

# ANAEROBIC AMMONIUM OXIDISING BACTERIA: SIGNIFICANT BIOLOGICAL SOURCE OF THE BACTERIOHOPANETETROL STEREOISOMER?

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## Introduction

Bacteriohopanetetrol (BHT) is a common bacteriohopanepolyol. The stereoisomer of this hopanoid (BHT isomer; Fig. 1) has been associated previously with anoxic environments (Sáenz et al., 2011). However, the biological source of the lesser occurring BHT isomer, so far, remains unknown. The exact stereochemistry of this isomer has yet to be determined. Possible configurations are shown in Fig. 1.

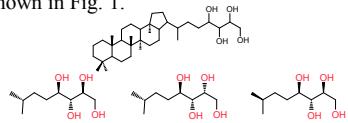


Figure 1. Bacteriohopanetetrol (BHT) structure and possible stereochemistries of the isomers.

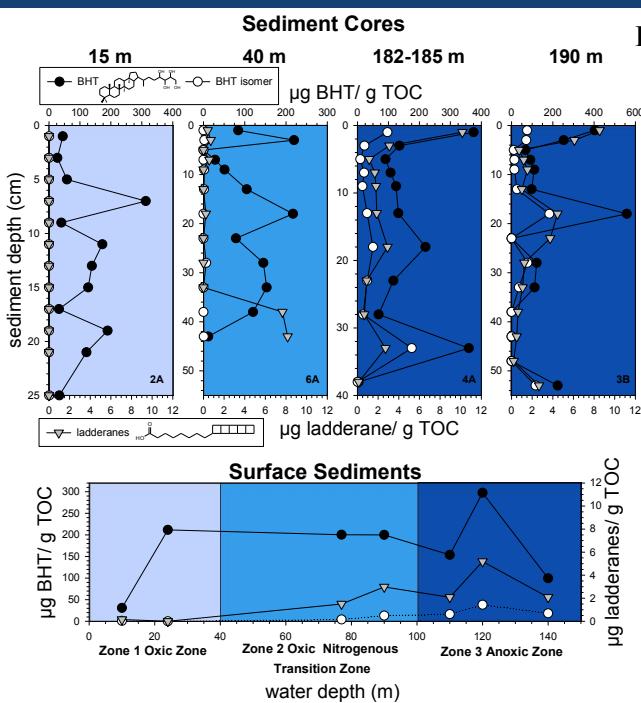


Figure 2. Ladderane and BHT concentrations in sediment cores and surface sediments (0-1 cmbsf) in Golfo Dulce.

## Environmental Occurrence of the BHT stereoisomer – Golfo Dulce

The BHT isomer has been observed in several anoxic environments, such as Golfo Dulce, a 200 m deep enclosure located in Costa Rica.

Golfo Dulce is physically cut off from mixing with the Pacific Ocean by a sill at 60 m, promoting the occurrence of anoxic conditions within the basin. There are three distinct chemical zones in the water column (Fig. 2). We observed that the distribution of the BHT isomer in Golfo Dulce sediments followed the same trend as distribution of ladderane fatty acids, biomarkers for anaerobic ammonium oxidation (anammox) (Sinninghe Damsté et al., 2002), indicating that the same biological source may be responsible for both lipid classes.

## Anaerobic Ammonium Oxidising (anammox) bacteria

Anammox is an important process in the marine nitrogen cycle. The anammox reaction is performed by Planctomycetes bacteria that contain an organelle-like structure called the anammoxosome, which is surrounded by a dense membrane made up of ladderane lipids (Fig. 3). Ladderane fatty acids are unique to the anammox bacteria and their presence in environmental samples is indicative of anammox. Although anammox bacteria are known hopanoid producers (Sinninghe Damsté et al., 2004), it wasn't until the co-occurrence of ladderanes and the BHT isomer was observed in Zone 3 (Fig. 2) that a link between BHT isomer production and anammox was considered.

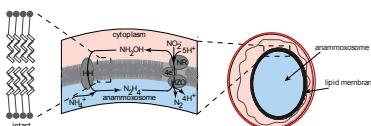


Fig. 3. Anammox cell structure, including the presence of the anammoxosome and ladderane lipids.

## Lipid composition of anammox

To determine whether anammox bacteria are responsible for BHT isomer synthesis, we investigated the lipid composition of three anammox cultures (Fig. 4).

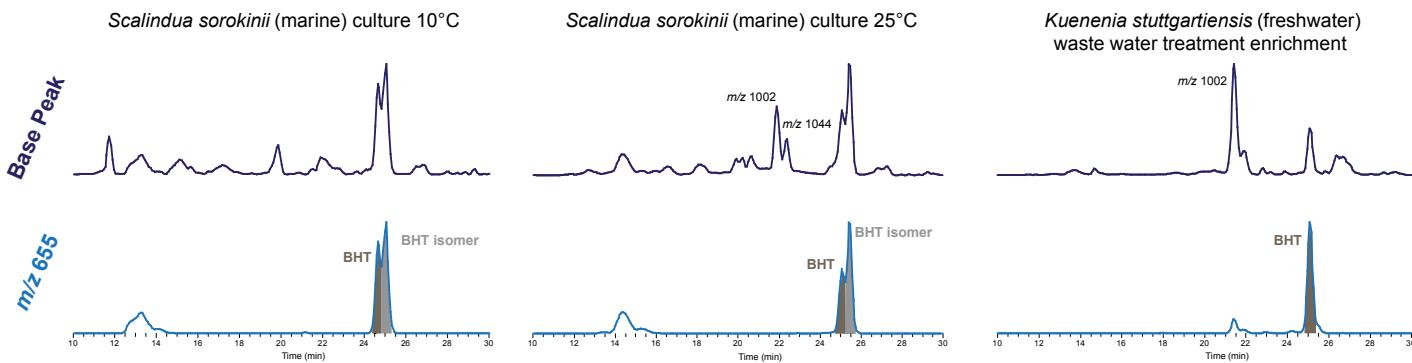


Fig.4. Base peak and mass chromatograms (m/z 655; BHT) for three anammox cultures: marine species *S. sorokinii* cultured at 10°C (a), marine species *S. sorokinii* cultured at 25 °C (b), and non-marine species *K. stuttgartiensis* enriched from a waste water treatment plant (c).

## Conclusion

Marine anammox bacteria appear to synthesise the BHT isomer, and seem to be responsible for at least a part of environmentally-observed BHT isomer occurrences. However, the apparent absence of BHT isomer in non-marine anammox species is as of yet unexplained.

The importance of anammox-produced BHT isomer needs to be investigated in other marine environments where anammox is known to be an important process (e.g. marine oxygen minimum zones, anoxic water columns, and anoxic sediments). Furthermore, the lipid composition of additional non-marine anammox species should be screened for BHT isomer in order to confirm that BHT isomer production is an exclusively marine anammox occurrence.

## References

[1] Sáenz et al., (2011) Organic Geochemistry 42, 1351-1362. [2] Sinninghe Damsté, et al. (2002) Nature 419, 708-712. [3] Sinninghe Damsté, et al. (2004) Organic Geochemistry 35, 561-566.