Introduction
Welcome to the 2nd e-bulletin of SABANA. This project was approved by the European Union’s Horizon 2020 Research and Innovation program, under the topic H2020-BG-2016-2017 Blue Growth: Demonstrating an ocean of opportunities, under the Grant Agreement No. 727874. The challenge is to build and operate a demonstration facility for producing biofertilizers/biopesticides and aquafeed at 5 ha scale. It provides a solution for three current key issues in the EU:

- Improvement of the safety and sustainability of food production in agriculture and aquaculture
- Contamination problems resulting from nutrients dissemination and scarcity (phosphorous)
- Minimization of greenhouse gas emissions from wastes (wastewater and flue gases)

You can find a video about the project at: https://youtu.be/2kpEyevr38E

Market analysis
Market analysis allows to identify agriculture and aquaculture as potential interesting applications for microalgae. On this markets the value of microalgae biomass is analogous to human consumption the size of the markets being including higher than for human consumption. Moreover regulation on these fields is simple, previous experiences confirming the utility of microalgae biomass on it.
WP1 Techno-economic analysis

Market analysis performed allows to identify potential applications of microalgae on agriculture and aquaculture fields. These markets require more sustainable and safe materials to replace chemicals or non sustainable raw materials, microalgae being validated as alternative. Moreover, there are commercial products based on macroalgae but although microalgae are more effective commercial products does not exist due to the low production capacity of actual industry of microalgal production.

Both the biostimulants and biopesticides market are continuously growing, specially in developed countries. Microalgae are included in all the reports as a potential source of this type of products

Aquafeed market require new sources of proteins and lipids replacing fish meal and fish oil microalgae being of the most promising. Only price and availability limits the application of microalgae in aquafeed.
WP2 Sustainability

We are more and more aware that production cause impacts on the environment, and it is important to design new production pathways, mainly in the frame of circular economy, that are genuinely committed to reduce these impacts. The reduction of impacts has to be quantified by numbers obtained according to robust and common methodologies, in which the input numbers and assumptions are transparent and declared. Life cycle assessment (LCA) is a multi-step procedure for calculating the lifetime environmental impact of a product or service according to a standardized and acknowledged procedure.

A basic estimation of the environmental impacts of microalgae production according to the SABANA model has been performed during the first year of the project. The analysis included all the production steps up to biomass harvesting, thus obtaining a biomass ready to be further processed.

System Boundaries

Life Cycle Inventory (LCI), is the assessment step where the energy and material inputs and outputs (including products, co-products, wastes and emissions) are identified, and quantified. It begins with flow diagrams of the system and its boundaries to determine the internal and external flows.

A complete inventory of inputs and outputs of the production steps of the SABANA model has been compiled and is available on the SABANA website. For the calculation of the Impact Categories is necessary to set a reference unit. The functional unit (FU) provides the reference to which all the data collected in the inventory are organized and normalized. Functional unit in this evaluation is the mass unit (kg) of dry microalgae produced. Different scenarios have been considered to highlight different situations and different level of downstream recovery, considering the combination of the water type used, the nutrients and the recirculation of water.

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WP3 Engineering
DEMO1 facility is being installed just attached to the University of Almeria, with access to freshwater, seawater and wastewaters, in addition to infrastructure and laboratories of the University.

Auxiliary facilities includes culture medium preparation, harvesting and processing of the biomass

Design of thin-layer and raceway reactors has been improved, up to three different units of each type being installed for evaluation inside a greenhouse. Size of these reactors range from 50 to 100 m\(^2\), it being fully automated.

Design and materials of raceway reactors has been improved to reduce the cost below 10 €/m\(^2\), a unit up to 500 m\(^2\) being installed for evaluation. Performance of paddlewheel system has been also enhanced minimizing the power consumption below 10 W/m\(^3\) but enhancing the mass transfer capacity.
WP4 Harvesting
Pilot scale trials has been performed to test and compare different algae harvesting technologies at Estación Experimental Las Palmerillas Fundación Cajamar (El Ejido, Almeria). Technologies includes a nozzle disc stack separator DA1 1-00-576, a ceramic module membrane unit HES 400A and a rotating ceramic disc membrane unit MSDS 090 on site.

