SPLASH

Sustainable Polymers from Algae
Sugars and Hydrocarbons

Lessons learned

Aim

Develop a new biobased industrial platform using microalgae as a renewable raw material for the production of renewable polymers.

B. braunii culture (Photo T. Bastet)
Vision:
Semi industrial production in 15-20 years
Algae: *Botryococcus braunii*

- Hydrocarbons
- (exo)Polysaccharides

- Systems biology
- Production / extraction / purification
- Conversion to renewable polymers
- Economics / Market / Sustainability

Biotechnology: Early stage research dimension: TRL<3
- Generate and transfer knowledge
- Interaction between basic research, pilot development and industry
- Multidisciplinary approach
Natural Algae
Botryococcus

Bio-tailoring of hydrocarbons and sugars production (genomics and metabolic engineering)

Green algae
Chlamydomonas

Biomass + Product

Poly-saccharides

Galactose, Fucose, Rhamnose, Glucose

Hydrocarbons

(Living) cell residue

Adipic acid

2-MeTHF

1,4-pentanediol

2,5-FDCA

Green naphtha

Ethylene, propylene

Polyesters, Copolyesters

Polyolefins

BIOTECH PRODUCTION

CHEMICAL CONVERSION

Process demonstration at pilot scale
Process integration, sustainability assessment, market analysis
Dissemination, exploitation and IP management
Characterisation of 16 strains of Bb at physiological and biochemical level

best candidates of race A & B identified

Race A, Sugars  CCALA778
Race B, Botryococcene  AC761
Understand and steer: Omics experiment

Changes in transcripts, proteins and metabolites levels in Race_A vs. Race_B cultures
Many parameters monitored:

- pH, CO$_2$, light, ...
- optical density
- chlorophyll content
- CDW, oDW
- hydrocarbon and sugar content
- colony size, morphology
- ...
Proteomics analysis: In-gel trypsin digestion then LC/MS

1189 protein groups can be quantified in e.g. early log phase between Race A and B
Genome-scale metabolic modelling of *Botryococcus braunii*

- **e.g.** botryococcene
- **e.g.** alkadienes/alkatrienes

*Botryococcus* genome-scale metabolic model
- ~1530 metabolic reactions
- ~1130 unique metabolites

- SPLASH metabolomics (sig. peaks) (Bielefeld CeBiTec)
- C,H,N,O elemental composition of cell pellets (WUR-BPE)
- Media compositions (WP2, WP3)
- Sugars profile of CCALA 778 (race A) (Arnoud, WP3 DLO-FBR [Aug 2014])

- Hydrocarbons GC-MS analysis (race B) (Slovy [March 2014])
- Hydrocarbon, lipid, and carbohydrate yields (Bielefeld CeBiTec [March 2014])

LifeGlimmer, Berlin
Wageingen University
Botryococcus 

Genome Scale Metabolic Model

Genes – annotation of genes of interest – enzymes and transporters – gene scale metabolic model – reaction fluxes

Flux Balance Analysis: 
Simulate the algae’s metabolism.

Useful for predicting the effect of growth medium, gene knockouts, knockins, and more, on the metabolism of the algae (e.g. maximizing lipid or sugar production)
Combined with proteomics data, differences starting to emerge

FBA with > 3fold differences

Race A > B
Race B > A

LifeGlimmer, Berlin
Wageningen University SSB
• Bb cultures contain also (needed) bacteria. It is a challenging organism to do genomics with.
  • Improved understanding comes from combining different omics approaches and also modelling

• Identifying common features between race A and race B has enabled us to find which of these are elevated/repressed in each race
  • these candidates might be most useful to target for manipulation

• Validation of putative genes to show they do what is predicted from sequence similarity is needed
  • need more time and we would have cracked it
Main objective

to develop innovative cultivation and downstream concepts for improved growth, product enhancement and integrated recovery of EPS and HCs.

Medium & process optimization
Large scale biomass production

Cultivation

Supersonic flow fluid processing
Milking with organic solvents
Supercritical CO₂
Combined microsieve extraction
Rotary vacuum drum

Hydrocarbons
Hydrocarbons
EPS

EPS : CCALA778
Hydrocarbons. Showa / AC761
Cultivation optimization - medium

Objective
- Improved productivity
- Lab versus Pilot: No Se allowed for at production at scale

**B. braunii strains**
- CCALA778
- AC761

**Literature review**
- 27 Botryo media
- 12 Algae media
- 3-level factorial: N, P, S, Fe, Mo
- 46 new media

**24-well experiments**
- Fast screening
- $P_{Cx}$, $P_{Carb}$, $P_{Hcs}$
- Best 4 media

**Roux flasks Validation**
- Insight in product accumulation
- $P_{Cx}$, $P_{Carb}$, $P_{Hcs}$
- Final media selected

Conclusion
- Se → minor role in biomass and product formation
- Final media selected for improved production

