Growth of Algae & Microbiome Cultures on Anaerobic Digester Centrate

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Research Program: ‘Greening’ Algal Biofuels Processes
Green & Sustainable Microalgal Biofuel & Bioenergy Production

Enhanced micro-algae production

Cement Production Plant
- Waste heat
- Organic C, N, & P

Wastewater Treatment Plant
- Waste heat
- Organic C, N, & P

Bio-oil, HMF, CMF Production
- HTL
- Organic C, N, & P

Bio-oil, Biodiesel
- Lipids

Anaerobic Digestion
- Biogas
- Syngas
- H₂
- Solid Residual

Bio-oil, Biojet Fuel
- Biopolymers
Green & Sustainable Microalgal Biofuel & Bioenergy Production

- Enhanced micro-algae production
  - Flue gas CO₂
  - Waste heat
  - Organic C, N, & P

- Bio-oil Extraction
  - CO₂ Induced Solvents
  - Organic C, N, & P
  - HTL

- Bio-crude, HMF, CMF Production
  - Waste heat
  - Bio-oil, Biojet Fuel Biopolymers

- Anaerobic Digestion
  - Biogas Syngas H₂
  - Solid Residual

- Lipids Biodiesel
  - Glycerol

- Cement Production Plant
  - Waste heat

- Wastewater Treatment Plant
  - Waste heat
  - Organic C, N, & P
"Growing Energy from Waste: A Natural Twist on Direct Potable Reuse"

dry-futures-pragmatic-category

ALGAE SYSTEMS
Demonstration Plant
Daphne, Alabama
[30.6311° N, 87.8864° W]

Wastewater → Treated Wastewater

Water

Dewatering Process

Floating Algae Bags

Algae Biosolids

Photos are from the Algae Systems demonstration plant in Mobile Bay, Alabama. Operational since 2012. Treating 40,000 gallons per day from the Daphne Municipal Utility and producing third-party certified bio-crude oil. Discharging water that exceeds water quality standards water that could be reused.

Treated Wastewater → Title 22 Water

WATER PRODUCTION

ENERGY PRODUCTION

+ Micro-Filtration
+ Reverse Osmosis
+ UV

Potable Water

+ Anaerobic Digestion
+ Cogeneration

Electricity

Bio-Crude Oil

Hydrothermal Liquefaction
Microalgae as an Alternative for Crude Oil

• Pros
  – No arable land required
  – Possible alternative to many petroleum-derived chemicals
  – Photoautotrophic, heterotrophic and/or mixotrophic
  – High yields (g/m²/Year)
  – Many possible by-products

• Cons
  – Water intensive
  – Fertilizer intensive
    • Peak phosphate
    • Competition with food crops
  – Low productivity (g/L/Day)
  – Harvesting is the technological challenge to be addressed
Wastewater Treatment & Microalgae

• Wastewater treatment
  – Infrastructure already in place
    • Water/solid separation machinery
    • Qualified personnel
  – Free access to water
  – Free access to macro and micro nutrients
  – Wastewater treatment credits
  – Mixotrophic cultivation
    • Higher yield and productivity
Microalgae & Wastewater Treatment

• Focus on algal biomass production
  – Optimization of culture condition
    • High biomass yield
    • Not necessarily ideal from a wastewater treatment perspective

• Focus on wastewater treatment
  – Tertiary treatment
    • Sequential process
    • Decrease total N and P of the discharged effluent
  – Enhancement of wastewater treatment system
    • Nutrient removal from anaerobic digester effluent
    • Decrease the nutrient load at the secondary treatment stage
Metro Vancouver Simplified Process

<table>
<thead>
<tr>
<th>Parameter</th>
<th>2013 Raw Influent</th>
<th>Average</th>
<th>2013 Effluent</th>
<th>Parameter</th>
<th>Average</th>
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<td>33.0</td>
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<tr>
<td>PO4-P (mg/L)</td>
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<td>2.43</td>
<td>PO4-P</td>
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<table>
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<th>Parameter</th>
<th>2013 Centrate</th>
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<td>Mg (mg/L)</td>
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<tr>
<td>NH3-N (mg/L)</td>
<td>1,385</td>
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<tr>
<td>PO4-P (mg/L)</td>
<td>207</td>
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</tbody>
</table>
Metro Vancouver Alternative Process

