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A novel procedure to detect low molecular weight compounds released by alkaline ester cleavage from low mature coal to assess its feedstock potential for deep microbial life.

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Abstract

In recent years the widespread occurrence of microorganisms was demonstrated in deep marine and terrestrial sediments. With this discovery inevitably the question of the potential carbon and energy sources for this deep subsurface microbial life arises. In the current study a new method for the investigation of low molecular weight (LMW) organic acids linked to the kerogen matrix is presented. These LMW organic acids form a potential feedstock for deep microbial populations. Twelve coal samples of different maturity (vitrinite reflectance (R_0) of 0.28 to 0.80%) from several coal mines on the North and South Island of New Zealand (NZ) were examined to assess the amount of bound LMW organic acids. Formate, acetate and oxalate were detected in significant amounts whereas the amounts of these compounds decrease with increasing maturity of the coal sample. This decrease of LMW

organic acids mainly correlates to the phase of diagenetic alteration of the organic matter characterized by the release of oxygen containing compounds. Concomitantly, it coincides with temperature conditions assumed to be still compatible with microbial life in the deep subsurface. First assessments of the feedstock potential and generation rates of LMW organic acids indicate that the NZ coals investigated exhibit the potential to feed deep terrestrial microbial life with appropriate substrates over geological time spans.

1 Introduction

It was long thought that life on Earth was restricted to the surface and the upper few meters of sediment (Morita and Zobell, 1955). In recent years the finding of deep microbial life in the subsurface opens the view to a fascinating new topic in today's microbiology and geoscience research (Kerr, 1997; Whiteman et al., 1998; Parkes et al., 2000; Horsfield et al., 2007). With the discovery of an ubiquitous deep biosphere on Earth inevitably the question on its potential carbon and energy sources arises.

In environments with little buried organic matter, for instance in igneous rocks like basalts and granites, lithoautotrophic microbial communities are able to synthesize small organic compounds from inorganic sources like radiolytically generated hydrogen gas and inorganic carbon dioxide, and these simple organic compounds can be utilized by other microorganisms. Therefore, such microbial communities form deep subsurface microbial ecosystems being independent from photosynthetically produced substrates (Stevens and McKinley, 1995; Fredrickson and Onstott, 1996; Lin et al., 2005, 2006; Sherwood-Lollar et al., 2006). In marine and terrestrial sedimentary basins the most obvious energy source is, of course, buried organic matter (Froelich et al., 1979; Stetter et al., 1993; D'Hondt et al., 2004). Caused by biotic but also abiotic alteration, the sedimentary organic matter undergoes a permanent restructuring process with increasing diagenesis, thereby releasing nitrogen, sulphur, and oxygen containing compounds (Sinninghe Damsté et al., 1989; Eglinton et al., 1992; Béhar et al., 1995; Putschew et al., 1998; Vandenbroucke and Largeau, 2007). Thus, organic carbon rich lithologies could act as potential "feeder" lithologies for the deep biosphere (L'Haridon et al., 1995; Krumholz et al., 1997; Horsfield et al., 2006).

Elucidation of the structural composition of sedimentary organic mat-

ter was and still is one of the most intriguing and challenging topics in organic geochemistry. To improve the understanding of the molecular composition of kerogen various analytical techniques were used such as nuclear magnetic resonance spectroscopy (NMR) (Derbyshire et al., 1989; Burgess and Schobert, 1998; Suggate and Dickinson, 2004; Werner-Zwanziger et al., 2005), infrared spectroscopy (IR) (Robin, 1975; Rullkötter and Michaelis, 1990; Lis et al., 2005) pyrolysis gas chromatography-mass spectrometry (Pyrolysis GC-MS) (Larter and Douglas, 1982; Horsfield and Larter, 1993; Khaddor et al., 2002; Killops et al., 2002; Sykes and Snowdon, 2002) and selective chemical degradation reactions (Schouten et al., 1993; Schaeffer et al., 1995; Höld et al., 1998; Putschew et al., 1998; Schaeffer-Reiss et al., 1998). These powerful analytic tools provided various information about the compositional structure of kerogen and its maturation history (Vandenbroucke and Largeau, 2007). Especially, for the elucidation of the different bonding types selective chemical degradation reactions have been demonstrated to be a highly sophisticated method (Rullkötter and Michaelis, 1990). Main decomposition steps are the cleavage of ester, ether, sulphur (Hofmann et al., 1992; Schaeffer et al., 1995; Höld et al., 1998) and carbon-carbon (Stock and Tse, 1983; Stock and Wang, 1986) bonds, providing valuable information on the internal bonding structures within the kerogen. Additionally, these techniques allowed insights into the chemical and structural variation within the kerogen during diagenetic alteration with increasing maturity.