During each trial, a wide selection of measurements was performed and captured to ensure analyzability of the data in regards to the comparability of the different harvesting technologies. The measurement of the fluorescence, which indicates the vitality of the living cells was essential to determine, which energy input (pump speed, velocity of flow, etc.) is safe to use without disrupting the cells. Also a lot of different process parameters (e.g. pore size of the used membranes, flow rate of the separator feed etc.) have been set to determine the best operation mode for each system.

Two different scenarios are considered regarding the application of the biomass and culture medium used.
WP5 Processing

Processing of the biomass includes cells disruption and operations requested to obtain final products from microalgae biomass both for agriculture and aquaculture. Different technologies are being evaluated for latter scale-up to DEMO1 and DEMO5.

Results confirm that cells disruption is highly consuming energy the utilization of high pressure homogenization and PEF being the most promising. Optimal processing is a function of biomass and culture conditions.

Pilot scale harvesting and processing systems are developed to evaluate the overall performance of the processes at real scale and to produce samples of products to be evaluated. Thus, up to 300 l reactors has been installed able to produce up to 1.000 l/day of biofertilizers.
WP6 Biology
Freshwater (21) and marine (5) green microalgae strains with potential plant biostimulating effect, and freshwater (15) and marine (15) cyanobacteria strains with potential antimicrobial activity against plant pathogens were evaluated. Plant biostimulating effect was detected with the “mung bean root development” bioassay in several strains (15), but three of them were the most potent plant biostimulants. The biostimulating strains increased the root weight of tomato and lettuce plantlet compared to the control significantly.

In general terms, a phytotoxic effect was observed when the extracts were applied at concentrations higher than 1 mg/mL. However, a biostimulating effect on the germination and radicle elongation was also detected when the seeds were treated with other strains at 2 mg/mL. Both high or too low germination have significant practical importance.

Freshwater cyanobacteria and green microalgae strains were screened for their activity against 7 fungal and 5 bacterial plant diseases. Up to 8 cyanobacteria and 5 green algae demonstrated antimicrobial activity, 3 of them showing biostimulating and antifungal activity, giving the chance to develop dual products.

Growth parameters of selected green microalgae were measured in laboratory and greenhouse culture systems either in mineral nutrient media or in diluted pig manure. All green algae were able to grow in pig manure diluted to 300 or 500 mg L\(^{-1}\) suspended dry solid contents. The average dry matter production in nutrient medium was about 0.8 g L\(^{-1}\), while in pig manure only 0.4 g L\(^{-1}\), which is valid also for the growth of the “valuable strains”.

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WP6 Biology

Regarding aquaculture, proteins and lipids, especially polyunsaturated fatty acids (PUFA) content of microalgae are of crucial importance. The biomass of five different microalgae species have been analyzed: *Scenedesmus almeriensis*, *Tetraselmis suecica*, *Nannochloropsis gaditana*, *Isochrysis galbana*, *Arthrospira platensis*. The proteins content ranged from 27.8 to 42.3%, and the lipids content varied from 5.9 to 16.1%. The amino acid profiles of microalgal biomass is similar to that of fish meal. The EAA/NEAA ratios (0.9) were close to the ratio found in standard fish meal (0.95). Microalgae biomasses do not contain protease inhibitors able to inhibit digestive proteases of both gilthead seabream (*Sparus aurata*) and Senegalese sole (*Solea senegalensis*).

<table>
<thead>
<tr>
<th></th>
<th>Crude protein (%)</th>
<th>Crude lipid (%)</th>
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</thead>
<tbody>
<tr>
<td><em>T. suecica</em></td>
<td>27.8 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.85 ± 0.30&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>I. galbana</em></td>
<td>31.3 ± 0.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.06 ± 0.50&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>A. platensis</em></td>
<td>36.8 ± 0.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.31 ± 0.76&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>S. almeriensis</em></td>
<td>37.9 ± 0.09&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.72 ± 0.30&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>N. gaditana</em></td>
<td>42.3 ± 0.09&lt;sup&gt;e&lt;/sup&gt;</td>
<td>13.52 ± 1.23&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Protein and lipid content in microalgal biomass (% dry weight).

