# SUSTAINABLE ALGAE BIOREFINERY FOR AGRICULTURE AND AQUACULTURE

# Sustainable Algae Biorefinery for Agriculture aNd Aquaculture



HORIZON 2020 European Union Funding for Research & Innovation

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

# **5th 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







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# **5th 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: <a href="https://youtu.be/2kpEyevr38E">https://youtu.be/2kpEyevr38E</a>

## 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 technoeconomic and sustainability analysis.













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### WP1 Techno-economic analysis

DEMO1 PRODUCTION facility has been completed and put in operation. Real figures from this 1.5 ha facility are used for techno-economic and sustainability analysis. Total cost of the facility was close to 1.2 M $\in$ , up to 65% corresponding to the build up of reactors and auxiliary facilities. Reactors up to 5.000 m<sup>2</sup> were installed and put in operation. The facility is used to produce microalgae biomass for both agriculture and aquaculture related applications, up to 70 tn/ha·year.





Report of requirements of locations for large scale microalgae production have been completed. The influence of environmental factors such as solar radiation and temperature, in addition to photobioreactor design and operation, and biology of the system have been analysed. The available knowledge has been integrated into a simulation tool useful to estimate the microalgae biomass productivity as a function of these major factors (environmental, engineering and biological).

Results confirm that although solar radiation is the energy driving the process, temperature is the most relevant factor determining the potential productivity. Only in South Europe it is possible to maintain biomass production all the year around, which is high relevant to achieve profitable processes.

Data confirm that productivity values up per than 100 tn/ha·year can be achieved under optimal conditions at adequate locations.



Location	Temp., °C	Solar rad., MJ/m2·day	Biomass conc., g/l	Productivity, g/m <sup>2</sup> ·day	Production, tn/ha- year	
Trebon	10.1	11.8	0.26	12.24	10.10	
Hungary	10.0	12.6	0.28	11.92	42.92	
Lombardy	12.8	14.7	0.35	17.65	63.53	
Canary Islands	20.7	18.9	0.49	33.23	119.63	
Almeria	17.9	19.6	0.51	28.96	104.26	



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# SABANA e-bulletin No.5, April 2020

**WP2 Sustainability** The SABANA Project has now its 1ha facility in operation, and the data are available for analysing the production Life Cycle Assessment in the facility.

The facility is working with freshwater and fertilizer supply, and is processing the biomass with High Pressure Homogenizer and hydrolysis to produce a valuable bio-stimulant, able to support crop production, increase yield and decrease the effects of stress on plants.



In figure is possible to have an overview of the different phases of the microalgae production in demo 1, that mainly contribute to the total impact for each category.

Electricity for cultivation (mixing and gas exchange) and processing is always one of the main contributor to impact categories.

Fertilizers, both N and P fertilizers, are the other relevant hotspot, as usual in the agricultural activity.



The eutrophication categories , that are related to the use and management of fertilizers and the release of nutrients in the environment, show a significantly lower impact (one order of magnitude) compared with that of traditional agriculture, thanks to the step wise addition of fertilizers, recirculation of growth medium and technological containment deployed in the microalgae production model.



In the left are presented the data of the 1 ha facility (yellow bar), that are here compared with the results coming from the use of wastewater as nutrients source (green), data by now simulated, as the activity will be performed in the second demo plant to be built.











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### **WP3** Engineering

DEMO1 PRODUCTION facility has been completed and operating. Design and construction of raceway reactors have been improved, in addition to the implementation of advanced control strategies. Facility includes auxiliary equipment for water and harvesting management, being capable to operate in full recirculation mode. Production of already selected strains is being evaluated.









Regarding DEMO INDUSTRIAL facility, **more than 10 different locations** have been studied in Spain and Portugal. Land availability and permissions requested were the major factors determining the selection of the right location for new DEMO scale.

The target is to develop robust and reliable demo facilities integrating the production of microalgae biomass with wastewater treatment, allowing to produce agriculture related products such as biofertilizers, on a sustainable mode.





Reactors of **10.000 m<sup>2</sup>** will be built in new demo scale, **larger scale worldwide**.

Different technologies and operation modes will be evaluated on the new facilities, **multiplying the replicability of SABANA project**.

Wastewater treatment capacity will be demonstrated all the year around only using real municipal **wastewate**r at the larger scale possible. Produced biomass will be used to produce **biofertilizers** improving the **sustainability** and yield of foods production by agriculture.











