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BioSyntex
MICROALGAE FOR A GREEN ECONOMY.

Sviluppo di ceppi microalgali in eterotrofia: una nuova frontiera per applicazioni nel settore alimentare e in agricoltura

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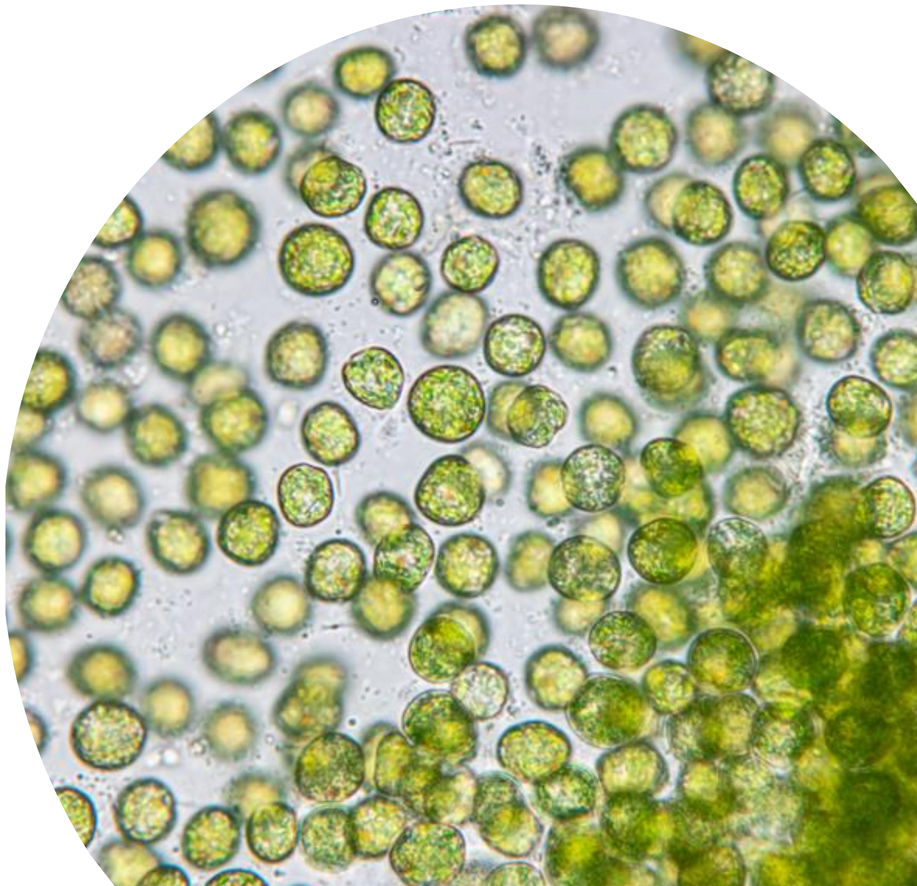


12a Conferenza sulla Chimica Sostenibile
«Collaborazione e innovazione per la transizione ecologica»
Federchimica, 5 Giugno 2025

About Us

Biosyntex

Founded in 2018 with a share capital € 2.36 million:

> **BF International S.r.l. (50%)**

> **FINIM S.p.A. (50%)**

«Green» biotechnology

The company operates as a supplier of technologies, services and products in the field of microalgae, cyanobacteria, and photosynthetic bacteria cultivation

Team

A multidisciplinary team (6 people) composed of biologists and biotechnologists works alongside universities and strategic partners to develop new applications using our microalgae biomasses and extracts.

