. 27	2. 67				
	5 000	586. 39	216. 38	802. 77	2. 71				
	10 000	147. 56	57. 87	205. 43	2. 55				

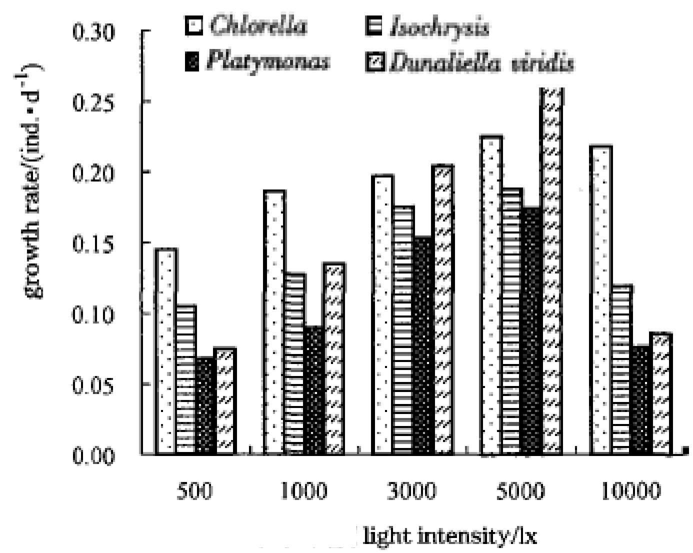
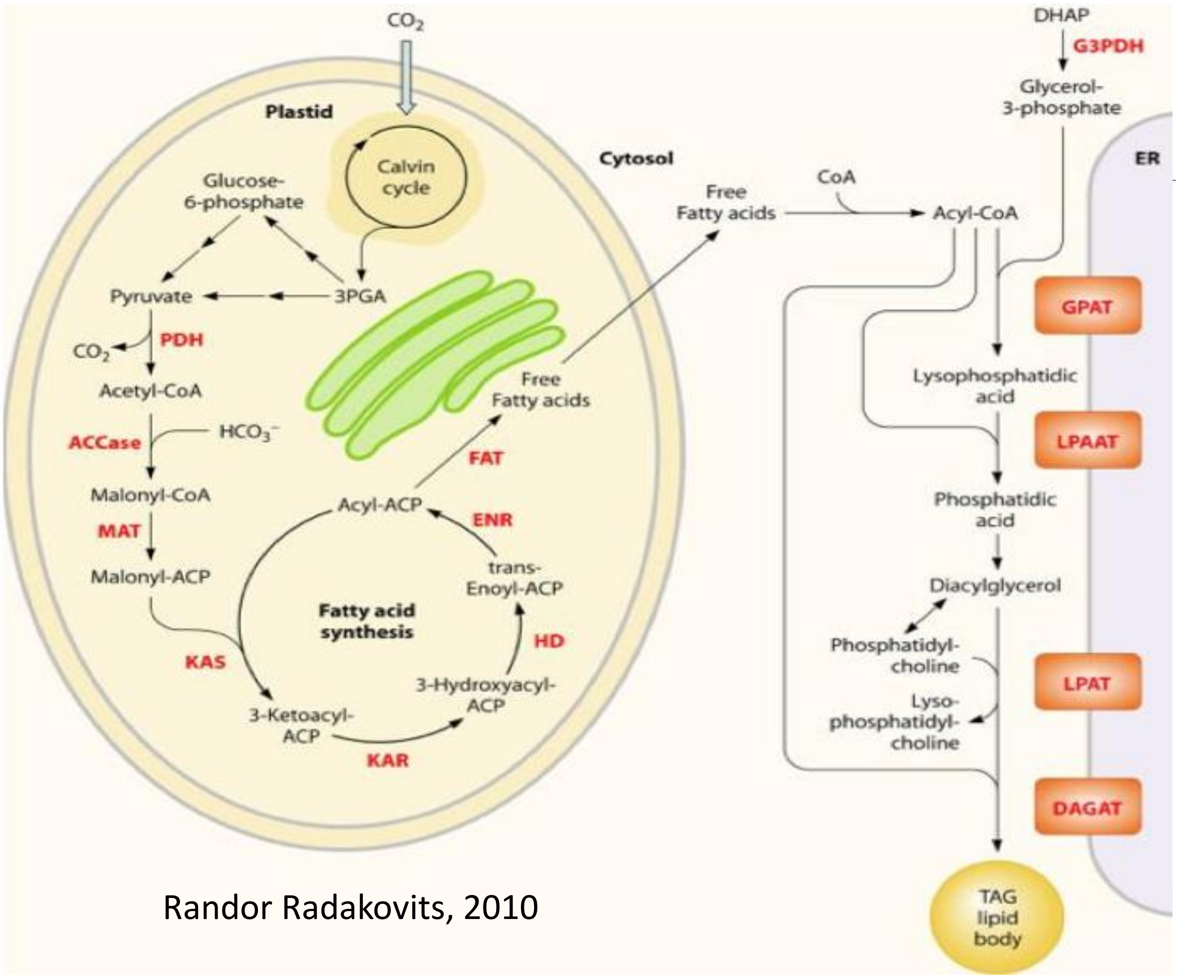


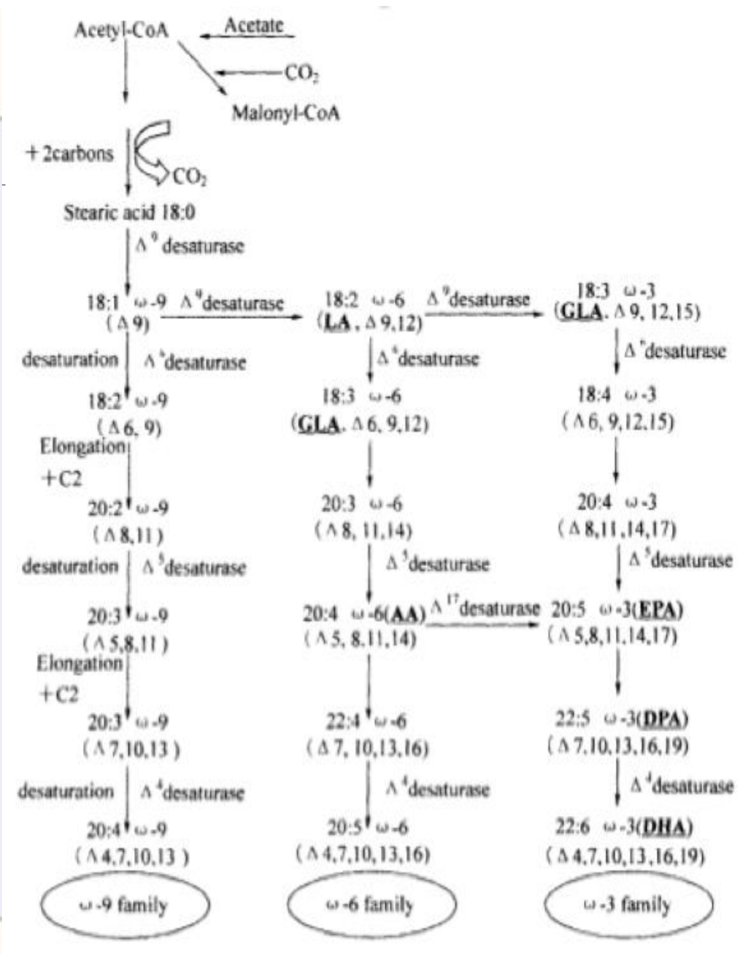
Fig. 2 The growth rate of four unicellular alga species under different light intensities

Lipid production in microalgae

lipid metabolism



Randor Radakovits, 2010

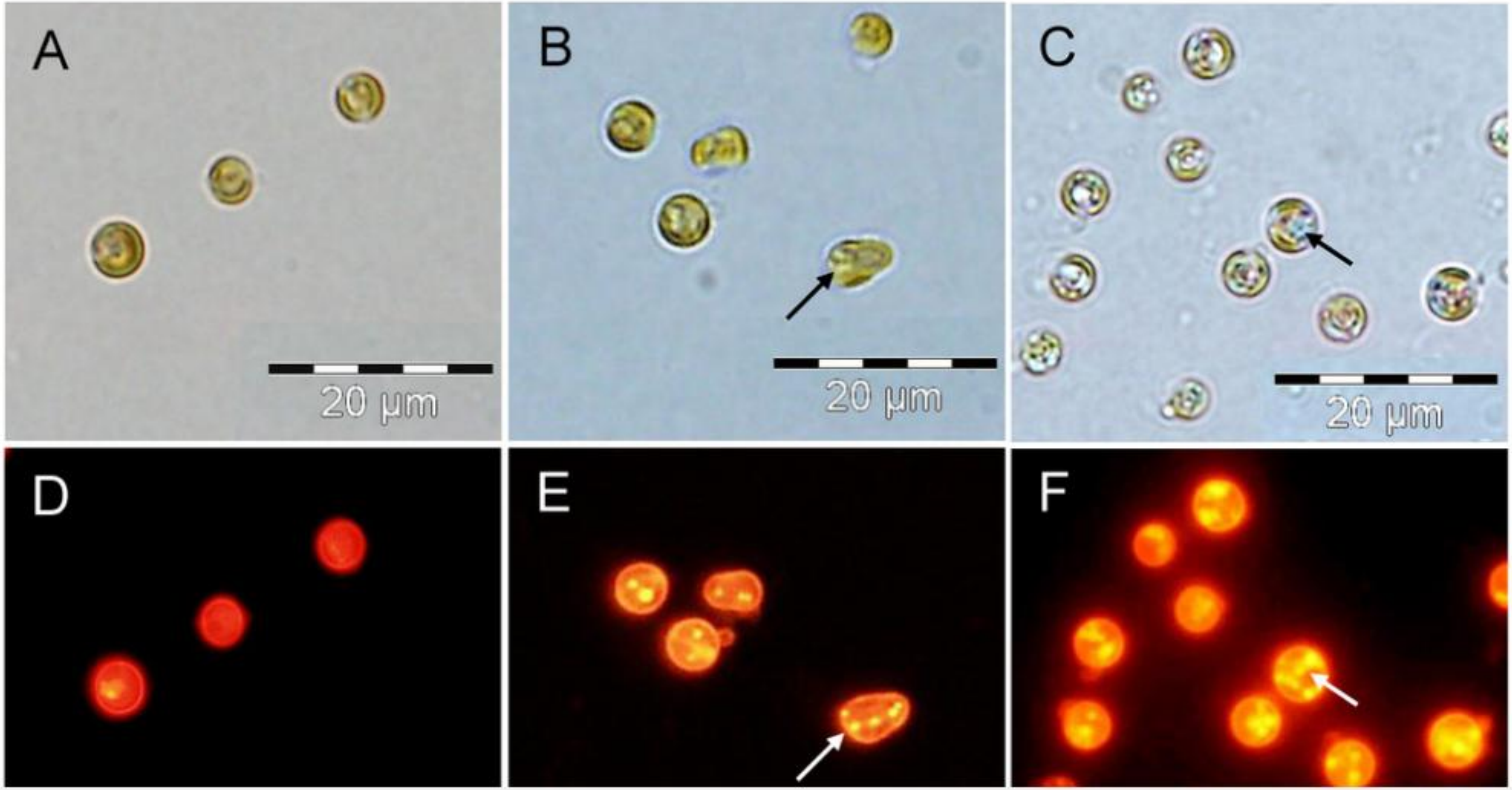


ACCase, acetyl-CoA carboxylase; ACP, acyl carrier protein; CoA, coenzyme A; DAGAT, diacylglycerol acyltransferase; DHAP, dihydroxyacetone phosphate; ENR, enoyl-ACP reductase; FAT, fatty acyl-ACP thioesterase; G3PDH, gycerol-3-phosphate dehydrogenase; GPAT, glycerol-3-phosphate acyltransferase; HD, 3-hydroxyacyl- ACP dehydratase; KAR, 3-ketoacyl-ACP reductase; KAS, 3-ketoacyl-ACP synthase; LPAAT, lyso-phosphatidic acid acyltransferase; LPAT, lyso-phosphatidylcholine acyltransferase; MAT, malonyl-CoA:ACP transacylase; PDH, pyruvate dehydrogenase complex; TAG, triacylglycerols.

