Université de Montréal
Laboratoire de développement de biocombustible

Algal Biofuels & Bioproducts in a Cold Climate - A Canadian Perspective

Dr. Patrick C. Hallenbeck

Département de microbiologie et immunologie

Université de Montréal

patrick.hallenbeck@umontreal.ca

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Summit on Industrial Biotechnology and Bioenergy
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Good points and Bad points

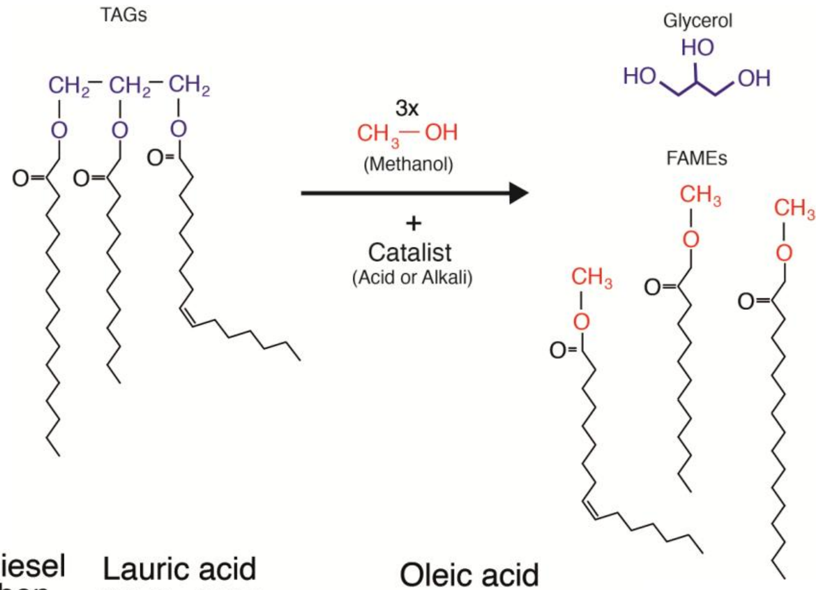
Proposed Advantages	Downsides
-Can be grown on marginal lands, urban areas or industrial parks: no competition with food production	-cultivation much more technologically challenging than traditional crops
-Rapid growth under optimal conditions	-optimal conditions, pH, temp, pCO_2, light intensity difficult to maintain -relatively easily over-run by “weeds” -subject to plagues of “pests”

Good points and Bad points

Proposed Advantages	Downsides
-High lipid content	-only a few species and under stringent conditions -High diversity of fuel quality/characteristics
-Sequester or mitigate CO_2 emissions from fossil fuel power plants	-“Enron style” repo 101 accounting, CO_2 immediately released when fuel combusted
-Production possible throughout the year	-Low productivity during winter months, heating may be necessary

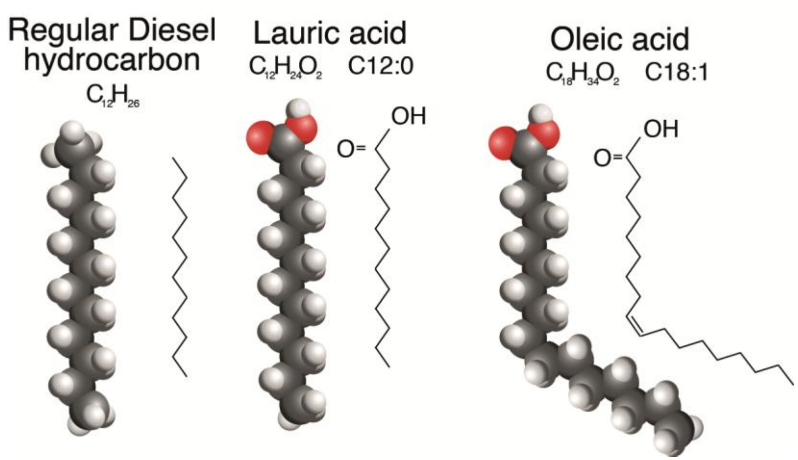
High lipid content

Under the right conditions some algae contain high [TAG]



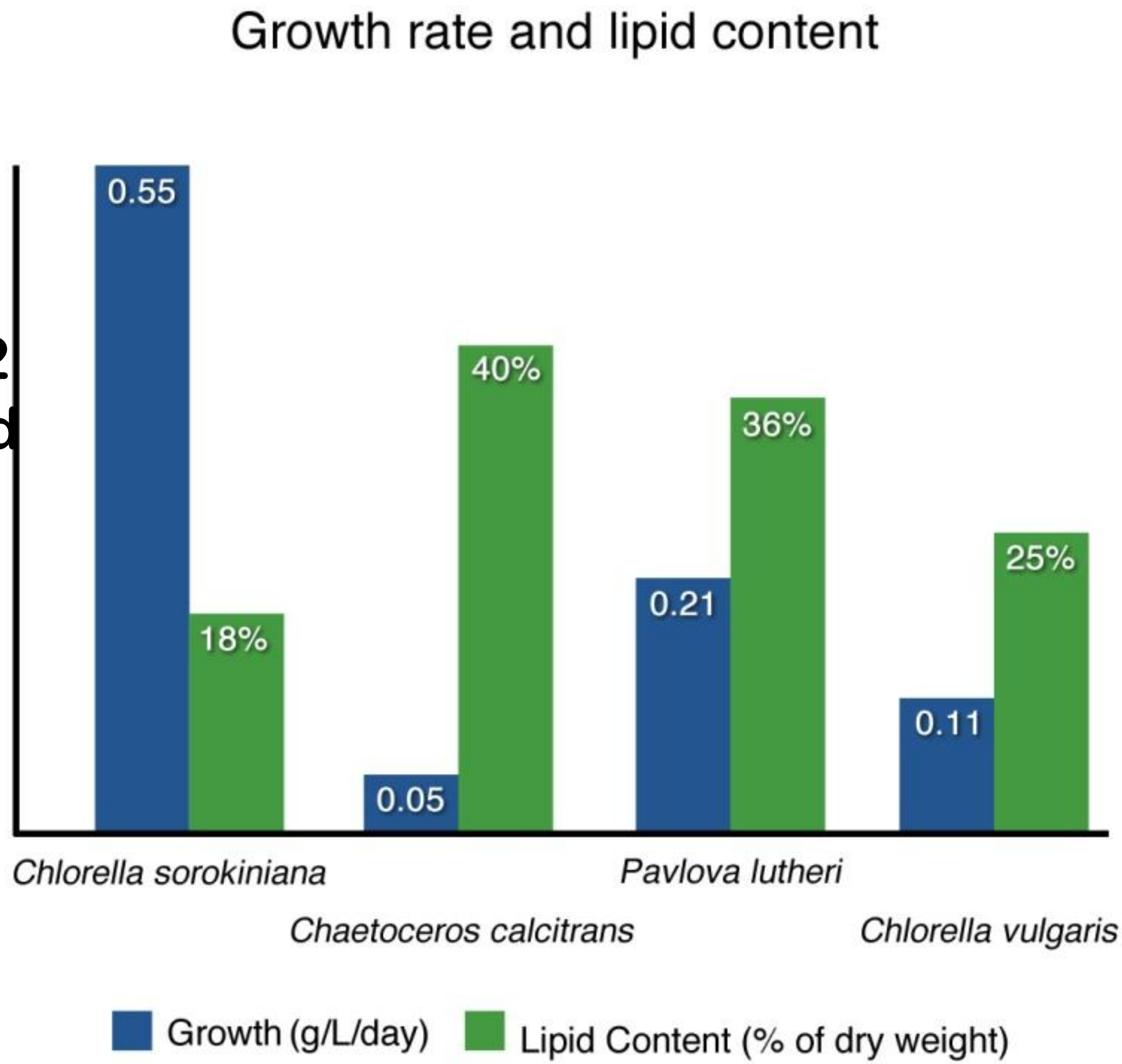
Enhancement of lipid production in different microalgae.

Species	Rich media	Nitrogen deficient
Chlamydomonas applanata	18%	33%
Chorella emersonii	29%	63%
Chorella minutissima	31%	57%
Chorella Vulgaris	18%	40%
Ettlia oleoabundans	36%	42%
Scenedesmus obliquos	12%	27%
Selenastrum gracile	21%	28%



• At saturating CO_2 fixation rates, fixed CO_2 can be used for cell growth or lipid production, not both

• Best strain needs to optimize both, trade-off



What are some of the limiting factors?

•Limitations in photosynthetic efficiencies

•High growth rate versus high lipid content

•Others; havestability, [CO_2] ↑ needed for optimal growth, etc.

You can't grow algae in the North, its too cold and there isn't enough sunlight!

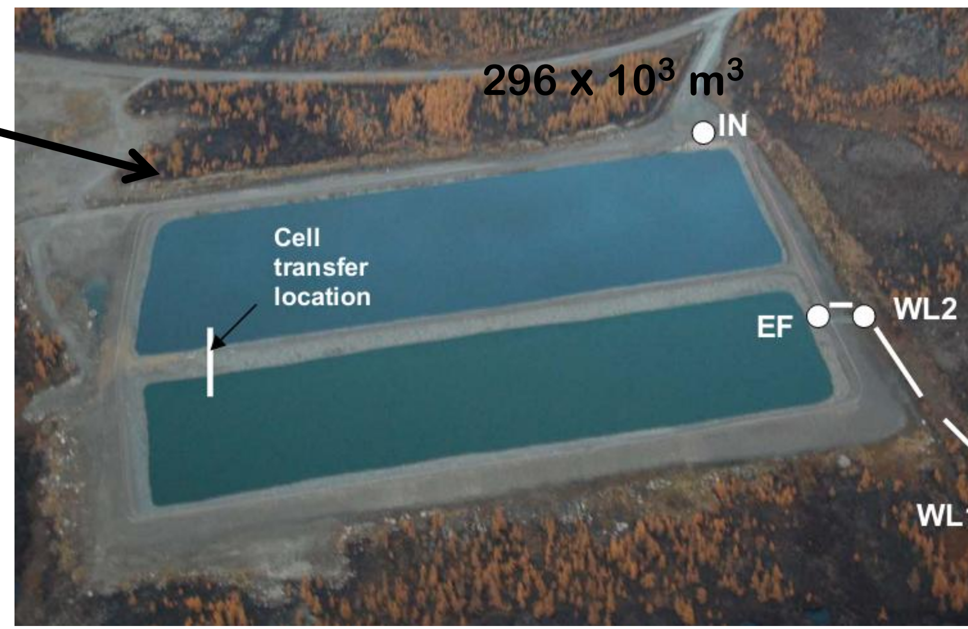
Challenges

- Low temperatures
- Short growing season/Low light

Canadian agriculture- a success inspite of the climate challenge

Crops (algae) adapted to local conditions need to be grown!!

Algae ponds in action in the Far North!!



