Aquatic biomass: downstream technologies to FAMEs

Angela Dibenedetto
Castro Marina
September 18th-24th 2011

A European Project supported within the Seventh Framework Programme for Research and Technological Development
Acquatic biomass

- Aquatic biomass is currently considered as an ideal feedstock for third generation biodiesel (biofuel) as they do not compete with food and feed crops and can produce higher amounts of oil than the terrestrial biomass. The cultivation of algae is also very attractive because of its potential to fix atmospheric CO$_2$ into biomass and for enhanced biological fixation (150x).
Biofuels

● **First generation:** “food” crops
  ● useful, but limited
  ● cannot produce enough biofuel without threatening food supplies and biodiversity
  ● not cost competitive with existing fossil fuels

● **Second generation:** lignocellulosic materials, residual biomass, residual non-food parts of crops
  ● Barrier: to extract useful feedstocks from woody or fibrous biomass, where the useful sugars are locked in by lignin and cellulose

● **Third generation:** aquatic biomass
  ● low-input, high-yield feedstock to produce biofuels
The aquatic biomass option

- A way to reduce the land utilization is the use of water environments
- Natural or artificial basins, salty or fresh waters can be considered for exploitation or resources recovery
  - Cultivation on marginal coastal areas or off-shore
  - Lagooning waste water for treatment
- Fisheries are interesting candidates coupling water treatment and aquatic biomass growing
- Municipal and process waters can be used, with the additional benefit of water treatment and better resource utilization
Terrestrial vs Aquatic

- Light efficiency 1.5-2.2%
- Requires land and water
- Productivity depends on soil quality (for a given plant)
- Soil additives may be required (environmental and economic costs)
- Biomass is generally rich in ligno-cellulosic components
- Seed plants are most used
- Open area more than greenhouse cultivation

- Light efficiency 6-8% (or higher when irradiated bioreactors are used)
- May not require land for cultivation (offshore ocean)
- Low lignocellulose content
- Richer in water
- Lipid/protein/polysaccharide content can be adjusted
- Easy to grow in bioreactors (light-temperature adjustment): decoupling from climatic conditions.
## Comparison of some sources of biofuel

<table>
<thead>
<tr>
<th>Crop</th>
<th>Oil yield (L/ha)</th>
<th>Land area needed (M ha)</th>
<th>Percent of existing US cropping area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>172</td>
<td>1540</td>
<td>846</td>
</tr>
<tr>
<td>Soybean</td>
<td>446</td>
<td>594</td>
<td>326</td>
</tr>
<tr>
<td>Canola</td>
<td>1190</td>
<td>223</td>
<td>122</td>
</tr>
<tr>
<td>Jatropha</td>
<td>1892</td>
<td>140</td>
<td>77</td>
</tr>
<tr>
<td>Coconut</td>
<td>2689</td>
<td>99</td>
<td>54</td>
</tr>
<tr>
<td>Oil palm</td>
<td>5950</td>
<td>45</td>
<td>24</td>
</tr>
<tr>
<td>Microalgae</td>
<td>136,900</td>
<td>2</td>
<td>1.1</td>
</tr>
<tr>
<td>Microalgae</td>
<td>58,700</td>
<td>4.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>

\textsuperscript{a} For meeting 50% of all transport fuel needs of the United States.
\textsuperscript{b} 70% oil (by wt) in biomass.
\textsuperscript{c} 30% oil (by wt) in biomass.
The interest towards the exploitation of aquatic biomass

- **Aquatic biomass include** macro-algae, micro-algae and emergents (plants).
  - **Macro-algae** commonly known as “seaweeds” are multicellular organism. They are often fast growing and can reach sizes of up to 60 m in length. They are mainly utilised for the production of food and the extraction of hydrocolloids.
  - **Micro-algae** are microscopic organisms. Diatoms are the dominant life form in phytoplankton and probably represent the largest group of biomass producers on earth. Green algae are especially abundant in fresh water. The main storage compound of these algae is starch, although oils can also be produced. The golden algae are similar to the diatoms and produce oils and carbohydrates.
  - **Emergents** are plants that grow partially submerged in bogs and marshes.
Productivity, lipid content and grown conditions

- The productivity of macroalgae under most performant conditions ranges from 150 to 600 tfw per hectar per year, that must be compared with the typical value for sugarcane that ranges from 70 to 170 tfw ha-1 y-1.