Important substrates for feeding microbial life are low molecular weight organic acids (Sansone and Martens, 1981, 1982; Sørensen et al., 1981; Jørgensen, 1982). Therefore, the cleavage of ester bound compounds is a promising tool to examine the potential of sedimentary organic matter of different levels of maturity to release organic acids which can potentially act as a feedstock for deep microbial life. The commonly used and well established procedure for the ester cleavage is alkaline hydrolysis (Putschew et al., 1998; Schaeffer-Reiss et al., 1998). However, due to the experimental preparation procedure the conventional ester cleavage methods are mainly focussed on the long chain, non-volatile ester cleavage products.

In this paper a novel analytical approach is presented to detect low molecular weight (LMW) organic acids from alkaline ester cleavage reaction of coal samples of different maturity. Simultaneously, analysis of the high molecular weight (HMW) product compounds (alcohols and fatty acids) can

be performed using this approach. However, results of this investigation will be published elsewhere. Using the developed approach a series of New Zealand (NZ) coal samples of continuous maturity from lignite to high volatile bituminous coal rank was investigated to determine the potential of these coals to release LMW organic acids (such as formic acid, acetic acid and oxalic acid) from macromolecular coal matrix forming a potential feedstock for deep microbial life.

2 Sample material

The North and South Island of New Zealand are home to coals covering a broad maturity range of almost continuous maturity (R_0 : 0.25 - 3.0%) from Cretaceous to Tertiary age in freshly exposed coal facies, coal mines and drill cores (Figure 1) forming the so called New Zealand Coal Band (Suggate, 2000; Killops et al., 2002; Sykes and Snowdon, 2002). Therefore, the New Zealand Coal Band provides one of the best maturity rank series in the world to examine natural maturation processes in coals. For the current study being part of the international DEBITS (Deep Biosphere in Terrestrial Systems) project we selected 12 samples from different coal locations on the North and South Island. Two of these samples were taken from the DEBITS-1 well, which was drilled within the scope of the DEBITS project on a meadow near the small village of Huntly on the North Island of New Zealand. The coals selected for this study cover a maturity rank range from lignites to high volatile bituminous coals (R_0 : 0.28-0.80%) and therefore, represent different feeding potentials. The sample material was part of several coalfields from four different coal bearing basins (Figure 1, Table 1): one sample from the Eastern Southland Basin being in the lignite rank range (R_0 : 0.28%), two coal samples from the DEBITS-1 well in the Waikato coal area also in the lignite rank range (R_0 : 0.29%), three additional samples from the Waikato Basin being in the sub-bituminous rank range (R_0 : 0.40 - 0.44%) and four coals from the West Coast Basin as well as two from the Taranaki Basin representing all high volatile bituminous coals (R_0 : 0.52 - 0.80%). The selected samples cover a time period from Late Cretaceous to Pleistocene age (Table 1).

Figure 1: Map of New Zealand, showing the location of the samples chosen for this study.

Sample	Basin	Coalfield / Sample location	Formation	Age	R ₀ %	TOC %	Formate gSed	Formate mg/ gTOC	Acetate gSed	Acetate mg/ gTOC	Oxalate gSed	Oxalate mg/ gTOC
G001978	Eastern Southland	Waimumu	Gore Lignite Measures	Oligocene - Miocene	0.28	52.44	16.5	31.4	3.4	6.5	0	0
G004541		DEBITS-1 (18.94 m)	Puketoka	Pliocene - Pleistocene	0.29	45.11	7.9	17.5	1.8	3.9	1.8	3.4
G004544	Waikato	DEBITS-1 (54.46 m)	Whangamarino	Miocene - Pliocene	0.29	37.87	10.8	28.7	1.2	3.3	2.2	5.7
G001982	Basin	Rotowaro	Waikato Coal Measures	Eocene - Oligocene	0.40	54.81	12.9	23.5	3.2	5.9	0	0
G001983		Maramarua			0.41	53.82	6.1	11.4	1.6	3	1.6	3
G001980		Rotowaro			0.44	61.16	4.5	7.4	1.4	2.2	1.5	2.5
G001996		Reefton			0.52	67.85	4.7	7	1.1	1.6	1	1.5
G001995	West Coast	Reefton	Brunner Coal	Eocene	0.52	67.76	4.3	6.4	1	1.5	0.8	1.2
G001989	Basin	Buller	Measures		0.69	73.33	2.5	3.5	0.7	0.9	0.7	1
G001993		Garvy Creek			0.76	77.73	1.6	2.1	0.5	0.7	0.6	0.7
G001994	Taranaki	Paturau River	Rakopi	Late Cetaceous	0.61	63.99	1.4	1.8	0.2	0.3	0.5	0.8
G001991	Basin	Ohanga-2 well	Mangahewa	Eocene	0.80	73.83	3.2	4.4	0.8	1.1	0.6	0.8

