SUSTAINABLE ALGAE BIOREFINERY FOR AGRICULTURE AND AQUACULTURE

SABANA

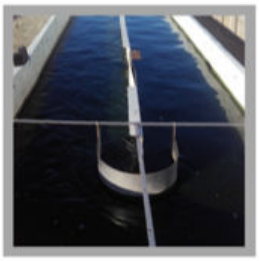
Sustainable Algae Biorefinery for Agriculture aNd Aquaculture

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

4th 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

SABANA e-bulletin No.4, May 2019

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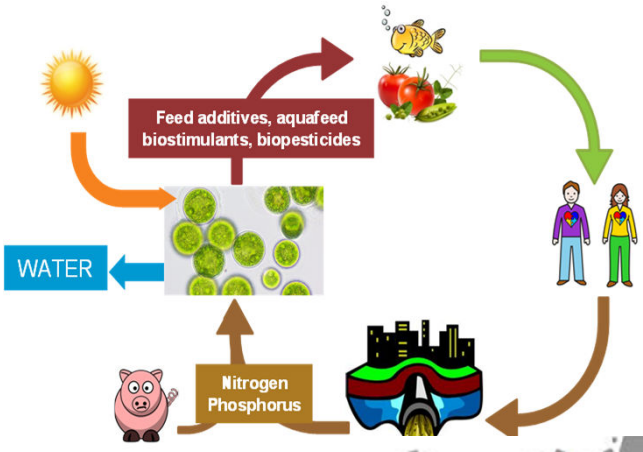
4th e-bulletin of SABANA PROJECT

Introduction

Welcome to the 4th e-bulletin of SABANA summarizing the achievements after 24 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:

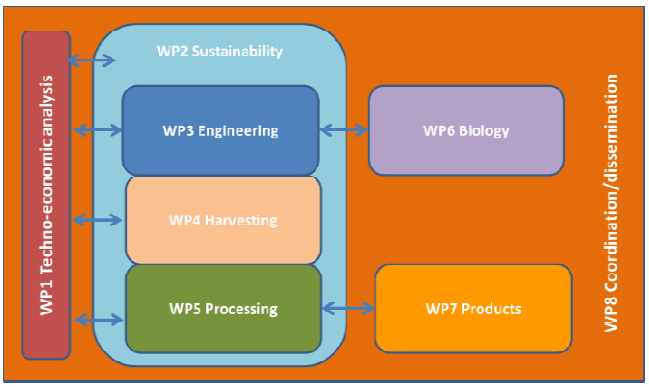
- Improvement of the safety and sustainability of food production in agriculture and aquaculture
- Contamination problems resulting from nutrients dissemination and scarcity (phosphorous)
- Minimization of greenhouse gas emissions from wastes (wastewater and flue gases)



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.

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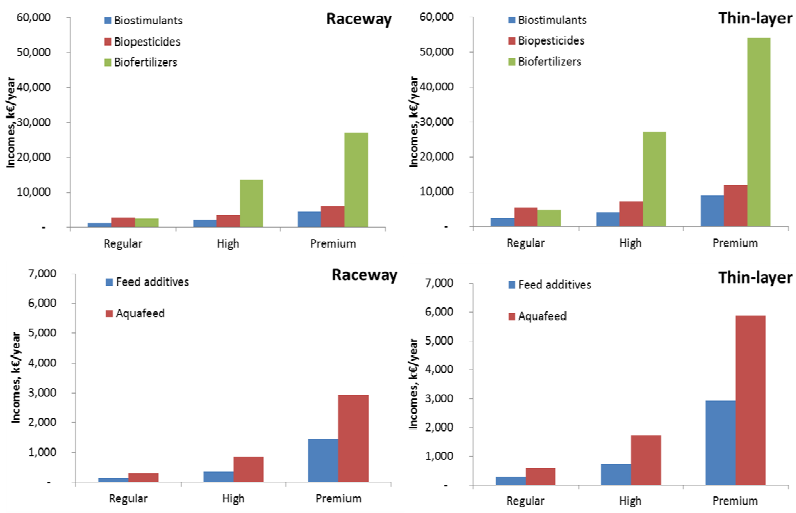
WP1 Techno-economic analysis

The marketability of microalgae related products for agriculture and aquaculture has been confirmed.

