OPTIMIZING *RALSTONIA EUTROPHA* H16 GROWTH AND ULTRASONIC CELL DISRUPTION

Anita Šalić[1], Ana Jurinjak Tušek[2], Sara Anđelović[1], Željka Ćurić[1], Bruno Zelić[1,3]

[1]University of Zagreb, Faculty of Chemical Engineering and Technology, Marulićev trg 19, HR-10000 Zagreb, Croatia
[2]University of Zagreb, Faculty of Food Technology and Biotechnology, Pierottijeva 6, HR-10000 Zagreb, Croatia
[3]University North, Trg dr. Žarka Dolinara 1, HR-48000 Koprivnica, Croatia

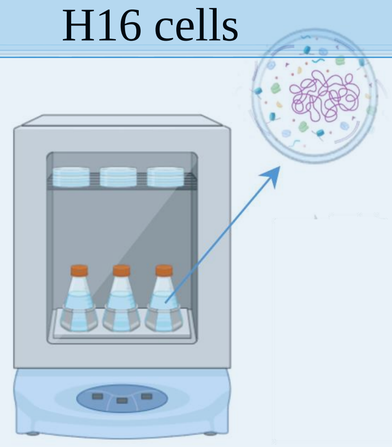
međunarodni znanstveno-stručni skup
Ružičkini dani
DANAS ZNANOST – SUTRA INDUSTRIJA
18. – 20. rujna 2024. I Vukovar, Hrvatska

Abstract: The growing global demand for clean and renewable energy sources has increased interest in biohydrogen, a versatile and environmentally friendly fuel that has the potential to significantly reduce dependence on fossil fuels. Due to its high energy content and the fact that its use produces no carbon emissions, biohydrogen is a promising alternative that is crucial for the transition to sustainable energy systems. *Ralstonia eutropha* H16 is a crucial microorganism for biohydrogen production as it can produce the enzyme hydrogenase, which plays an important role in hydrogen metabolism. The aim of this research was to increase biomass accumulation and maximize hydrogenase production by optimizing initial substrate and biomass concentrations. In addition, the study investigated the efficiency of cell disruption with ultrasound, a crucial step in the release of intracellular hydrogenase enzymes, by optimizing sonication parameters such as time, frequency and cell concentration. By refining both microbial growth and cell disruption, this research represents an effective strategy to increase hydrogenase yields, contributing to the development of biohydrogen as a viable and sustainable energy source on an industrial scale.

Project: IP-2022-10-2175
Source of funding: The Croatian Science Foundation
Project leader: Prof. Bruno Zelić, PhD
Project: Integrated micro-system for enzymatic biohydrogen production
Acronym: MicroBioH_2

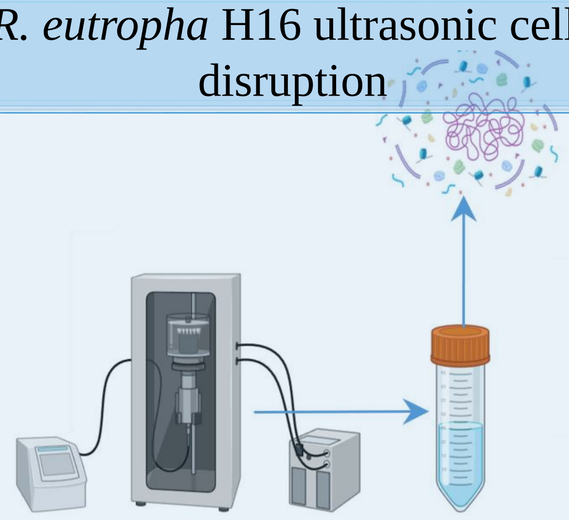
Experimental set-up:

Heterotrophic cultivation of *R. eutropha* H16 cells



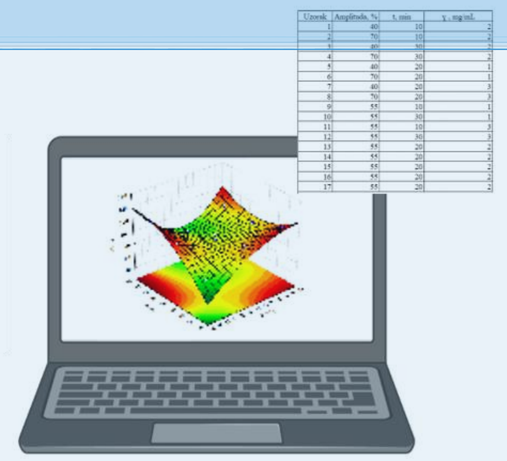
Heterotrophic cultivation of *R. eutropha* H16 was carried out (174 h) on four different media with different concentrations of fructose and glycerol.

R. eutropha H16 ultrasonic cells disruption



Ultrasonic cell disruption was performed using the Box-Behnken experimental design at three levels (-1, 0, 1).

