

# A thermodynamic analysis of the anaerobic oxidation of methane in marine sediments

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## ABSTRACT

Anaerobic oxidation of methane (AOM) in anoxic marine sediments is a significant process in the global methane cycle, yet little is known about the role of bulk composition, temperature and pressure on the overall energetics of this process. To better understand the biogeochemistry of AOM, we have calculated and compared the energetics of a number of candidate reactions that microorganisms catalyse during the anaerobic oxidation of methane in (i) a coastal lagoon (Cape Lookout Bight, USA), (ii) the deep Black Sea, and (iii) a deep-sea hydrothermal system (Guaymas basin, Gulf of California). Depending on the metabolic pathway and the environment considered, the amount of energy available to the microorganisms varies from 0 to 184 kJ mol<sup>-1</sup>. At each site, the reactions in which methane is either oxidized to HCO<sub>3</sub><sup>-</sup>, acetate or formate are generally only favoured under a narrow range of pressure, temperature and solution composition – particularly under low (10<sup>-10</sup> M) hydrogen concentrations. In contrast, the reactions involving sulfate reduction with H<sub>2</sub>, formate and acetate as electron donors are nearly always thermodynamically favoured. Furthermore, the energetics of ATP synthesis was quantified per mole of methane oxidized. Depending on depth, between 0.4 and 0.6 mol of ATP (mol CH<sub>4</sub>)<sup>-1</sup> was produced in the Black Sea sediments. The largest potential productivity of 0.7 mol of ATP (mol CH<sub>4</sub>)<sup>-1</sup> was calculated for Guaymas Basin, while the lowest values were predicted at Cape Lookout Bight. The approach used in this study leads to a better understanding of the environmental controls on the energetics of AOM.

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## INTRODUCTION

Because of its role as a greenhouse gas (Lashoff & Ahuja, 1990), much has been learned in recent years about the biogeochemical cycles influencing the sources, fluxes and sinks of methane on Earth (Kvenvolden, 1993; Mancinelli, 1995; Breas *et al.*, 2002; Whalen, 2005). In particular, considerable attention has been dedicated to better understand the consumption of methane in anoxic marine sediments, the largest known methane reservoir (Kvenvolden *et al.*, 1993). Although this process, referred to as the anaerobic oxidation of methane (AOM) (e.g. Hoehler *et al.*, 1994; Orphan *et al.*, 2001; Hinrichs & Boetius, 2002; Michaelis *et al.*, 2002; Orcutt *et al.*, 2005; Dale *et al.*, 2006; Jørgensen & Kasten, 2006; Nauhaus *et al.*, 2007), is responsible for oxidizing 90% of the methane produced *in situ* (Iversen, 1996; Reeburgh, 1996), little is known about the role of bulk chemical composition, temperature and pressure on the overall energetics of AOM and the suite of intermediate reactions

that have been hypothesized to occur during the oxidation of methane (Hoh & Cord-Ruwisch, 1997; Boetius *et al.*, 2000; Valentine & Reeburgh, 2000; Sørensen *et al.*, 2001). A thermodynamic approach is required to take into account mass-action effects that are generally ignored in biochemical descriptions of metabolic strategies (Hoh & Cord-Ruwisch, 1997), which are particularly important when these processes operate near equilibrium.

Since the pioneering work by Barnes & Goldberg (1976) and Reeburgh (1976), a variety of studies have addressed the energetics of AOM, and more specifically, determined the energy available to the organisms that catalyse the reactions describing methane oxidation. Several authors have reported thermodynamic calculations for AOM-related reactions at or near 25 °C and 0.1 MPa (Hoehler *et al.*, 1994; Schink, 1997; Boetius *et al.*, 2000; Valentine & Reeburgh, 2000; Sørensen *et al.*, 2001). Others (Hoehler *et al.*, 1998, 2002) have examined the effect of temperature and hydrogen concentration on sulfate reduction and methanogenesis in sediments



from Cape Lookout Bight. Kallmeyer & Boetius (2004) measured the rates of sulfate reduction and AOM in sediments from Guaymas Basin over a range of temperatures and pressures while Treude *et al.* (2005) investigated how variations in temperature and methane and sulfate concentrations affected the rate and distribution of AOM in Eckernförde Bay (Germany). However, these studies and many others that have examined or reviewed the thermodynamics of AOM (Martens & Berner, 1977; Hoehler *et al.*, 1994; Sørensen *et al.*, 2001) did not account for the large differences in temperature, pressure and bulk composition that can be found in the various sedimentary settings where AOM occurs.

The purpose of this study is to quantify the thermodynamic drive of the AOM process, including reactions involving potential intermediate compounds, and to assess the maximum amount of ATP that can be synthesized by AOM-based microbial communities. The latter allows for a better understanding of the role of past and present AOM communities on the methane cycle (Dale *et al.*, 2008) since the energy required to synthesize ATP from ADP and monophosphate is commonly used as a proxy for potential microbial growth (Hoehler, 2004). The approach builds on recent work on the thermodynamics of bioenergetic processes (Shock, 1992; McCollom & Shock, 1997; Amend & Shock, 1998, 2001; Spear *et al.*, 2005; Dick *et al.*, 2006; LaRowe & Helgeson, 2007). It uses recently available thermodynamic data and equation of state parameters to calculate the energetics of AOM reactions and the maximum amount of ATP produced by the microbes catalysing these reactions *in situ* (LaRowe & Helgeson, 2006a, b). Three study sites for which data were available in the literature were chosen for their diversity in temperature, pressure and bulk composition: a deep-sea hydrothermal system (Guaymas Basin), a deep-seated sediment in an enclosed marine basin (Black Sea), and a shallow, temperate coastal lagoon (Cape Lookout Bight).

## AOM AND ATP REACTIONS

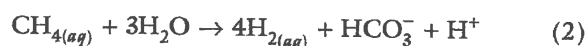
### AOM reactions

Numerous field- and laboratory-based studies support the hypothesis that AOM is coupled to the reduction of sulfate in a sediment depth interval known as the sulfate–methane transition zone (SMTZ) (for reviews see Hoehler & Alperin, 1996; Valentine & Reeburgh, 2000; Hinrichs & Boetius, 2002; Orcutt *et al.*, 2005; Jørgensen & Kasten, 2006). The overall process can be described by the following chemical reaction<sup>1</sup>:



However, because a single microorganism capable of catalysing Reaction 1 has not been discovered (Jørgensen & Kasten,

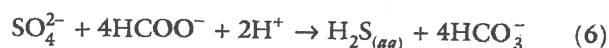
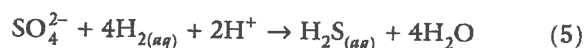
2006) while fluorescence *in situ* hybridization (FISH) and phylogenetic analyses (Orphan *et al.*, 2001, 2002; Hinrichs & Boetius, 2002) have revealed that methane-oxidizing archaea and sulphate-reducing bacteria are closely associated in the SMTZ, it has been postulated that a consortium of microbes catalyse AOM and sulfate reduction as distinct processes linked by shared intermediate chemical species such as  $\text{H}_2$ , formate ( $\text{HCOO}^-$ ) and acetate ( $\text{CH}_3\text{COO}^-$ ) (Hoehler *et al.*, 1994; DeLong, 2000; Hinrichs & Boetius, 2002; Michaelis *et al.*, 2002). For example, it has been suggested that methane could be oxidized via any combination of the following reactions (Valentine & Reeburgh, 2000; Sørensen *et al.*, 2001):



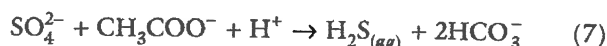
and/or



Syntrophic sulfate-reducing bacteria would then consume the products of Reactions 2–4, that is,



and/or



The sum of Reactions 2 and 5 is equal to Reaction 1, the overall AOM process. However, only 1 mole of formate is produced per mole of methane oxidized in Reaction 3, but 4 moles of formate are oxidized per mole of sulfate reduced in Reaction 6. Similarly, 1 mole of acetate is produced for every 2 moles of methane oxidized in Reaction 4 while the ratio of acetate oxidized per mole of sulfate is one in Reaction 7. Nevertheless, because 3 and 4 moles of  $\text{H}_2$  are also produced per mole of methane oxidized in Reactions 3 and 4, the intermediate reactions can be combined in such a way that the overall stoichiometric ratio of methane oxidized and sulfate reduced is the same as in Reaction 1. That is, 4(Reaction 3) + (Reaction 6) + 3(Reaction 5) = 4(Reaction 1) and (Reaction 4) + (Reaction 7) + (Reaction 5) = 2(Reaction 1). This combination is a direct consequence of the fact that eight electrons are transferred during the reduction of  $\text{SO}_4^{2-}$  to  $\text{H}_2\text{S}$  while only two and six electrons are transferred during the oxidation of formate and acetate to 1 and 2 moles of  $\text{HCO}_3^-$ , respectively. In order to quantify the respective influence of temperature, pressure and solution composition on the ability of microorganisms to extract energy from Reactions 1–7, the chemical affinity,  $A$ , was computed as described in the

<sup>1</sup> Chemical formulas followed by a charge or the subscript (aq) designate aqueous species.

