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The chemistry of death – Adipocere degradation in modern graveyards

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ABSTRACT

The formation of adipocere slows further decomposition and preserves corpses for decades or even centuries. This resistance to degradation is a serious problem, especially with regard to the reuse of graves after regular resting times.

We present results from an exhumation series in modern graveyards where coffins from water-saturated earth graves contained adipocere embedded in black humic material after resting times of about 30 years. Based on the assumption that this humic material resulted from in situ degradation of adipocere, its presence contradicts the commonly held opinion that adipocere decomposition only occurs under aerobic conditions.

To test our hypothesis, we collected black humic material, adipocere as well as soil samples above and below coffins from representative graves ($n = 7$). A comprehensive chemical analysis of the samples substantiated our in situ degradation theory. Element compositions and fatty acid mass spectra confirmed that the humic black material originated from the corpses. A van Krevelen diagram classified the excavated adipocere material as lipid, whereas the black humic material was closer to the carbohydrate region. Mass fragmentograms of the humic material revealed the presence of large amounts of saturated vs. unsaturated nC_{16} and nC_{18} fatty acids, which is typical for adipocere. In addition, the soil samples exhibited a lipid signature deriving primarily from plant waxes and root components (C_{20}–C_{32}). Solid-state ^{13}C NMR spectra of adipocere displayed well-resolved signals of saturated aliphatic chains and a signal that corresponded to carboxylic acid groups. The NMR spectra of the black humic material revealed signals characteristic of long aliphatic chains. The intensities varied in relation to the state of degradation of the sample, as did the signals of oxidized aliphatic chains, acetals and ketals, aromatic structures, esters and amids. The analyses confirmed that the black humic material was indeed derived from adipocere, so the assumption is that the components detected must have developed from aliphatic fatty acids via a number of oxidation and condensation processes.

We therefore propose the existence of chemical pathway(s) for the degradation of adipocere under poikiloaerobic conditions. Possible (biogeo)chemical reaction chains include (1) the autoxidation of fatty acids enhanced by haemoglobin, methaemoglobin and haemin, (2) the use of alternative electron acceptors, which leads to the formation of H_2S that then reacts abiotically with iron (from haemoglobin), rendering iron sulphide, and (3) the Maillard reaction. These findings are another step forward in understanding the chemistry of buried corpses.

1. Introduction

Adipocere (adipo = fat, cere = wax) was for the first time mentioned in scientific reports in 1786 when the now defunct Holy Innocents' Cemetery in Paris was closed and relocated [1,2]. The original cemetery was located in a wet area, which impaired the timely degradation of the corpses. There have since been numerous studies on the formation of adipocere, as it prevents the reuse of graves after the regular burial time of 20–40 years [3].

The formation of adipocere is a post-mortem process during which neutral fat (triglycerides; storage fats) in soft tissues is converted into saturated fatty acids [4–6]. The most abundant fatty

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acids found in adipocere are palmitic acid ($C_{16}H_{32}O_2$), followed by stearic acid ($C_{18}H_{36}O_2$) and myristic acid ($C_{14}H_{28}O_2$); triglycerides, hydroxy and oxo fatty acids (e.g. 10-hydroxystearic acid) are less abundant [7]. Typical chemical characteristics of these fatty acids are their high melting points (palmitic acid = 63 °C, 10-hydroxystearic acid = 81 °C) and low water solubility (e.g. stearic acid: 0.003 g l^{-1} at 20 °C), two factors that favour the persistence of adipocere in soil graves. The decomposition of adipocere is highly limited in the anaerobic conditions of waterlogged soils [8,9]. The blue Vivianite man "Brienzi" [10], the iceman "Ötzi" [11] and the "Roman child" [12] are prominent examples of the ability of adipocere to persist for several hundred years.

The formation of adipocere preserves corpses in a permanent firm cast. In a forensic context, adipocere can preserve injuries and thus help to determine the cause of a person's death [13,14]. On the other hand, the presence of adipocere makes it relatively difficult to determine the length of time corpses have been buried [15].

While the formation of adipocere has been intensively investigated, both under laboratory [6,16] and real conditions [17–19], only a few reports concentrate on its degradation. Although lipids such as stearic acid and palmitic acid are attractive energy sources for microbial decomposers, microorganisms require approximately three times more oxygen to metabolize these fatty acids than a carbohydrate molecule of roughly comparable size [20,21]. However, in well-drained soils, lipids turnover more quickly than other organic compounds [22]. The current consensus is that adipocere can only be degraded under aerobic conditions [23], and that it can do so in less than 10 years, provided the oxygen level is sufficiently high [24].

While over 30% of all Baden–Württemberg and Bavarian municipalities surveyed have reported substantial decomposition problems in their graveyards [25–28], the situation is nowhere near as critical as it could be. This suggests that there must be pathways other than just aerobic degradation of adipocere.

The first indication that adipocere can degrade in situ arose during a comprehensive exhumation series carried out in a graveyard in southwest Germany in 2006 [29]. A large number of the coffins that were opened contained adipocere as well as black humic material (Photo 1a and b). Morphologically similar material was observed on corpses exhumed in another graveyard in southern Germany in 2009 (data not published). The presence of black humic material had not previously been mentioned in most reports on adipocere-laden corpses. Only one case report [30] mentioned a "*thick layer of pitch-black clay*" covering the body of an adipocere corpse found inside a well-preserved coffin in a paddy field.

We postulate that adipocere can be degraded in situ under poikiloaerobic conditions over relatively short periods; depending on the environmental conditions degradation can range from complete to partial depletion. We hypothesize that the black humic material found in the above-mentioned coffins is a direct degradation product of the adipocere formed in situ and results from a combination of anoxic or facultatively anaerobic microbial and chemical processes. The present study therefore aims (1) to characterize the organic substances inside coffins (adipocere and black humic material) found on exhumed corpses, (2) to confirm that the origin of the black humic material is human and to exclude soil or plants as sources, and (3) to propose a chemical degradation pathway for adipocere as an alternative to aerobic microbial degradation.

