Co-products from Algae Biorefinery and Lignocellulosic Biomass

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Co-products from Algae Biorefinery
Approach for concomitant extraction of bio-oil and co-products.

**Approach A**

1. Screen different high oil content algae
2. Identify the value added products present in those algae
3. Develop a method to extract them along with oil

**Approach B**

1. Use any conventional biofuel method and upgrade it for the concomitant production of oil and co-products.
2. Identify the value added products that can be isolated by that method
3. Screen the algae which are fit for such method.
Sequential Subcritical Hydrothermal Extraction (SSHTE)
Advantages of the SSHTE method

Advantages

• Along with bio-oil this method is capable of efficiently removing the valuable algal polysaccharides and antioxidant compounds.

• Comparative analysis of the bio-oil produce by conventional direct hydrothermal liquefaction (DHL) showed that removal of polysaccharides is not significantly influencing the yield of bio oil.

• Comparative analysis of the DHL and the invented method showed DHL method lead to the high production of bio char. But in SSHTE method production of bio char is very less.

• This method isolated products of non-oil origin therefore, not effecting the yield of bio oil.
Plan of work to develop algal polysaccharides as a Co-products to reduce the biofuel cost

**Approach**

To identify the use of polysaccharides for the targeted industry:

Work should be divided into two broad groups:

1. Characterization of the isolated polysaccharides to identify the targeted industry for which it can be developed.

2. Screening of different valuable algal polysaccharides which can be extracted along with the bio oil by our method.
1. **Food industry**
   - Bio-emulsifier
   - Edible coating
   - Food Packaging
   - Tooth paste, Shaving cream

2. **Cosmetic Industry**
   - Antioxidants, antibacterial cream
   - Other skin enhancement lotion
   - Formulation of vaccines

3. **Pharmaceuticals**
   - Healing agent for burning as used in bandage
   - Immunomodulatory agents, anti-inflammatory agents
## Potential value added co-products from Algae

### Market estimations for micro algal products

<table>
<thead>
<tr>
<th>Product group</th>
<th>Product</th>
<th>Retail value (US$ x10^6)</th>
<th>Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass</td>
<td>Health food,</td>
<td>1250-2500</td>
<td>Growing</td>
</tr>
<tr>
<td></td>
<td>Functional food</td>
<td>800</td>
<td>Growing</td>
</tr>
<tr>
<td></td>
<td>Feed additive</td>
<td>300</td>
<td>Fast-growing</td>
</tr>
<tr>
<td></td>
<td>Aquaculture</td>
<td>700</td>
<td>Promising</td>
</tr>
<tr>
<td>Soil Conditioner</td>
<td>Astaxanthin</td>
<td>&gt;150</td>
<td>Growing</td>
</tr>
<tr>
<td>Coloring substances</td>
<td>Phycocyanin</td>
<td>&lt;10</td>
<td>Stagnant</td>
</tr>
<tr>
<td></td>
<td>Phycoerythrin</td>
<td>&gt;2</td>
<td>Stagnant</td>
</tr>
<tr>
<td>Antioxidant substances</td>
<td>β-carotene, tocopherol</td>
<td>&gt;280</td>
<td>Promising</td>
</tr>
<tr>
<td></td>
<td>Antioxidant extract</td>
<td>100-150</td>
<td>Stagnant</td>
</tr>
<tr>
<td></td>
<td>ARA, DHA, PUFA extracts</td>
<td>20</td>
<td>Growing</td>
</tr>
<tr>
<td>Special products</td>
<td>Toxins</td>
<td>1-3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isotopes</td>
<td>&gt;5</td>
<td></td>
</tr>
</tbody>
</table>