**Table 1** Temperatures, pressures and bulk compositions used to calculate the values of chemical affinity

	Black Sea (Station 7)	Guaymas Basin (Stations 1 and 2)	Cape Lookout Bight (Station A1)
Temperature (°C)	9 <sup>a</sup>	2.8–96.6 <sup>b</sup>	14.5 (winter)/27 (summer) <sup>c</sup>
Pressure (bars)	120 <sup>d</sup>	200 <sup>d</sup>	1 <sup>d</sup>
CH <sub>4</sub> (mM)	0.02–2.3 <sup>e</sup>	0.0014 <sup>f</sup> , 0.443 <sup>g</sup> , 14 <sup>h</sup>	0.09–1.65 <sup>i</sup>
SO <sub>4</sub> <sup>2-</sup> (mM)	4.3–14 <sup>e</sup>	16–29 <sup>j</sup>	0.1–28 <sup>i</sup>
H <sub>2</sub> S (mM)	1.5 <sup>k</sup>	0.5–1.1 <sup>j</sup>	1.5 <sup>j</sup>
pH	7 <sup>m</sup>	7 <sup>m</sup>	7 <sup>m</sup>
HCO <sub>3</sub> <sup>-</sup> (mM)	10 <sup>n</sup>	10 <sup>n</sup>	10 <sup>n</sup>
HCOO <sup>-</sup> (M) (formate)	10 <sup>-7</sup> , 10 <sup>-6</sup> , 10 <sup>-5o</sup>	10 <sup>-6</sup> , 10 <sup>-4</sup> , 10 <sup>-2o</sup>	10 <sup>-7</sup> , 10 <sup>-5</sup> , 10 <sup>-3o</sup>
CH <sub>3</sub> COO <sup>-</sup> (M) (acetate)	10 <sup>-7</sup> , 10 <sup>-6</sup> , 10 <sup>-5p</sup>	10 <sup>-6</sup> , 10 <sup>-4</sup> , 10 <sup>-2q</sup>	10 <sup>-7</sup> , 10 <sup>-5</sup> , 10 <sup>-3r</sup>
H <sub>2</sub> (M)	10 <sup>-10</sup> , 10 <sup>-9</sup> , 10 <sup>-8 s,t</sup>	10 <sup>-10</sup> , 10 <sup>-9</sup> , 10 <sup>-8 s,t</sup>	10 <sup>-10</sup> , 10 <sup>-9</sup> , 10 <sup>-8 u,t</sup>

<sup>a</sup>Jørgensen *et al.* (2001); <sup>b</sup>calculated from gradients given in Weber and Jørgensen (2002); <sup>c</sup>Hoehler *et al.* (1994); <sup>d</sup>calculated from depth below sea level and taken from the same source as the temperature data; <sup>e</sup>taken from Jørgensen *et al.* (2001) and shown in Fig. 1A; <sup>f</sup>seawater-sediment interface value taken from Teske *et al.* (2002); <sup>g</sup>intermediate value also used to generate Figs 4a–C, and 7C; <sup>h</sup>average value of venting fluid taken from Welhan (1988). All three of the methane concentrations given for Guaymas Basin were used to generate Fig. 3; <sup>i</sup>taken from Hoehler *et al.* (1994) and shown in Fig. 1C,D; <sup>j</sup>profile taken from Weber and Jørgensen (2001) ( $\text{SO}_4^{2-}$  – Station 2,  $\text{H}_2\text{S}$  – Station 1) and shown in Fig. 1B. The concentrations for  $\text{SO}_4^{2-}$  and  $\text{H}_2\text{S}$  shown in Fig. 1B are used to calculate values of A; <sup>k</sup>not reported for this site but this value is within the range reported for other Black Sea sediments reported by Bohrmann *et al.* (2003); <sup>l</sup>Hoehler *et al.* (1998) use an estimate of  $1.0 \pm 0.4$  mm for calculations involving Cape Lookout Bight sediments; <sup>m</sup>taken to be that of the interior of microbes (Voet *et al.*, 1999); <sup>n</sup>not reported at all depths at these sites, but taken to be constant over the depth profiles (Dale *et al.* 2006); <sup>o</sup>this range of values was taken in a first approximation to be equal to those of acetate; <sup>p</sup>Wellsbury and Parkes (1995) report that typical acetate concentrations in similar environments are between 0–15 mM; <sup>q</sup>Martens (1990) reports this range of acetate concentrations at Guaymas Basin at different locations than where the sulfate and methane profiles were taken. The higher concentrations used are expected in Guaymas because thermal alteration of organic-rich sediments are thought to undergo accelerated degradation leading to increases in dissolved organic compounds (Rushdi and Simoneit, 2002; Simoneit and Sparrow, 2002). Furthermore, in a set of experiments and observations, Wellsbury *et al.* (1997) report tens of mM of acetate in heated coastal sediments and warm sediment cores; <sup>r</sup>this range of values encompasses estimates by Hoehler *et al.* (1994) for Cape Lookout Bight (1 to 3 mM) at the same site and measurements by Sansone and Martens (1981 & 1982) in the area (~60–760 mM). The latter values were reported as acetate per liter of bulk wet sediment and were converted to traditional concentration units using the porosity data from Klump (1980); <sup>s</sup>Hoehler *et al.* (2002) note that a typical value for natural systems is  $7 \times 10^{-8}$  M, but a range of values were used here to illustrate the influence of  $\text{H}_2$  concentrations; <sup>t</sup>a concentration of  $\text{H}_2 = 10^{-9}$  M is used to calculate values of A for Reactions 3 and 4; <sup>u</sup>although from a different sample core, Hoehler *et al.* (1998) report  $\text{H}_2$  concentrations ranging from 1.5–12 nM and 0.5–3.7 nM in the summer and winter, respectively.

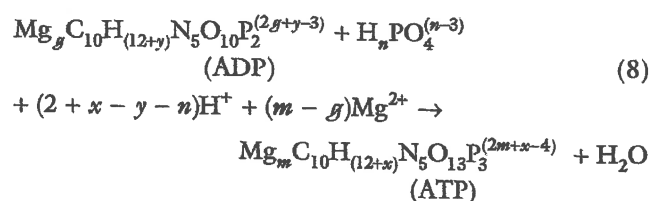
Appendix. Compositional data reported in the literature and summarized in Table 1 were used to characterize the activities of the reactants and products at the three sites.

### ATP reaction

Reactions 1–7 summarize oxidation–reduction processes that microorganisms can catalyse to harvest energy. Organisms convert much of this energy into ATP, a multipurpose biological molecule that can be used to promote otherwise energetically unfavourable reactions. Just as the Gibbs energy of Reactions 1–7 can vary as a function of temperature and pressure, the same is true for the synthesis of ATP (LaRowe & Helgeson, 2007). Therefore, quantifying the impact of temperature and pressure on AOM also requires an accounting of their influence on the energetics of biochemical reactions. Because of the universality of ATP, the thermodynamic potential for its synthesis is used here as a proxy for biochemical productivity. The method used to calculate the maximum amount of ATP that can be synthesized by AOM-microbial communities as a function of temperature and pressure is described as follows.

