Tackling *Nitrosomonas europaea* culture problems for future applications in inshore aquaculture [P4/13]

Jorge Padrão, Susana Cortez, Nicolina Dias, Ana Nicolau, Manuel Mota

Centre of Biological Engineering (CEB), University of Minho, Campus de Gualtar, Braga, Portugal

Worldwide aquaculture production represents nearly 160 million tonnes per year, roughly the double of captured fish. Technological improvements are required to provide and improve sustainability to the constant aquaculture production growth. Inshore aquaculture reduces dramatically the risk of infection of the cultured organisms, as sea water is treated before use. Contrariwise, to prevent the increase of operational costs, water must be recirculated multiple times. The high cumulative load of ammonium produced during the fish metabolism requires an especially efficient water treatment, namely for the denitrification process, which usually relies on biological consortium processes to be cost-efficient. Thus, nitrifying and denitrifying microorganisms must be further studied and their production scaled up in order to allow the development of novel technologies and fulfil the increasing demand of denitrification units. *Nitrosomonas europaea* is the most extensively studied ammonia oxidizing bacteria, being a ubiquitous nitrification organism. Its vital role in the nitrogen cycle is however impaired by the limited energy achieved by this inefficient source of energy, which is partially dedicated to fixate carbon from gaseous carbon dioxide, restricting biomass production. For scale up purposes the design of a culture medium with no precipitation of its constituents is essential, since inorganic debris may significantly impair downstream processing. Moreover, a non-precipitating medium allows a maximum bioavailability of all elements present in its recipe and improves recirculation effectiveness. A new formulation for *N. europaea* culturing was studied and optimized, fulfilling the addressed objective. This formulation was further tested using moderate pressure displaying positive results in biomass output. The higher *N. europaea* cell concentration allowed an immobilization in a latex based biocoating, which was evaluated for a possible denitrifying cartridge application.

Keywords: Nitrosomonas europaea, nitrification optimization, ammonia removal

Tackling *Nitrosomonas europaea* culture problems for future applications in inshore aquaculture

Jorge Padrão, Susana Cortez, Nicolina Dias, Ana Nicolau, Manuel Mota
Centre of Biological Engineering, University of Minho, *Campus* de Gualtar, Portugal

Key words: *Nitrosomonas europaea*, nitrification optimization, ammonia removal

Introduction

Worldwide aquaculture production reaches 160 million tonnes per year, roughly the double of captured fish. Technological improvements are required to provide and improve sustainability to the constant aquaculture production growth. To maintain inshore aquaculture in the vanguard, the high cumulative load of ammonium must be efficiently treated in order to recirculate the water multiple times. Thus, scale up production of nitrifying and denitrifying microorganisms has to be improved to fulfil the increasing demand of denitrification units.

Objective

Scale up strategies for *Nitrosomonas europaea, a* ubiquitous ammonia oxidizing bacteria:

- ❑ **Design of a non-precipitating culture medium**
  - **To maximize nutrient bioavailability**
  - **To inhibit the presence inorganic debris in down stream processes**
- ❑ **Application Moderate pressure**
  - **To increase dissolved oxygen**

