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Thermal stability of ladderane lipids as determined by hydrous pyrolysis

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ABSTRACT

Anaerobic ammonium oxidation (anammox) has been recognized as a major process resulting in loss of fixed inorganic nitrogen in the marine environment. Ladderane lipids, membrane lipids unique to anammox bacteria, have been used as markers for the detection of anammox in marine settings. However, the fate of ladderane lipids after sediment burial and maturation is unknown. In this study, anammox bacterial cell material was artificially matured by hydrous pyrolysis at constant temperatures ranging from 120 to 365 °C for 72 h to study the stability of ladderane lipids during progressive dia- and catagenesis. HPLC-MS/MS analysis revealed that structural alterations of ladderane lipids already occurred at 120 °C. At temperatures >140 °C, ladderane lipids were absent and only more thermally stable products could be detected, i.e., ladderane derivatives in which some of the cyclobutane rings were opened. These diagenetic products of ladderane lipids were still detectable up to temperatures of 260 °C using GC-MS. Thus, ladderane lipids are unlikely to occur in ancient sediments and sedimentary rocks, but specific diagenetic products of ladderane lipids will likely be present in sediments and sedimentary rocks of relatively low maturity (i.e., C_{31} hopane 22S/(22S + 22R) ratio <0.2 or ββ/(αβ + βα + ββ) ratio of >0.5).

1. Introduction

Anammox, the anaerobic oxidation of ammonium coupled to nitrite reduction with N_2 as the end product, is performed by chemolithoautotrophic bacteria identified as a distinct phylogenetic group within the *Planctomycetes* (Strous et al., 1999; Schmid et al., 2007). The anammox process was first detected in a wastewater treatment plant in 1995 (Mulder et al., 1995; Van de Graaf et al., 1995), and later it was also observed to be ubiquitous in the marine environment where it contributed significantly to the loss of inorganic nitrogen from the ocean (Devol, 2003; Kuypers et al., 2005; Hamersley et al., 2007). Anammox bacteria contain a separated intracytoplasmic compartment named the anammoxosome, where anammox catabolism was shown to take place (Sinninghe Damsté et al., 2002; Van Niftrik et al., 2004). The membrane of this "organelle" consists of unusual ladderane lipids forming a dense barrier which is thought to reduce the permeability of the membrane to small molecules, e.g. the toxic intermediate of the anammox reaction, hydrazine, which can easily permeate less dense bacterial membranes (Sinninghe Damsté et al., 2002). Ladderane lipids occur in a variety of different forms either ester or ether bound to the glycerol backbone, and contain three or five linearly fused cyclobutane rings (Fig. 1, structures I–IV), which is unprecedented in nature. Ladderanes have been applied as biomarkers for anammox activity in anoxic waters of the Black Sea (Kuypers et al., 2003), oxygen depleted zones of the ocean (Kuypers et al., 2003; Hamersley et al., 2007; Jaeschke et al., 2007) and in marine sediments (Hopmans et al., 2006).

During periods in the geological past when the world's oceans experienced water column anoxia, so-called oceanic anoxic events (OAEs), anammox has been proposed to be an important process in the marine nitrogen cycle, as a sink for fixed nitrogen (Kuypers et al., 2004). However, this hypothesis has remained untested so far. Fossil rem-

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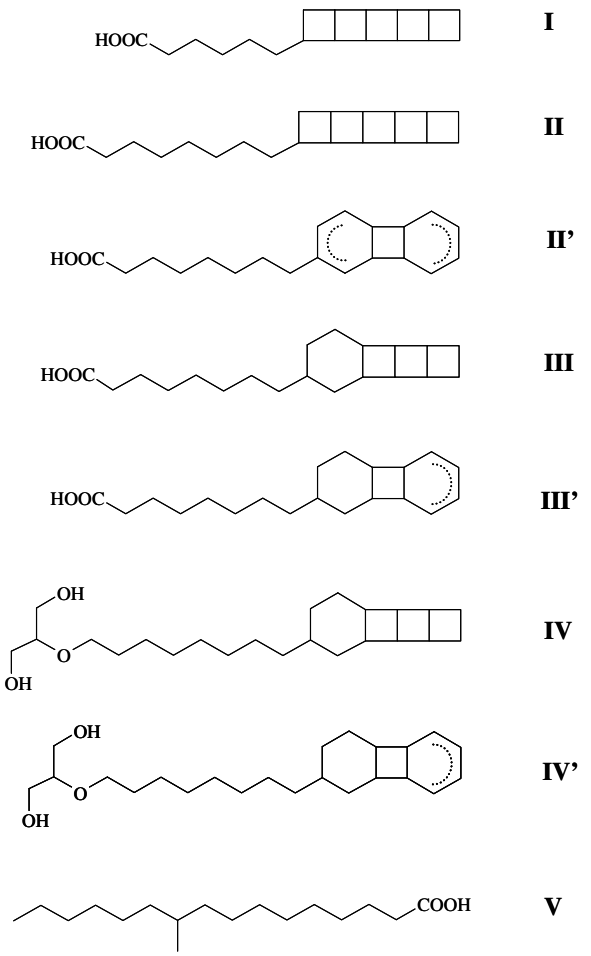