ATP synthesis can be quantified by accounting for the thermodynamic properties of individual species in stoichiometric and charge-balanced reactions. These species are represented in reactions by explicit chemical formulas whose thermody-

dynamic properties can be evaluated as a function of temperature and pressure. The synthesis of ATP from ADP and monophosphate can be written as:



where  $g$  and  $m$  refer to the number of moles of Mg atoms per mole of ADP and ATP, respectively,  $n$ ,  $y$  and  $x$  refer to the number of moles of H atoms per mole of phosphate and the reference basis species for ADP ( $\text{C}_{10}\text{H}_{12}\text{N}_5\text{O}_{10}\text{P}_2^{3-}$ , or  $\text{ADP}^{3-}$ ) and ATP ( $\text{C}_{10}\text{H}_{12}\text{N}_5\text{O}_{13}\text{P}_3^{4-}$ , or  $\text{ATP}^{4-}$ ), respectively. Values of the equilibrium constant for Reaction 8 can be calculated for a broad range of temperatures and pressures and combinations of values of  $g$  and  $m = 0, 1, 2$ ,  $n$  and  $y = 0, 1, 2, 3$ , and  $x = 0, 1, 2, 3, 4$  (see the Appendix for more details).

## SITE CHARACTERIZATION

Depth-dependent concentration profiles of several of the species that appear in Reactions 1–7 have been reported in the literature for the three sites discussed below. A selected set of measured species are presented in Fig. 1. Because concentration

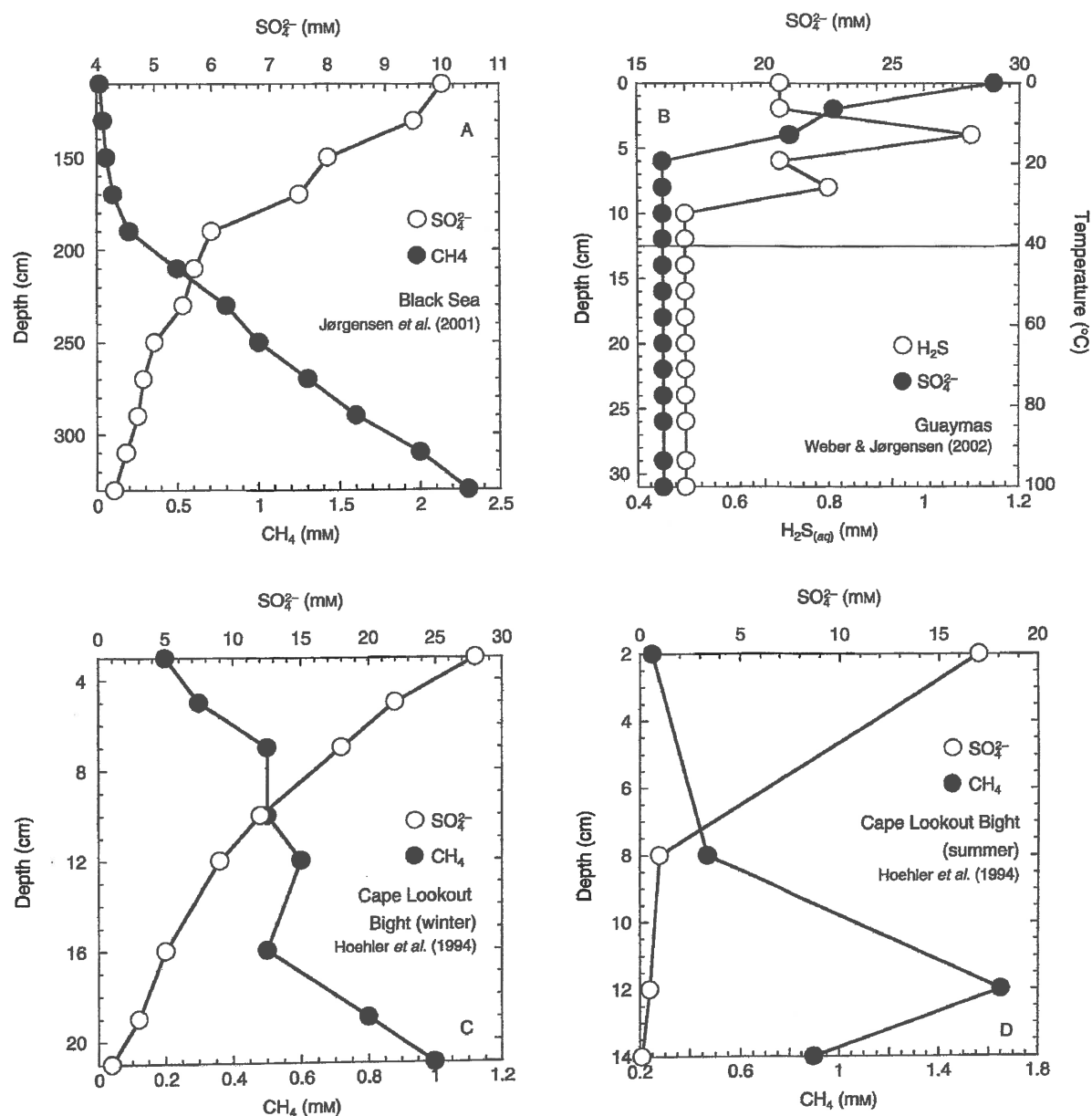


Fig. 1 Concentrations of selected species as a function of depth below the seafloor in the Black Sea (A), Guaymas Basin (B) and Cape Lookout Bight in the winter (C) and summer (D). The concentrations of sulfide and sulfate below the horizontal line in (B) were not measured and assumed equal to the concentration measured immediately above. Bibliographic references for these concentration profiles are given in each panel and Table 1.

profiles of hydrogen, formate and acetate are not available for these sites, a wide range of concentrations, constrained by typical values reported in the literature, have been used in the chemical affinity calculations.

### Black Sea

The Black Sea is the world's largest stratified water body, characterized by saline anoxic bottom waters separated from the brackish oxic surface waters by a stable halocline. The onset of salinization of the Black Sea began shortly after the last glacial maximum (10 ky ago) when Mediterranean water entered through the Bosphorus (Arthur & Dean, 1998). Today,

the deep waters are permanently sulfidic, and sulfate is the primary oxidant for organic matter in the sediments (Jørgensen *et al.*, 2001). AOM has been widely documented in the sediments and the water column of the Black Sea using  $^{13}\text{C}$  markers, archaeal lipids, 16S rRNA, incubation experiments and radiotracers (Reeburgh *et al.*, 1991; Jørgensen *et al.*, 2001; Michaelis *et al.*, 2002). The concentration profiles of sulfate and methane used for the chemical affinity calculations in the present study are taken from measurements at Station 7 (43°31'61 N, 030°13'33 E) in the study of Jørgensen *et al.* (2001) and are shown in Fig. 1(A). Here, methane transport towards the sediment surface occurs by molecular diffusion only and externally impressed fluid advection is absent.

## Guaymas Basin

Located in the Gulf of California, Mexico, Guaymas Basin is an active hydrothermal deep-sea spreading centre covered by a thick layer of rapidly accumulating ( $>0.1 \text{ cm year}^{-1}$ ) organic-rich sediments (2–4% organic C) (Simoneit *et al.*, 1979; Curray *et al.*, 1982; Von Damm *et al.*, 1985). The bottom waters are oxygen depleted ( $<80 \mu\text{M}$ ). The sediment temperature at the site analysed in this study increases from  $2.8^\circ\text{C}$  at the sediment–water interface to  $97^\circ\text{C}$  at 31 cm depth (Weber & Jørgensen, 2002). The hydrothermal fluids are essentially recirculated sea water rich in volatile fatty acids derived from pyrolysis of complex organic substrates at depths where the temperature exceeds  $100^\circ\text{C}$  (Martens, 1990). The sediments are sulfidic and anoxic throughout, and sulfate reduction rates may exceed  $2.0 \mu\text{mol cm}^{-3} \text{ day}^{-1}$  close to the sediment surface (Jørgensen *et al.*, 1992; Elsgaard *et al.*, 1994; Weber & Jørgensen, 2002; Kallmeyer & Boetius, 2004). Methane is present in the vent fluids at 12–16 mm with a  $\delta^{13}\text{C}\text{-CH}_4$  isotopic composition of around  $-50\text{‰}$ , consistent with a thermocatalytic origin (Welhan, 1988). Diagnostic lipid biomarkers with  $\delta^{13}\text{C}$  composition and 16S rRNA analyses indicate that methanotrophic archaea are active in Guaymas Basin (Teske *et al.*, 2002; Schouten *et al.*, 2003). Considered collectively, the available experimental evidence suggests that sulfate reduction coupled to AOM is an important barrier to methane efflux in this environment. The role of temperature, pressure and substrate characteristics on AOM in Guaymas basin has received sporadic attention (Jørgensen *et al.*, 1992; Elsgaard *et al.*, 1994; Weber & Jørgensen, 2002; Kallmeyer & Boetius, 2004), but the overall quantitative impact of these variables on the energetics of AOM has not yet been determined. The concentration profiles of sulfate and sulfide used in the present study were taken from measurements at stations 1 ( $27^\circ\text{N}$   $00.762$ ,  $111^\circ\text{W}$   $24.656$ ) and 2 ( $27^\circ\text{N}$

$00.764$ ,  $111^\circ\text{W}$   $24.558$ ) in Guaymas Basin (Weber & Jørgensen, 2002) and are shown in Fig. 1(B). The temperature gradient ( $2.8^\circ\text{C}$  to  $97^\circ\text{C}$  over 31 cm) was taken from Station 2. A range of methane concentrations, constrained by a measurement at the sediment–water interface (Teske *et al.*, 2002) and the average methane concentration of venting fluids at this site (Welhan, 1988) were used in the affinity calculations.

