ST-13 Bioeconomía

“La herramienta para el desarrollo de la economía circular”

APLICACIÓN DE TECNOLOGÍAS INNOVADORAS EN EL DESARROLLO DE UNA BIOREFINERÍA DE MICROALGAS Viable y Sostenible

Sonia Castañón de la Torre
“OVERCOMING THE BARRIERS TO DEVELOPMENT OF CULTURES OF MICROALGAE FOR ENERGY PURPOSES” (Ref: EFA217/11)

2012-2014

energreen

“NETWORK OF TECHNOLOGICAL CENTRES TO DEVELOP A BIOREFINERY FROM ALGAE” (Ref: EFA037/15)

2016-2019

CYCLALG

Suarez, S.; Urreta, I.; Izaguirre, J.K. and Castañón, S.

CONAMA 2016-ST-13 Bioeconomía: “La herramienta para el desarrollo de la economía circular”
IT IS ESTIMATED THAT THE WORLD’S ENERGY CONSUMPTION WILL HAVE GROWN 40% BY 2030. INCREASING ENERGY DEMAND FORECASTS, TOGETHER WITH GEOGRAPHICAL CONSUMPTION REDISTRIBUTION, WILL CONTRIBUTE TO THE FURTHER DEPLETION OF FOSSIL FUELS AND TO PUSH THEIR PRICES UP AS A RESULT OF A HIGHER SUPPLY-DEMAND IMBALANCE.

ENERGY DEPENDENCY IN THE EUROPEAN UNION CURRENTLY AMOUNTS TO 53% AND ON THE FACE OF THE STEADY ESCALATION IN ENERGY CONSUMPTION AND ENERGY IMPORTS, THE EU IS LOOKING AT THE CURRENT TRENDS WITH CONCERN.
THE IDEAL SOURCE OF BIOENERGY: MICROALGAE DO NOT COMPETE WITH FOOD CROPS

ENVIRONMENTAL BENEFITS AND SUSTAINABILITY: LESS FERTILE SOIL, LOWER DEMAND OF WATER RESOURCES AND HIGH CO₂ FIXATION.

ACCORDING TO THE HIGH GROWTH RATES AND OIL PRODUCTIVITY THAT SOME OF THESE SPECIES SHOW, IT IS ESTIMATED THAT THE AVERAGE BIODIESEL PRODUCTION FROM MICROALGAE COULD REACH TO A 20-TIMES HIGHER VALUE COMPARED TO OTHER OLEAGINOUS SOIL SPECIES (CHISTI, 2007).

<table>
<thead>
<tr>
<th>CROP</th>
<th>OIL PRODUCTION (L/Ha/YEAR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOY</td>
<td>450</td>
</tr>
<tr>
<td>CAMELINA</td>
<td>580</td>
</tr>
<tr>
<td>SUNFLOWER</td>
<td>950</td>
</tr>
<tr>
<td>JATROPHA</td>
<td>1,900</td>
</tr>
<tr>
<td>PALM</td>
<td>6,000</td>
</tr>
<tr>
<td>ALGAE</td>
<td>9,000-37,000</td>
</tr>
</tbody>
</table>

(PIENKOS, 2007)

THE MICROALGAE-BASED BIOREFINERY PROGRAMMES STARTED MANY YEARS AGO. IT IS TIME TO MOVE FORWARD IN THE DEVELOPMENT OF INDUSTRIALIZATION PROCESSES FOR MICROALGAE PRODUCTION, SPECIALLY FOR THOSE WITH BIOENERGETIC PURPOSES.
MICROALGAE CAN BE VALORIZED IN SEVERAL SECTORS AND MARKETS

- **FOOD and FEED**
  - Proteins
  - Starch
  - PUFAs
  - Antioxidants
  - Pre-biotic effects
  - Additives

- **NUTRACEUTICALS**
  - Body-care products
  - Carotenoids
  - Omega-3

- **CHEMICALS**
  - Pigments
  - Chemical building blocks
  - Reactants

- **BIOFUELS**
  - Biodiesel
  - Biogas
  - Bioethanol
  - Biohydrogen

- **AGRICULTURE**
  - Biofertilizer
  - Bioestimulant
  - Bioelicitor

- **BIODEPURATION**
  - Fossil CO2 up-take
  - Wastewater treatment
  - Bioremediation/biodepuration