### Market evaluation of macro algal polymers (From McHugh 2003)

<table>
<thead>
<tr>
<th>Product</th>
<th>Production (t y^(-1))</th>
<th>Algae harvested (t y^(-1))</th>
<th>Value (US$/ Mio)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrageenan</td>
<td>33,000</td>
<td>168,400</td>
<td>240</td>
<td>Mainly <em>Eucheuma</em> and <em>Kappaphycus</em></td>
</tr>
<tr>
<td>Alginate</td>
<td>30,000</td>
<td>126,500</td>
<td>213</td>
<td><em>Laminaria</em>, <em>Macrocystis</em>, <em>Lessonia</em>, <em>Ascophyllum</em> and other.</td>
</tr>
<tr>
<td>Agar</td>
<td>7,630</td>
<td>55,650</td>
<td>137</td>
<td>Mainly <em>Gelidium</em> and <em>Gracilaria</em></td>
</tr>
<tr>
<td>Nori</td>
<td>40,000</td>
<td>400,000 (wet only in Japan)</td>
<td>1500</td>
<td><em>Porphyra</em></td>
</tr>
</tbody>
</table>
### Products yield

#### Elemental Analysis

<table>
<thead>
<tr>
<th>Product</th>
<th>Temperature</th>
<th>SSHTE C%</th>
<th>SSHTE O%</th>
<th>SSHTE N%</th>
<th>DHL %</th>
<th>DHL (MJ/Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio-Oil</td>
<td>160°C</td>
<td>76</td>
<td>11</td>
<td>180°C</td>
<td>26%</td>
<td>/</td>
</tr>
<tr>
<td>Polysaccharide</td>
<td>180°C</td>
<td>0.78</td>
<td>14%</td>
<td>0.16</td>
<td></td>
<td>40.8</td>
</tr>
<tr>
<td>Bio-oil</td>
<td>300°C</td>
<td>72</td>
<td>11</td>
<td>300°C</td>
<td>27%</td>
<td>34%</td>
</tr>
<tr>
<td>Bio-char</td>
<td>300°C</td>
<td></td>
<td></td>
<td></td>
<td>2.40%</td>
<td>14.60%</td>
</tr>
</tbody>
</table>

1. Polysaccharide extracted from SSHTE total account for 40% based on dry algae weight whereas DHL shows higher carbon amount and less nitrogen amount compared with DHL.
2. Bio-oil yield from SSHTE is decreased compared with DHL.
3. Bio-char amount from SSHTE is fairly less than it from DHL.
## Chromatographic Characterization of Crude bio oil

