CHEMICAL PERMEABILIZATION OF *RALSTONIA EUTROPHA* H16 CELLS

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Abstract: With the aim of replacing the combustion of fossil fuels with more environmentally friendly processes, interest in the production of hydrogen through biotechnological processes has increased. Since the efficiency of biohydrogen production depends on the metabolic pathways, the selection of a suitable production microorganism is crucial. *Ralstonia eutropha* H16 is a bacterial strain that produces four hydrogenases, promising biocatalysts for hydrogen production. Conventional methods for obtaining intracellular products are often based on cell disruption processes (mechanical or non-mechanical). As an alternative, cell permeabilization methods offer a promising approach as they facilitate the selective release of intracellular products while preserving cellular integrity to a greater extent. In this study, different permeabilizing agents were used to enhance the extraction of enzymes from *R. eutropha* H16 cells. Agents such as EDTA, Triton X-100, SDS, CTAB, PEG and BSA were evaluated for their effectiveness in increasing the permeability of the cell membrane, thereby improving the efficiency of enzyme extraction.

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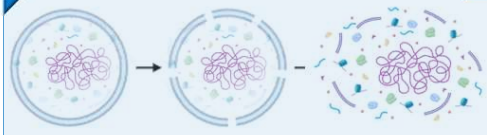
Project: Integrated micro-system for enzymatic biohydrogen production
Acronym: MicroBioH$_2$

Experimental set-up:

Heterotrophic cultivation of *R. eutropha* H16 cells

The heterotrophic cultivation of *R. eutropha* H16 was carried out on four different media that differed in the initial concentration of fructose and glycerol. The cultivation was monitored for 174 hours.

Cell wall permeabilization



The effectiveness of six permeabilizing agents (EDTA, Triton X-100, SDS, CTAB, PEG and BSA) was evaluated in independent batch experiments.

Data analysis

In each sample, the protein concentration and enzyme activity were measured in order to evaluated effectiveness of agents in increasing the permeability of the cell membrane.

Results:

The change in biomass concentration during cultivation is presented in Figure 1a. **Experiment 3**, in which glycerol (γ = 4 g/L) was added, showed the highest **biomass yield of 0.470 ± 0.134 g/L**.

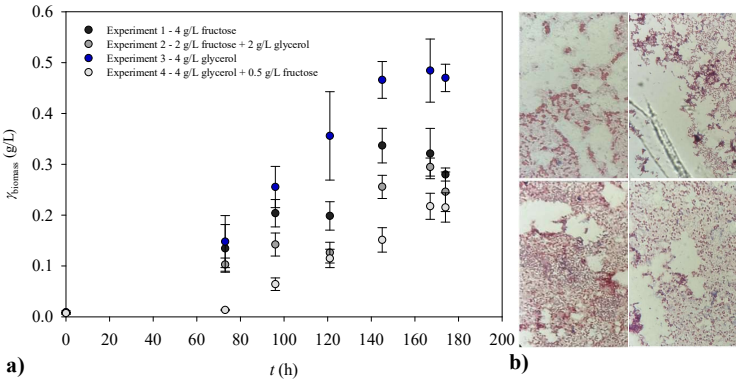


Figure 1. a) Change in biomass concentration during cultivation of *R. eutropha* H16 bacteria in four different media; b) Microscopic images of the cells after Gram staining under 1000x magnification

After successful cultivation, the cells were Gram stained (Figure 1b). It can be seen that all samples are stained red, indicating that they are **Gram-negative rod-shaped bacteria** and that the cells do not differ according to the medium in which they were grown, i.e. whether they used fructose, glycerol or both as substrate. It was also observed that the **bacteria live together in groups and probably form a biofilm**. To confirm biofilm formation, biomass samples were imaged using a scanning electron microscope (Figure 2).

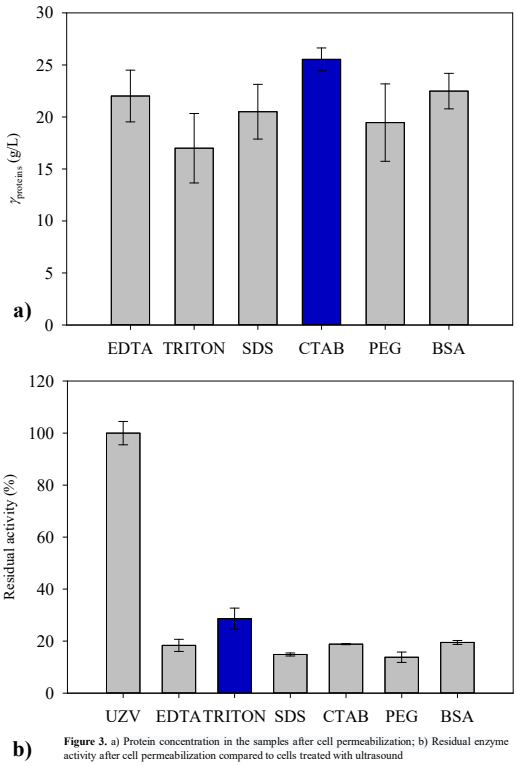


Figure 3. a) Protein concentration in the samples after cell permeabilization; b) Residual enzyme activity after cell permeabilization compared to cells treated with ultrasound

Of the agents tested, **the highest protein concentration was released with CTAB (25.536 ± 1.102 mg/mL)**, and the lowest concentration of 16.999 ± 3.338 mg/mL with Triton X-100 (Figure 3a).

The highest enzyme activity was measured in the sample that was permeabilized with **Triton X-100** (Figure 3b), which can be explained by the fact that each agent breaks down the cell wall differently, resulting in the release of higher or lower concentrations of proteins.

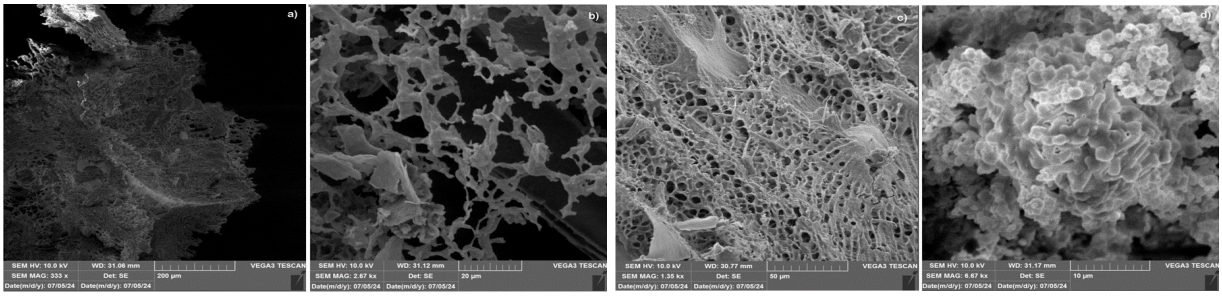


Figure 2. Photographs of samples from Experiment 3 on a scanning electron microscope at different magnifications a) 333x; b) 2670x; c) 1350x; d) 6670x

Conclusion:

R. eutropha H16 cells can be successfully permeabilized with CTAB, BSA, EDTA, SDS, PEG and Triton X-100 but further optimization is necessary due to the low enzyme activity.