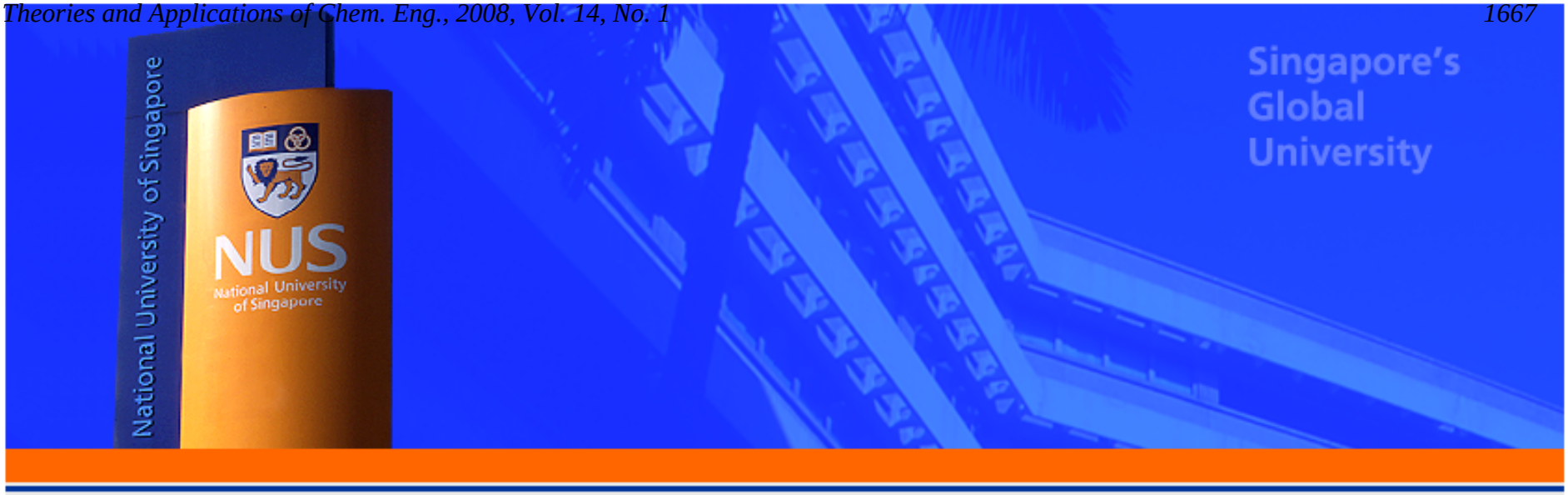

Screening of Microalgae Isolated from Marine Coastal Waters of Singapore for Production of Biodiesel Feedstock

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Tropical Marine Science Institute

NATIONAL UNIVERSITY OF SINGAPORE

- **Officially formed in April 1998 in response to a need for a centre of excellence in tropical marine science**
- **TMSI's marine research facility on St. John's Island was officially launched in September 2002**

Core research areas are:

- Environmental Sciences:
  Physical Sciences:
- Biological Sciences

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TMSI on St. John's Island

- **Facilities include**
  - laboratories (biofuels, analytical chemistry, biology, aquaculture)
  - Aquaria; mass algal culture facilities
  - Dormitories that can accommodate up to 20 people

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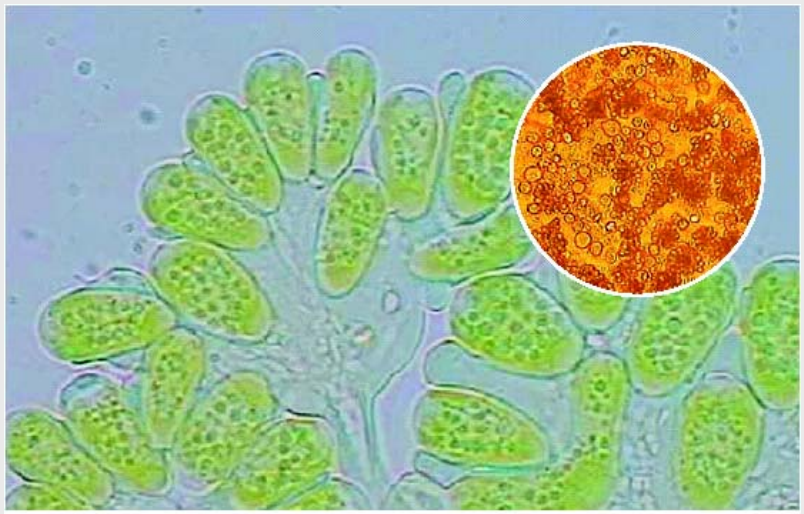
Biodiesel from Microalgae