- The lipid content of microalgae may largely vary with the species, typical values ranging around 20-40% of the dry weight with excellent records of 75-80% using particular stressing growth conditions.
Micro-algae

- Microalgae are currently cultivated commercially for fish nutritional scope around the world in several dozen small- to medium-scale production systems, producing a few tens to a several hundreds of tons of biomass annually.
- The main algae genera currently cultivated photosynthetically (e.g. with light energy) for various nutritional products are *Spirulina*, *Chlorella*, *Dunaliella* and *Haematococcus*. 
Microalgae - *Chlorella* and *Spirulina*

*Chlorella sp.* is a unicellular organism that can be found in almost any water environment (fresh water and marine). *Growth rate*: 26 g/m²·day dry weight. *Temperature*: 35–37 °C (depending on species). *pH*: Depends on species.

*Spirulina* is a multicellular, filamentous blue-green algae. Various commercial *Spirulina* production plants are currently in operation. *Growth rate*: 30 g/m²·day dry weight. *Temperature*: Optimum between 35–37 °C. Very tolerable to pH change.
Microalgae – *Haematococcus pluvialis* and *Dunaliella*

*Haematococcus pluvialis* is a freshwater species of *Chlorophyta*. It is usually found in temperate regions around the world.20 *Growth rate*: 9-13 g/m²·day dry weight.

*Dunaliella* is a type of halophile micro-algae especially found in sea salt fields. *Growth rate*: 1.65 g/m²·day dry weight. *Temperature* and *pH*: Depends on species.
Other microalgae of interest

*Ostreococcus* sp.
*Tetraselmis* sp.
*Botryococcus braunii*
*Chlamydomonas reinhardtii*
*Haematococcus pluvialis*
*Dunaliella* sp.
*Chlorococcum* sp.
*Neochloris oleobundans*
*Scenedesmus* sp.
*Desmodesmus* sp.
*Chlorella* sp.
*Parietochloris incisa*
*Prototheca* sp.

*Porphyridium cruentum*
Growing condition

- open ponds
  - raceways

- photo-bioreactors
Open ponds

- The culture in open ponds is more economically favourable, but rises the issues of land cost and water availability, appropriate climatic conditions, nutrients cost and production.
- Using open pond systems the nutrients can be provided through runoff water from nearby living areas or by channelling the water from waste water treatment plants.
- Some source of waste CO\(_2\) could be efficiently bubbled into the ponds and captured by the algae. The water is moved by paddle wheels or rotating structures (raceway systems), and some mixing can be accomplished by appropriately designed guides.
Types of ponds

- horizontal ponds and sloped cultivations ponds. Among horizontal ponds the most preferred are the raceways ponds, but there are also the circular ponds that are very expensive as they require high-energy consumption and it is difficult to obtain turbulence in the centre of the pond.
Raceway ponds

- lined with plastic or cement
- about 20 to 35 cm deep to ensure adequate exposure to sunlight.
- Presence of paddlewheels to provide motive force and keep the algae suspended in the water.

- The ponds are supplied with water and nutrients, and mature algae are continuously removed at one end.
Photobioreactor

- Using bioreactors, micro-algae can grow under light-irradiation and temperature controlled conditions, with an enhanced fixation of carbon dioxide that is bubbled through the culture medium.
Photobioreactor: Algae receive sunlight either directly through the transparent container walls or via light fibres or tubes that channel the light from sunlight collectors.
A 1000 L helical tubular photobioreactor at Murdoch University, Australia.
Capital Construction Costs for three different algal production systems

<table>
<thead>
<tr>
<th>Production systems</th>
<th>Costs ($/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open pond</td>
<td>76 000</td>
</tr>
<tr>
<td>Raceways</td>
<td>161 000</td>
</tr>
<tr>
<td>Photobioreactors</td>
<td>348 000</td>
</tr>
</tbody>
</table>
Key factors for growth

- **solar irradiation**
  - Both in ponds and in bioreactors the light availability is of paramount importance. Shadow or short light-cycles may cause a slow down of the growing process, conversely intense light (as may occur in desertic areas or bioreactors) does not guarantee a fast growth and may modify the cell functions.
Key factors for growth

- **Temperature, availability of land and water**
  - Semi- or tropical areas are the most practical
  - Evaporation rate (ER) may represent a problem in dry tropical areas (where the evaporation rate is higher than the precipitation rate)
  - A high ER increases salt concentration and pumping costs due to water loss and may have a cooling effect on the medium
  - Precipitation rate can cause dilution and a loss of nutrients and algal biomass,
  - High relative humidity and no winds may cause an increase of the temperature of the medium
A recent EU study has shown that the most suitable cultivation areas in Europe are:

- South of Spain
- South of Italy
- Greece

Such areas are not rich of fresh water!!!!!!!!
Key factors for growth

- **carbon dioxide sources**
  - The direct use of flue gases as CO$_2$ providers requires that algae should be resistant to the pollutants that are usually present in the flue-gas stream, namely nitrogen- and sulphur-oxides. Studies have shown that 150 ppm of NO$_2$ and 200 ppm of SO$_2$ do not affect the growth of some algal species.