Fig. 1. Structures of selected ladderane and other core lipids analyzed in this study. (I) C_{18}-[5]-ladderane fatty acid; (II) C_{20}-[5]-ladderane fatty acid; (II′) thermal degradation product of II; (III) C_{20}-[3]-ladderane fatty acid; (III′) thermal degradation product of III′; (IV) C_{20}-[3]-ladderane monoether; (IV′) thermal degradation product of IV; (V) 10-methyl-hexadecanoic acid.

nants of ladderane lipids have recently been found in a sediment core from the Arabian Sea, suggesting that anammox activity occurred over the last glacial cycle (Jaeschke et al., unpublished results). However, it is not known if ladderane lipids can be found in more ancient mature sediments and sedimentary rocks. Due to the highly strained conformation of the cyclobutane moieties, the structures of the ladderanes are probably altered during diagenesis and maturation. Indeed, the ladderane moiety has been found to be thermally labile, and conversion of ladderane lipids into thermally more stable degradation products has been observed during gas chromatographic analysis (Sinninghe Damsté et al., 2005).

Hydrous pyrolysis is a laboratory technique developed to simulate the natural maturation process by heating organic rich sedimentary rock in a closed reactor in the presence of liquid water at subcritical temperatures (<374 °C) for several days (Lewan et al., 1979; Lewan, 1993). Previous studies have shown that reactions involving biomarkers during hydrous pyrolysis experiments mimic those occurring during natural maturation (e.g., Koopmans et al., 1996). In this study, anammox biomass was artificially matured using hydrous pyrolysis to investigate the thermal stability of ladderane lipids and their possible degradation products, which can potentially be applied as molecular indicators for anammox activity in the geological past.

2. Material and methods

2.1. Sample description

Anammox cell material was obtained from a wastewater treatment plant of Paques B.V. (Balk, The Netherlands) located at the Waterboard Hollandse Delta (WHSD) in Rotterdam, The Netherlands. Coupled to partial nitrification, anammox bacteria convert ammonium and nitrite to dinitrogen gas in a cost effective and sustainable way. The material was filtered through a 0.5 mm sieve, freeze dried and homogenized. Fluorescence in situ hybridization (FISH) analysis confirmed the dominance of the anammox bacterium *Kuenenia stuttgartiensis* in the reactor (ca. 70% of the bacterial population) (Boumann et al., 2006).

2.2. Hydrous pyrolysis

A detailed description of hydrous pyrolysis experiments is given by Lewan (1993). In short, carburized 1 litre Hastelloy-C276 reactors were filled each with 75 g of anammox cell material and 500 g of distilled water. A 1 mm Cr–Ni screen was placed on top of the sample to prevent cell material from floating on the water surface during the loading of the experiment and product collection at the end of the experiment. Sample volume (based on an approximate sample density), reactor volume and amount of added water were calculated to ensure that the sample remains submerged in a liquid water phase throughout the experiment. After leak check, the remaining reactor volume was filled with helium at a pressure of 1.38 bars. Artificial maturation was accomplished by heating aliquots isothermally for 72 h at temperatures from 120 to 365 °C (Table 1). The temperatures were continuously monitored during the experiments at 30 s intervals with type J thermocouples. Standard deviations varied between 0.2 and 0.6 °C for the experiments, except for the experiments at 120 and 140 °C, which had standard deviations of 1.2 and 1.3 °C. After the experiments were completed and cooled to room temperatures (18–20 °C) within 24 h, the gas pressure and temperature were recorded and gas samples were collected in 30–50 cm^3 stainless steel cylinders. The remaining gas was vented and the reactor was opened to quantitatively collect the reaction products. Experiments at temperatures ranging between 260 and 350 °C generated an expelled oil, which was recovered from the water surface with a pipette. The reactor walls, reactor head and thermowell were rinsed with benzene to recover any sorbed oil films, which occurred in experiments starting at 200 °C. The water recovered from the reactor was filtered (0.45 μm) and an aliquot immediately analyzed for