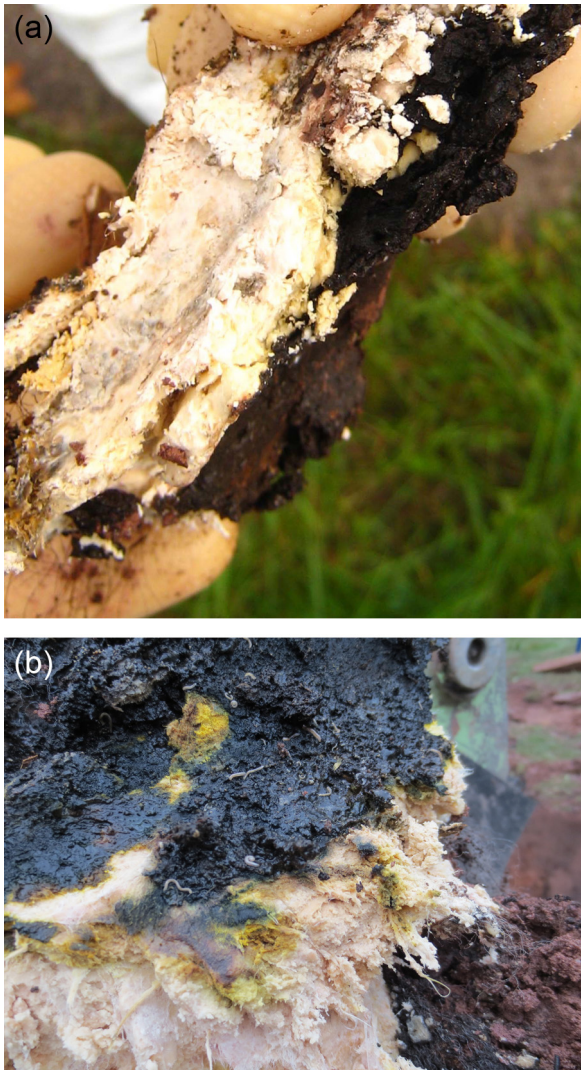


Photo 1. Adipocere plates derived from the buttocks of exhumed corpses. (a) Cross-section of an adipocere plate showing (i) the clear separation of black decomposition products and adipocere as well as (ii) degraded adipocere, which always commences on the surface. (b) Plan view of the surface of an adipocere plate.

2. Material and methods

2.1. Sites and sampling

The two graveyards under investigation are located in the Black Forest in southwest Germany (Table 1). In both graveyards, periglacial red sandstone covered with a predominantly sandy and clayey structure prevented the reuse of graves within the period prescribed as this particular cover deprived the soil of oxygen and led to the formation of adipocere. The exhumations were expected to provide information about the extent of adipocere formation.

Graveyard (1): In April 2006, 74 graves were opened after a resting time of 25 years (further details in [29]). Large parts of the bodies, including torsos and flat bones (mostly pelvic bones), were covered with adipocere. In addition, smaller adipocere chunks were found in 83% of all graves; they were embedded in humic and rooted black material that contained a large number of earthworms and artefacts (wood wool used as pillow filling, wood). Nine graves were selected for detailed characterization (Table 1).

Graveyard (2): In September 2009, five graves, located on an expanded area, were investigated. The graves, aged between 29 and >39 years, contained adipocere material (torsos, individual flat bones and chunks). Adipocere embedded in the dark, humic and moist black material of a double grave (the corpses were buried either in or before 1970; no exact information on burial

Table 1
Information about graveyards and samples under investigation.

Sample	Age at burial, sex	Burial duration (year)	Human remains (kg)	von Post degradation scale[c]	Observations
Graveyard (1), established in 1974 Location: Black Forest (Germany), 875 m a.s.l. Climate: MAT = 6 °C, MAP = 1200 mm Parent material = physically pre-weathered periglacial regolith of "Buntsandstein" (Triassic red sandstone), sandy and clayey texture					
F01[b]	80, f	26	3	H8	Black, trace of wood wool and roots, remains of soil fauna, sand
F02	n.k.	n.k.	n.d.	H4	Brown, wood wool, sand
F03[a,b]	57, m	27	3.8	H4	Brown, wood wool
F05[a]	89, f	26	14	H10	Dark black, traces of wood and roots
F07[b]	n.k.	n.k.	n.d.	H8	Brown-black, pieces of adipocere, wood wool, traces of roots, earthworms, millipedes
F09[a,b]	85, f	28	3.8	H9	Black, high density of roots
F10	68, f	27	2.8	H4	Brown, wood wool
F11	46, m	26	12	H6	Dark brown, roots, wood wool, sand
F23[a,b]	47, f	26	4	H10	Dark black, scratched directly from the adipocere surface, small visible bits of white adipocere material, sand, some soil fauna
Adipocere	47, f	26	4	–	Grey, adipocere material
Graveyard (2), established in the 18th century Location: Black Forest (Germany), 627 m a.s.l. Climate: MAT = 8 °C, MAP = 960 mm Parent material = physically pre-weathered periglacial regolith of "Buntsandstein" (Triassic red sandstone), sandy and clayey texture					
F04a[b]	n.k., m	>39	n.d.	H8	Brown-black, small pieces of white adipocere, traces of wood wool and roots, sand, soil fauna
F04b[b]	n.k., f	39	n.d.	H8	Brown-black, many pieces of white adipocere, traces of wood wool and roots, earthworms, soil fauna

f, female; m, male; n.k., not known; n.d., not determined.
[a] NMR samples.
[b] Samples for lipid extraction.
[c] von Post scale was developed for the classification of the degradation status of organic soils; H4, weakly decomposed; H6, well decomposed; H8, very strongly decomposed; H10, completely decomposed.

time is available) was investigated in detail in the present study (Table 1).