## Cape Lookout Bight

Cape Lookout Bight is a 10-m deep barrier lagoon on the coast of North Carolina, USA. This site is characterized by extremely high sedimentation rates ( $\sim 10 \text{ cm year}^{-1}$ ) and sulfidic sediments below a very thin oxic layer (Chanton *et al.*, 1987). Seasonal variations in water temperature from  $6^\circ\text{C}$  in winter to  $28^\circ\text{C}$  in summer lead to a seasonal vertical migration of the sulfate penetration depth between 8 and 25 cm depth (see Fig. 1C,D) (Klump & Martens, 1989; Hoehler *et al.*, 1994). There is no well-defined SMTZ, and methane concentration increases roughly linearly from the sediment–water interface down through the sulfate reduction zone. Sediment manipulation experiments have shown that AOM rates are sensitive to hydrogen concentration and seasonal temperature variations (Hoehler *et al.*, 1994). The concentration profiles of sulfate and methane used in the present study correspond to measurements at Station A-1 (Hoehler *et al.*, 1994) and are shown in Fig. 1(C,D) (winter and summer).

## RESULTS

### Chemical affinity of the overall AOM reaction

Values of the chemical affinities,  $A$ , for Reaction 1 (the net AOM process) are shown in Fig. 2. They are positive at all

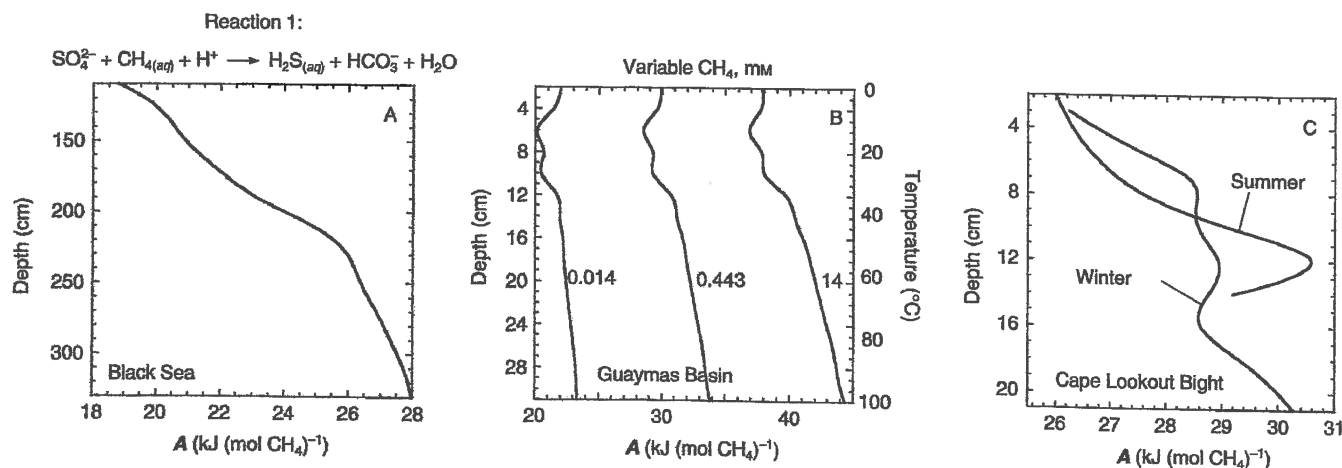


Fig. 2 Chemical affinities for the net anaerobic oxidation of methane (AOM) reaction (Reaction 1) as a function of sediment depth below the seafloor in the Black Sea (A), Guaymas Basin (B) and Cape Lookout Bight in the winter and summer (C). The three lines in (B) refer to values of  $A$  calculated for three methane concentrations (in mM). The concentrations of the other species used to generate these curves are given in Table 1.

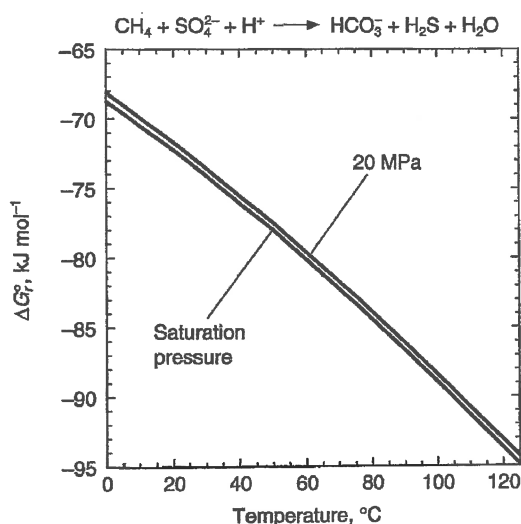


Fig. 3 Standard molal Gibbs energy of the net anaerobic oxidation of methane (AOM) reaction (Reaction 1) as a function of temperature at saturation pressure and 20 MPa.

sediment depths for all three sites, that is, the overall AOM reaction is always thermodynamically favoured and the overall trend is an increase in  $A$  with increasing depth. It should be noted that the sediment depths at which values of  $A$  were calculated are different for each site. The highest value is achieved in Guaymas Basin for the case of methane concentration = 14 mM. The greatest range, however, is found in the Black Sea sediments. In Cape Lookout Bight, the thermodynamic drive for Reaction 1 decreases as a function of depth for sediment depths >12 cm in the summer.

The different values of  $A$  shown in Fig. 2 are influenced by temperature, pressure and bulk composition. However, it can be seen in Fig. 3 that pressure has a minimal effect on the energetics of Reaction 1. In this figure, the standard molal Gibbs energy of the net AOM reaction ( $\Delta G_r^\circ$ ) is shown as a function of temperature at saturation pressure (the pressure at which water remains in the liquid phase: 0.1 MPa from 0 to 100 °C and up to 0.24 MPa at 125 °C) and at 20 MPa. Despite the large pressure difference between the calculated values of  $\Delta G_r^\circ$  for Reaction 1, the difference in the Gibbs energy of this reaction is less than 1 kJ mol<sup>-1</sup>. Temperature is of much greater significance for the overall energetics of AOM, whereby an increase from 0 °C to 125 °C increases the exergonicity of Reaction 1 from ~-68 kJ mol<sup>-1</sup> to ~-95 kJ mol<sup>-1</sup>, regardless of the pressure.

### Chemical affinity of intermediate AOM reactions

Because depth-dependent concentrations of the intermediate species, hydrogen, formate and acetate, are not available for the three sites considered here, a range of concentrations for each of these species was used to calculate the chemical affinity for Reactions 2–7 (see Table 1). The ranges chosen were based on single-depth measurements at these sites and, if not

available, at similar sites reported in the literature. For example, Hoehler *et al.* (2002) report a typical hydrogen concentration in 'most natural ecosystems' of  $7 \times 10^{-8}$  M, while Hoehler *et al.* (1998) report hydrogen concentrations ranging from  $0.5$ – $12 \times 10^{-9}$  M in Cape Lookout Bight, depending on the season. A  $H_2$  concentration range between  $10^{-10}$  to  $10^{-8}$  M was thus used in all chemical affinity calculations. In Guaymas Basin, thermal alteration of organic-rich sediments may lead to a high production of dissolved organic compounds (Rushdi & Simoneit, 2002; Simoneit & Sparrow, 2002). Similarly, Wellsbury *et al.* (1997) reported an increase in acetate concentrations by several orders of magnitude ( $\mu$ M to mM) by simply heating coastal marine sediments in the laboratory. Accordingly, and following Martens (1990), a wider range of higher concentrations of formate and acetate was used to calculate  $A$  for Guaymas Basin ( $10^{-2}$  to  $10^{-6}$  M) compared to the other sites ( $10^{-5}$  to  $10^{-7}$  M for the Black Sea and  $10^{-3}$  to  $10^{-7}$  M for Cape Lookout Bight).