MICROALGAE can be valorized in several sectors and markets, including food and feed, nutraceuticals, chemicals, biofuels, agriculture, and biodepuration.
ECONOMIC SUSTAINABILITY----MAIN BOTTLENECK TO OVERCOME

MARKET VOLUMEN vs. MARKET REVENUES

ECONOMIC DRIVING FORCE

NUTRA
FOOD
FEED
AGRICULTURE
BIOPOLYMERS
BIOFUELS

MARKET ECONOMIC REVENUE

MARKET SIZE

NECESSARY TO BE CONSIDERED (BIOREFINERY)

PRODUCTION COSTS

RELATIVE MARKET BENEFITS
ENERGREEN “OVERCOMING THE BARRIERS TO DEVELOPMENT OF CULTURES OF MICROALGAE FOR ENERGY PURPOSES”
THE PROJECT AIM IS THE READAPTING OF CONVENTIONAL MICROALGAE CULTURES TO OBTAIN MICROORGANISMS WITH HIGHER PRODUCTIVE POTENTIAL (OIL) AND SUITABLE FOR PRODUCING BIODIESEL IN A MORE COST EFFECTIVE AND ENVIRONMENTALLY SUSTAINABLE WAY.

......from cultivation to processing for biodiesel including the exploitation of waste material obtained from oil extraction, as biomolecules of 

✓ THE DEVELOPMENT OF MICROALGAE FARMING STRATEGIES TO OBTAIN ALGAE BIOMASS RICH IN LIPIDS

✓ THE EVALUATION OF ALTERNATIVE OIL EXTRACTION AND PROCESSING SYSTEMS

✓ The research of the potential of CO2 bio-fixation by microalgae from industrial gases with high CO2 content

✓ The evaluation of micro-algae and related extraction wastes used as raw material for the production of BIOGAS, anaerobic digestion and as a potential source of molecules of interest for other sectors
**CHALLENGE:**
LIPID PRODUCTIVITY INCREASE IN CULTURES.............means a reduction in the production costs

WE NEED STRAIN SELECTION FOR HIGH OIL CONTENT, SUITABLE FATTY ACID PROFILE FOR BIODIESEL CONVERSION, HIGH GROWTH RATE AND AN ECONOMICALLY PROFITABLE CULTURE SYSTEM DUE TO THE LOW ECONOMIC VALUE OF BIODIESEL (YEH & CHANG, 2011).

CHLORELLA: EASY CULTURE AND HIGH GROWTH RATE
Standard conditions: 20% LIPIDS and 3% FAMES in dry weight

SCENEDESMUS: HIGH GROWTH RATE, TOLERANT TO ENVIRONMENTAL CHANGES, RESISTANT TO CONTAMINATION WITH OTHER SPECIES
Standard conditions: 25% LIPIDS and 10% FAMES in dry weight
BIOMASS COMPOSITION INCLUDES PROTEINS, CARBOHYDRATES, LIPIDS AND FIBRE IN VARIABLE PROPORTIONS, MAKING IT A VERSATILE RAW MATERIAL

NUTRITIONAL AND ENVIRONMENTAL FACTORS APPLIED IN CULTURE CAN MODIFY DRAMATICALLY THE BIOMASS COMPOSITION AND HENCE, ITS ENERGY VALUE

“development of a culture strategy that allows improving the energy quality of microalgae biomass as biodiesel source”
THE LIMITATION OF NITROGEN AVAILABILITY CAUSES RESERVE LIPIDS STORAGE IN TAGs

NITROGEN STARVATION

THE LIMITATION OF NITROGEN AVAILABILITY CAUSES RESERVE LIPIDS STORAGE IN TAGs
ADVANCES IN THE CULTURE TECHNOLOGY ARE KEY FACTORS TO ACHIEVE COSTS REDUCTION AND IMPROVE PROFITABILITY FOR THE WHOLE VALUE CHAIN OF MICROALGAE CULTURE