Biofertilizers, in addition to provide an eco-friendly option, also maintain the soil and crop health with increased efficiency. Global Biofertilizers Market is expected to reach USD 1.88 Billion by 2020 at a CAGR of 14.0% from 2015 to 2020. The market was dominated by North America in 2014 and accounted for the largest share in the total biofertilizers market. The market for biopesticides is projected to reach USD 6.6 Billion by 2020 at a CAGR of 18.8% from 2015 to 2020. Biopesticides are used primarily as preventative measures for diseases in plants, made from naturally occurring substances that controls pests by nontoxic mechanisms and in an eco-friendly manner.

Related with aquaculture, the Asia-Pacific market topped the revenue chart of the global aquafeed industry, accounting for around 76.1% and 75%, both by value and volume respectively, of the total market. The global aquafeed market is expected to reach a value of 123 billion USD by 2019, at a CAGR of 12.1% from 2014 to 2019 by revenue, and by consumption value, it is projected to reach 89 billion USD by 2019, at a CAGR of 10.7% from 2014 to 2019.

The feed premix market is projected to reach USD 10.26 Billion by 2020, at a CAGR of 3.0%, as studied from 2015 to 2020. The market for feed premix products has a significant impact on the animal nutrition industry. Feed premix is broadly categorized based on their type into vitamins, minerals, amino acids, antibiotics, and others include enzymes, preservatives, organic acids, antioxidants, pigments, and flavors.



Techno-economic analysis already performed for a 5 ha facility clearly demonstrate the benefit of proposed technologies, especially when using thin-layer reactors due to the larger productivity on these systems versus conventional raceway reactors. Final balance is also a function of quality of end-products and its marketability, to focus in agriculture being much more interesting. Still these figures must be confirmed with market studies already in course by companies involved into the project.

For more information please visit: www.eu-sabana.eu
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WP2 Sustainability

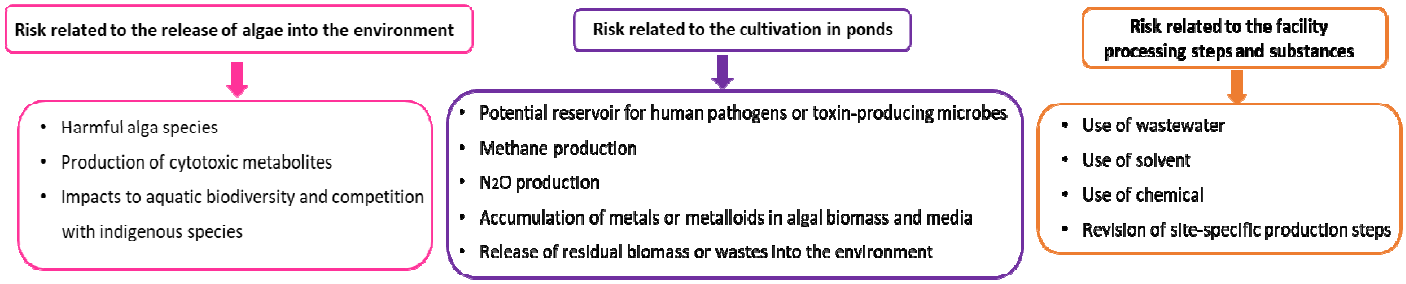
The development of large scale plants for the production of microalgae raises the need to understand, in addition to the benefits, all the risks connected with this new biotechnology. Agronomic approaches are a necessary element in R&D strategies in microalgae field to improve the state of cultivation technology: algal cultivation should be envisioned as a large-scale agricultural practice and not only as a biotechnology. Thus, outdoor field testing at meaningful scales will occur and interaction with environment will be significant, thus tools for evaluation, monitoring and mitigation are needed. In this way it will be possible to handle this new production with all the tools that can make it a successful and highly acceptable production.