Anaerobic conditions (presence of Fe^{2+}) in the grave soils under investigation were verified in the field using the α-α-dipyridyl test [31] during the exhumation process. The degree of decomposition of the black humic material inside the coffins was evaluated with the von Post scale of humification which ranks decomposed organic material on a scale from H1 (undecomposed) to H10 (completely decomposed) [32] (Table 1).

Adipocere and black humic material (adipocere sample F23, Table 1) were separated from one another using a scalpel. A distinct difference in colour (whitish grey and black) enabled the two to be clearly distinguished from one another. The samples were dried at 30 °C, freed from roots and subsequently ground for 1 min using a vibratory disc mill (T-100, Siebtechnik GmbH, Mülheim a. d. Ruhr, Germany) with an agate stone.

2.2. Elemental analyses

The C-, H-, S- and N-contents were determined through combustion of 2 mg sample material in CHNS mode. The O-contents of the organic (combustible) part of the samples were determined through combustion of 2 mg sample material in O mode using a vario EL cube (Elementar Analysensysteme GmbH, Hanau, Germany). Measurements were done in triplicates and detection was based on the materials' thermal conductivity. The soil samples (above and below the coffins) were analyzed in two replicates.

2.3. Fatty acid analysis

All samples from inside and from the area surrounding the coffins were Soxhlet-extracted with a 1:1 (v/v) mixture of dichloromethane/methanol. The collected extracts were saponified with 10% KOH in methanol. Total extracts were subsequently derivatized with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and analyzed by GC/MS using an Agilent 5975 MS coupled with an Agilent 6890 GC equipped with a HP-5MS column (30 m × 0.25 mm × 0.25 μm) and fitted with a programmable MMI (MultiMode Inlet) injector. Samples were injected splitless at 60 °C and the injector ramped to 320 °C within 2 min. The oven programme was 60–140 °C at 10°/min, followed by 4 °C/min to 320 °C and 25 min isothermal. Helium was used as carrier gas with a constant flow of 1.0 ml/min. Compound identification was performed using a reference mixture of fatty acid standards and comparison with the Wiley mass spectra library.

2.4. Solid-state NMR spectroscopy

2.4.1. General conditions

A 7.05 T Varian INOVA™ Unity (Varian Inc., Palo Alto, CA, USA) equipped with an HX Apex probe was used. The operating frequencies for ^{13}C and ^{1}H were 75.4 and 299.9 MHz, respectively. Samples were packed in 6 mm Pencil® rotors with Vespel® drive tips, and custom-made boron nitride inlays were used to position the samples in the homogeneous region of the sampling coil [33]. The samples were spun at 7500 ± 1 Hz and the temperature was kept constant at 25.0 ± 0.1 °C. All spectra were recorded with a sweep width of 25 kHz and an acquisition time of 30 ms. Decoupling was done using a SPINAL (Small Phase Incremental Alteration) sequence with a ^{1}H radio frequency (r.f.) field strength of 50 kHz, a phase of 4.5° and a pulse width of 11 μs. The acquired free induction decays (FID) were Fourier-transformed with Mestre-C (Version 4.9.9.9, Mestrelab Research, Santiago de Compostela, Spain) by applying an exponential filter function with a line broadening (lb) of 30 Hz.

Table 2
Element contents (mean ± standard error) and molar mass ratios of samples under investigation.

Sample	Element content (wt %)					Molar mass ratio	
	C	N	S	H	O	H/C	O/C
Graveyard (1)							
Adipocere[a]	71.3 ± 0.3	0.34 ± 0.05	0.36 ± 0.23	12.1 ± 0.2	15.5 ± 0.2	2.02	0.16
F01[b]	22.2 ± 0.3	2.10 ± 0.03	0.48 ± 0.01	3.24 ± 0.04	22.6 ± 0.2	1.75	0.76
F02	12.5 ± 0.2	0.66 ± 0.01	0.14 ± 0.03	1.87 ± 0.03	14.0 ± 0.3	1.78	0.84
F03[a,b]	34.1 ± 0.2	3.18 ± 0.05	0.53 ± 0.02	4.82 ± 0.08	34.6 ± 0.4	1.68	0.76
F05[a]	44.3 ± 0.1	5.60 ± 0.06	1.63 ± 0.03	6.21 ± 0.03	33.9 ± 0.1	1.67	0.57
F07[b]	39.7 ± 0.3	3.97 ± 0.08	0.80 ± 0.05	5.72 ± 0.07	31.5 ± 0.2	1.72	0.60
F09[a,b]	42.9 ± 0.2	3.39 ± 0.01	0.89 ± 0.05	5.74 ± 0.02	36.5 ± 0.3	1.59	0.64
F10	42.3 ± 0.2	1.21 ± 0.01	0.28 ± 0.04	5.48 ± 0.04	36.1 ± 0.3	1.54	0.64
F11	24.2 ± 0.4	2.35 ± 0.02	0.74 ± 0.06	3.33 ± 0.12	22.3 ± 0.4	1.64	0.69
F23[a,b]	35.6 ± 0.7	2.11 ± 0.05	0.41 ± 0.08	5.07 ± 0.08	31.5 ± 0.2	1.70	0.66
Graveyard (2)							
F04a	26.3 ± 0.1	1.91 ± 0.06	0.56 ± 0.06	3.83 ± 0.02	25.0 ± 0.1	1.74	0.71
F04b	38.8 ± 0.1	3.53 ± 0.02	0.98 ± 0.04	5.53 ± 0.04	32.2 ± 0.3	1.70	0.62
Grave soils							
Above coffins	0.15 ± 0.07	0.01	0.01				
Below coffins	0.06 ± 0.02	0.01	0.01				

[a] NMR samples.
[b] Samples for lipids extraction.