Low temperatures:

Possible solutions

- Find algal strains that are productive at low temperatures
- Co-locate production facilities with sources of waste heat

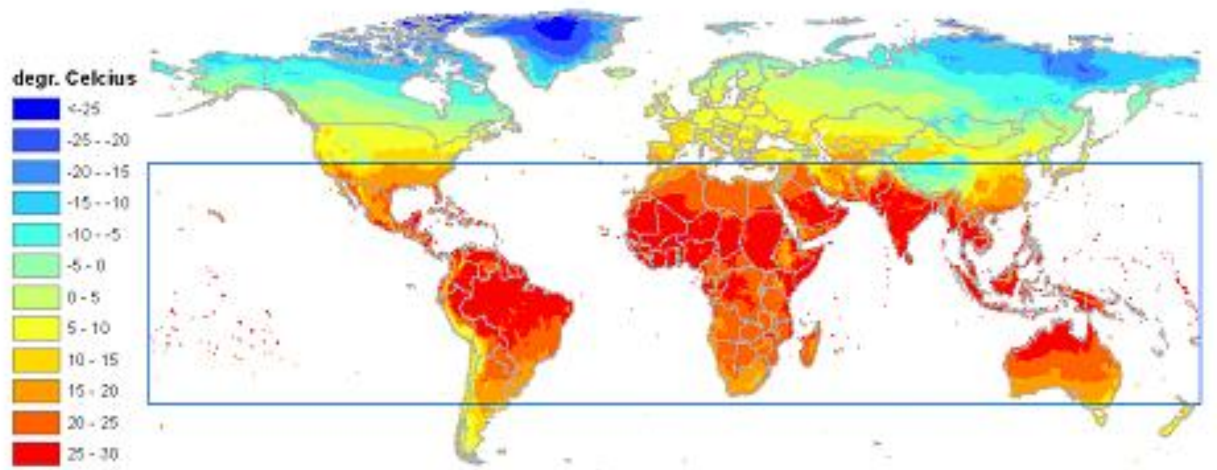
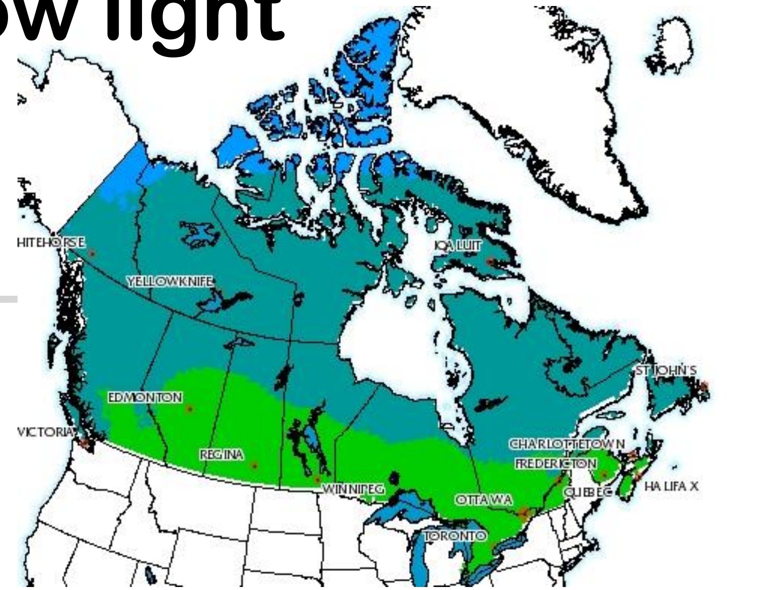


Figure 4.2: Temperature zones projected to be suitable for algae biofuel feedstock production corresponding to an annual average temperatures of above 15°C (Harmelen and Oonk, 2006).

Short growing season/Low light

possible solutions

annual



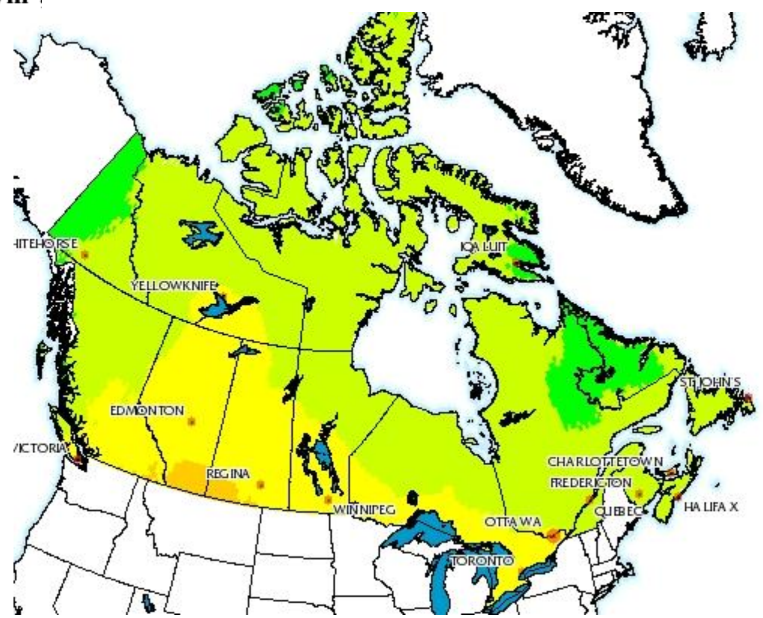
- Adopt three season growth –low OPEX/CAPEX required
- Use mixotrophic/ heterotrophic growth
- Need to select strains with proper characteristics

Legend

Mean daily global insolation (MJ/m^2

- 0-3 MJ/m^2 *(0-0.8 kWh/m^2)*
- 3-6 *(0.8-1.7)*
- 6-9 *(1.7-2.5)*
- 9-12 *(2.5-3.3)*
- 12-15 *(3.3-4.2)*
- 15-18 *(4.2-5)*
- 18-21 *(5-5.8)*
- 21-24 *(5.8-6.7)*
- 24-27 *(6.7-8.5)*
- 27-30 *(8.5-9.2)*
- 30+ *(9.2+)*

0 590 1180 1770 km



July

Characterization of microalgae native to Québec for biofuel production

Patrick C. Hallenbeck

Laboratoire de développement des biocarburants avancées
Département de Microbiologie et Immunologie
Faculté de Médecine
Université de Montréal

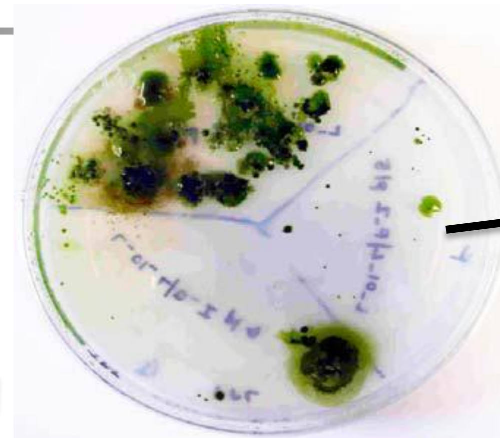
Laboratory for the development of advanced biofuels
Department of Microbiology and Immunology
Faculty of Medecine
University of Montreal

Sampling Locations

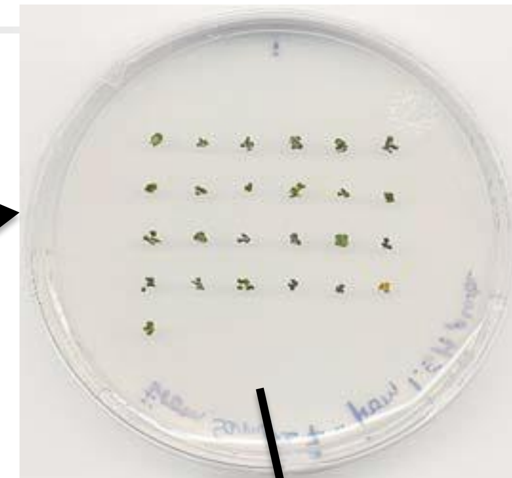
Traditional Isolation of Microalgae Species

1.Enrichment in liquid media

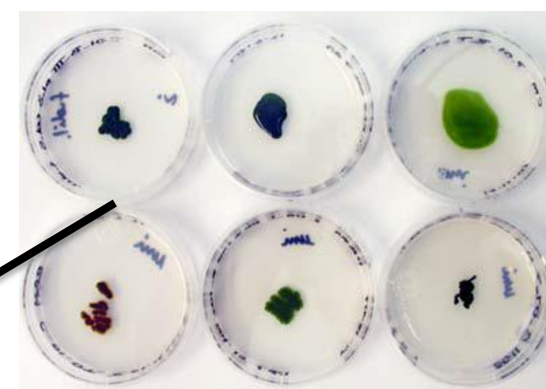
2. Direct plating

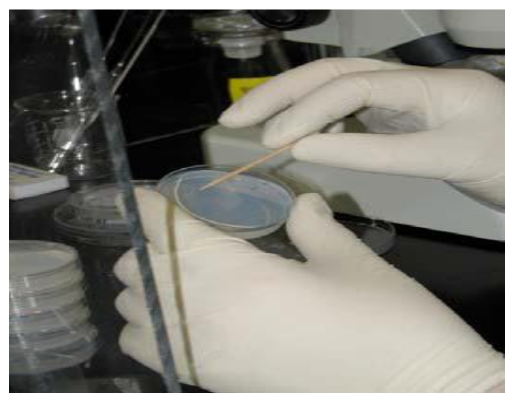


3. transfer to index plates



4. transfer to single plates
(Unialgal Strains)





Result per one site
~ 100 Strains

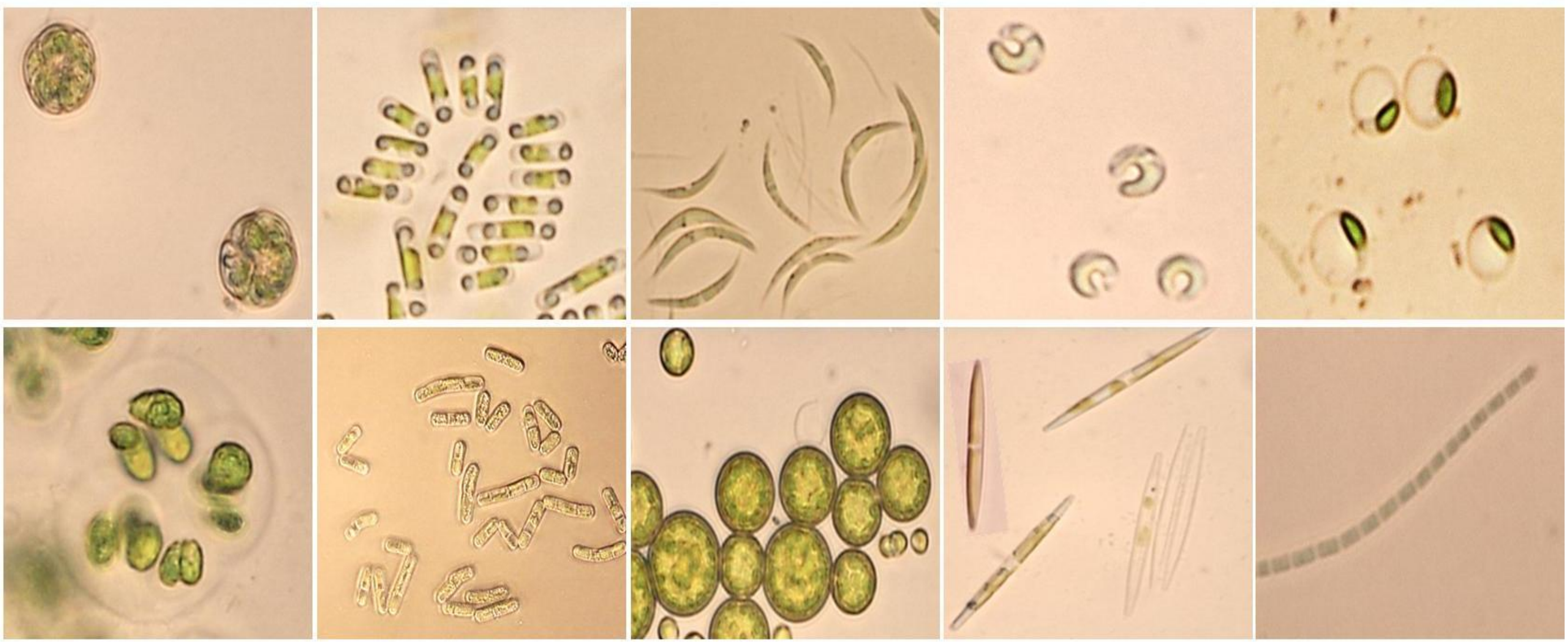
High throughput Isolation of Microalgae

Individual cells selected on the basis of fluorescence profile

FACS

Advantage: if there is growth in a well it is unialgal
Disadvantage: Not all survive (diatoms)

