

# SUSTAINABLE ALGAE BIOREFINERY FOR AGRICULTURE AND AQUACULTURE



## 6th e-bulletin of SABANA Project

The general objective of the SABANA project is to demonstrate the technical, environmental and social feasibility of producing valuable products for agriculture and aquaculture by using only marine water and wastewater as nutrients source.



IMPROVEMENT OF LARGE-SCALE PRODUCTION TECHNOLOGY



DEVELOPMENT OF INTEGRAL UTILIZATION OF BIOMASS PROCESSES



SCALE-UP AND DEMONSTRATION OF THE DEVELOPED TECHNOLOGY

## 6th e-bulletin of SABANA PROJECT

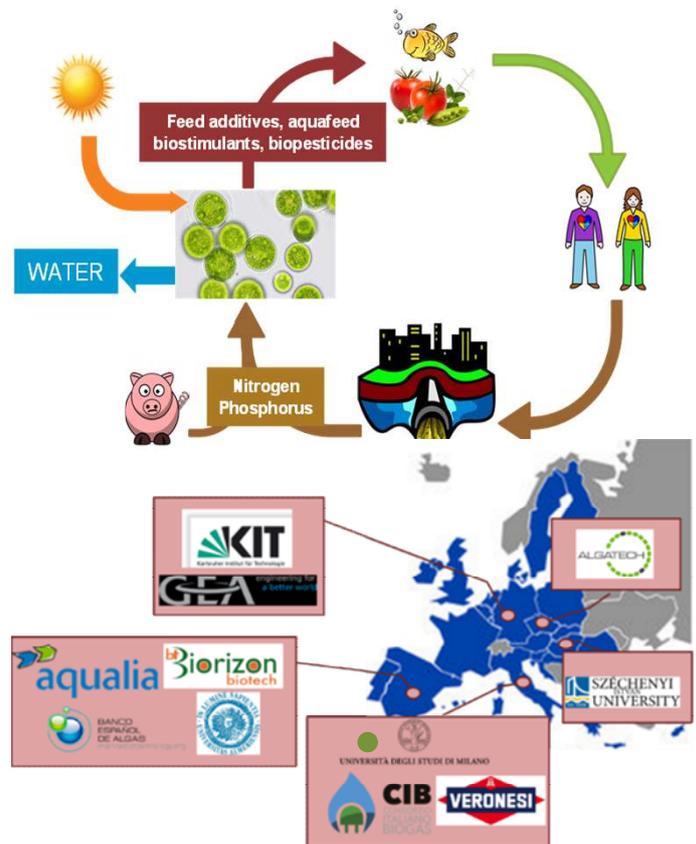
### Introduction

Welcome to the 5th e-bulletin of SABANA summarizing the achievements after 36 months of progress of the project. 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)

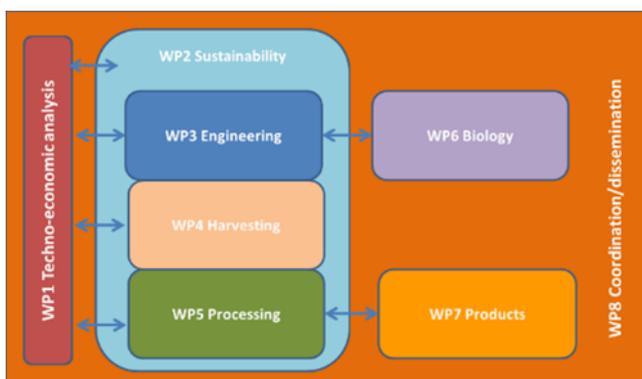
You can find a video about the project at:

<https://youtu.be/2kpEyevr38E>



### Partners

The project is led by the University of Almeria but major actors are the companies involved into the project (FCC Aqualia, GEA Westfalia, A.I.A. S.p.A., Biorizon Biotech) in addition to high reputation research centers at EU level (Karlsruhe Institute of Technology, Mikrobiologicky Ustav, Universita Degli Studi Di Milano, Univ. Las Palmas de Gran Canaria, Szechenyi Istvan University, Consorzio Italiano Biogas e Gassificazione). In addition to 11 partners from 5 EU countries, Fundacion Cajamar and IFAPA also collaborate in this project.

