**Photohydrogen production from cheese whey by recombinant strains of *Rhodobacter capsulatus***

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**Highlights**

* H2 production from cheese whey was modeled and optimized.
* Recombinant *R. capsulatus* IR3 strain containing the E. coli lacZ gene was used.
* Hydrogen production was obtained with cheese whey concentration of 90%.

**1. Introduction**

Hydrogen is considered as a clean energetic source [1]. Biological methods are a strong option to produce it because less energy than conventional methods is required, and waste materials or byproducts of the industry can be use as substrate. However, the efficiency of biological methods is low so many researches focus on increase it by optimized conditions and media [2]. Among the industrial effluents that can be use as substrate, cheese whey (CW) was selected due to it’s composition [3]. The photofermentation process stands out among the biological methodologies for its rates of substrate conversion. Photosynthetic non-sulfur bacteria catalyze the hydrogen production by the action of the nitrogenase [4,5]. In this study, a photofermentative process was used to produce hydrogen from CW with a recombinant strain of *Rhodobacter capsulatus*. The recombination allows the degradation of the lactose by the action of a plasmid containing β galactosidase gene under dependence of nifH promoter [6].

**2. Methods**

2.1 Bacterial strain and cheese whey

The bacteria used in this study was *Rhodobacter capsulatus* strain IR3::LacZ originated from a H2 superproducer strain IR3 [7]. Bacterial pre-culture medium was RCV, under anaerobic conditions. The cheese whey powder used was bought to CIMPA s.a.s a Colombian company. The CWP was solubilized in deionized water (1 kg CWP / 9 L water) and heat-pretreated.

2.2 Batch experiments

Reactors used for the batch experiments were 0.125 L glass square bottles, sealed hermetically with a rubber stopper and with a magnetic stirrer for mixing. The cultures were carried out in an illuminated incubator (lab-made) with temperature regulation at 30°C. Illumination was provided by a high-pressure Na lamp. Biogas produced volume was measured by water displacement.

2.3 Experimental Design

A Central composite face centered design of experiments was applied using the statistics software Design Expert 8.0 to evaluate the effects of 3 variables: **A.** Cheese whey concentration (%), **B.** Molybdenum concentration (µM), **C.** Light intensity (lx). The response studied was the H2 specific volumetric production (ml L-1).

**3. Results and discussion**

A quadratic model was the best fit for the relation between the three continuous parameters and the response (equation 1). The 3 variables studied were significant.

**Y (ml L-1) =** 5668 + 3118 A + 1265 B-867\*C+1227 B\*C - 2036 A2 (Eq1)

The model was statistically validated, and the 3D surface response and the cubic plot were used to describe the behavior of the study’s area. Maximum values of H2 production were observed at superior levels of CW concentration. This is a desirable result because the higher concentration of CW, the lower work volume and that increases the productivity of the process. The equation and plots indicate that the variable that influences the most the H2 production is the CW concentration. The interaction of Mo concentration and light intensity is also significant to the process.



**Figure 1** 3D and cubic representations of H2 specific volumetric production

**4. Conclusions**

The influence of the variables CW and molybdenum concentration and light intensity over the H2 production by *Rhodobacter capsulatus* IR3::LacZ was successfully determined and optimized with DOE methodology. The study led to improvements in the efficiency and productivity of the process. This methodology proves to be useful both for producing hydrogen and as a treatment for cheese whey. The consumption on lactose, after the bacterial action, can reach 98% reducing the environmental impact of this waste stream.

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