Table 1: Selected samples for the investigation of kerogen bound formate acetat and oxalke including informations about basin, coalfield, formation, age, vitrinite reflectance and TOC (data provided by R. Sykes, GNS Science (NZ)) and the detected amounts of formate, acetat and oxalate.

3 Experimental approach

As mentioned above, the commonly used procedures for alkaline ester cleavage are usually restricted to the recovery of high molecular weight cleavage products. In the modified approach, described below, we combine the conventional procedure covering the high molecular weight (HMW) products with a new procedure to detect the low molecular weight (LMW) products using ion chromatography (IC) (Figure 2).

3.1 Sample preparation

In order to remove the bitumen fraction from the coal samples the freeze dried and ground coal samples were pre-extracted with water using a Soxhlet extraction for two days, followed by a second Soxhlet extraction for three days with an azeotrope solvent mixture of acetone (38%), methanol (30%) and chloroform (32%). Prior to the ester cleavage the coal sample was additionally extracted with the reaction solvent (methanol) for one day to ensure that the compounds obtained after alkaline hydrolysis indeed represent the ester bound lipid fraction.

The cleavage reaction was conducted according to the method described by Höld et al. (1998). Two grams of the pre-extracted coal sample were suspended in 20 ml 1 N KOH solution in methanol and refluxed for 5 h. Optionally the cleavage reaction can also be performed in sealed vials placed in an oven at 100°C for 18 h. Afterwards, the suspension was cooled to room temperature and 50 g androstanone and 50 g erucic acid were added as internal standards to trace losses in the yield of the target compounds during subsequent sample treatment and to quantify the HMW cleavage products using gas chromatography-mass spectrometry (GC-MS). To remove the coal matrix the suspension was filtered, whereas the residue was washed three times with 50 ml of methanol. Subsequently, all filtrates were combined and split into two proportions. The first half was used for the investigation of the LMW ester cleavage products, the second half for the HMW products using the conventional analytical method described (Höld et al., 1998).

In the conventional method the next steps would be acidification of the filtrate with aqueous HCl to pH 3 to transform the alkali salts of the carboxylic acid into their corresponding acids, extraction of the acids with dichloromethane, removal of the organic solvent to dryness, methylation of the acids and preparation of a solution for GC-MS measurement. As far as LMW organic acids are concerned, this method is inadequate because small organic acids like formic and acetic acid are highly volatile and relatively polar. Thus, recovery problems for these compounds occur during the extraction of the water-methanol phase with dichloromethane and the subsequent removal of the solvent supporting the evaporation of the small volatile organic acids. The removal of the solvent is necessary, because ions have to be dissolved in water for ion chromatography. Additionally, the LMW organic acids cannot be measured directly from the reaction solution, because methanol and high amounts of hydroxyl ions interferes with the detection of small organic acids in the ion chromatography.

To overcome this problem in the proportion of the LMW ester cleavage product analysis, methanol was directly removed from the alkaline cleavage solution under reduced pressure. The organic acids precipitated as their corresponding potassium salts. Subsequently, the potassium salts were dissolved in water and the solution was adjusted to pH 8-10 with an aqueous solution of 5% HCl. Afterwards, the slightly alkaline solution was transferred into a volumetric flask and water was added up to 20 ml. This sample was used for ion chromatographic analysis of the LMW organic acids.