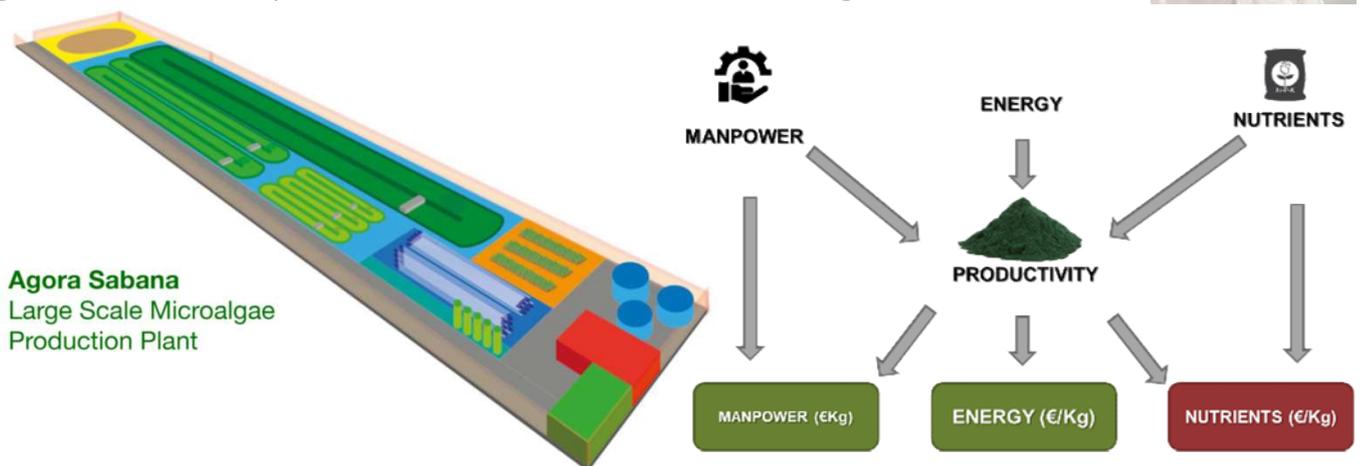


### Work packages:

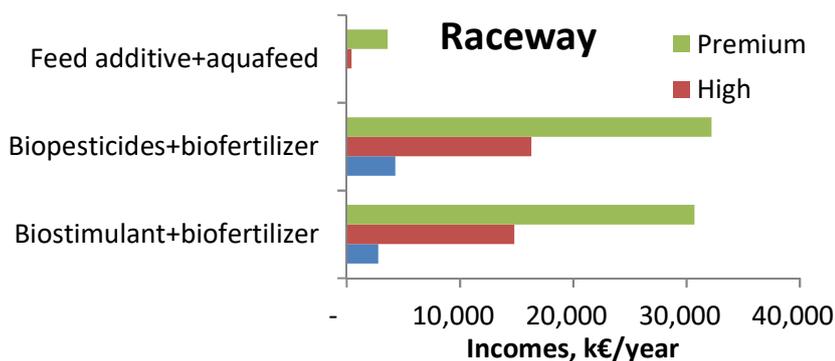
The work plan is divided in eight work packages combining scientific/technical research and innovation with market development and techno-economic and sustainability analysis.

### WP1 Techno-economic analysis

BIORIZON BIOTECH already complete the first demonstration plant of already developed technologies from SABANA project. Up to 1.7 ha of raceway and tubular reactors located inside a greenhouse are available. The facility is producing biomass while validating the developed technologies. Moreover, the facility is providing real figures about biomass production cost and main factors influencing it.