### GC Analysis for Bio-Oil

<table>
<thead>
<tr>
<th>Retention Time</th>
<th>Compound (DHL)</th>
<th>Compound (SSHTE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.7148</td>
<td>2-Cyclopenten-1,one,2-methyl-</td>
<td>/</td>
</tr>
<tr>
<td>14.442</td>
<td>2-Cyclopenten-1-one,2,3-dimethyl</td>
<td>/</td>
</tr>
<tr>
<td>15.057</td>
<td>Palmitic, C16:0</td>
<td>21.17 mg/g 57.48 mg/g 28.33 % FA 148.50 mg/g 30.02 % FA</td>
</tr>
<tr>
<td>16.799</td>
<td>Hexadecanoic acid, C16:1n9</td>
<td>5.34 % FA 14.50 mg/g 4.87 mg/g 25.53 mg/g 4.47 mg/g 24.94 mg/g</td>
</tr>
<tr>
<td>19.7</td>
<td>Stearic acid, C18:0</td>
<td>1.90 % FA 5.17 mg/g 2.37 mg/g 12.40 mg/g 2.51 mg/g 14.02 mg/g</td>
</tr>
<tr>
<td>34.335</td>
<td>Hexadecatrienoic acid, C16:3n4</td>
<td>0.00 mg/g 0.00 mg/g 7.35 mg/g 38.51 mg/g 7.84 mg/g 43.72 mg/g</td>
</tr>
<tr>
<td>46.777</td>
<td>Hexadecatrienoic acid, 1-Boc-10,3,7,12-trimethyl-</td>
<td>27.52 mg/g 74.76 mg/g 22.29 mg/g 116.88 mg/g 23.46 mg/g 130.89 mg/g</td>
</tr>
<tr>
<td>47.416</td>
<td>Linoleic acid, C18:2n6</td>
<td>0.00 % FA 84.00 mg/g 9.48 mg/g 49.68 mg/g 6.92 mg/g 49.77 mg/g</td>
</tr>
<tr>
<td>49.195</td>
<td>Pentadecanoic acid,14-methyl-methylester</td>
<td>90.93 mg/g 84.00 mg/g 12.40 mg/g 14.02 mg/g</td>
</tr>
<tr>
<td>49.918</td>
<td>Pentadecanoic acid,14-methyl-methylester</td>
<td>84.00 mg/g 12.40 mg/g 14.02 mg/g</td>
</tr>
<tr>
<td>50.641</td>
<td>Palmitic acid</td>
<td>9.00 mg/g 67.20 mg/g 9.48 mg/g 49.68 mg/g 6.92 mg/g 49.77 mg/g</td>
</tr>
<tr>
<td>50.664</td>
<td>Hexadecanoic acid</td>
<td>55.20 mg/g 67.20 mg/g 12.40 mg/g 14.02 mg/g</td>
</tr>
<tr>
<td>51.0</td>
<td>Heptadecanoic acid</td>
<td>55.20 mg/g 67.20 mg/g 12.40 mg/g 14.02 mg/g</td>
</tr>
<tr>
<td>51.4</td>
<td>Palmitic acid</td>
<td>55.20 mg/g 67.20 mg/g 12.40 mg/g 14.02 mg/g</td>
</tr>
<tr>
<td>53.2</td>
<td>Oleic acid</td>
<td>55.20 mg/g 67.20 mg/g 12.40 mg/g 14.02 mg/g</td>
</tr>
<tr>
<td>57.1</td>
<td>Linoleic acid</td>
<td>55.20 mg/g 67.20 mg/g 12.40 mg/g 14.02 mg/g</td>
</tr>
<tr>
<td>55.9</td>
<td>Linoleic acid ethylester</td>
<td>55.20 mg/g 67.20 mg/g 12.40 mg/g 14.02 mg/g</td>
</tr>
<tr>
<td>62.5</td>
<td>9-Octadecanamide,[z]-</td>
<td>55.20 mg/g 67.20 mg/g 12.40 mg/g 14.02 mg/g</td>
</tr>
<tr>
<td>62.7</td>
<td>Hexadecanoic acid,Z-12-</td>
<td>55.20 mg/g 67.20 mg/g 12.40 mg/g 14.02 mg/g</td>
</tr>
</tbody>
</table>

1. **Fatty acid** are the major composition in crude bio-oil. Palmitic acid and Hexadecatrienoic acid are the major component of Crude Bio Oil;
2. Crude oil from SSHTE and DHL do not have too much qualitative difference but with some quantitative difference;
3. The oil amount from both extraction methods were improved highly compared with the control group.
Advantages of the SSHTE method

Advantages

• Along with bio-oil this method is capable of efficiently removing the valuable algal polysaccharides and antioxidant compounds.

• Comparative analysis of the bio-oil produce by conventional direct hydrothermal liquefaction (DHL) showed that removal of polysaccharides is not significantly influencing the yield of bio oil.

• Comparative analysis of the DHL and the invented method showed DHL method lead to the high production of bio char. But in SSHTE method production of bio char is very less.

• This method isolated products of non-oil origin therefore, not effecting the yield of bio oil.
FT-IR analysis of the extracted polysaccharide

**Region Assignments**
- 3300-2700 1/cm – C-H stretching vibrations
- 1800-1500 1/cm – Characteristic bands for proteins
- 1700-1600 1/cm – Amide-I bands (due to C-O stretching)
- 1600-1500 1/cm – Amide-II bands (due to N-H bending)
- 1200-900 – C-O, C-C, C-O-C stretching vibration of polysaccharides
Characterization of the isolated polysaccharides to identify the targeted industry for which it can be developed.