Our Services

Algae Bank

> Collection > **40 strains**

> Available for partnerships

R&D Service

> Optimization of growth parameters

> Innovative microalgae applications

> Sampling of biomass and extracts

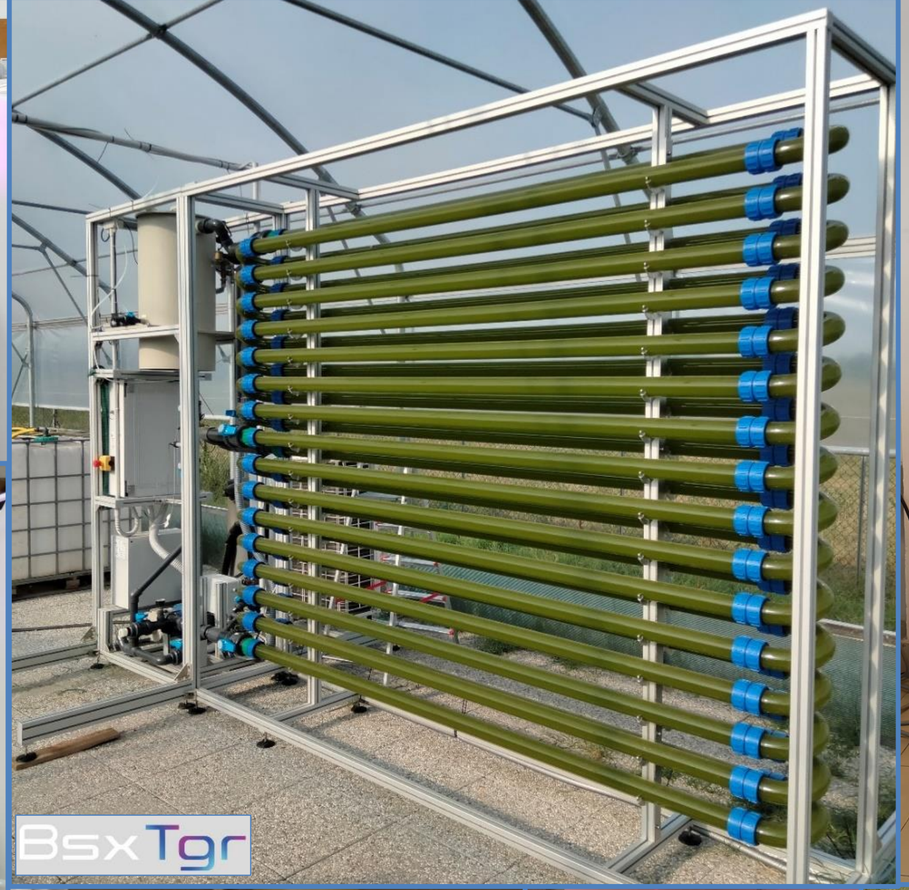
Consultancy

> Microalgae applications

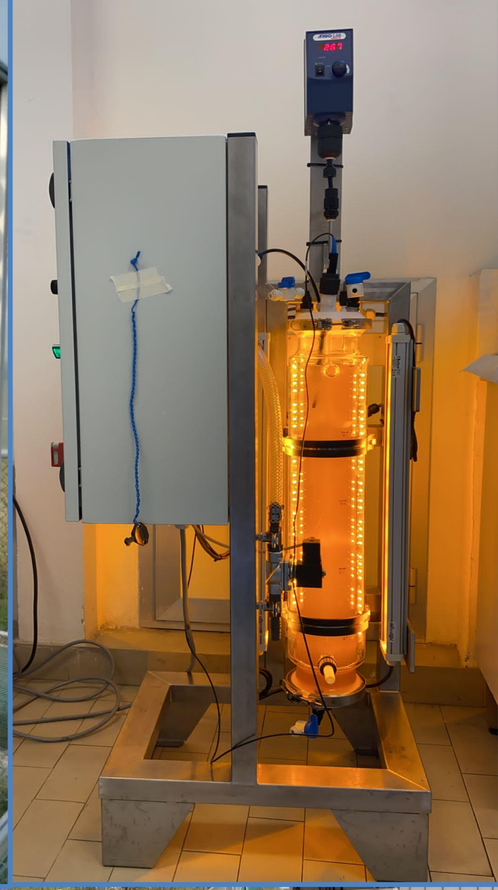
> Microalgae production

Our Products
MICROALGAE REACTORS

BioBox
A NOVEL COMPACT MICROALGAE REACTOR

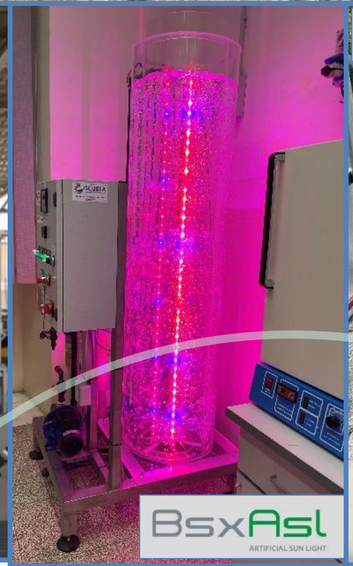


BsxTgr



BsxAsl
ARTIFICIAL SUN LIGHT

BioBosco
POWERED BY BIOSYNTEX



BsxAsl
ARTIFICIAL SUN LIGHT

BsxRwp

Our Products
MICROALGAE POWDER FOR FOOD & FEED

Specials	Natural food dyes	Fragrance & flavor	Techno-Functional highlights	Nutritional highlights	Applications in food
Yellow Chlorella	 Vibrant yellow color	Delicate, tart, yeasty and malt-like	• Emulsion capacity • Emulsion stability • Gel-forming capacity • Water retention prolong the shelf life of a product by lowering humidity and aw	High in protein, fibers and Vitamin B12	Egg and meat analogs, backery, patisserie, pasta, food supplement, smooths, soups, sauces, emulsions and enrichment of flour
White Chlorella	 No hue shifts	Delicate, malt-like, nuts and yeasty	• Emulsion capacity • Emulsion stability • Water retention prolong the shelf life of a product by lowering humidity and aw	High in protein, fiber and Vitamin B12, source of ALA (Omega-3)	Egg and meat analogs, backery, patisserie, pasta, food supplement, smooths, sauces and enrichment of flour