Lipid observation: Nile red fluorescent

Freddy Guihéneuf, 2013

Figure 4. Oil body/droplet formation in nitrate-depleted cells of *P. lutheri* batch-cultivated in the presence of different initial bicarbonate concentrations. Neutral lipid accumulation in lipid bodies was visualized in algal cells with the fluorescent dye Nile Red. Cells grown in the presence of 2, 9, and 18 mM bicarbonate (**A–D**, **B–E** and **C–F**, respectively). The arrows indicate lipid bodies.



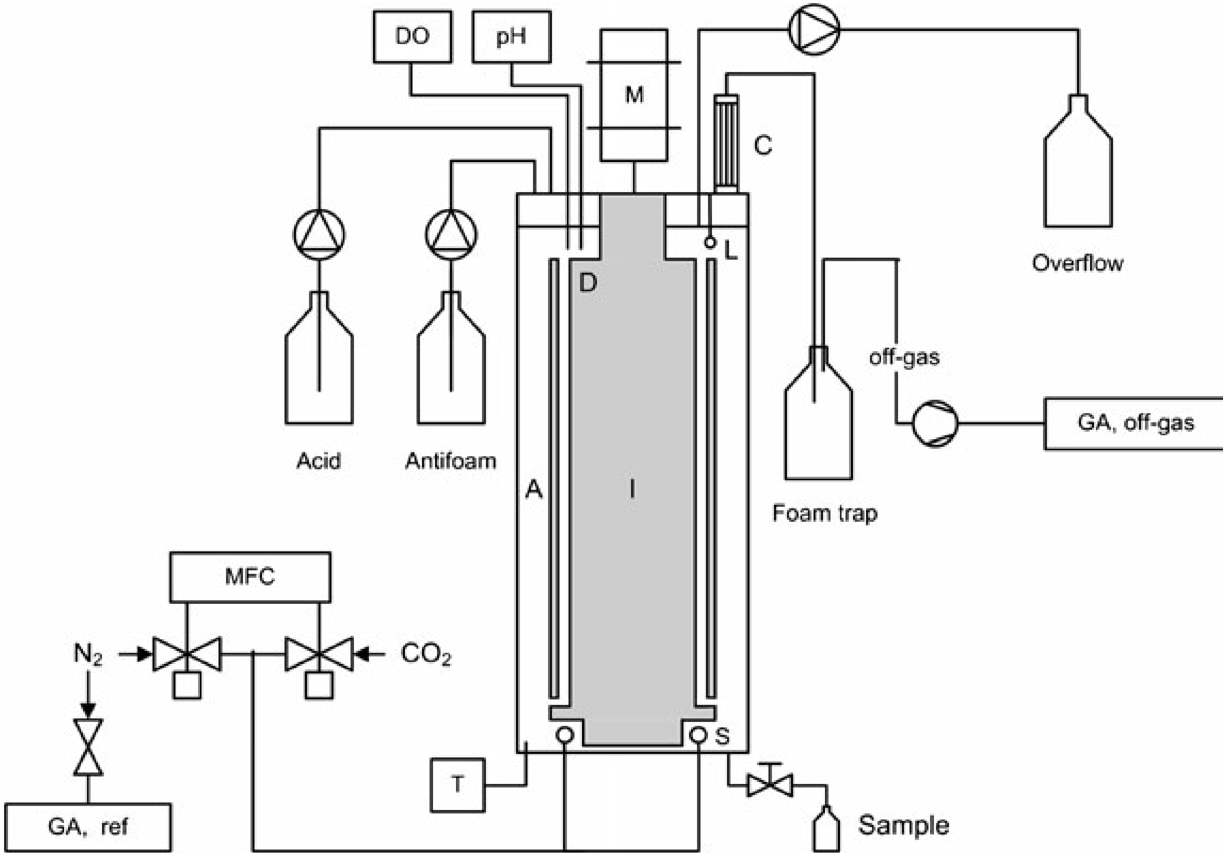
According to the pre-scan of excitation and emission characteristics of neutral lipid standards, the excitation and emission wavelengths of 530 nm and 575 nm were selected.

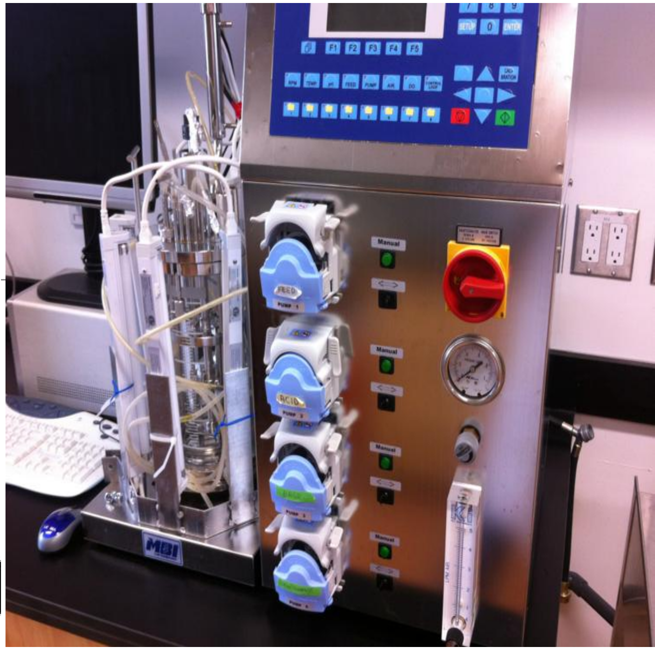
Culture process

Flask culture in photo incubator:

http://www.psi.cz/products/growth-chambers-and-incubated-shakers/

Photo-bioreactor





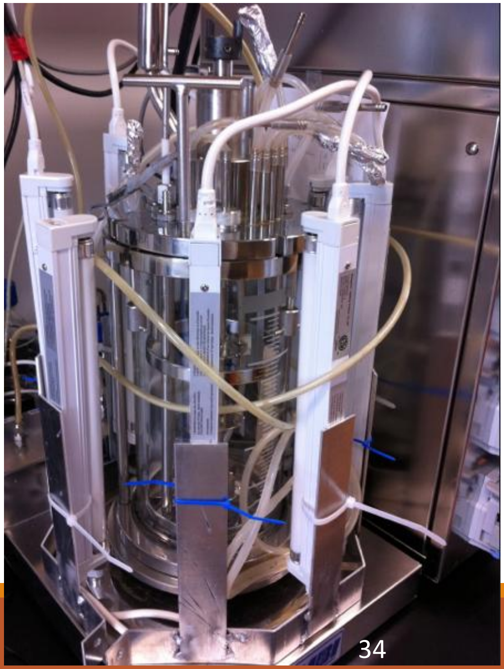


Fig. 2 Schematic overview of the photobioreactor. A: annular gap, C: condenser, D: internal down comer, DO dissolved oxygen sensor, GA gas analyzer, I inner cylinder, L spherical light sensor, M motor, MFC mass flow controllers for both N2 and CO2, pH pH control connected to acid pump, S sparger, T temperature control connected to cryostat and cooling jacket (not shown)

Laboratory scale:

what should we consider for choosing a photo bioreactor?

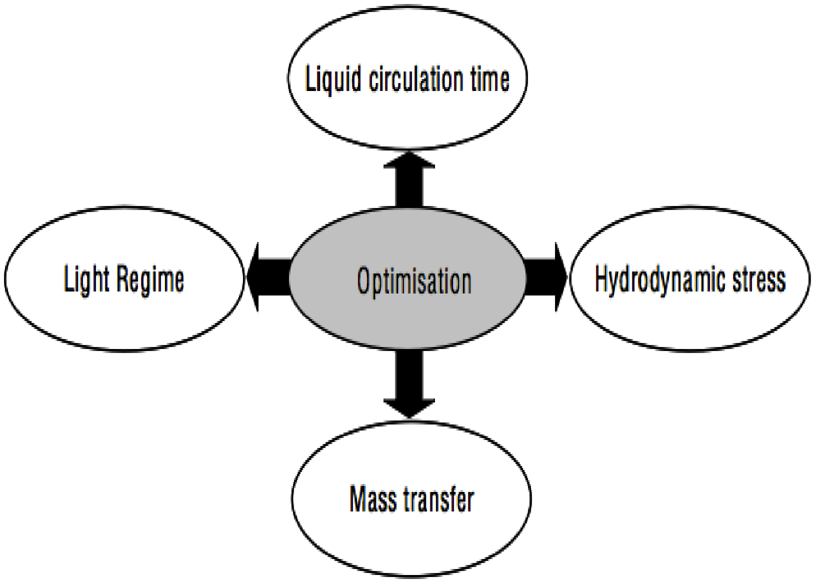


Figure 1.1. Scale-up parameters of photobioreactors.

The most critical scale-up and operational parameters:

Light penetrating

Mass transfer, mixing problem

O_2 inhibition problem

- Homogeneity of environmental conditions ,
- Meet the CO2 demand of the cells remove the oxygen produced

Shear force

These parameters are closely interrelated and they determine the productivity and efficiency of the system.

http://www.labs.chem-eng.utoronto.ca/allen/about/research-equipment/

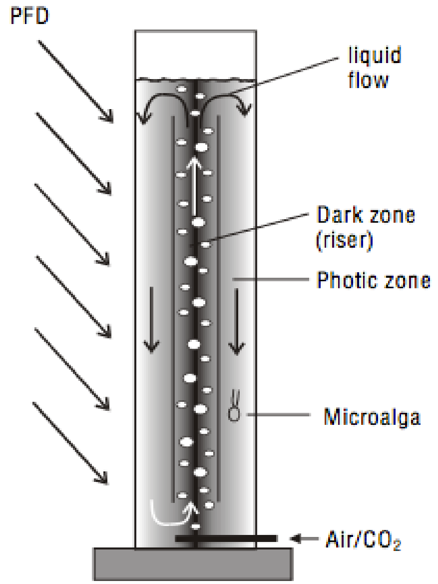


Figure 2.1. Air-lift loop reactor: liquid flow, light regime and resulting light gradient/dark cycles. PFD is the photon flux density.

http://www.kaznu.kz/en/4867

http://bbi-biotech.com/en/produkte/fermenter-bioreaktoren/algenbioreaktor-xcubio-pbr/

Air-lift bioreactor

Advantages:

