



The Effect of Copper Limitation on the archaeon *Nitrososphaera viennensis*

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Copper (Cu) is central to many enzymes in ammonia oxidizing archaea (AOA) including plastocyanins and multicopper oxidases¹. Although it is known that Cu plays an important role in AOA physiology and metabolism only recently have we begun to explore their response to Cu excess and limitation². In this study, we explored the physiological response of the AOA soil isolate, *Nitrososphaera viennensis* (EN76T)^{3,4} to Cu-limiting conditions. The chelator TETA [1,4,8,11-tetraazacyclotetradecane 1,4,8,11-tetraacetic acid hydrochloride hydrate] was used to lower bioavailable Cu²⁺ in culture experiments as predicted by thermodynamic speciation calculations (PHREEQC). Ammonium and nitrite concentrations were measured in these cultures over time using colorimetric assays. Cell abundance was determined by flow cytometry. Results show that *N. viennensis* is Cu-limited in nitrite production and growth at free Cu²⁺ concentrations below 6.18 x10⁻¹⁵ mol/l, as compared to standard conditions (3.26 x 10⁻¹² mol/L free Cu²⁺). Copper limited cultures recovered nitrite production and growth upon Cu addition. This Cu²⁺ limiting threshold is higher than in denitrifying bacteria (<10⁻¹⁶ mol/L)⁵ and similar to other AOA⁶, which also possess Cu containing enzymes.

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