<table>
<thead>
<tr>
<th>Media</th>
<th>Nitrate (mM)</th>
<th>Phosphate (mM)</th>
<th>Iron (μM)</th>
<th>Molybdenum (μM)</th>
<th>Selenium (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference Media</td>
<td>4</td>
<td>0.6</td>
<td>55</td>
<td>0.25</td>
<td>50</td>
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<tr>
<td>M11, B. braunii AC761</td>
<td>22</td>
<td>3.3</td>
<td>10</td>
<td>1</td>
<td>0</td>
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<td>M44m, B. braunii CCALA-778</td>
<td>22</td>
<td>0.6</td>
<td>40</td>
<td>2</td>
<td>0</td>
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</table>

Similar $P_{Cx}$ and $P_{Hcs}$ but no Se
- 2-fold $P_{Cx}$ and 3-fold $P_{Carb}$
Outdoor large scale cultivation + hydrocarbon formation / extraction

**Challenging:**
- Contamination
- Slow growth
- Biofilm attached in tubes
- Biomass settling

Biomass (600 L) used for cyclohexane extraction of hydrocarbons

hydrocarbons to be cracked
### Long list of solvents

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Structural formula</th>
<th>Log P (Octanol-Water partition coefficient)</th>
<th>Density g/ml (water =1)</th>
<th>Solvent miscibility in water</th>
<th>Solubility H2O (g/100ml)</th>
<th>Molecular weight (g/mol)</th>
<th>Melting point °C</th>
<th>Boiling point °C</th>
<th>Explosion limit (%volume in air)</th>
<th>MAC-value (ppm)</th>
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<tbody>
<tr>
<td>Trichloroethylene</td>
<td>C2HCl3</td>
<td>2.53</td>
<td>1.45</td>
<td>immiscible</td>
<td>1.28</td>
<td>131.4</td>
<td>-73</td>
<td>87.2</td>
<td>8-10.5</td>
<td>35</td>
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<td>Iso-propanol</td>
<td>C3H7OH</td>
<td>0.05</td>
<td>0.79</td>
<td>miscible</td>
<td>60</td>
<td>-89</td>
<td>82</td>
<td>2-12.7</td>
<td>400</td>
<td>400</td>
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<tr>
<td>Pentane</td>
<td>C5H12</td>
<td>3.26</td>
<td>0.63</td>
<td>immiscible</td>
<td>0.04</td>
<td>72.15</td>
<td>-130.5</td>
<td>36.1</td>
<td>1.4-8.3</td>
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<td>Methyl ethyl ketone</td>
<td>C4H8O</td>
<td>0.29</td>
<td>0.81</td>
<td>immiscible</td>
<td>27.5</td>
<td>72.11</td>
<td>-86</td>
<td>79.6</td>
<td>1.4-11.4</td>
<td>200</td>
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<tr>
<td>Methyl t-Butyl Ether</td>
<td>C5H12O</td>
<td>0.94</td>
<td>0.74</td>
<td>immiscible</td>
<td>26</td>
<td>88.15</td>
<td>-109</td>
<td>55.2</td>
<td>1-8</td>
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<td>Hexane</td>
<td>C6H14</td>
<td>3.76</td>
<td>0.65</td>
<td>immiscible</td>
<td>0.0095</td>
<td>86.2</td>
<td>-96</td>
<td>68.5</td>
<td>1.2-7.7</td>
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<td>Heptane</td>
<td>C7H16</td>
<td>4.27</td>
<td>0.68</td>
<td>immiscible</td>
<td>0.05</td>
<td>100.2</td>
<td>-91</td>
<td>98.1</td>
<td>1.05-6.7</td>
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<td>Diethyl Ether</td>
<td>C4H10O</td>
<td>0.8</td>
<td>0.71</td>
<td>immiscible</td>
<td>6.9</td>
<td>74.1</td>
<td>-116</td>
<td>35</td>
<td>1.7-48</td>
<td>400</td>
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<td>Ethyl Acetate</td>
<td>C4H8O2</td>
<td>0.73</td>
<td>0.9</td>
<td>immiscible</td>
<td>8.3</td>
<td>88.11</td>
<td>-83.6</td>
<td>77.1</td>
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<tr>
<td>Dichloromethane</td>
<td>CH2Cl2</td>
<td>1.25</td>
<td>1.3</td>
<td>immiscible</td>
<td>2</td>
<td>84.9</td>
<td>-96</td>
<td>40</td>
<td>100</td>
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<tr>
<td>1,2-Dichloroethane</td>
<td>C2H4Cl2</td>
<td>1.48</td>
<td>1.3</td>
<td>immiscible</td>
<td>0.8</td>
<td>99</td>
<td>-36</td>
<td>84</td>
<td>6.2-16</td>
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<td>Cyclohexane</td>
<td>C6H12</td>
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<td>immiscible</td>
<td>-</td>
<td>84.2</td>
<td>7</td>
<td>81</td>
<td>1.2-8.4</td>
<td>300</td>
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<tr>
<td>Chloroform</td>
<td>CHCl3</td>
<td>2</td>
<td>1.5</td>
<td>immiscible</td>
<td>0.8</td>
<td>119.4</td>
<td>-63</td>
<td>61</td>
<td>10</td>
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<tr>
<td>Butyl Acetate</td>
<td>C6H12O2</td>
<td>1.92</td>
<td>0.9</td>
<td>immiscible</td>
<td>0.7</td>
<td>116.2</td>
<td>-77</td>
<td>127</td>
<td>1.2-7.5</td>
<td>150</td>
</tr>
<tr>
<td>n-Butanol</td>
<td>C4H10O</td>
<td>0.9</td>
<td>0.8</td>
<td>immiscible</td>
<td>8</td>
<td>74.1</td>
<td>-89</td>
<td>118</td>
<td>1.4-11.3</td>
<td>50</td>
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<td>Benzene</td>
<td>C6H6</td>
<td>1.9</td>
<td>0.9</td>
<td>immiscible</td>
<td>0.18</td>
<td>78.1</td>
<td>6</td>
<td>80</td>
<td>1.2-8</td>
<td>10</td>
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<tr>
<td>Acetone</td>
<td>C3H6O</td>
<td>-0.2</td>
<td>0.8</td>
<td>miscible</td>
<td>100</td>
<td>58.1</td>
<td>-95</td>
<td>56</td>
<td>2.3-13</td>
<td>750</td>
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<tr>
<td>Dodecane</td>
<td>C12H26</td>
<td>6.98</td>
<td>0.75</td>
<td>miscible</td>
<td>170.34</td>
<td>-9.6</td>
<td>216.3</td>
<td>0.6</td>
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<tr>
<td>Hexadecane</td>
<td>C16H34</td>
<td>8.2</td>
<td>0.77</td>
<td>miscible</td>
<td>226.44</td>
<td>18</td>
<td>287</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>DCM:MeOH 1:1</td>
<td>Reference (extraction yield)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