Organic Spirulina Iron Plus	 Green-blue color	Same of standard Spirulina	• Oil absorption • Emulsion capacity • Water retention prolong the shelf life of a product by lowering humidity and aw	Source of bioavailable iron. Source of vitamin E. "Alpha - Tocopherol" deficiency leads to weakness and damage of erythrocytes. Enhance the production of ferritin, leading to a major iron accumulation. C-PC binds the iron present into the biomass, making it absorbable the level of the gastrointestinal system.	Sport food, Protein food, Energy bars, Food supplements

Classic	Natural food dyes	Fragrance & flavor	Techno-Functional highlights	Nutritional highlights	Applications in food
Green Chlorella	 Vibrant green color	Delicate, green, hint of the sea	• Oil retention • Gel-forming capacity • Water retention prolong the shelf life of a product by lowering humidity and aw	High in protein and fibers, source of Vitamin B12 and Zinc	Smoothies, backery, pasta, food supplement, soups, sauces and emulsions
Organic Spirulina	 Green-blue color	Delicate, green, vegetal and reminiscent of the sea	• Oil absorption • Emulsion capacity • Water retention prolong the shelf life of a product by lowering humidity and aw	High in protein, source of fibers, Vitamin B12, iron and ALA (Omega-3)	Supplementing nutrition, snacking, garnishing and finishing dishes

LAB & Yeast Fermented	Natural food dyes	Fragrance & flavor	Techno-Functional highlights	Nutritional highlights	Applications in food
Sour Chlorella/Spirulina	 Yellow, white and green color	Sour flavour. improved taste thanks to LAB & Yeast fermentation	• Emulsion capacity • Emulsion stability • Water retention prolong the shelf life of a product by lowering humidity and aw	Improved flavor & taste, higher digestibility, antioxidant and antinflammatory activity	Dairy, backery, food supplements, pasta

