

## Living microbial ecosystems within the active zone of catagenesis: Implications for feeding the deep biosphere

B. Horsfield <sup>a,\*</sup>, H.J. Schenk <sup>b</sup>, K. Zink <sup>a</sup>, R. Ondrak <sup>a</sup>, V. Dieckmann <sup>a</sup>, J. Kallmeyer <sup>a,1</sup>,  
K. Mangelsdorf <sup>a</sup>, R. di Primio <sup>a</sup>, H. Wilkes <sup>a</sup>, R.J. Parkes <sup>c</sup>, J. Fry <sup>c</sup>, B. Cragg <sup>c</sup>

<sup>a</sup> *GeoForschungsZentrum Potsdam, Telegrafenberg, 14473 Potsdam, Germany*

<sup>b</sup> *Forschungszentrum Jülich, 52425 Jülich, Germany*

<sup>c</sup> *Cardiff School of Biosciences, Cardiff University, Cardiff CF10 3TL, UK*

Received 24 November 2005; received in revised form 23 March 2006; accepted 23 March 2006

Available online 12 May 2006

Editor: Scott King

### Abstract

Earth's largest reactive carbon pool, marine sedimentary organic matter, becomes increasingly recalcitrant during burial, making it almost inaccessible as a substrate for microorganisms, and thereby limiting metabolic activity in the deep biosphere. Because elevated temperature acting over geological time leads to the massive thermal breakdown of the organic matter into volatiles, including petroleum, the question arises whether microorganisms can directly utilize these maturation products as a substrate. While migrated thermogenic fluids are known to sustain microbial consortia in shallow sediments, an in situ coupling of abiotic generation and microbial utilization has not been demonstrated. Here we show, using a combination of basin modelling, kinetic modelling, geomicrobiology and biogeochemistry, that microorganisms inhabit the active generation zone in the Nankai Trough, offshore Japan. Three sites from ODP Leg 190 have been evaluated, namely 1173, 1174 and 1177, drilled in nearly undeformed Quaternary and Tertiary sedimentary sequences seaward of the Nankai Trough itself. Paleotemperatures were reconstructed based on subsidence profiles, compaction modelling, present-day heat flow, downhole temperature measurements and organic maturity parameters. Today's heat flow distribution can be considered mainly conductive, and is extremely high in places, reaching 180 mW/m<sup>2</sup>. The kinetic parameters describing total hydrocarbon generation, determined by laboratory pyrolysis experiments, were utilized by the model in order to predict the timing of generation in time and space. The model predicts that the onset of present day generation lies between 300 and 500 m below sea floor (5100–5300 m below mean sea level), depending on well location. In the case of Site 1174, 5–10% conversion has taken place by a present day temperature of ca. 85 °C. Predictions were largely validated by on-site hydrocarbon gas measurements. Viable organisms in the same depth range have been proven using <sup>14</sup>C-radiolabelled substrates for methanogenesis, bacterial cell counts and intact phospholipids. Altogether, these results point to an overlap of abiotic thermal degradation reactions going on in the same part of the sedimentary column as where a deep biosphere exists. The organic matter preserved in Nankai Trough sediments is of the type that generates putative feedstocks for microbial activity, namely oxygenated compounds and hydrocarbons. Furthermore, the rates of thermal degradation calculated from the kinetic model closely resemble rates

\* Corresponding author.

*E-mail address:* [horsf@gfz-potsdam.de](mailto:horsf@gfz-potsdam.de) (B. Horsfield).

<sup>1</sup> Present address: University of Rhode Island, Graduate School of Oceanography, South Ferry Road Narragansett RI 02882, USA.

of respiration and electron donor consumption independently measured in other deep biosphere environments. We deduce that abiotically driven degradation reactions have provided substrates for microbial activity in deep sediments at this convergent continental margin.

© 2006 Elsevier B.V. All rights reserved.

*Keywords:* deep biosphere; Bio-Geo coupling; basin modelling; biogeochemistry; geomicrobiology; Nankai Trough

## 1. Introduction

The biosphere is the part of planet Earth inhabited by life. It occurs in three overlapping zones: the *lithosphere*, comprising soils, sediments and sedimentary rocks; the *hydrosphere*, that part of the lithosphere either covered by water or containing water (within pores and fractures); and the *atmosphere*, the gaseous envelope surrounding the Earth. Microorganisms make up a major component of the Earth's biomass because they can grow under a wide range of conditions and have diverse metabolisms. Aerobic bacteria use free oxygen to degrade and metabolise labile organic substrates, but where oxygen has already been consumed and cannot be replenished, for instance in fine grained sediments, anaerobic microorganisms take over degradation using other terminal electron acceptors (oxidisers) such as sulphate, nitrate, manganese, iron and carbon dioxide. Anaerobes are the dominant inhabitants of the lithosphere. Their abundance in sedimentary basins shows a general decrease with increasing depth, eventually petering out as organic matter becomes too recalcitrant to be degraded or because water, nutrients and terminal electron acceptors cannot be supplied or because temperatures are too high.

In recent years it has come to light that surprisingly large indigenous microbial populations with considerable diversity are present in sedimentary rocks at hundreds or in some cases thousands of metres depth [1–4]. The organic matter supporting microbial metabolism in deep subsurface environments probably originates within the deep subsurface itself though the mechanisms involved remain poorly understood [5]. Fermentation products from indigenous organic matter in shales could diffuse to microbial communities residing in juxtaposed porous facies, for example [6]. Moreover, elevated temperatures (30–90 °C) acting over short periods (*weeks and months*) increase the rate of microbial degradation in laboratory experiments [7,8]. In the case of buried sedimentary organic matter in the natural system, residence times at these elevated temperatures (30–90 °C and beyond) extend way beyond human timescales, lasting millions of years. This has two ramifications.

Firstly, the aforementioned *biological* fermentations should be able to take place throughout long periods of basin history, with rates being temperature dependant. Secondly, kinetically controlled *abiotic* organic maturation reactions such as condensation and elimination (zone of diagenesis), cracking and aromatisation (zone of catagenesis) become significant, converting recalcitrant organic matter such as kerogen into volatile products which include petroleum [9]. The intriguing question is whether all or some of the abiotic reaction products can be utilised by microorganisms as substrates in the deep biosphere [8,10]. While migrated or migrating thermogenic fluids are already known to sustain microbial consortia at gas hydrates [11,12], petroleum reservoirs [13] and surface expressions of leakage such as carbonate mounds [14,15], an in situ coupling of abiotic generation and microbial utilization has not been demonstrated up to now. Indeed, classical geochemical concepts state that the two should be separated from one another in time and space but, as alluded to above, our concept of the biosphere is being driven to include greater depth intervals. It is therefore likely that some overlap between the biosphere and zones of thermal cracking will occur. Indeed, microorganisms have been detected in geological systems up to 100 °C [65] and cultured up to 121 °C [16], and the approximate present day temperature for the onset of petroleum generation for a “typical” petroliferous basin (heating rate 1–5 K/Ma) lies between 70 and 120 °C [17–19].

In this paper we show that deep microbial ecosystems and active hydrocarbon generation occur together in the Nankai Trough, and demonstrate that the rates of thermal degradation closely resemble rates of respiration and electron donor consumption in deep biosphere environments. We deduce that abiotically driven degradation reactions can provide substrates for microbial activity in deep sediments at this convergent continental margin.

## 2. Geological framework of the Nankai Trough

The subduction boundaries of the Pacific and Philippine Sea plates form deep trenches east of the

