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Abstract
Single cell proteins (SCP) have emerged as an alternative protein source to partially alleviate the current problem of food scarcity. However, there is a need for the production of SCP from renewable and pathogen-free sources, such as gaseous streams. This study evaluated, for the first time, the use of enriched phototrophic purple bacteria (PPB) consortia for the production of SCP using H_2 and CO_2. The influence of pH (6-8.5), temperature (15-50 ºC) and light intensity (0-50 W m^{-2}) on the growth kinetics and biomass yields was investigated. Optimal conditions were found at a pH of 7, temperature of 25 ºC and light intensities over 30 $W \cdot m^{-2}$. High biomass yields (~ 1 g $COD_{biomass} \cdot g^{-1}$ $COD_{consumed}$) and protein yields of ~4 g protein$\cdot g^{-1}$ H_2 were achieved, regardless of the environmental conditions. The biomass had high protein contents (51–64 %w/w), potentially suitable as SCP. The biomass yields are amongst the highest ones reported from gaseous streams.

Keywords
Autotrophic growth; Environmental conditions; Hydrogen; Phototrophic Purple Bacteria; Single Cell Protein

INTRODUCTION

The sustainable production of food and feed is nowadays a serious global concern. Ever-growing population, climate change and limited natural resources call for the development of new sustainable food/feed alternatives (Zha et al., 2021). Proteins play a key role in human and animal diet as a source of nitrogen and essential amino acids (Ritala et al., 2017). The use of microorganisms as a protein-rich feedstock, the so-called single cell protein (SCP), is a promising alternative to plant or animal-based proteins, since microorganisms provide higher nitrogen recovery during protein synthesis (Puyol et al., 2017). The production of SCP has many environmental benefits compared to plant/animal proteins, such as lower land requirements and GHGs emissions, or a reduced water footprint (Alloul et al., 2022). Despite these advantages, SCP is currently produced from costly agricultural feedstocks or from unsustainable raw materials such as molasses, sucrose, starch, n-alkanes, methanol or natural gas.

Phototrophic purple bacteria (PPB) grown on pathogen-free sources are a potential SCP source, thanks to their high yields and their high contents on amino acids, pigments and vitamins. PPB exhibit a highly versatile metabolism, capable of performing anoxygenic photosynthesis, using solar light as energy source, and a wide range of electron/carbon donors such as organic compounds (photoheterotrophic growth), or H_2/H_2S with CO_2 as carbon source (litoautotrophic and photoautotrophic growth) (Capson-Tojo et al., 2020). H_2 from fermentation of organic waste, syngas, or generated via water electrolysis using surplus of electricity from renewable sources, and CO_2 from off-gases, represent promising electron and carbon sources for the sustainable and pathogen-free production of SCP. Spanoghe et al. (2021) proved, for the first time, the feasibility of the production of SCP from H_2 using pure PPB cultures. However, to the best of our knowledge, the potential of an enriched PPB community (without axenic conditions) growing photoautotrophically with H_2 as SCP source remains unexplored.

Here, the use of an enriched PPB consortia for the bioconversion of H_2 into SCP has been tested for the first time. The influence of environmental conditions (temperature, pH, light intensity) on microbial growth kinetics, protein contents, and microbial population, has been evaluated.

MATERIALS AND METHODS

An enriched PPB community grown photoheterotrophically from continuous photobioreactors used for wastewater treatment in Madrid (Spain) was used as a pre-inoculum. A series of batch enrichments (over 14) in Schott flasks of 500 mL were performed to obtain a PPB consortium able to achieve an efficient photoautotrophic growth. During each enrichment, aliquots of 20 mL from the previous culture were added to the Schott flasks, together with 180 mL of fresh medium. The medium was based on the mineral synthetic medium (MSM) proposed by Ormerod et al. (1961), modified to ensure photoautotrophic growth. The bottles were closed and the headspace was flushed with N_2 prior to H_2. The initial headspace pressure was adjusted to 1.3 bar. The flasks were covered with UV/VIS filters (Capson-Tojo et al., 2021) and incubated under continuous illumination at ~50 $W \cdot m^{-2}$ using infrared LED lights (850 nm). The flasks pressure was monitored daily. Once the pressure dropped below 1.0 bar, hydrogen was added. Each enrichment lasted for around one week.

Once the enrichment was stable and showed a constant performance, batch tests at different environmental conditions were tested. Assays at initial pH values of 6, 7 and 8.5, temperatures of 15, 25, 38 and 50 °C, and infrared light intensities of 0, 5, 15, 30 and 50 $W \cdot m^{-2}$ were carried out in triplicate, as described above for the enrichments (Figure 1). The gas composition and pressure were monitored 4-5 times per day. These data were used to calculate H_2 consumption rates. Liquid samples were also drawn at the beginning and at the end of each assay in order to determine biomass yields and productivities, crude protein contents, amino acids profiles and microbial communities.