Table 1
Analytical data for anammox biomass (75 g) artificially matured by hydrous pyrolysis at a range of different temperatures for 72 h

Temp (°C)	pH	Eh (eV)	Gas (g)	Exp oil (g)	Residue (g)	$\delta^{13}C$ (‰)	TOC (wt%)
Orig.	5.7	−135	0	0	75.0	−34.1	23.6
120	5.4	63	0.3	0	63.2	−34.9	19.7
140	5.6	−6	0.7	0	57.7	−35.3	15.3
160	6.3	−140	1.5	0	55.8	−35.4	14.5
180	6.9	−197	1.7	0	52.8	−36.1	15.4
200	7.5	−198	2.5	0.1	51.8	−36.0	14.0
220	7.3	−252	2.4	0.2	51.9	−36.3	14.1
240	7.6	−253	2.6	0.9	48.5	−36.3	11.5
260	7.7	−251	2.9	1.4	47.1	−36.4	11.5
280	7.8	−267	2.7	2.7	46.4	−35.8	8.9
300	7.9	−281	2.8	3.2	42.7	−35.4	6.0
320	8.0	−290	3.6	3.9	41.0	−34.8	4.9
335	7.9	−307	5.0	4.1	38.8	−34.5	4.0
350	7.7	−303	5.6	3.2	45.8	−34.0	3.4
365	7.9	−329	6.9	1.7	40.2	−33.7	4.3

pH and Eh at room temperature. The remaining water was kept to analyze water soluble compounds. The remaining cell material (i.e., residue) was removed from the reactor and freeze dried. More than 95% of the originally loaded material was recovered from all experiments as reacted water, expelled oil, generated gas and anammox residue. The product yields from the hydrous pyrolysis experiments are given in Table 1 with Eh and pH values for the recovered water. The deficit between the recovered products and the original 75 g of starting anammox biomass is attributed to the aqueous products dissolved in the recovered water (i.e., proteins, carbohydrates, calcium and phosphorous), which were not analysed.

2.3. Extraction and fractionation

Aliquots of the residue (2–3 g) were repeatedly ultrasonically extracted (5×) with a mixture of dichloromethane (DCM) and methanol (2:1v:v). The bulk of the solvent was removed by rotary evaporation under vacuum, and the extract was further dried over a Na_2SO_4 column to obtain the total lipid extract (TLE). The TLE was methylated with boron trifluoride (BF_3) in methanol to convert fatty acids into their corresponding methyl esters (FAMEs). To remove very polar components, the extract was subsequently eluted with ethyl acetate over a silica column. For HPLC-MS/MS analysis, an aliquot of the methylated TLE was dissolved in acetone and filtered through a 0.45 μm, 4 mm diameter PTFE filter. For gas chromatography (GC), GC-mass spectrometry (MS) and isotope ratio monitoring (irm) GC-MS analyses, another aliquot of the extract was silylated with bis (trimethyl) trifluoroacetamide (BSTFA) in pyridine at 60 °C for 20 min to convert alcohols in trimethylsilyl (TMS) ether derivatives, and the extract was dissolved in ethyl acetate. An aliquot of the separately collected oil that formed during experiments at 200–365 °C, was fractionated by column chromatography with Al_2O_3 into apolar and polar fractions by elution with hexane:DCM (9:1 v:v) and DCM:methanol (1:1 v:v), respectively. For quantification, a known amount of the internal standard (*anteiso*-C_{22} alkane) in ethyl acetate was added after silylation prior to GC analysis.

