Biogas to bioplastics, sustainable animal feed, and carbon sequestration - a new frontier for WRRFs
The people whose research I’m presenting:

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Waymouth
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Criddle Research Group

Meraz
Brandon
Averesch
Woo
El Abbadi
So many people, not enough fish (or cows or chickens or....)

Source: United Nations Department of Economic and Social Affairs, Population Division (2017)
Our Plastic Trajectory is Alarming

Annual plastic production (Tg/year)

Brandon and Criddle, 2019.
### What’s the Highest and Best Use of WW Resources?

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One m³ of wastewater

0.3 kg organic

0.7 kg methane = 0.07 kg

= 3.5 cf
Mineral-laden water emerging from a hydrothermal vent.
“Dark” Food Chains: Life without sunlight
“Dark” food chains aren’t just confined to the deep
Engineered “Dark” food chains at WRRFs

\[
\text{CH}_4 + 1.48 \text{O}_2 + 0.10\text{NH}_3 \rightarrow 0.10\text{C}_5\text{H}_7\text{O}_2\text{N} + 0.48 \text{CO}_2 + 1.79\text{H}_2\text{O} + 643 \text{kJ}
\]

*Energy from burning methane: \( \text{CH}_4 + 2\text{O}_2 \rightarrow \text{CO}_2 + 2\text{H}_2\text{O} + 802 \text{kJ} \)*
Very high volumetric protein production possible

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<th>product</th>
<th>growth conditions</th>
<th>protein productivity (kg protein/m³-day)</th>
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<tr>
<td>MOB microbial</td>
<td>air and methane fed fluidized bed reactor (N₂ as N-source)</td>
<td>0.00052¹¹²</td>
</tr>
<tr>
<td>protein</td>
<td>batch serum bottle incubation (ammonia N source)</td>
<td>2.8⁶⁹</td>
</tr>
<tr>
<td></td>
<td>high pressure (6 atm) Chemostat</td>
<td>60.5¹¹⁰</td>
</tr>
<tr>
<td>soybean</td>
<td>typical yield in midwest U.S. (1995–2012)</td>
<td>0.00054¹¹⁵</td>
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A (Hypothetical) Local Example
Oceanside Plant, San Francisco

15 MGD (dry weather)
390,000 CF Biogas/day
10 acres

780 tonnes protein/year
~9,000 gallon reactor
1,000 acres
At least two companies are selling animal feed from methanotrophs with EU approval.

But they use natural gas! GHG benefits debatable.

https://www.carbontrust.com/media/672719/calysta-feedkind.pdf
Why WRRFs?

• Biogas is renewable resource
• Large cooling capacity & heat demand
• Potential to recover nutrients & water

Challenges/Concerns:
• Efficient mass transfer of CH$_4$ and O$_2$ in H$_2$O
• Safe operations – pathogen barriers, explosions
• Unfamiliar market
### What if we make single cell protein?

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<td>0.07 kg methane</td>
<td>0.05 kg Single Cell Protein (SCP) @$1.00/kg</td>
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*compressed biomethane with 2015 state/fed credits*@$0.82/kg

**0.1 m³ methane ~3.5 cf (0.07 kg)**
Biogas as sustainable food?

The probable food chain would consist of three levels: microbial protein as a feed for animals, which are in turn consumed by humans. Research has explored the use of microbial protein for livestock (pigs, beef, and dairy cattle) and poultry (broiler chickens and laying hens).

Additional trophic levels are also possible: microbial protein could serve as feed for Daphnia, for example, which can then serve as live feed for aquaculture. However, each additional level reduces carbon use efficiency of the overall process, resulting in increased CO₂ production.

5.2. Integrated Resource Recovery Systems. Engineered dark food chains create interesting opportunities for recycling of water, carbon, and nitrogen (Figures 2 and 5). Treatment and reuse of water for SCP growth and for aquaculture sets the demand for imported water. Similarly, recycling of biogas carbon and nitrogen can offset requirements for imported carbon and nitrogen. At present, recirculation aquaculture practice includes technologies for water purification via solids removal, nitrogen removal via biofilters, reaeration, and disinfection. Configurations designed for resource recovery can be envisioned for SCP production.

