Growth of Algae & Microbiome Cultures on Anaerobic Digester Centrate

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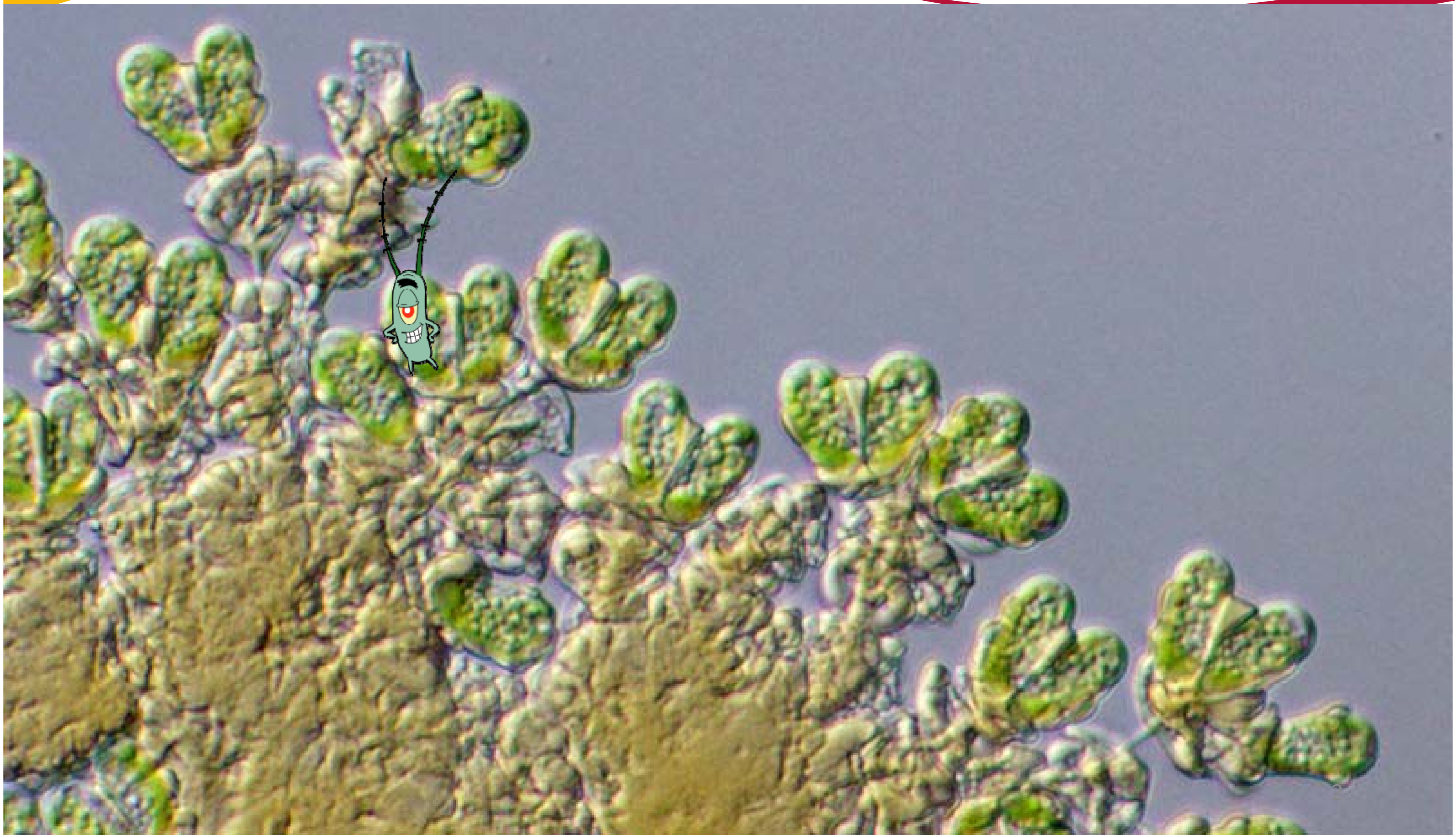
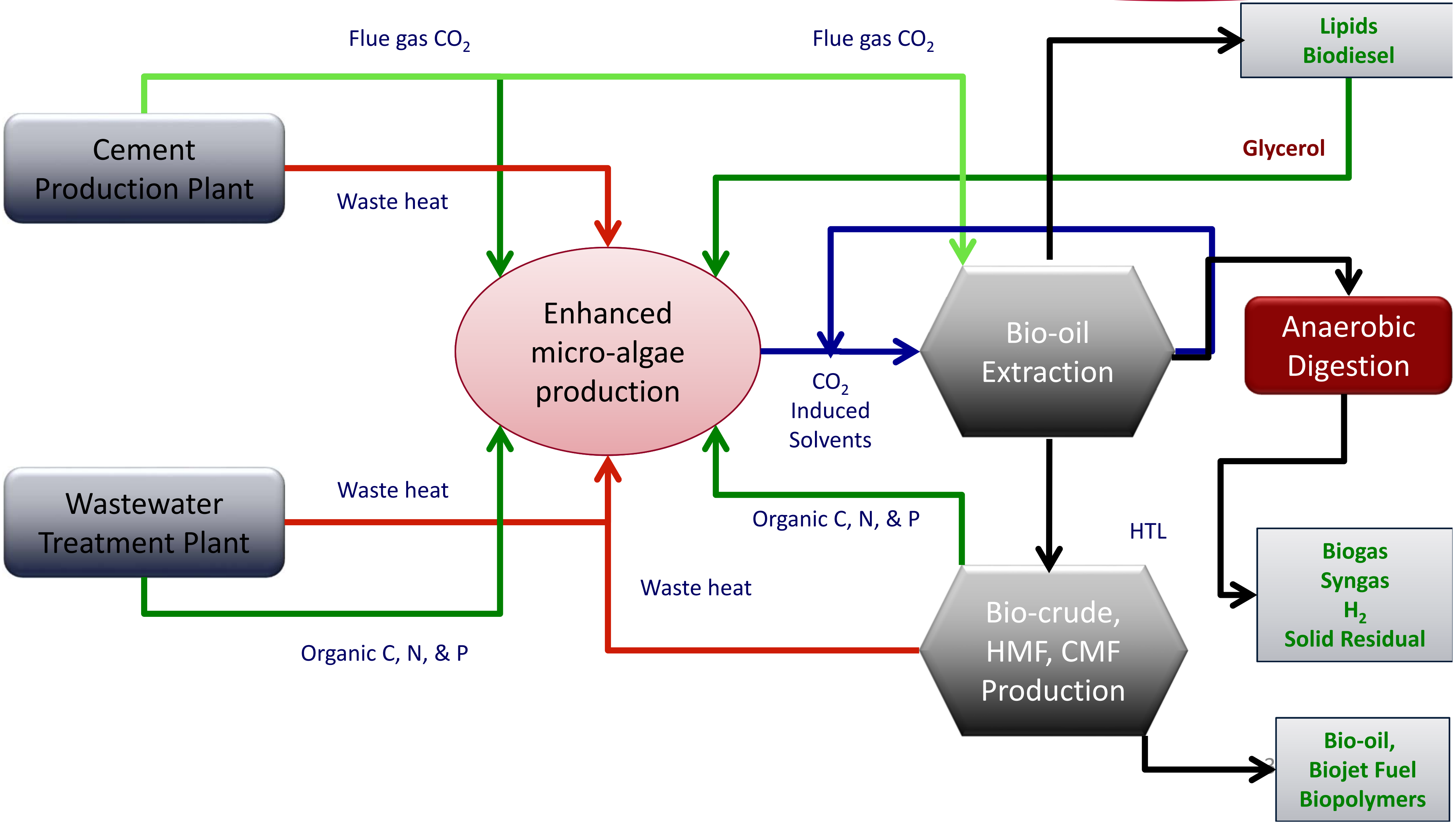
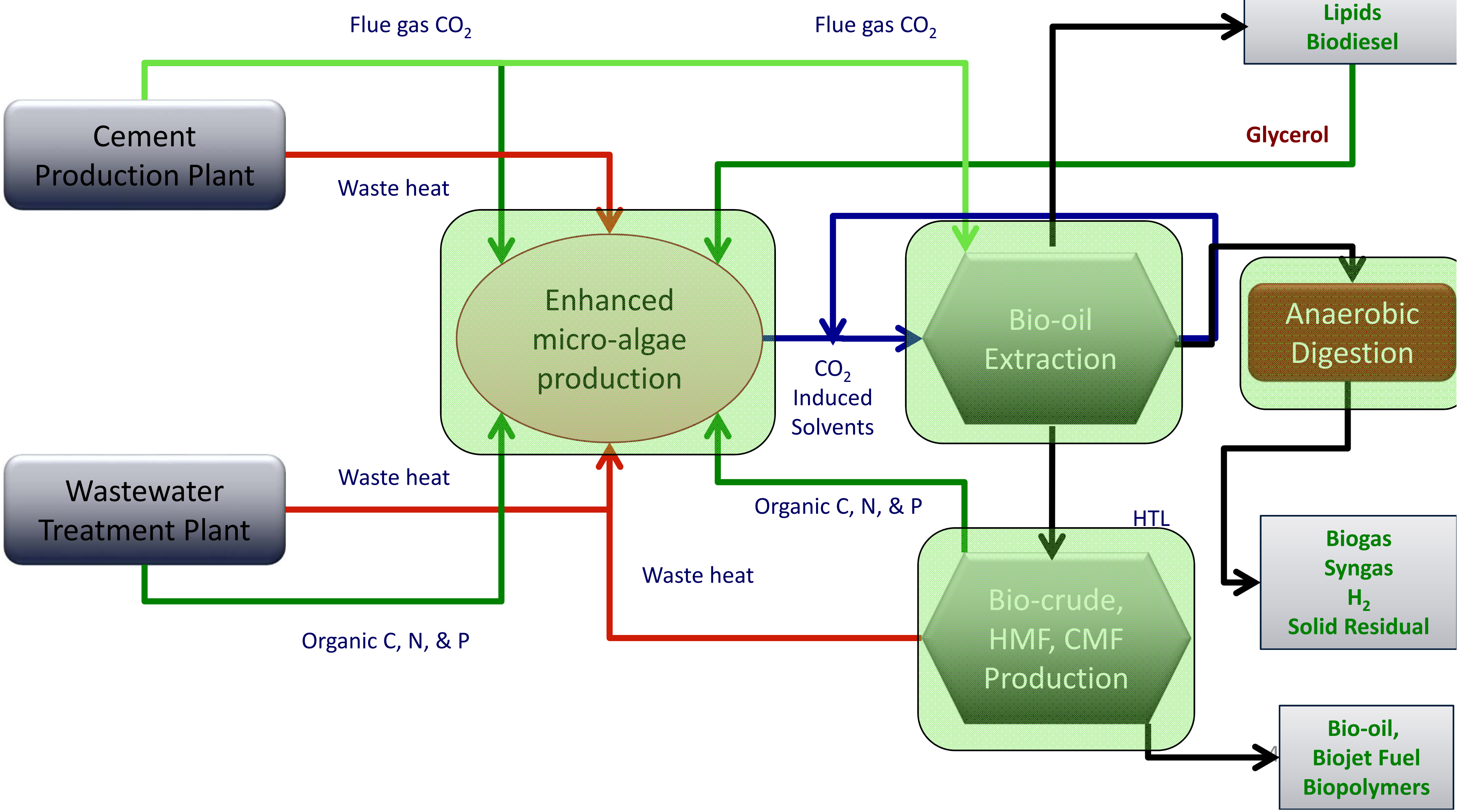
Research Program: 'Greening' Algal Biofuels Processes