Values of  $A$  for Reactions 2–4, describing methane oxidation to carbon compounds of varying average nominal oxidation state, are shown in Fig. 4. Each of the panels presents several curves that refer to values of  $A$  corresponding to different concentrations (M) of hydrogen, formate and acetate used in the calculations. Except for Guaymas Basin, the curves in Fig. 4 are constructed using the methane depth profiles shown in Fig. 1. In the case of Cape Lookout Bight (panels g–i), two sets of curves are shown, which correspond to solute concentrations and temperature conditions in winter (dashed lines) and summer (solid lines), respectively. Because the products of Reactions 3 and 4 include two types of reactive intermediate species (formate and hydrogen and acetate and hydrogen, respectively), ranges of the concentrations of both sets of species are presented in panels b, e and h (Reaction 3) and c, f and i (Reaction 4). At the Black Sea site, and for the selected concentrations of  $H_2$ , formate and acetate, the chemical affinities are mostly negative at all depths. Therefore, Reactions 2–4 are not thermodynamically favoured at this location, except under subnanomolar concentration of hydrogen for Reactions 2 and 4 (Fig. 4A,C) and subnanomolar hydrogen combined with submicromolar formate concentrations for Reactions 3 (Fig. 4B). In contrast, the values of  $A$  for Reactions 2–4 are positive at most depths in Guaymas Basin and an order of magnitude higher than at the other sites despite the fact that a higher range of acetate and formate concentrations are used. Negative values occur only at the shallow, cooler portion of the sediment profile if high hydrogen ( $10^{-8}$  M), acetate and formate concentrations are specified. Our calculations reveal that in Guaymas Basin (Fig. 4D–F) the thermodynamic drive for methane oxidation increases with depth and shows a strong vertical gradient in affinity. Since constant methane concentrations are employed, the gradient is exclusively due to the effect of the down-core temperature increase on the equilibrium constant,  $K$ , for these reactions.

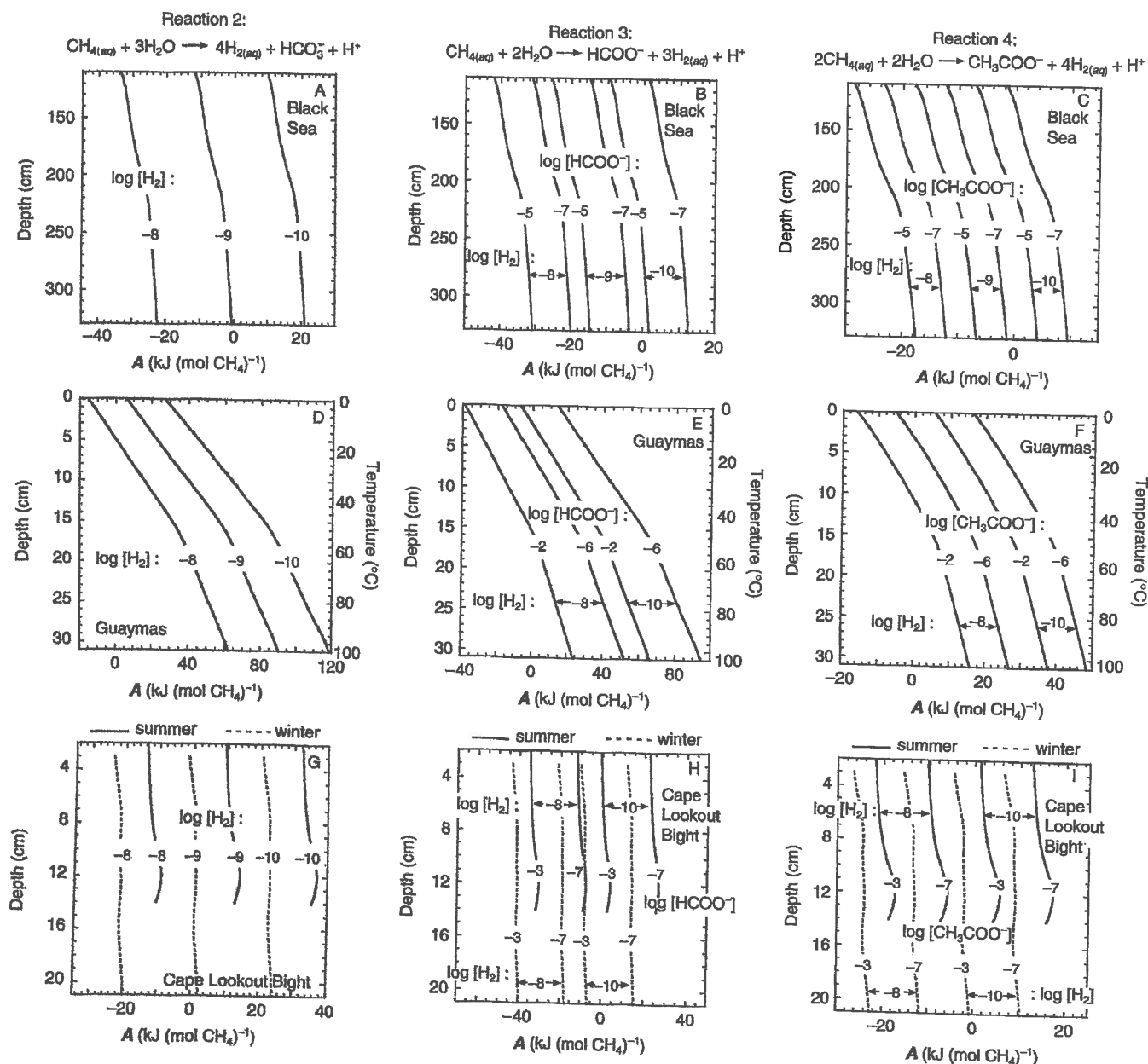


Fig. 4 Chemical affinities for Reactions 2–4 as a function of sediment depth below the seafloor in the Black Sea (top), Guaymas Basin (middle) and Cape Lookout Bight in the summer and winter (bottom). The reaction of interest is written above each column. The numbers that label the curves refer to the concentrations of H<sub>2</sub> (first column), formate and H<sub>2</sub> (second column) and acetate and H<sub>2</sub> (third column) used to calculate values of  $A$ . The concentrations of the other species used to generate these curves are given in Table 1. The temperature for Guaymas basin is labelled on the right sides of panels d–f.

The combined influence of temperature and composition can be observed at Cape Lookout Bight, where Reactions 2–4 are more favoured during the summer than in the winter. High hydrogen concentrations ( $10^{-8}$ ) lead to negative chemical affinities in the summer and winter at Cape Lookout Bight for Reactions 2–4 (Fig. 4G–I). Reactions 3 and 4 are only thermodynamically favoured at this coastal site if the hydrogen concentration is set to  $10^{-10}$  M, with lower formate and acetate concentrations accentuating this effect. In general, the thermodynamic drives for Reactions 2–4 to progress are favoured

by high temperatures (Guaymas Basin) and low concentrations of reactive intermediates. Also, because the stoichiometric coefficients for hydrogen are larger than they are for formate and acetate in Reactions 3 and 4, variations in hydrogen concentration influence the magnitude of  $A$  much more than variations in formate and acetate concentrations. In fact, the factor by which hydrogen concentrations quantitatively impact the values of  $A$  for Reaction 3 and 4 relative to formate and acetate, respectively, is the same as the stoichiometric ratio of these species in these reactions (since  $A$  is shown in kJ (mol