The work started first reviewing relevant projects and legislation related with microalgae and risk assessment. From this background the relevant areas of concern where outlined as listed below:

Specific Risks related to large scale microalgae facility	Measure to minimize impacts
Risk related to the cultivation in ponds	
Release of residual pollutants into the environment and human pathogens	Monitoring of water quality , fast algae growth and recirculation mode to allow high purification from pathogens and pollutants
GHG emission (CH_4 and N_2O)	Assure correct DO in the culture by proper mixing
Release of residual pollutants into the environment	Fast algae growth
Risk related to the release of algae into the environment:	
Harmful alga specie	Monitoring of algae population and bacteria
Production of cytotoxic metabolites	Detection of toxins when suitable condition for toxins producer strains occurs
Impacts to aquatic biodiversity and competition with indigenous species	Use of indigenous species or, when necessary, evaluation of the competitiveness of exogenous strain in SABANA environment

The specific steps for risk assessment in micro-algae facility like SABANA were outlined:

1. Identification of system boundaries and flows
2. Hazard identification, considering the main algae related topic and the site specific situation such as
   a. Raw materials and products
   b. Plant equipment and facility layout
   c. Operation environment
   d. Operational activitie
3. Identification of accident scenarios and target



4. Estimation of the accident risk: the likelihood of a specific event within a specified period and severity
5. Finally a critical ranking for likelihood and severity was performed and measures to minimize impacts were proposed.

Currently, legislation in the field of microalgae is limited to assessing safety for use in sectors such as food and feed. For this purpose, the SABANA project exploit its experience in the field by making available to technicians, decision makers and policy makers the description of risks and possible impacts as well as the guidelines to reduce them in installations of this type. This deliverable, as many of the SABANA project, has the ambition to support the commission's bodies in future regulatory and legislative actions in the field of microalgae.

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WP3 Engineering

DEMO PRODUCTION facility has been already completed at May 2019. After large efforts a 1.2 ha facility has been built, it consisting of bubble column and tubular photobioreactors to produce inoculum, in addition to four 100 m^2 reactors to scale-up the inoculum, four 1.000 m^2 raceway reactors for production and a large 5.000 m^2 raceway reactor as main producer reactor into the facility. The plant is fully automated, it working in continuous mode with full recirculation of water to save water and nutrients

The facility is able to operate with freshwater and sea water, in addition to use animal manure, digestate or wastewater as nutrients source. Main target is to verify the reliability of developed technologies, and to confirm the assumptions abut techno-economic analysis already performed.

Different strains previously checked into the DEMO R&D facility will be evaluated in order to confirm the reliability of producing these strains at industrial scale. The influence of environmental conditions and industrial operation will be evaluated. The challenge is to achieve a stable biomass production capacity of 60 tn/ha·year at biomass production cost below 5 €/kg.

First trials are currently in progress, also the performance of harvesting and processing units being checked. End-products to be obtained is planned to be tested in real field trials in the next period of the project… to to achieve a full industrial process.

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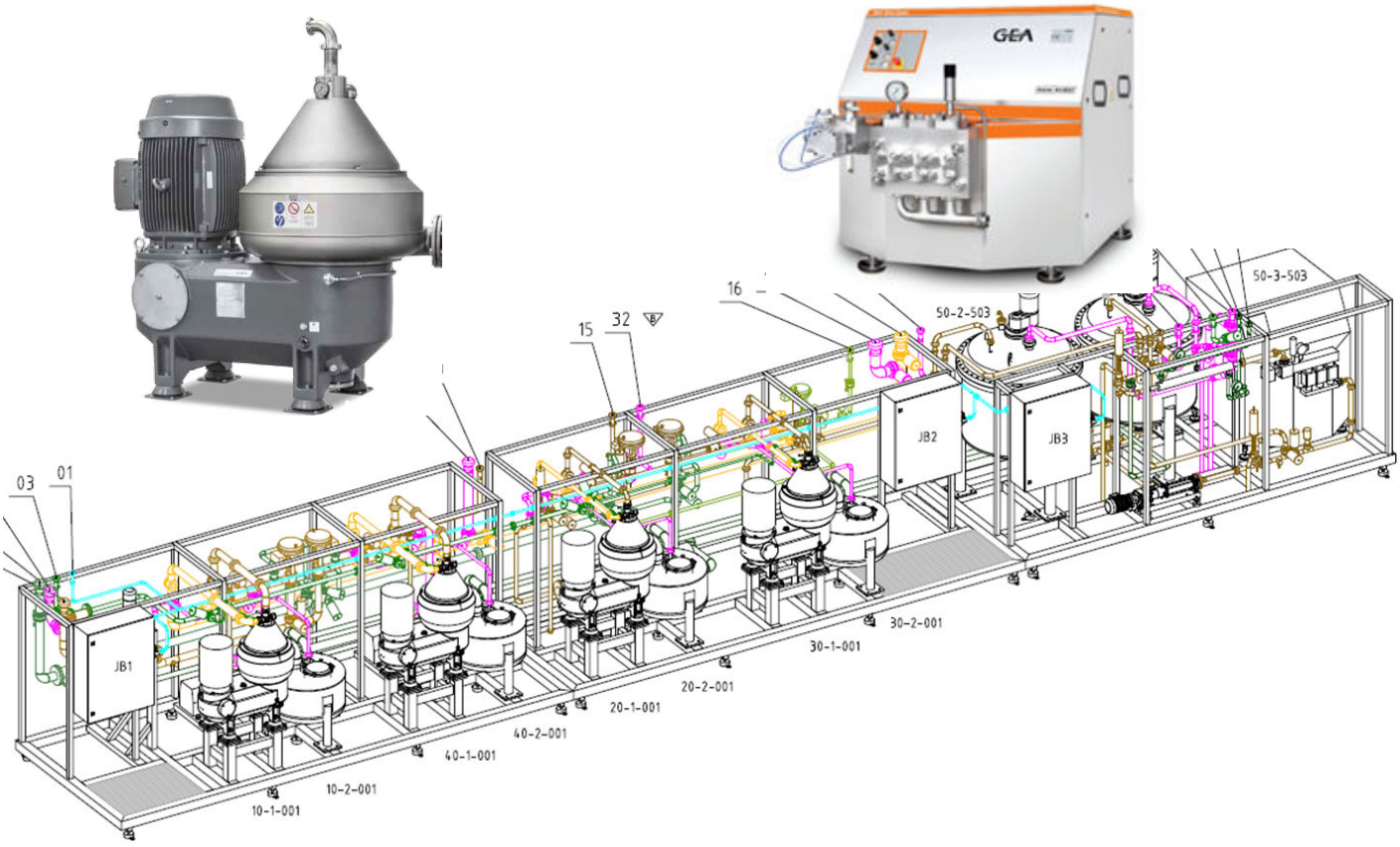
WP4 Harvesting