Our Products
ALGAE FINISHED PRODUCTS

Agriculture Biostimulant

Available on the market

Microalgae based food

(Crakers @ 6% White Chlorella)

Available on the market

Microalgae cosmetic Ingredients

R&D On course

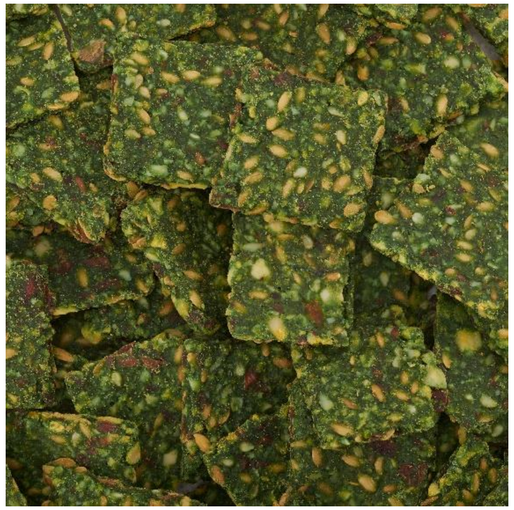
Phototrophic microalgae biomass

- ☺ High protein content (50-60 %)
- ☺ Rich in pigmets.
- ☺ Source of antioxidants, vitamins (Vit. B12).
- ☺ Complete A.A profile.
- ☺ Free from allergens.

...but:

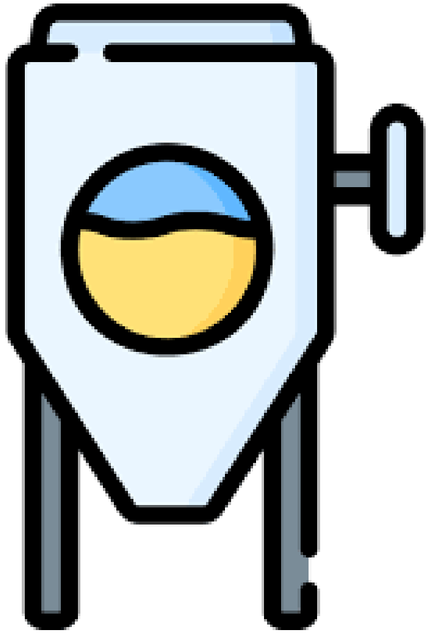
- ☹ Strong colors (green, brown).
- ☹ Strong Taste.
- ☹ Off-flavors.
- ☹ Expensive respect alternatives (eg. SCP, Soy, beans etc.)

Traditional (phototropic) microalgae for food & agri. applications…



…are limited by the green, typical algae flavors and
biomass cost

Could heterotrophic cultivation be the solutions?



FERMENTERS

- ✓ High productivity (g/L day)
- ✓ Cheaper than phototrophic at large scale (> 5000 kg/yr)
- ✓ Reduced plant foot-print
- ✓ Possibility to cultivate chlorophyll free strains

White Chlorella

Yellow Chlorella

Green Chlorella “H”

Objectives of the collaboration

1. Generate a stable axenic Chlorella Master Cell Bank, leaving from BSX's Chlorella strains.
2. Generate Chlorella mutants (yellow and white) to overcome typical «green» Chlorella constrains.
3. Development of a Fed-batch fermentation process (6.5 L)
4. Scale-up the Chlorella (WT and mutants) heterotrophic process up to 100L.