THE PROGRESSIVE NITROGEN LIMITATION IMPROVES FAME CONTENT INCREASING PRODUCTIVITY REGARDLESS OF THE DROP IN BIOMASS PRODUCTION
EFFECT OF CULTURE FACTORS IN THE BIOMASS COMPOSITION

**EFFECT OF NITROGEN LIMITATION**

- **Chlorella vulgaris**
  - Nitrogen Available
  - Nitrogen Limitation

- **Scenedesmus sp.**
  - Nitrogen Available
  - Nitrogen Limitation

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**NITROGEN LIMITATION LED TO AN INCREASE IN FAMES AND CARBOHYDRATES. RISING OF BOTH COMPOUNDS REPRESENTS AN IMPROVEMENT IN THE ENERGY VALUE OF THE BIOMASS**
<table>
<thead>
<tr>
<th></th>
<th>LABORATORY</th>
<th>GREENHOUSE</th>
<th>PILOT-PLANT</th>
</tr>
</thead>
<tbody>
<tr>
<td>% FAMEs C.vulgaris</td>
<td>16-19</td>
<td>11-13</td>
<td>9 - 13</td>
</tr>
<tr>
<td>% FAMEs Scenedesmus</td>
<td>30</td>
<td>22</td>
<td>10</td>
</tr>
</tbody>
</table>

**Biodiesel produced with**
- **99% DE FAMES**
- **90% FAMES**
- **80% FAMES**

<table>
<thead>
<tr>
<th></th>
<th>LABORATORY</th>
<th>GREENHOUSE</th>
<th>PILOT-PLANT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>99% Humidity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Phospholipids</td>
<td>20-40 %</td>
<td>1-2 %</td>
<td></td>
</tr>
<tr>
<td>- Glucolipids</td>
<td>10-20 %</td>
<td>0 %</td>
<td></td>
</tr>
<tr>
<td>- Triglycerides</td>
<td>30-50 %</td>
<td>98-99</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>LABORATORY</th>
<th>GREENHOUSE</th>
<th>PILOT-PLANT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>14-16% Humidity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Phospholipids</td>
<td></td>
<td></td>
<td>1-2 %</td>
</tr>
<tr>
<td>- Glucolipids</td>
<td></td>
<td></td>
<td>0 %</td>
</tr>
<tr>
<td>- Triglycerides</td>
<td></td>
<td></td>
<td>98-99</td>
</tr>
</tbody>
</table>
**CHALLENGE:**

**IMPROVE THE OIL EXTRACTION SYSTEM**

.............avoiding biomass drying, using non-toxic solvents

<table>
<thead>
<tr>
<th>1st STAGE: CONCENTRATION</th>
<th>2nd STAGE: EXTRACTION: SOLVENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓ DECANTATION</td>
<td>✓ HEXANE. NOT VERY EFFECTIVE. NEEDS DRYING</td>
</tr>
<tr>
<td>✓ FLOCCULATION</td>
<td>✓ CLO/ METHANOL. EFFECTIVE/ TOXIC. NEEDS DRYING</td>
</tr>
<tr>
<td>✓ FILTRATION</td>
<td>✓ SAPONIFICATION WITH ETHANOL AND HEXANE. NO NEED FOR DRYING</td>
</tr>
<tr>
<td>✓ CENTRIFUGATION**</td>
<td>✓ SUBCRITICAL WATER EXTRACTION: VERY LOW VALUES</td>
</tr>
<tr>
<td>✓ DRYING*</td>
<td></td>
</tr>
<tr>
<td>✓ LIOFILIZATION*</td>
<td></td>
</tr>
</tbody>
</table>

BIOMASS (3-5 g/l)

EXTRACTION OF **80-95 %** OF LIPIDS THAT CAN BE TRANSFORMED INTO BIODIESEL
**SELECTION OF SUITABLE STRAINS**

**ESTABLISHMENT OF INDOOR CULTURE CONDITIONS THAT ALLOW GOOD PRODUCTIVITY LEVELS (Fames>30% of Dry Weight)**

**HIGH EXTRACTION YIELDS (85-95%) FROM FRESH BIOMASS**

**BIOGAS AS A COPRODUCT: ENERGY ENRICHED BIOMASS**

**LIMITATION: OUTDOOR CULTURE CONDITIONS MAINTENANCE TO ACHIEVE SUITABLE PRODUCTIVITY LEVELS**

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**THE HETEROTROPHIC CULTURE SYSTEM WILL BE ABLE TO SOLVE THE PROBLEM OF LOW PRODUCTIVITY AND THE OPERATIONAL LIMITATIONS DURING THE SCALE-UP**