DEMO1 R&D facility is in full operation in regards of harvesting and processing with different technologies that can be used. Microalgae biomass is produced to gain final products e.g. for market evaluation and field tests.
In the R&D facility it can also be demonstrated that harvesting is an integral process step, that has to aligned according to the needs of further processing, whether agricultural or aquacultural products are produced. In alignment with the cultivation of biomass in different culture media, also the harvesting behaviour will be further monitored and optimized.

On the foundation of the knowledge gained in the R&D facility, the DEMO 1 PRODUCTION harvesting line was basic and detail engineered. The nozzle separator technology will be used for the purpose of algae harvesting and concentration. Furthermore this technology will be aligned with the high-pressure homogenization technology for cell disruption. The equipment will be installed on skid frame with all necessary auxiliary equipment, piping and instrumentation with full automization.



For DEMO 5 the technical specifications were evaluated and the planning and engineering phase will be kicked off. For DEMO 5 a harvesting concept with dissolved air flotation as a preconcentration step and separation for final concentration will be realized.

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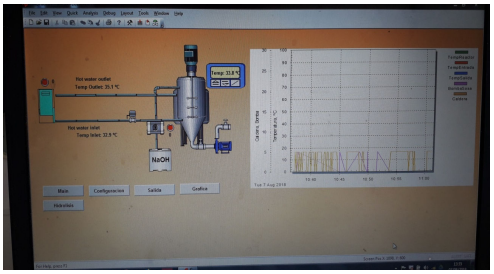
WP5 Processing

High Pressure Homogenization (HPH) and Pulsed Electric Field (PEF) processing have been identified as most energy-efficient processing methods for cell disruption. In both cases, the energy demand is 1 MJ/kgdw and less when fresh water microalgae slurries are treated. HPH exhibits advantages for processing of high conductivity marine strains, whereas PEF can recover multiple component fractions. Next step will be a comparison of both technologies on large scale.

For large scale PEF-processing at DEMO1 the mobile PEF-facility KEA was modified for processing of microalgae slurries at 100 gdw/ltr and conductivities in the range of 4-6 mS/cm. For this purpose a new generator core was designed and manufactured.

The facility consists of a 4 stage Marx-generator, shielded by the blue-colored housing, a slurry feed-pump, foreground, and pressurizing pump, right. The embedded image shows the treatment chamber, TC, and the pulse generator inside the facility. The minimum operating capacity is 550 ltr/h within an treatment energy range between 75 kJ/ltr and 150 kJ/ltr.