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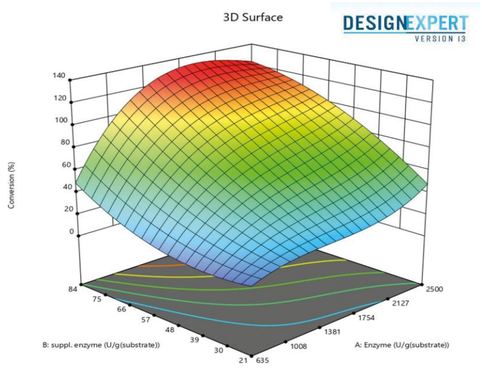
“ONE STOP SHOP” FOR PROCESS DEVELOPMENT, SCALE-UP AND MANUFACTURING

PROJECT MANAGEMENT

PROCESS DESIGN



MICROBIOLOGY AND FERMENTATION

DOWNSTREAM PROCESSING

BIOCATALYSIS

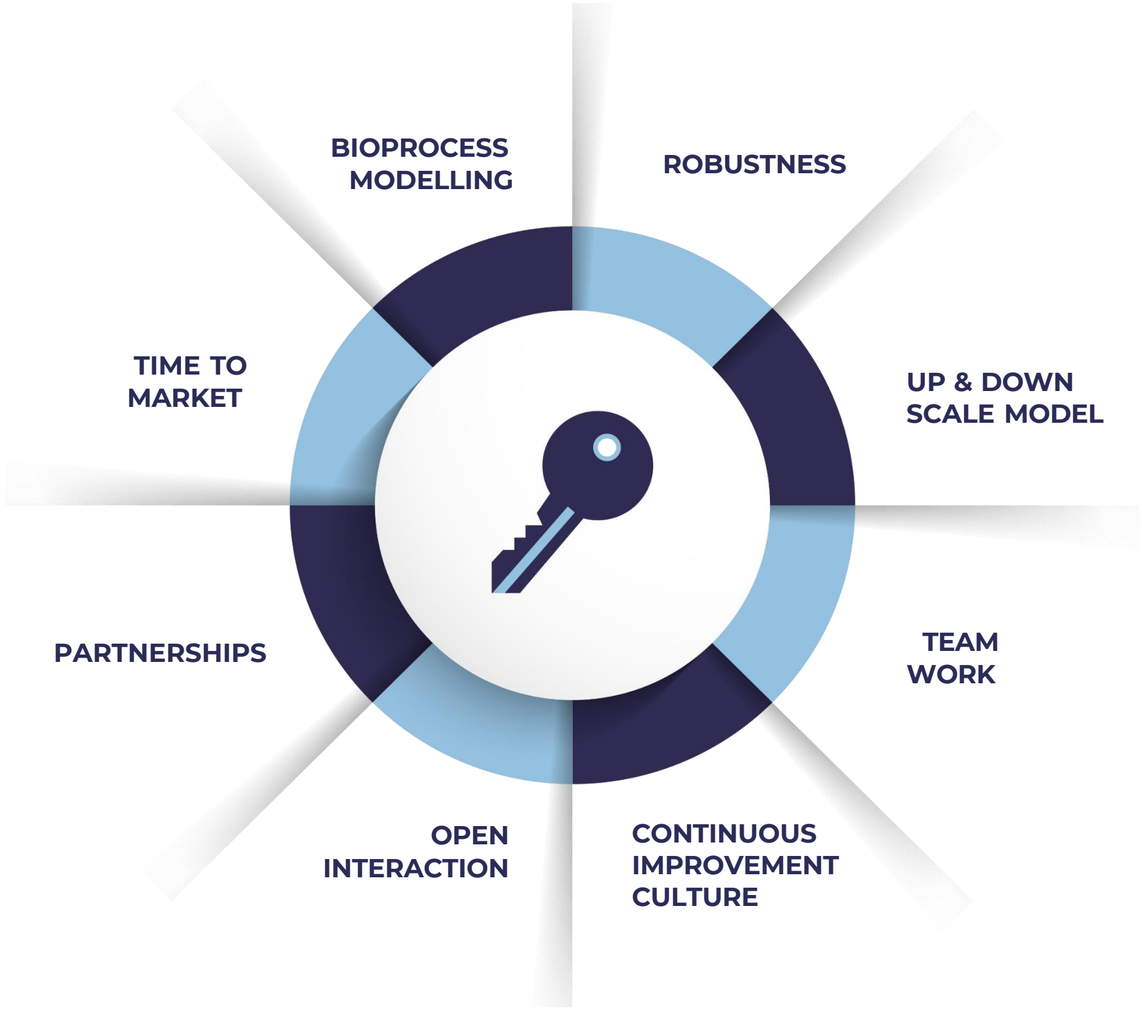
PILOT FACILITY FOR SCALE-UP

FULL SCALE INDUSTRIAL MANUFACTURING

OUR CDMO BUSINESS MODEL

We provide solutions to transform “Proof of Concept” into fully industrialized processes

- **Enzyme identification** and preliminary screening;
- **Enzyme engineering** and **evolution**;
- **Optimization of expression systems** for recombinant protein production;
- **Clone screening**;
- Wild-type **strain evolution**;
- Development of **fermentation** and **downstream processes**;
- **Scale-up from 1 liter to 1 m³**;
- Development of **bioconversion protocols**;
- **Mitigation of critical issues**, ensuring consistent and reliable production performance;
- **Techno-economic assessment and feasibility studies** for industrial production;
- Establishment of **long-term partnerships** for industrial manufacturing.



PILOT FACILITY FOR PROCESS SCALE-UP

6.5L
Consistency runs

150L
Pilot runs

1,500L
Demo scale run (upstream and downstream)