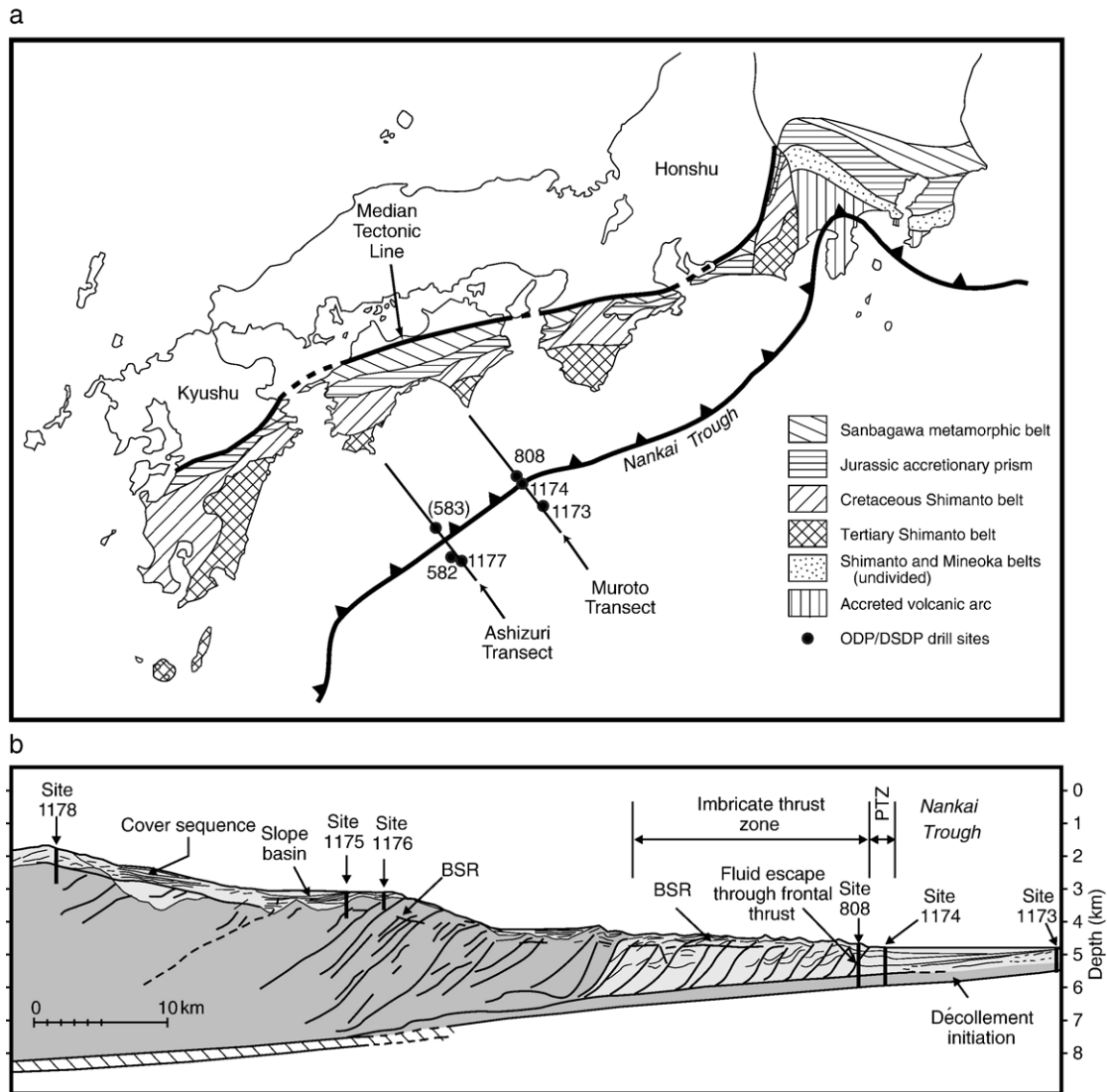


Fig. 1. a. Geologic map showing the Nankai Trough and the SW Japan forearc region with locations of the ODP sites used in this study [20]. b. Generalised cross section along the Muroto Transect, after Moore et al. [23]. PTZ=Protothrust Zone. BSR=Bottom Simulating Reflector.

Japanese Island arc system [20,21]. The Nankai Trough (Fig. 1a), located southeast of Shikoku Island, is the subducting plate boundary between the Shikoku Basin and the southwest Japan arc, and is an area of active sediment accretion. A sedimentary section, approximately 1 km thick, is accreted to or underthrust beneath the margin in the style of a fold and thrust belt, creating a large accretionary prism [20]. In the areas of ODP Leg 190, across the Muroto and Ashizuri Transects, the sedimentary section is divided into the undeformed Shikoku Basin and overlying trench fill, the protothrust zone, the imbricate thrust zone and the splay fault zone (Fig. 1b). Sedimentation started with the deposition of acidic

volcaniclastics followed by Middle Miocene to Middle Pliocene massive hemipelagite. From the Upper Pliocene to Lower Pleistocene, hemipelagites with tephra layers were deposited. During the Pleistocene the transition from hemipelagites to turbidite deposition took place followed by massive deposition of turbidites from the Pleistocene to Holocene. Seaward of the trench axis an additional unit is recognized below the Late Miocene hemipelagites which is characterized by a well-stratified sequence and correlated with Miocene turbidites. Variable but generally high heat flows have existed throughout the Cenozoic up to the present day near the Muroto Transect [20,21]. This anomalous behaviour has been attributed to both

Table 1  
Assignment of thermal conductivity (at 100 °C) to the various facies in Sites 1174, 1173, 1177

Facies in Nankai Trough [20]	Assumed thermal conductivity $W\ m^{-1}\ K^{-1}$ (100 °C)		
	1174	1173	1177
Trench-wedge	2.12 2.65 2.38 1.97	2.12	2.38
Trench basin transition	2.31	2.10	
Upper Shikoku Basin	2.31	2.10	1.94 2.10
Lower Shikoku Basin	2.33 2.38	1.94	1.94 1.97 2.37
Acidic volcanoclastic	2.10	2.13	2.10
Volcanic basement			1.97

The values were taken from Petromod© default lithologies.

hydrothermal circulation and the subduction of a former spreading ridge on the Philippine Sea plate, represented by the Kinan Seamounts; spreading only ceased at 15 Ma and its subsequent cooling has been restricted by a sedimentary blanket [22,23]. Rapid gradients in both temperature and thermal maturity occur. At ODP Leg 131 Site 808 the organic thermal maturity indicator vitrinite reflectance increases uniformly from 0.3% at the sediment–water interface to 0.7% at 1200 m [24], thereby indicating that petroleum-forming reactions are taking place towards the bottom of the drilled section. Indeed, both the Bernard Ratio [ $C_1/(C_2+C_3)$ ] and the stable isotopic composition of methane indicate shallow microbial gas gradually gives way to thermal gas with depth [25]. A similar scenario has unfolded at ODP Leg 190 Sites 1173 and 1174 where a change from microbial to thermal gas compositions accompanies rapidly increasing temperature [20]. The fact that living microorganisms are present up to 84 °C in these wells [26–31] makes the case all the more intriguing, and is the basis for considering (1) whether the biosphere actually overlaps with the zone of catagenesis at these locations, and (2) whether the living systems could be fed by the products of thermal degradation.

### 3. Samples and methods

We address these points by determining the temperature history of the study area using site-specific kinetic modelling, and comparing the rates of the thermal cracking reactions (putative substrate supply) with those quoted in the literature for respiration and anaerobic methane oxidation in deep biosphere environments.

#### 3.1. Numerical modelling

Conductive heat-flow is dominant in the Nankai Trough [23]. A 1D finite-element model of transient heat flow [32] was used to reconstruct the thermal history of the area under investigation, with input coming from numerous ODP and DSDP sites. ODP Site 1174 is located in the protothrust zone representing a zone of incipient deformation and initial development of décollement. ODP Sites 1173 and 1177 were drilled in the undeformed sediments of the trench (Fig. 1a,b). Basement temperatures at sites 1173 and 1174 are above 100 °C (“hot sites”), whereas that at 1177 is less than 70 °C (“cool site”). Several lithostratigraphic units are distinguished by ODP [20,23]: from top to bottom these are the Holocene slope apron facies, Holocene–Pleistocene trench-wedge facies, the Pleistocene trench–basin transition facies, the Lower Pleistocene–Upper Pliocene Upper Shikoku Basin facies, the Middle Pliocene–Middle Miocene lower Shikoku Basin facies and an acidic volcanoclastic facies. The thermal conductivities assumed for numerical modelling are given in Table 1. Present day heat flow values and downhole temperature data were taken from ODP publications [20,23]. The temperature data and published vitrinite reflectance results [20,24,33] were used to calibrate the thermal history.

#### 3.2. Organic matter typing

To provide an initial characterisation of the organic matter, 36 samples of whole round cores from Sites 1173, 1174 and 1177 were analysed by Rock-Eval pyrolysis [34] and LECO combustion. All determinations were performed in triplicate; the values in Table 2 are averages.

#### 3.3. Kinetic parameters

Three samples from the “cool site”, Site 1177, with highest TOC contents were chosen for determining the kinetic parameters of thermal degradation. Briefly, each of the three samples were subjected to programmed-temperature, open-system pyrolysis at linear heating rates of 0.1, 0.7 and 5.0 K/min [35]. The heating rates were considered low enough to ensure correct temperature measurements (thermocouple immediately above the sample,  $\pm 1$  °C) and sufficiently different for obtaining reliable frequency factors and unequivocal kinetic solutions [36]. A constant flow of argon (45 ml/min) transported all volatile pyrolysis products to a flame ionisation detector for the continuous measurement of bulk generation rates. A compilation of gross formation rate versus temperature for each heating rate was

Table 2  
Organic geochemical screening analysis of samples from ODP Leg 190

E-nr.	ODP Site	Depth (mbsf)	TC (%)	TOC (%)	TS (%)	S1 (mg/g rock)	S2 (mg/g rock)	S3 (mg/g rock)	$T_{\max}$ (°C)	HI (mg/g TOC)	OI (mg/g TOC)
49801	1173	4.15	0.72	0.58	0.10	0.29	1.42	0.13	545	243	22
49802	1173	98.29	0.57	0.46	0.10	0.06	1.22	0.09	542	268	19
49803	1173	193.29	1.12	0.51	0.34	0.05	0.38	0.13	548	74	24
49826	1173	296.24	0.76	0.37	0.20	0.05	0.25	0.10	548	66	27
49804	1173	296.99	0.43	0.38	0.34	0.04	1.93	0.04	548	507	10
49805	1173	402.69	0.58	0.33	0.21	0.02	0.27	0.09	547	82	27
49806	1173	497.69	0.55	0.35	0.30	0.01	0.44	0.08	546	124	21
49807	1173	497.69	0.32	0.33	0.33	0.02	1.21	0.02	538	362	5
49827	1173	496.76	0.35	0.33	0.11	0.01	0.73	0.03	486	221	10
49828	1173	604.50	0.46	0.38	0.33	0.01	0.28	0.06	544	73	17
49808	1174	2.65	0.79	0.73	0.69	0.27	1.63	0.09	547	224	12
49809	1174	306.70	0.97	0.61	0.21	0.05	0.33	0.09	416	55	16
49810	1174	412.60	0.58	0.44	0.12	0.04	0.54	0.06	541	123	14
49811	1174	499.55	1.21	0.37	0.08	0.03	0.11	0.12	409	30	32
49829	1174	598.30	0.51	0.40	0.19	0.03	0.43	0.06	542	107	15
49812	1174	598.85	0.43	0.34	0.29	0.01	0.35	0.04	538	103	12
49830	1174	704.50	0.69	0.39	0.29	0.01	0.12	0.06	409	30	16
49813	1174	703.15	0.45	0.30	0.38	0.01	0.29	0.04	542	95	13
49831	1174	799.07	0.56	0.39	0.13	0.01	0.10	0.06	418	27	14
49814	1174	796.54	0.94	0.32	0.33	0.01	0.08	0.06	411	26	20
49832	1174	901.10	0.29	0.29	0.06	0.01	0.40	0.03	485	136	10
49815	1174	901.70	0.27	0.26	0.05	0.01	0.48	0.02	518	186	6
49816	1174	1021.40	0.58	0.38	0.30	0.01	0.16	0.05	424	43	13
49833	1174	1020.70	0.37	0.35	0.28	0.01	0.24	0.04	534	69	12
49817	1174	1091.50	0.40	0.38	0.23	0.01	0.58	0.03	425	154	8
49818	1177	302.99	0.49	0.43	0.44	0.09	1.40	0.06	546	323	15
49819	1177	401.35	0.61	0.42	0.05	0.03	0.32	0.09	546	76	21
49820	1177	523.30	0.37	0.35	0.08	0.06	1.71	0.03	491	488	9
49821	1177	608.77	0.97	0.97	0.75	0.11	0.99	0.06	413	103	6
49834	1177	607.86	0.31	0.27	0.15	0.03	1.00	0.04	426	365	14
<b>49822</b>	<b>1177</b>	<b>687.15</b>	<b>2.77</b>	<b>2.57</b>	<b>1.33</b>	<b>0.20</b>	<b>1.86</b>	<b>0.18</b>	<b>426</b>	<b>72</b>	<b>7</b>
49835	1177	686.59	1.26	0.78	0.56	0.08	0.78	0.22	423	100	28
49836	1177	792.37	0.21	0.13	0.03	0.03	1.01	0.07	545	750	51
<b>49823</b>	<b>1177</b>	<b>744.88</b>	<b>1.38</b>	<b>1.19</b>	<b>1.13</b>	<b>0.08</b>	<b>1.03</b>	<b>0.31</b>	<b>424</b>	<b>87</b>	<b>26</b>
<b>49824</b>	<b>1177</b>	<b>744.88</b>	<b>1.71</b>	<b>1.44</b>	<b>0.84</b>	<b>0.13</b>	<b>1.68</b>	<b>0.37</b>	<b>424</b>	<b>117</b>	<b>26</b>
49825	1177	790.79	0.21	0.23	0.04	0.02	0.89	0.05	540	380	23