Carbon reuse can be enabled by dark food chains because CH₄ and CO₂ are at once feedstocks for microbial protein and products of wastewater treatment. Methanogens generate biogas from waste; methanotrophs consume the biogas to produce SCP; SCP is used to produce animal protein; and the loop is closed when the animal protein is consumed and converted again into waste.

Nitrogen recovery and reuse are likewise enabled by dark food chains because ammonium and nitrate are at once feedstocks for microbial protein production and products of wastewater treatment. Through recycle loops, nitrogen cycles that incorporate dark food chains can avoid loss of nitrogen through nitrogen recovery and its upcycling into animal protein (Figures 2c and 5).

Recovery of nutrients may be achieved in settings where domestic wastewater is treated, an alternative that has been successfully tested with urine streams in low-income settings. Further benefits are incurred by avoiding energy-intensive fixation of N₂ to NH₃ (37–45 MJ/kg N as NH₃) via the Haber-Basch process.

The minimum energy required for production of 1 kg of edible animal protein grown on microbial protein ranges from 606 to 850 MJ/kg-N protein (Table 2). Caution must of course be taken to avoid accumulation of toxic levels of ammonia within recirculation systems.

Heat is another resource that can potentially be harvested within combined microbial protein-aquaculture systems. As discussed in Section 4.3, microbial chemoautotrophy is accompanied by high rates of heat production. Heat pumps can be used to harness this energy. Heat can also be used at various disinfection stages in water treatment (for Advanced Treatment in Figure 2c), or for treatment of microbial protein. Specifically, heat can be used for both drying of cells and for reduction of total RNA content via activation of endogenous RNA degrading ribonucleases, an important consideration if SCP is to be used for human consumption (as discussed in Section 5.1).

VI. CONCLUSIONS AND RESEARCH GAPS

Engineered dark food chains have the potential to provide many benefits as a supply of microbial protein. Such food chains have the potential to decrease reliance upon fishmeal and enable high volumetric rates of productivity, thereby reducing land requirements and associated greenhouse gas emissions. Additionally, valuable coproducts, such as PHB, can be produced, enabling increased survival of aquaculture animals and reduced use of...
Our Plastic Trajectory is Alarming

Annual plastic production (Tg/year)

Brandon and Criddle, 2019.
PLASTICS IN THE MARINE ENVIRONMENT: WHERE DO THEY COME FROM? WHERE DO THEY GO?

TOTAL PLASTIC ENTERING THE MARINE ENVIRONMENT
12.2 Million tonnes per annum

BEACHES
2,000kg/ km² (5% of total)

OCEAN SURFACE
18kg/ km² (1% of total)*

LAND BASED - COASTAL
9 Million tonnes per annum

AT SEA
1.75 Mtpa

FISHING LITTER - 1.15

SHIPPING LITTER - 0.60

LAND BASED - INLAND - 0.50 Mtpa

225,000 tonnes/year

SEA FLOOR
70kg/ km² (94% of total)

**Bioplastics from waste – “Nature’s Plastic”**

**PHB: Poly-hydroxybutyrate**

**TEM image of PHB granules in bacteria**

**purified PHB powder**

[Chemical structure of PHB]

**PHA: Poly-hydroxyalkanoates**

[Diagram showing PHA’s and PHB]
Making PHB is normally a two-step process.

- **Vigorous mixing**

**Headspace:**
- $\text{CH}_4 + \text{O}_2$

**Medium:**
- Water + nutrients + methanotrophs
Two Phases

1) Repeating cycle (balanced growth) – cell replication for 12 hours
2) PHA production step (nutrient-limiting condition) – PHA accumulation for 12 hours
PHA Accumulation

A  Before PHA production (t = 0h)

B  After PHA production (t = 12h)
Poly-P concentration

product (P3HB) concentration

PHB levels up to 50 wt%

Jaewook Myung
Closer and sooner than you might think
**Other ways to add value**

**PHB limitations**

*Narrow processing window*

- **175°C** melting temperature
- **190°C** thermal degradation

*Brittle: elongation at break 5%*

**Add co-substrates to make PHBV**

- **110°C** melting temperature
- **210°C** thermal degradation

**Elongation at break 30%**

- **3HV fraction (mol%)**
- **Added valerate concentration (mg/L)**

*3D printing*

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<td>0.02 kg PHB bioplastic@$6.00/kg</td>
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Life Cycle of PHA Products