2.4. HPLC/APCI-MS/MS analysis

An aliquot of the methylated TLE was analyzed for ladderanes by high performance liquid chromatography coupled to positive ion atmospheric pressure chemical ionization tandem mass spectrometry (HPLC/APCI-MS/MS) as described by Hopmans et al. (2006) with some modifications. Specifically, separation was achieved using a Zorbax Eclipse XDB-C_8 column (3.0 × 250 mm, 5 μm; Agilent) and a flow rate of 0.18 ml/min MeOH. The source settings were: vaporizer temperature 475 °C, discharge current 2.5 μA, sheath gas (N_2) pressure 30 (arbitrary units), auxiliary gas (N_2) pressure 5 (arbitrary units), capillary temperature 350 °C, and source CID -10 V. Argon pressure was maintained at 1.5 mTorr in the second quadrupole. Ladderane lipids were quantified using external standard curves of the C_{20}-[5]- and C_{20}-[3]-fatty acids (Fig. 1, structures II and III) and the C_{20}-[3]-monoether (Fig. 1, structure IV) (Sinninghe Damsté et al., 2002; Hopmans et al., 2006). A detection limit (defined by a signal to noise ratio of 3) of 30–35 pg injected was achieved with this technique.

2.5. GC and GC/MS analysis

GC analysis was performed using a Hewlett-Packard 6890 instrument equipped with an on-column injector and a flame ionization detector (FID). A fused silica capillary column (25 m × 0.32 mm) coated with CP Sil-5 (film thickness 0.12 μm) was used with helium as carrier gas. The samples were injected at 70 °C. The GC oven temperature was subsequently raised to 130 °C at a rate of 20 °C/min, and then at 4 °C/min to 320 °C, which was held for 15 min. GC/MS analysis was carried out using a Finnigan Trace GC Ultra, Thermo Electron Corporation, interfaced with a Finnigan Trace DSQ mass spectrometer, using a mass range of *m/z* 40–800. GC conditions for GC/MS were the same as those described above for GC. The compounds formed during thermal destruction of the ladderane lipids were identified according to retention times and mass spectra described by Sinninghe Damsté et al. (2005). The reproducibility of concentration measurements based on duplicate analysis of the samples was between 1% and 7%.

2.6. $\delta^{13}C$ and TOC analysis

Total organic carbon (TOC) and carbon isotope ratios ($\delta^{13}C$) of the residual matter were measured against a benzoic acid laboratory standard (%C = 68.80, $\delta^{13}C = -28.1‰$), calibrated against the NBS22 standard ($\delta^{13}C = -30.03‰$), on a Carlo Erba Flash elemental analyzer coupled to a ThermoFinnigan Delta^{plus} mass spectrometer. All analytical results are reported in the usual δ notation, in per mil relative to the Vienna Pee Dee belemnite (VPDB) standard. The reproducibility of concentrations based on duplicate analysis of samples was better than 0.3‰ for $\delta^{13}C$ and better than 0.6% for TOC.

2.7. Compound specific isotope analysis

The $\delta^{13}C$ values of the ladderane lipids were determined using a ThermoFinnigan DELTA-C irm-GC-MS system. A fused silica capillary column (50 m × 0.32 mm) coated with CP-Sil 5 (film thickness 0.12 μm) was used with helium as carrier gas. The column conditions and temperature program were the same as described above for GC analyses. $\delta^{13}C$ values were corrected for the additional carbon added during the derivatization step (BF_3/methanol: $\delta^{13}C = -20.5‰$; BSTFA: $\delta^{13}C = -36.8‰$). The $\delta^{13}C$ values for individual compounds are reported in the standard delta notation against VPDB standard.

3. Results and discussion

3.1. HPLC-MS/MS analysis

To investigate the effect of thermal maturity on ladderane lipids, anammox biomass was artificially matured for three days using hydrous pyrolysis (Lewan, 1993) at temperatures ranging from 120 to 365 °C. Ladderane lipids were analyzed with HPLC/APCI-MS/MS using the transition of the protonated molecules to selected product ions of four ladderane lipids. This highly sensitive technique avoids thermal alteration of ladderanes upon gas chromatographic analysis (Hopmans et al., 2006) and has been used to detect ladderanes in environmental samples (Hamersley et al., 2007; Jaeschke et al., 2007). In the unheated sample the C_{20}-[3]-monoether (IV) is the most abundant component, followed by the C_{20}-[3]-fatty acid (III) in about 10 times lower concentration, and minor amounts of the fatty acids with a [5]-ladderane moiety (I, II) (Fig. 2a).