150 L and 1,500 L fermentation units ensure effective scale-up to obtain robust, reproducible, fully implemented industrial processes, and all downstream unit operations are scaled-up accordingly.

Key factors like energy and water consumption can be reliably measured, laboratory chemicals are replaced with bulk raw materials, and potential challenges are identified and addressed.

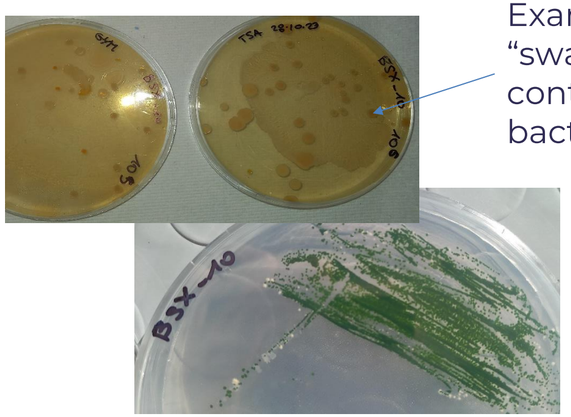
Additionally, product prototypes are produced in relevant quantities, enabling kilo-lab testing and comprehensive chemical and microbiological characterization.

OUR MAIN MARKET SECTORS

Industrial biotechnology solutions for a wide range of applications

Generation of stable axenic Chlorella strains

- N.3 BSX's Chlorella strains screened (**BSX.10 - BSX.11 - BSX.25**)
- Removal of symbionts and maintenance of vitality → Growth on different solid growth media for determination of the symbiont load.



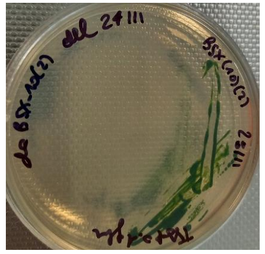
Example: "swarming" contaminating bacterium

Different strategies for obtainment of axenic strains:

Solid culture w/o **antibiotics** exposure

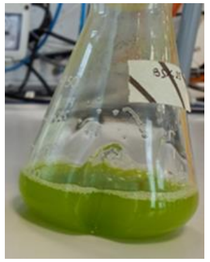
Selection of 1 colony from plate BG11 autotrophy with contaminants

Serial seeding on solid media

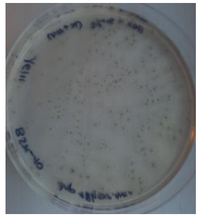


Growth on TSA heterotrophy

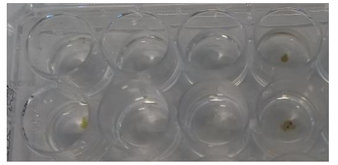
Liquid culture + **Antibiotics** exposure