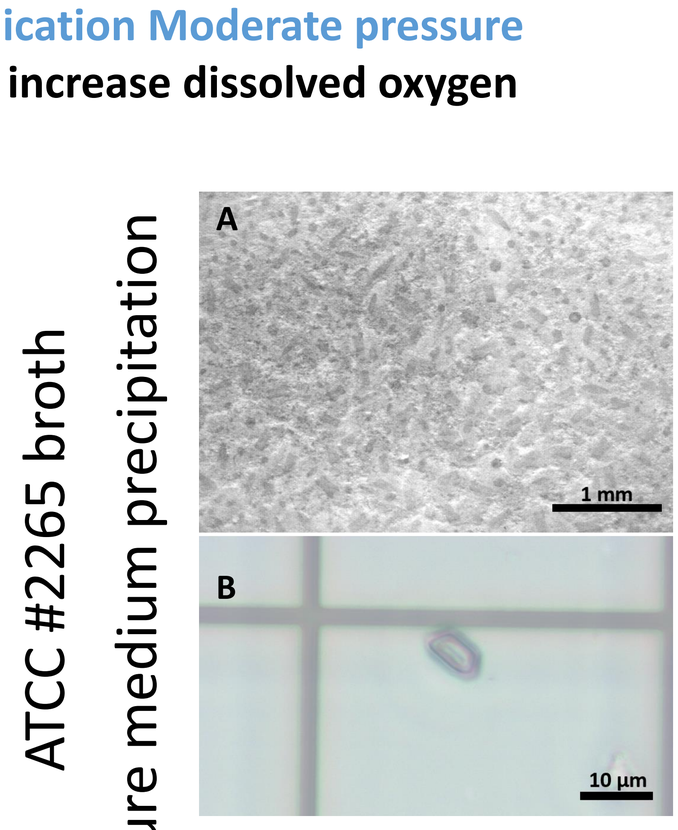

Figure 1 – A: orthorhombic crystals obtained after evaporation of the ATCC #2265 broth, **B:** an orthorhombic precipitate typically found in the ATCC #2265 broth

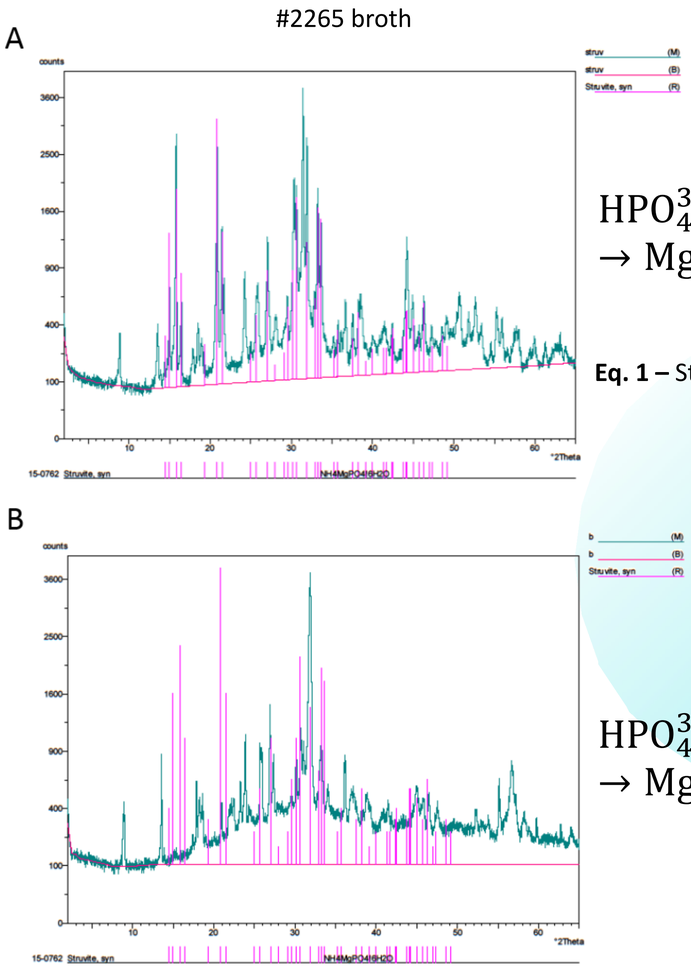

Figure 2 – XRD profiles of the salts obtained from the evaporation at room temperature of **A:** ATCC #2265 broth and **B:** modified medium.

$$HPO_4^{3-} + Mg^{2+} + NH_4^+ + 6H_2O \rightarrow MgNH_4PO_4 \cdot 6H_2O \downarrow + H^+$$

Eq. 1 – Struvite formation.