Excellent transfer of oxygen and other gases

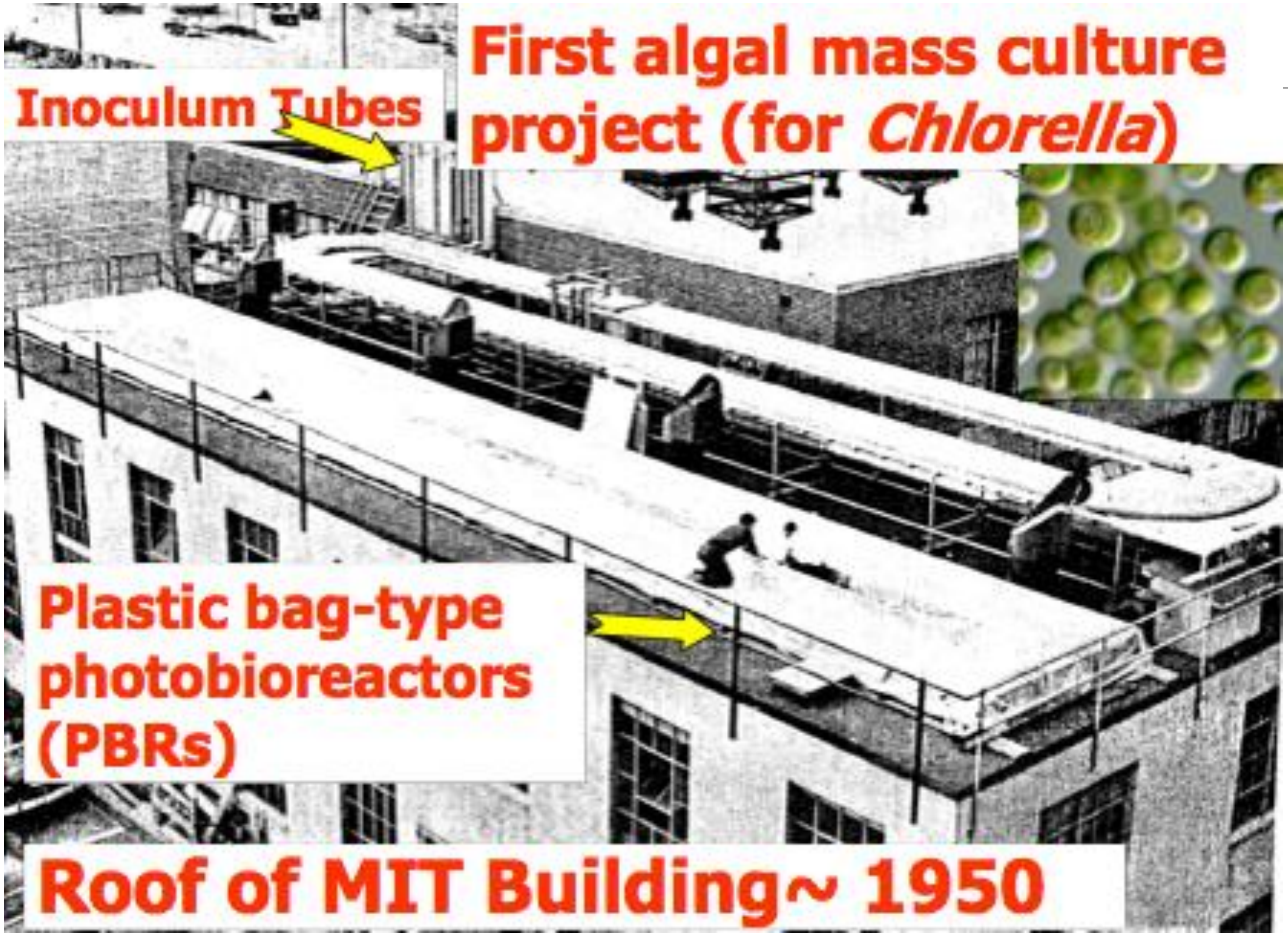
Mechanical simplicity of the bioreactor

May have an immobilized biomass (fluidized)-homogeneous

Air-lift bioreactor:
Oxygen transfer efficiency (kLa):

kLa = 0,32 $* \mathrm{U}_{sg}{}^{0.7}$ (s-1)
U_{sg}: the section surface of photobioreactor.

Pilot scale



John R. Benemann, 2011

Commercial scale

Raceway paddle wheel mixed high rate open ponds now the main (>99%) commercial production systems for microalgae

My project introduction

Problem identification

Biomass and lipid synthesis are in conflict: increasing lipid production always pay a cost of lower cellular growth. So the total lipid yield is seriously constrained, which becomes the main bottleneck in the research.

Lack of knowledge on algae cellular metabolism: Intracellular metabolism plays fundamental role in culture's behaviour, but there is still no relative comprehensive study in *Chlorella protothecoides*. So we have no detailed understanding of how the nutrition distributed in the metabolic processes, how does the related pathways competing with each other.

Lipid content was greatly different along with algae growth stages, culture conditions and nutritional states, which make the productivity quite unstable. **No dynamic tool to guide the metabolic process control.**

Objectives

Metabolic engineering for lipid improvement: establish a comprehensive dynamic metabolic model, to guide the metabolic process control.

1. Study metabolism characterization of *Chlorella protothecoides,* reconstruct cell metabolism network.

2. Establish the dynamic model, which can cover most metabolic pathways, key intracellular metabolites to describe cell dynamic behaviors. Introduce the regulation of energies and intermediates on the metabolic pathway.

3. Develop a cell engineering strategy based on the model prediction and guide our controlling process: model could help at defining optimal culture conditions and genetic engineering strategy.

Nutrient consumption can be predicted from the model

Production of fatty acids is regulated by acetyl CoA carboxylase (ACCase) (Post-Beittenmiller and Roughan 1992, Bao and Ohlrogge 1999).
Cyclotella cryptica ----(ACCase was overexpressed 2-3 folds) -----*C. cryptica* and *Navicula saprophila*

Fatty acid synthase has been suggested to be another rate-limiting regulator of lipid production and several studies have been performed where a single enzyme of the FAS complex is overexpressed (Roesler and Shintani 1997).

Overexpress of a certain key enzyme might not be sufficient to enhance the whole lipid synthesis. It tells us the interplay and metabolic balance of every metabolites should be considered as a whole. And preferable but balanced control of metabolic flux is essential to improve lipid synthesis. Metabolic modeling that can simulate flux of fatty acids through TAG biosynthetic pathways and intracellular metabolic networks should play an important part in developing strategies for future genetic manipulation

Methodology

Strain: *Chlorella protothecoides*

Culture conditions:

- Temperature: 28℃
- Basic medium: MBM; 1% Glucose in mixtrophic and heterotrophic culture
- Other condition: 5% CO_2, and 8-W fluorescent light are provided for autotrophic and mixtrophic culture

A M H

Autotrophy: A

Mixtrophy: M

Heterotrophy: H

Analysis methods:

1. Glucose, lactate, glutamine, glutamate analysis

Samples were automatically detected by YIS 2700 select, biochemistry analyzer

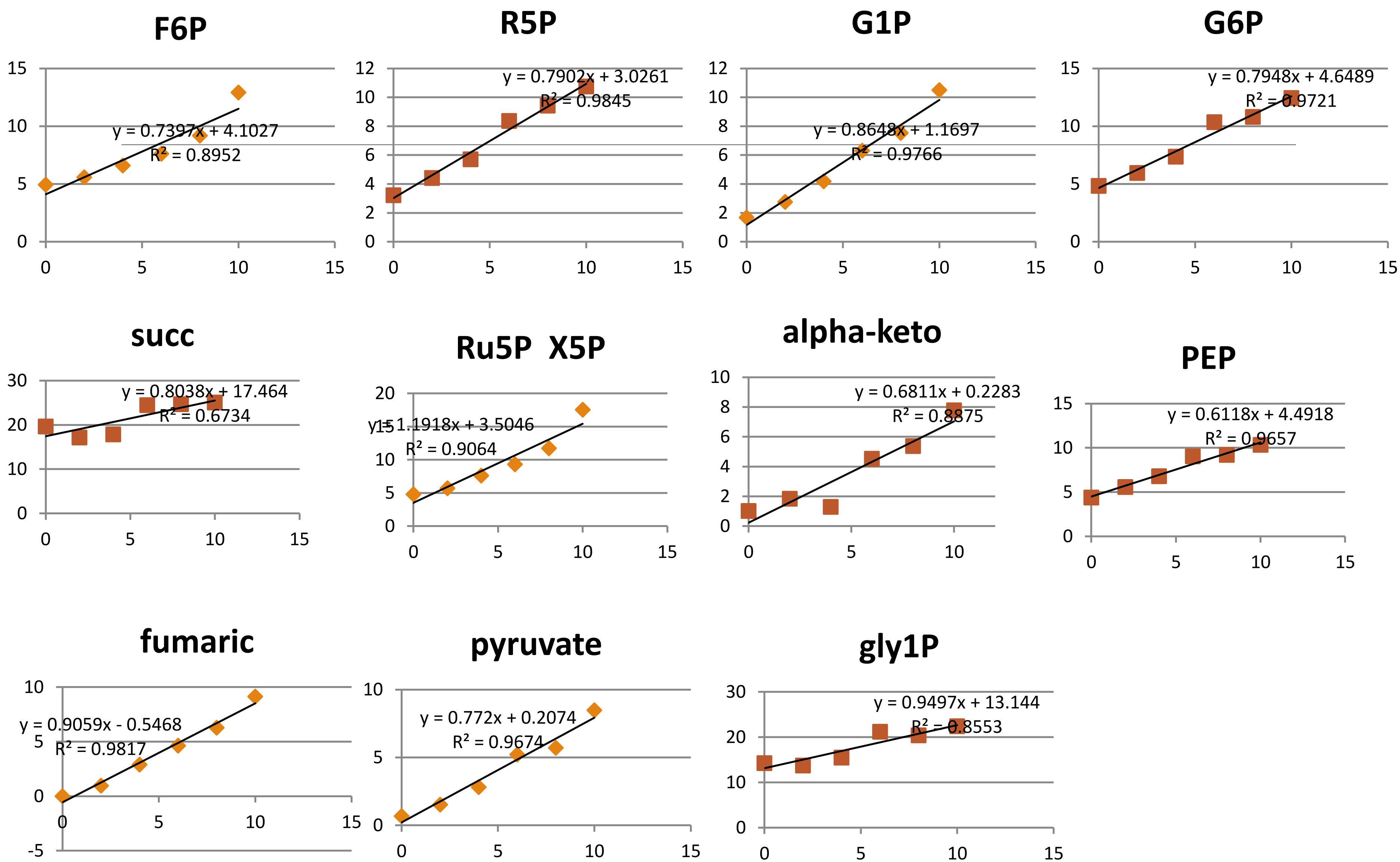
2. Lipid extraction and analysis: Acetone and sulfosalicylic acid; absorbance was read at 440nm by UV-VIS determination

3. Other intracellular metabolites: Methanol extraction and analyzed on HPLC-MS-MS

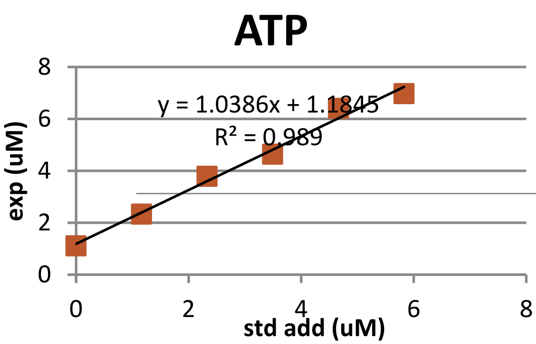
Glycolysis, photosynthesis, PPP, TCA: Sugar phosphate and organic acid: F6P R5P G1P G6P Succ Ru5P X5P alpha-keto PEP fumarate pyruvate Gly-1-P

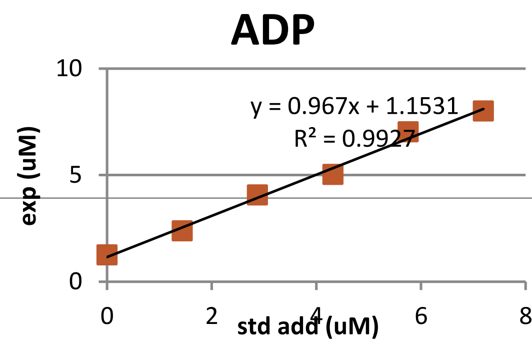
Oxidative phosphorylation, Energy flow: Redox+nucleartide: ATP ADP AMP UTP UDPG GTP NADPH

