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***Rhodobacter capsulatus* as a genetically engineered platform for converting waste into high-value compounds**

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Oral Presentations #9

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Rhodobacter capsulatus is a purple non-sulfur photosynthetic bacterium with remarkable potential for waste valorization owing to its metabolic versatility and its ability to produce hydrogen through nitrogenase activity. However, photofermentative H₂ production is constrained by physiological and regulatory mechanisms that limit both H₂ yields and the range of waste streams that can be exploited. Genetic engineering offers an effective strategy to overcome these limitations and develop strains adapted to specific residual feedstocks.

As a first approach, a $\Delta hupAB$ mutant was constructed by deleting the genes encoding the uptake hydrogenase, an enzyme responsible for consuming part of the H₂ produced by nitrogenase. Elimination of this competing pathway generated a H₂-overproducing strain capable of producing up to ten-fold more H₂ than the wild-type strain under diazotrophic conditions (Barahona et al., 2016).

The biotechnological potential of this engineered strain was evaluated using process water generated during the hydrothermal carbonization (HTC) of food waste (Díez et al., 2024). This effluent is characterized by a high organic load and represents an important environmental challenge. Photofermentation assays demonstrated that the $\Delta hupAB$ mutant efficiently utilized HTC process water as its primary carbon source while simultaneously producing H₂ and reducing the organic load of the effluent. Moreover, continuous operation for more than 600 h confirmed the long-term stability of the process and sustained H₂ production from this complex waste stream.

To further expand the applicability of this platform to ammonium-rich agro-industrial waste streams, a $\Delta hupAB \Delta draTG$ mutant is currently being developed. The DraT/DraG system controls the post-translational regulation of nitrogenase through reversible ADP-ribosylation of dinitrogenase reductase in response to ammonium availability (Masepohl et al., 2013). Disruption of this regulatory circuit is expected to maintain nitrogenase activity under ammonium-rich conditions, thereby extending photofermentative H₂ production to a broader range of agro-industrial effluents.

Overall, these results highlight the potential of *R. capsulatus* as a genetically engineered platform for waste valorization, where targeted modifications can be used to tailor cellular metabolism to diverse residual feedstocks and enhance sustainable biohydrogen production.

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