Figure 2: *Experimental procedure for analysis of LMWOAs released from coals by alkaline ester cleavage reaction.*

3.2 Ion chromatography

Prior to IC analysis the samples were diluted with water (1:10) and an ion exchange cartridge (Dionex On Guard II Ag/H) was used to remove high chloride contents. The cartridge contains a styrene based sulfonic acid that replaces the chloride ions by sulfate ions. In contrast to the chloride ions the sulfate ions did not interfere in the chromatogram with the target compounds because of their longer retention time. Test experiments showed that the use of the applied ion exchange cartridge had no effect on the organic acid concentration in the samples. All samples were analysed in replicate using a Dionex ICS 3000 ion chromatograph with a KOH eluent generator equipped with an ASRS Ultra II 2 mm suppressor and a Dionex conductivity detector. For anion separation the analytical column AS11HC (2 x 250 mm, Dionex Corp.) with a pre-column AG11HC was used at a temperature of 35°C. Samples were eluted using a KOH solution of varying concentration over time. The initial KOH concentration was 0.5 mM, held for 8 min. After 10 min, a concentration of 15 mM KOH was reached and kept constant for 10 min. After 30 min analysis time, a concentration of 60 mM KOH was reached, followed by a rapid increase to 100 mM reached after 30.2 min analysis time. At 32 min, the KOH concentration was again at the initial level of 0.5 mM and kept there for an additional 15 min to equilibrate the system. For quantification of organic acids, standards containing formic acid, acetic acid (suprapur, Merck, Germany) and oxalic acid (p.a., Merck, Germany) were measured in different concentrations every day. Standard deviation of sample and standard quantification is below 10% (determined from replicate analysis).

3.3 Method evaluation

To evaluate the general applicability of the presented methods especially in terms of compound recovery, test cleavage experiments were conducted using propyl formate, ethyl acetate (both Sigma-Aldrich, Taufkirchen, Germany), C_{18} , C_{26} and C_{30} fatty acid ethyl esters in known amounts of 2, 4, 6, 8 and 10 mg. However, these test experiments do not consider any adsorption effects of the coal matrix. The cleavage reactions were performed in methanol under reflux as described above. For comparison, another cleavage reaction method was tested, where the reaction was conducted in a closed glass vial

for 18 h in an oven using 5 ml 1N KOH in methanol (oven temperature 100°C) and 5 ml 1N KOH in water (oven temperature 120°C).

While propyl formate, ethyl acetate and octadecanoic acid ethyl ester was commercially available (Sigma-Aldrich, Taufkirchen, Germany), the longer chain fatty acid ethyl ester standards with 26 and 30 carbon atoms were synthesized from hexacosanoic and triacontanoic acid in a 10 ml sample vial according to a procedure published by Blau and Darbre (1993). To optimize the yields obtained by this method the closed reaction vials containing the respective acid, ethanol and thionyl chloride were additionally heated in water bath at 40°C until the solution became clear. The purity of the products was controlled by GC-MS analysis.

4 Results and discussion

4.1 Evaluation of the analytical method for the recovery of low molecular weight organic acids

The test cleavage experiments with different standard amounts show a retrieval of 69-79% for formate and ca. 73-85% for acetate using methanol as the reaction solvent under reflux conditions for 5 h (Figure 3). Conducting the experiments in a closed vial placed in an oven at 100°C for 18 h using methanol as solvent leads to a recovery of 63-84% of formate and 65-95% of acetate. In contrast the same experimental assembly using water as solvent leads to a recovery of 75-82% of formate and 84-88% of acetate. The retrieval of formate and acetate during the test experiments with methanol under reflux and water in a vial are relatively constant over the whole range of different standard amounts indicating that at least in the chosen range different compound amounts have no significant effect on the cleavage reaction. However, the recovery of the test experiments with water in a vial is slightly better. The recovery of formate and acetate in the methanol in a vial experiment using 8 mg and 10 mg is distinctly lower than in the experiments with 2 mg, 4 mg and 6 mg. It can be speculated that in the vial experiment side reactions like decarboxylation and chemical degradation are responsible for the lower recovery, which might be expected to be more significant at higher compound concentrations. However, this was not observed in the water in a vial experiment and also not in the methanol reflux experiment.

4 Results and discussion

Figure 3: Recovery of formate and acetate after ester cleavage of its ethylated congeners using different analytical ester cleavage conditions: with methanol (MeOH) under reflux, with MeOH in a closed vial in an oven at 100° C and with water in a closed vial in an oven at 120° C.

Thus, these lower yields might be more likely related to losses during sample processing.