High degree of diversity obtained, strains show many different, interesting characteristics



Screening for Biomass Production (Growth)

Use of Plate Reader

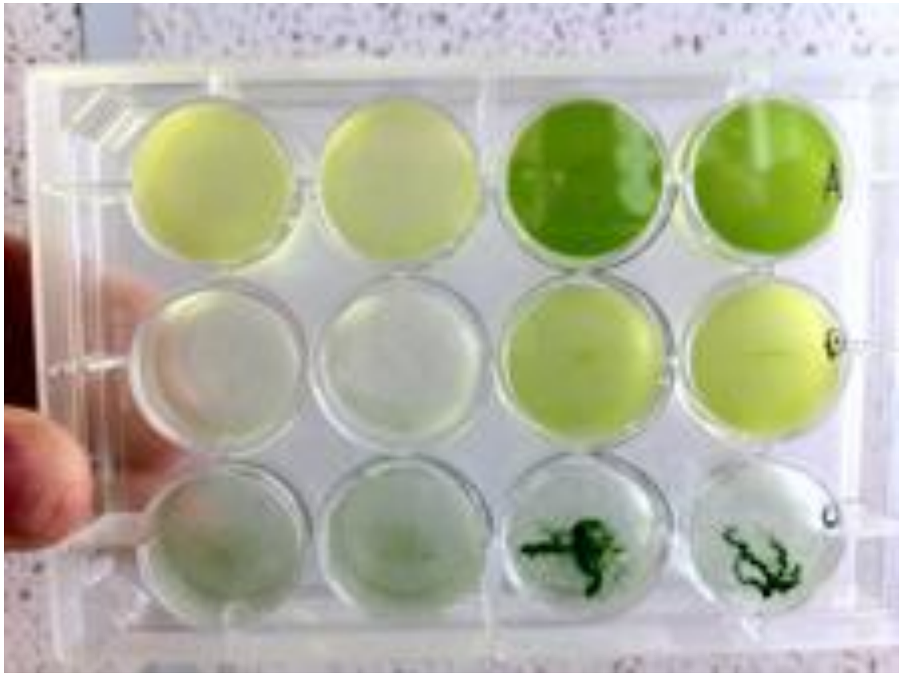
- Determine biomass (OD750)
- Determine Biomass Using Chlorophyll measurement

	1	2	3	4	5	6	7	8	9	10	11	12
A	506	532	523	857	783	771	343	340	347	236	235	246
B	13581	20845	15496	10913	12668	10202	4463	5383	4871	2290	2379	2566
C	322	326	328	311	306	312	272	273	270	307	305	300
D	2124	2366	2377	2690	2689	2689	2582	2089	2492	2438	2781	2335
E	256	265	268	310	301	300	257	260	264	329	328	323
F	2279	2350	2994	3381	3398	3985	3002	2313	2700	3617	3628	2928
G	289	288	296	308	311	310	312	309	303	263	259	260
H	2507	3044	2762	2123	2301	2245	2215	2155	1854	4235	3054	4505

Characterization : Growth dominance

Defined Media

Waste water



Different temperatures

Different salinities

Characterization : Growth dominance

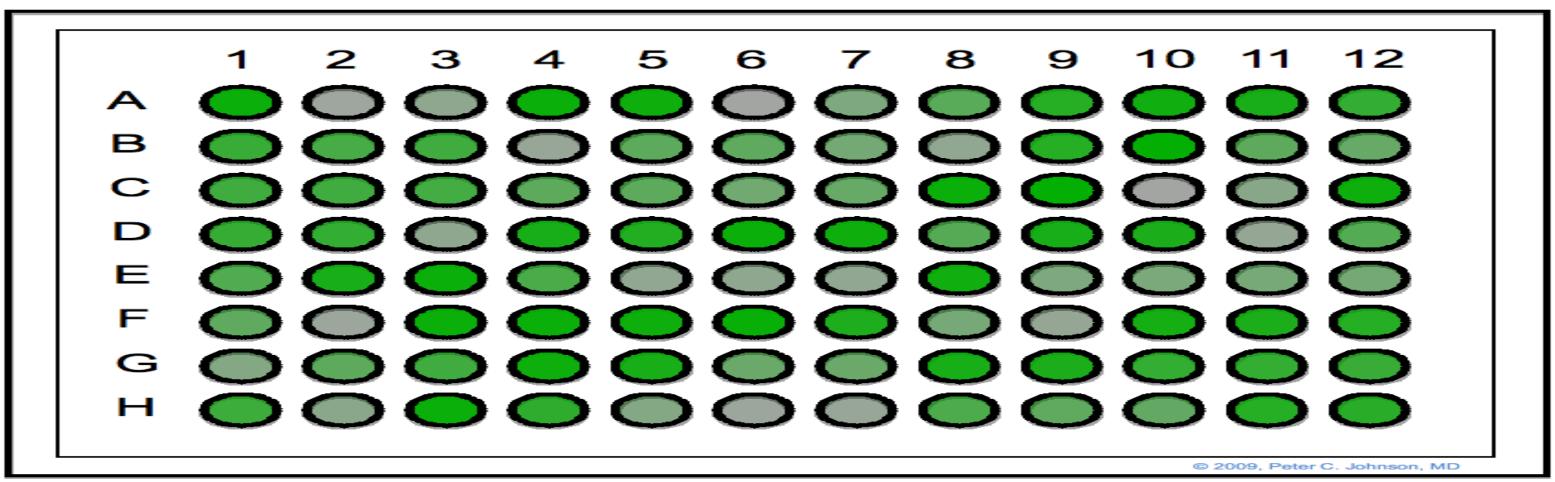
Result per one site

Medium

- 8 °C
- 22 °C

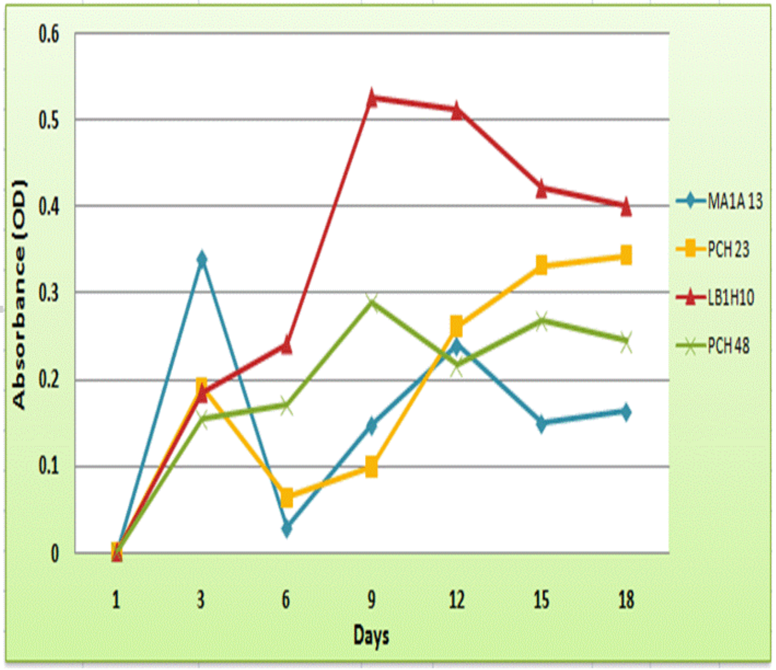
Waste Water

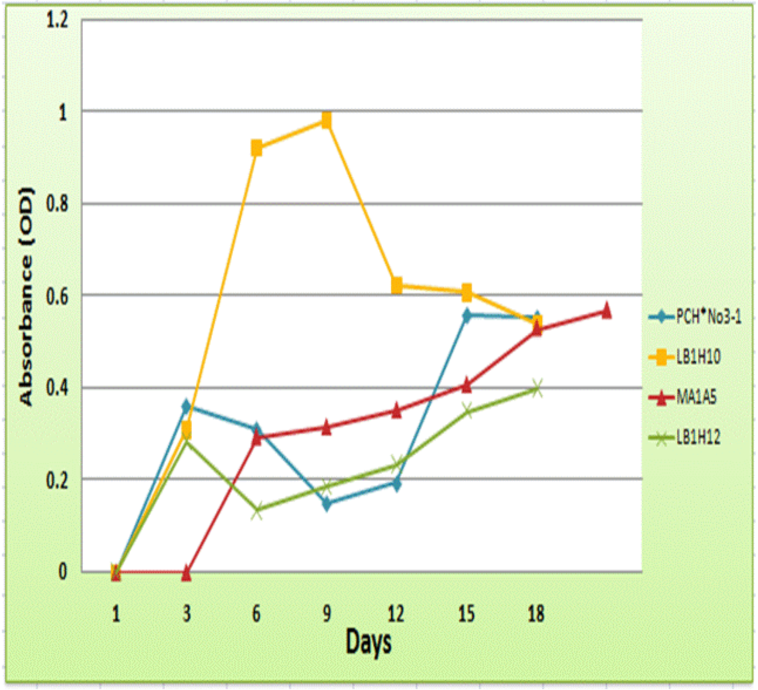
- 8 °C
- 22 °C



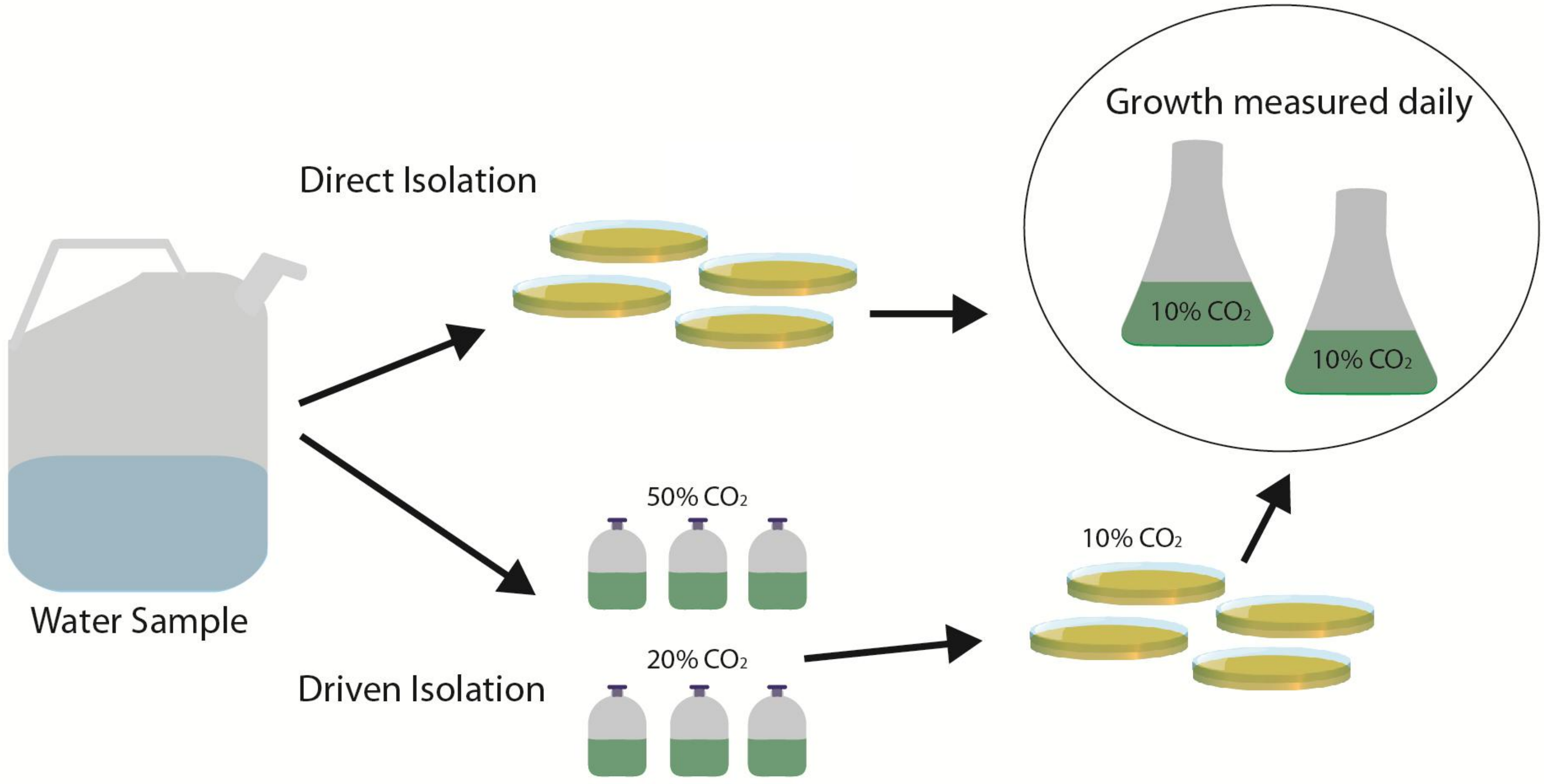
Some strains grow well on wastewater, either 8C or 22C