- **nutrients (N- and P-compounds)**
  - The use of waste water (sewage, fisheries, some industrial waters) rich in N-, and P-nutrients is an economic option with a double benefit represented by recovery and utilization of useful inorganic compounds, and the production of clean water that, finally, can be reused or discharged into natural basins.
A LCA study has demonstrated that if nutrients are added and purchased on the market the C- and economic balance is negative!

Energy for nutrient production
Cost of chemicals
Harvesting

- Micro-algae, due to their size and, sometimes, fragility, demand for sophisticated equipment and handling operations.

- The choice of harvesting methods depends on:
  - Type of algae that has to be harvested (filamentous, unicellular, etc),
  - Whether harvesting occurs continuously or discontinuously
Harvesting

The mainly used technologies are:

- **Centrifugation** *(very popular)*
- **Sedimentation,*
- **Filtration** *(use of tubular, capillary or hollow-fiber membranes).*
  - The size of the pore decreases in the order from tubular (5-15 mm) to capillary (1 mm) to hollow-fiber (0.1 μm) and the risk of plugging increases with the decrease of the pore diameter.
- **Flocculation** *(metal compounds, cationic polymers and natural polymers such as chitin).*
Macroalgae

- The big advantage of macro-algae is their huge mass production.
  - The productivity of natural basins is in the range 1-20 kg m\(^{-2}\) y\(^{-1}\) dry weight (10-150 tdw ha\(^{-1}\) y\(^{-1}\)) for a 7-8 month culture.

- Macro-algae are very effective in nutrients (N, P) uptake from sewage and industrial waste water.
  - The estimated recovery capacity is 16 kg ha\(^{-1}\) d\(^{-1}\).

- In Europe macro-algae are grown in experimental fields, and natural basins.

- Also in a colder climate, macro-algae grow at an interesting rate. For example, in Denmark the Odjense Fiord produces ca. 10 kt dw d\(^{-1}\) of biomass equivalent to ca. 10 t per year per ha.
Macroalgae

- The photosynthesis of macro-algae is saturated at different levels of carbon dioxide (500-2000 ppm)
  - with CO$_2$ concentration up to five times the atmospheric concentration, under the correct light conditions and nutrient supply, they may grow with the same or better performance they show in natural environments.

- Macro-algae require less sophisticated techniques for growing: coastal farms are the most used techniques.

- The world market of seaweeds is close to 40 Mt/y.
Macroalgae

*Chaetomorpha linum* 1 cm
- Unattached; coastal lagoons; both hemispheres

*Ulva lactuca* Areschoug
- Attached; coastal lagoons; both hemispheres

*Codium vermilara*
- Attached and unattached; coastal lagoons; boreal hemisphere

*Pterocladia capillacea*
- Attached; coastal lagoons; both hemispheres

*Gracilaria bursa-pastoris*
- Attached and unattached; coastal lagoons; both hemispheres
Cultivation

- Few genera have been commonly cultivated for many years such as: *Laminaria, Porphyra, Undaria, Gracilaria, Euchema, Ulva and Chondrus*.
- The seaweed harvested from natural stocks has decreased significantly, while cultivated seaweed has sharply increased.
- The overall amount of seaweed harvested has almost doubled in the last 10 years to 15 million wet tonnes (FAO, 2006).
Harvesting

Hand Harvesting of Ascophyllum
(M Guiry/Algaebase)

Scoubidou System Used in France (CEVA)

Harvest of Drift Seaweed at Sacca di Goro, Italy (Internet)
Macroalage: a waste to convert

Sargassum floating in the Venice bay
(Picture: www.algaebase.org)

Drift Ulva spp (CEVA)
Emergents or aquatic plants

*Egeria densa*
*Eichhornia crassipes*
*Elodea canadensis*
*Lagarosiphon major*
*Lemna minor*

With respect to micro- and macro-algae, plants may contain a larger content of cellulosic materials and require different technologies for their treatment and use for energetic purposes.
Adapt the technology to the biomass

- **Macroalgae**
  - Low lipid content $\rightarrow$ Chemicals, Biogas
  - High lipid content $\rightarrow$ Lipid and chemicals extraction $\rightarrow$ Biodiesel, $H_2$
  - Residual biomass $\rightarrow$ Syngas $\rightarrow$ Chemicals, $H_2$

- **Microalgae**
  - High lipid content $\rightarrow$ Lipids $\rightarrow$ Biodiesel, $H_2$
  - Residual biomass $\rightarrow$ Syngas $\rightarrow$ Chemicals, $H_2$
Where are located the products?