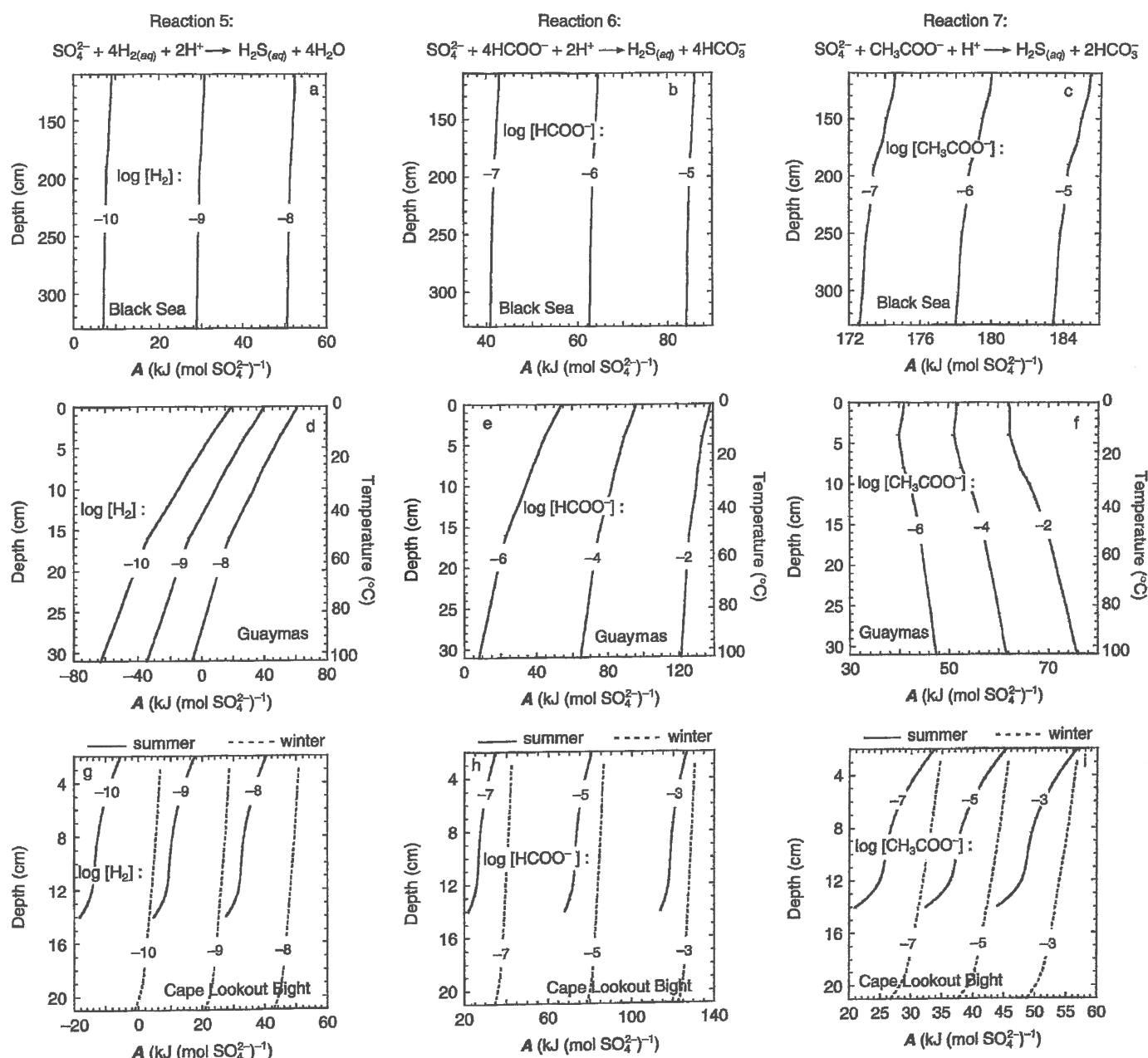


Fig. 5 Chemical affinities for Reactions 5–7 as a function of sediment depth below the seafloor in the Black Sea (top), Guaymas Basin (middle) and Cape Lookout Bight in the summer and winter (bottom). The reaction of interest is written above each column. The numbers that label the curves in each of the panels refer to the concentrations of  $\text{H}_2$  (first column), formate and  $\text{H}_2$  (second column) and acetate and  $\text{H}_2$  (third column) used to calculate values of  $A$ . The concentrations of the other species used to generate these curves are given in Table 1. The temperature for Guaymas basin is labelled on the right sides of panels d–f.

$\text{CH}_4$ )<sup>-1</sup>, this magnitude is not immediately apparent for Reaction 4 (Fig. 4C,F,I) in which 2 moles of methane are oxidized per mole of acetate produced).

Values of  $A$  for sulfate reduction coupled to the oxidation of hydrogen, formate and acetate (Reactions 5–7) are shown in Fig. 5, and are considerably higher than those obtained for the reactions producing the intermediates from methane oxidation (Fig. 4). Sulfate reduction coupled to the oxidation of the intermediate species leads to positive values of chemical affinity everywhere in the Black Sea and at nearly all depths in the Cape Lookout Bight sediments (the exception being a low

concentration of hydrogen ( $10^{-10}$ ) during the summer, Fig. 5G). This is also the case for Reactions 6 and 7 in Guaymas Basin. In contrast, the values of  $A$  for Reaction 5 at this site are only positive in the shallow parts of the sediment, especially for high  $\text{H}_2$  concentrations ( $>10^{-10}$  M), and negative in the deeper portion of the core where the temperature is higher (Fig. 5D). In general, the thermodynamic drive for sulfate reduction is strong due to large positive values of the equilibrium constant,  $K$ , for these reactions (not shown). Variations in temperature and solution composition have a smaller effect on Reactions 5–7 than on Reactions 2–4.

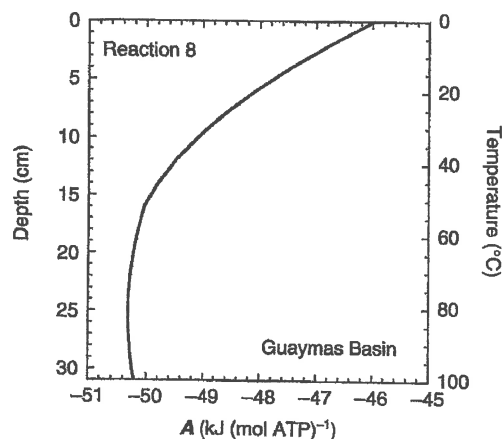


Fig. 6 Chemical affinities for the synthesis of ATP (Reaction 8) as a function of sediment depth below the seafloor in Guaymas Basin.

### ATP production in AOM communities

Values of  $A$  for ATP synthesis at Guaymas Basin are shown in Fig. 6 for constant total concentrations of ATP, ADP, magnesium, monophosphate and protons. Due to the steep thermal gradient, the chemical affinity decreases first with depth from about  $-46 \text{ kJ (mol ATP)}^{-1}$  at the sediment–water interface to a minimum of nearly  $-50 \text{ kJ (mol ATP)}^{-1}$  at ~25 cm. Thereafter,  $A$  increases very slightly with depth. Because the total concentrations of the reactants and products in the ATP reaction remain constant, the difference in the  $A$  values is a result of the temperature-dependent speciation of monophosphate, ADP and ATP (see Appendix). Therefore, because the range of temperature variation is minimal in the Black Sea and Cape Lookout Bight sediments, the values of  $A$  for ATP synthesis are nearly constant at  $-46.8 \text{ kJ (mol ATP)}^{-1}$  and  $-48.1 \text{ kJ (mol ATP)}^{-1}$  at Cape Lookout Bight in the winter and summer, respectively, while it is  $-46.4 \text{ kJ (mol ATP)}^{-1}$  in the Black Sea.

The amount of ATP that can be synthesized in sedimentary environments can be calculated assuming that the AOM-microbial communities acquire energy by catalysing the net Reaction 1. In this case, the energy available for AOM communities will vary not only as a function of the different temperature and pressure conditions, but also as a function of the concentration profiles of the species involved in this net reaction (Fig. 1). Using these measurements, the maximum potential for cellular metabolism, growth and reproduction is determined by calculating the maximum number of moles of ATP produced per mole of methane oxidized via Reaction 1 as a function of depth at each site (Fig. 7). The resulting ratio of ATP/CH<sub>4</sub> shows a range of values within and between the three sites, with the highest values ( $0.67 \text{ mol ATP (mol CH}_4\text{)}^{-1}$ ) at 30 cm in the Guaymas Basin sediments and lowest values at 100 cm in the Black Sea sediments ( $0.4 \text{ mol ATP (mol CH}_4\text{)}^{-1}$ ). At Cape Lookout Bight, ATP production varies from 0.54 to

$0.64 \text{ mol ATP (mol CH}_4\text{)}^{-1}$  in the summer and from 0.56 to  $0.64 \text{ mol ATP (mol CH}_4\text{)}^{-1}$  in the winter. The greatest range of variation is calculated for the Black Sea ( $0.4$  to  $0.6 \text{ mol ATP (mol CH}_4\text{)}^{-1}$ ) because the extensive SMTZ permits coexistence of methane and sulfate over a large depth interval.

