# TRACING THE ACTIVITY OF METHANOGENIC ARCHAEA IN MARINE SEDIMENTS BY LIPID RADIO ISOTOPE PROBING (LIPID-RIP)

T.W. Evans[1, *], S. Coffinet[1, *], M. Könneke[2], M. Elvert[1], J.-S. Lipp[1], and K.-U. Hinrichs[1]

[1]Organic Geochemistry Group, MARUM - Center for Marine Environmental Sciences, University of Bremen, Germany
[2]Marine Archaea Group, MARUM, University of Bremen, Germany
[*]*The corresponding authors equally contributed to this work: thomas.evans@uni-bremen.de, scoffinet@marum.de*

Studies investigating microbial communities in subsurface sediments have mostly concentrated on the abundance of cells, DNA or lipids to quantify the size and phylogenetic relationships of the microbes (e.g. Parkes et al., 1994; Schippers et al., 2005; Lipp et al., 2008), but these techniques do not allow to trace the microbial activity in these energy starved environments. To this aim, lipid stable isotope probing experiments of various marine sediments were performed (e.g. Takano et al., 2010; Lin et al., 2013; Lengger et al., 2014). However, these studies showed only minor or no uptake of the different $^{13}$C-labelled substrates, even after long incubations in subsurface sediments. We implemented radio isotope probing (RIP) to improve the labelling sensitivity due to the extremely low natural abundances of $^{14}$C. This technique was applied in order to quantify lipid biosynthesis by methanogens in marine sediments. Methanogenesis is the final step in the remineralization of organic matter and one of the major microbial processes in marine sediments (Reeburgh, 2007). The direct link between specific lipids and methanogenesis is difficult given that the major membrane lipids, such as glycerol dialkyl glycerol tetraethers (GDGTs) and diphytanyl diethers (archaeols), are widespread among Archaea. The application of RIP will help to trace archaeal lipids that are actively synthesized during methanogenesis.

We performed a RIP incubation experiment to track the activity and the lipid biosynthesis of methanogenic Archaea in marine sediments from the Rhone delta via $^{14}$C incorporation into specific lipid groups. Sediments from three different depths (0-12, 80-85 and 135-140 cmbsf) were incubated with either $^{14}$C-bicarbonate (DIC) or 2-$^{14}$C-acetate (ACE) as carbon source, and $H_2$ as major energy source. Bacterial growth was inhibited by addition of antibiotics. As a control, sediment from each depth and with each carbon source was incubated with a headspace composed of $N_2$ instead of $H_2/CO_2$. Additionally, a second set of sediment slurries was amended with non-labelled methanol together with $H_2$ and with $^{14}$C-DIC or $^{14}$C-ACE as carbon source to track the use of non-competitive, methylated substrates for methanogenesis.

Gas measurements during the incubation revealed high methane production in all samples with a $H_2/CO_2$ headspace (w/wo methanol) compared to the controls with $N_2$ headspace (Fig 1A and B). Nevertheless, $^{14}$C-incorporation into the total lipid extracts was detected in all incubated samples (Fig.1C and D). For all depths, ACE was more strongly incorporated into the lipids than DIC, possibly indicating the preference of microorganisms to a heterotrophic lifestyle. Surface samples showed a more than two-fold higher incorporation of $^{14}$C into the total lipid extract than the deeper sediments for both carbon sources amended. Moreover, methanol induced an enhanced microbial activity in the surface sediments, particularly in presence of ACE, a phenomenon not observed in the subsurface incubations.

These preliminary results suggest that methanogenesis is the major metabolic process in our incubations and seems to be carried out by different microbial communities according to depth in the sediment from the Rhone river delta. We will present the radioisotope incorporation into specific archaeal lipid pools such as GDGTs and archaeols in order to start

constraining archaeal lipids in marine sediment that are potentially originating from methanogenic archaea.

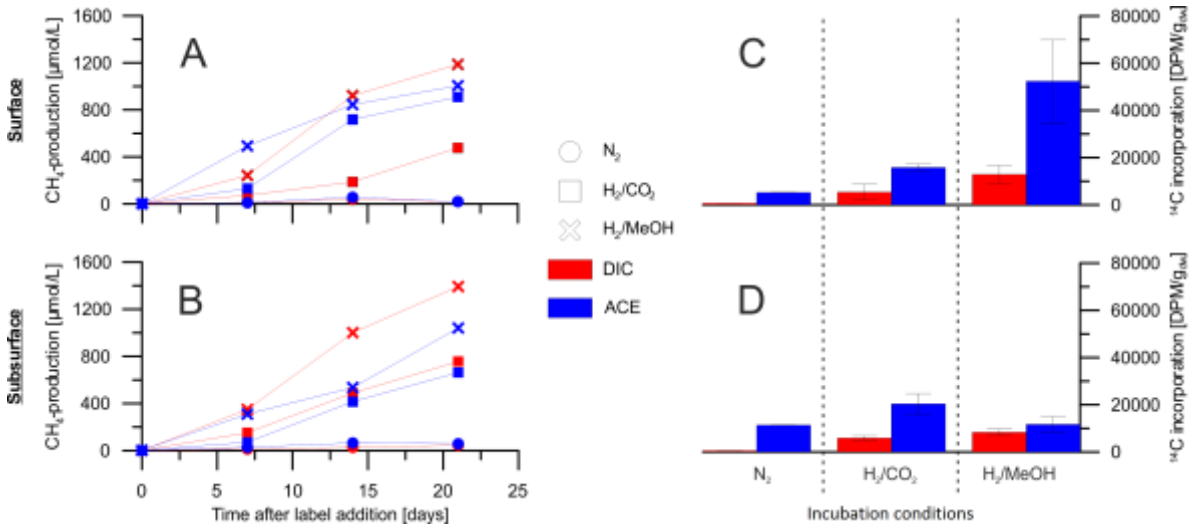


***Figure 1:*** *methane production (A-B) and $^{14}C$-incorporation of the two carbon sources in disintegrations per minute (DPM; C-D), bicarbonate (DIC, in red) and acetate (ACE, in blue), in the total lipids extracts from methanogenic incubation experiments. Anoxic slurries with surface (A and C; 0-12 cmbsf) or subsurface (B and D; 135-140 cmbsf) sediment were incubated with $H_2$ or $H_2$ plus methanol (MeOH) as major energy source for 21 days at 28°C in the dark.*

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