Three samples from the “cool site” 1177 marked in bold were analysed by pyrolysis to determine kinetic parameters of thermal degradation.

TC=Total Carbon (%).

TOC=Total Organic Carbon (%).

TS=Total Sulphur (%).

S1=Thermovaporised Organic Compounds (mg/g rock).

S2=Organic Products from Pyrolysis (mg/g rock).

S3=CO<sub>2</sub> from Pyrolysis (mg/g rock).

$T_{\max}$ =Temperature of Maximum Generation Rate of S2 (°C).

HI=Hydrogen Index (S2\*100/TOC).

OI=Oxygen Index (S3\*100/TOC).

analysed assuming 48 first-order parallel reactions with activation energies regularly spaced between 35 and 82 kcal/mol and a single frequency factor for each sample. The kinetic parameters, when integrated with the thermal history [9] of Sites 1173 and 1174, were used to calculate the rates and timing of petroleum generation at these “hot” locations.

## 4. Results and discussion

### 4.1. Burial and temperature histories

The burial histories of all three sites are shown in Fig. 2. Site 1174 is characterized by a moderate rate of subsidence (ca. 50 m/Ma) during the Miocene and



Pliocene followed by extremely rapid burial (ca. 600 m/Ma) during the Quaternary. At the other two sites Miocene–Pliocene subsidence is about the same (30–50 m/Ma), but Quaternary subsidence is appreciably lower (75–100 m/Ma). Surface heat flow on the trough

floor varies between 130 and 200 mW/m<sup>2</sup>, variability being attributed to the nature of the subducted Philippine Sea Plate and fluid release from the accretionary prism [22,23,37,38].

Various heat flow scenarios were modelled. According to the relationship between heat flow and the age of oceanic crust [39,40] average heat flow for sites on the Muroto (Sites 1173, 1174; 17 Ma) and Ashizuri (Site 1177; 27 Ma) Transects would be expected to be 125 and 95 mW m<sup>-2</sup>, respectively. However, temperature and vitrinite reflectance trends [21,24,33] are often not reproduced using this model (Fig. 3). The same is true of smectite–illite conversion [22]. Additional heat is required throughout the recent geological past reflecting the increasing tectonic activity in the region of the frontal thrust. In this regard, growth of the seaward-most part of the prism took place very rapidly, with 40 km of accretion within the past 2 Myr, and most notably, the frontal thrust was drilled in 365 mbsf in Hole 808C where outer marginal trench wedge sediments about 0.46 Ma old are overthrust on lower axial trench wedge facies about 0.28 Ma old [20].

A modelled conductive heat pulse of 180 mW m<sup>-2</sup> acting during that part of the Quaternary associated with rapid accretion (<0.4 Ma) brings about a good match for Site 1174. A similar situation applies to Site 1173 (not shown); 200 mW m<sup>-2</sup> over the past 0.4 Ma gives a good match between predicted and measured temperatures. 180–200 mW m<sup>-2</sup> fall within the range of surface heat flow measurements. The heat-flow at Site 1177 appears to conform to the prediction of the Parsons and Sclater Model [40], namely 95 mW m<sup>-2</sup>. We have assumed that mainly conductive heat transport has thermally matured the organic matter in the sediments from the bottom upwards [24,25,33]. The influence of episodic lateral fluid flow on heat transport, though subsidiary [23], still needs to be clarified using 2D and 3D basin modelling.

The temperature fields superimposed on the subsidence curves in Fig. 2 are calculated from heat flow history and thermal conductivity as a function of lithology and degree of compaction. Heating rates of up to 130 K/Ma occurred during the most recent and rapid phase of burial at Site 1174, values that are ten to sixty times higher than in most petroliferous basins (1–20 K/Ma) [41].

#### 4.2. Organic matter composition and kinetic parameters

According to Table 2, and in accordance with ODP data [20], the organic matter content of all samples is extremely low, most TOC values lying below 1%, and the average being 0.52%. Kerogens are of Types II and

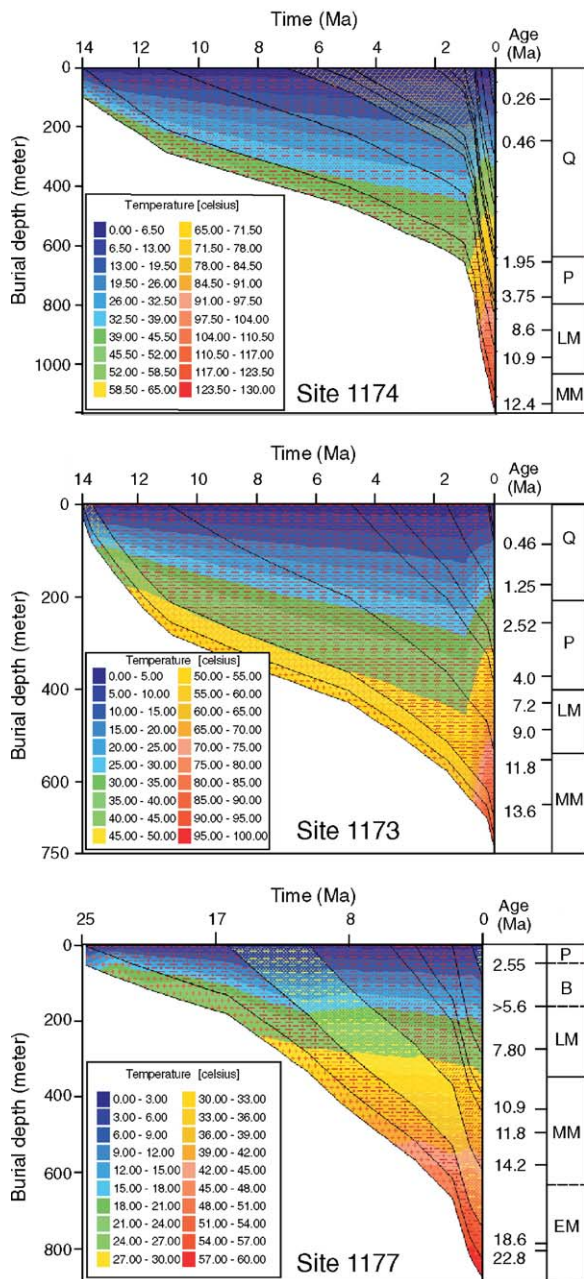


Fig. 2. Burial and temperature histories of ODP Leg 190 Sites 1174, 1173 and 1177 using a basal heat flow which varies as a function of the age of the oceanic lithosphere but increases during the Quaternary. Q=Quaternary; P=Pliocene; LM=Late Miocene; MM=Middle Miocene; EM=Early Miocene; B=Barren.

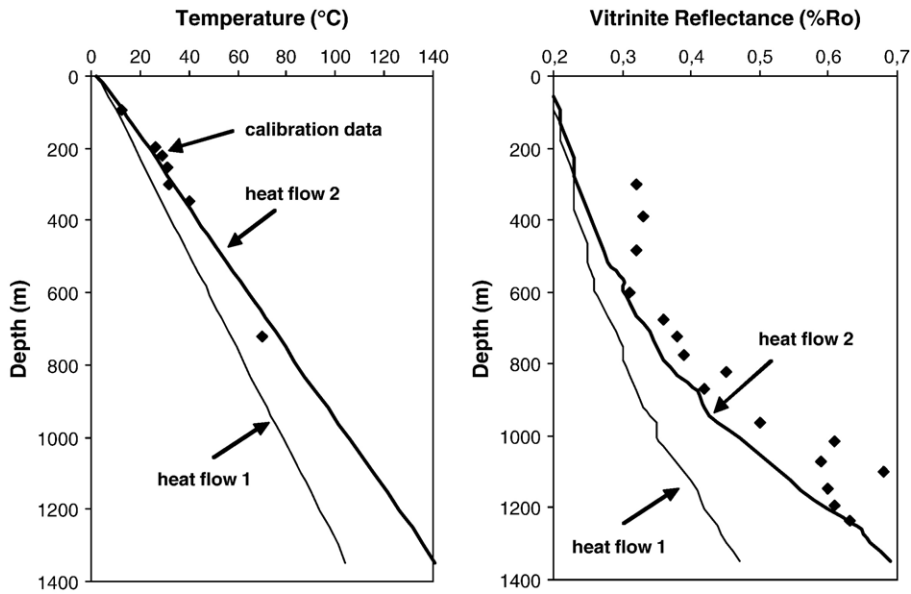


Fig. 3. Testing of two heat flow scenarios for Site 1174, using (a) calculated and measured temperature at the site and (b) calculated and measured vitrinite reflectance for the nearby (4 km) Site 808 [24]. Heat flow 1 is calculated according to the heat flow versus age relationship of Lister, 1977 [39]. Heat flow 2 includes an additional enhanced heat flow ( $180 \text{ mW m}^{-2}$ ) during the Quaternary [21].