Although the cleavage reaction with water shows slightly higher recoveries, we decided to use the method with methanol under reflux for 3 reasons: (1) the mixing of the coal matrix with methanol and therefore with the reagents was much better than with water, an effect not considered in our test experiments with only 5 selected standard compounds. (2) The separation of the reaction solution from the coal matrix by filtration is much more time consuming when water is used as the solvent. (3) Long chain fatty acid experiments indicate that the ester cleavage reaction is not complete using water as solvent which is inadequate when the investigation of both low and high molecular weight fatty acids is intended. In a test experiment C_{18} , C_{26} and C_{30} fatty acid ethyl esters were treated with 1 N KOH solution in water under reflux for 5 h. Non-cleaved ethyl esters were detected in DCM extract of the reaction mixture, whereas the amount of ethyl esters increased with increasing chain length (C_{18} : 6.1%, C_{26} : 9.3% and C_{30} : 11.1%). These findings might reflect differences in the solubility of the investigated fatty acids

in water becoming poorer with the increasing aliphatic character of longer chain fatty acids.

A problem with the use of methanol as a solvent could be the transesterification reaction (Otera, 1993) leading to methyl esters instead of corresponding potassium salts of the acids. This would mean that a proportion of the LMW organic acids could be lost as highly volatile methyl esters during the removal of methanol under reduced pressure. Transesterification takes place when the attacking nucleophile is a methanolate instead of a hydroxyl ion. Methanolate ions simply form when hydroxyl ions are solved in methanol. For instance, Caldin and Long (1954) showed for a solution of 0.1 N NaOH in 99% ethanol that 96% of the solved base was ethanolate. Transesterification reaction is used e.g. for the production of biodiesel from vegetable oils (Schuchardt et al., 1998). Comparing the cleavage experiment curves in Fig. 3 it can be recognized that the recoveries for the water experiments (where no transesterification can take place) are slightly better than for the methanol experiments. However, this difference is only small and might also derive from operational variations instead of transesterification. An indication that transesterification is not a significant side reaction in the applied procedure is that no ethyl and methyl esters of long chain fatty acids (C_{18} , C_{26} and C_{30}) can be detected in the GC run of the organic solvent extracts directly obtained from the alkaline ester cleavage solution without derivatisation (methylation). The lack of ethyl esters indicate complete saponification and the lack of methyl esters that transesterification plays not a significant role. After methylation of the extract with diazomethane the saponified fatty acids can be detected as their methyl esters.

To understand why transesterification might not play a role, it has to be noted that every possible transesterification product can again be attacked by another nucleophile. This would result again in a methyl ester in the case of another methanolate or if the nucleophile is a hydroxyl ion into a free acid or under alkaline conditions into the corresponding potassium salt. The deprotonated acid is not reactive anymore for a nucleophile attack due to the mesomeric stabilisation of the carboxylic ion (Exner, 1988). Thus, the deprotonated acid forms a trap for the competing chemical reactions. In contrast to industrial processes like the production of biodiesel by transesterification (Schuchardt et al., 1998), where KOH is only used in catalytic amounts (1-2 mol%) compared to the initial educt esters (vegetable oils), in the applied geo-

chemical reaction scale the nucleophile is used in a large excess and therefore might cause complete saponification.

Thus, the observed losses of the target substances in the test experiments (Figure 3) might more likely be the result of product losses during the diverse sample treatment steps in the applied complex analytical method. Finally, it has to be mentioned that the test experiments do not consider any adsorption effects of the coal matrix concerning the ester cleavage products which might occur in natural samples.

4.2 LMW organic acids linked to NZ coals of different maturity

The 12 NZ coal samples of different maturity (Table 1) were investigated applying the developed analytical approach. Using ion chromatography the ester cleavage products formate, acetate and oxalate were detected in significant amounts in almost all samples investigated (Figure 4). The main compound released during the ester cleavage experiment is formate, followed by acetate and oxalate. During the diagenetic phase the amount of ester linked formate rapidly decreases with increasing maturation of the coals. During early catagenesis the decrease slows down and remains low from a maturity level of about 0.6% R_0 , the onset of the oil window of the coals from the New Zealand Coal Band (Sykes and Snowdon, 2002). For acetate and oxalate the same trend is indicated, although oxalate was not detected in all samples investigated. The higher variation in the amounts of the released low molecular weight fatty acids during the diagenetic stage is most likely the result of a higher variability in the organofacies of the individual coals of lower maturity, which even might occur in coal samples from the same coalfield. Sykes and Snowdon (2002) also show a high variability in the bitumen index data for the New Zealand coals of lower maturity indicating differences in the organofacies of the investigated coals.