Green & Sustainable Microalgal Biofuel & Bioenergy Production

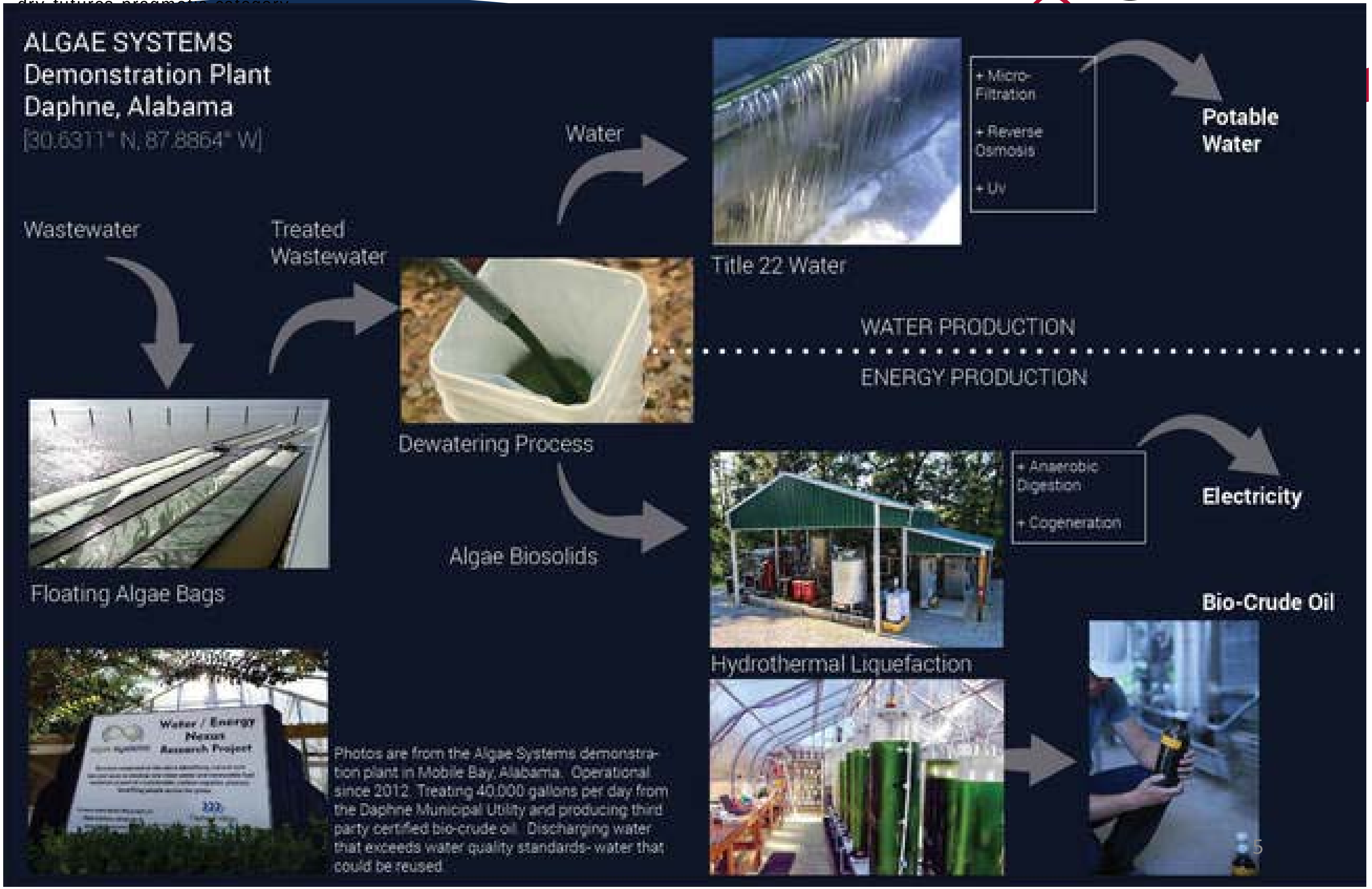


Green & Sustainable Microalgal Biofuel & Bioenergy Production



"Growing Energy from Waste: A Natural Twist on Direct Potable Reuse"

http://archinect.com/news/article/137816376/growing-energy-from-waste-a-natural-twist-on-direct-potable-reuse-an-honorable-mention-in-



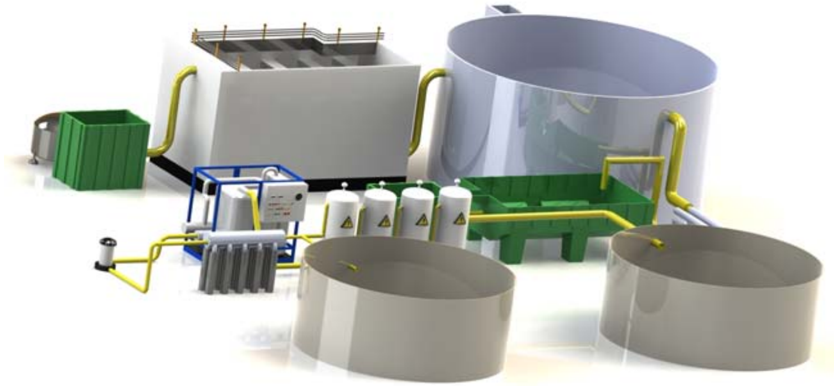
Microalgae as an Alternative for Crude Oil

- Pros
  - No arable land required
  - Possible alternative to many petroleum-derived chemicals
  - Photoautotrophic, heterotrophic and/or mixotrophic
  - High yields ($g/m^2/Year$)
  - 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