- **The cell surface-membrane**
  - Polymers
    - Polysaccharides (cellulose and cellulose-like)
    - Alginate (cell wall in seaweeds)
    - Carrageenans (cell wall in red-algae)

- **Intracellular space**
  - Lipids
  - Aminoacids
  - Other organics
  - PHA
Macroalgae adaptation: Research program

● Eco-physiological characterization of the selected algae.
● Standardization of the culture conditions.
● Definition of the photosynthetic capacity as function of the temperature and light intensity.
● Optimization of the conditions for growing algae in ponds.
● Sensitivity to:
  ● carbon dioxide concentration
  ● NOx and SOy level in the fed gas
● Use of waste water from fisheries
Influence of pH on the algal growth

✓ The pH of the culture medium depends on the amount of CO\textsubscript{2} bubbled. A pH = 6 is usually reached that may affect the growth.

✓ The response to pH is not the same for all seaweeds.

✓ It is, thus, necessary to select species which growth is not much pH-dependent.

✓ Alternatively, CO\textsubscript{2} must be bubbled under controlled conditions.
Resistance to NOx and SOy

✓ A major constraint is the purification cost of carbon dioxide
✓ Therefore, the direct use of flue gases is compulsory in order to avoid such costs
✓ The resistance of algae to NOx and SOy is a fundamental requisite
✓ 150 ppm of NOx and 200 ppm of SOy do not affect the algal growth
The influence of the growing conditions

● Influence of N, P
  ● They affect the rate of growing

● Influence of temperature
  ● It influences the rate of growing

● Influence of CO$_2$ concentration
  ● Working btwn + 0.038-10 % in the gas phase
  ● Influences the lipid content and their distribution

● Physical stresses can drive the product distribution
The must for aquatic biomass exploitation

● Mastering of the product-entropy distribution
  ● Maximization of the yield of production of a class of products: specialty chemicals (high value), starch and sugars (bioethanol), proteins (feed for animals), lipids (biodiesel), biogas, soil additive