Influent → Primary treatment → Primary effluent channel → Secondary treatment → Effluent

Primary treatment:
- Primary effluent channel
- Sludge

Secondary treatment:
- Thickener
- AD
- Centrate
- Inject CO₂

Microalgae Treatment Process:
- Microalgal biomass
- Co-digestion Or Processed by 3rd party

Biosolids
Generation of Adapted Microbiomes

Microbiome Source
(Annacis WW Effluent + Centrate)

10% Centrate

- Enrichment Process
- Filtration Process

Raw

Filtered \( \phi 2.7 \mu m \)

MVA10

MVB10

20% Centrate

Raw

Filtered \( \phi 2.7 \mu m \)

MVA20

MVB20

Microbiomes
Performance of Algal Monoculture vs Algal-Based Microbiomes

• Generation of Adapted Algal-Based Microbiomes
  – Annacis WWTP (Vancouver, BC)
  – Secondary Effluent as Microbiome Source

• Four Microbiomes
  – Filtered, Non-filtered,
  – Enrichment with 10% or 20% Centrate

• Two Monocultures
  – Chlorella Sp.
  – Scenedesmus Sp.

• Different Concentrations of Centrate
  – 5% - 10% - 20% - 35%

• Evaluated Nutrient Removal
  – Phosphate, Ammonium, Nitrate, Nitrite
Mean value of biological triplicates.
Error bars are shown when the variation of the values are significant.
Performance of Different Strains/Microbiomes

- Monocultures underperformed compared to all adapted microbiomes
- Microbiomes derived from raw effluent (MVA10 and MVA20) consistently outperformed microbiomes produced with filtered effluent (MVB10 and MVB20)
- Biomass production at 20% of centrate
  - MVA10: 1.7g/L DCW in 9 days (0.19g/L/day)
  - MVA20: 1.8g/L DCW in 8 days (0.22g/L/day)
- Biomass production at 35% of centrate
  - MVA10 and MVA20: 1.8g/L DCW in 7 days (0.25g/L/day)
Growth Performance per Type of Consortium

Mean value of biological triplicates. Error bars are shown when the variation of the values are significant.
Growth Performance Under Different Centrate Concentrations

- No significant difference between unfiltered adapted microbiomes MVA10 and MVA20
- The centrate adapted microbiomes consistently underperformed when the centrate concentration was below 10%
- Adapted Microbiome from filtered effluent enriched with 20% centrate (MVB20) underperformed when cultivated on 5% centrate
- No significant difference in performance between growth on 20% or 35% of centrate
Mean value of biological triplicates.
Error bars are shown when the variation of the values are significant.
Nutrient Removal

• Ammonium Removal
  – MVA10 outperformed other microbiomes and monocultures
  – Minimum concentrations reached by day 7
  – Monocultures and adapted microbiomes could not completely remove ammonium

• Nitrate/Nitrite Removal
  – Control was stable throughout the experiment
  – after 10 days, nitrate/nitrite concentrations were barely detectable
  – All except MVB10 showed a peak above the control on the fourth day. Presumably due to nitrification
  – All adapted microbiomes exhibited faster nitrate/nitrite removals than the monocultures
Nutrient Removal

• Phosphate Removal
  – Adapted microbiomes exhibited faster phosphate removals than monocultures
  – Phosphate concentrations stable after 7 days for all adapted microbiomes
  – Adapted unfiltered microbiomes MVA10 and MVA20 were more efficient in the removal of phosphate than adapted filtered microbiomes MVB10 and MVB20
  – Monocultures presented a steady removal rate of phosphate
Variations in Light Intensity & CO₂ Supplementation
• At low CO₂ concentrations, light intensity did not limit growth performance
• Higher CO₂ concentrations did not necessarily improve the performance of the adapted microbiomes
• *C. vulgaris* under performed all adapted microbiomes under all conditions
• Unfiltered adapted microbiomes (MVA) were generally more robust
## Microbiome Analysis

