EVALUATION OF THE POTENTIAL FOR HYDROGEN PRODUCTION OF A PHOTO-TROPHIC MIXED CULTURE USING ORGANIC ACIDS AS CARBON SOURCE

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Introduction. Biological hydrogen (H_2) production has been mainly developed via anaerobic digestion (AD). However the main drawback of this process is that besides H_2, some organic acids are obtained as final products. Those can be used as feedstock for other processes [1].
Phototrophic bacteria of the *Rhodobacter* and *Rhodopseudomonas* genus have been identified as potential H_2 producers since they have the metabolic machinery for its production form organic acids. However H_2 is produced by a complex enzymatic system which is not yet fully understood. Additionally, the use of mixed phototrophic cultures for the hydrogen production has the potential to increase the yield when the final products of AD are used as substrate [2].
The aim of this project was to evaluate H_2 production by mixed phototrophic cultures when feeding with organic acids.

Methods. Three mixed cultures taken from a Winogradsky column were tested to evaluate their potential for H_2 production. First each culture was activated in a complex media using acetate as carbon source [1]. Then a modified RCVB media was used for massive biomass production. A nitrogen-limited media was used for hydrogen production. All cultures were performed in serological bottles and grown at 30°C, initial pH 6.8 and 150 rpm. To obtain anaerobic conditions the systems were initially flushed with Ar. Acetate and butyrate were tested as only carbon sources (2 g/L). Biomass was measured by OD (660 nm) and adjusted to dry weight with a standard curve previously done. H_2 content in the gas phase was measured by TCD chromatography. Concentration of acetate was determined with HPLC system.

Results. After the activation process, all cultures showed the capability for growing on both acetate and butyrate. For all cultures (C2, C4 and C5) an increase in biomass concentration was observed (Table 1) when media contained NH4+. However, no H_2 was determined due to the presence of $NH4^+$, which has been reported to inhibit this process. When the cultures were grown in free-nitrogen medium, hydrogen production was observed and biomass production decreased. These results are summarized in Table 1.

Table 1. Biomass and H_2 production yields

Medium	C2		C4		C5	
	Yxs, g_x/g_s	Y_{H2}, mL/g_s	Yxs, g_x/g_s	Y_{H2}, mL/g_s	Yxs, g_x/g_s	Y_{H2}, mL/g_s
Non-limited	0.69	0.00	0.75	0.00	0.33	0.00
Nitrogen-limited	0.54	0.25	0.75	0.45	0.63	0.46
Nitrogen-free	0.20	**15.38**	0.03	10.25	0.18	2.87

The H_2 and biomass production profile obtained with C2 is shown in Figure 2. This culture reached the highest hydrogen concentration (75%) compared with those obtained with the other cultures. H_2 production was associated to growth. The maximum production was obtained between 300 and 455 h of culture.

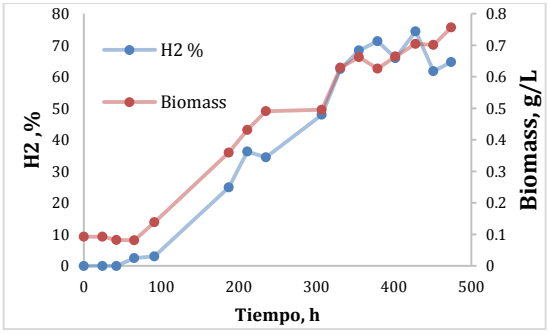

Fig.2 Hydrogen production and biomass growth

Conclusions. All phototrophic cultures tested were able to grown on acetate and butyrate. When growing on acetate and free-nitrogen media, culture C2 showed the highest H_2 production.

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