Although there are some variations, an overall trend of a decreasing amount of the ester cleavage products formate, acetate and oxalate can be observed with increasing maturity and thus indicate a continuous loss of kerogen linked small organic acids during maturation of the organic matter. The lignite sample from the Waimumu coalfield (Eastern Southland Basin) showed the highest amounts of formate and acetate with up to 31.4 mg/g TOC and 6.4 mg/g TOC, respectively, but no oxalate. The highest amounts of oxalate were detected in the sample taken from the DEBITS-1 core from

Figure 4: Formate, acetate and oxalate concentrations liberated by ester cleavage reaction from New Zealand coals of different maturity. The onset of early catagenesis was deduced from Vu et al., in prep. The zone between 0.5 and 0.6% R_0 is suggested to be the onset where temperature conditions are incompatible with microbial life.

the Wahangamarino formation at 64.46 m depth (Waikato Basin). The sub-bituminous coals from Waikato Basin show still high but strongly decreasing amounts of formate and acetate and oxalate (exception G001982) with increasing maturity. This trend can also be followed in the high volatile bituminous coals in the West Coast Basin until finally reaching low values of about 2-4 mg/g TOC formate and 0.1-1 mg/g TOC acetate and oxalate at maturity levels higher than 0.6% R_0 for coal samples from the West Coast and Taranaki Basins. Thus, the data indicate that the main decrease of LMW organic acids is associated with diagenetic structural alteration processes characterised by the loss of oxygen containing compounds.

Temperature is thought to be a limiting factor for the occurrence of microbial life in the deep subsurface. Deduced from investigations in petroleum reservoirs, to date temperatures between 80 and 90°C are suggested to form the limit temperatures for deep microbial activities (Connan, 1984; Wilhelms et al., 2001; Head et al., 2003). Using the kinetic model of Sweeney and Burnham (1990) with an average heating rate for geological systems of 2°C per Ma, for the current study temperatures between 80 and 100°C are calculated for the maturity level between 0.5% and 0.6% R_0 . Thus, conditions being incompatible with microbial life are suggested to commence during the later stage of early catagenesis.

The overall decrease of oxygen bearing compounds coincides with observation on diagenetic restructuring processes of kerogen obtained by other analytical methods. For instance, the evolution pathway of buried organic matter plotted in a van Krevelen diagram indicates as well an enhanced loss of oxygen containing compounds during diagenetic maturation by the preferential decrease of the atomic O/C ratio relative to the H/C ratio (Tissot and Welte, 1984). For the selected sample material of the NZ coals the loss of oxygen was shown in a van Krevelen diagram by Vu (2008). Furthermore, infrared spectroscopy of kerogen samples with increasing maturity reveals the loss of compounds containing a CO moiety as indicated by the decrease of the CO vibration at ca. 1700 cm⁻¹ (Robin, 1975; Tissot and Welte, 1984; Rullkötter and Michaelis, 1990; Lis et al., 2005). Concomitantly, these infrared analysis show that the decrease in the CO vibration continues up to 3% R_0 as reported for the Sarufutsu coals (Takeda and Asakawa, 1988). This might indicate that the release observed in Figure 4 will proceed slowly due to thermogenic degradation processes at maturity levels higher than covered

	Minimum	Maximum
CO_2 respiration rates¹		
mmol/l/a	$1 * 10^{-6}$	$1 * 10^{-3}$
mg/l/a	$4.4 * 10^{-5}$	$4.4 * 10^{-2}$
stoichiometric factor for conversion of LMWOA to CO_2		
formic acid (M = 46.03)		1.05
acetic acid (M = 60.05)		1.36
oxalic acid (M = 90.04) ²		1.02
feedstock [mg/l/a]		
formate	$4.62 * 10^{-5}$	$4.62 * 10^{-2}$
acetate	$5.98 * 10^{-5}$	$5.98 * 10^{-2}$
oxalate	$4.49 * 10^{-5}$	$4.49 * 10^{-2}$
av. density of sediment [kg/l]		
		approx. 2
feedstock [mg/g/a]		
formate	$2.31 * 10^{-8}$	$2.31 * 10^{-5}$
acetate	$2.99 * 10^{-8}$	$2.99 * 10^{-5}$
oxalate	$2.24 * 10^{-8}$	$2.24 * 10^{-5}$

Table 2: Assessment of required formate, acetate and oxalate respiration rates to sustain deep microbial life (modified after (Horsfield et al., 2006)). ¹ CO_2 respiration rates from D'Hondt et al. (2002) ²one oxalate provides two CO_2 .

by the current study.