2.4.2. $^{13}C(^{1}H)$ Cross polarization (CP) experiments

A 90° ^{1}H pulse of 4 μs was applied, followed by a cross polarization (CP) spinlock pulse of 1 ms. The r.f. field applied to ^{13}C during CP was kept constant at 47.5 kHz, while the ^{1}H r.f. field strength was set at 40 kHz with a ramp of 6 kHz in order to take into account inhomogeneities of the Hartmann–Hahn condition [34]. Between 256 and 50,000 transients (nt) with a recycle delay (d1) of 3 s (except adipocere sample: d1 = 15 s) were acquired.

3. Results

The typical sandy clay loam soil texture in combination with strong lateral water flow (punctiform water outflow on the pit wall) led to a high level of moistness in all grave soils. The soils surrounding the coffins were grey in colour and associated with strong reducing conditions, which were identified by a positive Fe^{2+} test (Table 1).

The black material inside the coffins was moist and greasy. The adipocere was of a greyish, off-white colour. The black material either occurred together with small plates and chunks of adipocere or covered the adipocere (Photo 1). The degree of decomposition of the black humic material inside the coffins ranged from H4 (weakly decomposed) to H10 (completely decomposed) on the von Post scale (Table 1).

3.1. Elemental composition

The mean concentration of carbon and hydrogen in the adipocere material investigated was considerably higher than that of the black humic material (713 vs. 330 g C kg^{-1} and 121 vs. 46 g H kg^{-1}; Table 2). The humic material revealed higher mean concentrations of sulphur (S), nitrogen (N) and oxygen than the adipocere material (6.7 vs. 3.6 g S kg^{-1}; 28 vs. 3.4 g N kg^{-1}; 291 vs. 155 g O kg^{-1}). The soil matrix surrounding the coffins displayed far lower C and N concentrations than the organic substance contained in the coffins. The soil material directly above the coffins investigated contained 1.5 ± 0.7 g C kg^{-1} and 0.1 g N kg^{-1}, the material below the coffins 0.6± 0.2 g C kg^{-1} and 0.1 g N kg^{-1} (Table 2). The black humic material of the two graveyards had a very similar element concentration despite the fact that there was a considerable difference in resting time (25 vs. >39 years). Four samples (F01, F02, F11 and F04a; Tables 1 and 2) had much lower C-, and H-contents than the other samples due to the presence of sand that diluted the organic material. This was also reflected in the lower quantities of O originating from the organic (combustible) part.

The mean molecular mass ratio between H and C of the humic material was lower (1.68 vs. 2.02) and the O/C ratio (0.68 vs. 0.16) higher than that of adipocere (Table 2).

The adipocere material could be clearly classified as lipid in the van Krevelen diagram (Fig. 1). The decomposition products, on the other hand, were close to the carbohydrate region.

3.2. Fatty acid analysis

The extract yields of total lipids derived from 7 grave sites (Tables 1 and 2) were on average 44,000 ppm for the black humic material in contrast to only 135 ppm for soils above and 73 ppm for soils below the coffins (equal to 120, 130 and 82 mg per g total organic carbon).

The soil samples exhibited a lipid signature that was primarily derived from plant waxes and root components, as indicated by the dominance of long-chain lipids (C_{20}–C_{32}) with an even number of carbon atoms. Lipid classes were dominated by n-fatty acids,

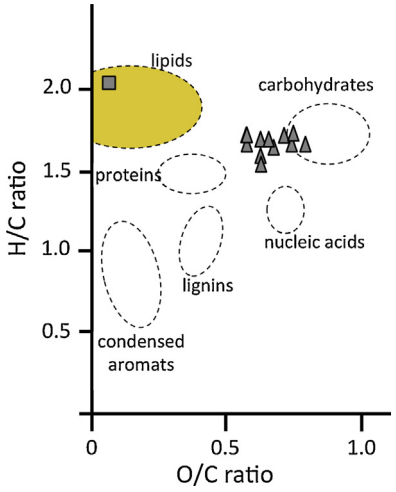


Fig. 1. Positioning of the samples in the van Krevelen diagram (square: adipocere; triangles: decomposed material).

