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Bio-Hydrogen, the CNR activity in the framework of AdP-PNRR program

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The program

According to the European Recovery & Resilience policies launched to react to COVID-19, Italy launched its own "Piano Nazionale di Recupero e Resilienza – PNRR" (National Plan of recovery and resilience) supported by EU. Within the actions, a significant effort for research was considered, and hydrogen technologies were considered as the training technologies for de-carbonisation and future energy market.

In particular, the Minister for Energy Transition (now renamed Minister of Environment and Energy Security) launched the research agreement "AdP - Research and Development of technologies for the hydrogen supply chain" 8AdP-Hydrogen), with an investment of 110 M€. The program foreseen an intensive collaboration between the three main research bodies involved in energy and environmental research (ENEA, CNR and RSE). The aims are: reinforce the collaboration between the three research bodies, and speed-up the technology transfer to industry of hydrogen technologies. For this reason the AdP-Hydrogen research program covers all the aspects of hydrogen value chain, including the new promising early stage hydrogen technologies that could be of interest for the national hydrogen value chain.

Bio- Hydrogen line

The program is divided in Topics, Work Packages and Line of Activity, where each research body contribute with research activities that are complimentary each other. In this framework the Line of Activity 1.1.30 "Development of sustainable biological processes and bioreactors for the production of hydrogen with bacteria and/or photosynthetic microorganisms from by-products and biomass of the agro-food industry" (SusBioH), falls within the new promising approaches for sustainable hydrogen production.

As clearly exposed in the title the SusBioH research activity aim is the valorisation of waste water by producing hydrogen as a high added value product. This approach will allow the concomitant depuration and reuse of waste water with the production of hydrogen and, possibly other valuable products, thus reducing the today energy consumption for waste water treatment.

In this way the SusBioH research activity will contribute the four UN Sustainable development goals:

Goal 6 – Clean water and sanitation

Goal 7 – Affordable and clean energy

Goal 9 – Industry, innovation and infrastructures

Goal 11 – Sustainable cities and communities.

LA 1.1.30 - Development of sustainable biological processes and bioreactors for the production of hydrogen with bacteria/photosynthetic microorganisms from by-products and biomass of the agro-food industry

The research activity was started in July 2022 and foreseen:

- Chemical and biochemical studies on anaerobic hypertemophiles bacteria of the Thermotogale order for bio-hydrogen production and CO2 capture. - ***CLF***

- Study of micro-algae and cianobacters for the production of hydrogen from waste water. - ***BF***

- Photo-fermentation of organic compound from polluting water and soil. - ***FF***

- Development of materials for bacteria and micro-organism support, linkage and growth. - ***MIC***

- Development of a new concept bio-reactor prototype for hydrogen production for demonstrating technology application potentialities and its transfer to industry. - ***BRT***

The LA 1.1.30 actors

The LA 1.1.30 activity is carried out by 5 Institutes of Consiglio Nazionale delle Ricerche (CNR)

***Istituto di Chimica Biomolecolare (CNR-ICB)**, Via Campi Flegrei, 34, 80078 Pozzuoli (NA), Italy - LA coordinator and in charge for CLF activity.*

***Istituto di Tecnologie Avanzate per l'Energia "Nicola Giordano" (CNR-ITAE)**, via Salita S. Lucia sopra Contesse 5, 98126 Messina, Italy – In charge for BRT activity*

***Istituto Processi Chimico Fisici (CNR-IPCF)**, Via Orabona, 4, 70126 Bari, Italy – In charge for FF activity*

***Istituto Polimeri, Compositi e Biomateriali (CNR-IPCB)**, Via Campi Flegrei, 34 - fabbricato 70, 80078 - Pozzuoli (NA), Italy – In charge for MIC activity*

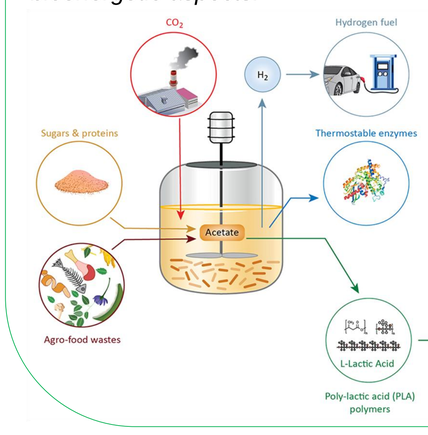
***Istituto di Scienze e Tecnologie Chimiche "Giulio Natta" (CNR-SCITEC)**, Via Mario Bianco 9, 20131 Milano, Italy – in charge for BF activity*