● Coupling the fuel-chemicals production in order to obtain the best economical value (the cost of fuel alone would be prohibitive)
  → terrestrial plants 700->2000->4800 €/ha
<table>
<thead>
<tr>
<th>Strain</th>
<th>M/F</th>
<th>Protein</th>
<th>Carbohydrates</th>
<th>Lipids</th>
<th>Nucleic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Scenedesmus obliquus</em></td>
<td>F</td>
<td>50 - 56</td>
<td>10 - 17</td>
<td>12 - 14</td>
<td>3 - 6</td>
</tr>
<tr>
<td><em>Scenedesmus quadricauda</em></td>
<td>F</td>
<td>47</td>
<td>-</td>
<td>1.9</td>
<td>-</td>
</tr>
<tr>
<td><em>Scenedesmus dimorphus</em></td>
<td>F</td>
<td>8 - 18</td>
<td>21 - 52</td>
<td>16 - 40</td>
<td>-</td>
</tr>
<tr>
<td><em>Chlamydomonas rheinhardii</em></td>
<td>F</td>
<td>48</td>
<td>17</td>
<td>21</td>
<td>-</td>
</tr>
<tr>
<td><em>Chlorella vulgaris</em></td>
<td>F</td>
<td>51 - 58</td>
<td>12 - 17</td>
<td>14 - 22</td>
<td>4 - 5</td>
</tr>
<tr>
<td><em>Chlorella pyrenoidosa</em></td>
<td>F</td>
<td>57</td>
<td>26</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td><em>Spirogyra sp.</em></td>
<td>F</td>
<td>6 - 20</td>
<td>33 - 64</td>
<td>11 - 21</td>
<td>-</td>
</tr>
<tr>
<td><em>Dunaliella bioculata</em></td>
<td>M</td>
<td>49</td>
<td>4</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td><em>Dunaliella salina</em></td>
<td>M</td>
<td>57</td>
<td>32</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td><em>Euglena gracilis</em></td>
<td>F</td>
<td>39 - 61</td>
<td>14 - 18</td>
<td>14 - 20</td>
<td>-</td>
</tr>
<tr>
<td><em>Prymnesium parvum</em></td>
<td>M</td>
<td>28 - 45</td>
<td>25 - 33</td>
<td>22 - 38</td>
<td>1 - 2</td>
</tr>
<tr>
<td><em>Tetraselmis maculata</em></td>
<td>M</td>
<td>52</td>
<td>15</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td><em>Porphyridium cruentum</em></td>
<td>M</td>
<td>28 - 39</td>
<td>40 - 57</td>
<td>9 - 14</td>
<td>-</td>
</tr>
<tr>
<td><em>Spirulina platensis</em></td>
<td>F</td>
<td>46 - 63</td>
<td>8 - 14</td>
<td>4 - 9</td>
<td>2 - 5</td>
</tr>
<tr>
<td><em>Spirulina maxima</em></td>
<td>F</td>
<td>60 - 71</td>
<td>13 - 16</td>
<td>6 - 7</td>
<td>3 - 4.5</td>
</tr>
<tr>
<td><em>Synechoccus sp.</em></td>
<td>M</td>
<td>63</td>
<td>15</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td><em>Anabaena cylindrica</em></td>
<td>F</td>
<td>43 - 56</td>
<td>25 - 30</td>
<td>4 - 7</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: M/F indicates marine or freshwater species.
<table>
<thead>
<tr>
<th>Products from microalgae</th>
<th>Product</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biomass</strong></td>
<td>Biomass</td>
<td>Health food, Functional food, Feed additive, Aquaculture, Soil conditioner</td>
</tr>
<tr>
<td><strong>Coloring substances &amp; Antioxidants</strong></td>
<td>Xanthophylls (astaxanthin and canthaxanthin), Lutein, β-carotene, Vitamins C and E</td>
<td>Food and feed additives, Cosmetics</td>
</tr>
<tr>
<td><strong>Fatty acids-FA</strong></td>
<td>Arachidonic acid-AA, Eicosapentaenoic acid-EPA, Docosahexaenoic acid-DHA, γ-linolenic acid-GCA, Linoleic acid-LA</td>
<td>Food additive</td>
</tr>
<tr>
<td><strong>Enzymes</strong></td>
<td>Superoxide dismutase-SOD, Phosphoglycerate kinase-PGK, Luciferase and Luciferin, Restriction enzymes</td>
<td>Health food, Research, Medicine</td>
</tr>
<tr>
<td><strong>Polymers</strong></td>
<td>Polysaccharides, Starch, Poly-β-hydroxybutyric acid-PHB</td>
<td>Food additive, Cosmetics, Medicine</td>
</tr>
<tr>
<td><strong>Special products</strong></td>
<td>Peptides, Toxins, Isotopes, Aminoacids (proline, arginine, aspartic acid), Sterols</td>
<td>Research, Medicine</td>
</tr>
<tr>
<td>Class of products</td>
<td>Chemicals</td>
<td>Extraction technology</td>
</tr>
<tr>
<td>-------------------</td>
<td>-----------</td>
<td>-----------------------</td>
</tr>
<tr>
<td><strong>Proteins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Aminoacids</strong></td>
<td></td>
<td>Phenol-acetic acid-water</td>
</tr>
<tr>
<td><strong>Lipids</strong></td>
<td></td>
<td>Sc-CO₂, organic solvent, liquefaction, pyrolysis</td>
</tr>
<tr>
<td><strong>Essential oils</strong></td>
<td>Geraniol, geranyl formate or acetate, cytronellol, nonanol, eucalyptol</td>
<td>Distillation</td>
</tr>
<tr>
<td><strong>Sterols</strong></td>
<td>Cholesterol</td>
<td></td>
</tr>
<tr>
<td><strong>Pigments: chlorophylls, carotenoids, xanthophylls</strong></td>
<td>Isoprenoids</td>
<td>Solvent extraction</td>
</tr>
<tr>
<td><strong>Amines</strong></td>
<td>Methylamines, ethylamines, propylamine, isobutylamine</td>
<td></td>
</tr>
<tr>
<td><strong>Inorganic compounds</strong></td>
<td>Iodides, bromides, sulphates, nitrates, etc</td>
<td></td>
</tr>
</tbody>
</table>
Macroalgae vs Microalgae