- Some species of algae are suited to bio-diesel production due to their high oil content (some well over 50 per cent oil), with extremely fast growth rates.
- The National Renewable Energy Laboratory in the US has performed research on harvesting bio-diesel from algae farms.
- According to some estimates, the yield (per acre) of oil from algae is over 20 times the yield from the best-performing plant/vegetable oils.

Feedstock	Litres/hectare
Soybean	375
Rapeseed	1,000
Mustard	1,300
Jatropha	1,590
Palm oil	5,800
Algae	95,000 !!

(US National Renewable Energy Lab data)



The algae *Botryococcus braunii* under magnification, showing many of the natural oil particles in the algal cells. The inset shows the particles under x500 magnification. Jun Qin/Flinders University

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Research Interests

"Working on fast growing tropical species of marine microalgae as an alternative feedstock for biodiesel production"

- Optimization of algae growth and lipid production under phototrophic conditions
- Enhanced algal harvesting and lipid extraction
- Enhancement of lipid production with the use of metabolic engineering
- Genetic manipulation of algae to enhance lipid production

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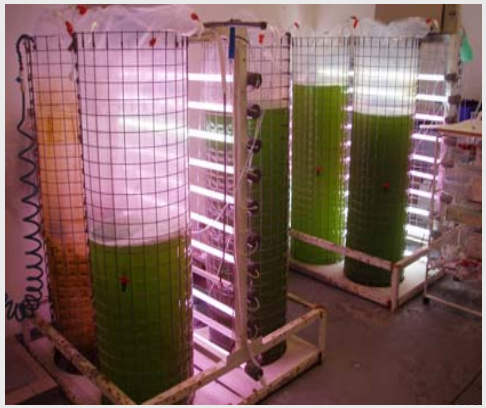
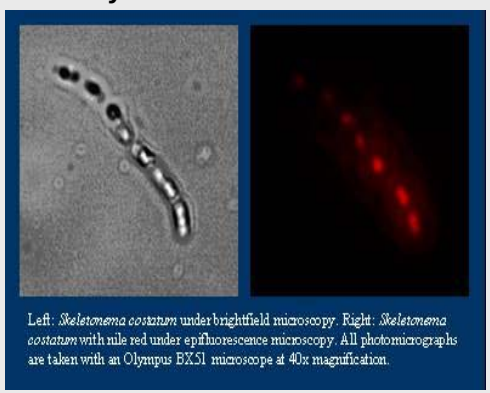
Research Facilities at TMSI



Mass algal culture

Flow cytometer

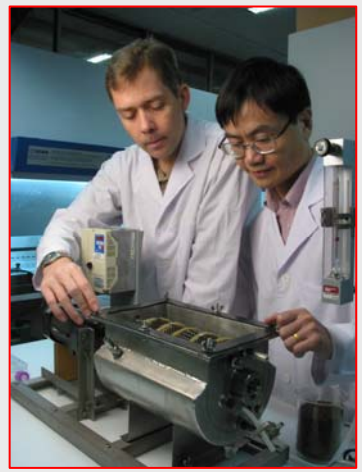


Left: *Skeletonema costatum* under brightfield microscopy. Right: *Skeletonema costatum* with nile red under epifluorescence microscopy. All photomicrographs are taken with an Olympus BX51 microscope at 40x magnification.