In the sample from the 120 °C experiment additional peaks appeared (Fig. 2b, II′, III′, IV′) which may represent degradation products of the ladderane lipids (see below) whereas the original ladderane lipids were already low in abundance when compared to the starting material, with a loss of about 99% (I), 95% (II), 77% (III), and 78% (IV), respectively. At a hydrous pyrolysis temperature of 140 °C none of the ladderane lipids in their original form could be detected, suggesting that these compounds were completely degraded (Fig. 2c). Analysis of the early eluting compounds formed at these temperatures (II′, III′, IV′) by MS/MS in data-dependent mode revealed the same protonated molecular ion and almost identical fragmentation patterns as the intact ladderanes, signifying similar structures. The elution times of these components were earlier by ca. 1 min compared to the parent ladderane, indicating that these compounds are more polar. Collectively, this suggests that these newly formed compounds represent ladderanes in which one of the cyclobutane rings opened, resulting in the formation of a double bond within the ladderane moiety. This is similar to what has been observed for ladderane lipids during GC analysis, i.e., cleavage and internal proton shifts of bonds within the [5]-ladderane moiety leading to a moiety comprised of one cyclobutane ring with two condensed cyclohexenyl groups (Fig. 1, structure II′) (cf. Sinninghe Damsté et al., 2005). Transformation of two cyclobutane rings into one cyclohexene ring was further suggested for the lipids containing the [3]-ladderane moiety (Fig. 1, structure III′). This is supported by HPLC-MS/MS analysis of isolated standards of these products formed during GC analysis. Sinninghe Damsté et al. (2005) showed that these products have similar mass spectra and retention times as those formed in the 120 and 140 °C hydrous pyrolysis experiments. For the C_{20}-[3]-ladderane monoether (Fig. 1, structure IV) no isolated degradation products existed but almost identical fragmentation patterns of the two peaks detected with

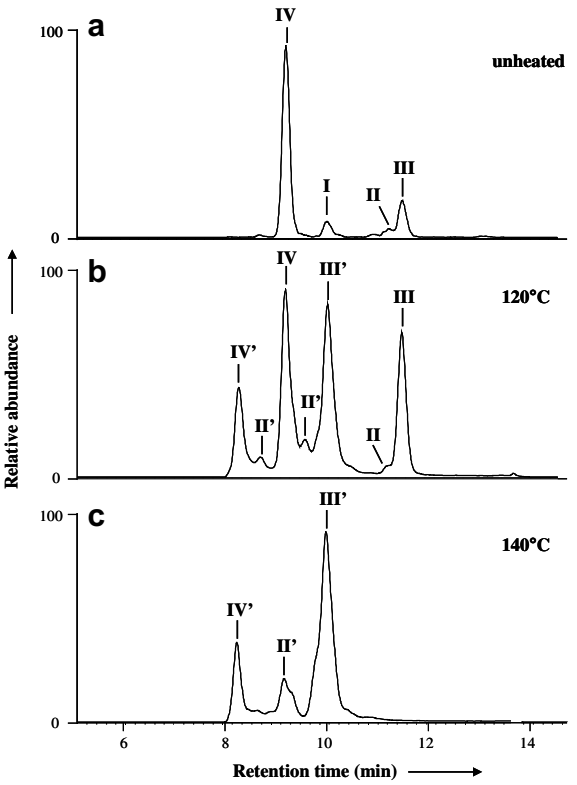

Fig. 2. HPLC base peak chromatograms showing the distribution of ladderane lipids (analyzed as their methyl ester derivatives) in biomass (comprised of 70% of anammox bacteria) derived from a wastewater treatment plant. (a) Original, and artificially matured for 72 h at (b) 120 °C, (c) 140 °C. I–IV corresponds to structures in Fig. 1. II′–IV′ are possible thermal degradation products of ladderane lipids, II′ and III′ are those identified by Sinninghe Damsté et al. (2005).

selective reaction monitoring (Fig. 2b, IV and IV′) also suggests ring opening and formation of a thermally more stable product (Fig. 1, structure IV′) in the same way as shown for the C_{20}-[3]-ladderane fatty acid.

These results indicate that the diagenetic products of ladderanes formed during hydrous pyrolysis experiments are the same as those formed during GC analysis. At 120 °C, already 87% of the C_{20}-[5]-ladderane fatty acid is transformed into the thermally more stable form, whereas for the C_{20}-[3]-ladderane fatty acid and the C_{20}-[3]-ladderane monoether this is 49% and 47%, respectively. Thus, ladderane lipids are transformed at the very early stages of maturation, which suggests that original ladderanes are unlikely to be present in mature sediments and sedimentary rocks.