Isolate	Growth Rate (day^{-1})
Wastewater 8° C	
MA1A 13	1.943
PCH 23	1.751
LB1H 10	1.74
PCH 48	1.681
Wastewater 22° C	
PCH*NO3-1	1.963
LB1H 10	1.913
MA1A 5	1.896
LB1H 12	1.883



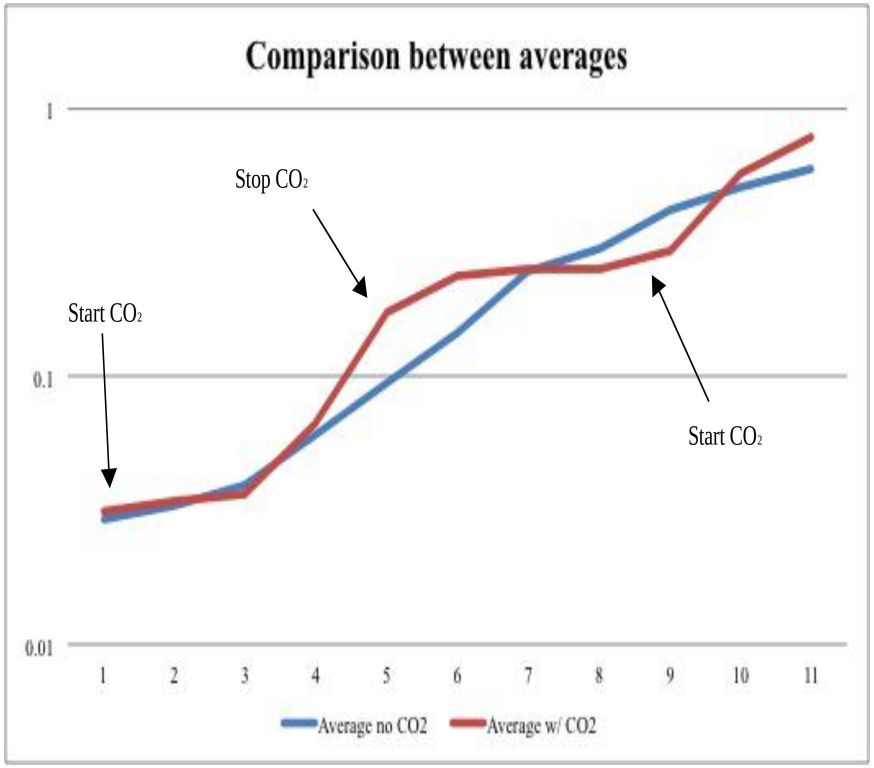


Selection of perfoming strains using CO_2 enrichment



Selection of perfoming strains using CO_2 enrichment

Growth rate			
Isolation with [CO_2]		Isolation without [CO_2]	
B17	2.03	1.06	B9
A19	1.48	0.85	A7
A11	1.37	0.72	B10
A14	1.21	0.66	A6
A17	1.17	0.65	A8
Avg *	1.26	0.66	Avg *



Evaluation of the capacity of microalgae native to Quebec to use xylose or glycerol as alternative carbon source

Four strains showing different patterns of response:
PCH 36 mobilizes both substrates
PCH25 is indifferent
PCH05 and PCH06 show apparent inhibition

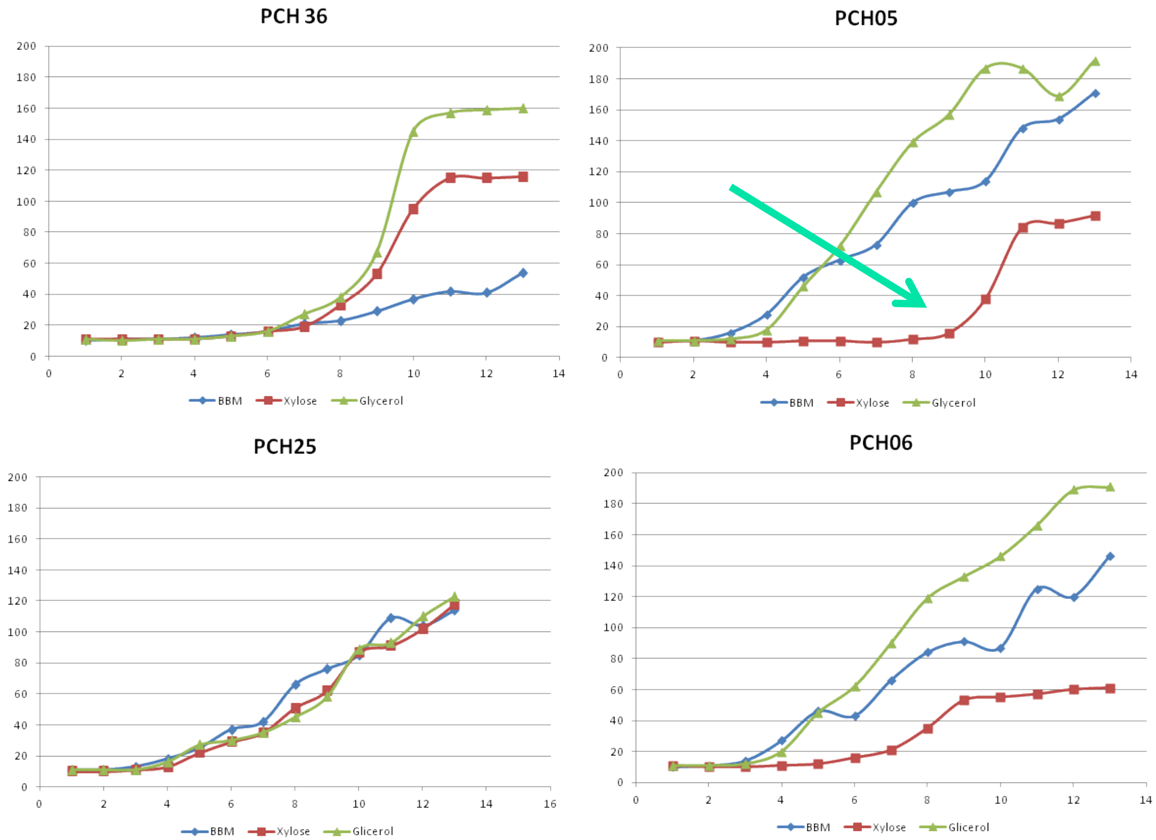
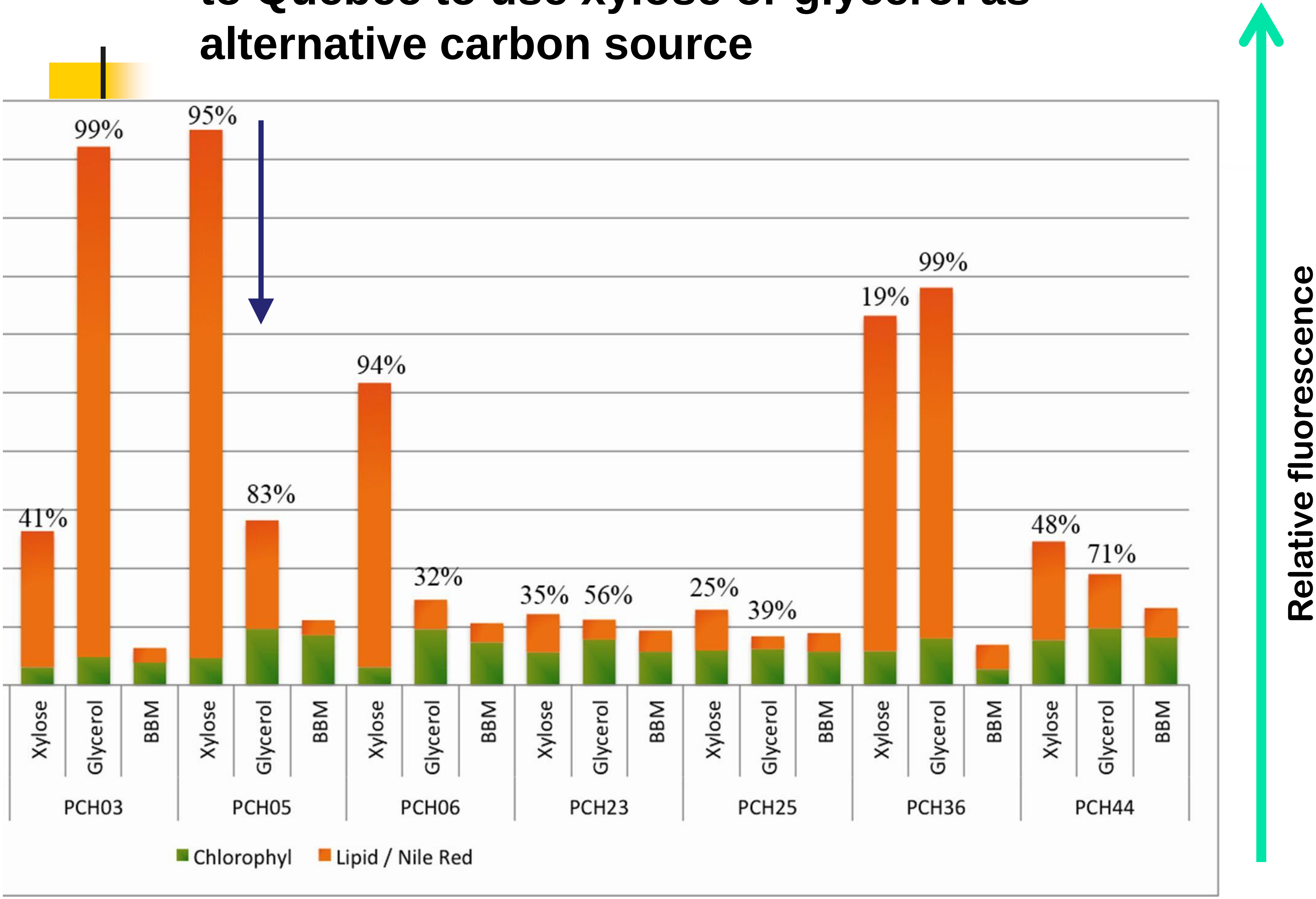


Fig 1: Linear growth curve of four strains showing different patterns of response to the presence of sugar. PCH 36 were able to mobilize both types of sugars, PCH25 showed itself indifferent while PCH05 and PCH06 showed some sort of inhibition of chlorophyll synthesis.