Data confirms previous estimations, identifying manpower, energy and nutrients as the major factors contributing to the biomass production cost. Values up to 6.3 €/kg were validated in spite of the non 100% utilization of the facility. It is expected in the coming months to maintain a higher operation of the plant to achieve the target of 60 t/ha-year versus the actual 16 t/ha-year. Labour and nutrients are the major contributions to the production cost, then in the next months more automatization and recycling systems will be used to reduce these costs.



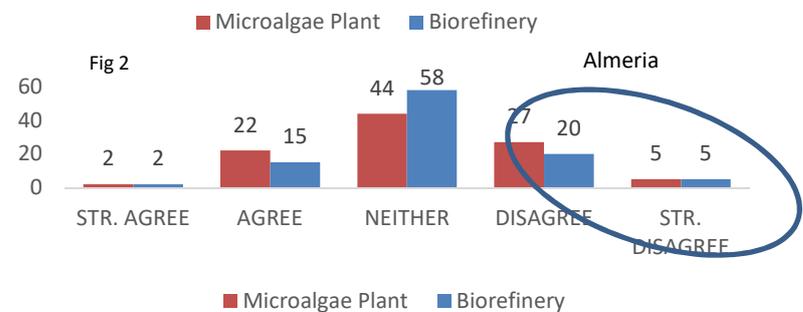
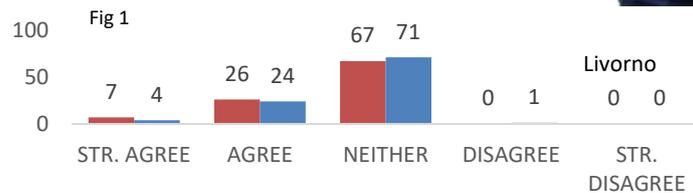
Business plan already confirm the agriculture and aquaculture markets as the most promising for microalgae related applications.

It must be noted that products form the project are being already validated first results being highly positive. Moreover, demand of these products is really high and new products providing benefits for both agriculture and aquaculture markets have been already developed and patented. Innovations related with these processes are being completed to start their commercial development.

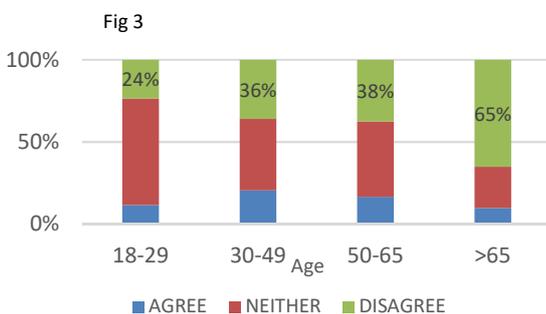
**WP2 Sustainability** An aspect that is becoming increasingly important in the social sustainability, is the perception of citizens and consumers with respect to new technologies. The potential benefits of any innovative biotechnological process will only be realized if society accepts the science and new products as safe, effective and desirable. One of the aims of the SABANA project was precisely the dialogue and the transmission of concepts such as “circular economy” and “nutrient recovery” as safe to civil society.



We carried out a survey on the perception and social acceptability of microalgae production chain on common citizens. The total interviews were 400: 200 in Almeria and 200 in Livorno, randomly chosen to constitute a balanced sample by gender and age group. In figure 1 and 2 is reported the distribution of answers to the question: How much do you agree with the construction of a microalgae facility plant? And a biorefinery?



In Livorno respondents did not have a formed opinion about microalgae (around 70% of undecided). Both Almeria and Livorno citizens were more concerned and in disagreement to the construction of a microalgae plant in the hypothesis of having the plant built nearby. In Almeria undecided respondents were lower and the opposing higher than in Livorno (see fig 2).



Demographic analysis (fig 3) outlined that older people (over 65 years) are the ones opposing more (more that 65% when the respondent suppose the plant will be built close to them) and also low education level is related with higher opposition. The younger sector (18-29 years), on the other hand, is less in favour than middle age sector (30-49 and 50-65 years) and more undecided.