After cell disruption a stirred tank reactor has been developed to transform the biomass into end-products such as biostimulants, biopesticides and feed additives. The reactor has a capacity of 300 L per batch although it can be also used in continuous mode. Automatic control of reactor conditions is carried out by SCADA specifically designed for these processes. The performance of the reactor has been evaluated in real scale using biomass from existing reactors.



Patents about biostimulant and biopesticides production process has been already completed. Quality and stability of end-products has been also already verified. Now field trials are being performed by companies already involved into the project to confirm the advantages of obtained end-products.

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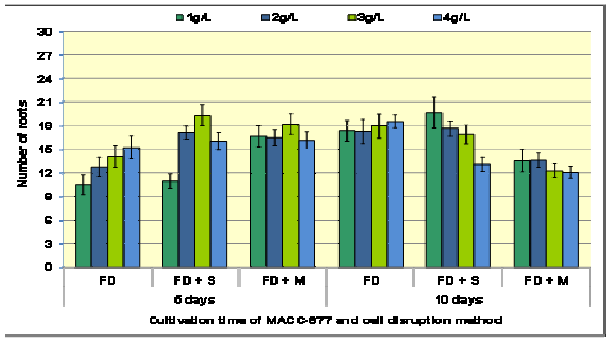
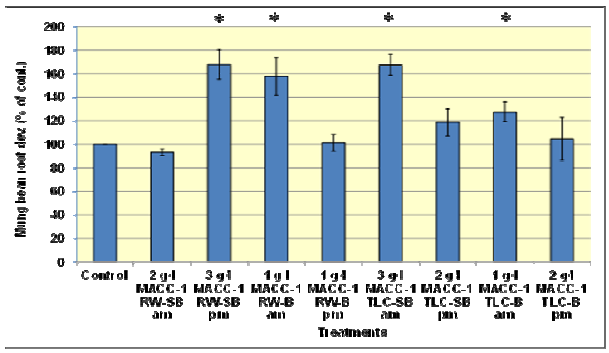
WP6 Biology

Selected plant biostimulating and/or antimicrobial microalgae strains were cultivated in nutrient solution or in pig manure (PM) in laboratory or outdoor conditions in raceway ponds (RW) or in thin-layer-cascade (TLC) culture systems in batch (B) or semi-batch (SM) cultivation mode. The harvested biomass was freeze-dried (FD), freeze-dried and sonicated (FD+S) or freeze-dried and milled (FD+M) to detect the effect of different cell disruption methods on the bioactivity.

In another experimental set the harvested biomass was ultra-sonicated and either hydrolysed with enzyme into peptides/amino acids (with EH) or not (without EH). Both samples were either centrifuged or not before starting the bioassays.



Auxin-like activity of microalgae biomass samples in 1, 2, 3 and 4 g/L concentrations



Auxin-like activity of the microalgal strain, MACC-1 cultivated in nutrient medium in outdoor systems and harvested in the morning (am) or in the afternoon (pm)

Mung bean bioassay was used to measure the auxin-like activity of the samples, which indicates the plant biostimulating effect. The agar diffusion biassay demonstrated the antimicrobial activity against specific plant pathogens. The man objective was to compare the bioactivity of the laboratory biomass samples with biomass samples produced outdoor in different culture conditions and treated with various methods. Selected results are presented in the figures, which show the main tendency of the bioactivity.

It was concluded, that: (1) freeze-drying of biomass was sufficient to release plant biostimulating compounds from the cells; (2) biomass produced in nutrient medium under laboratory conditions was the most effective, while biomass grown outdoor in pig manure the less effective plant biostimulant; (3) biomass produced in PM showed the strongest antimicrobial effect.

The biomass samples of selected microalgae produced under controlled laboratory conditions and in outdoor culture systems maintained their plant biostimulating and antimicrobial activity. However, the bioactivity of the biomass should be detected before marketing because of the non-predictable variations in the bioactivity.