Fluorescence staining of microalgae

- Mass algal culture suite;
- Full Microscopy suite;
- Mass seawater flow system (filtered, sterile);
- Fluorescence staining and spectrometry suite;
- Flow cytometry (cell counting and isolation);
- Analytical suite (inc. GCMS-MS and LC MS-MS);
- Photobioreactors;
- Transesterification micro-reactor, rotating biological contactor (for biocatalysis).

Growth reactor



Rotating biological contactor

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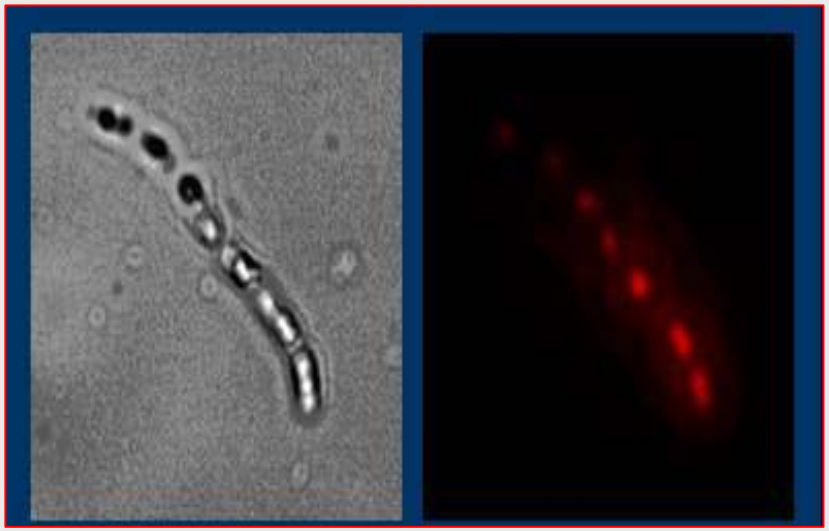
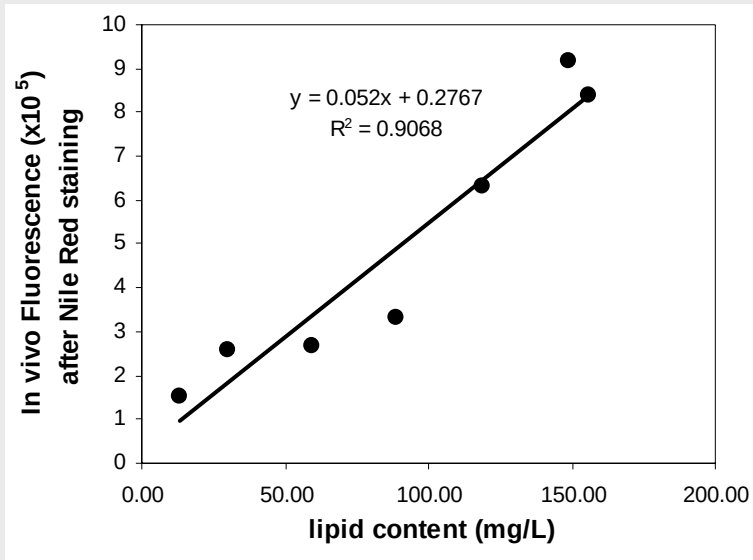


Bright field microscopy Epifluorescence microscopy



Lipid Characterization

- **Total lipid: Gravimetric method**
- **Neutral lipid: Fluorescence by Nile Red**
- **Lipid Profile: One step transesterification followed by GC-MS analysis**

➢Ubiquitous in the marine environment, particularly in the waters of Singapore

Figure: Photographs of different strains taken after Nile Red staining

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Gravimetric lipid content of 10 local microalgae strains