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Microalgae</th>
<th>Macroalgae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growing season</td>
<td>250-280 d</td>
<td>210-240 d</td>
</tr>
<tr>
<td>Productivity, dw</td>
<td>33-50 t ha(^{-1})</td>
<td>10-70 t ha(^{-1})</td>
</tr>
<tr>
<td>Lipid content</td>
<td>20-75 % dw</td>
<td>0.3-32 % dw</td>
</tr>
<tr>
<td>Production cost</td>
<td>100/5000 $ t(^{-1}) dw</td>
<td>100 $ t(^{-1}) dw</td>
</tr>
<tr>
<td>Heat value GJ t(^{-1})</td>
<td>21 GJ t(^{-1}) dw</td>
<td>12.2–20 GJ t(^{-1}) dw</td>
</tr>
<tr>
<td>Energy cost</td>
<td>0.56 $ MJ(^{-1})</td>
<td>0.05-0.6 $ MJ(^{-1})</td>
</tr>
</tbody>
</table>
Technologies available for the treatment of the biomass

• Extraction (pressure, solvent)
• Thermal (pysolysis, liquefaction, gasification)

• Treatment with SCF
  • Extraction with sc-CO$_2$, also in presence of co-solvents
  • sc-H$_2$O gasification (Syngas, H$_2$-CO)

• Fermentation (alcoholic, anaerobic) (ethanol, bio-gas, bio-hydrogen)
Extraction

- The extraction of chemicals from micro- and macro-algae may require different technologies due to the different size and quality of the cell membrane of the algae.
- Depending on the species-strain, the cell membrane can result to be very hard, so that crushing of the membrane is recommended prior to the extraction.
  - Such crushing is quite effective if performed at low temperature, typically the liquid nitrogen temperature (183 K).
  - This will obviously increase the cost of the extracted oil and lower its net energetic value.
● The extraction by using organic solvents is very often used.
  ● This may have a drawback due to the retention of solvent, manipulation of it, treatment at the end of the extraction phase.
● Supercritical carbon dioxide (scCO$_2$) may substitute the organic solvent as it has some unique advantages and is considered a good candidate for algae treatment because it is a non-toxic and fully “green” solvent.
  ● anhydrous materials are recommended (water content below 5%), so energy should be consumed to dry the biomass.
  ● the cellular wall has to be broken in order to increase the extraction yield (it is possible to use liquid nitrogen, or a different method).
  ● sometimes methanol can be added as co-solvent in order to increase the extraction yield.
# Oil content of some microalgae

<table>
<thead>
<tr>
<th>Microalga</th>
<th>Oil content (% dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Botryococcus braunii</em></td>
<td>25–75</td>
</tr>
<tr>
<td><em>Chlorella sp.</em></td>
<td>28–32</td>
</tr>
<tr>
<td><em>Cryptothecodinium cohnii</em></td>
<td>20</td>
</tr>
<tr>
<td><em>Cylindrotheca sp.</em></td>
<td>16–37</td>
</tr>
<tr>
<td><em>Dunaliella primolecta</em></td>
<td>23</td>
</tr>
<tr>
<td><em>Isochrysis sp.</em></td>
<td>25–33</td>
</tr>
<tr>
<td><em>Monallanthus salina</em></td>
<td>&gt;20</td>
</tr>
<tr>
<td><em>Nannochloris sp.</em></td>
<td>20–35</td>
</tr>
<tr>
<td><em>Nannochloropsis sp.</em></td>
<td>31–68</td>
</tr>
<tr>
<td><em>Neochloris oleoabundans</em></td>
<td>35–54</td>
</tr>
<tr>
<td><em>Nitzschia sp.</em></td>
<td>45–47</td>
</tr>
<tr>
<td><em>Phaeodactylum tricornutum</em></td>
<td>20–30</td>
</tr>
<tr>
<td><em>Schizochytrium sp.</em></td>
<td>50–77</td>
</tr>
<tr>
<td><em>Tetraselmis sueica</em></td>
<td>15–23</td>
</tr>
</tbody>
</table>
Amount (L) of oil per hectare per year of different type of biomass including microalgae

<table>
<thead>
<tr>
<th>Biomass</th>
<th>Yield (L ha(^{-1}) y(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>170</td>
</tr>
<tr>
<td>Soybeans</td>
<td>455 to 475</td>
</tr>
<tr>
<td>Safflower</td>
<td>785</td>
</tr>
<tr>
<td>Sunflower</td>
<td>965</td>
</tr>
<tr>
<td>Rapeseed</td>
<td>1200</td>
</tr>
<tr>
<td>Jatropha</td>
<td>1890</td>
</tr>
<tr>
<td>Coconut</td>
<td>2840</td>
</tr>
<tr>
<td>Palm</td>
<td>6000</td>
</tr>
<tr>
<td>Microalgae</td>
<td>47250 to 142000</td>
</tr>
</tbody>
</table>
The quality of bio-oil