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THE FRAMEWORK PROGRAMME FOR RESEARCH AND INNOVATION
HORIZON 2020

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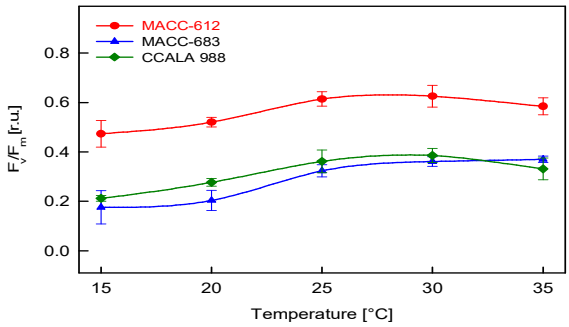
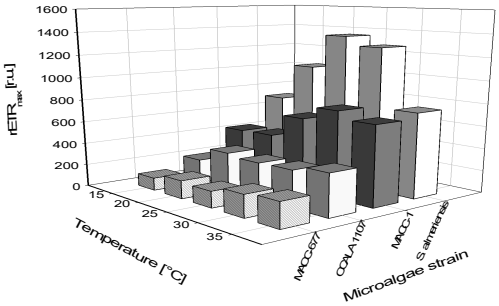
WP6 Biology

One of project objectives is to develop and select fast, robust and easy-to-measure techniques to optimise growth regimes of outdoor microalgae cultures. In this respect photosynthesis monitoring techniques – chlorophyll fluorescence and oxygen production – were tested in-situ and off-situ to correlate photosynthetic performance with growth. We have verified that selected fluorescence variables – maximum PSII photochemical yield Fv/Fm, electron transport rate rETR, fast fluorescence induction kinetics as well as dissolved oxygen concentration (DOC) well indicate the physiological status of microalgae culture and represent reliable markers to estimate culture performance.

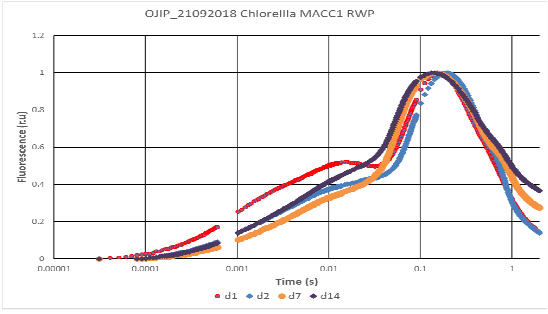
The rETR variable measured in-situ well corresponds to the build-up of DOC in the culture and its growth. This important conclusion concerns the problem of high DOC in fast-growing microalgae cultures. High photosynthetic activity producing high DOC can secure high growth rate. There exists clear correlation: in low activity cultures the build-up of DOC, changes of Fv/Fm and rETR are smaller, and these cultures will grow slowly. On the contrary, high DOC build-up, high rETR and high Fv/Fm characterize highly productive cultures. Namely, a 20-30% decrease of fluorescence photochemical yield at midday is inevitable symptom in highly-productive cultures



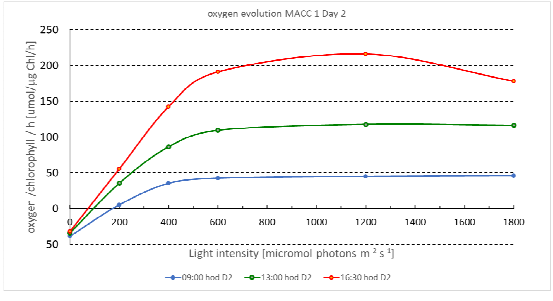
Temperature dependence in the maximum PSII photochemical yield Fv/Fm measured off-situ by Chl fluorescence in laboratory microalgae cultures



Temperature dependence in the maximum electron transport rate rETR measured off-situ by Chl fluorescence in laboratory microalgae cultures



Changes of fast fluorescence induction kinetics measured off-situ in the morning on several days in outdoor cultures of Chlorella MACC-1 grown in a raceway pond



Light-response curves of photosynthetic oxygen production measured at various daytimes in outdoor cultures of Chlorella MACC-1 grown in a raceway pond

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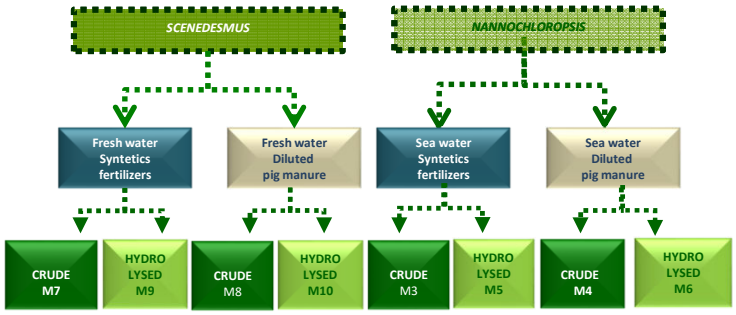
WP7 Products