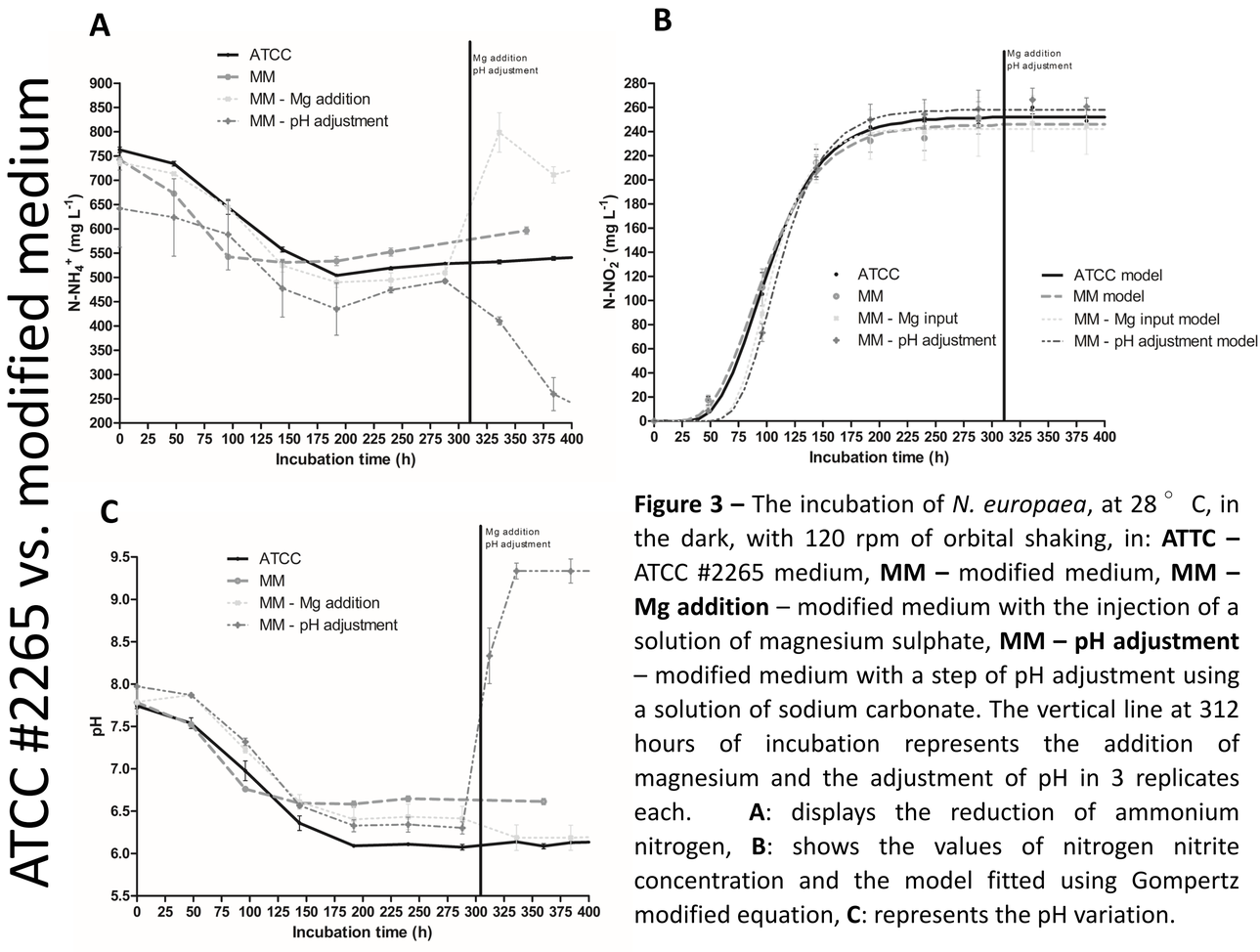

Figure 3 – The incubation of *N. europaea*, at 28 ° C, in the dark, with 120 rpm of orbital shaking, in: **ATTC –** ATCC #2265 medium, **MM –** modified medium, **MM – Mg addition** – modified medium with the injection of a solution of magnesium sulphate, **MM – pH adjustment** – modified medium with a step of pH adjustment using a solution of sodium carbonate. The vertical line at 312 hours of incubation represents the addition of magnesium and the adjustment of pH in 3 replicates each. **A**: displays the reduction of ammonium nitrogen, **B**: shows the values of nitrogen nitrite concentration and the model fitted using Gompertz modified equation, **C**: represents the pH variation.