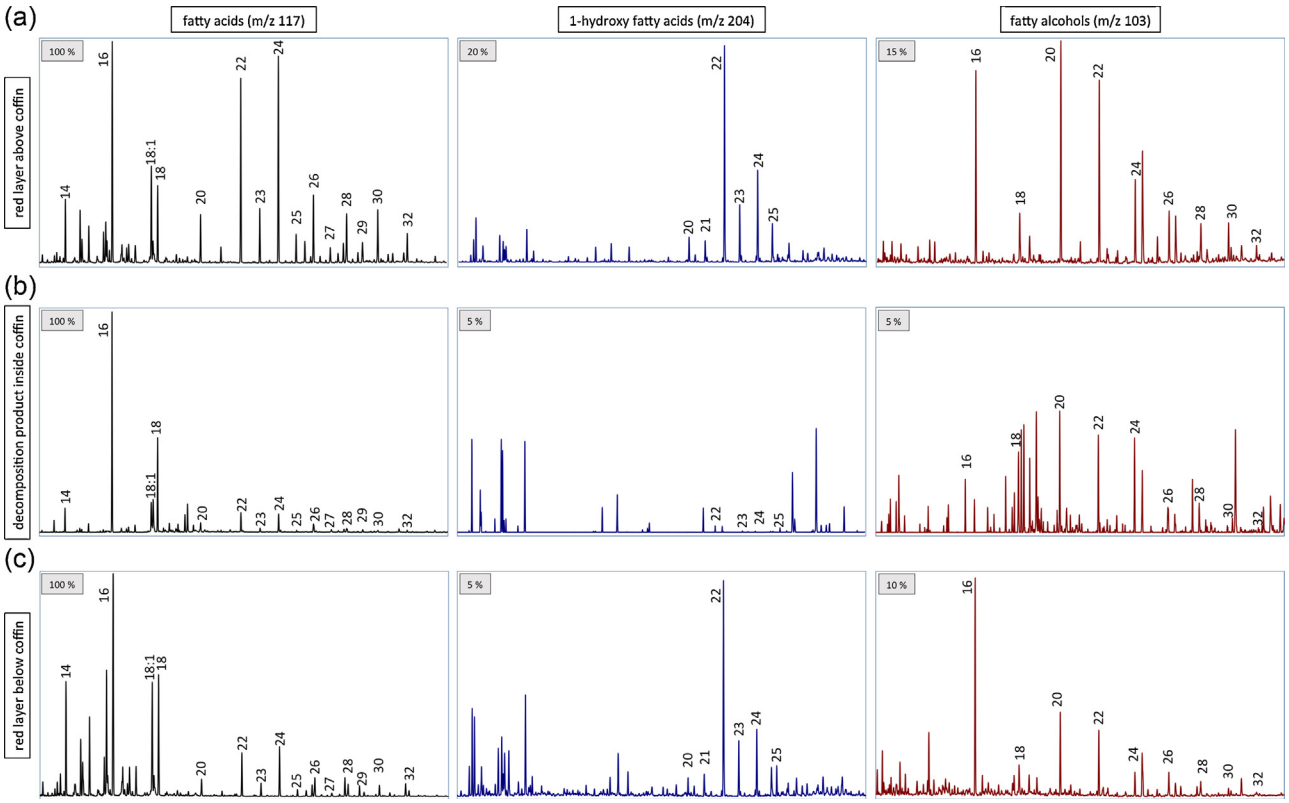


Fig. 2. Mass fragmentograms of mass $m/z = 117$ (fatty acids), $m/z = 204$ (hydroxy fatty acids) and $m/z = 103$ (n-alcohols). Three sampled horizons of grave F07. Numbers above peaks indicate carbon atoms within corresponding lipid. Fatty acid notation includes the carbon number followed by the number of double bonds. Relative intensities are normalized to the highest peak (hexadecanoic acid).

followed by terminal n-hydroxy fatty acids and n-alcohols. The most abundant n-fatty acid was nC_{24}; the most abundant n-hydroxy fatty acid was nC_{22} and the most abundant n-alcohol was nC_{20}.

An example of the compound distribution patterns of the black humic material is given for sample F07 from graveyard 1 (Fig. 2). The n-fatty acids are depicted by the mass fragment of $m/z = 117$, the hydroxy fatty acids by $m/z = 204$ and the n-alcohols by $m/z = 103$. In addition to the long-chain wax lipids, short-chain microbial components (C_{14}–C_{18}) were abundant in n-fatty acids and n-alcohols. Short-chain hydroxy fatty acids were not observed. Fragmentogram $m/z = 204$ revealed sugars to be early eluting compounds. The short chain fatty acids were dominated by nC_{16}, which was the only isomer in the black humic material.

In contrast, the soil samples above and below the coffins contained high level of mono-unsaturated $C_{16:1}$ fatty acids, presumably of microbial origin. The C_{18} fatty acids were composed of mixtures of saturated and mono-unsaturated isomers, with significantly higher amounts of mono-unsaturated components in the soils where saturated and unsaturated isomers occurred at equal concentration (Fig. 2). Hexadecanol was the only short-chain alcohol that was present in high amounts (Fig. 2).

3.3. NMR spectra

Fig. 3 displays the CP/MAS spectra of an adipocere sample and some black humic materials. Table 3 gives an overview of the signal assignments in ^{13}C NMR.

The NMR spectrum of the adipocere sample displayed a peak at 14 ppm, which is due to terminating methyl groups; the peak at 24 ppm belongs to methylene groups that are close to the terminating methyl groups and the 32 ppm peak was generated by methylene groups inside aliphatic chains. Methylene groups adjacent to carboxyl functions or hydroxylated carbons resonated at 38 ppm. The two signals at 180 and 181 ppm were due to carboxyl groups and/or their salts. The signal at 71 ppm documents the presence of scattered >CHOH groups. Furthermore, a few unsaturated –CH=CH– groups were present and generated a small peak at 130 ppm. Aromatic structures also resonated at this chemical shift, but were unlikely to be present due to the nature of the sample.

Sample F23 was scratched directly from the surface of the adipocere material and contained small chunks of the latter, as shown in the spectrum. The aliphatic region displayed well-defined peaks at 14, 24 and 32 ppm; a shoulder originating from the acid groups was visible at 179 ppm. Apart from the adipocere-related signals, the 45–90 ppm region (C–O, C–N) was very prominent and two clear peaks were present at 102 ppm (acetal C) and 172 ppm (ester, amid C). The 110–160 ppm region

Table 3
^{13}C NMR chemical shift assignment according to Wilson [60] and Bortiatynski et al. [61].

Shift region (ppm)	Chemical structure
0–45	Linear and cyclic alkyl C (–CH_3, >CH_2, >CH–, >C<)
45–60	Aliphatic C–N
50–58	Methoxyl C
45–90	Aliphatic C–O (carbohydrates, alcohols)
90–110	Acetal-/ketal-C (anomeric C in carbohydrate)
100–140	Aromatic and olefinic C
140–160	Substituted aromats (aryl-O, aryl-N)
160–185	Carboxyl C (acid, ester) and amid C
185–220	Carbonyl C (aldehyde, keto)

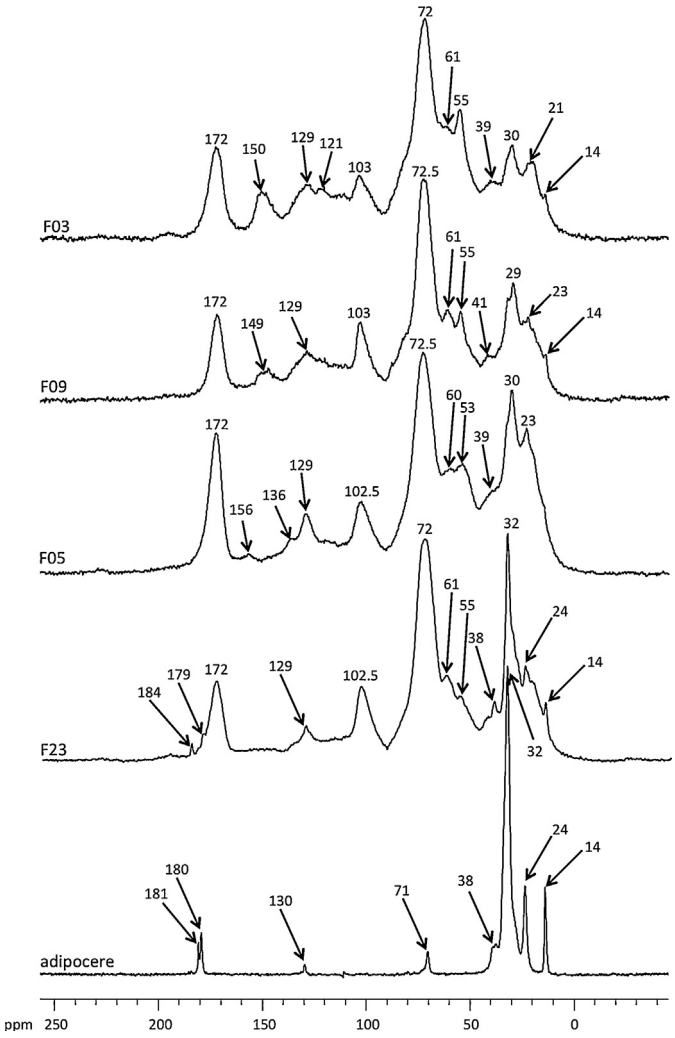


Fig. 3. 13C(1H) cross polarization NMR spectra of selected samples (all signal intensities are graphically normalized to the signal at 72 ppm; does not apply to the adipocere sample).

contained a smeared signal of aromatic structures with a sharper signal at 129 ppm sitting on top of it, which could be due to unsaturated –CH=CH– groups.

The black humic material of samples F09, F05 and F03 did not contain any visible adipocere pieces. The spectra of these samples displayed a reduced main peak in the aliphatic region (32 ppm). Furthermore, the aliphatic signals were no longer well-defined. The reduction of the main methylene signal and the broadening of the signals at 14 and 24 ppm are due to shorter aliphatic chains and/or more ramified aliphatic structures. The broadening of the resonance at 38 ppm indicates a higher content of aliphatic carbons adjacent to hydroxylated carbons, whose presence in turn was evident from the distinct signals at 72 ppm (>CHOH) and 61 ppm (–CH_2OH). The signal at around 55 ppm is typical for methoxy groups (–O–CH_3). The 110–160 ppm region mainly contained aromatic structures and unsaturated >C=C< bonds. A distinct resonance at 129 ppm could be seen in the samples F23 and F05, where the intensity of the aliphatic region was less reduced than in samples F09 and F03. Conversely, the 129 ppm signal of samples F09 and F03 was much broader and less distinct. The smearing of this region might be due to the emergence of various conjugated systems, inducing the delocalization of π electrons.

4. Discussion

4.1. Fatty acid analysis

The lipid composition of the three investigated layers, i.e. the black humic material inside the coffins vs. soils above and below the coffins, revealed big differences in the source materials as well as in microbial overprinting. The most abundant lipid class of n-fatty acids occurred ubiquitously and may derive from a variety of biological sources. The n-fatty acid distribution pattern differed substantially between the three samples, indicating a much higher plant-derived input in the soil layers than in the black humic layer inside the coffins, which showed almost no plant-derived components, except for some nC_{22} and nC_{24} isomers. These two n-fatty acids are primarily enriched in subterranean root waxes [35,36] but, depending on plant type, may also originate from subaerial intra- and extracuticular waxes. The highest relative abundance of wax fatty acids was noted for the soil layer above the coffins, which may have received the largest input of surface-derived material during excavation and placement of the coffin. This soil layer exhibited the greatest abundance of roots. Plant wax input into the soil layers could be substantiated further on the basis of the terminal hydroxyl fatty acid distribution, which again revealed a high proportion of root-coating waxes and suberinic polyesters, as indicated by their nC_{22} and nC_{24} dominance [35,36]. The origin of the long-chain alcohols from plant waxes was evident from the dominance of long-chain components, which contributed around 10–15 percent of the total lipid pool in the soil layers, but less than 5% in the black humic material inside the coffins. The plant-derived lipids were most abundant in the soil above the coffins that was richest in root-derived lipids, then in the soil below the coffins and occurred only in traces in the adipocere horizon.

Microbial lipids, as indicated by the presence of hexadecenoic and octadecenoic acids, occurred in typical concentrations in the soil layers and are a sign of organic soil matter decomposition. The black humic material deviated from this typical distribution pattern by showing no unsaturated C_{16} fatty acids at all and only minor amounts of $nC_{18:1}$. This was taken as evidence that the saturated hexadecanoic and octadecanoic acids that dominate the fatty acid distribution with a cumulative proportion of approx. 60% of the total fatty acids derive from triacylglycerols esterified with saturated fatty acids. Such triacylglycerols constitute the body fat of Animalia, including humans. Natural hydrolysis of fat esters releases these components into the environment; saponification upon sample preparation tends to enhance this process. The high abundance of saturated vs. unsaturated nC_{16} and nC_{18} fatty acids in this black layer strongly supports the likelihood of its origin from adipocere. The fatty acid fingerprint is highly compatible with that determined for adipocere from a retreating glacier in British Columbia, Canada [37] and from a peat bog in Ireland [38]. Mid-chain substituted hydroxy fatty acids proposed as molecular markers for adipocere [39] were not detected in this study nor mentioned in the last two literature references. Their effective application as diagnostic markers for adipocere in natural soils and sediments cannot therefore be confirmed.