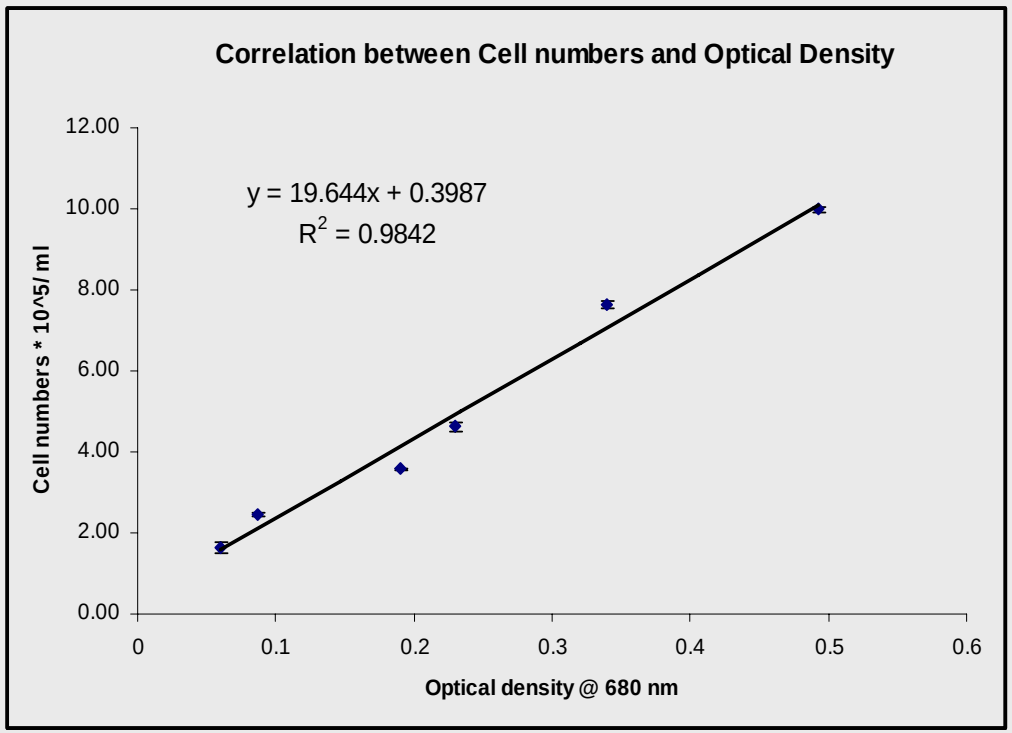
Local strain	Lipid concentration ±SD (mg/L)	Lipid content ± SD (%,w/w dry biomass)
B1 (40)	41.75±2.75	44.52±1.72
1	8.33±1.75	9.49±1.65
4	13.78±4.96	17.79±3.75
5	2.78±2.06	0.17±0.09
18	6.46±2.32	2.65±0.39
19	4.11±6.49	0.31±0.49
30	4.56±0.26	1.58±0.21
34	9.04±0.71	16.27±4.03
35	2.23±1.64	2.22±1.61
37	2.06±0.42	-

Marine Microalgae

- Chain-forming (Small cells linked with external tubes)

Optimization of growth rate for Diatom, B1

- Modification of culture parameters was compared w.r.t. f/2 media
- Optical Density of the culture at 680nm was used as an alternative tool to determine cell concentrations.