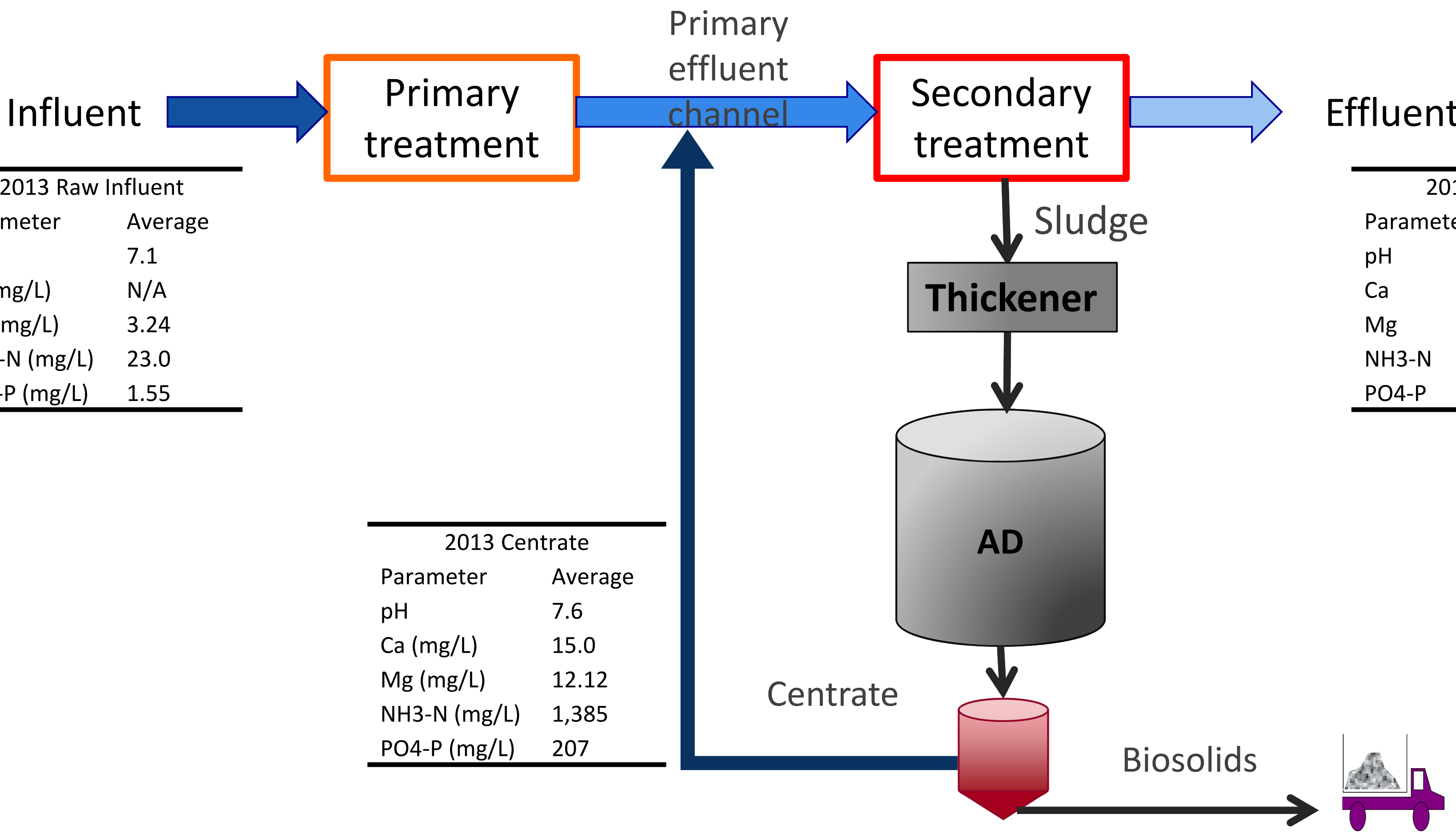


Microalgae & Wastewater Treatment

- 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

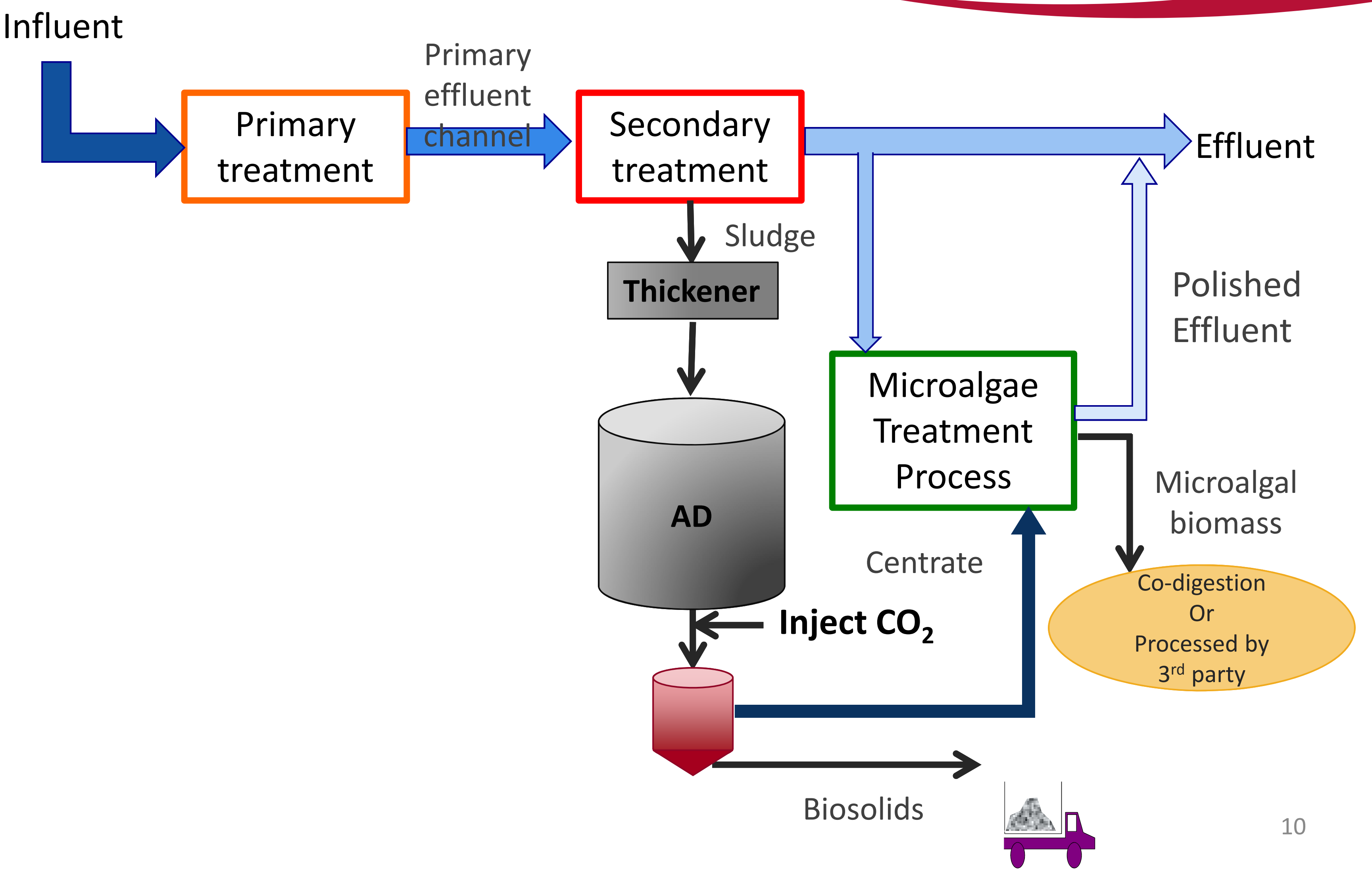


2013 Raw Influent	
Parameter	Average
pH	7.1
Ca (mg/L)	N/A
Mg (mg/L)	3.24
NH3-N (mg/L)	23.0
PO4-P (mg/L)	1.55

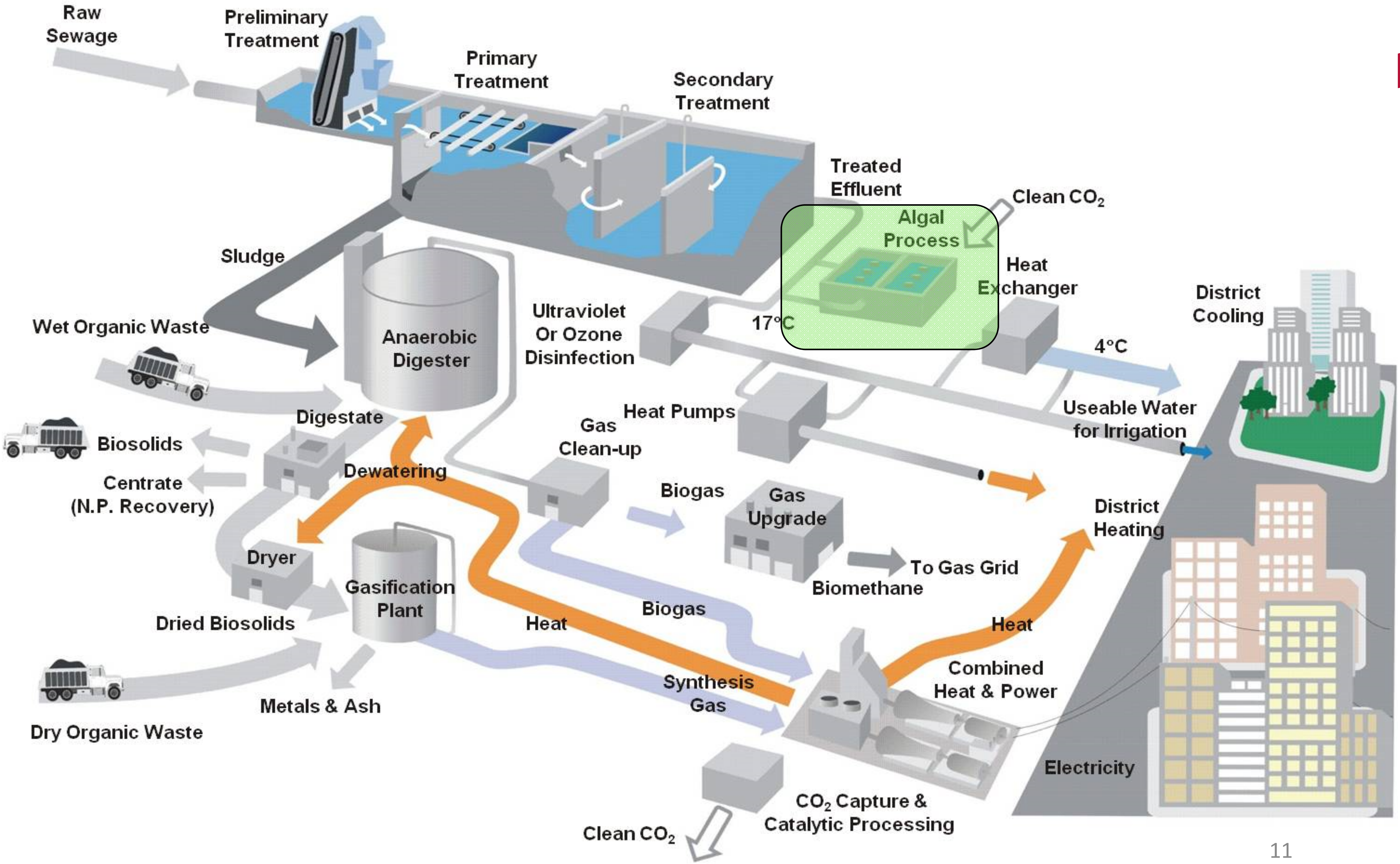
2013 Effluent	
Parameter	Average
pH	7.7
Ca	N/A
Mg	3.26
NH3-N	33.0
PO4-P	2.43