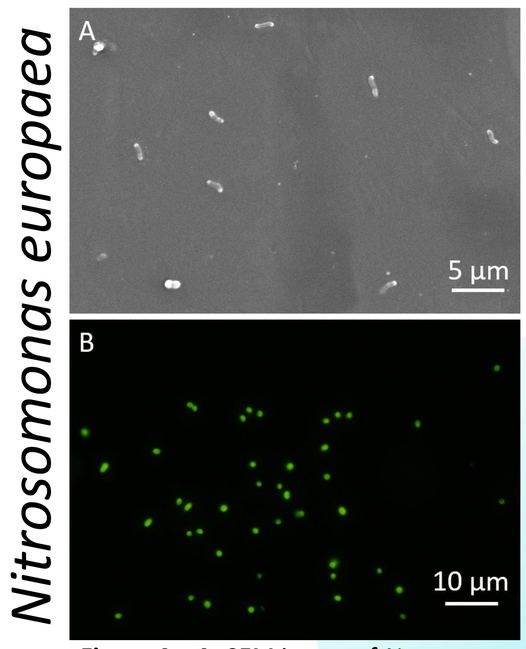

Figure 4 – A: SEM image of *N. europaea* cells cultured in modified medium, **B:** epifluorescence microscopy image of *N. europaea* cultured in modified medium after hybridization with Nsm156 labelled with 6-FAM fluorophore.

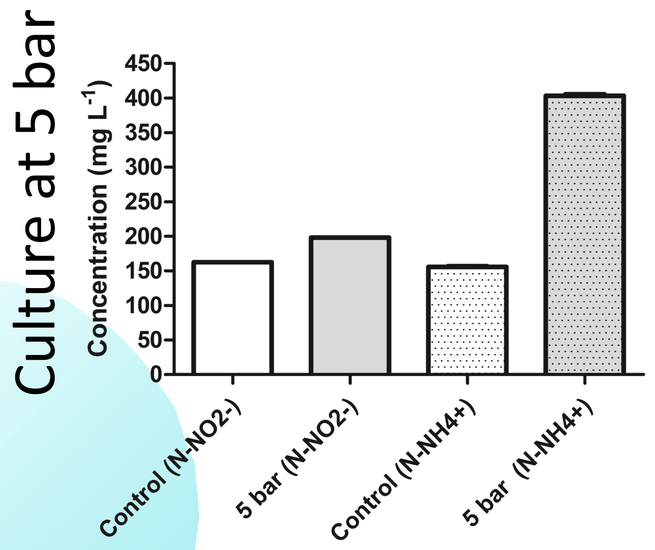

Figure 5 – *N. europaea* nitrogen nitrite ($N\text{-}NO_2^-$) concentration and ammonium ($N\text{-}NH_4^+$) concentration achieved in a shaken culture (control), in opposition to a static culture at 5 bar.

Conclusion

The modification of the standard ATCC culture medium for *N. europaea*:

- ❑ **Successfully avoid precipitation**
- ❑ **Did not impaired the *N. europaea* growth kinetics**

The application of moderate pressure (5 bar):

- ❑ **Displayed an improvement of the *N. europaea* growth**
- ❑ **Prevented the ammonia volatilization (main energy source)**

Acknowledgments
Research supported by the Portuguese Foundation for Science and Technology (Fundação para a Ciência e Tecnologia – FCT) under the scope of the strategic funding of UID/BIO/04469/2013 unit and COMPETE 2020 (POCI-01-0145-FEDER-006684) and also the Project RECI/BBB-EBI/0179/2012 (FCOMP-01-0124-FEDER-027462). The authors also acknowledge the support of BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte.