



## 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<sup>1</sup>) on H<sub>2</sub> 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 wide 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<sub>2</sub>-fixing type, such as *A. variabilis*, are highly promising candidates for development of photobiological H<sub>2</sub>-producing systems, owing to their capacity to generate H<sub>2</sub> under autotrophic conditions using sunlight as energy source<sup>2</sup>.

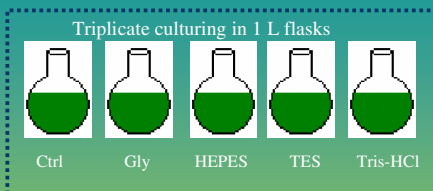
In order to achieve significant H<sub>2</sub> production rates over long time periods, the strains used should be selected according to their specific hydrogen metabolism but the overall cultivation conditions in a photobioreactor are also to be improved. Together with nitrogen source and temperature, 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)<sup>3</sup>
- 800 mL cultures performed in 1L flasks.

- All cultivation parameters duly controlled:
  - ✓ Temperature: 23 ± 0.5°C
  - ✓ pH ≈ 7.10 (of the culture medium, in the beginning of each experiment)
  - ✓ Air-flow: 50 L/h
  - ✓ Light intensity : 3 μmol·m<sup>-2</sup>



## Experimental Procedures

Measurement of:  
1. OD<sub>680 nm</sub>  
2. pH

Statistical analyses

Selection of high-growth rate and pH stability

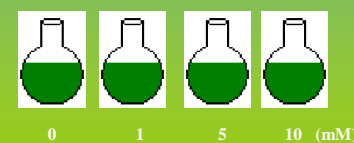


Overall aspect of culture setup

Statistical analyses

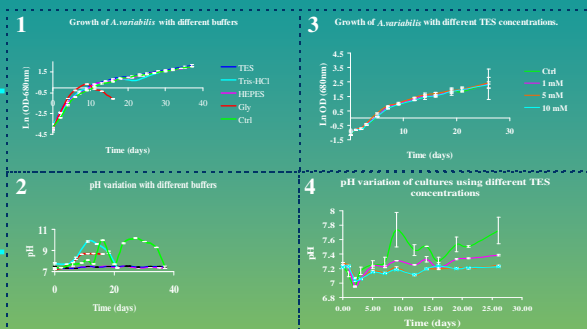
Measurement of:  
3. OD<sub>680 nm</sub>  
4. pH

Triplicate TES culture (in 1 L flasks), tested at different concentrations



## RESULTS AND DISCUSSION

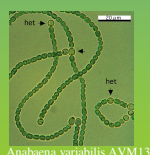
- The *Anabaena variabilis*-AVM13 culture in Tris-HCl buffer exhibited an irregular growth rate.
- Glycylglycine buffer permitted the highest initial growth rate, but within a few days a dramatic loss of viability took place.
- Ctrl, HEPES and TES buffers showed similar growth rates throughout time,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



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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

## 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.

## ACKNOWLEDGEMENTS

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- <sup>2</sup>Tamagnini, P., R. Axelsson, P. Lindberg, F. Oxelfelt, R. Wünschiers, and P. Lindblad. 2002. Hydrogenases and hydrogen metabolism of cyanobacterium. Microbiol. Mol. Rev. 66: 1-20
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