2013 Centrate	
Parameter	Average
pH	7.6
Ca (mg/L)	15.0
Mg (mg/L)	12.12
NH3-N (mg/L)	1,385
PO4-P (mg/L)	207

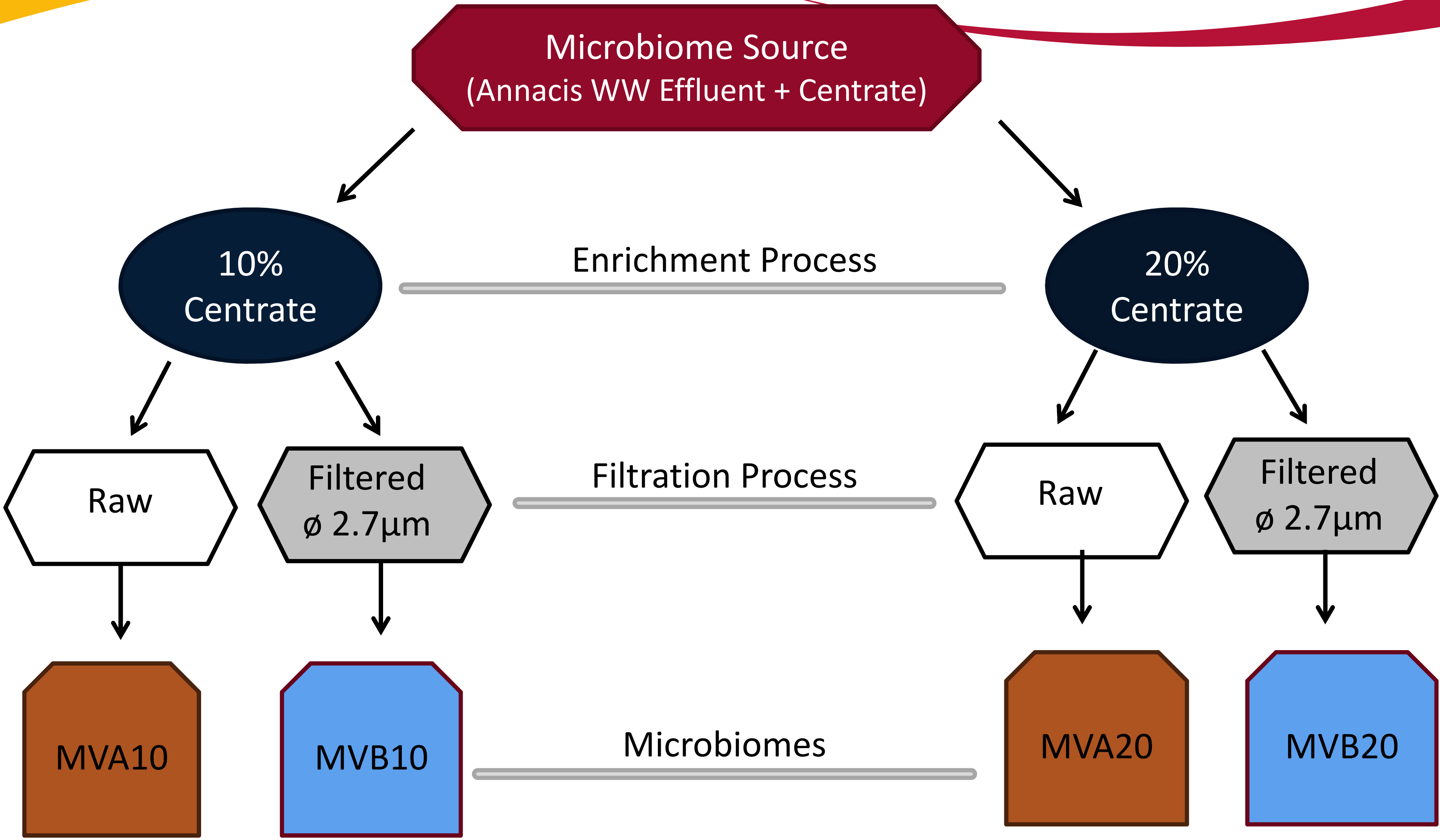
Metro Vancouver Alternative Process



Potential Future for Wastewater Treatment Plants - Vancouver, BC



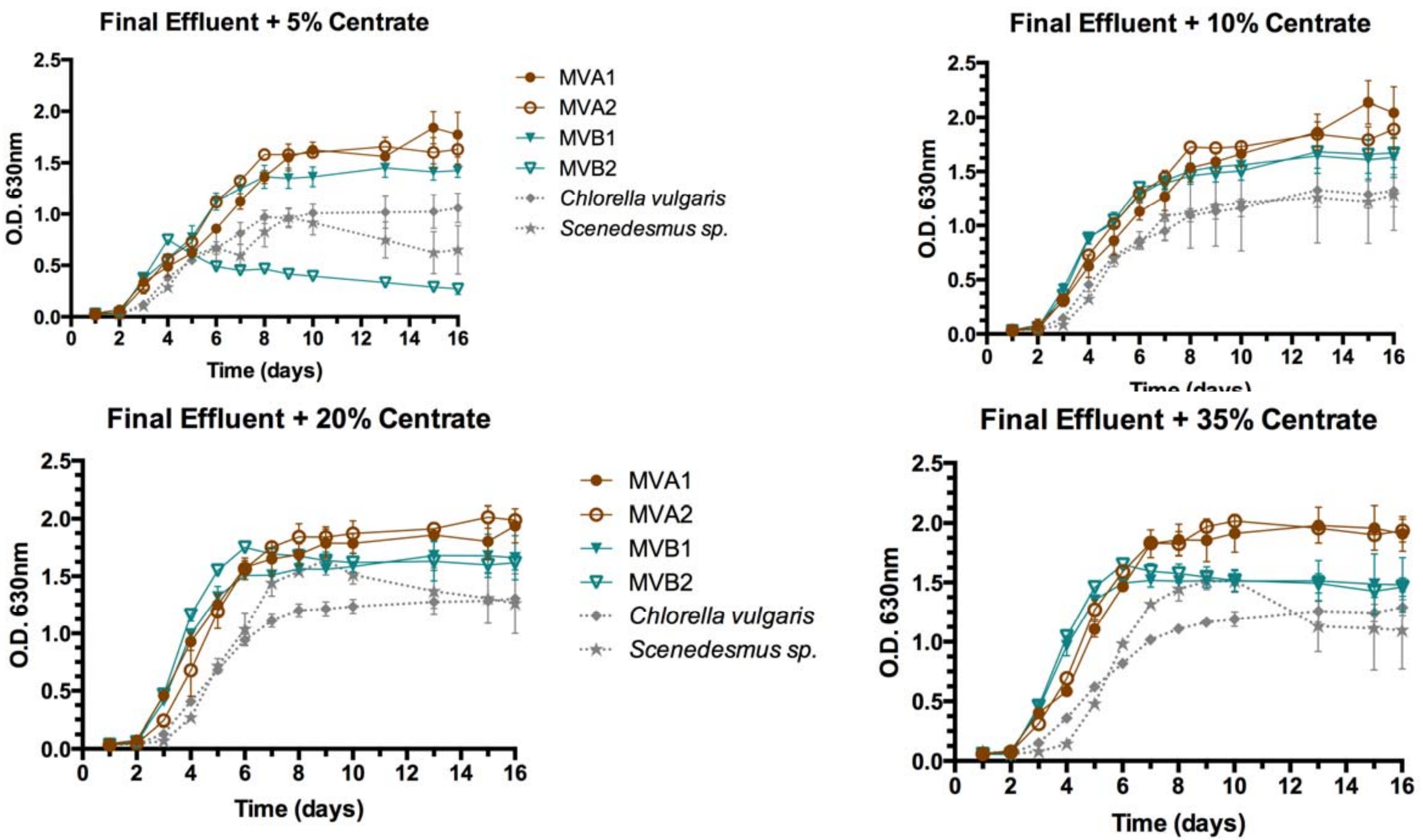
Generation of Adapted Microbiomes



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

Growth Performance Per Media Composition