Incubation in medium BG11 + Mix Antibiotics 24, 48, 72h time of antibiotics exposure



Seeding on solid media BG11



Liquid growth in 24 well plates BG11 medium

Random Mutagenesis and Selection of Chlorophyll-Deficient Mutants

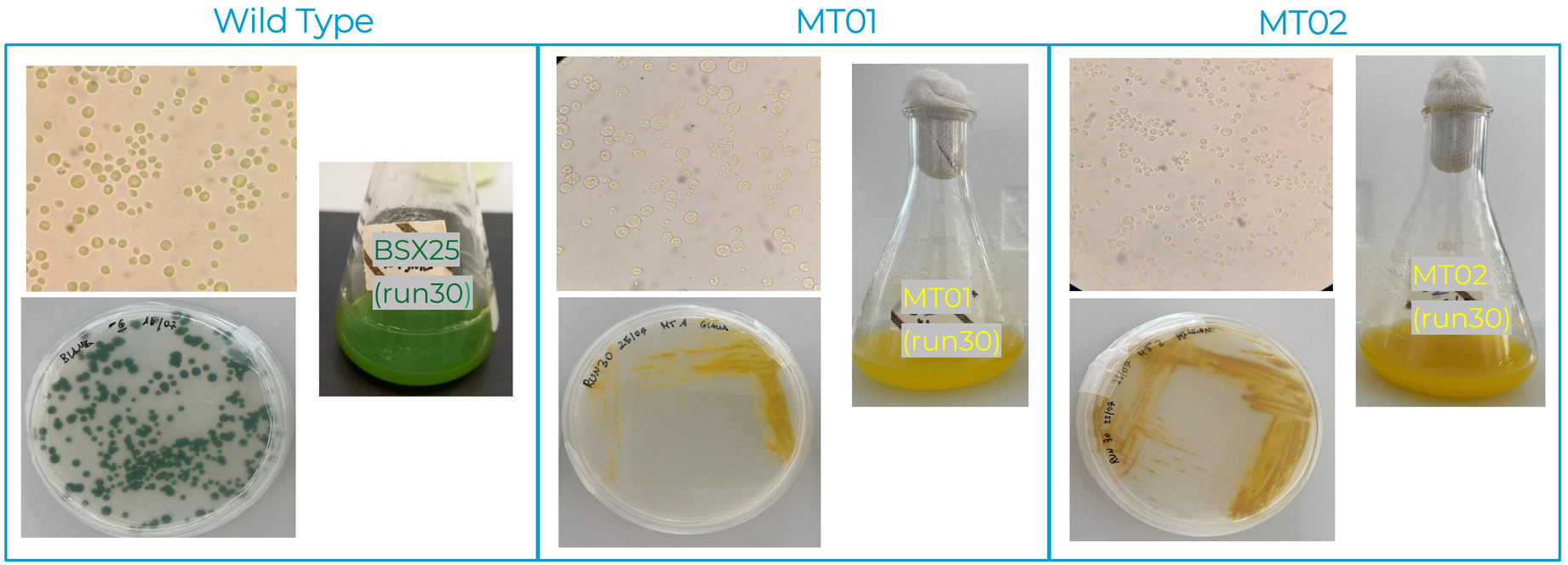
- Chlorella BSX.25 strain
- Two-step **chemical mutagenesis** applied
- Final mutants selection and confirmation of the stability of the mutagenesis by repeated sub-cultivations on solid media

1° Round of Mutagenesis (on green wild type Chlorella):

- Mutagenesis: Ethyl methane sulfonate (**EMS**) at six concentrations (150–400 mM).
- Mutants Selection: by visual inspection for pigment deficient (yellow) colonies on Plate Count Agar

2° Round of Mutagenesis (on yellow mutant):