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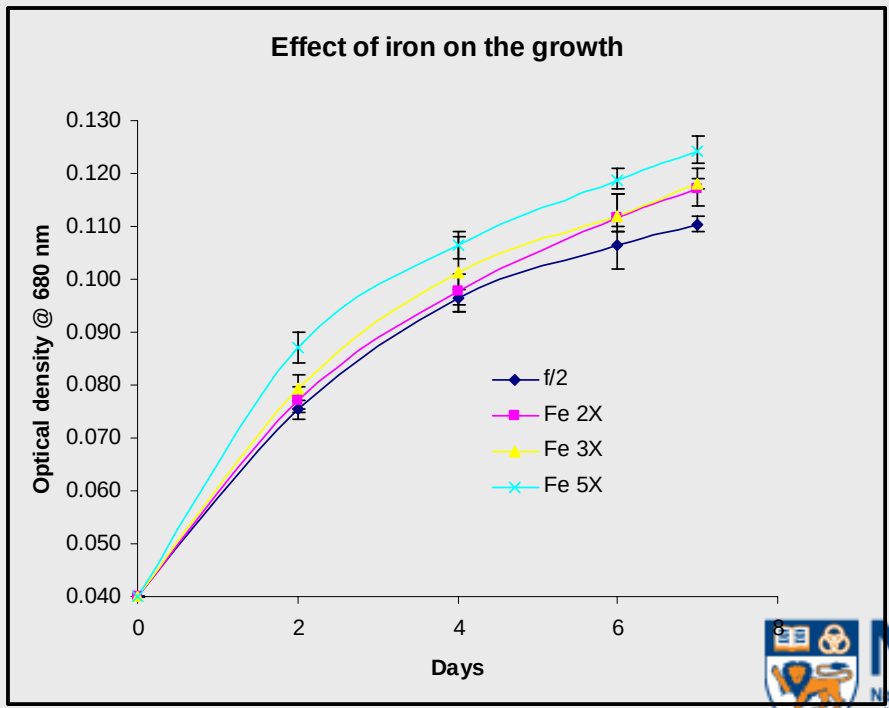
Optimization of diatom B1

Effect of iron

- In f/2 media ferric chloride was used (as 1×10^{-5} M)
- Effect of iron on the growth rate was studied by increasing the iron concentration of f/2 media by 2X, 3X and 5X times.
- Ferric Citrate was added to balance the iron concentration.

Media	Iron Conc. (M)
f/2	1×10^{-5}
Fe 2X	2×10^{-5}
Fe 3X	3×10^{-5}
Fe 5X	5×10^{-5}



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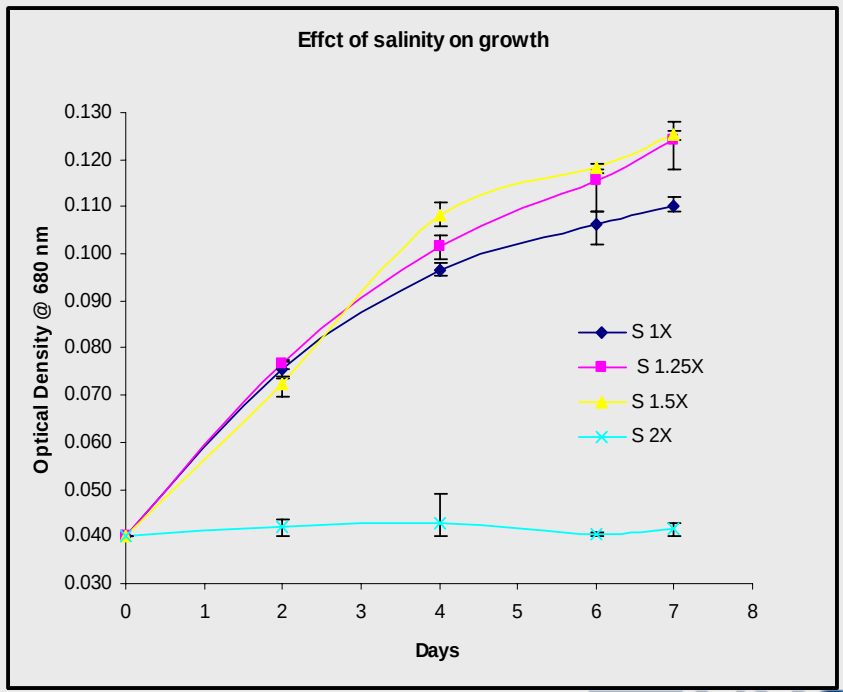
Optimization of diatom B1 cont.