3.2. GC and GC-MS analysis

Since HPLC-MS/MS analysis indicated that ladderane lipids were quickly transformed into diagenetic products similar to those formed during GC analysis, we also analysed lipids using GC and GC-MS. In the unheated sample the distribution of ladderane lipids was similar to that revealed by HPLC analysis, i.e., the thermally stable product of the C_{20}-[3]-ladderane monoether (Fig. 1, structure IV′; Fig. 3, peak 9) formed upon GC analysis was highest in concentration, followed by a broad peak representing the thermally stable products of the C_{20}-[3]-ladderane fatty acid (Fig. 1, structure III′; Fig. 3, peak 6), which is two times lower in concentration. Broad peaks representing the thermally stable products derived from the two fatty acids with the [5]-ladderane moieties are in the lowest concentrations (Fig. 1, structure II′; Fig. 3, peaks 4 and 5). At a hydrous pyrolysis temperature of 120 °C the concentration of these lipids already decreased by 70–100% compared to the unheated sample (Fig. 4a). The products of the C_{18}-[5]-ladderane fatty acid (Fig. 1, structure I) were below detection limit using GC-MS at the lowest hydrous pyrolysis temperature, whereas the C_{20}-[5]-ladderane fatty acid products (Fig. 1, structure II) were detectable until 180 °C. Remarkably, the broad peak shape of the C_{20}-[3]-ladderane fatty acid (Fig. 3a, Peak 6) evident in the original sample disappeared at 120 °C and instead a sharp peak was observed (Fig. 3b, peak 6) with the same mass spectrum. This suggests that opening of the cyclobutane rings and conversion into thermally more stable products had already occurred during hydrous pyrolysis, in good agreement with the HPLC-MS/MS results. With increasing artificial maturation temperatures the concentration of the diagenetic products derived from the [3]-ladderane moiety further decreased, and were below detection limit at 280 °C (Fig. 4a), indicating that all compounds were thermally degraded. Thus, GC and GC-MS analysis revealed that a high percentage (70–100%) of the ladderane lipids already disappeared at low temperatures but that the thermally more stable ladderane degradation products were still detectable at 260 °C.

To study the effect of thermal maturity on ladderane fatty acids in comparison to normal fatty acids we analyzed the 10-methylhexadecanoic acid in the unheated and artificially matured anammox biomass. The 10-methylhexadecanoic acid (Fig. 1, structure V, Fig. 3, peak 3), although not unique to *Planctomycetes* (Londry et al., 2004), has been used as an indicator for the presence of anammox bacteria in a freshwater lake (Schubert et al., 2006). Compared to the starting material a 40% decrease in concentration of the 10-methylhexadecanoic acid is observed at 120 °C (Fig. 4a). A further decrease is observed with increasing temperatures but low concentrations of the fatty acids are still detectable at 350 °C. Thus, compared to the conventional fatty acids, ladderane fatty acids have a much lower thermal stability that attests to the thermal instability of the structurally strained cyclobutane moieties.