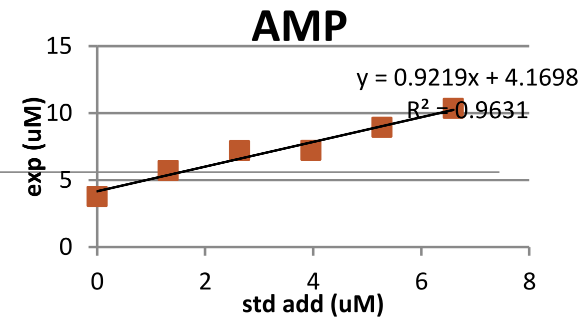
Sugar phosphate and organic acids: Glycolysis, photosynthesis, PPP, TCA

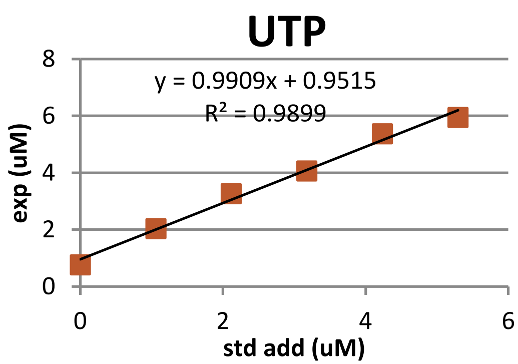


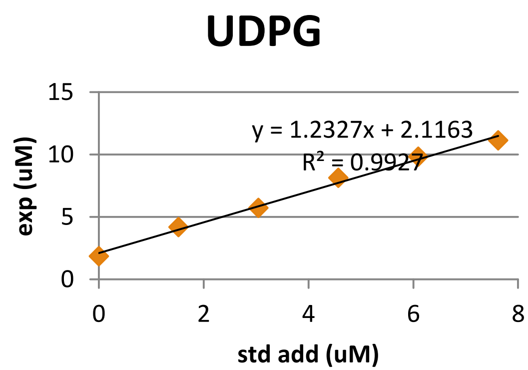
Energy state, Redox nucleotide: Oxidative phosphorylation, Energy flow

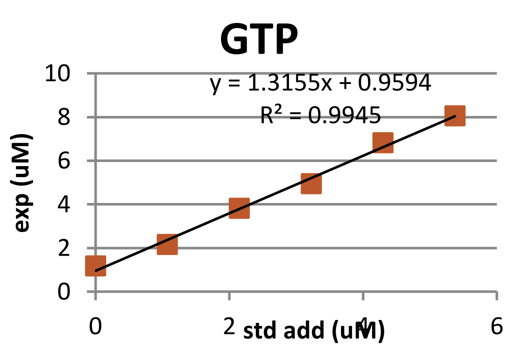


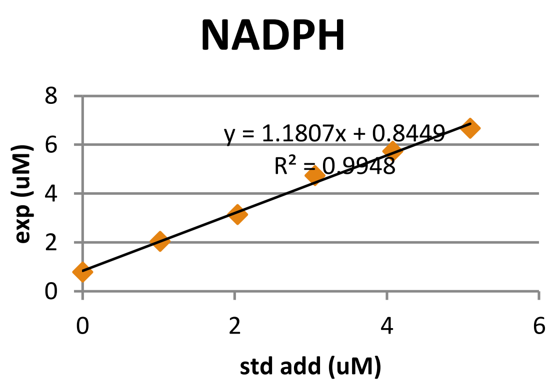












Preliminary results

Chapter 1: Culture characterization

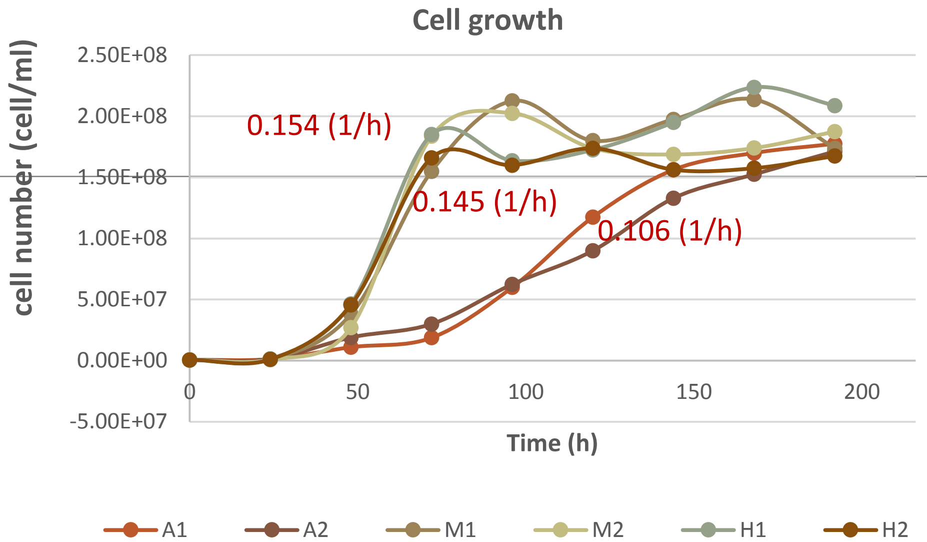
Culture behavior

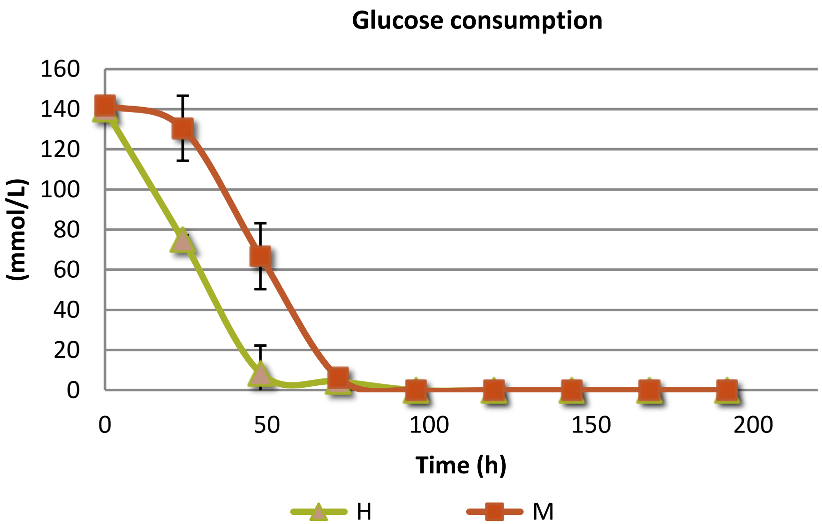
Biomass:

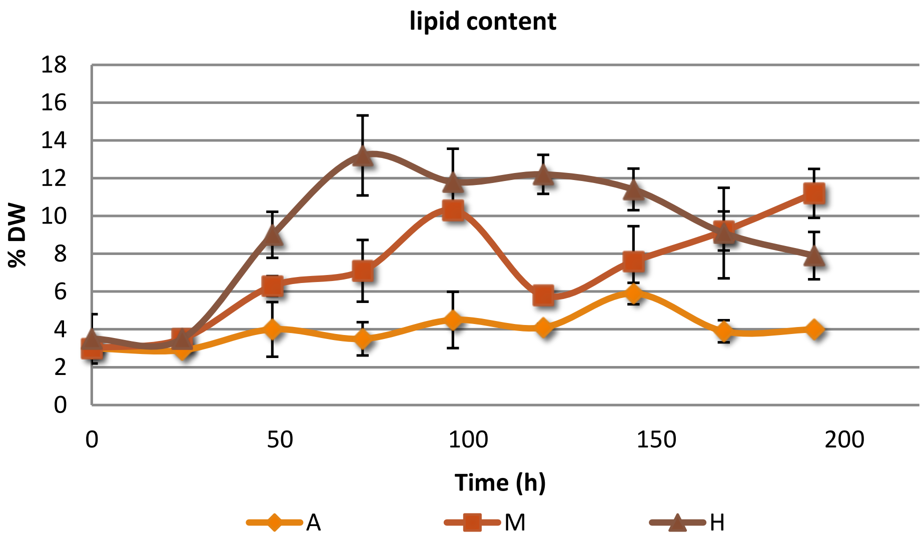
H: 10.32gDW/L

M: 9.54gDW/L

A: 5.04gDW/L



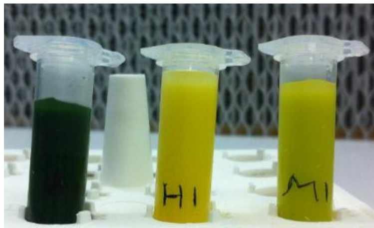




Chlorophyll synthesis

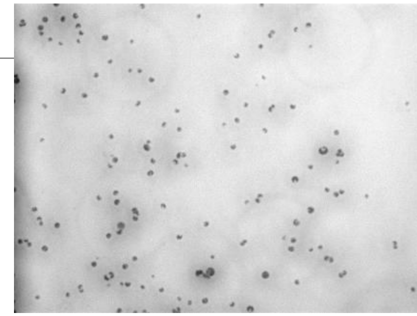
White light-normal cells

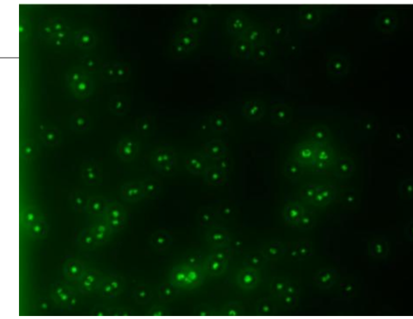
Chlorophyll-fluorescent-365nm excitation