4.3 Feedstock potential of the NZ coals of different maturity

The data presented suggest that there is a progressive release of LMW organic acids with ongoing maturation. Thus, LMW organic acids are permanently liberated from the coals into the surrounding sediments. Therefore, the LMW organic acids linked to the coal matrix form a potential feedstock for deep microbial life. To estimate this feedstock potential, CO_2 respiration rates of 10^{-6} to 10^{-3} mmol per litre and year, reported by D'Hondt et al. (2002) for deep-

aquifer systems (Table 2), were compared to the amounts of LMW organic acids released from the NZ coals of different maturity (Table 3) following a calculation presented by Horsfield et al. (2006). The CO_2 respiration rates in mg per litre per year (Table 2) were converted into the required formate, acetate and oxalate feedstock in mg/g sediment per year using a stoichiometric conversion factor from CO_2 to the corresponding LMW organic acid and an approximate sediment density of 2 kg/l. Afterwards these data were compared to the detected formate, acetate and oxalate amounts released during the ester cleavage experiments corrected for the average amounts of these compounds at maturity levels higher than 0.55% R_0 . At higher maturity levels the decrease flattens representing an amount of LMW organic acids which is not released during diagenetic and early catagenetic transformation of the organic matter (non released residue). The minimum feedstock potential was calculated from the maximum respiration rate and vice versa. Depending on minimum and maximum respiration rates, organic acid species detected and maturity level of the investigated coals the feedstock potential of the NZ coals is highly variable. It ranges from several tens of thousand years to several hundred of million years (Table 3).

Because of the differences in the organo facies of the coals and the approximate age classifications, generation rates for small organic acids from the NZ coals are difficult to determine. Therefore, such calculations should be regarded as a first estimate only. Between sample G001978 of Oligocene to Miocene age (ca. 23 Ma; R_0 0.28%) and sample G001995 of approximately Middle Eocene age (ca. 43 Ma; R_0 0.52%) about 12.2 mg/g sediment formate and 2.4 mg/g sediment acetate was released during 20 Ma. For oxalate the generation rate was calculated between sample G004544 of Miocene to Pliocene age (ca. 5 Ma; R_0 0.29%) and G001995 (see above). According to this 1.4 mg/g sediment oxalate was released during ca. 38 Ma. This correspond to generation rates of $6.1 \cdot 10^{-7}$ mg/g sediment per year formate, $1.2 \cdot 10^{-7}$ mg/g sediment per year acetate and $3.7 \cdot 10^{-8}$ mg/g sediment per year oxalate, falling into the range of deep biosphere utilisation rates (2), although at the lower end. However, metabolic rates in the deep biosphere are assumed to be on a very low level (Parkes et al., 2000). Thus, these approximate estimations show that during diagenetic alteration the organic carbon rich formations can provide, indeed, a potential substrate feedstock, being large enough to sustain deep microbial life over geological time spans.

Sample No.	Eastern Southland		Waikato				West Coast	
	G001978	G004541	G004544	G001982	G001983	G001980	G001996	G001995
R_0	0.28	0.29	0.29	0.40	0.41	0.44	0.52	0.52
Available acids [mg/g sediment]								
Formate	14.3	5.7	8.6	10.7	3.9	2.3	2.5	2.1
Acetate	2.8	1.2	0.6	2.6	1.0	0.8	0.5	0.4
Oxalate	-	1.2	1.6	-	1.0	0.9	0.4	0.2
Estimated feedstock potential [min. value in thousand years - max. value in million years]								
Formate	619	257	372	463	169	100	108	91
Acetate	94	40	20	87	33	27	17	13
Oxalate	-	54	71	-	45	41	18	9

Table 3: Calculated feedstock potential of the New Zealand coals investigated. Minimum values are calculated from maximum respiration rate, maximum values are calculated from minimum respiration rate of each compound. "Available acids" are calculated from the detected acids (Table 3) subtracted by an average residue amount, which appears not to be released during diagenesis. This "average non-released residue" was estimated from coal samples with $R_0 > 0.55\%$: for formate 2.2 mg/g sediment, for acetate 0.6 mg/g sediment and for oxalate 0.6 mg/g sediment.

4.4 Mechanisms for the release of LMW organic acids from the coal matrix

In contrast to free organic compounds being part of the bitumen fraction and being partly dissolved in the pore water, the ester linked LMW organic acids, investigated here, are not directly available to act as a feedstock for deep microbial life. However, the presented data show that these compounds are gradually released during increasing maturation. Thus, there must be processes activating this part of the organic matter. Although, we can only speculate on these processes, both abiotic as well as biotic processes are conceivable.