III (Fig. 4). Samples with lower Hydrogen Index (HI) values tend to have the higher TOCs. For Sites 1173 and 1174, exposed to thermal stress, original HI may have been higher. Site 1177 has been least affected by maturation, and it is from here that three samples were

selected for measurement of kinetic parameters; all three contained Type III organic matter (Fig. 4).

It should be noted that  $T_{\text{max}}$  values are extraordinarily high ( $>500 \text{ }^\circ\text{C}$ ) throughout the entire section (Site 1173) or interspersed with lower values (Sites 1174, 1177),

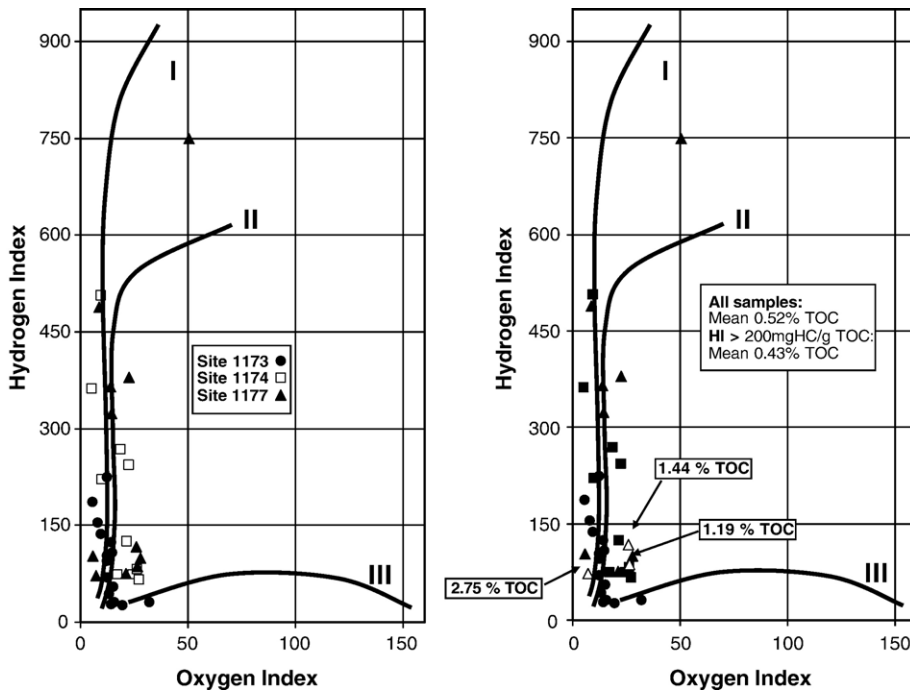


Fig. 4. Pseudo van Krevelen diagram used for distinguishing kerogen types. The three samples chosen for kinetic analyses are shown on the right.

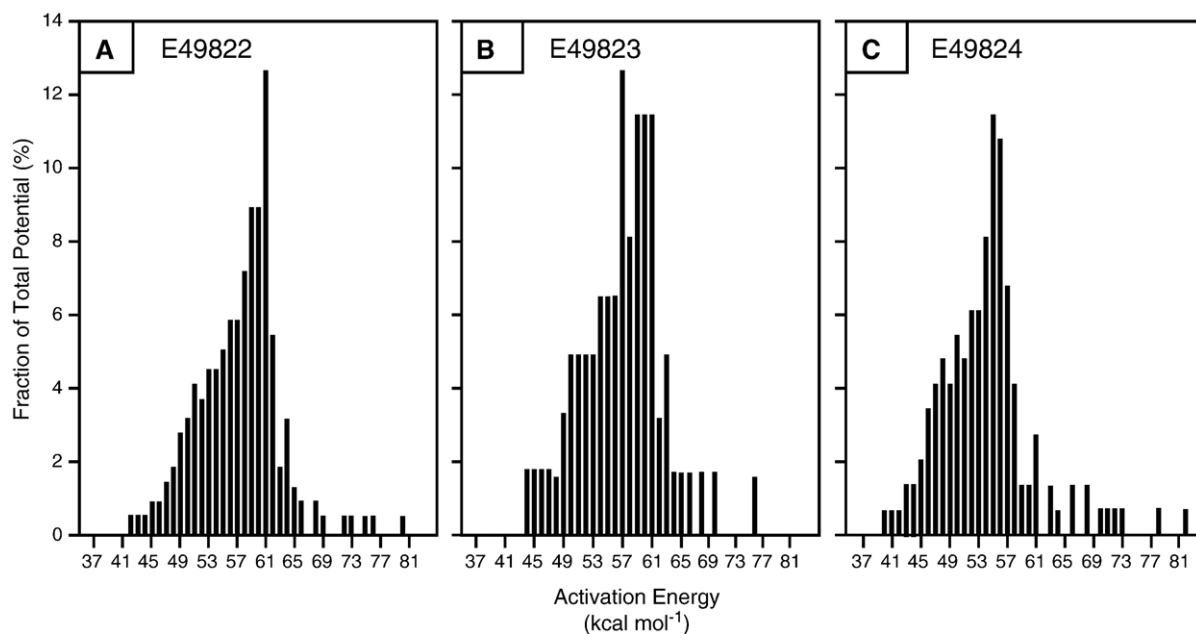


Fig. 5. Activation energy distributions for samples from ODP Leg 190 Site 1177: A. Sample E49822, with frequency factor of  $4.0 \times 10^{17} \text{ min}^{-1}$ . B. Sample E49823 with frequency factor of  $3.8 \times 10^{17} \text{ min}^{-1}$ . C. Sample E49824 with frequency factor of  $1.4 \times 10^{16} \text{ min}^{-1}$ .

and that HI is not inversely correlated with  $T_{\text{max}}$ . These phenomena can be directly traced to the extreme broadness and flatness of the S2 peak (numerator in the HI); for replicate analyses of a given sample,  $T_{\text{max}}$  can be low or high depending upon very small differences in peak shape. The components contributing to the broad S2 peak are elaborated on below.

Kinetic parameters for the three samples are shown in Fig. 5. All three activation energy distributions are extremely broad, reflecting the very heterogeneous nature of the sedimentary organic matter (Type III kerogen). Aromatised reworked particles [24] and plant detritus oxidised in the water column (settling time approximately 1–5 years according to Stokes' Law), both of which are thermally stable, help to account for higher activation energy potentials and for the high  $T_{\text{max}}$  values seen throughout the entire sample set (Table 2). Less altered organoclasts mainly of terrigenous origin account for potentials with lower activation energies. While bacteria have been reported in these sediments [27–29], their contribution to the activation energy distributions is very minor (0.00001–0.03%) judging by bacterial cell counts [20] and typical cell densities [42].

The kinetic parameters for two of the three samples were essentially identical (E49822 and E49823). Therefore, only E49823 and E49824 are presented below in the modelling of hydrocarbon generation.

### 4.3. Hydrocarbon generation in nature

Integrating kinetic parameters and thermal histories provided predictions of petroleum generation at Sites 1173 and 1174 (Fig. 6). At Site 1174 the thermal transformation of the sedimentary organic matter becomes significant at 500 m below sea floor (5300 m below sea level). This prediction is substantiated by gas logging profiles [20], which recorded the presence of predominantly dry microbial gas in the shallower parts of the borehole, thermogenic wetter gas in the deepest part and a mixing zone in between. A similar profile has been published for Site 808, 4 km to the northwest [20]. At Site 1173 transformation is predicted to start at around 300 m below sea floor (5100 m below sea level), and again there is good agreement with the gas compositions from logging [20]. In accordance with the principles of chemical kinetics, the shallow onset of generation has been brought about by extremely labile organic components being exposed to high temperatures over geological time. The high heating rate (130 K/Ma) may also have caused some parts of the kerogen which usually remain involatile at lower heating rates, to be cracked into volatile products [43].

We conclude that the shallow thermal gases in ODP Leg 190 Nankai Trough sediments are indeed generated in situ and have not migrated into the section from elsewhere.