48h

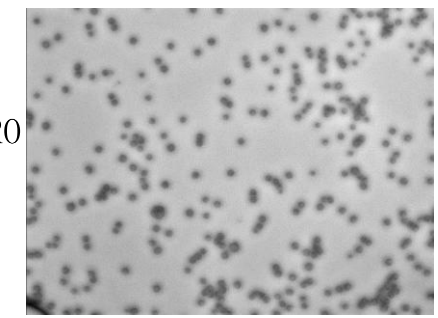
AUTO

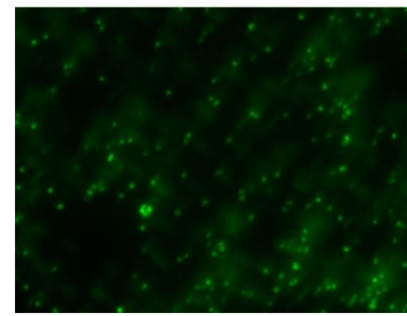


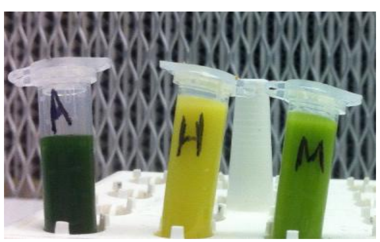


120h

MIXTRO

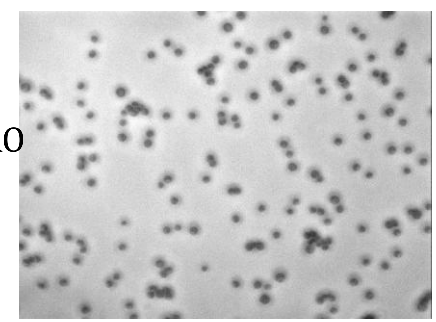


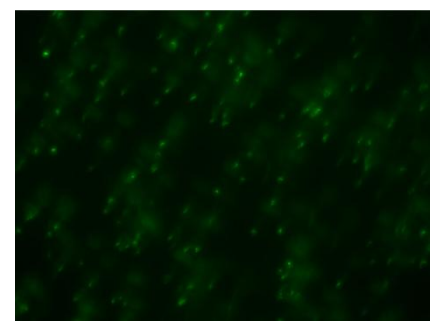




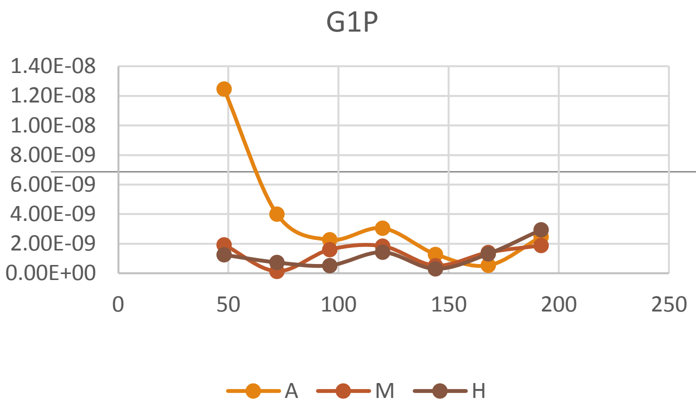
144h

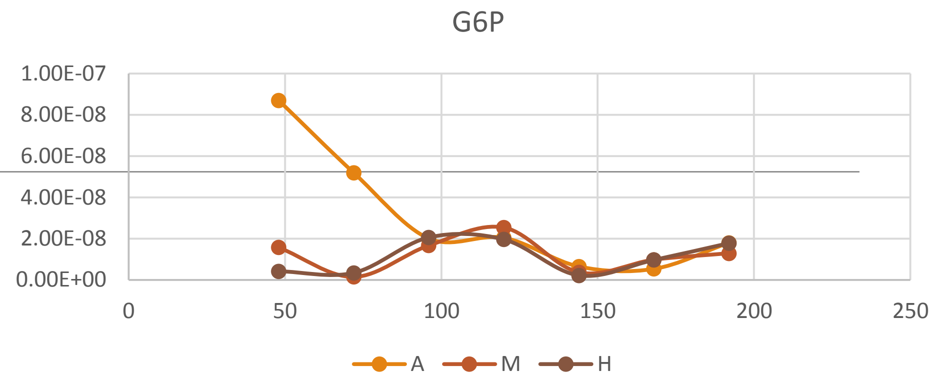
HETERO

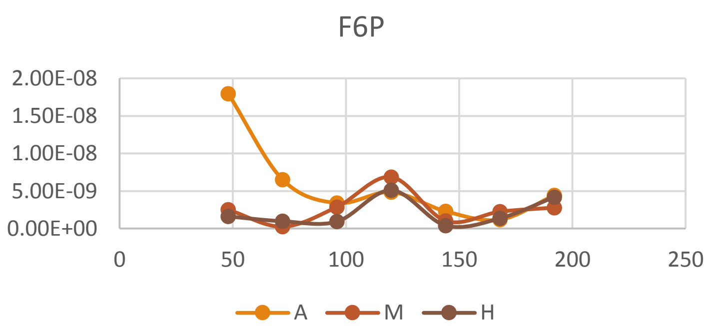


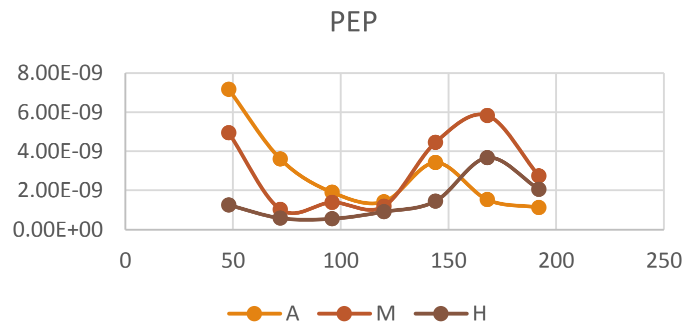


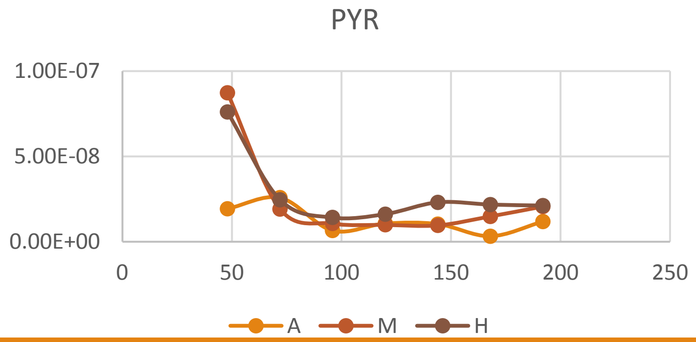
Glycolysis and gluconeogenesis





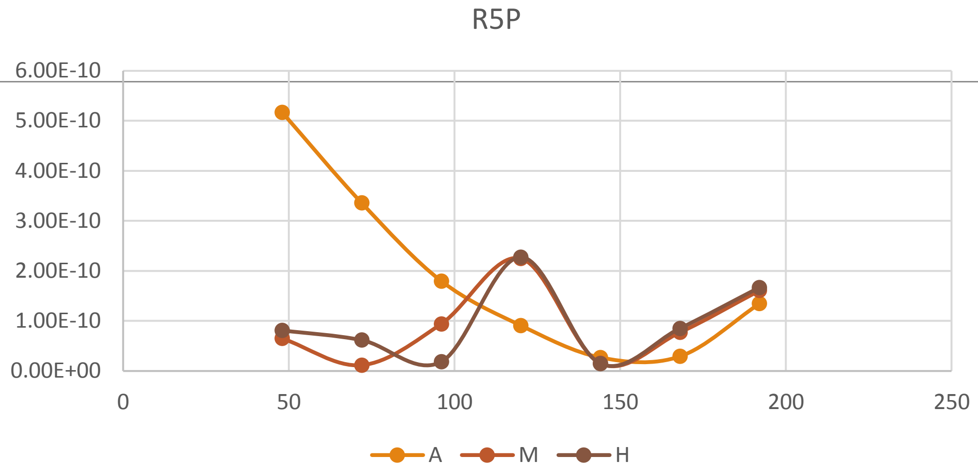


