PRIN: PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE – Bando 2022

Prot. 20225WLFFR

BIOpolymers from agri-food waste digestates for SMART release bioFERTilisers: BIOSMARTFERT

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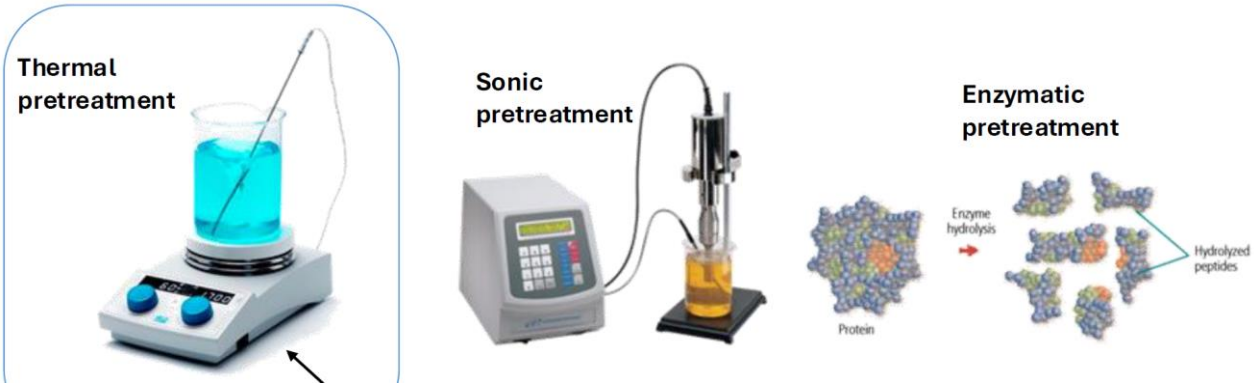
SUMMARY OF ACTIVITIES CARRIED OUT IN THE FIRST 18 MONTHS

WP1 - VFAs PRODUCTION FROM DIGESTATE AS FEEDSTOCK FOR PHA PRODUCTION (UNIPI)

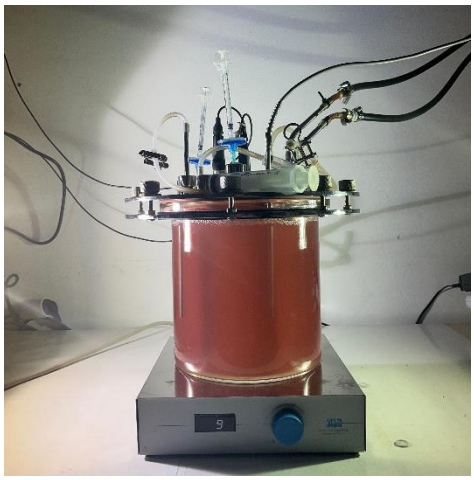
In the framework of WP1, "dark fermentation" tests were carried out to increase the availability of carbon in the form of volatile fatty acids in a possible feedstock useful for producing volatile fatty acids precursors of PHAs. The agro-food organic waste was pre-treated, before the "dark fermentation", with thermal, sonic, and enzymatic pre-treatments to increase the soluble carbon and therefore increase the content of volatile fatty acids. The tests were carried out for 5 days in one-liter reactors at a temperature of 37 °C.



To define the experimental tests, the RSM (Response Surface Methodology) method was used, and therefore, optimum curves were determined for the pre-treatment time and temperature parameters. Samples produced by thermic treatment from agri-food waste were analyzed preliminarily by NMR spectroscopy through the compared analysis of bidimensional homonuclear (COSY, TOCSY, ROESY) and heteronuclear (HSQC, HMBC) experiments in D2O and CDCl3. Low-molecular weight components (acetic acid, butyric acid, valeric acid/caproic acid, 4-aminobytiric acid, 5-aminovaleric acid, 1,4-diaminobutane) have been identified.

WP2 – PHA PRODUCTION (CNR-IRET)

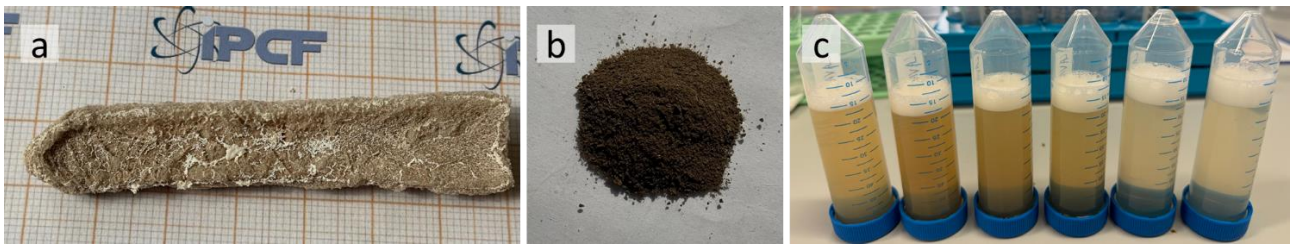
For WP2, the development of red bacterial cultures for PHB production was carried out on a small scale in 0.2 L photobioreactors. Cell growth for PHB production was performed using acetate, propionic acid, or butyric acid as a carbon source. In addition, medium red bacterial cultures were performed with acetate and glucose as carbon source in a 4.0 litre photobioreactor.