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### **WP4 Harvesting**

For the DEMO 1 PRODUCTION facility the harvesting line was detail engineered and built. The harvesting line consists of 4 nozzle separators type SDA 40 (GEA algaeprime®) and can flexible be operated to meet different demands of culture capacities (up to 60 m<sup>3</sup>/h) and a washing of the biomass, if required. Furthermore a high-pressure homogenizer type Ariete NS 3030H for cell disruption is considered (related to WP 5). All equipment is installed on a skid frame with all necessary auxiliary equipment, piping and instrumentation with full automization.





For DEMO 5 the engineering and construction phase kicked off. The DEMO 5 harvesting concept consists of dissolved air flotation units (Type DAFAST<sup>®</sup> from FCC Aqualia) as a preconcentration step and a decanter (GEA sludge decanter pro 1500) for the final biomass concentration to 21 %DS.













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# **WP5** Processing

The energy consumption for PEF-treatment of the freshwater species *Scenedesmus almeriensis* for biofertilizer production by subsequent enzymatic hydrolysis (EH) has been demonstrated to compare well with the energy demand of HPH. A clear advantage of PEF-treatment is the preservation of the cell shape which allows easy mechanical separation of biomass residuals, which enables recovery of multiple components by cascade processing, e.g. recovery of lipids *and* amino-acids.



In the first treatment sequence enzymatic hydrolysis can be performed after PEF-treatment and lipid extraction can be done subsequently. In this case, the kinetics of the degree of hydrolysis DH is well comparable to the degree of hydrolysis after HPH treatment.

Alternatively, when applying the reversed sequence #2, i.e. lipid extraction before enzymatic hydrolysis, achievable degree of hydrolysis values are significantly lower. Even control values (untreated) are higher than PEF-treated values. This can be explained by protein losses during lipid extraction. Lipid yields in both PEF-assisted treatment sequences are well comparable and allow recovery of 75% of total lipids. Lipid extraction from untreated biomass after EH is also high.



This indicates that enzymatic hydrolysis also permeabilizes plasma membranes. Regarding EH yields, treatment sequence No. #1 is the most efficient PEF-assisted cascade processing pathway.















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#### WP6 Biology

Activities has been developed to better understand the composition behaviour of microalgae/bacteria consortia. Results show as the dilution rate is a major factor determining it. Increasing the dilution rate the biomass of microalgae reduces but the daily productivity increases. The bacteria biomass and daily production increases with the dilution rate, a maximum being achieved at 0.6 1/day, then reducing. In summary, dilution rate determines the overall biomass productivity, but also the ratio between microalgae and bacteria biomass.



low activity



Samples from different reactors show as only in the tubular photobioreactor (1T) was a purity culture of Scenedesmus, while in the other samples (2RW, 3RI and 4RH) Scenedesmus disappeared and took place the genus Desmodesmus with various species. The algal community of 2RW (Raceway fed with synthetic medium) and 4RH (Raceway fed with slurry) are very similar in composition. Consequently, the composition of the microalgal community is influenced by the type of photoreactor but not by the type of medium used to feed the culture. The PCoA analysis shows that the bacterial composition of 1T sample is very distant from the other 3 samples. The dominant species are all photosynthetic cyanobacteria. The only exception is the Flavobacterium aquatile (flavobacteria), which has been found in the close tubular system

100% 80% 60% 40% 20% 0% 1T 2RW 3RI 4RH Desmodesmus sp1 Monoraphidium sp Desmodesmus armatus Chytridiales desmodesmus sp2 Desmodesmus communis Scenedesmus almeriensis Vorticella Chlorella sp Desmodesmus sp3 Oocystella Scenedesmus guadricauda Desmodesmus costato-granulatus New.ReferenceOTU382 Desmodesmus pannonicus Fungi sp chlorella sp Didymogenes Bacteria with high activity Bacteria with

Debris

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Algae community

Bacterial metabolic activity was measured. Percentages of high (alive) bacteria were observed to be always higher than low (death) bacteria. Percentage of high metabolic activity bacteria decreased during the experimental period in SDC cultures while those were increased in control f/2 medium. In general, percentages of low metabolic activity bacteria were increasing during the experimental period in both f/2 and SDC.