DEMO R&D is producing biomass samples of *Scenedesmus almeriensis* and *Nannochloropsis gaditana* strains to be evaluated as dietary ingredient for feeding juvenile Siberian sturgeon (*Acipenser baerii*). Microbiological and nutritional analysis of microalgae-based experimental aquafees demonstrate their suitability for feeding fish. Preliminary data show good palatability and adequate zootechnical performance. Aquafeed supplemented with hydrolysed *Nannochloropsis* yield the better results in term of growth performance, though no statistically significant difference is evidenced respect to group fed on a microalgae-free diet.

Microalgae species (selection, production and evaluation) → **Aquafeeds (formulations, production and evaluation)** → **Feeding trials and growth performance**

Experimental diets

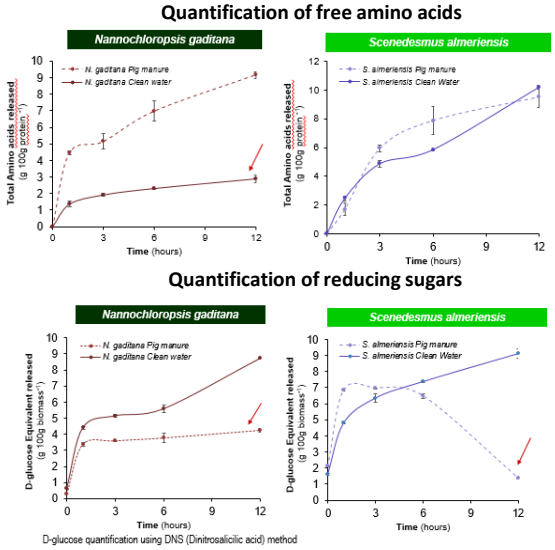


Microalgae have been used for partial replacement (10%) of fish meal and fish oil, and as functional ingredient for increasing sustainability and profitability in the aquaculture sector.

Feeding trial

Diet	IBW (g)	FBW (g)	Feed Intake (g)	SGR (%)	FCR	SURVIVAL%
M2- Control (algae free)	12,8	44,2	346,2	3,09	0,69	100
M3- *Nannochloropsis*, raw clean	13,3	46,5	346,5	3,13	0,75	85,4
M4- *Nannochloropsis*, raw pig	12,3	43,9	327,0	3,18	0,69	95,8
M5- *Nannochloropsis*, hydrol. clean	12,8	43,0	319,0	3,03	0,73	95,8
M6- *Nannochloropsis* hydrol. pig	13,4	48,2	354,7	3,20	0,65	95,8
M7- *Scenedesmus*, raw clean	12,5	40,8	320,1	2,96	0,71	97,9
M8- *Scenedesmus*, raw pig	12,7	40,5	319,8	2,90	0,72	100,0
M9- *Scenedesmus*, hydrol. clean	12,3	39,9	322,7	2,95	0,74	97,9
M10- *Scenedesmus*, hydrol. pig	12,6	40,2	329,5	2,90	0,75	97,9

Hydrolysis of microalgae biomass



Microalgae have been submitted to a previous enzymatic treatment to increase nutrient availability for carnivorous fish. The hydrolysis of *Nannochloropsis* grown on pig manure releases high content of free amino acids.

All experimental feeds offer good growth performance, with average Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR) of 2.95 ± 0.22 and 0.74 ± 0,06 respectively.

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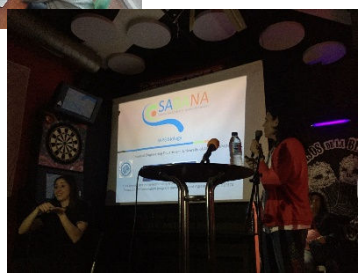
WP8 Dissemination

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 new website.



Hundreds of students and professionals visit the facilities of SABANA project along the project. Moreover more than 60 researchers perform stays at our facilities on that time, and continue… New projects and collaborations raised from these stays…

SABANA project already perform a Massive Online Open Course, which in his first edition has been coursed for more than 3.000 students around the world.

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

SABANA project has been granted with the Prize of Social Development for the Social Council of Andalusian Universities, also receiving visits of politicians and represents of civil society.