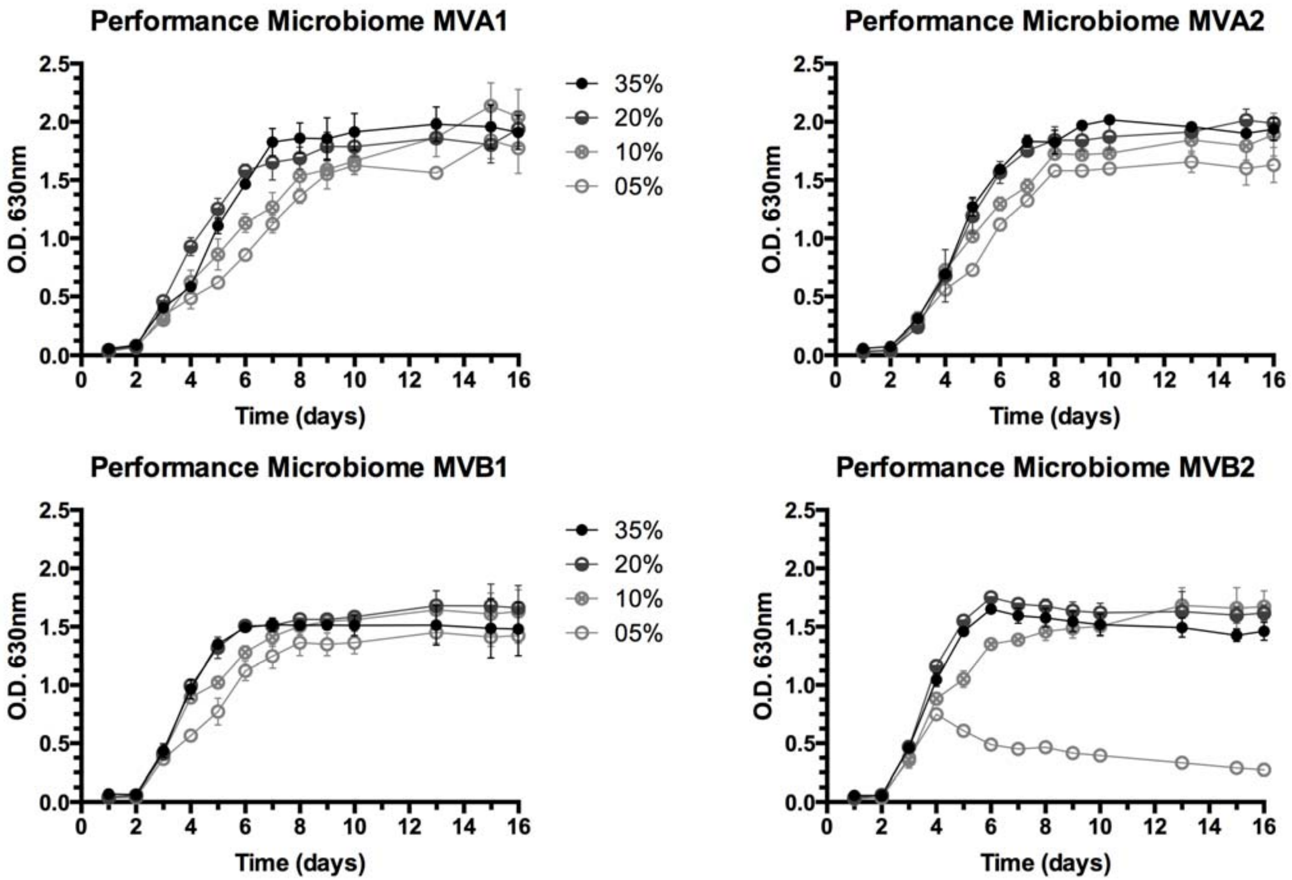


Mean value of biological triplicates.
Error bars are shown when the variation of the values are significant

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.
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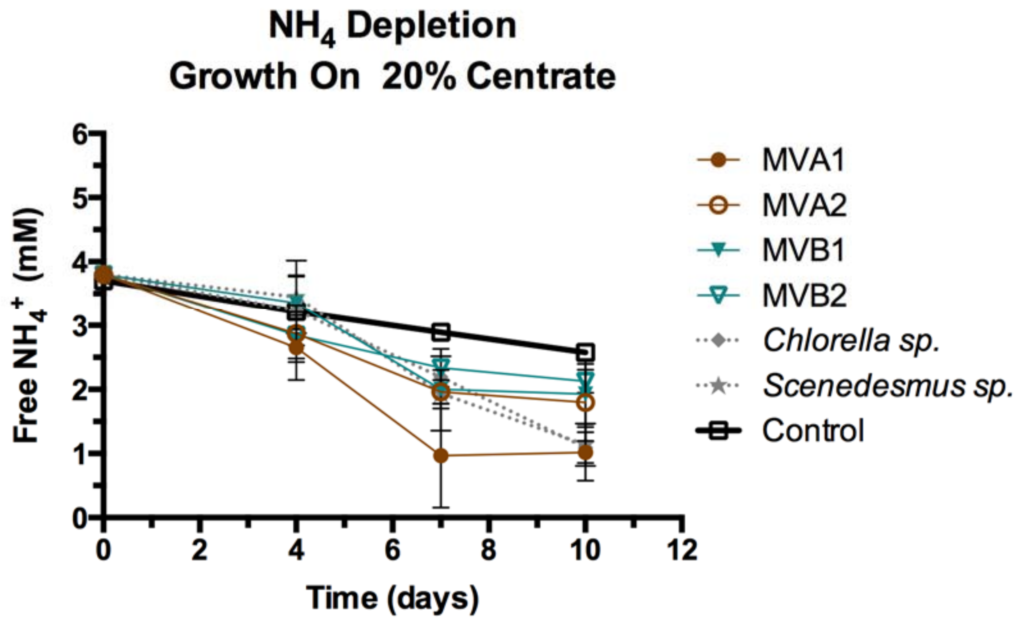
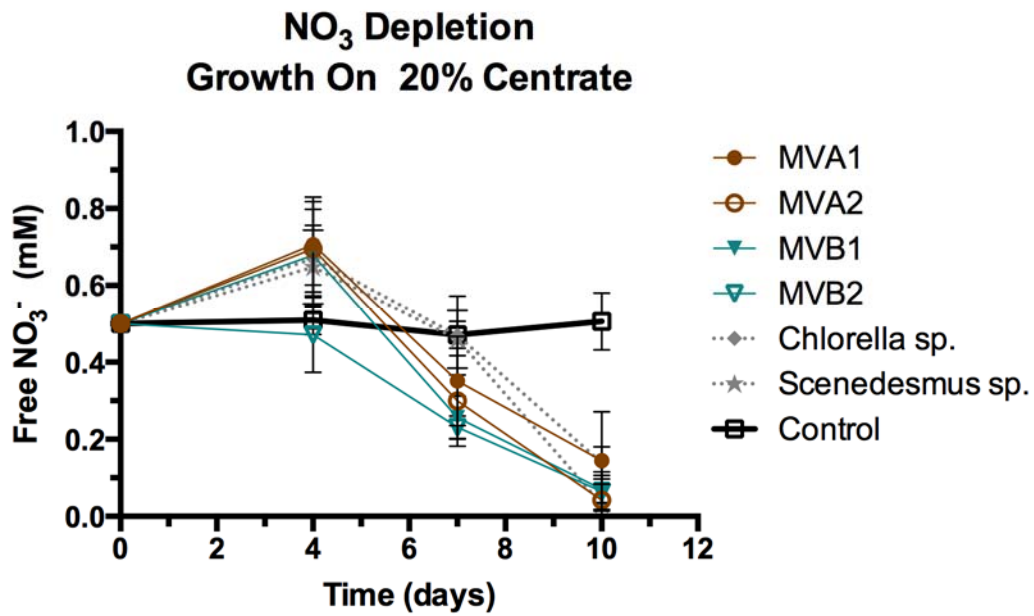
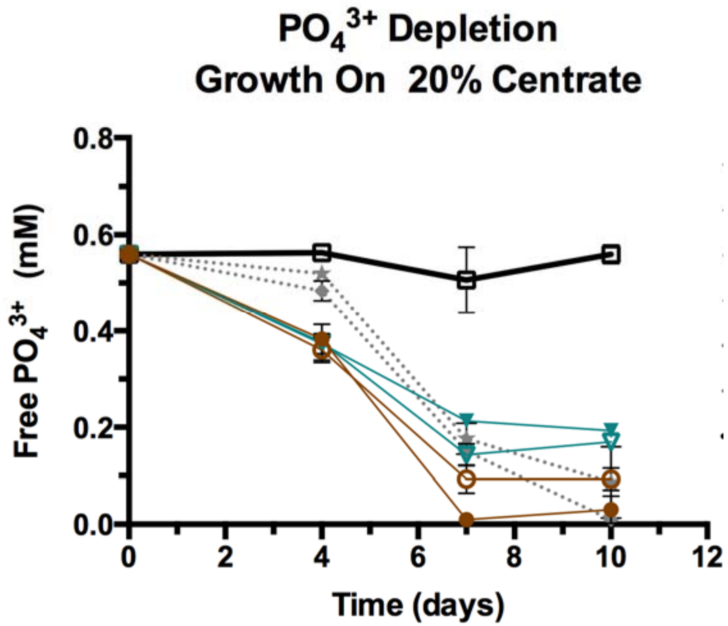
Growth Performance Under Different Centrate Concentrations