## DISCUSSION

### AOM and intermediate species

The values of chemical affinity shown in Fig. 2 indicate that there is a strong thermodynamic drive for the net AOM reaction (Reaction 1) at all depths in each of the sediment cores considered here. However, because it is currently hypothesized that a consortium of microbes is required to catalyse the oxidation of methane and the reduction of sulfate in anoxic environments by the production and consumption of one or more intermediate species, we have also tested the viability of various two-step reaction pathways in environments characterized by different solution compositions, pressures and temperatures. If the oxidation of methane and the reduction of sulfate are indeed carried out by separate organisms, energy must be provided for the microbial group catalysing each of the individual reactions producing or consuming intermediate species. Using the range of concentrations of intermediate species summarized in Table 1, the chemical affinity computations suggest that CH<sub>4</sub> can be oxidized to either HCO<sub>3</sub><sup>-</sup>, formate, or acetate (Reactions 2–4, respectively) only in some portion of the sediment cores (Fig. 4). For Reactions 2–4, low reactive intermediate species concentrations are required to achieve positive  $A$  values in the Black Sea and Cape Lookout Bight sediments, and are favourable over a significantly wider concentration range in Guaymas Basin where the temperature increases rapidly as a function of depth. In contrast, sulfate reduction coupled to the oxidation of these intermediate species (Reactions 5–7) is strongly favoured at all three sites except for Reaction 5 in the deeper portion of the Guaymas Basin sediments where temperature is high and in Cape Lookout Bight under low hydrogen concentrations (Fig. 5). Although the ranges of concentrations of hydrogen, formate and acetate used in the  $A$  calculations cover a broad and realistic set of compositional conditions, constraining further the thermodynamic calculations demands, together with sulfate and methane, depth-dependent profiles of reactive intermediate concentrations.

The large range of temperatures, pressures and bulk compositions that have been used to quantify the thermodynamic feasibility of two-steps in AOM reaction pathway provide a reference frame to discuss results from similar investigations. For example, based on thermodynamic and kinetic calculations, Sørensen *et al.* (2001) have concluded that a consortium of anaerobic methane-oxidizing microorganisms cannot utilize hydrogen or acetate as reactive intermediates. These results were obtained at 25 °C, presumably 0.1 MPa,

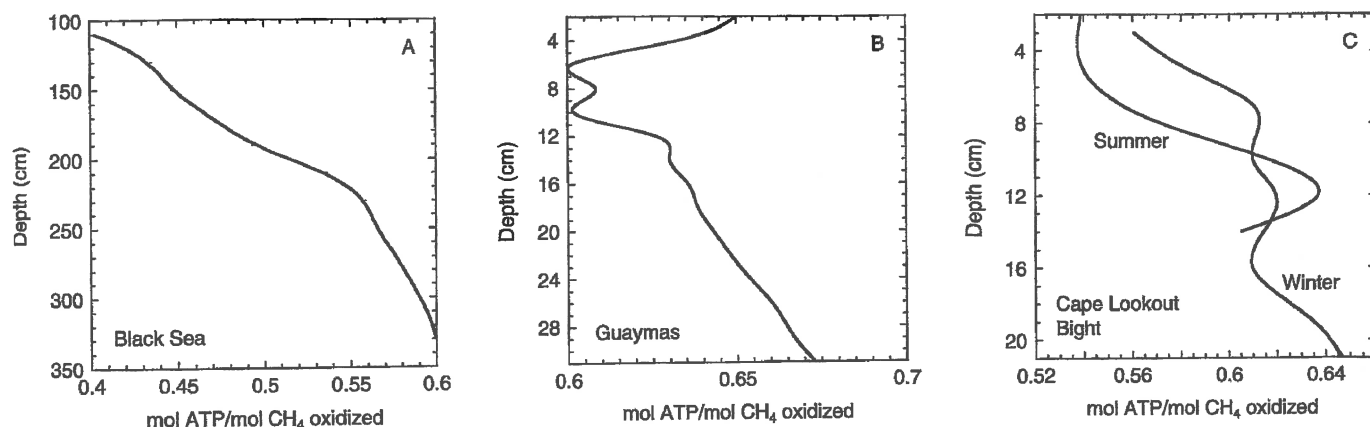


Fig. 7 Maximum number of moles of ATP that can be produced per mole of methane oxidized via Reaction 1 as a function of sediment depth below the seafloor in the Black Sea (A), Guaymas Basin (B), and Cape Lookout Bight in the winter and summer (C).

using constant concentrations of most of the reactants and products and fixed values of activity coefficients. However, the panels in Figs 4 and 5 show that a thermodynamic drive for methane oxidation and sulfate reduction coupled to any of the intermediate species may exist at least in some portion of the sediment cores considered in this study. Yet, its magnitude is highly dependent on substrate concentrations and, in the case of Guaymas Basin, temperature. Increasing the concentrations of hydrogen, formate and acetate decreases the thermodynamic drive of Reactions 2–4 at all locations. However, high concentrations of intermediate species ( $10^{-8}$  M for hydrogen and  $10^{-2}$  M for the organic acids) are not sufficient to thermodynamically inhibit these reactions at the high temperatures found in the deeper section of the Guaymas basin sediment core. In incubation experiments from a methane seep sediment (Hydrate Ridge), Nauhaus *et al.* (2002) found no conclusive evidence that hydrogen, formate or acetate were acting as intermediate compounds in AOM communities, and concluded that these species are unlikely intermediates in AOM. Instead, to account for the light isotopic fractionation found in the putative AOM consortium of methane-oxidizing archaea and sulfate-reducing bacteria, they suggested that the sulfate reducers simply assimilate  $\text{CO}_2$  or other carbon-containing waste compounds produced by the methane oxidizers. In another study, Orphan *et al.* (2002) identified two groups of methane-oxidizing archaea (ANME-1 and ANME-2) living in bacteria–archaea consortia but also in monospecific aggregations, that is, archaea communities not closely associated with bacteria. More recently, Moran *et al.* (2007) failed to observe an inhibitory effect of high hydrogen concentration on methane oxidation in AOM communities and suggested that rather than hydrogen, methylsulfides could serve as AOM intermediate species.

The results presented here reveal that, from a strict thermodynamic perspective, reactive intermediate species are not required for AOM to proceed, and, under certain conditions may actually preclude methane oxidation if the reactive

intermediate species reach inhibiting concentrations. For example, it is thermodynamically unfavourable for methane to be oxidized to  $\text{HCO}_3^-$ , formate or acetate in the Black Sea site for the range of reactive intermediates that were used in the present study. Therefore, if a consortium of organisms is required for AOM (Boetius *et al.*, 2000), the role of the different groups of microbes might be more complex than that of one oxidizing  $\text{CH}_4$  and another reducing  $\text{SO}_4^{2-}$ . For example, one organism could perform the net AOM reaction alone, while the other could catalyse a reaction that does not affect the concentrations of  $\text{CH}_4$  and  $\text{SO}_4^{2-}$ , but that is nonetheless closely associated with the AOM microbes.

### AOM and ATP

Although the rate at which organisms can catalyse low-energy yielding catabolic reactions might serve as a limiting factor for growth, it has been shown both experimentally (Nauhaus *et al.*, 2007) and theoretically (Dale *et al.*, 2006) that AOM microbes have a doubling time of several months and therefore microbial growth rates determined *in situ* might only be 1% (Konhauser, 2007) or less (Madigan *et al.*, 1997) of those observed in the laboratory where optimized growth conditions are established. This extremely sluggish, yet finite, microbial growth supports the notion (Kleerebezem & Stams, 2000; Adams *et al.*, 2006; LaRowe & Helgeson, 2007) that as long as there is a positive chemical affinity for a given chemical reaction, microorganisms could be capable of catalysing the process to generate ATP. In this study, we have shown that the maximum amount of ATP that can be generated from the catalysis of methane oxidation coupled to sulfate reduction (Reaction 1) depends on the temperature, pressure and bulk composition of the environment in which the organism is living, and therefore varies significantly from site to site and within each site. It is important to note that the ATP yields calculated here are maximum values because all of the energy from Reaction 1 is assumed to be channelled into

ATP production. Also, because depth distributions of measured AOM rates show that the overall drive for AOM may also be determined by the kinetics of methane oxidation (Regnier *et al.*, 2005), high ATP yields predicted from thermodynamic calculations do not necessarily imply that AOM will occur. Furthermore, the variation in ATP production calculated here is almost entirely due to the differences in methane and sulfate concentrations because the compositions of microbial interiors are assumed to be constant. Larger variations could thus arise due to cellular-level changes in compositions with depth. Currently, the constant total concentration assumption is necessary because the intracellular composition of the relevant species are currently not known for the microbes living at these sites, and are poorly known in general (see the Appendix for selected species concentrations). More data on the composition of cellular fluids will help constrain further the energetics of ATP synthesis.

