083. TOWARDS BIOHYDROGEN OVERPRODUCTION AND VALORIZATION OF FOOD WASTE BY GENETIC ENGINEERING OF *RHODOBACTER CAPSULATUS*.

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Introduction

More than 80% of the global energy demand is met by fossil fuels, mainly oil, gas, and coal, contributing to environmental concerns and global warming. In response, researchers are actively exploring sustainable energy sources. Hydrogen gas (H2) offers eco-friendly attributes and is a potential future energy carrier. Photofermentation by microorganisms encompasses a wide variety of microbial processes that combine the properties of oxygenic or anoxygenic photosynthesis with the activity of the enzymes responsible for producing H2: hydrogenases and nitrogenases. Purple non-sulfur bacteria (PNSB), such as Rhodobacter capsulatus, are the most common microorganisms that produce H2 by photofermentation via nitrogenase (Barahona et al. 2022). PNSBs have several advantages over other H2 bioproduction systems, such as high substrate conversion efficiency, using a wide variety of carbon sources, the possibility of operating at ambient conditions, reducing energy consumption, and the high purity of the hydrogen produced. Despite all these advantages, higher production rates and cheaper carbon feed sources are required to compete with conventional methods.

In this study, we genetically redesign R. capsulatus to enhance H2 production rates. Additionally, we are focused on optimizing and improving the overall process by valorizing food waste's material and energy content.

Materials and Methods

In the experiments focusing on H2 production by R.capsulatus, both the wild-type strain (WS) and a mutant in the uptake [NiFe] hydrogenase were assayed. The structural genes of the uptake hydrogenase (hupA and hupB) were removed to avoid the consumption of the H2 produced by the Mo-nitrogenase, generating the ΔhupAB mutant, according to Barahona et al. (2016).

The process water (PW) generated from the hydrothermal carbonization (HTC, 180 ºC, 1 h) of food waste (75 gCOD/L; 3.4% w/w carbohydrates) and a minimal RCV medium (30 mM DL- malate and 10 mM (NH4)2SO4) were used as a substrate in photofermentation process.

Experiments were conducted in continuously illuminated batch cultures with an N2-saturated atmosphere for 94 hours at 30 oC under the following conditions: RCV, RCV without nitrogen, RCV without malate supplemented with PW, PW with phosphate buffer saline at pH 6.8 (PBS), and PW

only. The vials were inoculated with anaerobically grown R. capsulatus cells to an initial OD600 of 0.02. The PW was added to an initial COD concentration of 2.88g/L and pH was adjusted to 6.8 when necessary.

Gas and liquid samples were taken daily from each vial to determine the gas composition (percentage of H2 and CO2), gas pressure, soluble COD, absorbance, nitrogen, and phosphate concentrations.

Results and discussion

Our results showed that R. capsulatus WS exhibits low rates of H2 accumulation under all analyzed conditions (Fig. 1A). As expected, the highest levels of H2 production (almost 110 mL of H2/ gCOD) were observed under the condition in which the hupAB mutant was growing in RCV medium without a nitrogen source (Fig 1B). In this condition, the Mo-nitrogenase is fully derepressed from the beginning of the experiment and catalyzes the reduction of N2 into NH3 in a reaction that also produces, at a minimum, one mol of H2 per mol of reduced N2. This hydrogen remains unconsumed due to the deletion in the uptake hydrogenase. We also observed H2 production under conditions where PW was used as a substrate. The H2 production within the initial 24-hour period was similar than observed in the RCV without malate condition however, hydrogen levels decreased by 90% compared to the maximum production (Fig. 1B). This drop could be attributed to a nitrogen source in the process water, resulting in reduced nitrogenase enzyme activity, as well as to a very rapid depletion of carbon sources that can be utilized by *R. capsulatus*.

After obtaining these results, our experiments focused on optimizing the photofermentation process through adaptation to a continuous system and by genetically reengineering the *hupAB* mutant to enhance nitrogenase activity with trace amounts of ammonia. Nevertheless, we demonstrate that *R. capsulatus* can produce green H2 from PW obtained from food waste.

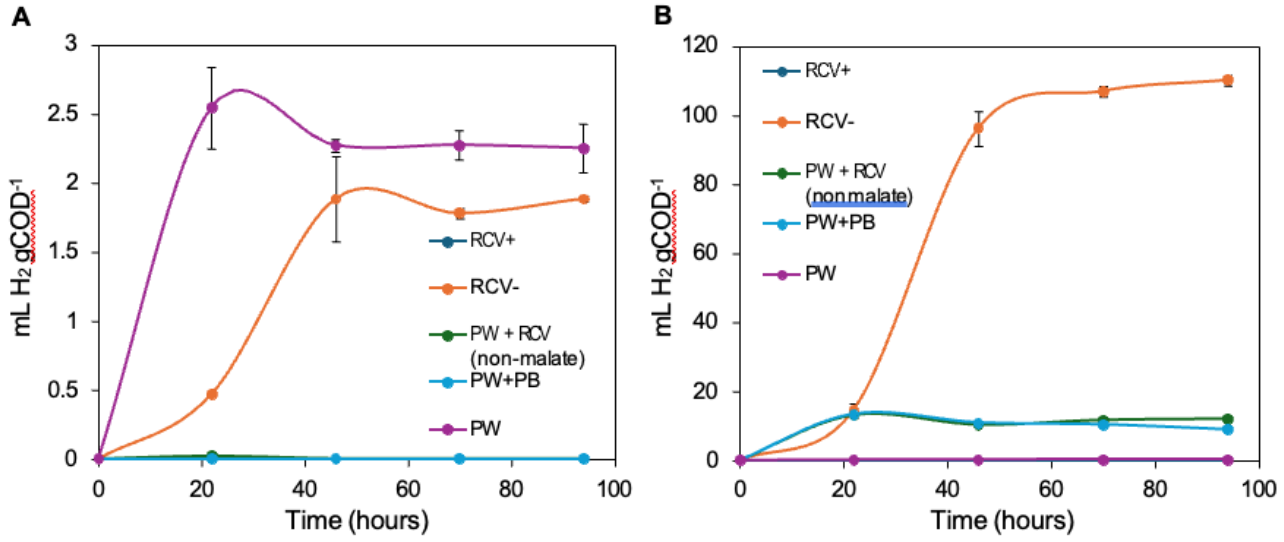

Fig. 1. Specific hydrogen production yield (mL H_2 $gCOD^{-1}$ added) via photofermentation by R. capsulatus WT (A) and hupAB mutant (B) in all conditions assayed: RCV (dark blue), RCV without nitrogen (brown), RCV without malate supplemented with PW (green), PW with PBS (blue), and PW only (purple). Error bars are 95% confidence intervals for the means from triplicate measurements.

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