Process optimization



Estimation of the parameters of the mathematical model of the cultivation process and optimization of ultrasonic cell disruption.

Results:

R. eutropha H16 was first cultivated on two different media, one containing γ = 4 g/L fructose and the second containing γ = 4 g/L glycerol. Based on the change in the concentrations of biomass and substrate, the parameters of the mathematical model of the cultivation process were estimated from the experimental results by non-linear regression. The results are shown in Figure 1.

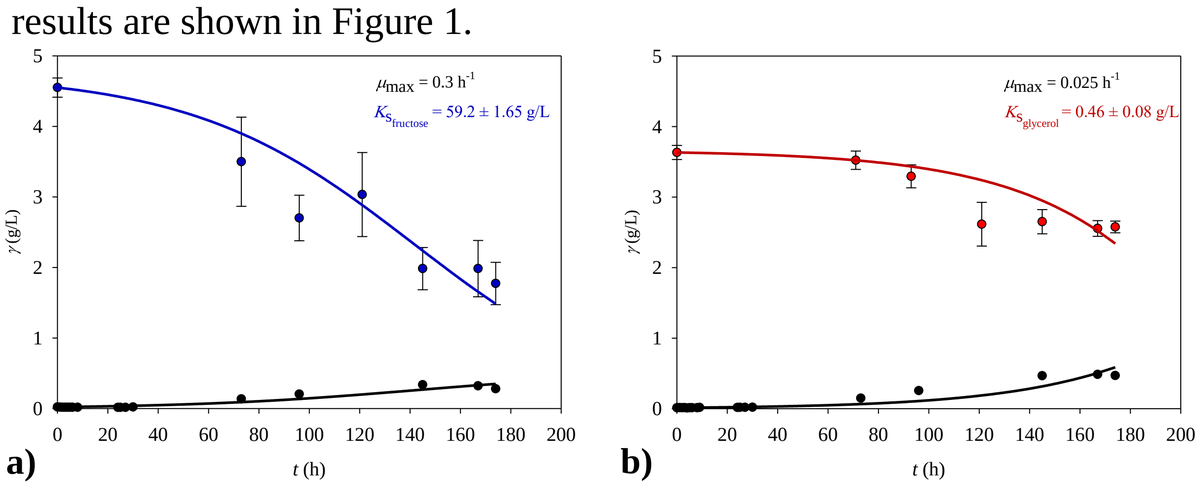


Figure 1. Dynamic change in biomass concentration (●) in media containing a) fructose (●) and b) glycerol (●) (● experimental results, ▬ mathematical model)

In the next step, the mathematical model for the batch reactor was proposed and the estimated parameters were validated using two independent experiments (Figure 2).

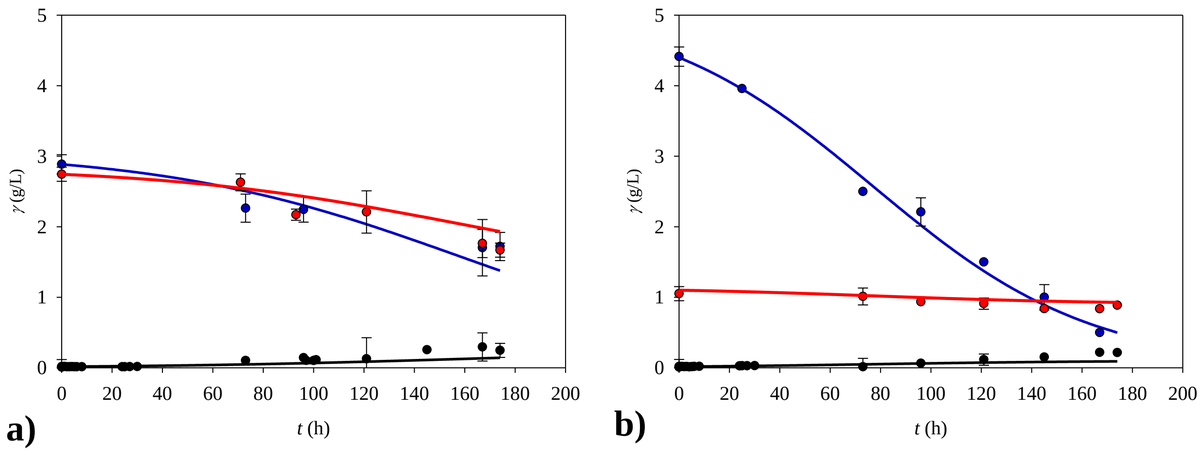


Figure 2. Dynamic change in the concentrations of biomass (●), fructose (●) and glycerol (●) in two independent experiments with different initial concentrations of the substrates a) 2.7 g/L fructose and glycerol and b) 4.5 g/L fructose and 1 g/L glycerol

The proposed and validated model was used for further process optimization.