4.2. NMR spectra

The spectrum of the pure adipocere sample corresponded with that of fatty acids and hydroxylated fatty acids, respectively, and hence confirms the sample's lipid nature as demonstrated by the van Krevelen plot (Fig. 1).

The joint presence of signals at 55 and 150 ppm in soil samples is often due to lignin structures. Both signals were very prominent in samples F09 and F03. From the description of these samples (Table 1) it becomes clear that these lignin signals were in fact

generated by lignin structures originating from wood wool in F03 (stuffing material of the inner coffin bedding) and a particular high amount of plant roots in F09. The fact that both signals were significantly reduced in samples F05 and F23 further confirms this.

Part of the signal intensity between 45 and 90 ppm might be due to simple alcohols and amines generated through the putrefaction of human tissues. However, another potential source of this dominating signal is the autoxidation of the fatty acids from the adipocere material, which generates perhydroxylated and hydroxylated carbons and carbonyl functions such as aldehyde and keto groups. This autoxidation of the aliphatic chains furthermore produced a signal shift from the pure aliphatic region to the region of substituted aliphatics (45–90 ppm) and, theoretically, to the region of aldehydes and ketones (185–220 ppm), which also explains the signal reduction in the aliphatic region.

Aldehyde and keto groups react with hydroxyl groups to form acetals, ketals or cyclic hemiacetals, which generated the distinct signal at around 102 ppm. The lack of an aldehyde/keto signal at around 200 ppm suggests that this reaction completely consumed the aldehyd and keto groups formed. In soil science, the acetal signal at 102 ppm is usually assigned to the anomeric C (i.e. cyclic hemiacetal) of the cellulose components. In the present case, the structures generated are similar to those in carbohydrates, but not as regular. This is because the oxidation of the fatty acids is random and will only produce "pseudo-carbohydrates", unlike the controlled synthesis of cellulose in plants. This was also confirmed by the position of the decomposition products in the van Krevelen diagram. The O/C ratio of these samples was lower than the ratio found in carbohydrates, indicating that the chains are less substituted with hydroxyl groups than common carbohydrates.

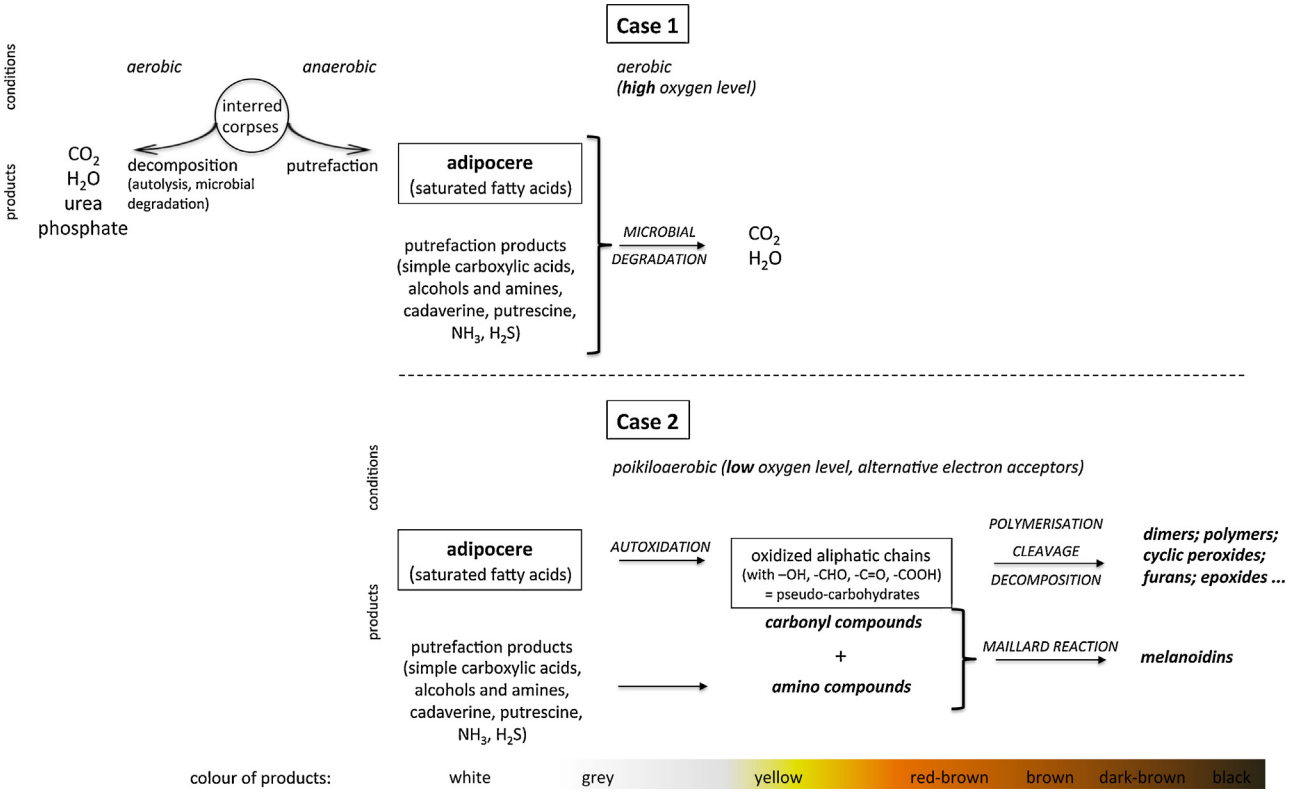
Judging from the aliphatic region, samples F23 and F05 seem to be less advanced in their degradation and the distinct signal at 129 ppm was probably due to isolated double bonds (i.e. localized π electrons) that are not yet part of a conjugated system. The carboxyl groups of the fatty acids most likely reacted with hydroxyl groups to form ester groups or lactones (i.e. cyclic esters), producing a shift of the carboxyl signal (around 180 ppm in the adipocere spectrum) to 172 ppm. The large signal intensity suggests that further groups resonating in this area were formed as the adipocere decomposed.