• Characterization has been divided into 4 groups

1. Identifying the polysaccharides as suitable bio emulsifier by testing their rheological and emulsifying properties under different condition (pH, NaCl concentration, different concentration of polysaccharides and temperature)

2. Testing the bio surfactant property of the polysaccharides by evaluating the effect of this compound in minimizing the surface tension of distill water

3. Characterization different bio material property like the storage modulus, loss modulus and material hardening, tensile property to evaluate its probable use of the compounds as a industrial polymer.

4. Evaluation of the bio activity and further purification of the crude polysaccharides to develop finer compounds for pharmaceutical uses.
Emulsifier

Emulsifier is a substance which can be used to produce an emulsion out of two liquids that normally cannot be mixed together (such as oil and water). Surfactants and emulsifiers are indispensable components of daily life.

Use of emulsifier
• Pharmaceutical
• Cosmetic
• Petroleum
• Food industries

Market Value
• The surfactant industry now exceeds US$ 9 billion per year (Desai and Banat, 1997).

Source of emulsifier
• Most of these compounds are of petroleum origin, which are not easily biodegradable and their manufacturing processes and by-products can be environmentally hazardous.

Drawback of petroleum originated emulsifier

Increased environmental awareness and strict legislation has made environmental compatibility of surfactants an important factor in their applications for various uses.
Bio emulsifier

• Several different microbial products that exhibit surface-active properties have been identified in the past.

• These biosurfactants are produced by certain bacteria and by a number of yeasts and filamentous fungi.

• They include low-molecular-weight glycolipids, lipopeptides and high-molecular-weight lipid-containing polymers such as lipoproteins, lipopolyssacharide-protein complexes and polysaccharide-protein-fatty acid complexes.

• These are readily biodegradable and can be produced in large amounts by microorganisms and thus are not dependent on petroleum-derived products.

• The success of biosurfactant production depends on the development of cheaper processes and the use of low cost raw materials, which account for 10-30% of the overall cost.
Formation of emulsion

<table>
<thead>
<tr>
<th>Percentage of Emulsion (E 24)</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>60.1 %</td>
<td>Corn oil</td>
</tr>
<tr>
<td>61.5 %</td>
<td>Benzene</td>
</tr>
<tr>
<td>75.0 %</td>
<td>Hexane</td>
</tr>
</tbody>
</table>

Corn oil  Benzene  Hexane
Future Goal

- Screening of algae containing more new relatively temperature resistant co-products, that have higher value when used as functional compounds than merely as biofuel molecules.

- Further standardization of the SSHTE method for cost effective separation of those compounds from algae biomass.

- Alteration of the concentrations and compositions of those compounds through species selection and varying culture conditions.

- Product development for targeted industry
High Value Lignin-derived Co-products
Chemical characterization of the bio-degraded lignin and metabolites released during chemical and biological pretreatment to the ascertain structural relationship with the native lignin macromolecular assembly

Investigate physical, chemical and biological properties of the lignin derived co-products to evaluate potential application as fine chemicals, antioxidant and high valued products
Degradation product of G-Lignin
Degradation product of S-Lignin
Bacterial Species of Interest for Lignin Deconstruction and Bio-degradation

S. Viridosporus – ATCC 39115 - $255
• BSL 1 – Grows in Yeast Malt Extract Agar, 26°C

P. Putida – ATCC 33015 - $205
• Grows in Benzoate Medium, 30°C

Rhodococcus sp. 43230 – unavailable
• Study obtained from Dr. Eltis, UBC, Canada
Proposed Pathway for Microbial degradation of Lignin

Adapted from: Ahmad et al., 2010, Molecular Biosystems, 6, 815-821
Proposed Integrated Process for Producing Biopolymers and Fine Chemicals from Bio-processed Lignin

1. Biological pretreatment
2. Enzymatic hydrolysis
3. Oxidized Phenolics
4. Membrane filtration
5. Size exclusion
6. Ion exchange
7. Polymeric Lignin Fragment
8. Sugars and Carbohydrates
9. Bio-ethanol and Lipids
10. Polymeric Blends, Elastomers, Rigid foams
11. Phenolics, Vanillate, Fine Chemicals