Evaluation of the capacity of microalgae native to Quebec to use xylose or glycerol as alternative carbon source



Strategies

Strains like PCH05

•Could be used for hetertrophic conversion of cheap or no cost fixed carbon substrates to lipid

•Could be used in a two stage process:
 -Autotrophic growth to obtain sufficient biomass
 -Second stage feeding with direct conversion of fixed carbon to lipid with little or no increase in biomass

What can be done?

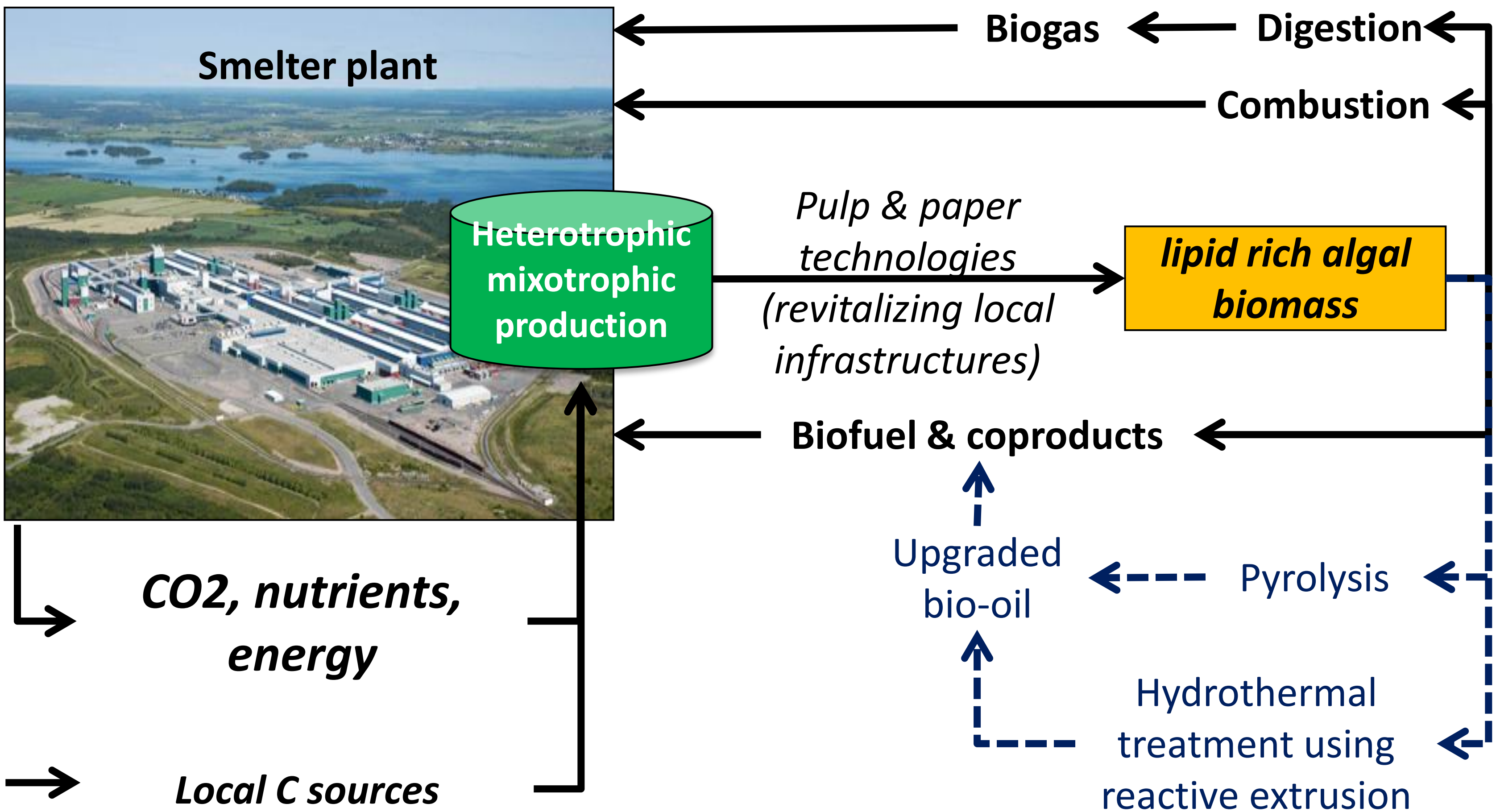
Challenges	Workarounds
-Large amounts of water needed	-Use wastewater or brackish
-Small size, difficult to harvest	-Develop novel harvesting technologies • Screens (large species) • Natural sedimentation • Add flocculating agents
-High water content, dewatering challenging	-Develop novel downstream processing • Novel extraction strategies • In situ transesterification • Engineered strains
-Require high levels of nutrient input	-Use waste water, agricultural run-off

Dr. Simon Barnabé (Université de Québec à Trois-Rivières) is heading a project: RTA-UQTR-AlgaLabs-CSPP

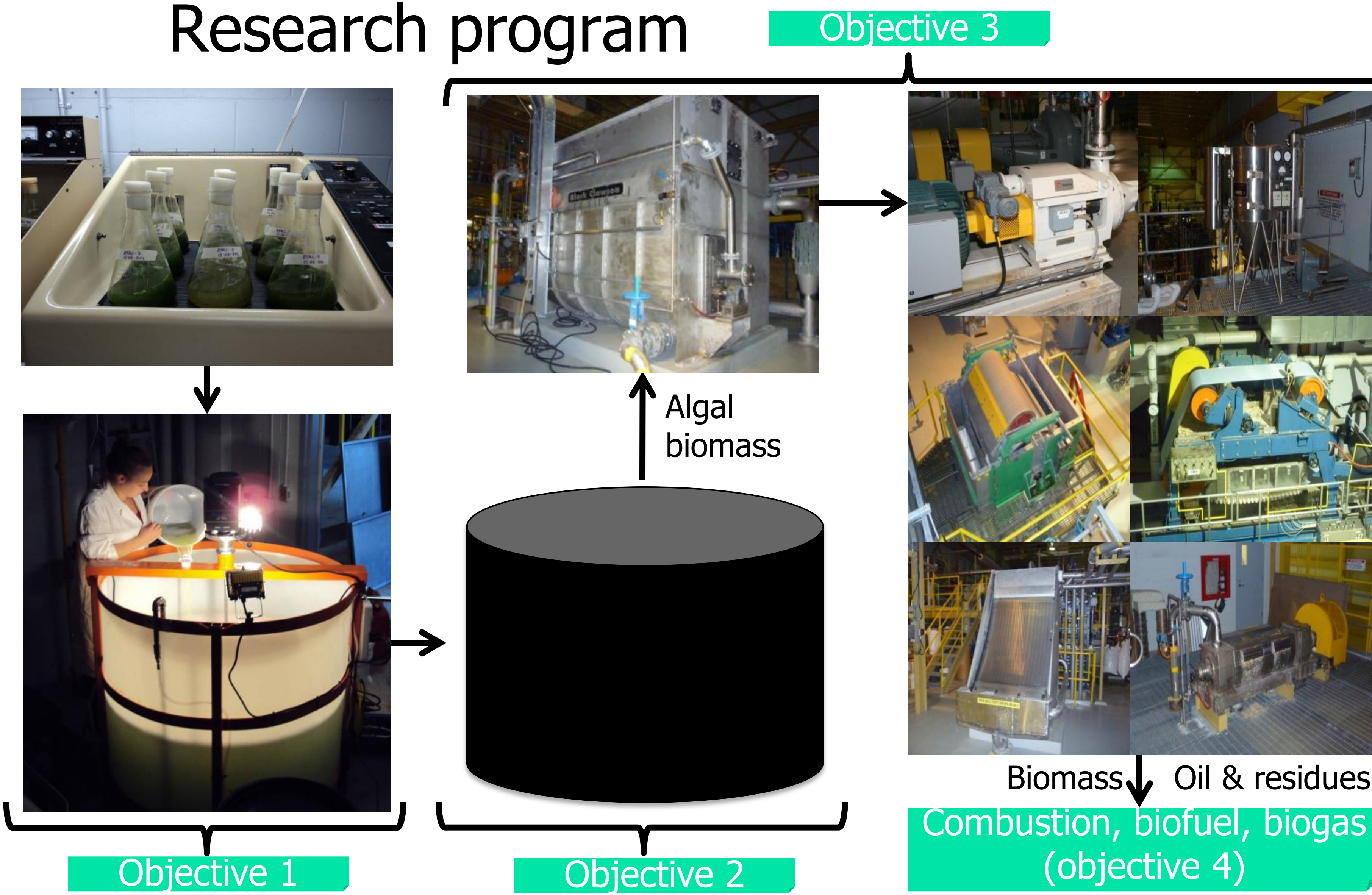
Why does RTA consider algal biomass as a source of renewable energy and fuel ?

- Difficulty in securing adequate supply of forest biomass despite its abundancy in the area
- Why not produce its own biomass by growing algae using its waste nutrients and waste energy ?
    - ✓ A way to secure biomass
    - ✓ Partnership with an algae producer is needed
    - ✓ Local synergy with cheap C source & nutrient suppliers

A co-location multi-sectorial approach



Research program



Preliminary results

- Adapted and robust native algal strain
- Growth medium using smelter wastewater and *in situ* and local C sources

	Productivity
Biomass	0,97 $g{\cdot}L^{-1}{\cdot}j^{-1}$
Neutral lipids	0,03 $g{\cdot}L^{-1}{\cdot}j^{-1}$
% lipids	13%

- Still looking for abundant and locally available C sources
- Harvesting and dewatering experiments in progress
- Production in Alga-Fuel™ 10m³ tank starting soon (Dec 2012)

CO_2-Induced BioMicroalgal Bio-oil Extraction

Dr. Pascale Champagne, P.Eng., D.WRE

Department of Civil Engineering &
Department of Chemical Engineering

October 10-12, 2012 – Vancouver (Canada)

Feasibility of Bio-Oil Production from Microalgae

Research developments needed to improve the feasibility of bio-oil production from Microalgae