- 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

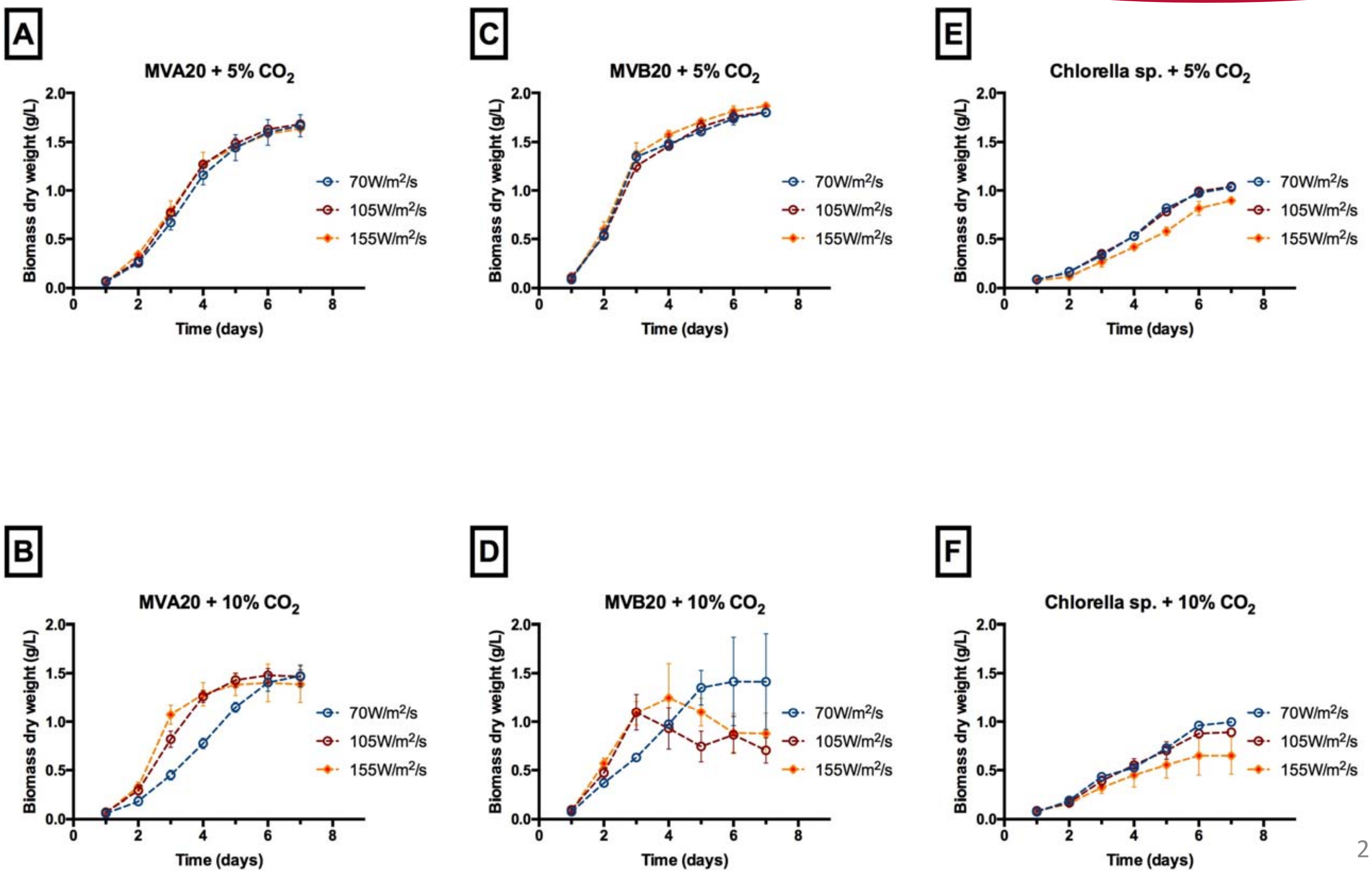
Nutrient Removal

- 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 micobiomes MVA10 and MVA20 were more efficient in the removal of phosphate than adapted filitered microbiomes MVB10 and MVB20
  - Monocultures presented a steady removal rate of phosphate

Variations in Light Intensity & CO_2 Supplementation



Variations in Light Intensity & CO_2 Supplementation

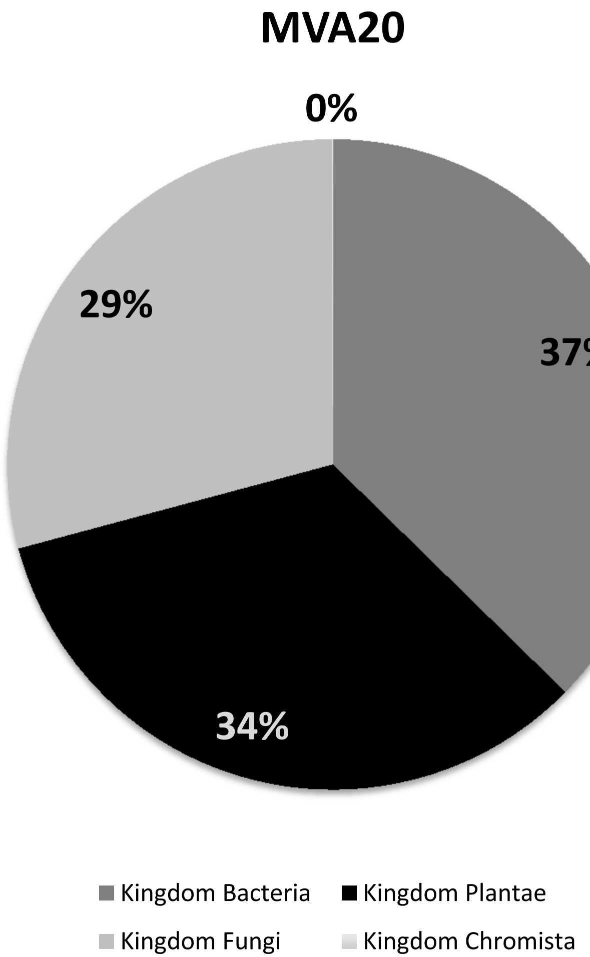
- At low CO_2 concentrations, light intensity did not limit growth performance
- Higher CO_2 concentrations did not necessarily improve the performance of the adapted microbiomes
- *C. vulgaris* under performed all adapted microbiomes under all conditions
- Unfiltered adapted microbiomes (MVA) were generally more robust

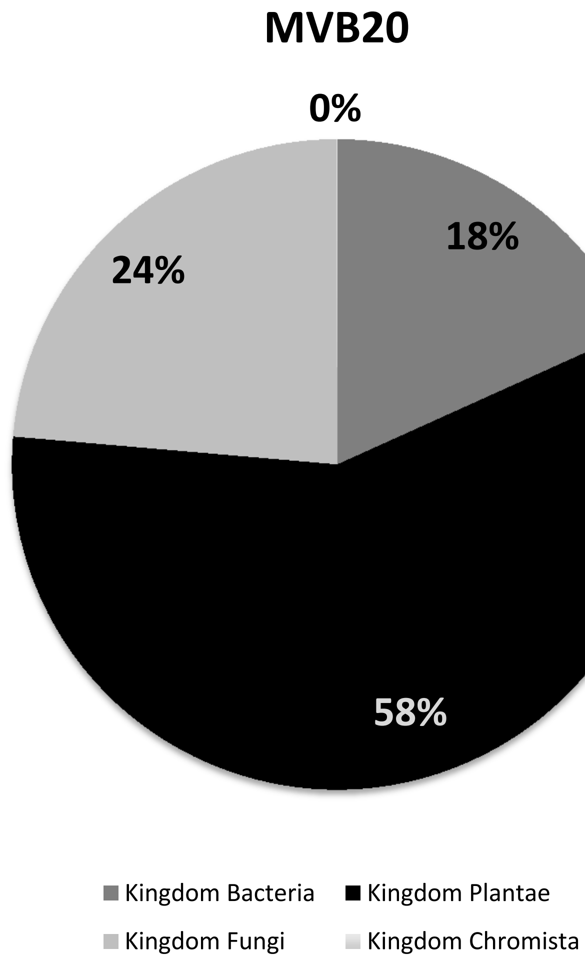
Microbiome Analysis

Taxon	Microbiome MVA20	Microbiome MVB20
Kingdom Bacteria	11,245 (37.3%)	9,015 (18.3%)
Kingdom Plantae	10,088 (33.5%)	28,700 (58.1%)
Kingdom Fungi	8,775 (29.1%)	11,658 (23.5%)
Kingdom Chromista	9 (0.03%)	13 (0.03%)