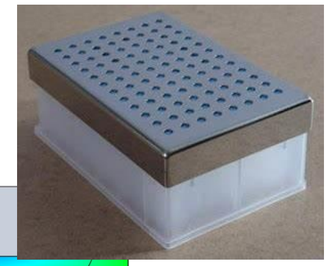
- EMS treatment: 200 mM.
- For stabilization of the mutagenesis

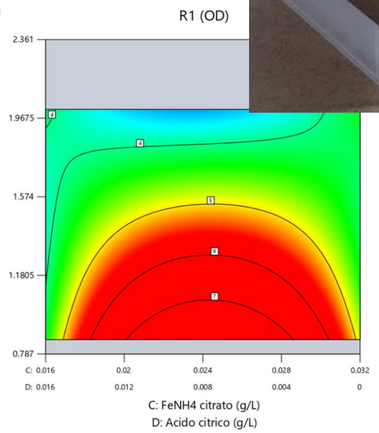


Medium screening – optimization of biomass and protein content %

DoE (Design of Experiment) / RSM (Response Surface Methodology)

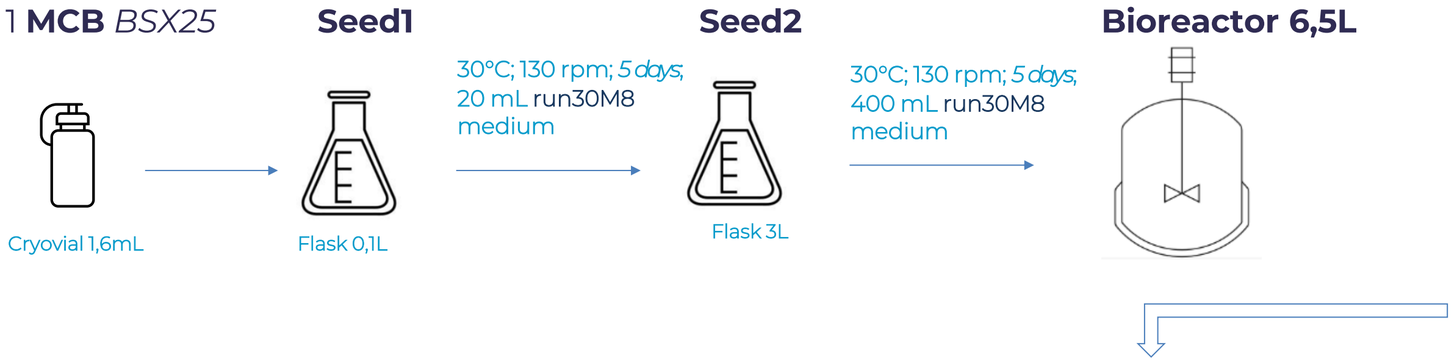
> **Carbon** source : 4 glucose concentrations tested → 4 molar ratio C:N
> **Nitrogen** source: 3 different types of N at different level of concentration ($NaNO_3$, NH_4Cl, and Urea)
> **Phosphorus** source: 3 concentrations K_2HPO_4 (Buffer) → 3 molar ratio N:P
> Optimization of Ca, Mg, Fe, and other nutrients





Development of a Fed-batch fermentation process

> *Chlorella sorokiniana green* **BSX25 heterotrophic growth**
> **Optimized medium** + M8 trace element



100x of typical Chlorella **phototropic productivity**

	Fermentation
C/N/P molar ratio	10/1/0.1
Final Volume [L]	3,86
Total Glucose consumed - S [g]	590
Final Biomass - X [g]	250,1
Maximum biomass conc. [g/L]	69,0
Biomass productivity [g/L/d]	18,7
Max Volumetric productivity	**27,4 g/L day**
Average Growth rate μ [h^{-1}]	0,053
Yield (biomass/glucose)	
Y X/S [g/g]	0,42

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Grazie per l'attenzione!

CONTATTI

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