INFLUENCE OF MEDIUM BUFFERS ON GROWTH OF THE CYANOBACTERIUM *ANABAENA VARIABILIS* AVM13

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OBJECTIVES

✓ In attempts to optimize culturing conditions, the interference of various buffers used during growth of *Anabaena variabilis* (AVM13 – *hup*- mutant strain[1]) on H_2 photoproduction has been studied. The present work describes the influence of four different buffers upon growth of the *A. variabilis*: Glycylglycine - Gly (5 mM), (N-[2-Hydroxyethyl]piperazine-N´-[2-ethanesulfonic acid] - HEPES (10 mM), N-tris[Hydroxymethyl]methyl-2-aminoethane-sulfonic acid - TES (10 mM) and Tris(hydroxymethyl)aminomethane hydrochloride - Tris-HCl (6.35 mM), as compared with no buffer at all (Control - Ctrl).

✓ The buffer that presented the best performance in terms of growth rate in the exponential phase (obtained under appropriate growth conditions) and pH stability, was further tested at several concentrations, with the goal of reducing processing costs and avoid possible interferences on *A. variabilis* growth.

INTRODUCTION

World wite attention has increasingly focussed on alternatives to fossil fuels – hydrogen is one such potential alternative for large scale production of power in the future. Cyanobacteria, especially of the N_2-fixing type, such as *A. variabilis*, are highly promising candidates for development of photobiological H_2-producing systems, owing to their capacity to generate H_2 under autotrophic conditions using sunlight as energy source [2].

In order to achieve significant H_2 production rates over long time periods, the strains used should be selected according to their specific hydrogen metabolism but the overall cultivation conditions in a photobioreactor are also to be improved. Together with nitrogen source and temperature, a crucial processing parameter is pH, which may be stabilized via addition of a buffer to the medium. Therefore, the aim of this work was to optimize buffer type and concentration, in order to achieve high pH stability at low cost.

MATERIAL AND METHODS

Culture conditions

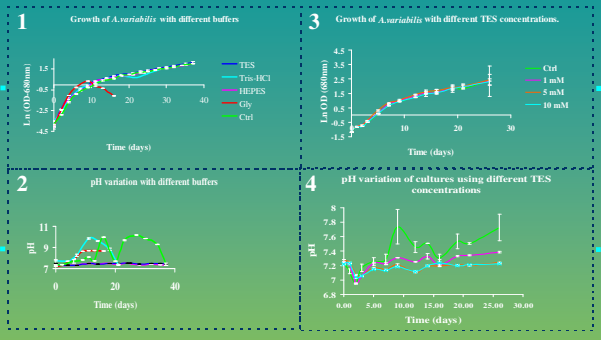
- *Anabaena variabilis* strain AVM13 (Thomas Happe-Ruhr-Universität-Bochum),
- Cultivation medium: BG11o (Stanier et al. 1971)[3]
- 800 mL cultures performed in 1L flasks.
- All cultivation parameters duly controlled:
  - ✓ Temperature: 23 ± 0.5ºC
  - ✓ pH ≈ 7.10 (of the culture medium, in the beginning of each experiment)
  - ✓ Air-flow: 50 L/h
  - ✓ Light intensity : 3 $\mu mols^{-1}m^{-2}$

Overall aspect of culture setup

Experimental Procedures

RESULTS AND DISCUSSION

- The *Anabaena variabilis*-AVM13 culture in Tris-HCl buffer exhibited an irregular growth rate.
- Glycylglycine buffer permitted the highest initial growth rate, but within a few days a dramatic loss of viability took place.
- Ctrl, HEPES and TES buffers showed similar growth rates throughout time, but the best performance concerning high growth rate in the exponential phase was obtained using TES.

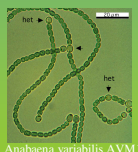


- The growth rate of the cyanobacterium was not affected by TES concentration.

- The TES concentrations presenting the higher buffering capacity were 5 and 10 mM.
- No significantly differences between 5 and 10 mM of TES were observed, so the former was selected for further studies.

- Both HEPES and TES presented the highest pH stability (buffering capacity).

TES, which presented the best performance concerning growth rate in the exponential phase and necessary buffering capacity, was selected for subsequent experiments at several concentrations



Anabaena variabilis AVM13

CONCLUSIONS

✓ **TES** has proven an efficient buffer for growth of *A. variabilis*.

✓ No effects on cell viability in cultures buffered with different concentrations of TES were observed.

✓ A concentration of **5 mM** was enough to maintain pH stable during 26 d.

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