Targets

- *Description of the regulation and interaction of metabolic pathways related to hydrogen production in Thermotogales. Definition and lab validation of at least one bio-waste process suitable for large scale application.*
- *Definition of protocols for the cultivation of commercial microalgae and cyanobacteria in conditions that allow the production of hydrogen, and related procedures for the preparation of the culture bed starting from waste water from food industries.*
- *Selection of photosynthetic microbial strains and consortia capable of producing hydrogen and electricity from waste water. Definition of the general characteristics of suitable bio-reactors for their application and its proof of concept realization.*
- *Development of new materials and hydrogels based on polysaccharides, proteins and synthetic polymers to immobilize microorganisms for hydrogen production.*
- *Design, realization and demonstration of a new bio-reactor for thermophile bacteria applying the developed know-how and suitable for continuous run, scalability and stack ability. Including the definition of the control system and operative protocols.*

CLF activity

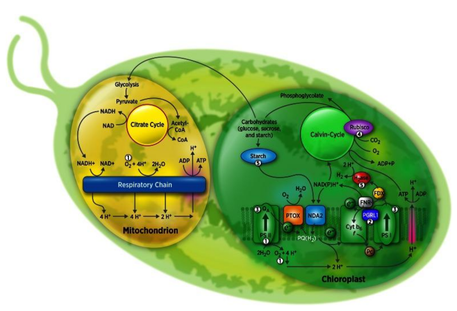
Capnophilic Lactic Fermentation (CLF), CNR-patented pathway (EP2948556B1) in hyperthermophilic anaerobic bacteria Thermotoga neapolitana, allow the conversion of carbohydrates into bio-H2 and L-lactic acid, recycling of CO2 by reductive carboxylation of acetate. The activity will concern bio-organic chemistry studies of the CLF to define the biochemical and cellular mechanisms underpinning bio-H2 production, including the biosynthetic pathways, the regulation of hydrogenase and other key enzymes of the process and the bioenergetic aspects.



The metabolic studies will be the basis for the development of the microbial platform for the technological exploitation of CLF for the production of hydrogen with high yields and the valorization of sugar-based biowastes.

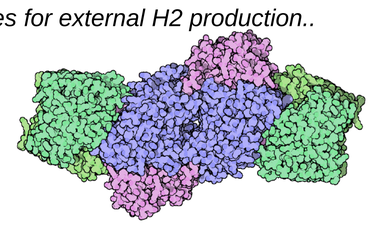
BF activity

Biophotolytic hydrogen production by microalgae and cianobacteria for energy purposes, attracts attention of researchers because it offers the highest theoretical light energy conversion efficiency. Unfortunately, hydrogenase enzymes are irreversibly inactivated when exposed to molecular oxygen, so optimization of the process is needed to fully exploit this technology. Various chemical and physical parameters, such as nutrients requirements, cellular redox potential as well as light regimes impact H_2 yield. Aim of the activity is to study and tailor nutrient requirements and growth conditions of the microalgal C. reinhardtii for H_2 production. Moreover, the growth of microalgae on waste water will be assessed to evaluate the indirect biophotolysis for H_2 production.



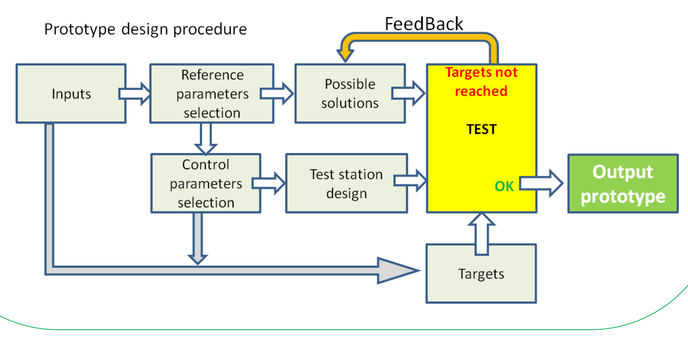
FF activity

The activity involves investigations on the biological production of green hydrogen(H_2) from biowaste using anoxygenic photosynthetic bacteria (purple and green) via photofermentation (FF) of organic compounds or through the production of photocurrents for H_2 production processes at the electrode. The activity will concern: selection of strains of photosynthetic anoxygenic bacteria, including facultative ones, hyperproducing H_2 from collections of microorganisms and polluted environments; standardization of H_2 production by optimizing pH, salinity, soil, heavy metal content, sulphur content, visible and NIR lighting; assembly of a small photobioreactor with previously selected microorganisms and in the most favourable growth conditions for the production of H_2; characterization of by-products and wastewater from the dairy, tomato and oil industries; laboratory tests of the photobioreactor in (1) direct H_2 production mode and in (2) electrochemical mode with electrodes that maximize the interaction with microorganisms towards anodes for external H2 production..