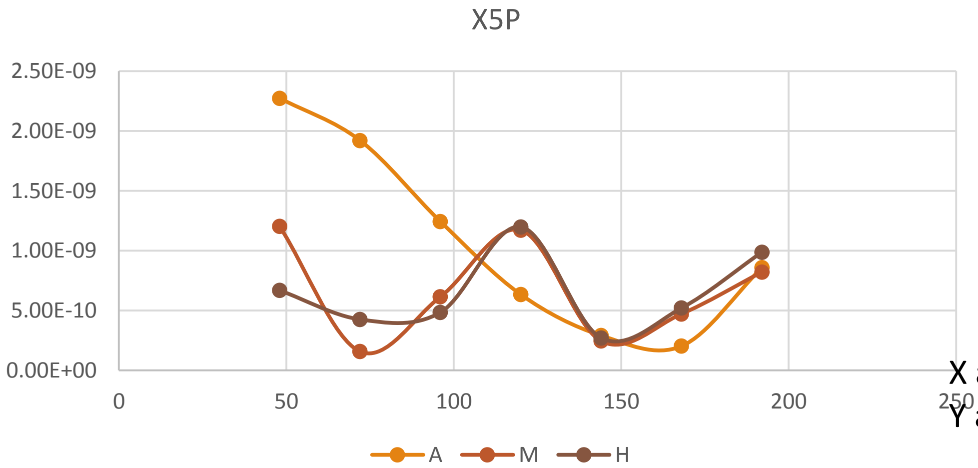




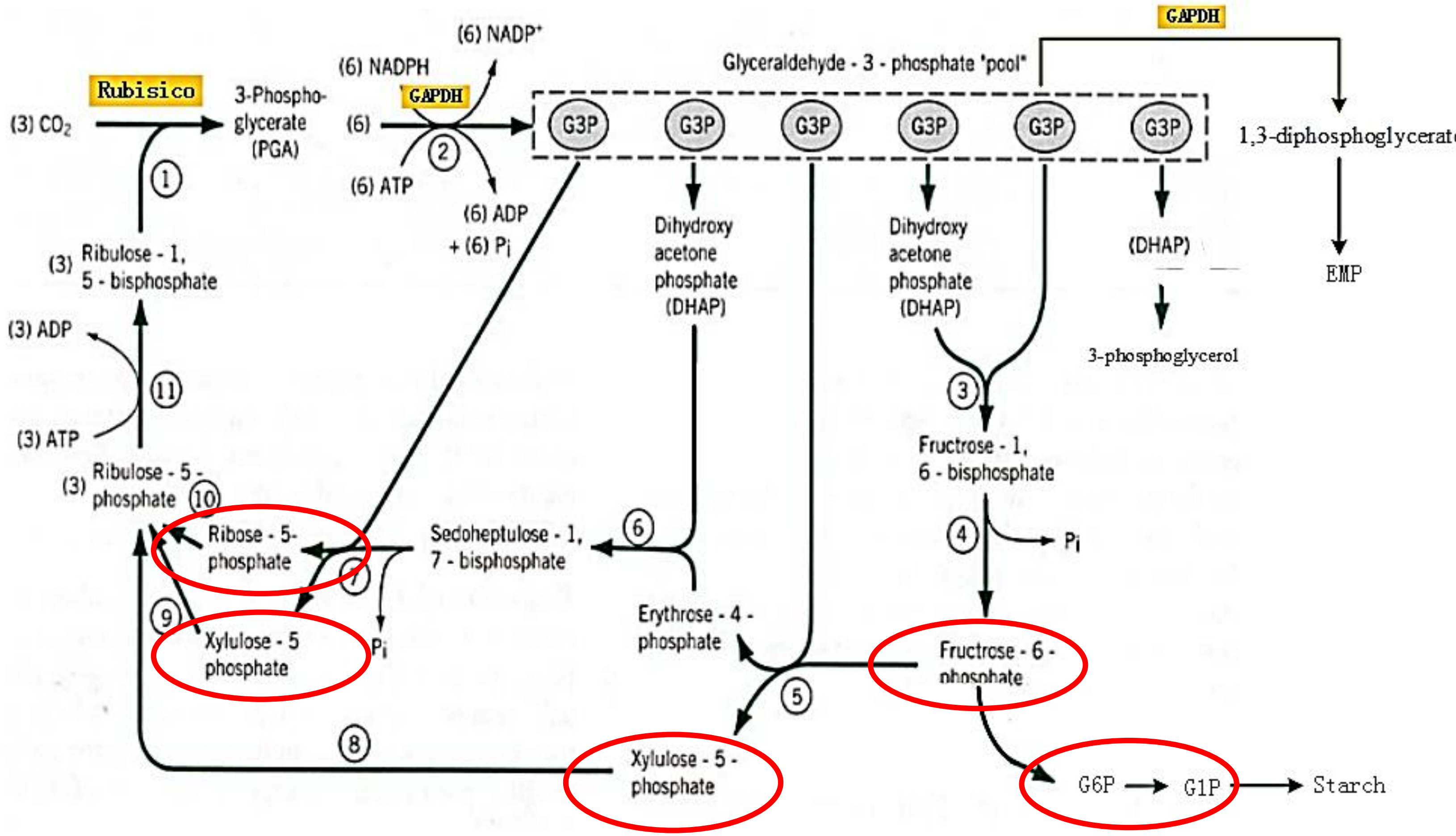
X axle: time-h
Y axle: [C]-nmol/cell

PPP and Calvin cycle



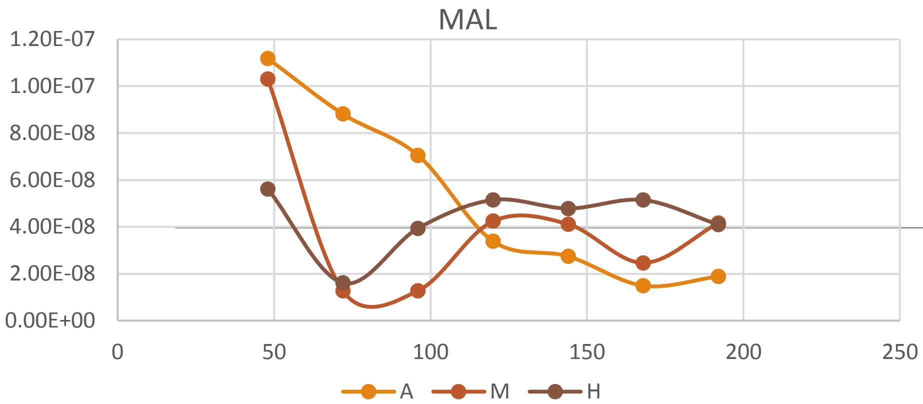


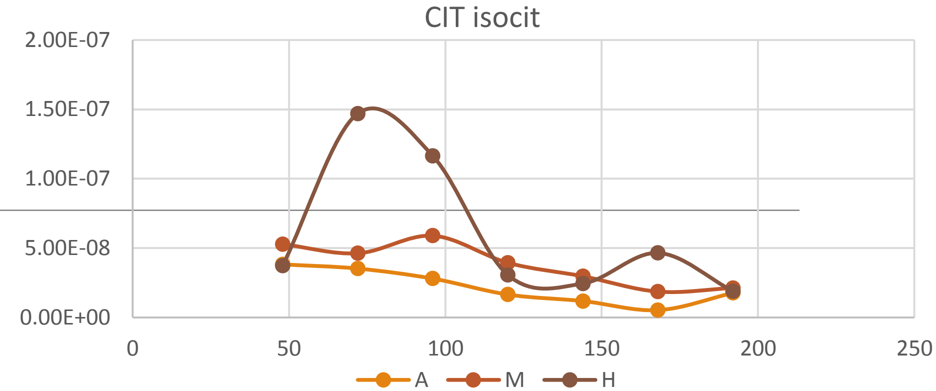
X axle: time-h
Y axle: [C]-nmol/cell

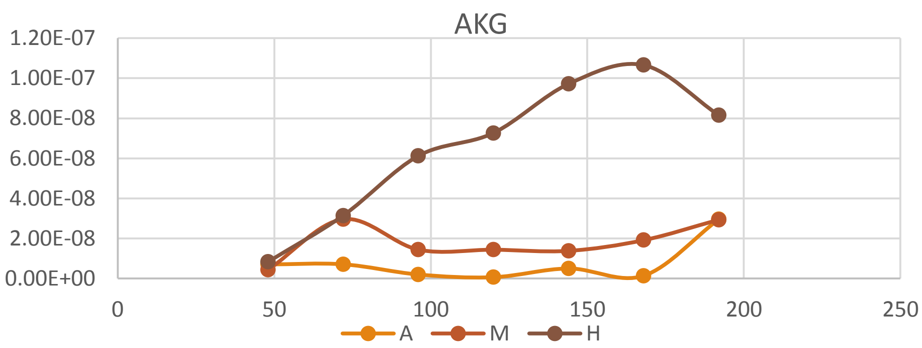


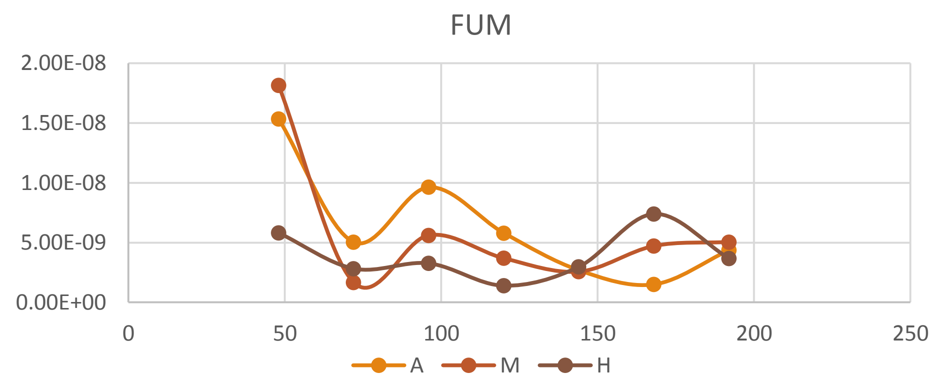
Intermediate metabolites concentration in gluconeogenesis pathway are much higher than that of mixtrophy and heterotrophy, this accumulation further extended to the PPP pathway on R5P and X5P;