① Conventional recycling → Leads to downcycling

② Methane-PHA recycling → Long feedstock supply chain

③ Precursor-PHA recycling → No downcycling → Quick regeneration (hours vs. days)
Toward Cradle-to-Cradle Materials

- **CO₂**
  - Photosynthesis: 1 - 10 years
  - Biomass
  - Cradle-to-grave circular lifecycles
  - Fossil carbon feedstocks
    - >10⁶ years

- **RENEWABLE H₂**
  - RENEWABLE CARBON FEEDSTOCKS

- **Microbial Degradation**
  - Bioreactors
  - 880 THOUSAND TONNES
    - Global annual production, 2017

- **Physical / Chemical Recycling**
  - Recovery of resin materials
  - 840 MILLION TONNES
    - Global annual production, 2015

- **Environmental Degradation**
  - Leakages
    - ~35%

- **Incineration**
  - 12%
  - Accumulate in landfills or the environment
    - ~44%

- **Downcycling**
  - 2 - 3% process losses
  - 9% recycled

- **Closed-loop Recycling**
  - 1 - 2% closed-loop recycling

- **Downcycling Losses**
  - 5% downcycling losses

- **CO₂**
  - Global annual production, 2017
  - ~44% in landfill or the environment

Brandon and Criddle, 2019.
The inventors surprisingly found that hydroxybutyrate and poly-hydroxybutyrate, have a great potential for uses in animal feed for modulation of the gut flora...for suppressing or inhibiting pathogenic bacteria in the gastrointestinal tract.

"The inventors surprisingly found that hydroxybutyrate and polyhydroxybutyrate, have a great potential for uses in animal feed for modulation of the gut flora...for suppressing or inhibiting pathogenic bacteria in the gastrointestinal tract"
**Applied Microbial and Cell Physiology**

Poly-β-hydroxybutyrate (PHB) increases growth performance and intestinal bacterial range-weighted richness in juvenile European sea bass, *Dicentrarchus labrax*

Peter De Schryver - Amit Kumar Sinha - Prabesh Singh Kunwar - Kartik Baruah - Willy Verstraete - Nico Boon - Gudrun De Bock - Peter Bossier

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**Veterinary Microbiology**

Poly-β-hydroxybutyrate (PHB) accumulating *Bacillus* spp. improve the survival, growth and robustness of *Penaeus monodon* (Fabricius, 1798) postlarvae

Joseph Leopoldo Q. Laranja, Gladys L. Ludevese-Pascual, Edgar C. Amar, Patrick Sorgeloos, Peter Bossier, Peter De Schryver

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**Effect of poly-β-hydroxybutyrate on Chinese mitten crab, *Eriocheir sinensis*, larvae challenged with pathogenic *Vibrio anguillarum***

L. Sui, J. Cai, H. Sun, M. Wille and P. Bossier

1. Tianjin Key Laboratory of Marine Resources & Chemistry, Tianjin University of Science & Technology, Tianjin, China
2. Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Ghent, Belgium

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**Sea Bass**

**Crabs**

**Shrimp**
### What if we used methane to make prebiotic?

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0.07 kg methane

Prebiotic

$0.20
Many other possible products from methane

$1300/kg
Protects against UV-induced damage

Methane as a feedstock

- **Opportunities**
  - Cheap and abundant - not tied to food.
  - No need for cultivation and harvesting of crops.
  - Consistent feedstock enables consistent production of a “base” polymer (P3HB).
  - Diverse copolymers can be made by adding to the P3HB base

- **Challenges**
  - Doubling times 4-12 hours
  - Two-step process required.
  - Low methane solubility decreases mass transfer rates
  - Heat must be removed at high specific growth rates.
  - Explosion hazards.
  - Co-substrates are typically expensive.
Cradle-to-Cradle Materials Could Fix Carbon

Brandon and Criddle, 2019.