In *vitro* protein hydrolysis of microalgae biomass was assessed. Results confirm that microalgae are valuable protein sources for feeding gilthead seabream and Senegalese sole.

<table>
<thead>
<tr>
<th></th>
<th>Aspartic acid</th>
<th>Glutamic acid</th>
<th>Alanine</th>
<th>Cysteine</th>
<th>Glycine</th>
<th>Serine</th>
<th>Proline</th>
<th>Tyrosine</th>
<th>Arginine*</th>
<th>Phenylalanine*</th>
<th>Histidine*</th>
<th>Isoleucine*</th>
<th>Leucine*</th>
<th>Lysine*</th>
<th>Methionine*</th>
<th>Threonine*</th>
<th>Valine*</th>
<th>Total NEAA</th>
<th>Total EAA</th>
<th>Ratio EAA/NEAA</th>
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<tbody>
<tr>
<td><em>T. suecina</em></td>
<td>9.62</td>
<td>11.36</td>
<td>5.99</td>
<td>1.21</td>
<td>6.45</td>
<td>4.36</td>
<td>3.76</td>
<td>2.94</td>
<td>6.57</td>
<td>5.88</td>
<td>1.84</td>
<td>4.11</td>
<td>6.95</td>
<td>3.77</td>
<td>1.32</td>
<td>4.63</td>
<td>5.02</td>
<td>45.71</td>
<td>40.09</td>
<td>0.88</td>
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<tr>
<td><em>S. almeriensis</em></td>
<td>8.48</td>
<td>10.23</td>
<td>7.55</td>
<td>0.80</td>
<td>5.93</td>
<td>3.83</td>
<td>3.70</td>
<td>2.77</td>
<td>5.22</td>
<td>4.68</td>
<td>1.60</td>
<td>3.92</td>
<td>7.29</td>
<td>4.26</td>
<td>1.24</td>
<td>4.55</td>
<td>4.93</td>
<td>43.29</td>
<td>37.70</td>
<td>0.87</td>
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<tr>
<td><em>I. galbana</em></td>
<td>8.92</td>
<td>11.44</td>
<td>5.81</td>
<td>0.85</td>
<td>5.39</td>
<td>3.90</td>
<td>3.37</td>
<td>2.84</td>
<td>5.49</td>
<td>4.75</td>
<td>1.56</td>
<td>4.52</td>
<td>6.75</td>
<td>4.11</td>
<td>1.47</td>
<td>4.09</td>
<td>4.67</td>
<td>42.53</td>
<td>37.40</td>
<td>0.88</td>
</tr>
<tr>
<td><em>N. gaditana</em></td>
<td>7.62</td>
<td>10.61</td>
<td>5.89</td>
<td>0.83</td>
<td>5.30</td>
<td>3.66</td>
<td>7.12</td>
<td>2.84</td>
<td>5.79</td>
<td>4.52</td>
<td>1.66</td>
<td>4.32</td>
<td>6.89</td>
<td>4.31</td>
<td>1.30</td>
<td>4.06</td>
<td>4.96</td>
<td>43.86</td>
<td>38.30</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Amino acid composition (g 100 g protein<sup>-1</sup>) of fishmeal and the different microalgal biomasses. NEAA: non essential amino acids; EAA: essential amino acids.
WP8 Dissemination
SABANA project sign agreement with Andalusian Regional Institute for Agriculture and Aquaculture for the development of microalgae biotechnology.

Young researchers from different countries participate on SABANA project

SABANA project participate on 2nd Training School EUALGAE

SABANA project participate on “European Night of the Researchers”

SABANA project participate on symposiums and courses at Hungary and Spain, among others...

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