Temperature is most likely one of the crucial factors. In the upper sediments buried organic matter passes through intense biodegradation processes. It is thought, that these processes initially lead to an increase of the recalcitrant proportion of the organic matter (Wellsbury et al., 1997; Parkes et al., 2007). However, with increasing burial depth ambient temperature increases (on average about 30°C/km; (Allan and Allen, 1998)) affecting the stability of chemical bonds. The thermal supply of energy to the sedimentary system with depth increases the probability that the required activation energy for the cleavage of chemical bonds is achieved (Quingley and Mackenzie, 1988). During diagenesis this might support abiotic bond cleavage of potential substrates e.g. by hydrolysis and, thus, gradually activate the buried organic matter increasing its bioavailability. These thermally supported processes might therefore stimulate deep microbial activity (Parkes et al., 2007).

Parkes et al. (2007) simulated thermally supported alteration in long term laboratory experiments and showed continuously stimulated bacterial activity, indicating temperature induced activation of recalcitrant organic matter. Investigating coastal sediments using temperature heating experiments Wellsbury et al. (1997) showed that the bioavailability of buried organic matter increases already at relatively low temperatures (30-40°C) indicated by enhanced release of acetate into the pore water. In coal environments the activation of recalcitrant organic matter by increasing geothermal heat with increasing burial depth additionally might be supported by catalytic effects in the pore water system. Lignites and coals contain high amounts of humic and fulvic acids. The kerogen itself bears free carboxylic acid groups and acidic phenol hydroxy groups. Furthermore, oxidation of pyrite in coals can cause low pH values of pore waters, an effect known

from weathering of coals in coal mines, leading to the so called acid mine drainage (AMD), a problem for surface ecosystems located near coal mines (Olson et al., 1979; Christensen et al., 1996; Ludwig and Balkenhol, 2001; Blodau, 2006). Therefore, the pore water in coal seams often represents an acidic environment. In the presence of water and catalytic amounts of acids, esters form a chemical equilibrium with the hydrolysed organic acids and alcohols. The hydrolysed acids and alcohols can be removed by pore water flow or might simply be consumed by microorganisms. Therefore, acidic conditions in the pore water system are supposed to support a constant substrate release from coal matrices.

From early catagenesis to the main catagenetic phase the relevance of thermogenic bond-cracking continuously increases. For example, acetate was found to be associated with thermogenic alteration of buried organic matter as a process of the formation of fossil fuel and represents a principal energy source for the deep production of bacterial methane (Cooles et al., 1987; Strapoc et al., 2008). However, thermogenic processes might not only support the release of ester-bond LMW fatty acids as acids but also as CO_2 or in their reduced forms like methane and higher hydrocarbon gases.

Furthermore, it was shown that microorganisms are able to support the degradation of organic matter using extracellular enzymes (Arnosti et al., 1998; Arnosti and Jørgensen, 2003). Although, such processes were so far only observed in marine or lacustrine sediments during early diagenesis, it is conceivable that this is another appropriate way for microorganisms to activate potential substrate sources in deep terrestrial systems as well.

5 Conclusion

In the current paper a novel analytical approach was developed to receive low molecular weight compounds from alkaline ester cleavage reaction of coal samples of different maturity to assess the feedstock potential of these coals for deep microbial life. Test experiments on the applicability of the new method show best results in terms of recovery of low molecular weight organic acids and high molecular weight (HMW) fatty acids as well as analytical handling when using methanol as the reaction solvent under reflux or in a closed vial in an oven.

Twelve coal samples showing a maturity range from lignite to high volatile

bituminous coals and taken from different locations on the North and South Island of New Zealand were investigated for ester linked LMW organic acids forming an potential substrate feedstock for deep microbial life. In almost all samples significant amounts of formate, acetate and oxalate were detected, whereas the amounts releasable from the coal matrix decrease with increasing maturity. The main decrease of LMW organic acids is associated with the diagenetic phase reflecting structural alteration processes characterised by the loss of oxygen containing compounds. Concomitantly, this phase coincides with temperature condition where life in the deep subsurface is assumed to be still possible. First estimations of the feedstock potential and the generation rates of LMW organic acids indicate that the investigated NZ coals contain a substrate feedstock being large enough to sustain deep terrestrial microbial life over geological time spans.

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