<table>
<thead>
<tr>
<th></th>
<th>Phototrophic culture (real data)</th>
<th>Heterotrophic culture THEORETICAL DATA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biomass (g dw L⁻¹)</strong></td>
<td>2.6±0.1</td>
<td>14</td>
</tr>
<tr>
<td><strong>Productivity (mg dw L⁻¹ d⁻¹)</strong></td>
<td>289±14</td>
<td>2000</td>
</tr>
<tr>
<td><strong>Lipids (%DW)</strong></td>
<td>22.8±0.4</td>
<td>40-50</td>
</tr>
<tr>
<td><strong>TAGs (%DW)</strong></td>
<td>19.4±2.8</td>
<td>40-50</td>
</tr>
<tr>
<td><strong>Productivity (mg TAG L⁻¹ d⁻¹)</strong></td>
<td>54±3.1</td>
<td>600-800</td>
</tr>
</tbody>
</table>

**LIMITATIONS:**

- COST OF CULTURE MEDIA
- USE OF CARBON SOURCES DESTINATED FOR FOOD

**PARAMETERS CONTROL DURING THE SCALE-UP**
Promote the use of microalgae as renewable energy source through the improvement of their economic viability and the sustainability of the process under the basic premise of “zero residues”

How?

- Proposing an scheme based on the circular economy, where the generated wastes are used as nutrient source in the same culture process.
- By diversifying the added value products that are obtained in the Chemical, Energy, and Agriculture and Livestock industries.
- Creating a Dynamic Map that will allow the cross-border cooperation and the complementarity between economic activities.
Specific Actions
URBAN SOLID WASTE
450 kg person\(^{-1}\) year\(^{-1}\)

- negative environmental impacts
- raw material waste
- greenhouse gas emissions
- large investments
- poorly efficient processes
- Low value added products are generated; difficult management

50% organic waste

MONOSACCHARIDES

PROTEIN/Aminoacids
### BIOMASS CHARACTERIZATION

- **Protein Hydrolysis**
  - 50 °C; pH: 7

- **Filtering**

### PROTEIN AMINOACIDS

- Supernatant
  - [AA] (mg/mL)
  - 19

### CARBOHYDRATES

- Supernatant
  - [glucose] (mg/mL)
  - 79

### PROTEIN HYDROLYSIS

- % Hydrolysis
  - USW¹
  - Soybean Waste: 76
  - Rape Seed Waste: 83.5
  - Sunflower Waste: 73
  - Microalga Waste: 78

### CARBOHYDRATE HYDROLYSIS

- % Hydrolysis
  - USW¹
  - Rape Seed Waste: 92
  - Sunflower Waste: 87
  - Microalga Waste: 96

### Filtered Waste

- Waste 1
  - 120 °C, 5-20 min

### Media Culture

- Waste 2
Table 1. Hydrolysates chemical composition.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Hydrolysate</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Algae</td>
<td>Rapessed</td>
</tr>
<tr>
<td>Total N (%)</td>
<td>0.99</td>
<td>0.89</td>
</tr>
<tr>
<td>Free Amino N (%)</td>
<td>5.50</td>
<td>5.44</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>0.7</td>
<td>5.56</td>
</tr>
<tr>
<td>Carbon (%)</td>
<td>12.60</td>
<td>6.68</td>
</tr>
<tr>
<td>Phosphorus (%)</td>
<td>0.23</td>
<td>0.17</td>
</tr>
<tr>
<td>Potassium (%)</td>
<td>0.11</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Table 2. Culture media composition

<table>
<thead>
<tr>
<th>Composition</th>
<th>100 ml Erlenmeyer</th>
<th>5 L Fermentor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen (g/L)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total N</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Glucose (g/L)</td>
<td>10</td>
<td>30-40</td>
</tr>
<tr>
<td>Sea salts (g/L)</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>