Effect of salinity

- f/2 media was prepared in normal seawater
- Salinity was increased to 1.25X, 1.5X and 2X times of normal seawater and its effect on growth rate was compared with normal seawater.
- Supplemented seawater (Salinity 2X times) was added accordingly to normal seawater to adjust the salinity

Supplemented Seawater	
Chemicals	**Amounts (in 1L seawater)**
NaCl	23 gm
$MgCl_2.6H_2O$	5 gm
Na_2SO_4	4 gm

Media	Salinity ppt
f/2 or S 1X	33
S 1.25X	41.25
S 1.5X	49.5
S 2X	66



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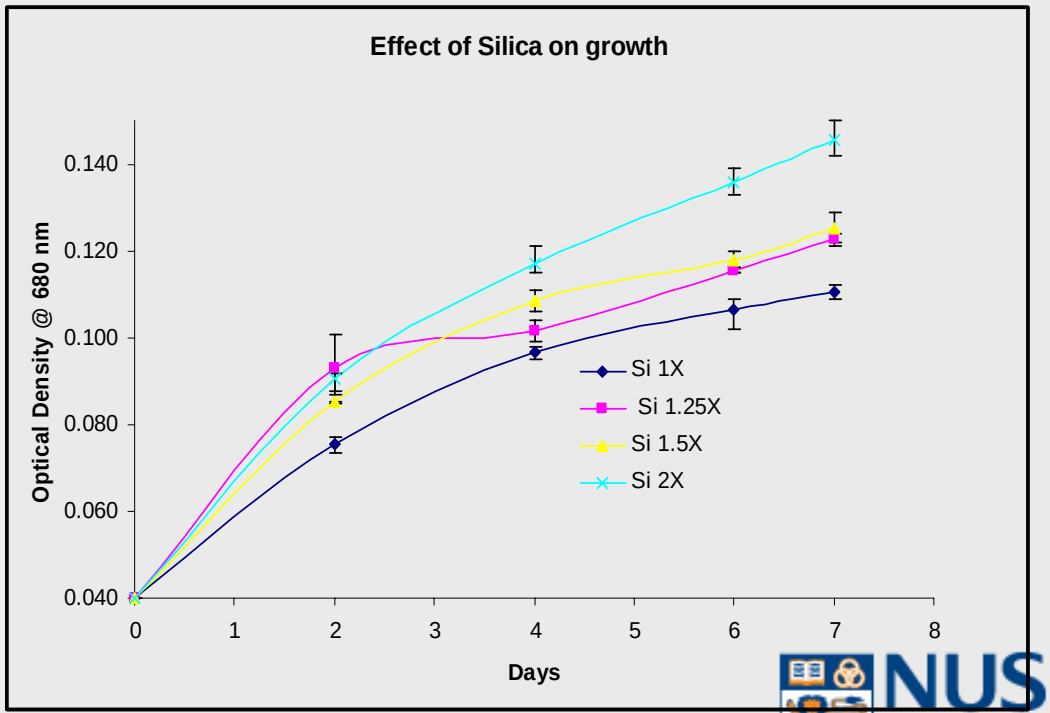
Optimization of diatom B1 cont.

Effects of Silica

- In f/2 media silica conc. was 1.07 x 10^{-4} M
- Silica concentration of f/2 media was increased by 1.25X, 1.5X and 2X times and compared with f/2.
- In all media Sodium Silicate was used to adjust the silica concentration.

Media	Silica Conc. (M)
f/2 or Si 1X	1.07 x 10^{-4}
Si 1.25X	1.34 x 10^{-4}
Si 1.5X	1.60 x 10^{-4}
Si 2X	2.14 x 10^{-4}



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Lipid Profile and Quantification

Strain: B1

- Total lipid: 50±2% of dry biomass
- Neutral lipid: 37±3% of dry biomass

Lipid Profile

FAMEs	% of FAMEs
C14 : 0	10.3
C15 : 0	0.16
C16 : 0	33.04
C16 : 1	36.78
C18 : 0	0.21
C20 : 5	16.04
Total Unsaturated	52.82
Total Saturated	47.18