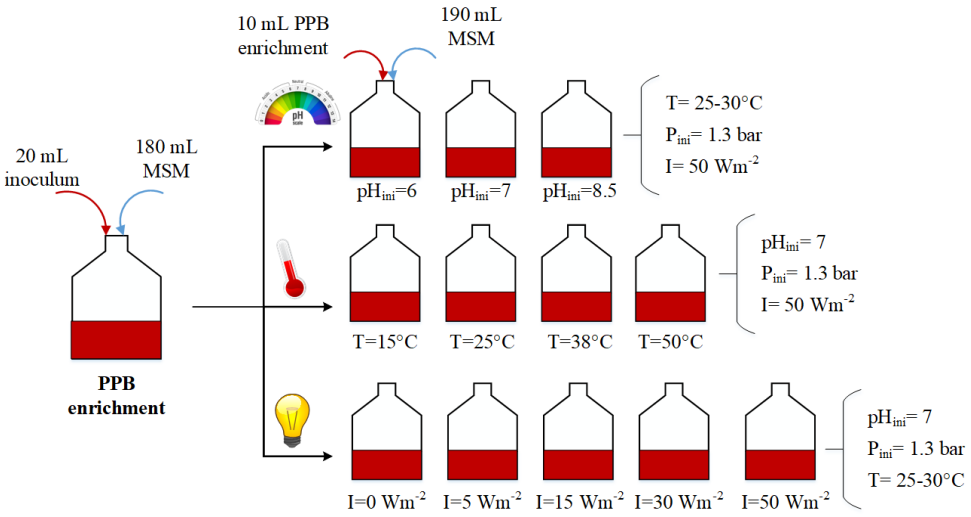

Figure 1. Simplified experimental procedure followed for the evaluation of the influence of environmental conditions on the bioconversion of H_2 into SCP using an enriched PPB culture.

RESULTS

After 2-3 enrichments, H_2 consumption was observed, confirming the ability of the mixed PPB culture for growing photoautotrophically. Population analyses (16S r-RNA sequencing) of two enrichment samples after seven cycles confirmed that the consortium was dominated by PPB (> 80%), with *Rhodobacter* sp. and *Rhodopseudomonas* sp. as dominant genera. Consistent biomass yields of 0.9-1.0 g $COD_{biomass} \cdot g^{-1}$ $COD_{consumed}$ were obtained during the last six enrichments.

Environmental conditions had a noticeable impact on H_2 consumption, impacting biomass growth, kinetics, and protein production (Figure 2, Table 1). The highest H_2 consumption was observed at initial pH of 7, (with a final value of 8.4 due to CO_2 consumption). The most unfavourable initial pH was 8.5. The highest H_2 consumption was observed at 25ºC, with 38ºC decreasing the growth kinetics and 50ºC inhibiting growth. Temperatures down to 15ºC also reduced H_2 consumption. Light limitation was observed at intensities lower than 30 $W \cdot m^{-2}$ but similar results were obtained for both 30 and 50 $W \cdot m^{-2}$. Biomass yields close to 1 g $COD_{biomass} \cdot g^{-1}$ $COD_{consumed}$ and protein yields over 4 g protein$\cdot g^{-1}$ H_2 were achieved in almost all the conditions tested (Table 1). These proteins yields were higher than those obtained using pure cultures (Spanoghe et al., 2021). High protein contents (> 50% w/w) were achieved, in agreement with PPB grown photoheterotrophically (Hülsen et al., 2022a, 2022b). Similar C and N contents in the biomass (42 and 9 %w/w) were obtained (Table 1).