### 5. Occurrence of viable organisms

Geomicrobiological and biogeochemical data for Sites 1173 and 1174 are presented in Figs. 7 and 8. Rates of

potential total microbial methanogenesis [23] at the two sites were measured in separate experiments using  $^{14}\text{C}$ -radiolabeled bicarbonate, methanol, hexadecane, benzoate and acetate. One-way analysis of variance between

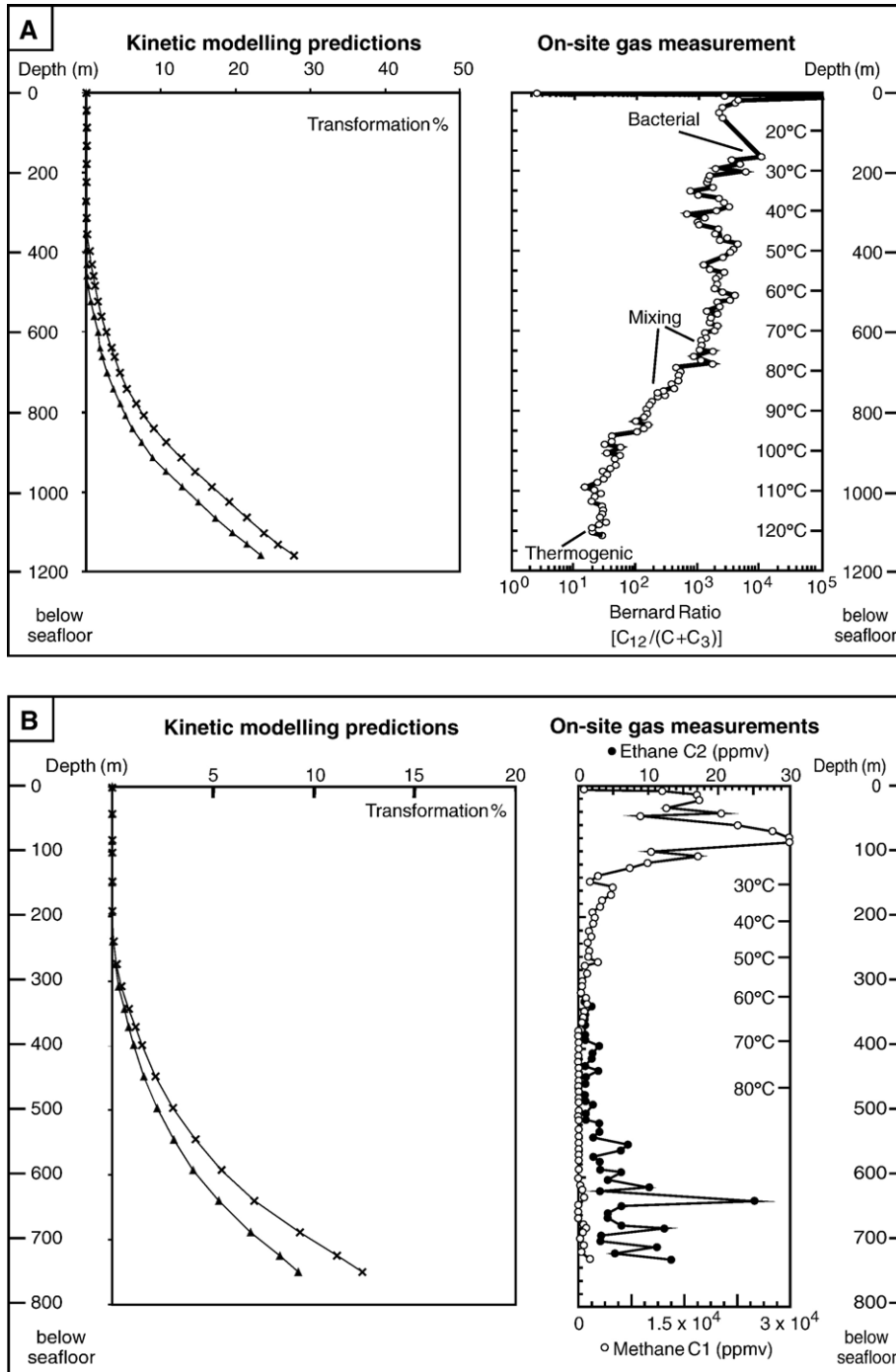


Fig. 6. Kinetic modelling predictions (left) and on-site gas measurements [20] (right) for (A) Site 1174 and (B) Site 1173. The labelling of bacterial, mixing and thermogenic for on-site gas measurements comes from ODP [20]. Generation curves were calculated using temperature history and the kinetics of organic compound generation using samples from ODP Leg 190 Site 1177 (Crosses = sample E49824; Triangles = sample E49823).

groups (ANOVA) shows that there is no significant difference between any of the mean total (summed from individual measurements for each substrate) methanogenesis rates at any of the depths, the reason for this being the high variability (mean standard deviation =  $1.59 \log_{10}$  units) of the measured rates and the small number of measurements ( $n=3$  at each depth). The values for methanogenesis are rather low in most cases ( $0.0015$ – $102.0 \text{ pmol d}^{-1} \text{ cm}^{-3}$ ) and the actual values are almost the same as those from the Woodlark Basin, another deep, low organic carbon site [44] and the equatorial Pacific Ocean (ODP sites 1225 and 1226, unpublished results). However, the trends still match the acridine orange direct count (AODC) trends and so are probably genuine; for example, for site 1174 the correlation between the mean  $\log_{10}$  AODC and rates of methanogenesis was significant ( $r=0.819$ ;  $P=0.024$ ). Methanogenesis also decreases to a minimum at approximately the same depth at which thermal generation of hydrocarbons becomes significant. At Site 1174 the AODC values decrease significantly ( $P<0.001$ ) with depth to about 550 mbsf, and increase thereafter. This agrees with the predicted generation of volatile organic matter below this depth. At Site 1173 the evidence for response of AODC to thermogenically

produced organic matter is not as clear. Although there is a significant increase in counts below about 460 mbsf there is no evidence above this depth.

Intact phospholipids are unambiguously indicative of living cells, as they are rapidly degraded even without the influence of microorganisms [45]. Intact phospholipids have been detected in Nankai Trough sediments to temperatures at least as high as  $84 \text{ }^\circ\text{C}$  [26]. Both the regular phospholipids and, in higher concentrations, lysophospholipids (e.g. lysophosphatidylcholines) have been detected. Diglyceride mass fragments of various phospholipids were assigned to fatty acyl side-chains of typical chain length ( $\text{C}_{14}$ ,  $\text{C}_{16}$ ,  $\text{C}_{18}$ ,  $\text{C}_{20}$  FA) and degree of unsaturation (zero, one or two double bonds). The enhanced occurrence of lysophospholipids was not explained completely by the authors, but a response to increasing thermal and ecological stress was suggested. As shown in Figs. 7 and 8, their occurrence overlaps with the generation curves. Microorganisms have been cultured from these deep Nankai Trough sediments [30,31].

We conclude that there is overlap between active catagenesis and living systems in the Nankai Trough, zones which are classically depicted as being separated

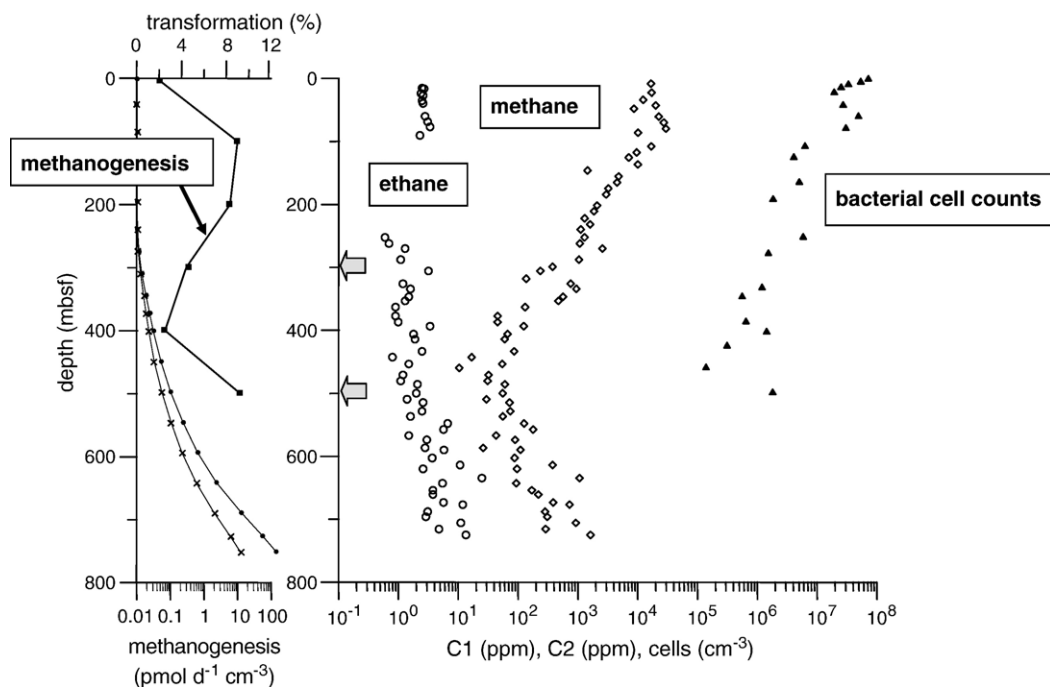


Fig. 7. Site 1173 geomicrobiology and biogeochemistry summary. Left panel: Generation curves from kinetic modelling (Crosses = sample E49824; Triangles = sample E49823) and experimentally determined combined rates of potential total methanogenesis (marked by squares; rates for all substrates summed). Right panel: On-site gas measurements in ppm [20] for methane (diamonds) and ethane (circles), and AODC total counts in  $\log_{10} \text{ cm}^{-3}$  (triangles). The arrows point to depths where intact phospholipids (PL) have been detected [26]. Note that while all depths are given in metres below sea floor, an additional 4800 m of water must be considered when considering pore pressure.

by kilometres of depth [46,47]. The key question is whether the two systems, bio- and geo-, are actually coupled. We have used the near-surface biosphere as an analogue to begin considering this possibility.

### 5.1. Feeding the deep biosphere — compositional considerations

In the near-surface biosphere, macromolecular components are decomposed into more simple molecules by hydrolytic and fermentative processes and subsequently these compounds, for instance short-chain fatty acids, alcohols, methylated amines and hydrogen [5], are utilised by other anaerobic microorganisms as substrates. If another source for these small functionalised compounds were to be made available, for example from condensation, elimination, aromatisation and cracking reactions (abiotic), instead of primary fermentation (biotic), the deep biosphere could become decoupled from the shallow surface biosphere as long as the other requirements for life, such as water and nutrients, were met [8].