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Microbiome MVA20</th>
<th>Microbiome MVB20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kingdom Bacteria</td>
<td>11,245 (37.3%)</td>
<td>9,015 (18.3%)</td>
</tr>
<tr>
<td>Kingdom Plantae</td>
<td>10,088 (33.5%)</td>
<td>28,700 (58.1%)</td>
</tr>
<tr>
<td>Kingdom Fungi</td>
<td>8,775 (29.1%)</td>
<td>11,658 (23.5%)</td>
</tr>
<tr>
<td>Kingdom Chromista</td>
<td>9 (0.03%)</td>
<td>13 (0.03%)</td>
</tr>
</tbody>
</table>

Microbiome Analysis

MVA20

- Kingdom Bacteria: 37%
- Kingdom Plantae: 29%
- Kingdom Fungi: 34%
- Kingdom Chromista: 0%

MVB20

- Kingdom Bacteria: 58%
- Kingdom Plantae: 18%
- Kingdom Fungi: 24%
- Kingdom Chromista: 0%
Microbiome Analysis: Kingdom Plantae

Microbiome MVA

Microbiome MVB
Conclusions

• Centrate adapted microbiomes exhibited higher biomass productivities than monocultures when cultivated in secondary wastewater effluent enriched with centrate

• Adapted microbiomes produced by raw secondary wastewater are more robust than microbiomes produced from filtered secondary wastewater effluent

• Centrate adapted microbiomes exhibited higher or equivalent nutrient removal capabilities

• Unfiltered adapted microbiomes (MVA) were generally more robust and less sensitive to fluctuations in light intensity and CO$_2$ concentrations.
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QUESTIONS?
Biofuels: A Current Need

- Transportation sector uses 28% of the primary energy
- 71% of the petroleum is used for the transportation sector
- US imports 60% of its needs
- Canada imports 55% of actual needs
- The Canadian Renewable Fuels regulations:
  - 2% of renewable fuel in diesel
  - 5% of renewable fuel in gasoline
Primary Energy Consumption by Source & Sector

(Quadrillion Btu)

Source

Percent of Sources

Total = 97.5

Percent of Sectors

Transportation
26.9
(28%)

Industrial
21.5
(22%)

Residential & Commercial
10.7
(11%)

Electric Power
38.4
(39%)

Sector

Petroleum
35.1
(36%)

Natural Gas
26.6
(27%)

Coal
18.1
(19%)

Renewable Energy
9.3
(10%)

Nuclear Electric Power
8.3
(8%)
Crude Oil Dependence: Economical Impacts

- Peak oil may be close
- Political instability of major oil exporters
- Challenging technology required for new oil reserves
  - Canadian Oil Sands
  - Brazilian Pre-salt
- Instability in crude oil prices
Microalgae as an Alternative for Crude Oil
Methods For Nutrient Detection

• Nutrient Removal
  – Ammonium: Nessler’s Reaction
  – Nitrate/Nitrite: Diphenylamine Method
  – Phosphate: Malachite Green Method
• Analysis of performance under variation of:
  – Light Intensity
    • 70W/m²/s; 105W/m²/s and 155W/m²/s
  – CO₂
    • 5% and 10%

• All variable tested independently
Microbiome Analysis

- DNA analysis made for microbiomes prepared with filtered and non-filtered wastewater secondary effluent enriched with 20% centrate

  - Total DNA extracted
    - Modified bead beater method
      - +DTAB; +chloroform; +buffer

  - DNA purification
    - Silica adsorption method

  - Total DNA sequencing
    - 454 Sequencing Technology
    - Three sets of primers
      - Aiming for Fungi, Bacteria and Algae
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Utilities Kingston