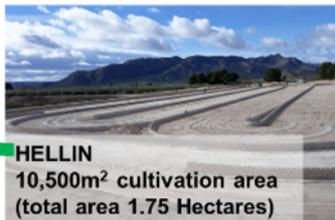
Finally, in both the locations after a simple explanation of what a microalgae plant is and does, a good share of respondents changed idea turning to be in favour. These findings provide important information on the type of message and the target audience to address communication campaigns for social acceptance.

### WP3 Engineering

BIORIZON BIOTECH already complete the first demonstration plant from SABANA project. Up to 1.7 ha of raceway and tubular reactors located inside a greenhouse are in operation to produce biomass for agriculture and aquaculture related applications. Using clean water and fertilizers the plant is managing up to 2.000 m<sup>3</sup> of microalgae cultures. A large effort was devoted to ensure the quality and reliability of the process.



AQUALIA is completing the last two demonstration facilities to be located in Merida and Hellin. In both cases the biggest raceways in Europe and around the world will be constructed, up to 10.000 m<sup>2</sup> per single reactor. Lar efforts were devoted to the selection of the final Locations, information from these new facilities providing useful information for the project. The new facilities will be an excellent demonstration of microalgae related processes especially linked to wastewater treatment.



On the new facilities some advances already developed on previous projects will be validated and demonstrated at large scale, information from these previous facilities also helping to improve the operation of the new ones. On this way Europe is in front of worldwide research on microalgae field.



### WP4 Harvesting

The first demonstration equipment for harvesting was already developed by GEA and installed at demonstration facility developed by Biorizon Biotech in Almeria. Nozzles separators were selected to build a complete system capable to process more than 40 m<sup>3</sup>/h of microalgae culture, including washout of the biomass and cell disruption. The facility allows to process microalgae for different applications.



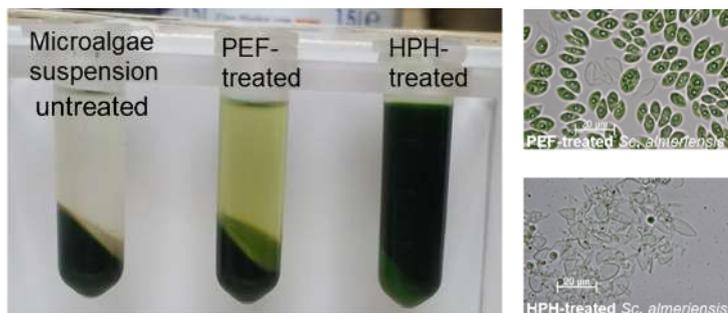
The GEA algaeprime SDA 40 harvesting line have four separators, and the skid includes pre-installed pipes, pumps, valves and automation panels. The separation skid consist of two skid frames and is connected to the GEA Ariete NS 3030H homogenizer skid for inline processing. Power consumption below 0.8 kWh/m<sup>3</sup> and overall production capacity higher than 4 m<sup>3</sup>/day of concentrated sludge are achievable using this equipment, as example of large process for medium/high value applications.

For low value applications a combination of dissolved air flotation (DAF) plus decanter centrifuge was selected. A DAF unit with capacity up to 200 m<sup>3</sup>/h was designed, the utilization of flocculants at this stage being minimized. The final sludge contains up to 40 g/L of microalgae biomass. Dewatering is performed in a decanter skid unit GEA sludgepro 1500. Beside the decanter, the skid includes pre-installed pipes, a feed pump, a sludge pump, valves, an automation panel, and a flocculant dosing unit. The skid operation will be handed over for full biomass production afterwards, therefore a training of the staff is considered. The main goal in 2021 is to gain data from the production systems regarding: (i) harvesting and concentration performance, (ii) data for the LCA und sustainability reports.



### WP5 Processing

High pressure homogenizer was selected as more suitable technology for large scale processes. A GEA Ariete NS 3030H homogenizer was included into the skid already installed in the facilities of Biorizon Biotech in Almería. Beside the homogenizer, the skid includes pre-installed pipes, pumps, valves, buffer tanks and an automation panel.