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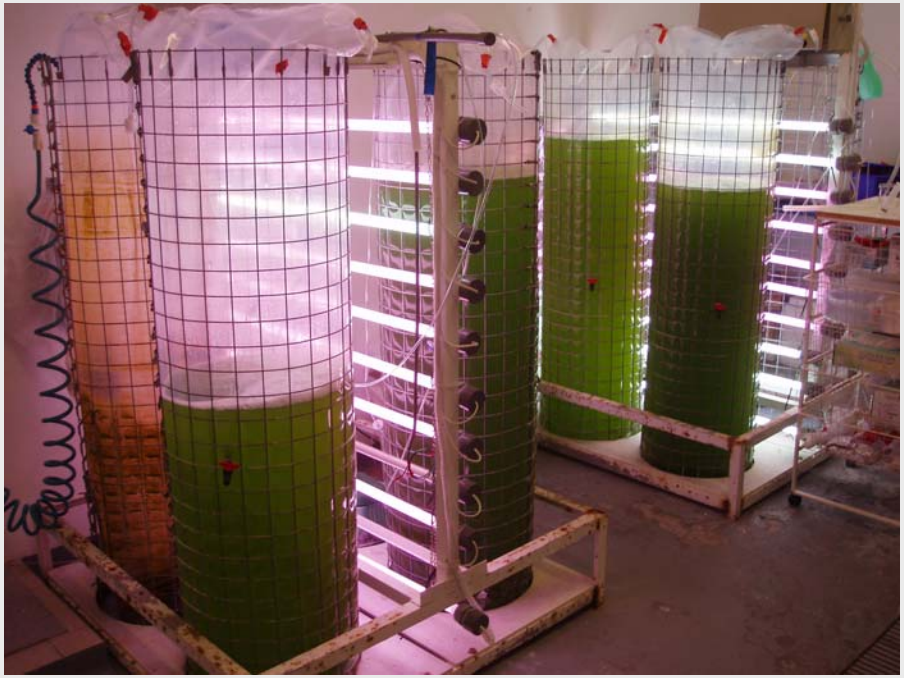

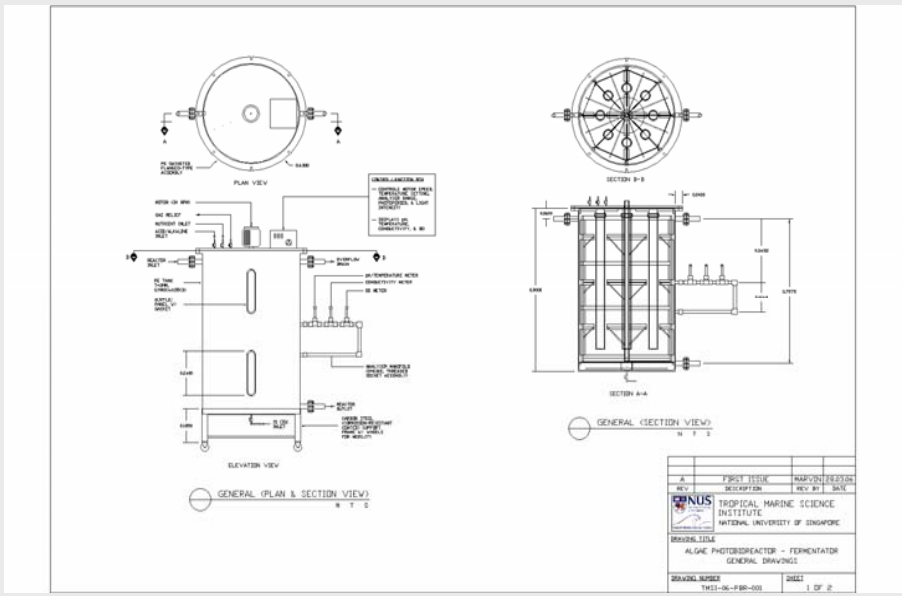

Algal Mass Culture & Photobioreactors

- Variable parameters (light intensity, temperature, photoperiod, pH, gas flux, rotational speed)
- Designed specifically for optimization of algae growth and increased harvest