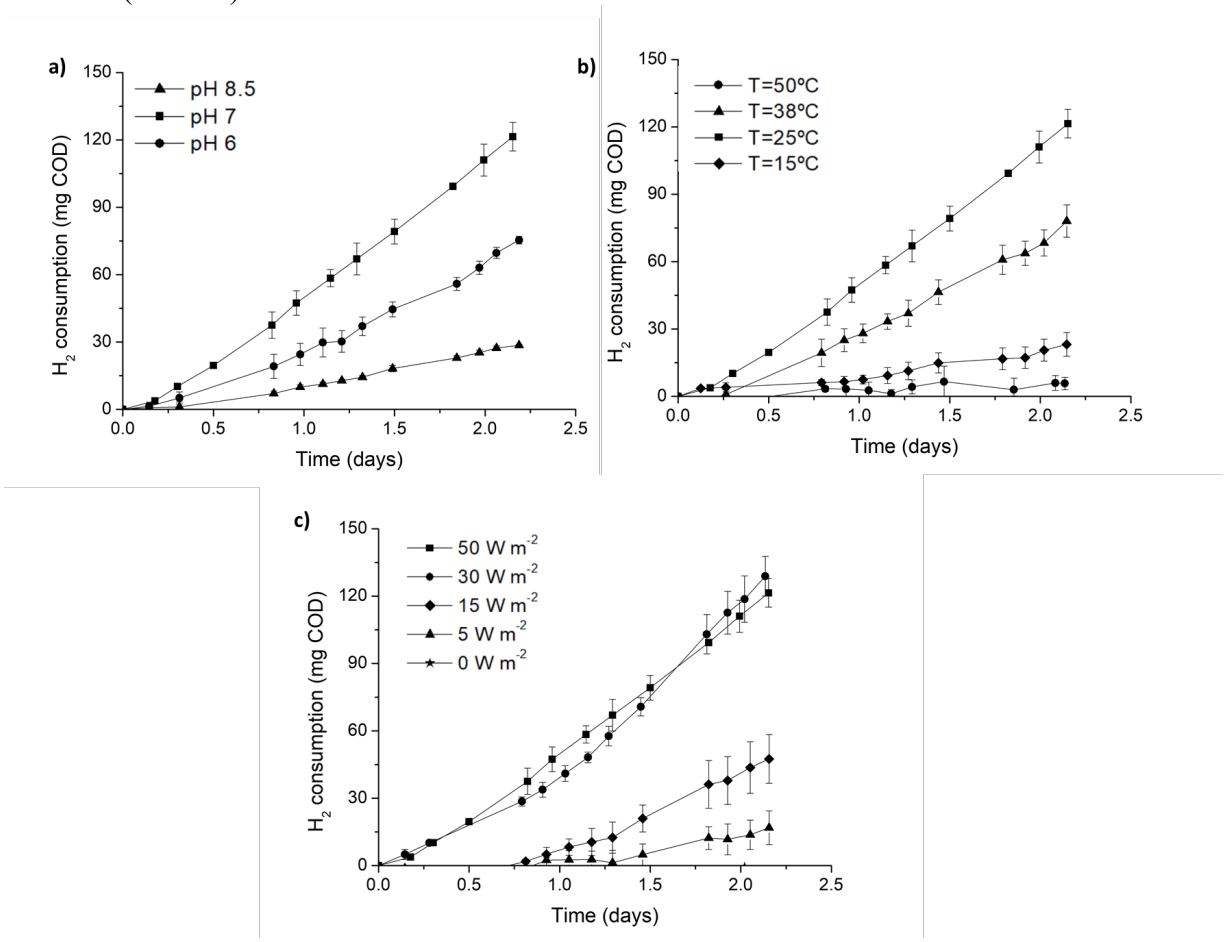

Figure 2. Time course of H_2 consumption by the enriched PPB culture at a) different pH values, b) different temperatures and c) different light intensities. Each data point shows the average and standard deviation (n=3).

Table 1. Main results obtained along with the corresponding standard deviation (n=3) during the batch tests.

	pH		**Temperature (°C)**		**Intensity ($W \cdot m^{-2}$)**	
	6	**8.5**	**15**	**38**	**30**	**50***
Biomass yield ($g\ COD_{biomass} \cdot g^{-1}\ COD_{consumed}$)	1.00±0.07	0.93±0.01	0.85±0.07	0.99±0.07	1.05±0.01	1.04±0.02

Biomass production rate ($mgCOD \cdot L^{-1} \cdot d^{-1}$)	202±37	77±26	56±44	182±59	378±72	351±60
Protein content of biomass (%w/w)	-	56±5	54±2	51±3	64±2	51±2
Protein yield (g protein$\cdot g^{-1}$ H_2)	-	3.9±0.3	4.4±0.6	4.3±0.5	4.9±0.8	4.2±0.3
C content of biomass (%w/w)	42±1	41±2	41±1	41±1	43±3	41±2
N content of biomass (%w/w)	8±1	10±0	8±0	9±0	10±1	10±0

*These values also corresponded to the pH 7 and temperature 25°C tests.

CONCLUSIONS

This study demonstrated, that PPB consortia can be efficiently used for production of SCP from H_2. Neutral pH, temperatures of ~25ºC and light intensities higher than 30 $W \cdot m^{-2}$ are the best conditions for biomass growth. High biomass yields and protein contents were achieved. The high yields and rates achieved confirm the great potential of enriched PPB cultures for H_2 valorisation.

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