* N is provided by hydrolysates AH and RH or by Yeast Extract
Figure 1. Biomass productivity and lipid content in a 4-days culture experiment. (A). Biomass productivity was enhanced by using hydrolysates compared with yeast extract (control). (B). Lipid content in biomass was also enhanced, specially for the DHA-producing microalga *S. limacinum*. 
Figure 1. Biomass productivity and lipid content in a 4-days culture experiment. (A). Biomass productivity was enhanced by using hydrolysates compared with yeast extract (control). (B). Lipid content in biomass was also enhanced, specially for the DHA-producing microalga *S. limacinum*.
<table>
<thead>
<tr>
<th></th>
<th>C. Protothecoides</th>
<th>Rapeseed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FATTY ACIDS</strong></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Mirystic (C14:0)</td>
<td>0,87</td>
<td>tr.</td>
</tr>
<tr>
<td>Pentadecanoic (C15:0)</td>
<td>0,19</td>
<td>-</td>
</tr>
<tr>
<td><strong>Palmitic (C16:0)</strong></td>
<td>13,18</td>
<td>5</td>
</tr>
<tr>
<td>Palmitoleic (C16:1)</td>
<td>0,28</td>
<td>0,3</td>
</tr>
<tr>
<td>C16:2 ??</td>
<td>0,03</td>
<td>-</td>
</tr>
<tr>
<td>Margaroleic (C17:1)</td>
<td>0,18</td>
<td>-</td>
</tr>
<tr>
<td><strong>Stearic (C18:0)</strong></td>
<td>3,00</td>
<td>2,2</td>
</tr>
<tr>
<td><strong>Oleic (C18:1)n-9</strong></td>
<td>63,09</td>
<td>57</td>
</tr>
<tr>
<td><strong>Linoleic (C18:2)n6</strong></td>
<td>13,90</td>
<td>20,5</td>
</tr>
<tr>
<td>Linolenic (C18:3)n3</td>
<td>1,90</td>
<td>9</td>
</tr>
<tr>
<td>Arachidic (C20:0)</td>
<td>0,31</td>
<td></td>
</tr>
<tr>
<td>Gadoleic C-20-1</td>
<td>0,09</td>
<td>4,4</td>
</tr>
<tr>
<td>c-22-5 n-6</td>
<td>0,56</td>
<td></td>
</tr>
<tr>
<td>c-22-6 n3</td>
<td>2,42</td>
<td></td>
</tr>
<tr>
<td><strong>100,00</strong></td>
<td></td>
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</tr>
</tbody>
</table>

**ADEQUATE PROFILE FOR BIODIESEL**
✓ SELECTION OF SUITABLE STRAINS (*Chlorella protothecoides*)
✓ ESTABLISHMENT OF CULTURES CONDITIONS THAT ALLOW GOOD PRODUCTIVITY LEVELS (Fames>50% of Dry Weight). SCALE UP
✓ HIGH EXTRACTION YIELDS (85-95%) FROM FRESH BIOMASS
✓ CIRCULAR ECONOMY: REUSE OF AGROFOOD WASTES (SUSTAINABILITY) AND RESIDUAL BIOMASS (“CRADEL TO CRADEL”, PREMISE OF “ZERO RESIDUES”)
✓ ADDED VALUE PRODUCTS

✓ POSSIBLE INDUSTRIALIZATION PROCESSES?? LIFE CYCLE ASSESSMENT
Chlorella protothecoides

Biomass 200 Kg DW
9.5% Protein; 1.5% N

3 Kg N

EXTRACTION
40.5% Oil
81 Kg

Residue 120 kg DW
15.73% Protein; 2.51% N ---- 3 Kg N

HYDROLYSIS
60% hydrolysis

Hydrolysate
1.8 Kg N
(+ Glc soluble)

Remaing solids

Nitrogen Available
Nitrogen Limitation
The project has been 65% cofinanced by the European Regional Development Fund (ERDF) through the Interreg V-A Spain-France-Andorra programme (POCTEFA 2014-2020). POCTEFA aims to reinforce the economic and social integration of the French–Spanish–Andorran border. Its support is focused on developing economic, social and environmental cross-border activities through joint strategies favouring sustainable territorial development.