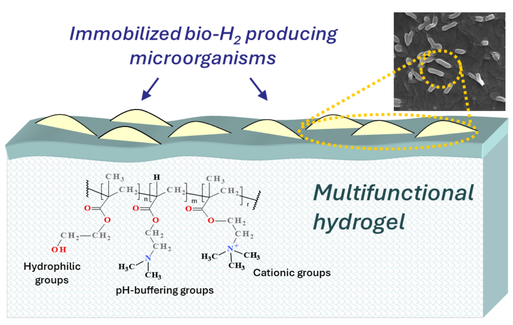
BRT activity

A prototype bio-reactor (BRP) for hyperthermophilic anaerobic bacteria will be developed for the production of bio-H2 in continuous flow. Looking at the large scale application an innovative approach compared to traditional systems will be used, this with the aim to minimize volumes and maximize the results obtained in the CLF activity, creating the conditions for technology transfer to companies. Furthermore, a rough evaluation on the adaptability of the basic concepts of the prototype to the case of photosynthetic microorganisms will be carried out. Guidelines for the development of the prototype will be the use of low cost and no critical raw materials, easy scalability, minimisation of the controls, high quality of products minimising post treatments.



MIC activity

Polymer matrices for cell immobilization (MIC) will be synthesized for the development of high-performance systems for the production of bio-H_2. In particular, hydrogels based on polysaccharides, hydrophilic proteins and acrylic with tailored interaction with microbial cells polymers will be prepared. The stability and possibility of recycling of the matrices for continuous production systems will also be evaluated. Functionalized and stimuli-responsive hybrid polymers will be developed to optimize culture conditions (adhesion, surface charge, pH-buffering capacity, selective adsorption of by-products) and bio-H_2 production, simplifying the management procedures of the bio-reactor.



Preliminary results

The activities developed in line with the project plan.

***CLF**: Screening of different species of the genus Thermotoga and Pseudothermotoga revealed that CLF pathway is only retained in Thermotoga neapolitana, T. neapolitana subsp. capnolactica (DSM33003, proprietary CNR strain) and a sister strain Thermotoga. sp. strain RQ7 (naturally competent and with complete genome sequence). Gene expression levels of key CLF genes and analysis of fermentation products provided important elements for the elucidation of metabolic pathways related to hydrogen and lactic acid production. Experiments were also carried out using ^{13}C-glucose-labelled precursors to trace carbon during fermentation and to study its relationship with hydrogen biosynthesis.*

***FF**: The growth conditions of red and green anoxygenic photosynthetic microorganisms were investigated on the basis of lighting conditions: intermittent natural and artificial light, including halogen lamps and some LEDs of different colors (blue, red, green). The results indicate that with the same electrical consumption, the best condition is that of three LEDs used simultaneously.*

***BF**: After optimizing the growth of the Chlamydomonas reinhardtii strains and the common Nostoc cyanobacterium, the analysis of their production capacity began. The activity therefore involved the development of gas chromatography methods for measuring the quantities of hydrogen produced and the possibility of simultaneously determining the quantity of oxygen consumed is being evaluated.*

***MIC**: some natural polymers, starch, pectin and albumin have been selected and used for the development of hydrogels with high porosity and thermal stability. The latter have been characterized in terms of swelling, hydrothermal stability at 80 °C and morphology, and will be used for cell immobilization and biohydrogen production tests. Furthermore, hydroxyacrylic-based hydrogels, with a cationic charge and controlled degree of swelling, were prepared and cross-linked.*

***BRP**: From the analysis of the international state of the art, including a selection of non-peer reviewed and informative technical articles, we have come to the conclusion that there are no bio-reactors specifically designed for the production of hydrogen and that, to have some references technical, it will be necessary to refer to bio-reactors developed for other purposes. Therefore, the prototype design and verification protocol has been defined. The main parameters to be controlled and those to be measured in the testing phase and the benchmarking values to evaluate the quality of the prototype have been defined .*

ACKNOWLEDGEMENT

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