4.3. Hypothesis on possible chemical adipocere degradation pathways

As stated in the introduction, adipocere is formed under anaerobic conditions and degradation has only been observed when environmental conditions changed to an aerobic milieu that allows microbial degradation [23,24,40,41]. Up until now, the only degradation pathway considered plausible involves the microbial degradation under aerobic conditions, which ends with the formation of water and carbon dioxide (Scheme 1, case 1).

In the following, we would like to propose a further degradation pathway, i.e. the chemical degradation of adipocere at low oxygen levels. We base our hypothesis on the observation that abundant black material was present on the adipocere, which is clearly of human origin (see above) and which obviously stems from the underlying adipocere as no other source material was present (Scheme 1).

It is unlikely that under the poikiloaerobic (alternating anaerobic/aerobic) conditions of the graveyards investigated, oxygen levels will ever be high enough for efficient aerobic microbial degradation. As already outlined by Schoenen and Schoenen [41], the oxygen supply in deeper soil layers is very limited and not sufficient to completely degrade a corpse. However, autoxidation of the fatty acids starts during the long resting time and proceeds under the poikiloaerobic conditions present. An induction period [42,43] precedes the perceptible degradation of fats during which the quality of the fats remains unchanged over a prolonged period of time. After this induction period, the autoxidation rate then accelerates exponentially. Furthermore, haemoglobin, methaemoglobin and haemin are very



Scheme 1. Possible degradation pathways of adipocere under aerobic and poikiloaerobic conditions.

effective catalysts of fat autoxidation and much more effective in low concentrations than comparably low concentrations of inorganic iron salts [44,45].

The autoxidation of fats is a free-radical process, which starts with the abstraction of a hydrogen atom, preferably adjacent to a double bond. The NMR spectra show that the adipocere only contained a small amount of unsaturated fatty acids. Once the concentration of the initially formed radicals reaches a threshold value, the reaction rate is accelerated autocatalytically [46]. This pyramidal effect implies that relatively few initiating radicals are needed to break down a large amount of fatty acids [47]. For more details on autoxidation of fats (see supplementary section). The products of the autoxidation include dimers, polymers, keto, hydroxy and epoxy compounds as well as cyclic compounds such as furans, aldehydes and alcohols [47–49].

A further adipocere oxidation pathway under anoxic conditions proceeds through alternative electron acceptors such as NO_3^- or SO_4^{2-}. Facultatively anaerobic bacteria such as *Enterobacter* are able to use nitrate for the oxidation of organic matter. At very low redox potentials, obligate anaerobes such as *Desulfobacter* sp. use sulphate as an electron acceptor for this purpose [50]. An indicator of the latter is the intense black colour of the degradation material. During sulphate reduction in the presence of organic matter, H_2S forms and reacts abiotically with iron (e.g. from haemoglobin) to form iron sulphide. This reaction is well known from anoxic wetland soils such as paddy fields which develop an intense dark brown to black colour due to iron monosulphide precipitation [50,51].

The Maillard reaction is another possible consecutive reaction of the intermediate products of the fat autoxidation. The Maillard reaction is usually described as a reaction between reducing sugars and amino acids during typical cooking procedures such as roasting, baking or frying. However, it is neither restricted to these two well-defined classes of chemical components nor does it only occur at temperatures above 100 °C. Maillard himself [52,53] noted that the reaction also takes place at room temperature, but at a greatly reduced rate. He also suggested that the reaction plays a major role in the formation of humic substances [54,55]. The soil science community largely agrees that the Maillard reaction only contributes marginally to the formation of organic soil matter. To food chemists, however, it is a key reaction when it comes to the ageing and degradation of vegetable and animal food products. For more details on the Maillard reaction (see the supplementary section).

In the present case, autoxidation produced oxidized aliphatic chains containing hydroxy, keto and aldehyde groups. During putrefaction, the nitrogen-containing compounds of the human body are usually transformed into the diamine compounds cadaverine and putrescine. Red-brown to black-brown melanoidins are the typical end products of the Maillard reaction; their structures can also vary considerably. Intermediate products can contain cyclic furans, furanons, pyranons and other heterocyclic compounds such as pyrrols, oxazols and thiazols or polymeric forms of aldimines or ketimines.

The chemical shifts of aldimines and ketimines lie between 150 and 179 ppm [56,57]. The prominent spectral signal at 172 ppm (Fig. 2) might therefore also be due to the presence of aldimine or ketimine structures. However, the formation of heterocycles or aromatic structures as reported in papers on the structure of melanoidins [58,59] cannot be deduced from the spectra of the decomposition products. This could be due to the fact that in the present case the starting materials are oxidized aliphatic chains rather than structurally well-defined carbohydrates such as cellulose or starch. As the chains are probably not regularly hydroxylated, it is possible that the intermediates lack reaction partners in the proper position in the chain and the formation of heterocycles is hindered.

5. Conclusion

The black humic material covering the adipocere in a number of waterlogged graves was purely organic (with occasional sand admixture) and the analysis of the fatty acid profiles ascertained the material to be of human origin. The black material was therefore a direct degradation product of the underlying adipocere. The decomposition of the adipocere was triggered through alternating anaerobic/aerobic conditions which induced the autoxidation of the fatty acids. The oxidized products then underwent further condensation or degradation.

The degradation pathway hypothesis presented here does not of course encompass the complete degradation of the adipocere and is meant to be considered as one of the number of options. Further processes involved are the microbial use of the sugar-like materials as energy source (which require lower amounts of oxygen for microbial degradation than adipocere), the conversion of the decomposition products, possibly also the adipocere itself, by worms (present in a number of graves) and the enzymatic degradation by fungi.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.forsciint.2015.09.010.

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