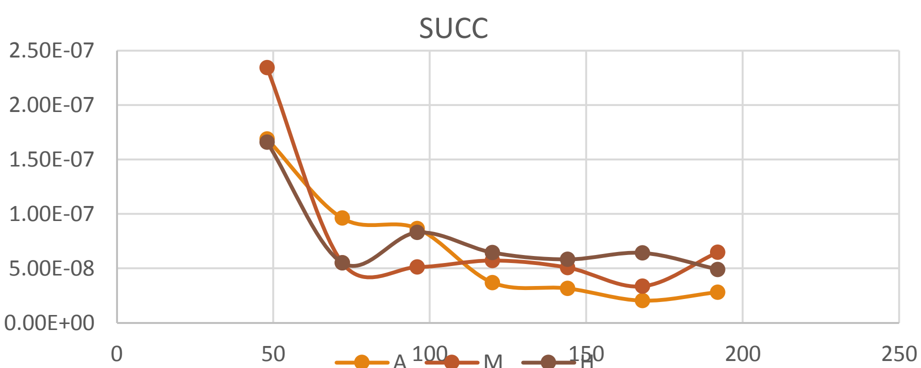
TCA cycle



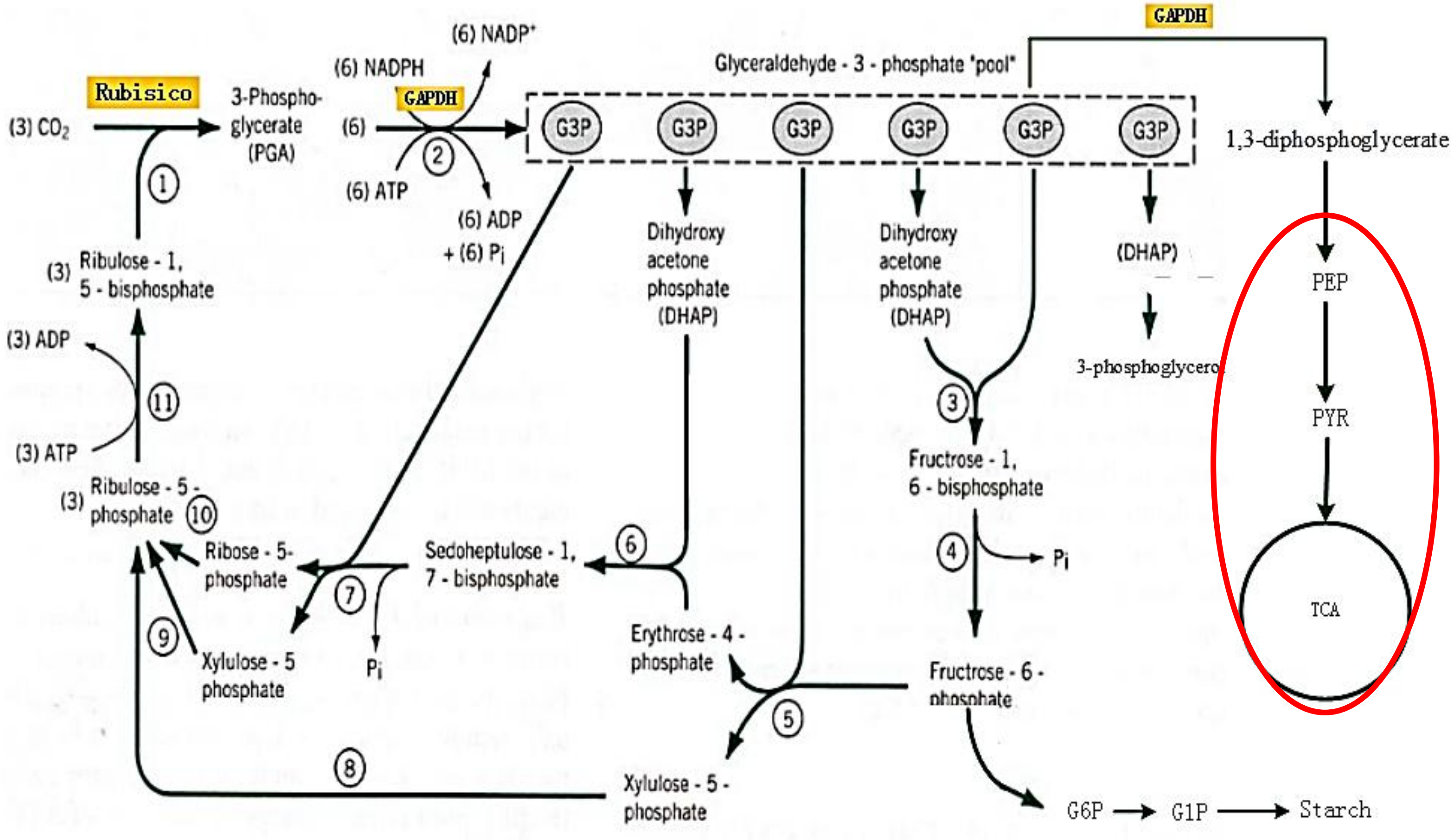






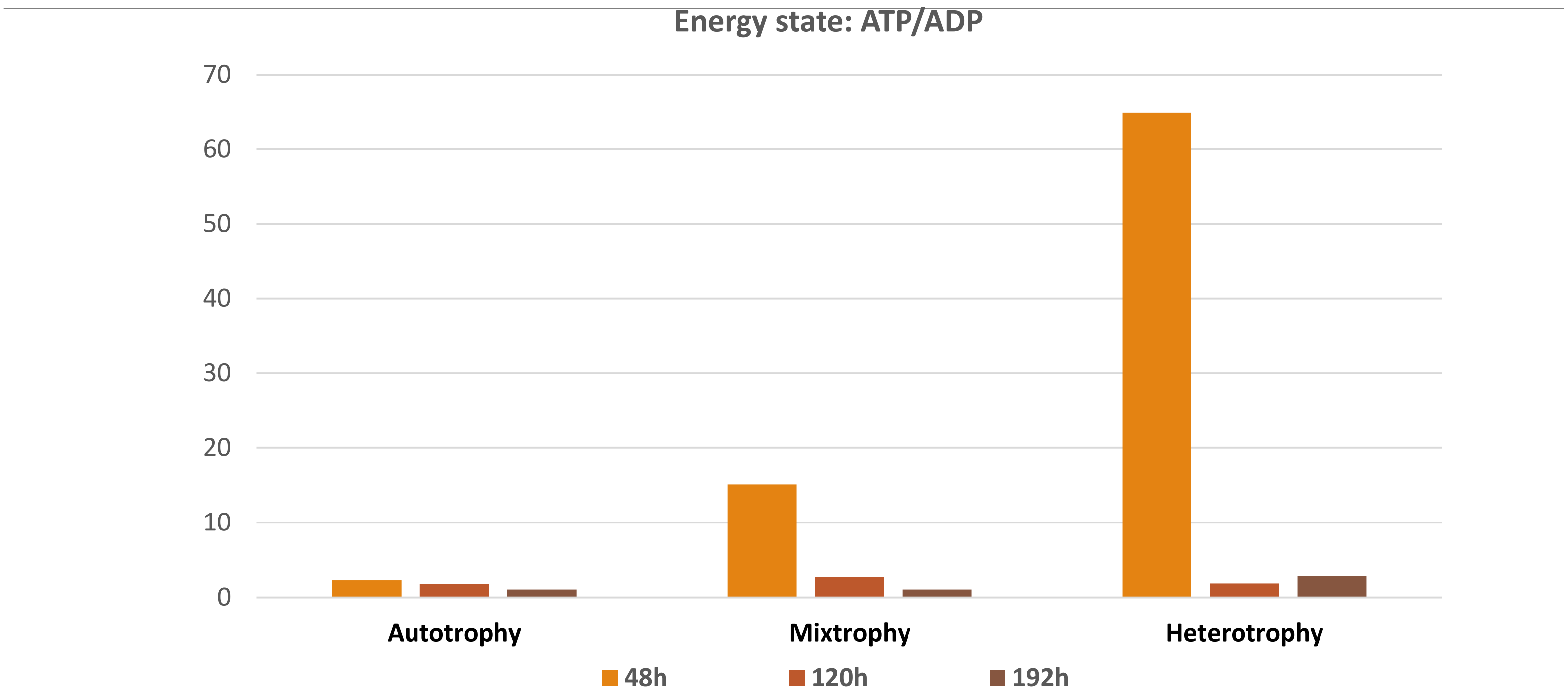


X axle: time-h
Y axle: [C]-
nmol/cell



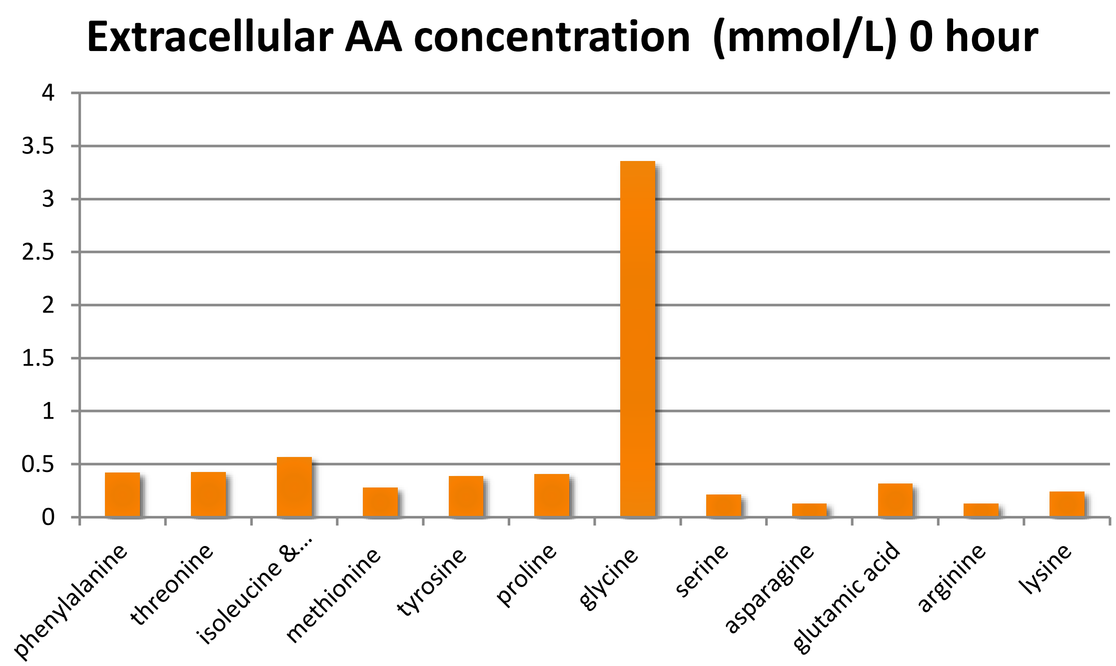
TCA cycle is the starting point for many anabolisms, such as lipid, protein and so on. Therefore, this result explains the metabolic basis that lipid content was significantly higher in heterotrophy and mixtrophy culture than in the autotrophy culture.

Energy state： oxidative phosphorylation pathway

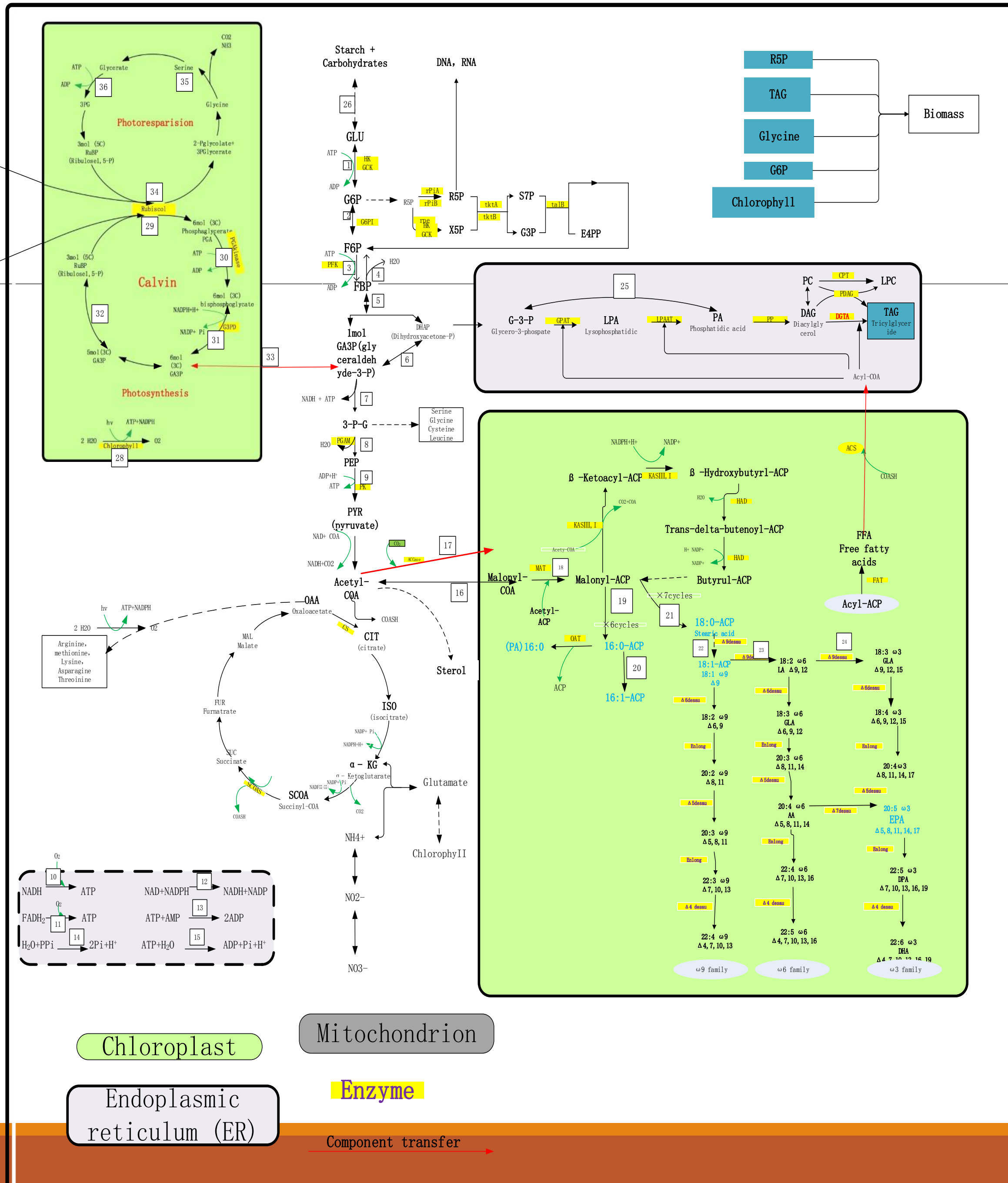


Energetic level of the cells within time

Amino acids: Nitrogen source metabolism



Metabolic pathway



Conclusion:

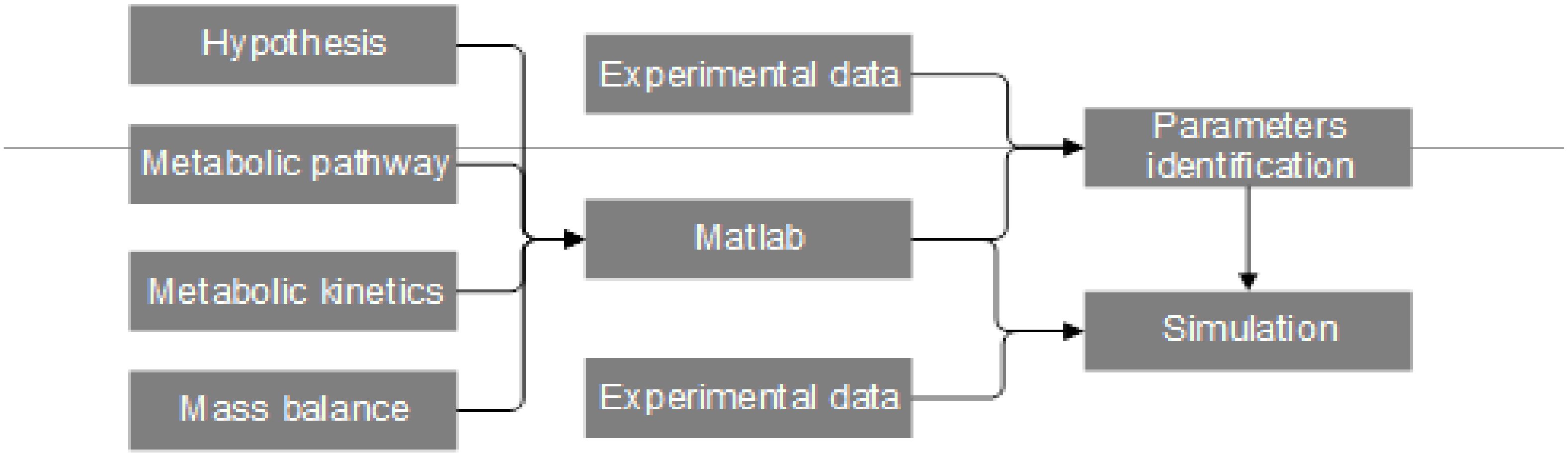
1. Developed culture characterizations of the three metabolic strategies in *Chlorella protothecoides.*

2. Developed analysis methods, tracked variation of metabolites on central metabolic pathways, studied flux distribution in three culture strategy and collected data for model development.