The 4.0 L photobioreactor used for the cultivation of photosynthetic bacteria

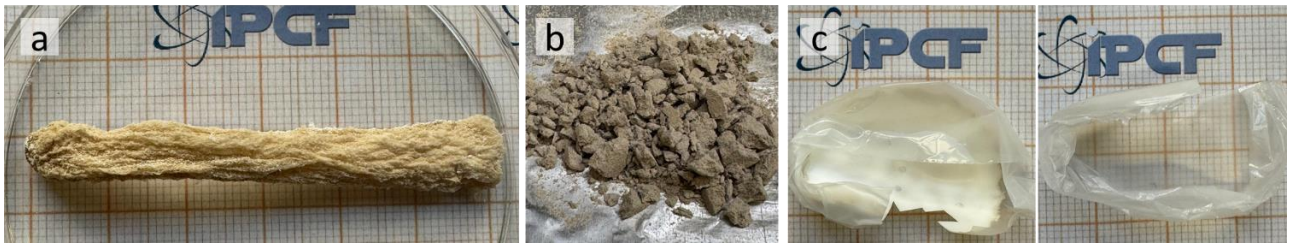
WP3 – PRODUZIONE DEL FERTILIZZANTE SMART (ESTRAZIONE PHA E SINTESI DI IDROGEL E PELLET) (CNR-IPCF, UNIPI)

A preliminary characterization was performed on three biomass samples containing polyhydroxyalkanoates (PHA), obtained from mixed microbial cultures (MMC) subjected to three different experimental conditions. The samples, previously ground and homogenized, were analyzed by ATR-FTIR spectroscopy, to identify any residues of compounds deriving from the biomass feed substrate. Based on the results obtained, the samples were subjected to repeated washing and recovery phases (3–5 cycles, depending on the starting matrix). The comparison between the initial mass and that obtained after purification allowed a first estimate of the yields of the treatment process.



a) Biomass as received after freeze-drying; b) Homogenized biomass before washing; c) Washing water

The purified biomass was subjected to freeze-drying and solvent extraction, followed by filtration and recovery of the product by solution casting. This procedure was iterated until white or visually colourless polymeric films were obtained.
The efficiency of the extraction process was determined by comparing the mass of the extracted PHA with that of the purified sample and with the initial weight of the biomass.
The films obtained in sufficient quantities were characterized by ATR-FTIR spectroscopy, differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA).



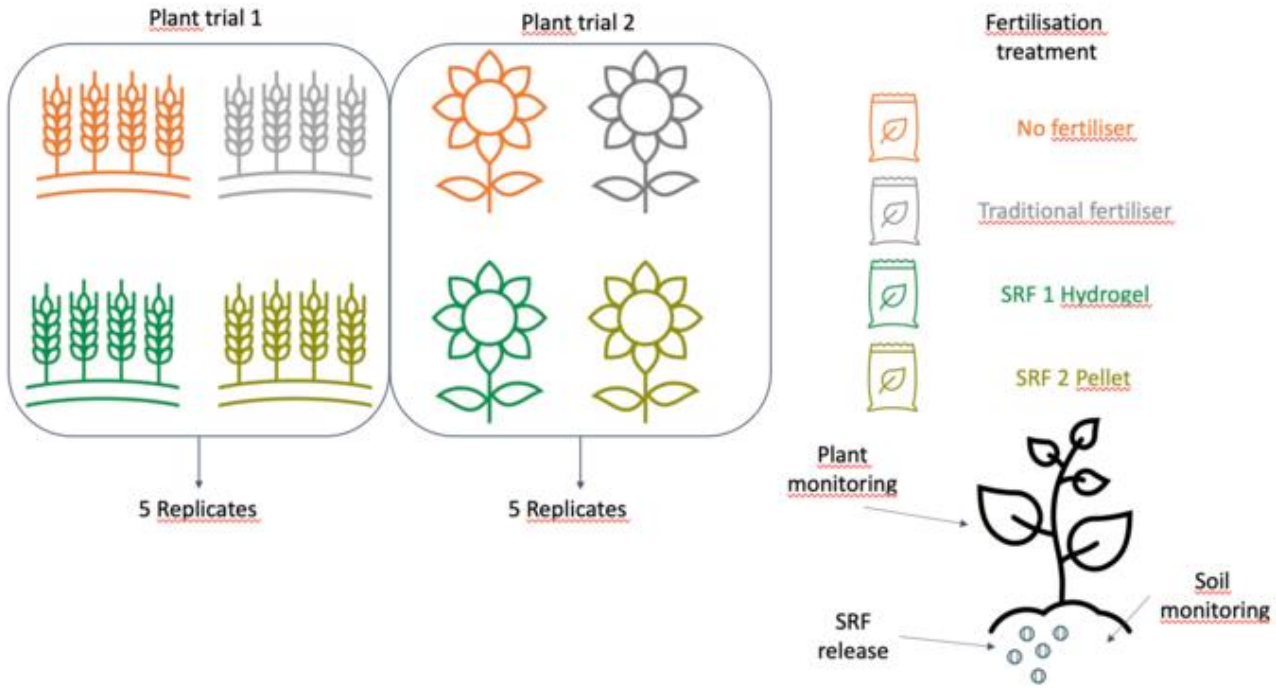
a) Biomass after washing and freeze-drying; b) Biomass after homogenization and extraction; c) PHA films obtained after extraction