There is already a wealth of published elemental, spectroscopic and pyrolytic data to demonstrate that indeed the required types of low molecular weight compounds can be generated abiotically during diagenesis and catagenesis in most sedimentary basins. The

dramatic fall in O/C, particularly for terrestrial organic matter, points to a significant release of oxygen-containing species such as  $\text{CO}_2$ , organic acids and  $\text{H}_2\text{O}$  to pore systems [48]. A weight loss of about 15% is associated with these changes, and is mainly related to the progressive elimination of the  $\text{C}=\text{O}$  group from ester and ketone groups [49]. Analytical pyrolysis has revealed that kerogens thermally degrade to yield many types of low molecular weight compounds including hydrocarbons, ketones, alcohols, nitriles and thiols [50–52]. Sulphur- and oxygen-containing compounds are abundant in the pyrolysis products of low maturity carbonates [53,54], their decrease thereafter being due to generation and release or incorporation in condensed ring systems [55]. The hydrous pyrolysis of kerogens produces copious quantities of acetate and acetic acid, which have been viewed both as direct natural gas precursors via thermal cracking as well as potential substrates for methanogenic activities [8,56,57]. Terrigenous organic matter in particular, the type present in the Nankai Trough, is thought to aromatise yielding free hydrogen, a co-substrate for  $\text{CO}_2$  reduction, beginning at low rank [58–60].

Based on generalised *compositional considerations* we conclude that the abiotically formed low molecular weight organic substrates generated from Type III

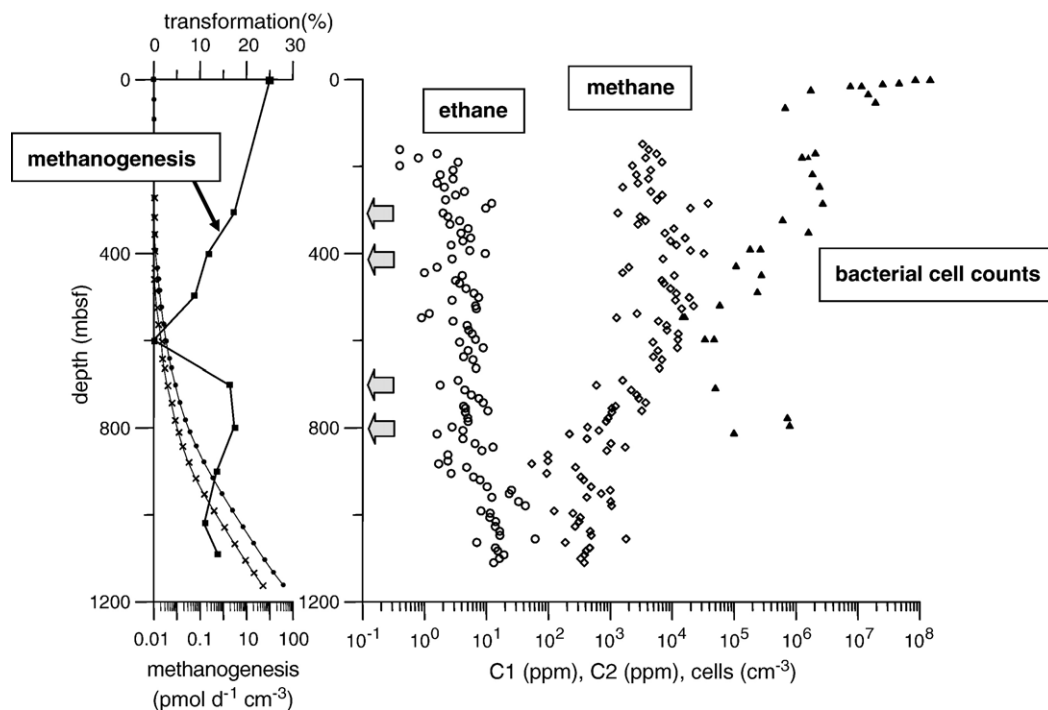


Fig. 8. Site 1174 geomicrobiology and biogeochemistry summary: symbols are identical to Fig. 7.

Table 3

Calculations of putative substrate generation from abiotic maturation as compared with rates of deep biosphere respiration [5,6] and anaerobic methane oxidation [61]

Nankai Trough — abiotic maturation of organic matter from kinetic modelling		
Rates of abiotic generation		
<i>Hydrogen Index in mg/g TOC</i>		100
<i>TOC in %</i>		1
<i>Transformation Ratio</i>		0.1
<i>Timeframe in Ma</i>		1
<i>Volatiles generated in mgHC/g TOC/Ma</i>		10
<i>Volatiles generated in mgHC/g TOC/yr</i>		$1 * 10^{-5}$
<i>Volatiles generated in mgHC/g sediment/yr</i>		$1 * 10^{-7}$
Madison aquifer — deep biosphere microbial activity [5,6]		
	Maximum	Minimum
Rates of respiration		
<i>CO<sub>2</sub> production in mmol/l/yr</i>	$1 * 10^{-3}$	$1 * 10^{-6}$
<i>CO<sub>2</sub> production in mg/l/yr</i>	$4 * 10^{-2}$	$4 * 10^{-5}$
<i>Stoichiometry</i>	0.68	0.68
Rates of OM utilisation (compare with abiotic maturation of organic matter see above)		
<i>Feedstock in mg/l/yr</i>	$2.72 * 10^{-2}$	$2.72 * 10^{-5}$
<i>Density</i>	2.00	2.00
<i>Feedstock in mg/kg sediment/yr</i>	$1.36 * 10^{-2}$	$1.36 * 10^{-5}$
<i>Feedstock in mg/g sediment/yr</i>	$1.36 * 10^{-5}$	$1.36 * 10^{-8}$
Rates of OM utilisation (compare with anaerobic sulphate reduction see below)		
<i>Assumed bacterial counts/ml</i>	$1 * 10^6$	$1 * 10^6$
<i>Feedstock in µg/cell/yr</i>	$2.72 * 10^{-8}$	$2.72 * 10^{-11}$
<i>Feedstock in mol/cell/yr</i>	$6.80 * 10^{-16}$	$6.80 * 10^{-19}$
Deep sea sediments [61]		
Anaerobic sulphate reduction in mol/cell/yr		
<i>ODP 798B (Japan Sea)</i>		$2.60 * 10^{-16}$
<i>ODP 681C (Peru Margin)</i>		$6.40 * 10^{-18}$
<i>ODP 1175 (Nankai Trough)</i>		$7.50 * 10^{-15}$

organic matter are of the type that can be utilised by microorganisms.

### 5.2. Rates of potential substrate provision

Because both the chemical reactions and biological ecosystems occur together, transport distances are very short (micrometres to metres), and hence *rates* of putative substrate supply from our kinetic modelling can be compared directly with rates reported in the microbiological literature. Calculations are presented in Table 3.

Considering the biological system first, CO<sub>2</sub> respiration rates between  $10^{-3}$  and  $10^{-6}$  mmol per litre per year have been reported in deep-aquifer sediments [5]. If a reduced organic carbon (ROC) substrate is assumed

(average composition CH<sub>2</sub>O; 30 g/mol) the rate of substrate utilisation would then amount to between approximately  $1.36 * 10^{-5}$  to  $1.36 * 10^{-8}$  mg ROC per g sediment per year. These values, converted into mol cell<sup>-1</sup> year<sup>-1</sup> are closely similar to mean sulphate reduction rates in the anaerobic methane oxidation zone [61]. We deduce that the range  $10^{-5}$  to  $10^{-8}$  mg substrate per g sediment per year can be taken as a good order of magnitude approximation of deep bacterial rates of substrate utilisation. Biomass production can be neglected in the calculation because the system is operating under survival conditions.

The next question is whether the products of catagenesis are generated at compatible rates. Microorganisms occur within the uppermost part of active catagenesis, namely <10% conversion (Figs. 6–8), and essentially all generation has taken place over the last 1 Ma. Within these boundary conditions, the yield of organic products generated per unit weight of sediment depends on the amount and type of organic matter deposited. Considering a case of 1% TOC with a total generative potential (Hydrogen Index) of 100 mg/g TOC,  $1 * 10^{-5}$  mg organic compounds are generated from every g TOC per year on average during the Quaternary, or  $1 * 10^{-7}$  mg per g sediment per year. This rate,  $1 * 10^{-7}$  mg organic compounds per g sediment per year, falls within the quoted range  $1.4 * 10^{-5}$  to  $1.4 * 10^{-8}$  mg substrate per g sediment per year for sulphate reduction and organic matter oxidation in the aforementioned deep biosphere settings. Even allowing for adsorption losses, which remove higher molecular weight compounds preferentially [62], the rates of supply are still within the assumed range of deep biosphere utilisation rates, but now at the lower end.

A steady state system of abiotic substrate provision and biological utilisation can be invoked. This kinetically driven flux can of course be expected to operate in addition to, and not instead of, purely biological fermentation [20,63]. For instance, it has been proposed that the décollement zone penetrated at Site 1174 may have been the locus of episodic fluid flow [63], stimulating bacterial activity. The temperature activation of indigenous sedimentary organic matter [7] might also operate. That bacterial populations at ODP Leg 190 are lower than the ODP average [20], likely reflects the extremely low concentrations of indigenous organic carbon available as a substrate provider (Table 2).

## 6. Inferences and outlook

The above match between rates of deep biosphere respiration and abiotic substrate generation is not restricted to the Nankai Trough. For instance, a coal with a

TOC of 70% and Hydrogen Index of 200 mg/g TOC at the start of catagenesis generates  $1.4 \times 10^{-6}$  mg per g sediment per year (2 K/Ma) during the first 15% of transformation, as compared to the range  $1.4 \times 10^{-5}$  to  $1.4 \times 10^{-8}$  mg substrate per g sediment per year for deep biosphere settings. Again, putative feeding and utilisation rates match. It appears, therefore, that in a steady state system the carbon-related metabolic needs of deep microorganisms can be met quantitatively by the extremely slow thermal degradation of an immense pool of recalcitrant organic carbon (global mass  $10^{16}$  tonnes [47]).