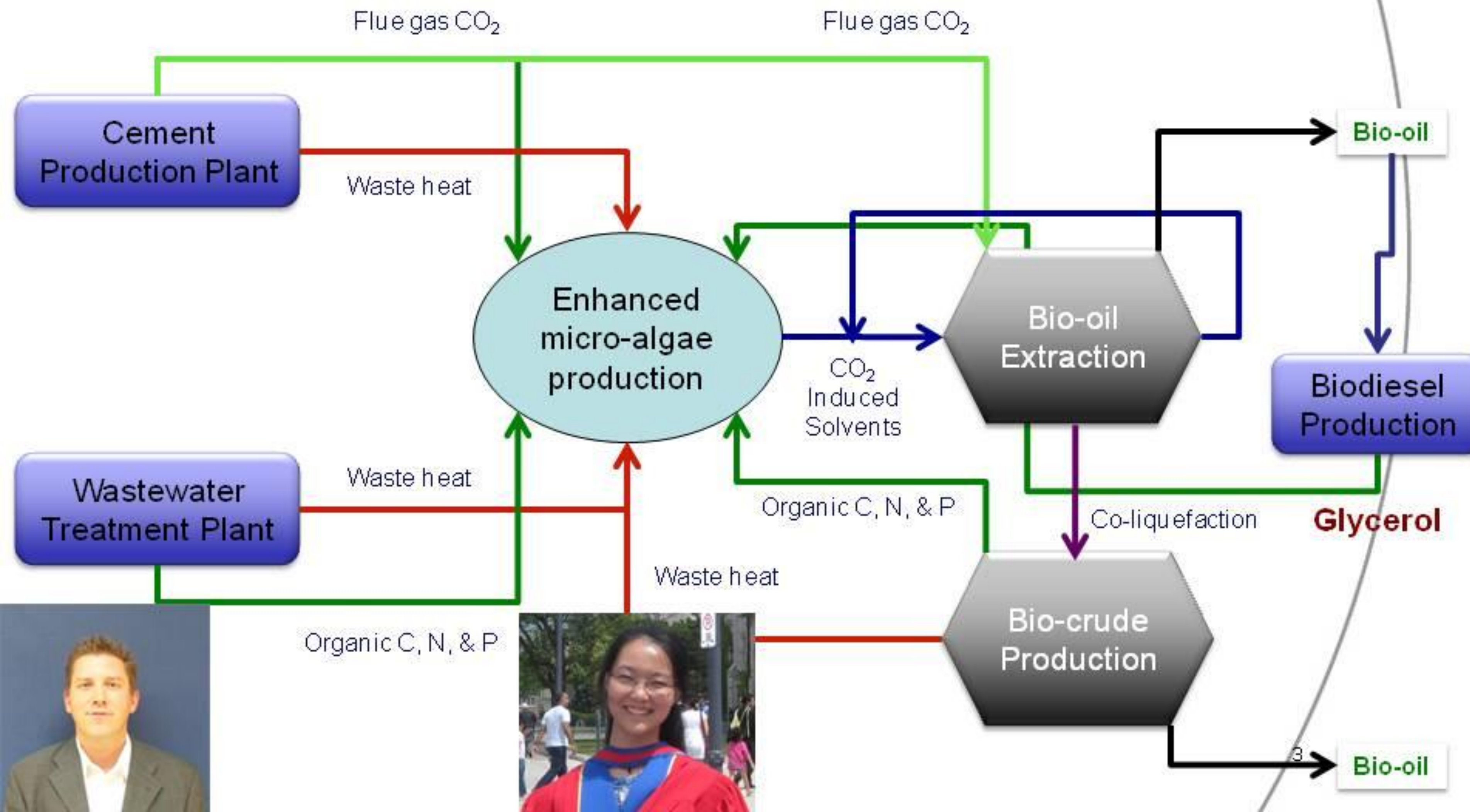
- Enhanced cultivation for bio-oil production
- Improved harvesting strategies
- Efficient dewatering processes
- Environmentally sound & sustainable bio-oil extraction approaches

Improve the energy requirements for these these processes

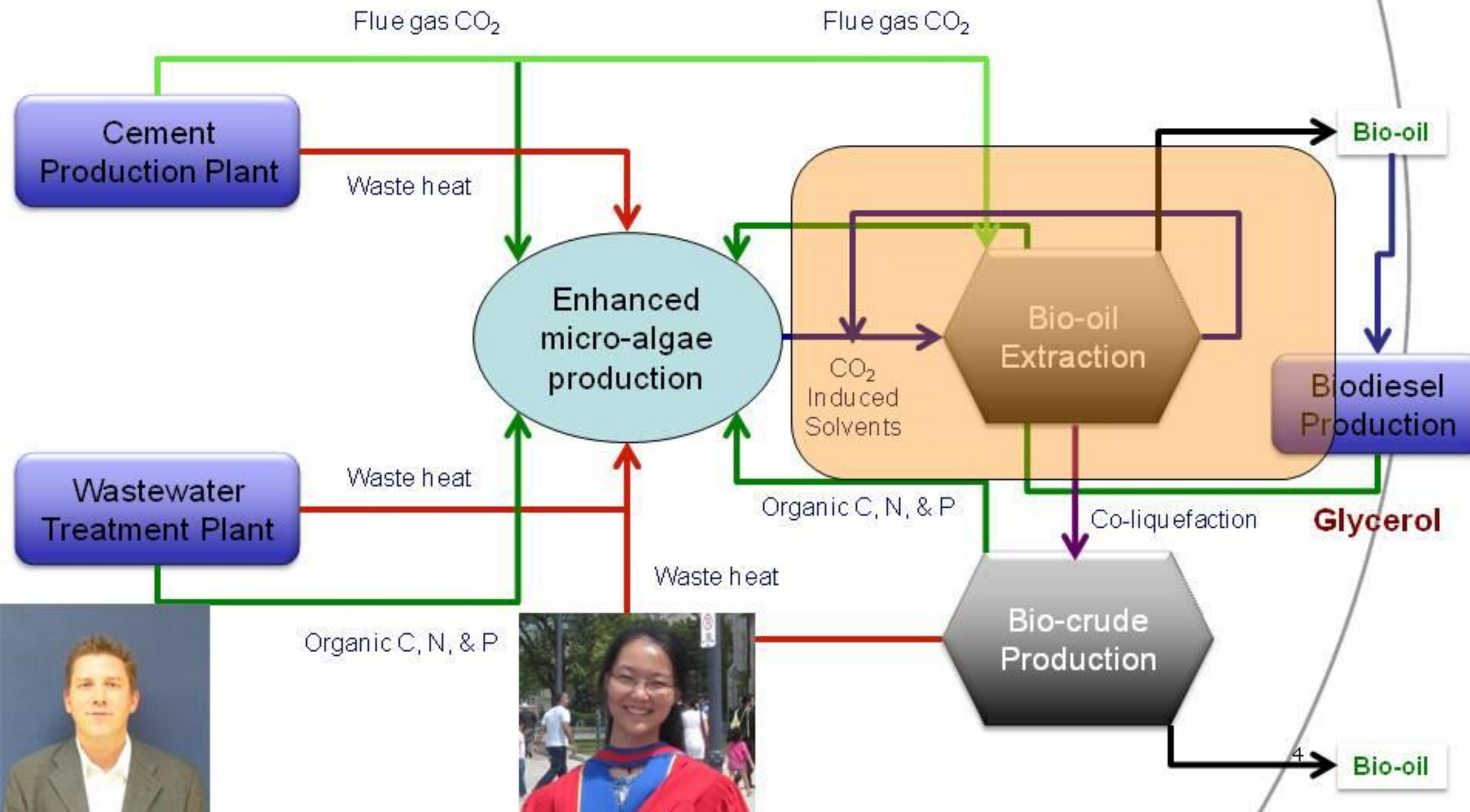
Extracting bio-oil from algal slurries

Cooney, M.; Young, G.; Nagle, N. *Separation & Purification Revs.* **2009**, *38*, 291-325.

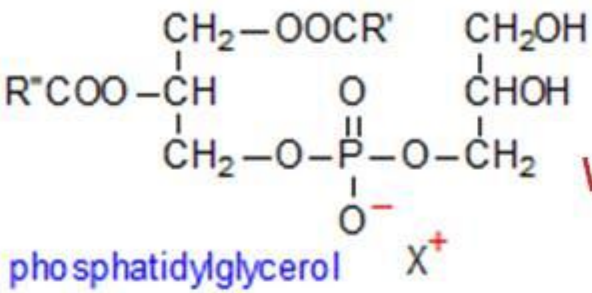
Green & Sustainable Microalgal Bio-oil Production



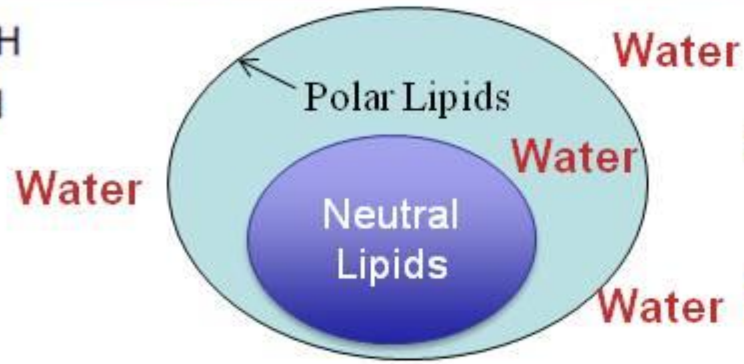
Green & Sustainable Microalgal Bio-oil Production

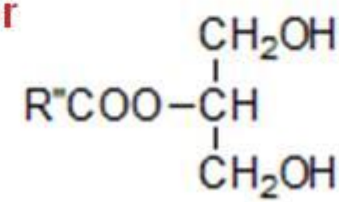


Overcoming the Bio-Oil Extraction Challenge



phosphatidylglycerol

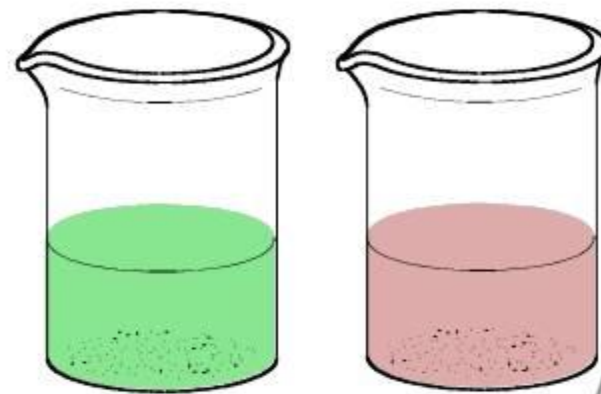




2-monoacylglycerol

Co-solvent extraction strategies

- organic-organic
- IL-alcohol
- CO_2 switchable polarity (SPS)-alcohol

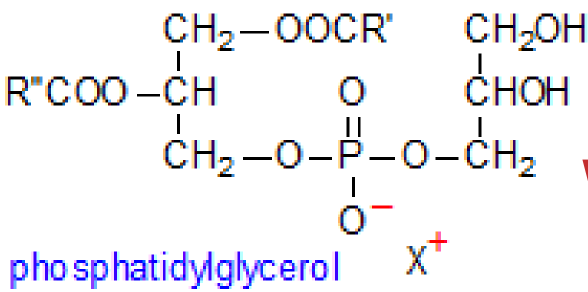


low polarity (oil-like) solvent

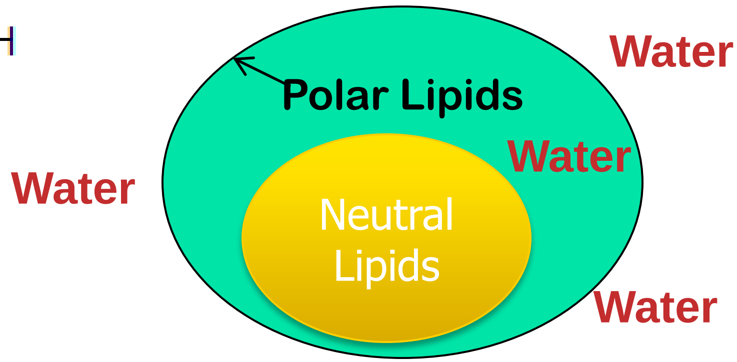
high polarity (water-like) solvent

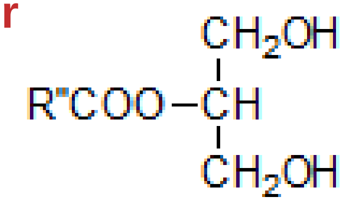
Halim R, Gladman B, Danquah MK, Webley PA *Bioresour Technol* **2011**, *102*, 178.
Cooney MJ, Young G, Nagle N *Sep Pur Rev* **2009** *38*, 291.
Young G, Nippgen F, Titterbrandt S, Cooney MG *Sep Pur Technol* **2010**, 72, 118.
Samori C, Torri C, Samori G, Fabbri D, Galletti P, Guerrini F, Pistocchi R, Tagliavini E *Bioresour Technol* **2010**, *101*, 3274.