However, only Pulsed Electric Field (PEF) technology allows to optimize the breakage of the cells to enable the multistep recovery of intracellular components. By this reason a pilot scale system was developed to perform a new campaign of experiments at the already existing demonstration facilities from the project located in Spain.

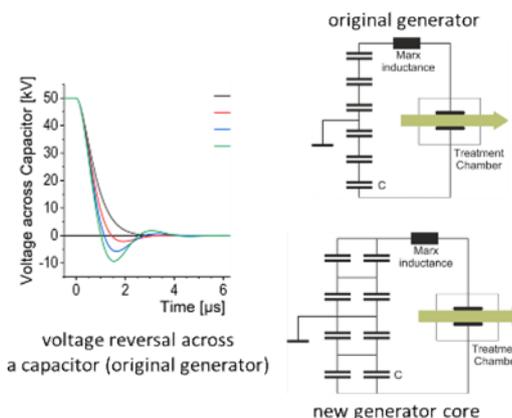
The new equipment was optimized about energy consumption and robustness:

- Pulse energy: 1050 J
- Pulse repetition frequency: up to 45 Hz
- Output voltage: 300 kV
- Rated mass flow: 600 L/h, 100 gdw/L, 150 kJ/ltr



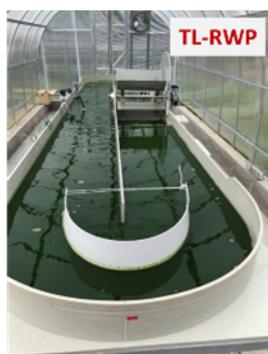
Additionally, new enzymatic reactors were developed to produce end products to be evaluated as part of the project. It includes:

- Automatic control of processing by SCADA
- Temperature control by oil jacket heating
- pH control by admixture of 1M NaOH
- Stirring regulated by inverter



## WP6 Biology

Pilot scale systems already installed at Trebon (Czech Republic) were used to study the effect of culture conditions and harvesting time both in the biomass production and quality of produced biomass. Results confirm the biopesticide effect of produced biomass. Moreover, the effects are much more relevant when the biomass is produced using wastewater and pig manure as nutrients source.



- 5 m<sup>2</sup>
  - Culture layer of ~ 15-20 mm
  - Paddle wheel – gentle mixing
  - Flow speed about 0.2 m s<sup>-1</sup>
- 5 m<sup>2</sup>
  - Culture layer ~ 7-10 mm
  - Centrifugal or paddle-wheel pump
  - Flow speed about 0.5 m s<sup>-1</sup>

Sample	Antimicrobial effect of algal samples against phytopathogenic fungi (inhibition %)			
	<i>Pythium ultimum</i>	<i>Fusarium oxysporum</i>	<i>Rhizoctonia solani</i>	<i>Phytophthora capsici</i>
Laboratory control	54.12	30.00	25.64	19.40
RW batch AM	0	<b>42.86</b>	8.93	3.70
RW batch PM	0	17.26	21.43	11.25
RW semi batch AM	0	1.19	0	12.16
RW semi batch PM	0	32.14	10.12	0
TLC batch AM	0	37.50	25.60	34.56
TLC batch PM	0	22.62	11.90	15.92
TLC semi batch AM	0	37.50	11.31	14.94
TLC semi batch PM	0	33.33	23.81	19.60
Pig manure (150 mg L <sup>-1</sup> NH <sub>3</sub> ) UNIMI	<b>40.48</b>	<b>41.67</b>	<b>46.43</b>	<b>60.00</b>
RW wastewater AM	33.28	31.58	28.8	46.92
RW wastewater PM	35.62	21.96	44.23	41.42
TLC wastewater AM	25.66	28.70	27.20	41.38
TLC wastewater PM	34.25	26.84	42.33	50.37