The implications of this are far-reaching. Microbial growth in natural systems is strongly limited by substrate supply, the exponential growth curve being written:

$$dN/dt = \mu \cdot N \cdot (C-N)/C$$

where  $N$  is the number of organisms present,  $\mu$  is the growth rate, and  $C$  is the capacity, referring to a limiting substrate, to which growth of  $N$  is possible [64]. If the substrates utilised by deep microbial ecosystems are abiotically generated, then kinetic expressions for these abiotic reactions will govern  $C$ . Most published models for the thermal decomposition of kerogen are applied in the search for petroleum. Our results suggest the same models, utilising either bulk volatiles (undifferentiated gas and oil) or compositional, may be applied to predict feedstock generation for deep microbial ecosystems.

## 7. Conclusions

Sites drilled as part of ODP Leg 190 are characterised by very high heat flows during Late Quaternary rapid sediment accretion. Sedimentary organic matter is present in low concentration throughout the stratigraphic section. Kinetic parameters of hydrocarbon generation for the richest sampled layers are consistent with a highly heterogeneous and in part thermally labile macromolecular structure.

The results of kinetic modelling, in situ gas calibrations,  $^{14}\text{C}$ -radiolabelling of methanogenesis, bacterial cell counts and the presence of intact phospholipids together point to abiotic thermal degradation reactions going on in the same part of the sedimentary column as where a deep biosphere exists.

Compositional considerations indicate the type of organic matter found in Nankai Trough sediments could be an ideal source of low molecular weight oxygenated compounds and hydrogen, known to be substrates for methanogenesis.

The rates of thermal degradation closely resemble rates of respiration and electron donor consumption in

deep biosphere environments, pointing to a steady state supply and utilisation system.

We deduce that abiotically driven degradation reactions can provide substrates for microbial activity in the same or closely juxtaposed deep sediments at this convergent continental margin.

## Acknowledgements

This research used samples provided by the Ocean Drilling Program (ODP). ODP is sponsored by the U.S. National Science Foundation (NSF) and participating countries under management of Joint Oceanographic Institutions (JOI), Inc. Funding for this research was provided by the 5th Framework EU-sponsored project *DeepBUG* (EVK3-CT-1999-00017). We thank our colleagues from the University of Bristol (UK), Max Planck Institute for Marine Microbiology (Germany), and the Université de Bretagne Occidentale (France) for scientific discussions.

## References

- [1] R.J. Parkes, B. Cragg, S.J. Bale, J.M. Getliff, K. Goodman, P.A. Rochelle, J.C. Fry, A.J. Weightman, S.M. Harvey, Deep bacterial biosphere in Pacific Ocean sediments, *Nature* 371 (6496) (1994) 410–413.
- [2] I.V. Zlatkin, M. Schneider, F.J. de Bruijn, L.J. Forney, Diversity among bacteria isolated from the deep subsurface, *J. Ind. Microbiol.* 17 (1996) 219–227.
- [3] D.B. Ringelberg, S. Sutton, D.C. White, Biomass, bioactivity and biodiversity: microbial ecology of the deep subsurface: analysis of ester-linked phospholipids fatty acids, *FEMS Microbiol. Rev.* 20 (1997) 371–377.
- [4] R.A. Noble, J.F.H. Henk, Hydrocarbon charge of a bacterial gas field by prolonged methanogenesis: an example from the East Java Sea, Indonesia, in: B. Horsfield, et al., (Eds.), *Advances in Organic Geochemistry, Org. Geochemistry*, vol. 29, 1998, pp. 301–314.
- [5] Lovley, Chapelle, Deep subsurface microbial processes, *Rev. Geophys.* 33 (1995) 365–381.
- [6] P.B. McMahon, F.H. Chapelle, Microbial production of organic acids in aquitard sediments and its role in aquifer geochemistry, *Nature* 349 (1991) 233–235.
- [7] P. Wellsbury, K. Goodman, T. Barth, B.A. Cragg, S.P. Barnes, R.J. Parkes, Deep marine biosphere fuelled by increasing organic matter availability during burial and heating, *Nature* 388 (1997) 573–576.
- [8] R.J. Parkes, B.A. Cragg, P. Wellsbury, Recent studies on bacterial populations and processes in seafloor sediments: a review, *Hydrogeol. J.* 8 (2000) 11–28.
- [9] H.-J. Schenk, B. Horsfield, B.M. Krooss, R.G. Schaefer, K. Schwochau, Kinetics of petroleum formation and cracking, in: D.H. Welte, B. Horsfield, D.R. Baker (Eds.), *Petroleum and Basin Evolution*, Springer Verlag, Heidelberg, 1997, pp. 231–270.
- [10] D.D. Rice, G.E. Claypool, Generation, accumulation, and resource potential of biogenic gas, *Bull. Am. Assoc. Pet. Geol.* 65 (1) (1981) 5–25.



- [11] F.S. Colwell, M.E. Delwiche, D. Blackwelder, M.S. Wilson, R.M. Lehman, T. Uchida, Microbial communities from core intervals, JAPEX/JNOC/GSC Mallik 2L-38 gas hydrate research well, in: S.R. Dallimore, T. Uchida, T.S. Collett (Eds.), Scientific Results from JAPEX/JNOC/GSC Mallik 2L-38 Gas Hydrate Research Well, Mackenzie Delta, Northwest Territories, Canada, Bulletin, vol. 544, Geological Survey of Canada, 1999, pp. 189–195.
- [12] K. Mangelsdorf, R.M. Haberer, K.G. Zink, V. Dieckmann, H. Wilkes, B. Horsfield, Molecular evidence for the occurrence of a Deep Biosphere at the Mallik 5L-38 Gas Hydrate Research Well, in: S.R. Dallimore, T.S. Collett (Eds.), Scientific Results from the Mallik 2002 Gas Hydrate Production Research Well Program, Mackenzie Delta, Northwest Territories, Canada, Bulletin, vol. 585, Geological Survey of Canada, 2004, p. 11.
- [13] A. Wilhelms, S.R. Larter, I. Head, P. Farrimond, R. di Primio, C. Zwach, Biodegradation of oil in uplifted basins prevented by deep-burial sterilization, *Nature* 411 (2001) 1034–1037.
- [14] J. Naeth, R. di Primio, B. Horsfield, R.G. Schaefer, P.M. Shannon, W.R. Bailey, J.P. Henriot, Hydrocarbon seepage and carbonate mound formation: a basin modelling study from the Porcupine Basin (offshore Ireland), *J. Petrol. Geol.* 28 (2) (2004) 43–62.
- [15] V.A.I. Huvenne, W. Bailey, P.M. Shannon, J. Naeth, R. Di Primio, J.P. Henriot, B. Horsfield, H. de Haas, A. Wheeler, K. Olu-Le Roi, The Magellan mound province in the Porcupine Basin, *Int. J. Earth Sci.* (2005), doi:10.1007/s00531-005-0494-z.
- [16] K. Kashefi, D.R. Lovley, Extending the upper temperature limit for life, *Science* 301 (2003) 934.
- [17] V. Dieckmann, B. Horsfield, H.-J. Schenk, D.H. Welte, Kinetics of petroleum generation and cracking by programmed-temperature closed-system pyrolysis of Posidonia Shale, *Fuel* 77 (1998) 23–31.
- [18] V. Dieckmann, Modelling petroleum formation from heterogeneous source rocks: the influence of frequency factors on activation energy distribution and geological prediction, *Mar. Pet. Geol.* 22 (2005) 375–390.
- [19] E.W. Tegelaar, R.A. Noble, Kinetics of hydrocarbon generation as a function of the molecular structure of kerogen as revealed by pyrolysis-gas chromatography, in: N. Telnaes, G. van Graas, K. Oygard (Eds.), Advances in Organic Geochemistry 1993, vol. 22, Pergamon, Oxford-New York, 1994, pp. 543–574.
- [20] G.F. Moore, A. Taira, A. Klaus, L. Becker, B. Boeckel, B.A. Cragg, A. Dean, C.L. Fergusson, P. Henry, S. Hirano, T. Hisamitsu, S. Hunze, M. Kastner, A.J. Maltman, J.K. Morgan, Y. Murakami, D.M. Saffer, M. Sanchez-Gomez, E.J. Sreaton, D.C. Smith, A.J. Spivack, J. Steurer, H.J. Tobin, K. Ujiie, M.B. Underwood, M. Wilson, New insights into deformation and fluid flow processes in the Nankai Trough accretionary prism: results of Ocean Drilling Program Leg 190, *Geochem. Geophys. Geosyst.* 2 (10) (2001) (129/2001GC000166).
- [21] R. Ondrak, R. di Primio, Reconstruction of the burial and temperature histories of 5 ODP/DSDP wells in the Nankai Trough via basin modeling, *Terra Nostra* 2002/4 (2002) 425–430.
- [22] G.A. Spinelli, M.B. Underwood, Modeling thermal history of subducting crust in Nankai Trough: constraints from in situ sediment temperature and diagenetic reaction progress, *Geophys. Res. Lett.* 32 (L09301) (2005), doi:10.1029/2005GL022793.
- [23] G.F. Moore, H. Mikada, J.C. Moore, K. Becker, A. Taira, Legs 190 and 196 synthesis: deformation and fluid flow processes in the Nankai Trough accretionary prism, in: H. Mikada, G.F. Moore, A. Taira, K. Becker, J.C. Moore, A. Klaus (Eds.), Proc. ODP, Sci. Results, vol. 190/196, Ocean Drilling Program, College Station TX, 2005, pp. 1–26.
- [24] U. Berner, J. Koch, Organic matter in sediments of Site 808, Nankai accretionary prism, Japan, in: I.A. Hill, A. Taira, J.V. Firth, et al., (Eds.), Proc. ODP Sci. Results, vol. 131, Ocean Drilling Program, College Station TX, 1993, pp. 379–385.
- [25] U. Berner, E. Faber, Light hydrocarbons in sediments of the Nankai accretionary prism (Leg 131, Site 808), in: I.A. Hill, A. Taira, J.V. Firth, et al., (Eds.), Proc. ODP Sci. Results, vol. 131, Ocean Drilling Program, College Station TX, 1993, pp. 185–193.
- [26] K.G. Zink, H. Wilkes, U. Disko, M. Elvert, B. Horsfield, Intact phospholipids-microbial “life markers” in marine deep subsurface sediments, *Org. Geochem.* 34 (6) (2003) 755–769.
- [27] C.J. Newberry, G. Webster, B.A. Cragg, R.J. Parkes, A.J. Weightman, J.C. Fry, Diversity of prokaryotes and methanogenesis in deep subsurface sediments from the Nankai Trough, *Ocean Drilling Program Leg 190, Environ. Microbiol.* 6 (3) (2004) 274–287.
- [28] G. Webster, C.J. Newberry, J.C. Fry, A.J. Weightman, Assessment of bacterial community structure in the deep sub-seafloor biosphere by 16S rDNA-based techniques: a cautionary tale, *J. Microbiol. Methods* 55 (2003) 155–164.
- [29] G. Webster, R.J. Parkes, J.C. Fry, A.J. Weightman, Widespread occurrence of a novel division of bacteria identified by 16S rRNA gene sequences originally found in deep marine sediments, *Appl. Environ. Microbiol.* 70 (2004) 5708–5713.
- [30] L. Toffin, A. Bidault, P. Pignet, B.J. Tindall, A. Slobodkin, C. Kato, D. Prieur, *Shewanella profunda* sp. nov., isolated from deep marine sediment of the Nankai Trough, *Int. J. Syst. Evol. Microbiol.* 54 (2004) 1943–1949.
- [31] L. Toffin, G. Webster, A.J. Weightman, J.C. Fry, D. Prieur, Molecular monitoring of culturable bacteria from deep sea sediment of the Nankai Trough, Leg 190 Ocean Drilling Program, *FEMS Microbiol. Ecol.* 48 (2004) 357–367.
- [32] Petromod 1D (IES®).
- [33] P.K. Mukhopadhyay, J. Rullkötter, R.G. Schäfer, D.H. Welte, Facies and diagenesis of organic matter in the Nankai Trough sediments, Deep Sea Drilling Project Leg 87a, in: H. Kagami, D.E. Karig, W.T. Coulbourn, et al., (Eds.), Initial Reports of the Deep Sea Drilling Program, vol. 87, U.S. Government Printing Office, Washington, 1986, pp. 877–889.
- [34] J. Espitalié, G. Deroo, F. Marquis, La pyrolyse Rock-Eval et ses applications. Troisième Partie, *Rev. Inst. Fr. Pétrole* 41 (1986) 73–89.
- [35] R.G. Schaefer, H.J. Schenk, H. Hardelauf, R. Harms, Determination of gross kinetic parameters for petroleum formation from Jurassic source rocks of different maturity levels by means of laboratory experiments, *Org. Geochem.* 16 (1–3) (1990) 115–120.
- [36] H.J. Schenk, B. Horsfield, Kinetics of petroleum generation by programmed-temperature closed- versus open-system pyrolysis, *Geochim. Cosmochim. Acta* 57 (1993) 623–630.
- [37] M. Yamano, J.-P. Foucher, M. Kinoshita, A. Fisher, R.D. Hyndman, Heat flow and fluid flow regime in the western Nankai accretionary prism, *Earth Planet. Sci. Lett.* 109 (1992) 451–462.
- [38] D.M. Saffer, B.A. Bekins, Episodic fluid flow in the Nankai accretionary complex: timescale, geochemistry, flow rates, and fluid budget, *J. Geophys. Res.* 103 (1998) 30351–30370.
- [39] C.R.B. Lister, Estimators for heat flow and deep rock properties based on boundary layer theory, *Tectonophysics* 41 (1977) 157–171.
- [40] B. Parsons, J.G. Sclater, An analysis of the variation of ocean floor bathymetry and heat flow with age, *J. Geophys. Res.* 82 (1977) 803–827.