CO_2 Induced Solvents: Overcoming the Bio-Oil Extraction Challenge

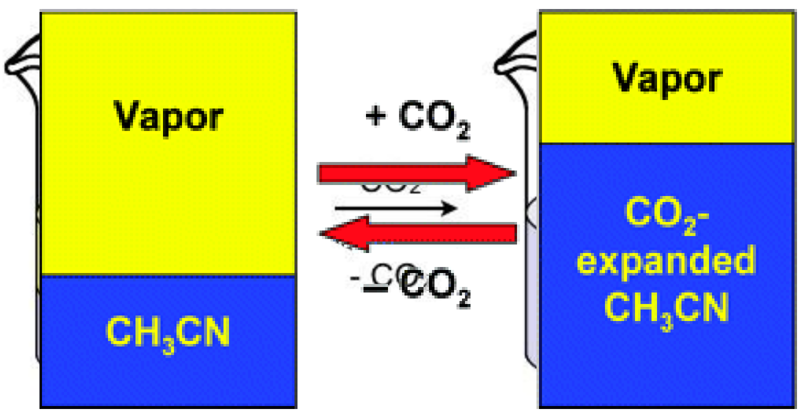


phosphatidylglycerol





2-monoacylglycerol



CO_2 Induced Solvents

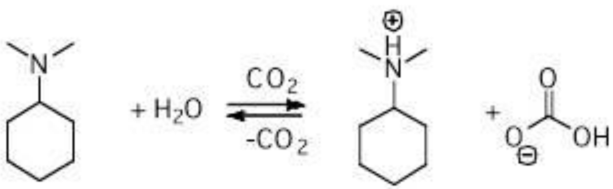
- Switchable Solvents (SHS)
- Gas-expanded liquids (CXL)

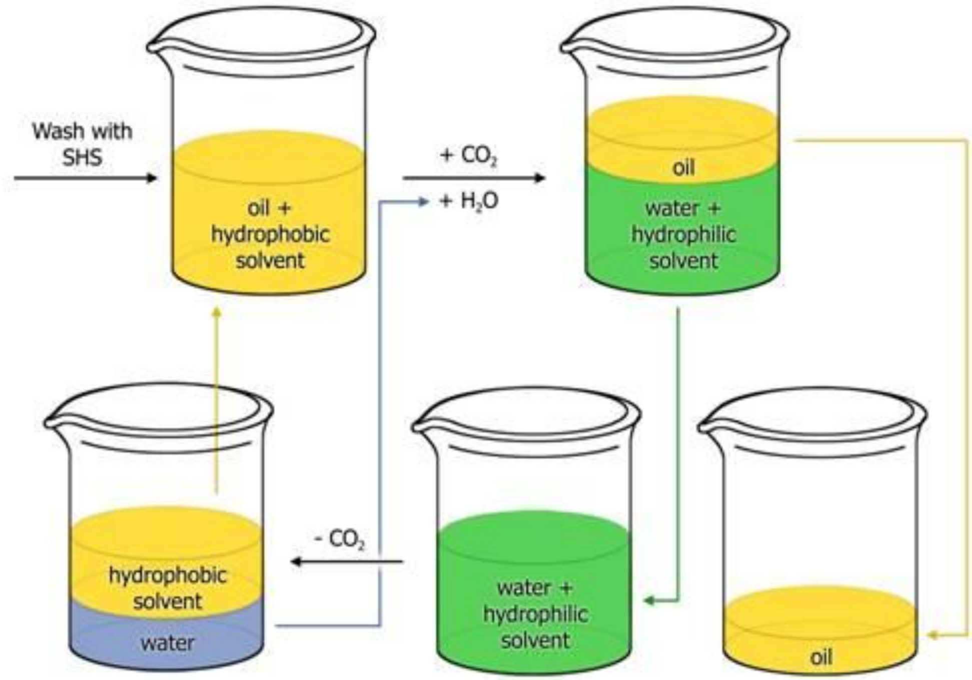
Jessop PG, Subramaniam B *Chem Rev* **2007**, *107*, 2666.
Ford JW, Janakat ME, Lu J, Liotta CL, Eckert CA *J. Org Chem* **2008**, ***73***, 3364.
Phan L, Brown H, Peterson T, White J, Hodgson A, Jessop PG *Green Chem* **2009**, *11*, 53.

Microalgal Bio-oil Extraction Using Switchable Hydrophilicity Solvents

Queen's University

Botryococcus braunii



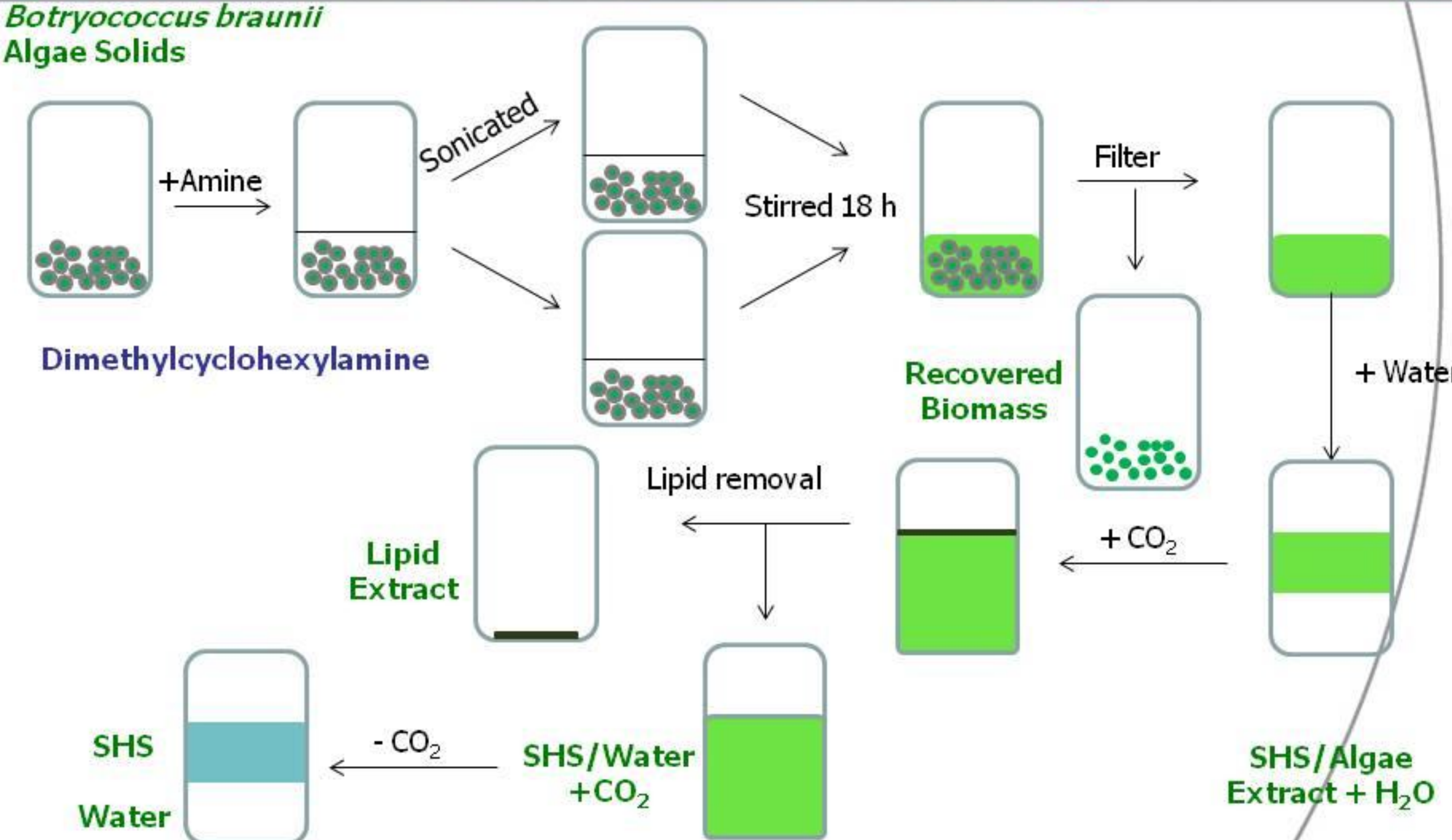


Dimethylcyclohexylamine

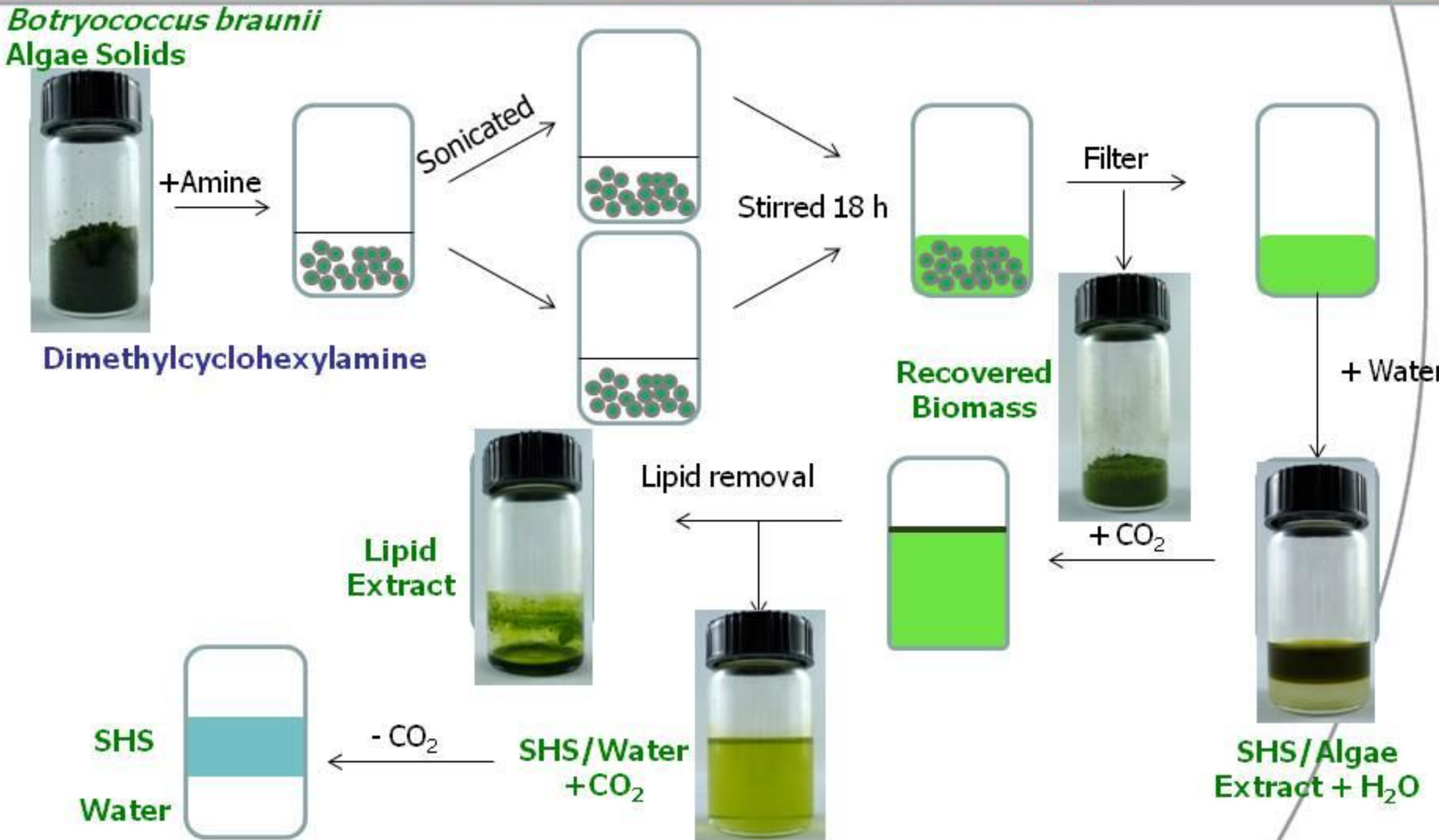
- low toxicity
- commercially available
- exhibits the properties required for an SHS

Boyd A, Champagne P, McGinn P, MacDougall K, Melanson J, Jessop P. *Bioresour Technol*, **2012**, 118, 628