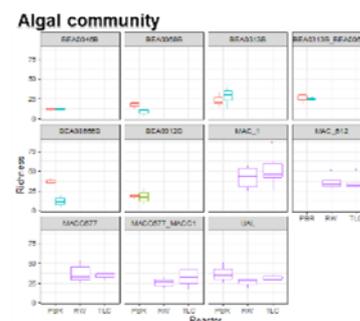
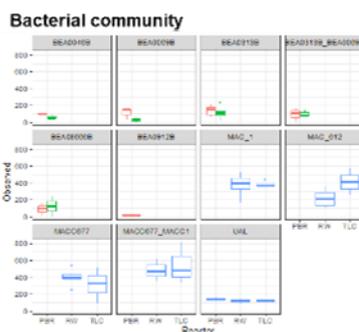
The performance of marine strains at pilot scale was also evaluated, in addition the comparison between the utilization of fertilizers and wastewaters as nutrients source being included. Similar results to those obtained in freshwater were obtained, it confirming that also marine strains are useful for agriculture related applications of microalgae. The utilization of wastewater as nutrients source not only improve the sustainability of production step but also increase the biopesticide effect of biomass.



Triplicates and control (100 L)      Photoreactors for the acclimatization to the centrate and outdoor conditions (30, 70 and 80 L)      Triplicate cultures for long scale and volume (400 L)

Sample	Antimicrobial effect of algal samples against phytopathogenic fungi (inhibition %)			
	<i>Pythium ultimum</i>	<i>Fusarium oxysporum</i>	<i>Rhizoctonia solani</i>	<i>Phytophthora capsici</i>
Laboratory control f/2	0	1.23	0	5.36
PBR 400 L f/2	1.00	0	0	15.00
PBR 100 L f/2 AM	0	3.73	2.38	14.22
PBR 100 L f/2 PM	0	-2.99	0	49.53
Centrate 5%	<b>41.67</b>	15.27	5.04	<b>66.39</b>
PBR 400 L centrate 1%	9.00	32.00	29.00	46.00
PBR 100 L centrate AM	0	35.82	42.26	35.07
PBR 100 L centrate PM	0	<b>37.31</b>	<b>45.24</b>	48.02

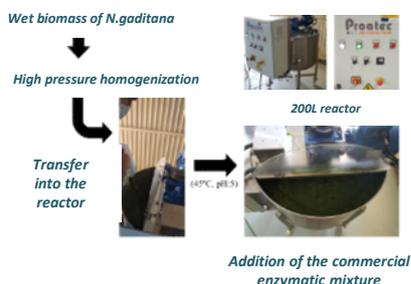
The biodiversity of samples from pilot scale reactors was analysed. Metagenomics tools were utilized on this study to identify the most abundant species of both microalgae and bacteria. Microalgae cultures were only partially contaminated by other microalgae when using wastes as nutrients source whereas no pathogens were found in the cultures.



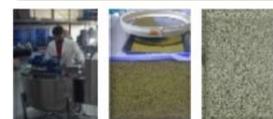
### WP7 Products

Research on potential application of microalgae obtained by biorefineries in aquaculture products was continued and new feeding trial at commercial-scale on *S. aurata* carried out. For this purpose *N.gaditana* biomass was produced and subjected to hydrolysis treatment and five complete diets were formulated to be grossly iso-proteic and iso-lipidic and a control diet (C), prepared using a blend of conventional animal and vegetal protein sources. All the test diets were prepared by replacing the 5% of protein and lipid supplied by the blend of conventional protein and lipid-rich ingredients with microalgae and coined respectively: *N. gaditana* grown on Synthetic Medium, Crude (CNSM-C), *N. gaditana* grown on Pig Manure, Crude (NPM-C), *N. gaditana* grown on Synthetic Medium, Hydrolyzed (CNSM-H), *N. gaditana* grown on Pig Manure, Hydrolyzed (NPM-H). In vivo trial is currently in progress on marine species *S.aurata* at Istituto Spallanzani (Italy) facilities. According to experimental design, nine hundred *S. aurata* juveniles (body weight 36g) were randomly allocated among 15 square-shaped, fiber-glass tanks volume 500-L, included in an indoor, partially re-circulating marine water system. Diets are offered in two daily meals with a fixed feed ratio during the entire period of experimentation. At the end of feeding trial, growth parameter and somatic indices will be determined and some fish for each group will be evaluated for fillet proximate composition, oxydation parameters and GUT *status*.