### Short list of solvents:
- Cyclohexane
- Hexane
- Pentane
- Dichloromethane

### Hydrocarbon “milking”

#### Viability tests
- 100% solubility solvent
- Longer contact times in 2-phase system

#### Milking experiments
- Ongoing
Exopolysaccharide “milking”

Outdoors
Scale: 4 x 40 litre

Results so far:
Efficiency of EPS extraction ~12%
To be optimized
Making algae based polymers (Chemical conversion)

- Converting hydrocarbons into ethylene and propylene (cracking)
  • Monomers for polyethylene (PE) and polypropylene (PP)

- Converting carbohydrates into polyesters
  • Carbohydrate hydrolysis to monosaccharides
  • Conversion of monosaccharides into
    – Diols
    – Di-carboxylic acids
• Overall yields starting from monosaccharides
  – 1,4-pentanediol: 12%
  – Adipic acid: >29%
  – 2,5-FDCA: >50%

• Some novel polyesters were produced by WFBR

  » Potential in soft-block polymers and glues

  1,4-pentanediol + dimethyl adipate $\xrightarrow{\text{Ti(OiPr)₄}}$ [Polymer]

  » Potential in fibre applications

  1,4-pentanediol + dimethyl-FDCA $\xrightarrow{\text{Ti(OiPr)₄}}$ [Polymer]
• Further research on Avantium’s YXY technology to produce PolyEthylene Furanedicarboxylate (PEF) as a replacement for PET
  – Ropes and nettings for marine, fishing and offshore applications
• Conclusions

✓ It is possible to hydrocrack algae based **hydrocarbons**
  ✓ Tested as blend in organic solvent
  ✓ Use hydrocarbons for higher value applications

✓ Polyesters can be chemically produced from algae based **sugars**

✓ Novel biobased polyesters were successfully prepared
  ➢ Yields of building block must be improved
  ➢ Separation of the different monosaccharides is a major challenge
4 main scenarios: specialties and commodities

1. EPS plant
   - 20,000 T EPS/y; €6/kg
   - 200 T EPS/y; €15/kg

2. HC plant
   - 10,000 T HC/y; €4/kg

3. PS derivative plant
   - 1,4 Propane diol plant
   - FDCA plant

4. Ethylene/propylene plant
   - 100,000 T/y Eth/Prop; €1/kg

LCA and economics currently under study
Botryococcus braunii makes very interesting products. It is very complex and not an axenic culture (impact on omics).

Tools developed for combining different omics approaches and also modelling.

Botryococcus braunii’s products can be extracted continue but process improvements are required.

Novel biobased polyesters were successfully prepared.

Yields of building block must be improved.

Separation of the different monosaccharides is a major challenge.

Aim for high value products.
Lessons learned

- Understanding and steering of product formation in complex consortia.
  - Biology

- Optimize products formation combined with product isolation
  - Bio-Technology

- Harvest “unique” algal products for specific, defined markets
  - Technology and Market

- Address scale up issues
  - Biology, Technology, Market
Project Info

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http://www.eu-splash.eu/
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