The pretreatment, extraction and purification procedures just described were used for a larger set of 15 biomass samples containing PHA, deriving from MMC cultures treated in five different experimental conditions, each analyzed in triplicate. Also in this case, the polymeric films obtained were subjected to characterization by ATR-FTIR, DSC and TGA. The same procedure is in progress on a batch of red bacteria biomass rich in PHB.
The 15 samples were analyzed via 1H and 13C NMR spectroscopy. The proton spectra were used for determining the monomer ratio (FV/FB), while the analysis of the carbon spectra was focused on the determination of the content of diads and triads V-centered, with the aim to gather information on the distribution of butyrate and valerate units. Given the strong superimposition between the signals

belonging to different diads or triads in the carbon spectral regions analyzed, the relative intensity of each resonance was determined by deconvolution. The degree of randomness of diads and triads were calculated from the data collected from 13C NMR analysis, and the sequence distribution of the two monomers within the PHA polymeric chain was analyzed by following three statistical models: Bernoullian statistics, a first-order Markovian model, and a model suitable for a mixture of two Bernoullian random copolymers.

WP4 - EVALUATION OF SMART-RELEASE FERTILISERS AGRONOMIC PROPERTIES (CNR-IRET)

In WP4, the digestates obtained from the three different pretreatments (thermal, sonic and enzymatic) were characterized. Furthermore, waiting for the SMART fertilizers, the experimental layout for the planned agronomic trials was set up using lettuce and tomato as target species.



WP5 - MATHEMATICAL MODELLING; LCA ANALYSIS AND ECONOMIC EVALUATION (UNICAMPANIA, UNIPI)

The project's activities to date have focused on the development and optimisation of the biological processes of Dark Fermentation (DF) and Photo Fermentation (PF), as well as the creation of predictive mathematical models to simulate and optimise the yield of these processes.
The experimental activities aimed at the production of SRF, conducted to date, have been designed using Response Surface Methodology (RSM). RSM is an empirical methodology that uses mathematical and statistical techniques to relate two or more input variables. The methodology was used both for the design of the DF experiments aimed at choosing the best feedstock pre-treatment (low-temperature thermal, sonic and enzymatic), and for the interpretation of the results.

The mathematical modelling work focused on the development of two codes for the simulation of DF and PF processes. These models are based on Anaerobic Digestion Model No. 1 (ADM1), and subsequent modifications, and were developed using MATLAB R2023b software. The model is designed to predict laboratory results, in particular VFA and PHB production, based on substrate characteristics and operating conditions.

In order to construct the LCA related to the treatment chain, the purification and extraction procedures adopted were summarized and analyzed. In particular, the volume of solvents used, the duration of the operations and the energy consumption of the laboratory equipment used during the entire sample manipulation process, up to the final obtaining of the PHA films, were taken into consideration.

WP6 - DISSEMINATION AND COMMUNICATION - PROJECT MANAGEMENT (ALL UNITS)

The BIOSMARTFERT project and its results were presented at the following events:

-BIOECONOMY DAY 23 MAY, GIORNATA DI STUDIO DEI RICERCATORI UNIPI, PISA, ITALY (UNIPI)

-SIDISA 2024, XII INTERNATIONAL SYMPOSIUM ON ENVIRONMENTAL ENGINEERING, 1-4 OCTOBER, PALERMO, ITALY (UNIPI)

-BRIGHT 2024, LA NOTTE DEI RICERCATORI, 27 SEPTEMBER 2024, AREA DELLA RICERCA DI PISA, ITALY (CNR)

-SEMINARIO “FROM BIOMATERIALS TO SUSTAINABLE AGRICULTURE, COLLABORATING FOR A COMMON ONE HEALTH OBJECTIVE”, 1 DECEMBER 2024 AREA DELLA RICERCA DI PISA, ITALY (CNR)

-MEETING IPCF 2025, 18-20 MARCH 2025, AREA DELLA RICERCA DI PISA, ITALY (CNR)