### N. GADITANA BIOMASS HYDROLYSIS



### EXPERIMENTAL DIETS PRODUCTION



### FEEDING TRIAL AT COMMERCIAL SCALE

900 *S. aurata* juveniles



Field trials were also performed to evaluate the benefits of microalgae hydrolysate in the yield and quality of fruits in different crops. Different formulations were evaluated it being observed as both fruits yield and quality largely increases when providing microalgae based products. These behaviour was observed including in largely different crops such as tomato, pepper, but also in grapes, olive trees, orange, and maize. The benefits/cost ratio of using microalgae based products in agriculture provide additional incomes for farmers, at the same time that increasing the quality, safety and sustainability of foods.



### Parameters evaluated:

1. Fruit weight.
2. Firmness.
3. Total Soluble Solid Content ("Brix).
4. Yield.
5. Assimilated Calcium.
6. Roots weight

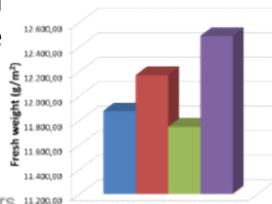
Quality parameters



### Formulations evaluated:

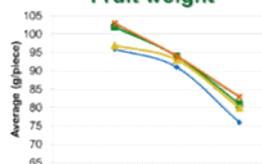
- T1: Conventional mineral fertilisation.
- T2: Microalgae hydrolysed (8%).
- T3: Microalgae hydrolysed (4%).
- T4: Microalgae hydrolysed (8%) enriched with calcium.

### Yield per treatment



Treatment	Formulation	Yield vs Mineral fertilisation (%)
T1	T1 (Mineral fertilisation)	0
T2	Microalgae hydrolysed (8%)	2,45
T3	Microalgae hydrolysed (4%)	-1,08
T4	Microalgae hydrolysed (8%) + calcium	5,14

### Fruit weight



### Firmness





This project has received funding from the European Union's Horizon 2020 Research and Innovation program under the Grant Agreement No. 727874

# SABANA e-bulletin No.6, December 2020

## WP8 Dissemination

In the last year the covid-19 pandemic have a strong effect in the normal live of citizens around the world. This also affect the development of SABANA related activities both in dissemination and transference. Thus, personal activities were replaced by online meetings and webinars. In spite of these difficulties activities continues and from the last meeting already developed in Milano a new one was planned to be performed online.

In the past March 8<sup>th</sup> we celebrate the "day of the women". From SABANA we recognize the large contribution of women scientific to the project. We are proud to have young scientific from largely different countries around the world, all of them providing relevant advances as part of their research activities.



Information about SABANA project is published online in different journals for specialized and general public.

La UAL introduce microalgas como ingredientes de panes y galletas

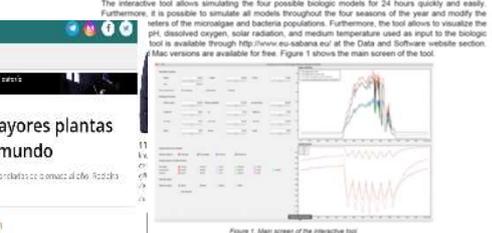


IWA SPECIALIST GROUP NEWSLETTER

July 2020



IWA INSTRUMENTATION CONTROL AND AUTOMATION



Last advances from SABANA project are disseminated in webinars, conferences and online courses, in addition to social networks.



More than 3.000 students already attend the Massive Online Open Course available at MiriadaX platform.



MIRIADA X



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Contact: [info@sabana.eu](mailto:info@sabana.eu)