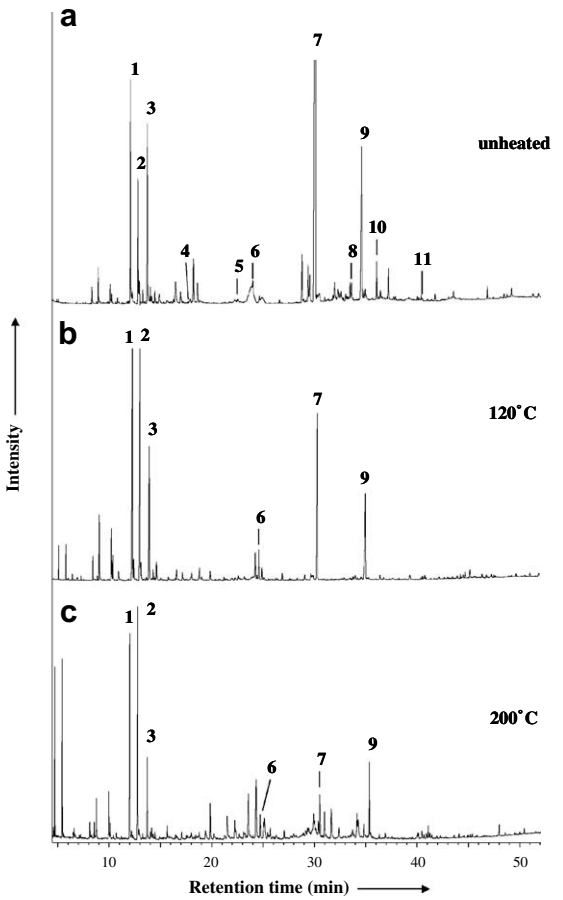

Fig. 3. Gas chromatograms of the total lipid fractions (analyzed as their methyl ester and TMS derivatives) in anammox biomass derived from a wastewater treatment plant. (a) Original biomass, and samples artificially matured for 72 h at (b) 120 °C, (c) 200 °C. Compound numbers represent (1) *iso*-hexadecanoic acid; (2) *n*-C_{16}; (3) 10-methyl-hexadecanoic acid; (4) C_{18}-[5]-ladderane fatty acid; (5) C_{20}-[5]-ladderane fatty acid; (6) C_{20}-[3]-ladderane fatty acid; (7) squalene; (8) C_{27} hopanoid ketone; (9) C_{20}-[3]-ladderane monoether; (10) diploptene; (11) diplopterol.

3.3. Stable carbon isotopic composition and TOC

Chemolithoautotrophic anammox bacteria have been shown to possess a carbon fixation pathway (the acetyl-CoA pathway) (Strous et al., 2006) that strongly fraction-

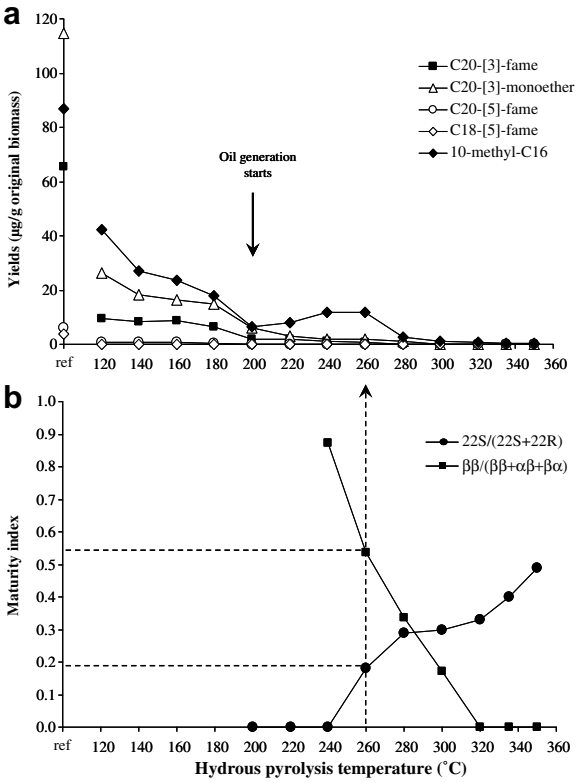

Fig. 4. Concentration and ratios of compounds as a function of hydrous pyrolysis temperature. (a) The most abundant ladderane lipids and the 10-methyl-hexadecanoic acid as analyzed by GC; (b) diagram showing two hopane maturity parameters, the ββ/(ββ + αβ + βα) C_{31} homohopane ratio and the 22S/(22S + 22R)−17α,21β(H) C_{31} homohopane ratio as a function of maturation temperature. The stippled line indicates the temperature and maturity index where diagenetic products of ladderane lipids were still detectable by GC-MS.

ates against ^{13}C, resulting in distinct isotopic signatures, i.e., ladderane lipids are depleted by as much as 47‰ relative to the carbon source (CO_2) of anammox bacteria (Schouten et al., 2004). The stable carbon isotopic composition of the C_{20}-[3]-ladderane fatty acid and the C_{20}-[3]-ladderane monoether analyzed in this study also revealed substantially depleted $\delta^{13}C$ values of −48.9‰ and −48.7‰, respectively, for the unheated sample (Table 2), whereas the bulk isotopic composition of the anammox starting material has a more ^{13}C enriched $\delta^{13}C$ value of −34.1‰ (Table 1). The stable carbon isotopic composition of the 10-methylhexadecanoic acid with values of −44.8 to −43.4‰ (Table 2) is also substantially depleted in ^{13}C compared to other bacterial lipids ($\delta^{13}C$ = ca −20‰ to −30‰) (Schmid et al., 2003), which strongly suggests that this lipid originated from anammox bacteria. With increasing hydrous pyrolysis temperatures the bulk biomass $\delta^{13}C$ values become isotopically lighter by about 2‰, which is probably due to the loss of thermally labile functional groups enriched in ^{13}C, i.e., proteins and carbohydrates, whereas $\delta^{13}C$ values of the ladderane lipids become slightly more enriched by about 2–4‰. With increasing temperatures (>260 °C) thermal cleavage of preferentially