SHS Extraction Procedure



SHS Extraction Procedure



CO_2 expanded methanol (CXL/GXL)

Queen's University

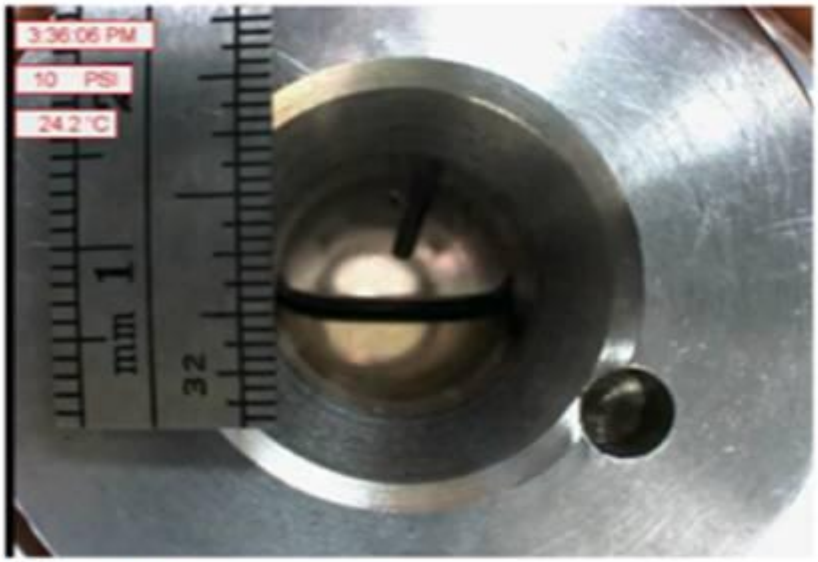


Figure 2a:
methanol in pressure gauge at 10 PSI (~ 0.7 atm)

Video

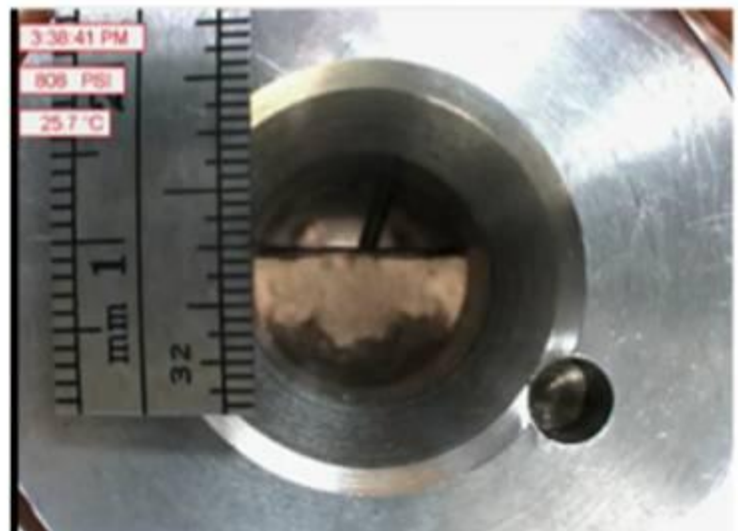


Figure 2b:
methanol under 808 PSI (~55 atm) CO_2 exhibits significant expansion

GXL/CXL (Methanol) – Experimental Procedure

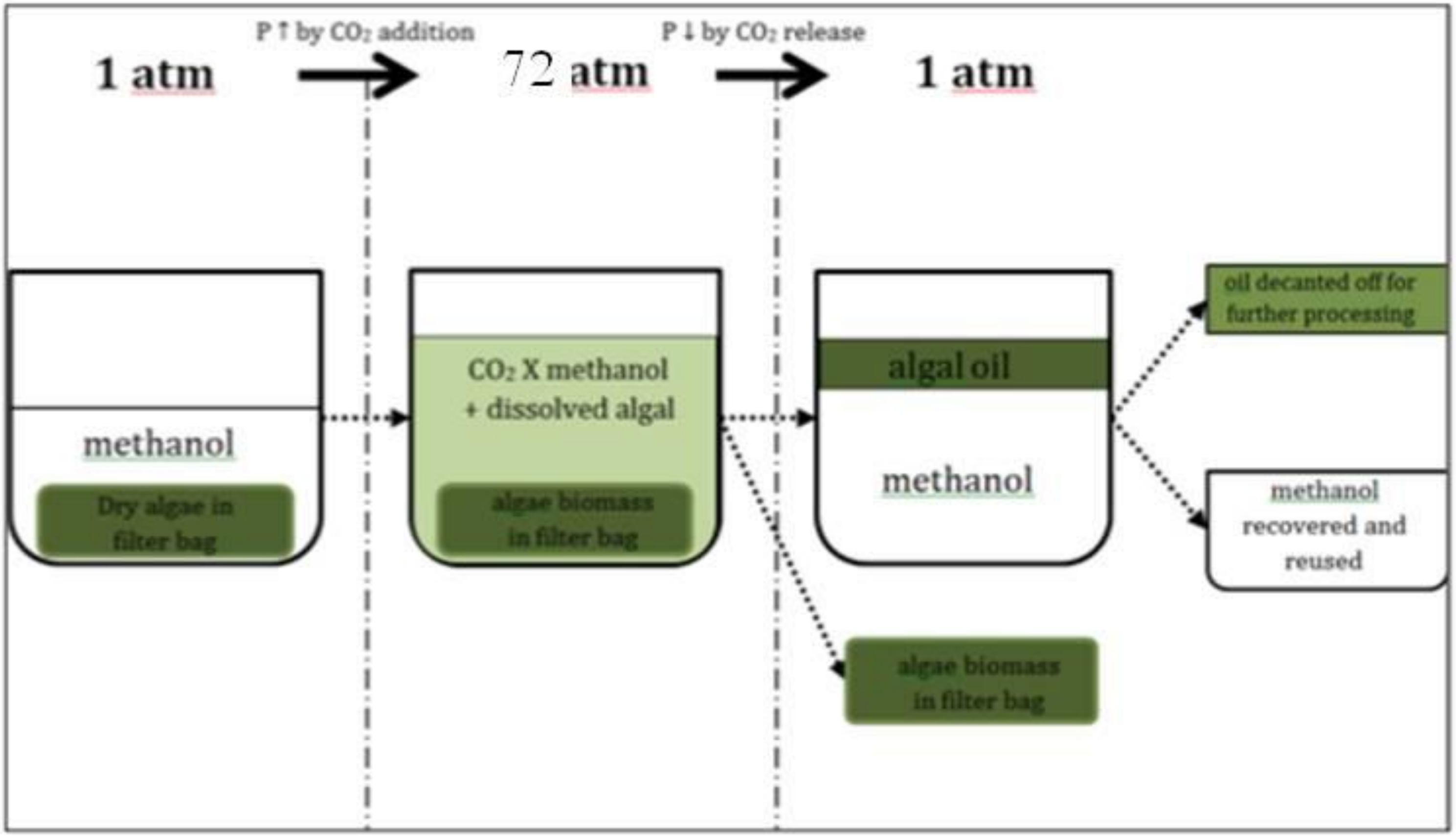
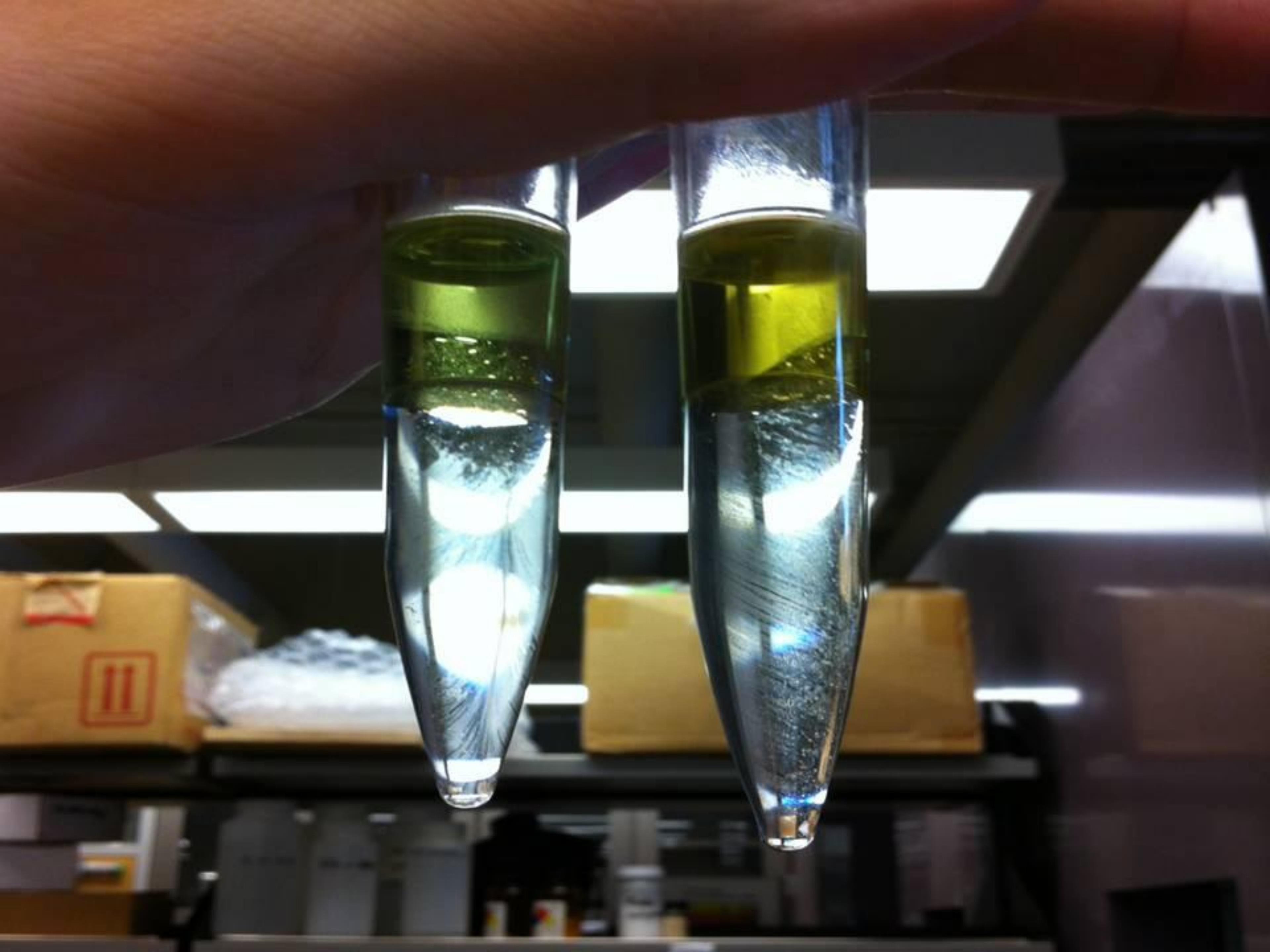


Figure 7: schematic depiction of the ideal GXL extraction process

Mezneric et al. 2011

GXL/CXL (Methanol) - Experiment



Conclusions - Microalgal Bio-Oil Extraction Using CO_2 Induced Solvents

- SHS and GXL for the extraction of lipids from microalgae have energy saving advantages
- Drying of SHS solvent is not required for the extraction since SHS is used in the presence of water
- SHS and GXL solvent is also removed/recovered without distillation as CO_2 is used to protonate the SHS allowing for phase separation from the lipids
- Use of CO2-expanded methanol may decrease the amount of methanol (or other polar solvent) needed for the extraction

Université de Montréal

Thank you for your attention!

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sur la nature
et les technologies
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Dr. Serge Guiot, Biotech. Res. Inst.
Drs. Pat McGinn, Steven O'Leary, NRC