Distribution of fatty acids in lipids present in some macro-algae.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Species and Percentage of a given compound in the species</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Compound</strong> N C&lt;sub&gt;atoms/unsaturated bonds&lt;/sub&gt; of</td>
<td><strong>Fucus sp</strong></td>
</tr>
<tr>
<td>Saturated C&lt;sub&gt;12→C&lt;sub&gt;20&lt;/sub&gt;</td>
<td>15.6</td>
</tr>
<tr>
<td>Mono-unsaturated C&lt;sub&gt;14→C&lt;sub&gt;20&lt;/sub&gt;</td>
<td>28.55</td>
</tr>
<tr>
<td>Poly-unsaturated C&lt;sub&gt;16/2→C&lt;sub&gt;16/4&lt;/sub&gt;, C&lt;sub&gt;18/2→C&lt;sub&gt;18/4, C&lt;sub&gt;20/2&lt;/sub&gt;</td>
<td>55.86</td>
</tr>
</tbody>
</table>
Bio-oil, such as extracted, can be directly used in thermal processes or in combustion, but cannot be used in diesel engines as it presents a Low Enthalpy Value-LHV (8-12 MJ/kg) and high viscosity.

To the latter use it can be converted into bio-diesel through using different techniques, for example a transesterification reaction in order to increase to 36 MJ/kg the LHV.
FUELS production technologies

There are three basic conventional ways for the production of methyl esters from oils and fats:

- Base catalysed transesterification of the oil (triglycerides) with methanol;
- Direct acid catalyzed esterification of the free fatty acids (FFA) with methanol;
- Conversion of the oil to FFA followed by their esterification.

New approaches (our area of operation):

- Direct conversion of biomass into FAMEs
- Conversion of the extracts into hydrocarbons (hydrocraking)
Biodiesel from triglyceride oils

1. Multi-step reaction mechanism: Triglyceride ↔ Diglyceride ↔ Monoglyceride ↔ Methyl esters + glycerine
2. Affected by various factors depending upon the reaction conditions used such as: the reaction temperature, the ratio of alcohol to vegetable oil, the amount and the type of catalyst, the mixing process and the raw oils used.
Critical parameters of the process are

- **Presence of water**
  - at high temperatures water can hydrolyze the triglycerides and form free fatty acids (require a different catalyst than triglycerides) and unwanted soaps.

- **Complete reaction**

- **Removal of glycerol (now watery glycerol is formed that requires energy for the recovery)**

- **Removal of the catalyst**

- **Removal of excess alcohol**
When the starting material contains amount of FFAs that exceed 0.1-0.5 wt%, a direct base catalysed trans-esterification of the lipid fraction significantly decreases the biodiesel yield due to the formation of soap and water (Reaction a-b).

\[ \begin{align*}
\text{a)} & \\
R-C-OH + MOH & \rightarrow R-C-O^{-}M^{+} + H_{2}O \\
\text{b)} & \\
R-C-OR + H_{2}O & \rightarrow R-C-OH + ROH \\
M = \text{Na, K}
\end{align*} \]
The bio-oil extracted by micro-algae contains large amount (up to 19%) of FFAs and require two different types of catalysts (acid and basic catalyst) used in a two-stage process. This means that the acid catalyst from the first stage of esterification of FFAs has to be removed before the basic catalyst is added in the second stage for the trans-esterification of lipid.
New catalysts are needed:

- Heterogeneous (mesoporous solids)
- Bi-functional (acid-base)
  - multi-purpose highly active,
  - recoverable,
  - reusable
Simultaneous trans-esterification and esterification: an example

- *New mixed oxides* based on calcium, cerium and aluminium have been used as catalysts in the simultaneous direct trans-esterification of lipid and esterification of FFAs present in oil extracted from *aquatic biomass* (*Nannochloropsis sp* microalgae).

<table>
<thead>
<tr>
<th>Catalysts</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 CaO CeO₂</td>
</tr>
<tr>
<td>0.5 CaO CeO₂</td>
</tr>
<tr>
<td>CaO CeO₂</td>
</tr>
<tr>
<td>12 CaO 7 Al₂O₃</td>
</tr>
<tr>
<td>12CaO 7Al₂O₃ 3 CeO₂</td>
</tr>
<tr>
<td>12CaO 7Al₂O₃ 7 CeO₂</td>
</tr>
<tr>
<td>12CaO 7Al₂O₃ 12 CeO₂</td>
</tr>
</tbody>
</table>
• The conversion of bio-oil (lipids plus FFAs) into FAMEs was carried out using catalysts characterized by a different number of basic and acid sites.