The optimization of ultrasonic cell disruption was performed using the Box-Behnken experimental design at three levels (-1, 0, 1) to improve the release of intracellular bioproducts while minimizing disruption of the cell integrity.

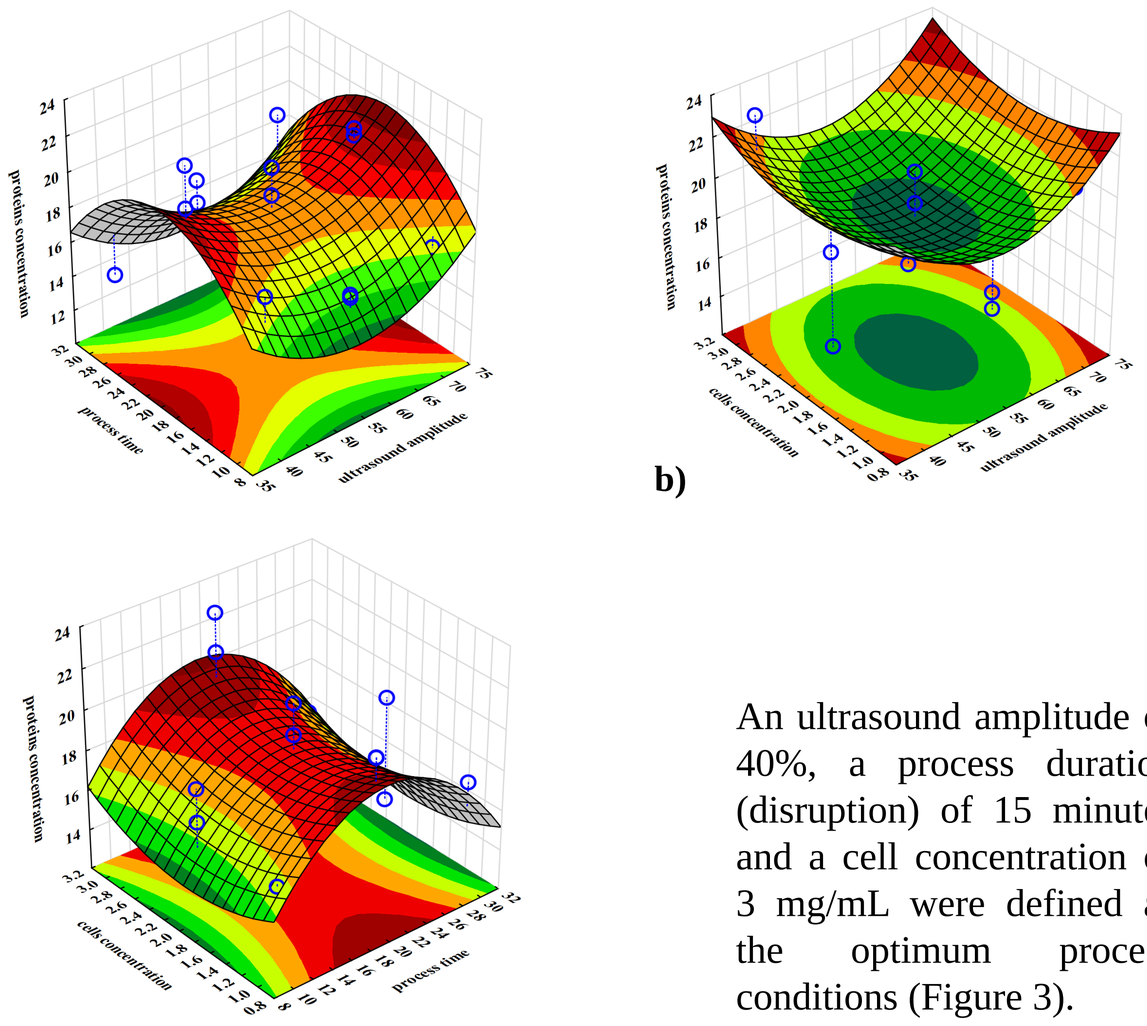


Figure 3. 3D representation of the optimal conditions for the protein extraction process (ultrasound amplitude, process time and cell concentration) - a) process time and ultrasound amplitude, b) cell concentration and ultrasound amplitude and c) cell concentration and process time

An ultrasound amplitude of 40%, a process duration (disruption) of 15 minutes and a cell concentration of 3 mg/mL were defined as the optimum process conditions (Figure 3).

Under optimum process conditions, a protein content of 68 % of the total dry cell weight was achieved.

Conclusion:

In this study, the heterotrophic cultivation of *R. eutropha* H16 on synthetic media was successfully optimized, focusing on the use of fructose and glycerol as substrates. The Box-Behnken experimental design facilitated the optimization of cell disruption with ultrasound. Overall, this study provides a robust framework for improving biohydrogen production with *R. eutropha* H16 and provides valuable insights to optimize both growth and cell disruption for sustainable energy production.