Table 2
Stable carbon isotopic ($\delta^{13}C$) data for selected lipids obtained from anammox biomass

Lipid	$\delta^{13}C$ (‰ vs VPDB)		
	Unheated	120 °C	200 °C
iso-C_{16}	−50.9	−49.2	−49.4
C16:0	−41.7	−42.9	−42.0
10-Me-C_{16}	−44.8	−43.7	−43.3
C20-[3]-FA	−48.9	−48.0	−45.1
C20-[3]-Monoether	−48.7	−45.6	−45.4

All fatty acids were analyzed as methyl esters (FAME).

^{12}C–^{12}C bonds during oil and gas generation leads to isotopically heavier $\delta^{13}C$ values of the biomass (−33.7‰ at 365 °C). However, the ^{13}C depleted signal is mainly preserved also at higher maturity, and this characteristic feature may be used in the assignments of diagenetic products of anammox lipids.

3.4. Implications for the occurrence of ladderane lipids in ancient sediments

These results can be used to make predictions of the occurrence of ladderane lipids in ancient sediments. The 22S/(22S + 22R) ratio and the ββ/(αβ + βα + ββ) ratio for the C_{31} homohopanes, which are generated from bacteriohopanetetrol and possible other hopanepolyol derivatives present in anammox biomass (Sinninghe Damsté et al., 2004) during artificial maturation, are generally used biomarker maturity parameters with maximum values of ca. 0.6 and 0, respectively (Seifert and Moldowan, 1980; Van Duin et al., 1997). They can thus be used to examine the degree of thermal maturation of ladderane lipids in our hydrous pyrolysis experiments (Fig. 4b). Ladderane lipids already disappear at 140 °C where no αβ or 22S hopanoid isomers have yet formed, and therefore, these lipids are not expected to be found even in relatively immature ancient sediments or sedimentary rocks. Thermal diagenetic products of ladderane lipids containing the [3]-ladderane moiety were detected at hydrous pyrolysis temperatures of ⩽260 °C, which corresponds to a 17α,21β(H)-hopane 22S/(22S + 22R) ratio of ca. 0.2. This ratio corresponds to pre-oil generation maturities (Peters et al., 2005) and suggests that ladderane lipids are only expected to be present in sediments and sedimentary rocks with a relatively low level of thermal maturity. At 260 °C, the thermally unstable 17β,21β(H) isomer of the C_{31} hopane is still present (Fig. 4b), which is another indication of the relatively low level of thermal maturity of ladderane lipids revealed by our artificial maturation experiments. Therefore, reconstruction of past anammox activity based on diagenetic products of ladderane lipids is only feasible for relatively immature sediments and sedimentary rocks. Since ladderane lipids are likely to be present in low abundance in the depositional environment (e.g., 5 to <0.5 ng/g sediment; Jaeschke et al., unpublished results) and a large part (>70%) of them are lost during the earliest stages of maturation, new analytical methods targeting diagenetic products of ladderanes will need to be developed in order to trace their presence in ancient sediments.

4. Conclusions

Artificial maturation of anammox bacterial biomass by hydrous pyrolysis revealed that ladderane lipids rapidly undergo structural modification due to breakdown of the condensed cyclobutane rings at temperatures as low as 120 °C, forming thermally more stable compounds. Thermally stable products derived from ladderane lipids with a [3]-ladderane moiety were detectable by GC-MS from hydrous pyrolysis experiments at temperatures as high as 260 °C, which corresponds to C_{31} 17α,21β(H)-homohopane 22S/(22S + 22R) ratios of 0.2 or ββ/(αβ+βα+ββ) ratios of >0.5. These diagenetic products might, in combination with their highly ^{13}C depleted carbon isotopic signature, constitute suitable biomarkers for the detection of past anammox activity in immature ancient sediments and sedimentary rocks.

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