- [41] M.N. Yalcin, R. Littke, R.F. Sachsenhofer, Thermal history of sedimentary basins, in: D.H. Welte, B. Horsfield, D.R. Baker (Eds.), *Petroleum and Basin Evolution*, Springer Verlag, Heidelberg, 1997, pp. 71–167.
- [42] K.M. Fagerbakke, M. Heldal, S. Norland, Content of carbon, nitrogen, oxygen, sulfur and phosphorus in native aquatic and cultured bacteria, *Aquat. Microb. Ecol.* 10 (1996) 15–27.
- [43] H.J. Schenk, B. Horsfield, Using natural maturation series to evaluate the utility of parallel reaction kinetics models: an investigation of Toarcian shales and carboniferous coals, Germany. *Advances in Organic Geochemistry 1997*, *Org. Geochem.* 29 (1–3) (1998) 137–154.
- [44] P. Wellsbury, I. Mather, R.J. Parkes, Geomicrobiology of deep, low organic carbon sediments in the Woodlark Basin, Pacific Ocean, *FEMS Microbiology, Ecology* 42 (1) (2002) 59–70.
- [45] W.W. Christie, in: W.W. Christie (Ed.), *Advances in Lipid Methodology — Two*, The Oily Press, Dundee, 1993, pp. 195–213.
- [46] B.P. Tissot, D.H. Welte, *Petroleum Formation and Occurrence*, Springer, Berlin, 1984, 699 pp.
- [47] S.D. Killops, V.J. Killops, *An Introduction to Organic Geochemistry*, John Wiley & Sons, New York, 1993.
- [48] B.P. Tissot, B. Durand, J. Espitalié, A. Combaz, Influence of nature and diagenesis of organic matter in formation of petroleum, *Bull. Am. Assoc. Pet. Geol.* 58 (1974) 499–506.
- [49] P.G. Rouxhet, P.L. Robin, Infrared study of the evolution of kerogens of different origins during catagenesis and pyrolysis, *Fuel* 57 (1978) 533–540.
- [50] C.E. Rovere, P.T. Crisp, J. Ellis, P.D. Bolton, Chemical characterization of shale oil from Condor, Australia, *Fuel* 62 (1983) 1274–1282.
- [51] M.A. Wilson, R.P. Philp, A.H. Gillam, T.D. Gilbert, K.R. Tate, Comparison of the structures of humic substances from aquatic and terrestrial sources by pyrolysis gas chromatography–mass spectrometry, *Geochim. Cosmochim. Acta* 47 (1983) 497–502.
- [52] B. Horsfield, Practical criteria for classifying kerogens: some observations from pyrolysis–gas chromatography, *Geochim. Cosmochim. Acta* 53 (1989) 891–901.
- [53] J.S. Sinninghe-Damsté, T.I. Eglinton, J.W. deLeeuw, P.A. Schenck, Organic sulphur in macromolecular sedimentary organic matter. 1. Structure and origin of sulphur containing moieties in kerogen, asphaltenes and coal as revealed by flash pyrolysis, *Geochim. Cosmochim. Acta* 53 (1989) 873–889.
- [54] J.S. Sinninghe-Damsté, M.E.L. Kohlen, B. Horsfield, Origin of low molecular weight alkylthiophenes in pyrolysates of sulphur-rich kerogens as revealed by micro-scale sealed vessel pyrolysis, *Org. Geochem.* 29 (1998) 1891–1903.
- [55] B. Horsfield, The bulk composition of first-formed petroleum in source rocks, in: D.H. Welte, B. Horsfield, D.R. Baker (Eds.), *Petroleum and Basin Evolution*, Springer Verlag, Heidelberg, 1997, pp. 335–402.
- [56] W.W. Carothers, Y.K. Kharaka, Aliphatic acid anions in oil-field waters; implications for origin of natural gas, *Bull. Am. Assoc. Pet. Geol.* 62 (12) (1978) 2441–2453.
- [57] T. Barth, A.E. Borgund, A.L. Hopland, Generation of organic compounds by hydrous pyrolysis of Kimmeridge oil shale — bulk results and activation energy calculations, *Org. Geochem.* 14 (1) (1989) 69–76.
- [58] D.F. Payne, P.J. Ortoleva, A model for lignin alteration. Part 1. A kinetic reaction-network model. *Organic Geochemistry* 32, *Org. Geochem.* 32 (9) (2001) 1073–1085.
- [59] D.F. Payne, P.J. Ortoleva, A model for lignin alteration. Part II. Numerical model of natural gas generation and application to the Piceance Basin, Western Colorado, *Org. Geochem.* 32 (2001) 1087–1101.
- [60] A.D. Carr, J.E. Williamson, The relationship between aromaticity, vitrinite reflectance and maceral composition of coals: implications for the use of vitrinite reflectance as a maturation parameter. *Advances in Organic Geochemistry*, *Org. Geochem.* 16 (1–3) (1989) 313–323.
- [61] S. D’Hondt, S. Rutherford, A.J. Spivack, Metabolic activity of subsurface life in deep-sea sediments, *Science* 295 (2002) 2067–2070.
- [62] E.I. Sandvik, W.A. Young, D.J. Curry, Expulsion from hydrocarbon sources: the role of organic absorption. *Advances in Organic Geochemistry*, *Org. Geochem.* 19 (1–3) (1991) 77–87.
- [63] A.J. Spivack, M. Kastner, B. Ransom, Elemental and isotopic chloride geochemistry and fluid flow in the Nankai Trough, *Geophys. Res. Lett.* 29 (14) (2002) 4.
- [64] B. Schink, Prokaryotes in the biosphere, in: J.W. Lengeler, G. Drews, H.G. Schlegel (Eds.), *Biology of the Prokaryotes*, Thieme, Stuttgart, 1999, pp. 721–811.
- [65] B.B. Jørgensen, M.F. Isaksen, H.W. Jannasch, Bacterial sulfate reduction above 100 °C in deep-sea hydrothermal vent sediments, *Science* 258 (1992) 1756–1757.