The freshwater cyanobacterium (MACC-612) and the marine green alga (BEA-0313B) have the highest auxin-like activities and it doesn't change significantly with the increase of the biomass concentrations. Biomass samples of sewage-grown strains, including MACC-612 show increased antimicrobial activities against fungal plant pathogens. Either direct antifungal effect of accompanying bacteria/fungi inhibit the pathogens or they act as elicitors to induce the production of antimicrobial compounds of microalgae

6 J		Auxin- and cytokinin-like activity							
MACC-612	0.5	0.5 g L <sup>-1</sup>		1 g L <sup>-1</sup>		2 g L <sup>-1</sup>		3 g L <sup>-1</sup>	
	IBA	KIN	IBA	KIN	IBA	KIN	IBA	KIN	
Laboratory control	0.5		>0.7		0.5		0.5		
TLC reg1	0		0.3		0.3		0.5		
TLC reg2	0.3		0.3		0.5		0.3		
RW wastewater AM	0	<0.3	0	<0.3	0	<0.3	0	<0.3	
RW wastewater PM	0	0	0	0	0	<0.3	0	<0.3	
Sample		Antimicrobial effect of algal samples against phytopathogenic fungi (inhibition %)							
MACC-612	Pyth ultin	ium num	Fusa oxyspe	rium orum	Rhizo sol	ctonia ani	Phytop cap	ohthora osici	
Laboratory control	0		24.95		29.41		27.67		
TLC reg1	0	0		27.36		28.57		44.44	
TLC reg2	0	0		19.84		40.48		34.03	
RW wastewater AM	31.93	31.93		31.71		28.80		40.00	
RW wastewater PM	29.79	29.79		39.65		28.81		34.48	
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# WP6 Biology

A simultaneous study of biomass productivity of marine microalgae using centrate, from a wastewater sludge anaerobic digestion unit, and f/2 as a control medium was performed at the Spanish Bank of Algae (BEA) in Gran Canaria. Four native microalgae, two eukaryotes and two cyanobacteria from the Collection at the Spanish Bank of Algae, were selected from 100 different strains tested. Outdoor experiments were performed in 100-400 L-PBR and semi-continuous mode at dilution 0.2 1/day.





Results on productivity, nutrient removal capacity, bacterial growth, biomass nutritional composition, microbiology and heavy metal accumulation (below thresholds established by regulations) support the possibility of producing valuable microalgae biomass for further industrial applications, as evaluated by SABANA, by depurating wastewater (centrate) and controlling bacterial activity on a large scale. Eukaryotic microalgae SABGC0071B, SABGC0072B and cyanobacteria SABGC0069B and SABGC0070B presented not only effective yields in biomass productivity and removal of contaminants, but also possibilities for agriculture applications (biostimulant and/or antifungal activities). Methodologies and results on wastewaters bioremediation with these selected strains have been submitted for evaluation as a patent register.





SAB-GC-0313 SAB-GC-0912 SAB-GC-0069 SAB-GC-0866 SAB-GC-0046

Productivity (g DW/L·d) and bacterial growth rate (cfu/mL·d) of BEA-strains in f/2 control medium and centrate in the experiments performed outdoor at pilot scale (semicontinuous mode during at least 20 days of incubation in tubular 100-400L-PBR)











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#### WP7 Products

Regarding aquaculture applications, three main products from large-scale microalgal biorefinery have been developed to be used as **potential ingredients** or **additives** in aquafeeds.







Preliminary data confirm the potential for using microalgae additives, and raw and hydrolyzed biomass obtained from a biorefinery in aquafeeds, which ensure nutrient balanced diets, and adequate digestive functionality and fillet nutritional quality. Anyway, among the various products tested, hydrolyzed N. qaditana biomass grown on pig medium offered the most interesting results in growth performance terms of in although Siberian sturgeon, the utilization of this organic medium without a pre-treatment involves risk of pathogen presence.

Regarding agriculture related applications different laboratory and *in vivo* trials were performed. Performance of different microalgae strains was evaluated. Results confirm benefits already reported in previous trials, the right selection of microalgae and downstream strategy being highly relevant.



Real field trials are also in progress with different crops both in protected and unprotected conditions. Food production and quality, in addition to overall growth performance and tolerance to stress and pathogens is being evaluated in long term trials under real conditions.











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# **WP8** Dissemination

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SABANA project participate in the most relevant events on microalgae field, in addition to that concerning nutrients recovery or bioeconomy. Information about dissemination activities performed is available at the website. Strong activities have been also performed in social networks such as facebook, twitter, Instagram

**SABANA** 

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A large number of news/press releases, in addition to participation in fairs and exhibitions have been



SABANA project already perform a Massive Online Open Course, which in his second edition has been coursed for more than 2.000 students around the world.



https://www.youtube.com/watch?v=-eg TyaMUNY

SABANA project establish a large number of collaborations with companies and EU projects.





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