<table>
<thead>
<tr>
<th>Cat</th>
<th>n_B/n_A</th>
<th>BET (g/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CeO₂·3%Al₂O₃</td>
<td>0.53</td>
<td>43.6</td>
</tr>
<tr>
<td>CeO₂·10%Al₂O₃</td>
<td>0.69</td>
<td>57.8</td>
</tr>
<tr>
<td>CeO₂·40%CaO</td>
<td>1.16</td>
<td>10.5</td>
</tr>
<tr>
<td>12CaO·7γAl₂O₃</td>
<td>1.27</td>
<td>40.1</td>
</tr>
</tbody>
</table>

n_B = basic sites; n_A = acid sites
FAMEs production from bio-oil extracted from microalgae

Operative conditions: 1 h, 353 K, MeOH/oil= 150/1

FAMEs> 96%
FFA<0.5%

Bio-oil used

CeO2
0.1 CaO CeO2
0.5 CaO CeO2
CaO CeO2

Operative conditions: 1 h, 353 K, MeOH/oil= 150/1
New approach: from biomass to biodiesel

Reactive extraction ---
direct one-pot conversion
of biomass into FAMEs

Methylpalmitoleate
Methylpalmitate
Methyloleate
Methylstearate
Methylmiristate

M. Aresta, A. Dibenedetto, M. Ricci Italian Patent to ENI 2009
Summary

<table>
<thead>
<tr>
<th>Resource Type</th>
<th>Biomass Generation</th>
<th>Harvesting</th>
<th>Pre-treatment</th>
<th>Downstream Processing</th>
<th>Market</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macroalgae (Seaweeds)</td>
<td>Natural stocks Aquaculture Nearshore Offshore</td>
<td>Manual Mechanisation</td>
<td>Cleaning Dewatering Desalination</td>
<td>Biogas Bioethanol Biorefinery Residues</td>
<td>Logistics Infrastructure Engines</td>
</tr>
<tr>
<td>Microalgae (Phytoplankton)</td>
<td>Cultivation Photo bioreactor Open ponds Species selection</td>
<td>Filtration Sedimentation Centrifuge Flocculation</td>
<td>Dewatering Drying</td>
<td>Biodiesel (lipids) Fermentation (biomass) Biorefinery Residues</td>
<td>Logistics Infrastructure Engines Aviation</td>
</tr>
</tbody>
</table>
How to assess the environmental and economic benefits?

- A tool is necessary that may take into consideration the many parameters and can evaluate the effects of changing the growing conditions and the product entropy.

- Development of the LCA methodology for the comparison of the performance of the biomass.

- Software for an easy numerical treatment.
System description.

Legend:
- **System boundaries**
- **Subsystems**
- **Processes**
- **Elementary flux**
- **LCA**

- **Raw material**
- **Auxiliary material**
- **Energy**

- **Nutrient supply**
  - **Biomass cultivation**
  - **Biomass harvesting**
  - **Biomass drying**

- **Gas supply**

- **By-products**

- **Emissions into air, water, soil**

**Processes**:
- Biomass conversion
- BIO-OIL treatment
- BIO-DIESEL
Scenario 1 – Integrated system for biodiesel production

System boundaries

Power plant

- Flue gas
  - MEA
  - NH$_3$
  - Energy, heat

- CO$_2$
- Ethylene oxide
- NH$_3$
- Energy, heat

Wastewater

Algae production

- CO$_2$
- Energy
- CO$_2$

Algae harvesting and drying

- Effluent

Algae conversion (sc-CO$_2$)

- LHV=8-12 MJ/kg
- Waste from reaction
- Biogas/Incineration

BIO-OIL

- Energy
- Hydroxide
- MeOH
- Acid
- Glycerol
- Waste from reaction

BIO-DIESEL

FU = 1 MJ BIO-DIESEL
Scenario 2 – System functions: biodiesel production, electricity production and wastewater treatment

- **Power plant**
  - **Flue gas**
  - **Flue gas cleaning**

- **Biomass cultivation**
  - **Biomass conversion**
  - **BIO-OIL**
    - **BIO-OIL treatment**
      - **BIO-DIESEL**

- **Waste water treatment plant (WWTP)**
  - **Waste water**
  - **E, nutrients, pesticides**
    - **E, solvents, heat**

- **Energy**
  - **NaOH**
  - **MeOH**
  - **HCl**

- **System boundaries**
  - **FU = 1 MJ BIO-DIESEL**

- **Bio-diesel production**
Thank you very much for your kind attention