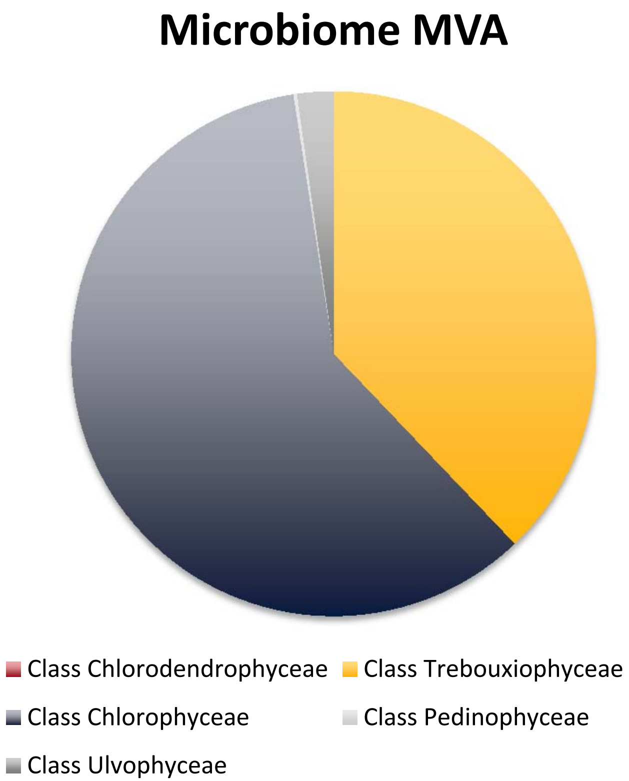
1. **Ruggiero MA**, **Gordon DP**, **Orrell TM**, **Bailly N**, **Bourgoin T**, **Brusca RC**, **Cavalier-Smith T**, **Guiry MD**, **Kirk PM**. 2015. A Higher Level Classification of All Living Organisms. PLoS ONE **10**:e0119248–60.

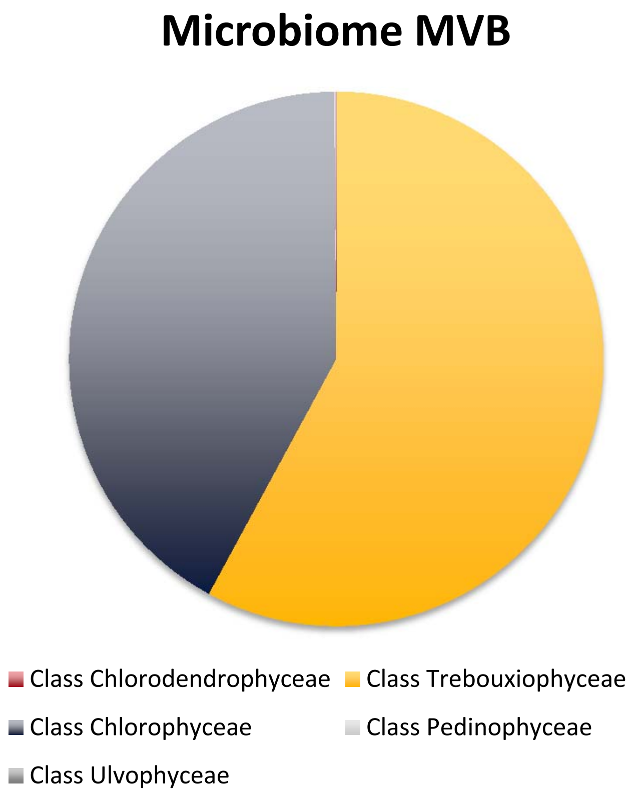
Microbiome Analysis





Microbiome Analysis: Kingdom Plantae





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
- Centrated 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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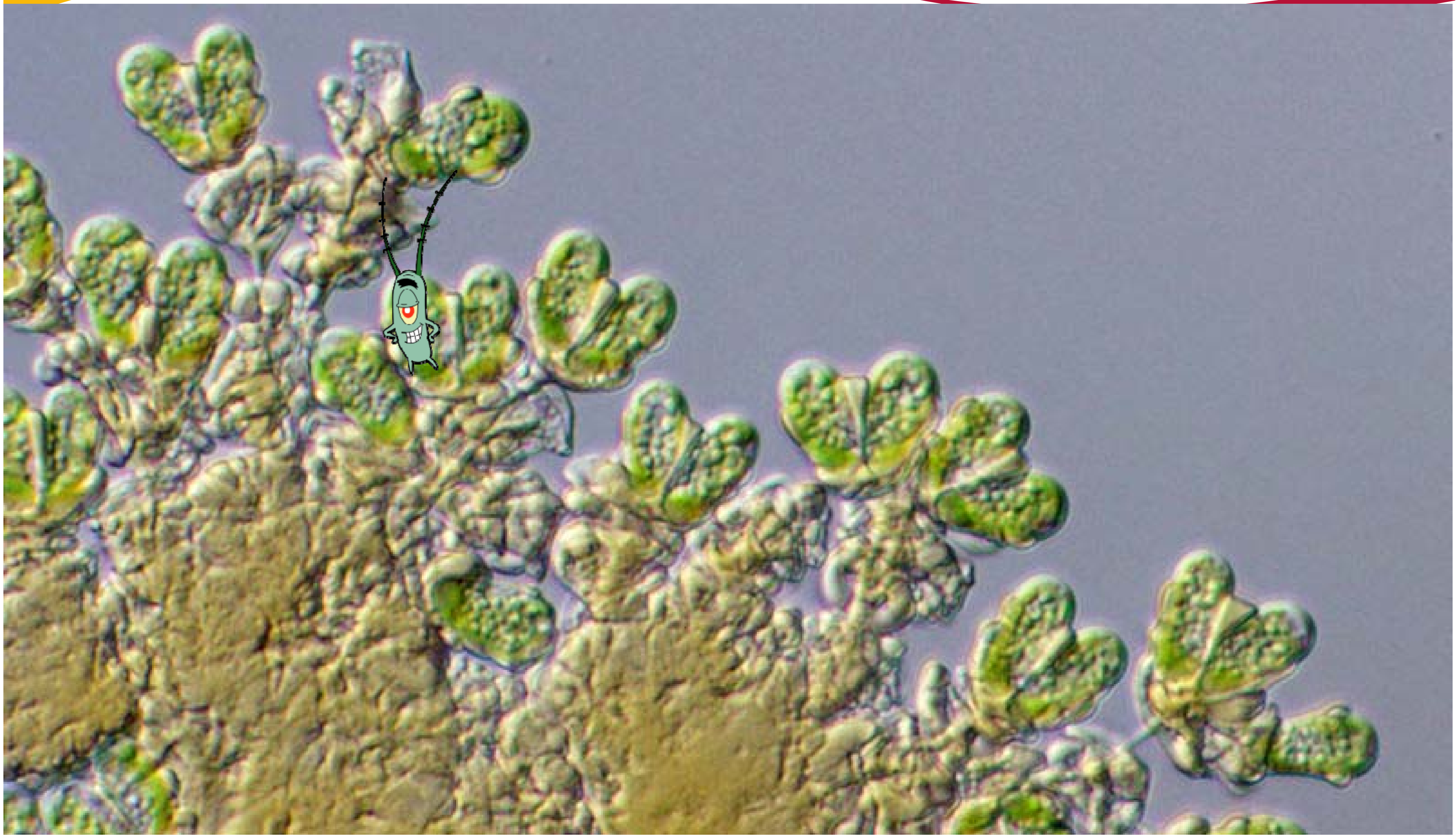
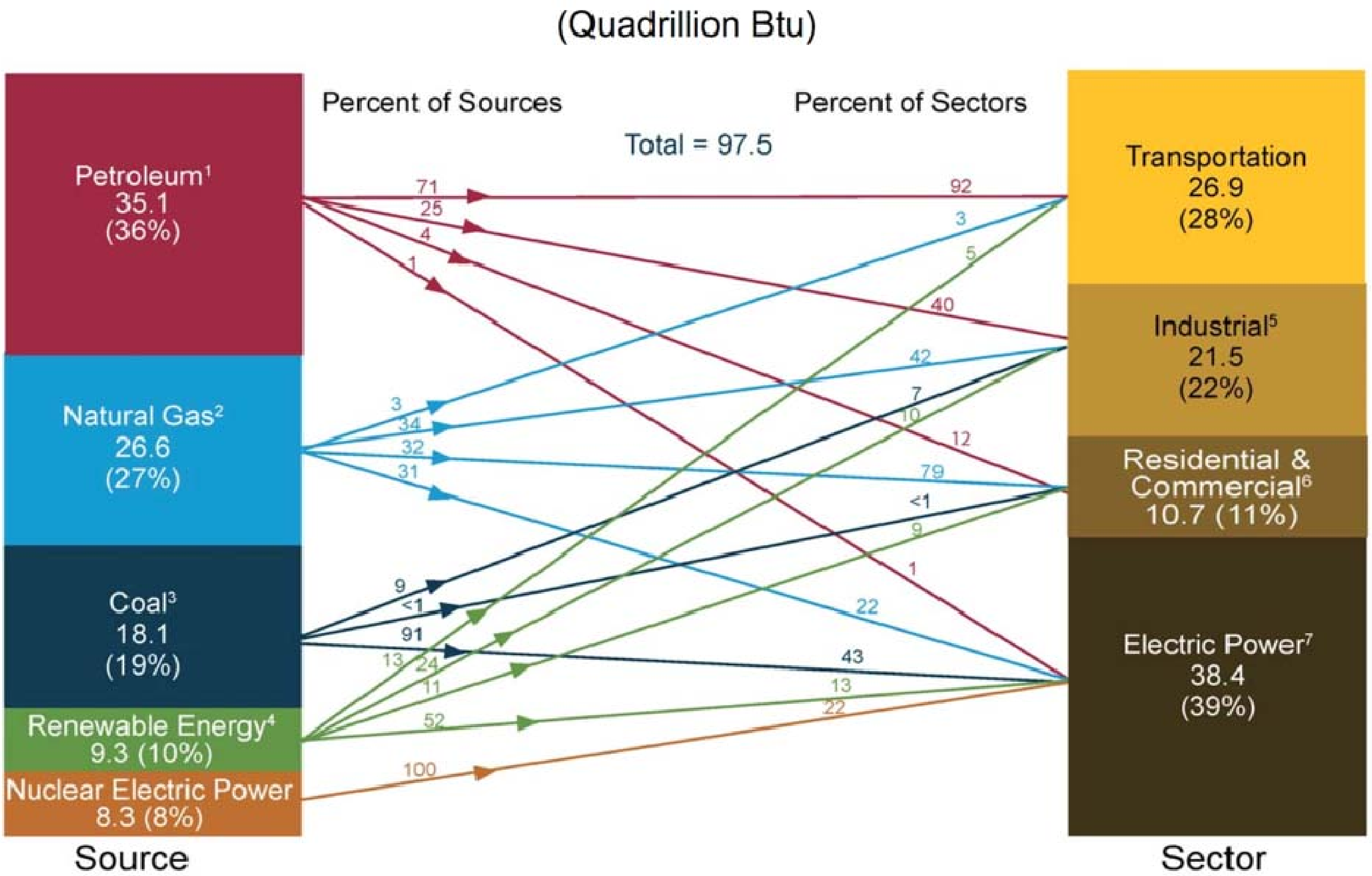
QUESTIONS?