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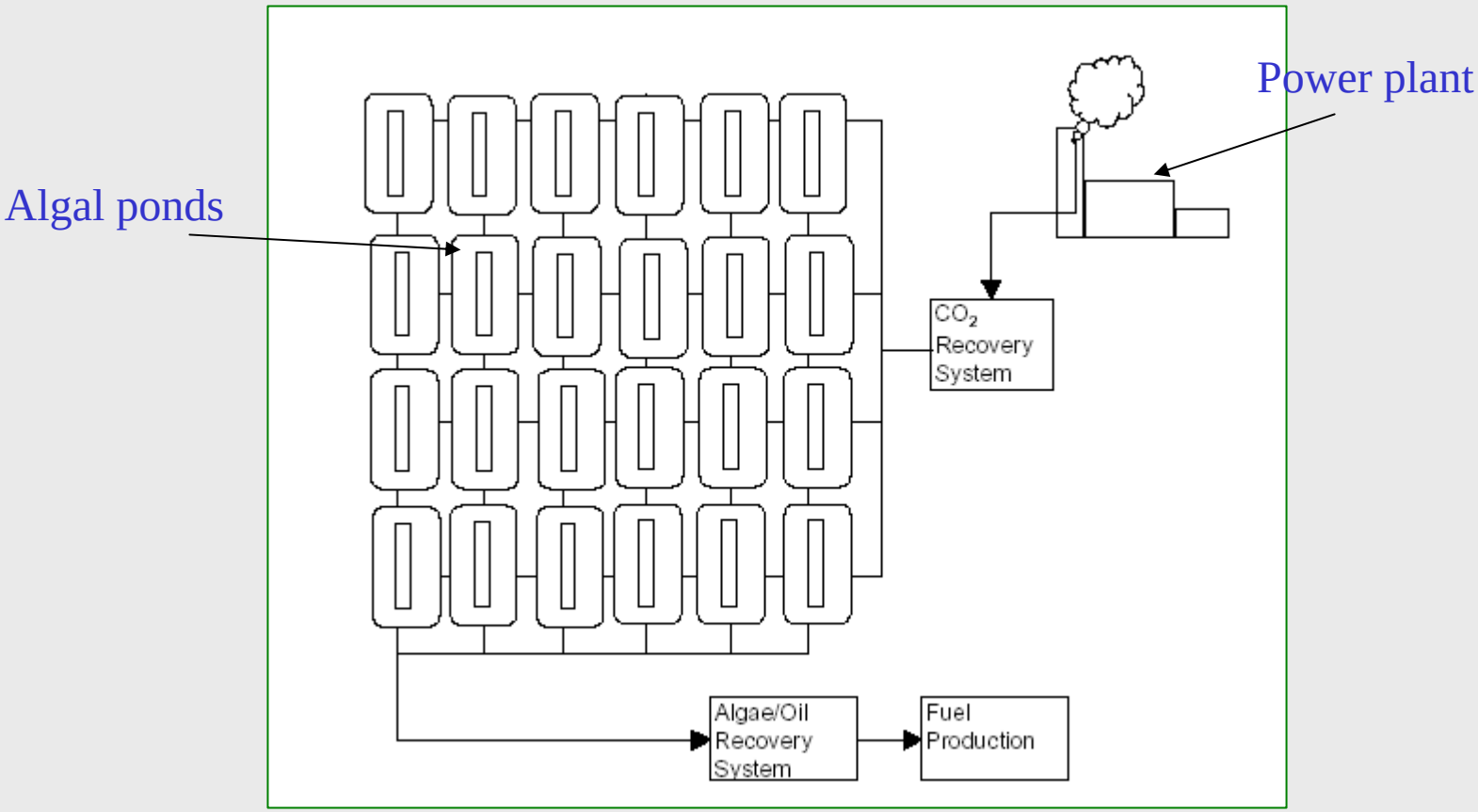
Carbon Capture & Sequestration, Biofuels



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