## CONCLUSIONS

Solution chemistry and thermodynamics have been used to quantify the energetics of the reactions describing anaerobic oxidation of methane in three different environments. We have shown that (i) temperature sharply affects the energetics of these reactions, (ii) pressure has little influence and (iii) depending on the site and the sediment depth, the variable concentrations of the reactants and products may lead to significant variation in the thermodynamic drive of the net AOM reaction. Furthermore, in an effort to determine whether AOM is catalysed by a single organism or by a consortium of separate methane-oxidizing archaea and sulfate-reducing bacteria, the energetic feasibility of using hydrogen, formate and acetate as intermediate species in such a syntrophic community has also been tested. Since the concentrations of these intermediate species are not well-known for the particular sediment profiles used in this study, the thermodynamic calculations were performed over a broad range of likely values that are constrained by the data available in the literature. The results of these calculations suggest that, except in sediments characterized by a significant flow of heat from hydrothermal sources or by very low concentrations of reactive intermediates, methane oxidation coupled to the production of these intermediate species is thermodynamically unfavourable. In contrast, the reactions that describe the reduction of sulfate coupled to the consumption of the same intermediate species are generally favoured substantially.

The maximum amount of ATP that can be produced by the net AOM reaction at each of the study sites has been calculated in order to determine how pressure and temperature influence the speciation of ATP, ADP and monophosphate, and thus, the energetic cost of producing ATP *in situ*. Our results reveal that a thorough thermodynamic analysis of a particular biogeochemical system provides a means to quantify the

influence of environmental parameters on the energetics of reactions and aids analyses of hypotheses concerning the dominant processes defining coupled methane-sulfate cycles.

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## APPENDIX

Chemical affinity ( $A$ ) is used to quantify the thermodynamic potential associated with chemical reactions. At constant temperature and pressure, the chemical affinity (De Donder,



1920, 1927; De Donder & Van Rysselberghe, 1936; Kondepudi & Prigogine, 1998) of a given reaction can be expressed as:

$$A = -\frac{dG}{d\xi} = -\sum_i \mu_i \nu_i = RT \ln\left(\frac{K}{Q}\right) \quad (9)$$

where  $G$  corresponds to the Gibbs energy of the system,  $\xi$  designates the reaction progress variable for the reaction,  $R$  stands for the gas constant,  $T$  represents the absolute temperature in Kelvin,  $K$  refers to the equilibrium constant,  $\mu_i$  indicates the chemical potential of the  $i$ th species in the reaction and  $\nu_i$  stands for the stoichiometric reaction coefficient of the  $i$ th species in the reaction. Finally,  $Q$  denotes the reaction quotient, which is given by

$$Q = \prod_i a_i^{\nu_i} \quad (10)$$

where  $a_i$  corresponds to the activity of the  $i$ th species in the system. Values of  $K$  were calculated using the standard molal thermodynamic properties and revised HKF equations of state parameters for the species of interest (Johnson *et al.*, 1992; LaRowe & Helgeson, 2006a,b) together with the revised HKF equations of state (see Helgeson & Kirkham (1974a,b, 1976); Helgeson *et al.* (1981); Tanger & Helgeson (1988); Shock & Helgeson (1990)) using the SUPCRT92 software package (Johnson *et al.*, 1992). Positive values of  $A$  indicate that the reaction of interest is thermodynamically favoured such that the activities of the reactants decrease and those of the products increase. Chemical affinity is used because, as first shown by (De Donder, 1920),  $A$  represents the change in Gibbs energy of the system caused by an infinitesimal increment of reaction progress. Chemical affinity is thus the actual driving force for reactions to proceed in the direction that leads to an overall minimum Gibbs energy for the system, regardless of whether the actual minimum is achieved in natural reaction processes (LaRowe & Helgeson, 2007).

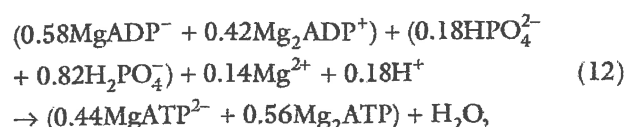
Because the marine sediment pore waters are relatively dilute, the value of  $a_i$  for  $H_2O$  was taken to be equal to unity at any pressure and temperature. The activity values of the other species were calculated using molal concentrations ( $m_i$ ) together with:

$$a_i = \gamma_i m_i \quad (11)$$

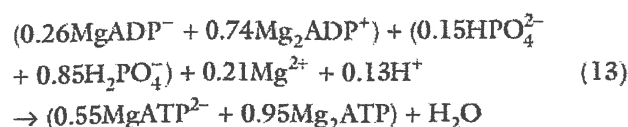
where  $\gamma_i$  represents the stoichiometric activity coefficient of the  $i$ th species. Values of  $\gamma_i$  for charged species were calculated using the extended Debye-Hückel equation (Helgeson, 1969), while those for neutral species were computed with the Setchénow equation (Oelkers & Helgeson, 1990) using the HCh software package (Shvarov & Bastrakov, 1999; Shvarov, 1999). Values of  $a_i$  used to evaluate Eqn. 10 for the species shown in Reactions 1–7 were calculated using Eqn. 11 with the values of  $m_i$  given in Table 1.

The standard state adopted here for aqueous species other than  $H_2O$  corresponds to that of unit activity in a hypothetical one molal solution referenced to infinite dilution at any pressure and temperature. The standard molal thermodynamic properties of charged aqueous species are consistent with the hydrogen ion convention (see LaRowe & Helgeson (2007)). The standard molal Gibbs energies and enthalpies required to calculate values of  $K$  are expressed as apparent standard molal Gibbs energies and enthalpies of formation (Benson, 1968; Helgeson, 1969; Helgeson & Kirkham, 1974b, 1976).

In order to quantify the energy required to synthesize ATP, the total concentrations of ADP, ATP, phosphate and magnesium and the pH were estimated for intracellular microbial fluids. The concentrations of total phosphate, ADP and ATP were set to 4 mM (Voet *et al.*, 1999), 1 mM and 2 mM (Thauer *et al.*, 1977), respectively. The pH was set equal to 7 and the total initial concentration of  $Mg^{2+}$  was taken as 50 mM. This value of  $Mg$  was chosen since cellular concentrations higher than seawater (52.82 mM) (Millero, 2002) are unlikely because they are bound to macromolecules (Fagerbakke *et al.*, 1999). For the same reason, the intracellular ionic strength was assumed to be equal to sea water (0.7). The speciation calculations were also carried out using the standard molal thermodynamic properties and revised HKF equations of state parameters for individual ADP and ATP species (LaRowe & Helgeson, 2006a,b) and the HCh Gibbs energy minimization software package (Shvarov & Bastrakov, 1999; Shvarov, 1999). Values of  $A$  for Reaction 8 were then calculated using Eqns 9 and 10. The values of the  $n$ ,  $x$ ,  $y$  and  $z$  coefficients in Reaction 8 vary as a function of temperature, pressure and pH. For example, the explicit formulation for Reaction 8 that was used to calculate the chemical affinity for the synthesis of ATP at Guaymas basin at 3 cm depth in the sediment, corresponding to 10 °C, 20 MPa and pH = 7, can be written as:



where  $MgADP^-$ ,  $Mg_2ADP^+$ ,  $MgATP^{2-}$  and  $Mg_2ATP$  represent mono- and dicomplexed magnesium adenosine di- and triphosphate species. Under the same pressure, pH and pMg, but at 97 °C, that is, 31 cm at the same site, Reaction 8 becomes



Therefore, temperature alone alters significantly the ratio of species in what is classically considered to be the same chemical reaction.