Biofuels: A Current Need

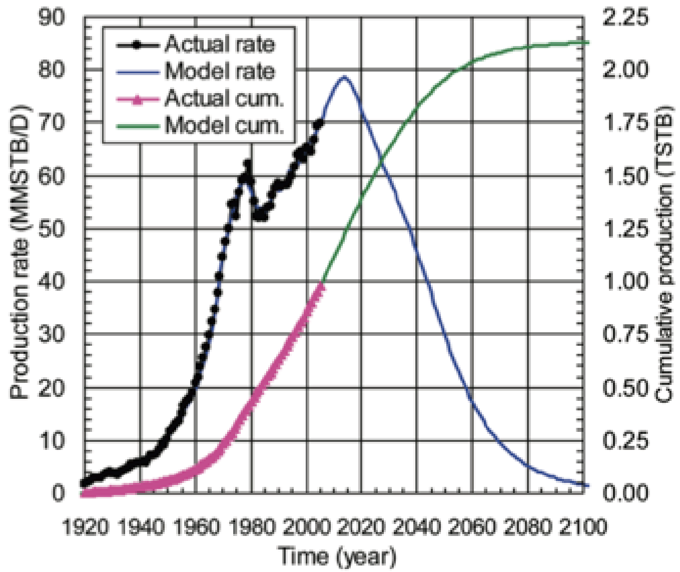
- Transportation sector uses 28% of the primary energy
- 71% of the petroleum is used for the transportation sector
- US imports 60% of its needs
- Canada imports 55% of actual needs[3]
- The Canadian Renewable Fuels regulations:
  - 2% of renewable fuel in diesel
  - 5% of renewable fuel in gasoline

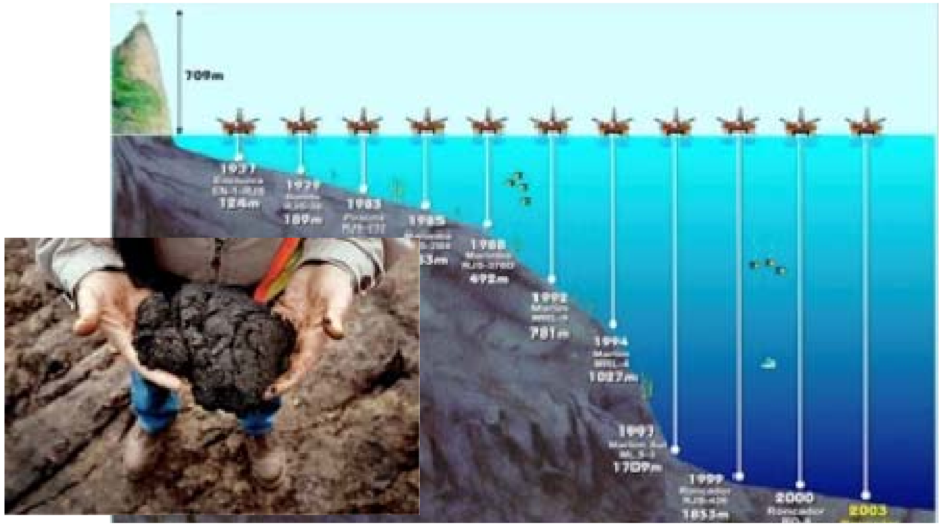
Primary Energy Consumption by Source & Sector



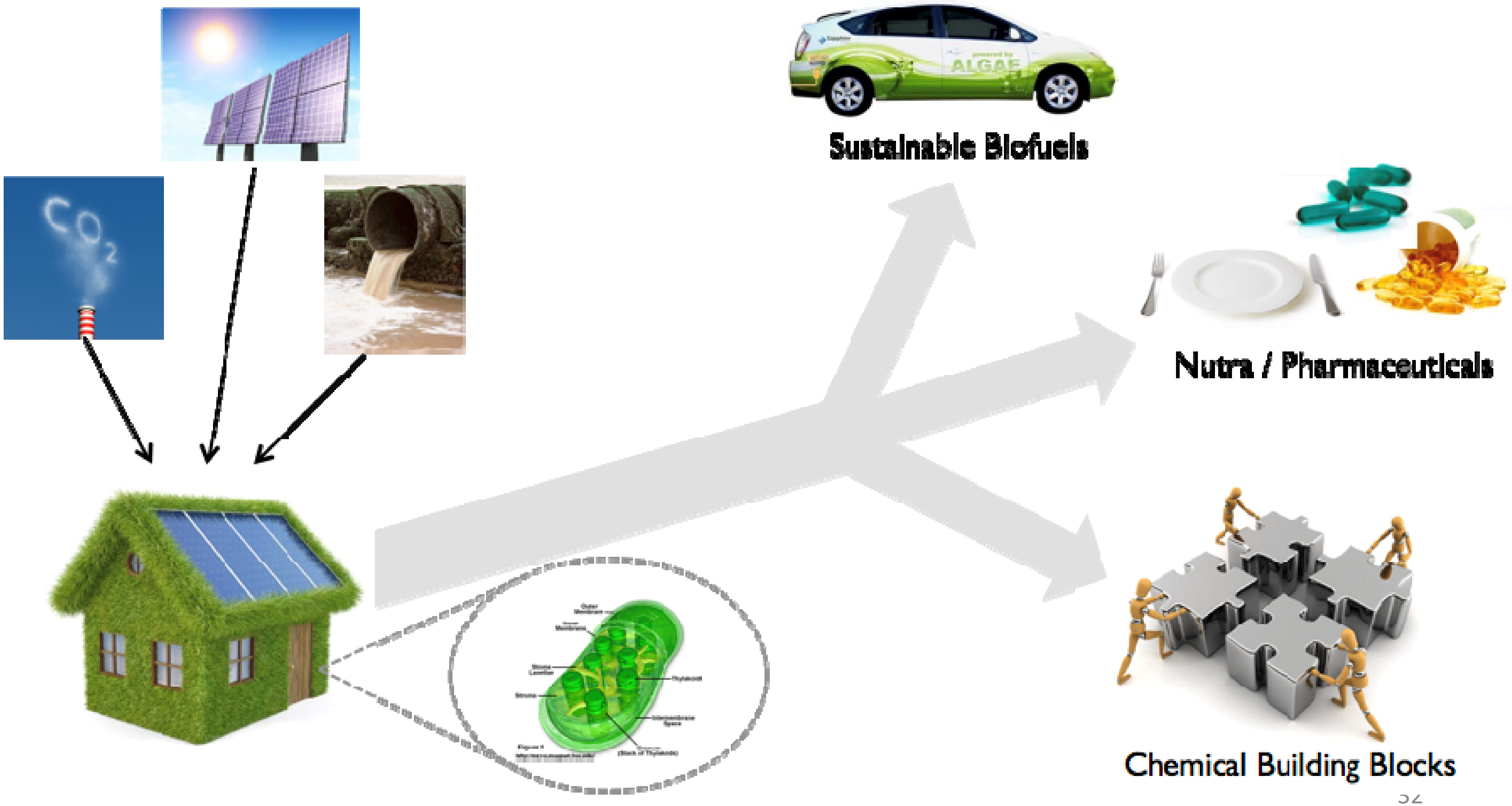
Crude Oil Dependence: Economical Impacts

- 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

Variations In Light Intensity & CO_2

- Analysis of performance under variation of:
  - Light Intensity
    - 70W/m^2/s; 105W/m^2/s and 155W/m^2/s
  - CO_2
    - 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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