3. Established *Chlorella protothecoides*' metabolic pathway based on databases.

Chapter2: Model development

Model development:



Model hypothesis:

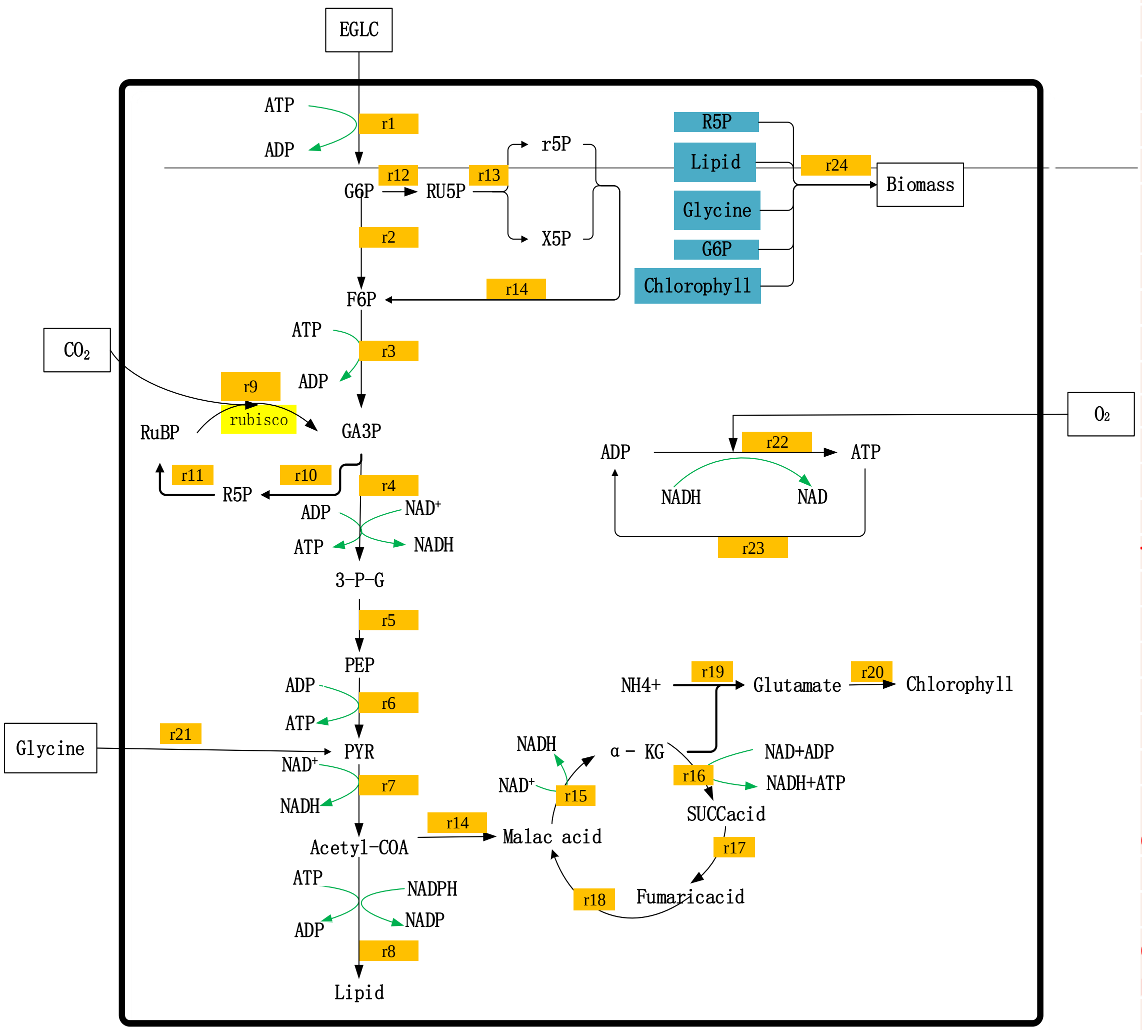
We consider only carbon metabolism in the primary work, nitrogen and energy metabolism will be added in our model later.

We assume G6P, glycine, lipid, R5P and chlorophyll make contribution to biomass growth.

we assume all the reactions are only one direction, reverse reactions will be considered later.

No other regulation in the metabolism process, this will also be considered in our latter work.

Metabolic network and stoichiometric:



Glycolysis	
r1	EGLC+ATP----G6P+ADP
r2	G6P ---F6P
r3	F6P+ATP ----GA3P+ADP
r4	GA3P+ADP+NAD----3PG+ATP+NADH
r5	3PG ----PEP
r6	PEP+ADP -----PYR+ATP
r7	PYR+NAD -----Acetyl-COA+NADH
Lipid synthesis	
r8	Acetyl-COA+ATP+NADPH ---- lipid+ADP+NADP
Photosynthesis	
r9	RuBP---- GA3P
r10	GA3P -----R5P
r11	R5P -----RuBP
PPP pathway	
r12	G6P -----Ru5P
r13	Ru5P ----- R5P+X5P
r14	R5P+X5P ---- F6P
TCA cycle	
r15	Acetyl-COA ----- Malac
r16	Malac +NAD -----α- KG+NADH
r17	α- KG _NAD_ADP-----Succac+NADH+ATP
r18	Succac ------Fumaric
r19	Fumaric ------ Malac
r20	α- KG+NH4 -----Glutamate
Chlorophyll synthesis	
r21	Glutamate----Chlorophyll
r22	Glycine----PYR
Oxidative phosphorylation	
r23	ATP ---- ADP
r24	ADP +NADH------ ATP+NAD
Biomass synthesis	
r25	R5P+lipid+Glycine+G6P+Chlorophyll ---- X

Model functions:

1. Biochemical reaction kinetics: Multi-Michaelis-Menten equation

$$V = V_{\max}\left[\prod_{i=1}^{N_S}\frac{C_{S_i}}{K_{mSi} + C_{S_i}}\right]$$

2. Cell growth: Multi-Monod-equation

$$V_{\_growth} = V_{\_growthmax} \times \frac{R5P}{R5P + K_{m\_R5P}} \times \frac{Lipid}{Lipid + K_{m\_lipid}} \times \frac{Glycine}{Glycine + K_{m\_glycine}} \times \frac{G6P}{G6P + K_{m\_G6P}} \times \frac{Chlophyll}{Chlorophyll + K_{m\_Chlorophyll}}$$

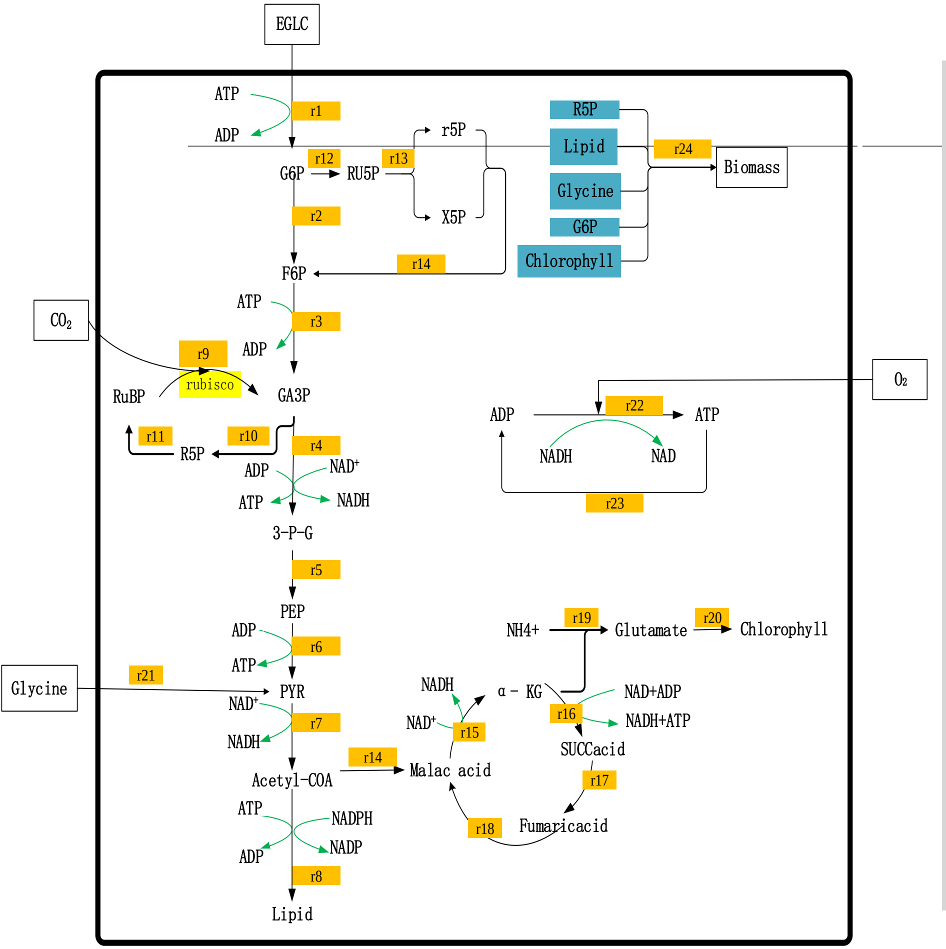
3. Mass balance

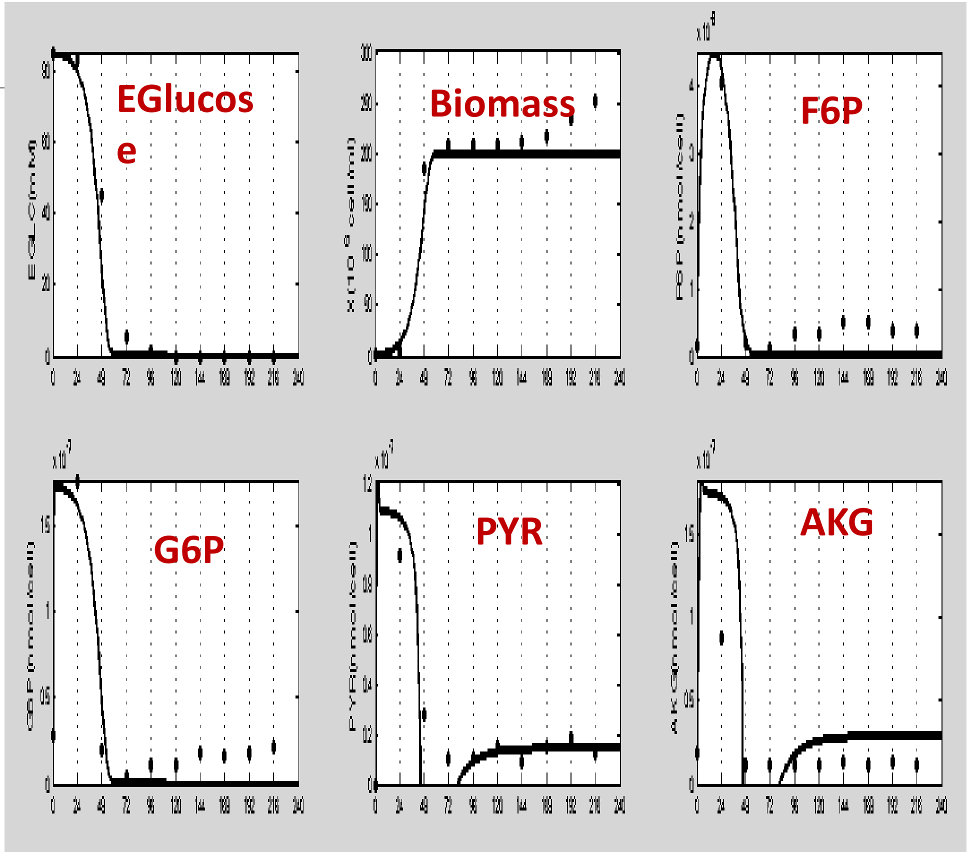
$$\frac{d[C_{S_i}]}{dt} = \left(\sum_{i=1}^{M} V_{input_i} - \sum_{j=1}^{N} V_{output_j} - \mathrm{a} \times V_{growth} - \right) \times Biomass$$

Contribution to Biomass

Extracellular

Simulation result:





Simulation results of model and experimental data

Future work

Nutrition strategy based on the model prediction

- Analysis of the flux distribution and identify key enzymes and nutrient limitations along the time profile.
- Develop a real-time control nutritional strategy to improve lipid production based on model prediction.
- Predict hypothesis of knocking off relevant genes, which will also provide a further strategy to improve the lipid production.

Acknowledgement

Thanks for my supervisors Prof. Mario Jolicoeur, Jean-Sébastien Deschênes, Réjean Tremblay support my research.

Thanks for our technician Jingkui Chen and other partners' help in my work.

Thanks for the supports from the organizations.

Bio-P²

Canada Research Chair on the Development of Metabolic Engineering Tools

Ressources Aquatiques Québec RAQ

POLYTECHNIQUE MONTRÉAL LE GÉNIE EN PREMIÈRE CLASSE

UQAR

Lab course plan

- Medium preparation: modified basal medium
- Inoculation: $1.0*10^6$cell/ml
- Microscope observation: cell size, cell density, chlorophyll
- Cell harvest: 3600rpm, 5min, wash cells.
- lipid analysis: Acetone and sulfosalicylic acid; absorbance was read at 440nm by UV-VIS determination

Merci !