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Quantification of adipocere degradation with and without access to oxygen and to the living soil

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ABSTRACT

Adipocere is formed from body fat in moist and oxygen-deficient decay conditions. The persistence of adipocere may cause problems for the reuse of graves after the expiration of statutory resting times in some countries. Up to now, no quantitative data existed on the persistence of adipocere in either aerated or anoxic conditions. We investigated the rate of degradation (disappearance) of adipocere in five different samples from human corpses. The experimental incubation was (a) in water without air contact, (b) in water with access to air, (c) in physiological saline with access to air, (d) on sterilized quartz sand, (e) in vitro on living soil, and (f) buried 15 cm deep in field soil. The weight loss of the samples was determined after 215 (293) days and half-lives were calculated under the assumption of simple first-order kinetics. Furthermore, the nitrogen content and the fatty acid composition of the adipocere samples were analyzed.

The results revealed half-lives that differ between the adipocere samples from 11 to 82 years under anaerobic conditions (mean of all samples, 37 years). In air, the half-life of adipocere was reduced to about one tenth, ranging from 0.7 to 10 years (mean of 2.8 years for all samples incubated in aerated physiological saline, mean of 4.0 years for all samples incubated on living soil in the laboratory). Burying adipocere in a biologically active field soil resulted in half-lives of disappearance from 1.2 years to 2.1 years (mean, 1.5 years). The N content of the adipocere samples ranged between 1.9 and 6.7 mg N g^{-1}. The sample with the highest N content was also that with the lowest half-life of disappearance in all types of incubation. The fatty acid analysis of the samples revealed a composition typical of adipocere, with a clear dominance of saturated acids (palmitic, myristic and stearic acid) over unsaturated ones. The variation of fatty acid composition between the different adipocere samples could only be attributed partly to their age and the burial conditions.

It can be concluded that the aeration of adipocere-laden corpses will lead to a disappearance of adipocere (and hence restitution of the decay process) within a time span of several years.

1. Introduction

Adipocere is a solid white substance which is formed from body fat during decay processes in moist and anaerobic conditions [1,2]. Adipocere develops as an accumulation of unsaturated fatty acids (FAs) at the surface of anoxic hydrated zones [3]. By microbial beta oxidation and hydrogenation, the unsaturated FAs in adipose tissue (mainly oleic and palmitoleic acid [4]) are converted to palmitic and myristic acid, which have a considerably higher melting point than their unsaturated precursors (63 °C in palmitic acid vs. 16 °C in oleic acid). The unsaturated FAs move to the surface of the water phase, where they may solidify and form an exchange barrier. This has been observed in laboratory experiments [5,6]. Palmitic acid is the main constituent of adipocere, which also contains smaller amounts of stearic and myristic acid [7–9]. In wet conditions, hydroxystearic acid is also present as a typical component of adipocere [10,7].

Adipocere formation is associated with an inhibition of decay, which may cause problems in such countries as Germany, where graves are generally reused after a certain resting period (often 25 years) [3,5]. In the case of disturbed decomposition in graveyards, measures to aerate the graves are offered commercially to promote the degradation of adipocere and the decay of the corpse in general [11]. There have been attempts to use adipocere in the determination of the post mortem interval [12]. The gas chromatographic assay of adipocere-specific fatty acids in soil samples has also been proposed to characterize grave soil [13]. While the formation of adipocere has been studied extensively [5,10,14], to our knowledge

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there are only two studies that explore the degradation of adipocere [15,16]. With the exception of [16], there are no quantitative data on the rate of adipocere degradation either under anaerobic or under aerobic conditions. The extent to which soil biota are involved in the process of adipocere is also not known.

We investigated the degradation of adipocere samples at different incubation regimes in the laboratory and in the field. In particular, the half-lives of adipocere disappearance (DT50) were calculated, and the following hypotheses were tested: (a) adipocere disappearance is accelerated under aerobic conditions and (b) the presence of a natural community of soil animals promotes the disappearance of adipocere.

2. Material and methods

Five samples of human adipocere collected at reburials were investigated. Table 1 shows the burial periods, and the state of the corpses and graves at the time of exhumation.

The samples taken from the coffin were placed in air-tight plastic bags and stored in the dark at 4 °C for a time period varying from 14 days to 6 months. At the start of the experiment, small pieces were taken from every sample and prepared to monolithic specimens with a weight of 0.05–0.17 g.

The decomposition of these specimens was studied in six treatments:

(a) incubation in demineralized water without air contact (one specimen per sample);
(b) incubation in demineralized water with air contact (one specimen per sample);
(c) incubation in physiological saline with air contact (one specimen per sample);
(d) laboratory incubation on sterilized quartz sand (two specimens of samples A–C; one specimen of samples D and E);
(e) laboratory incubation on living soil with mesofauna (three specimens of samples A–C and E; four specimens of sample D);
(f) field incubation in living forest soil (five specimens of samples A–D; four specimens of sample E).

Treatments (a)–(c) were incubated in Petri dishes. An initial microflora was set by adding 1% of cleaned, filtered sewage water at the start. In treatments (d) and (e), 210 ml glass vessels with a screw top were each filled with approximately 60 g tempered quartz sand and sifted soil (2 mm mesh) from the Ah-horizon of a mull-type forest soil. The water content was adjusted to 65% water holding capacity. In every vessel, two specimens were placed in an open plastic tube (mini container) on top of the substrate (see photograph in online supplementary data). A hole in the lid, covered with 0.2 mm mesh nylon gauze, was provided for aeration. To reduce evaporation, the vessels were kept in a closed water bath. The specimens were wetted with a few drops of tap water every 2–4 weeks, and visually controlled. The experiment was terminated after 215 days. The adipocere specimens were dried at 36 °C in an exsiccator until weight constancy was established and the weight loss during incubation was determined.

In the field incubation (treatment (f)), the adipocere from laboratory incubations (d) and (e) was reused. The dried specimens were placed in Eisenbeis mini containers [17], covered by 2 mm mesh nylon gauze, placed in supporting rods and placed horizontally at a depth of 15 cm in the soil of the same site from where the soil for the laboratory incubation was taken. The site contains a group of old beech (*Fagus sylvatica*) and oak (*Quercus robur*) trees with a dense herb layer of yellow archangel (*Lamiastrum galeobdolon*), situated in a park. The soil is a cambisol with humus form mull. Analytical determination of the Ah-horizon revealed 7.1% organic carbon, 0.38% total nitrogen, pH 6.5, 9% clay and 65% sand. After 293 days (2006/07/06 to 2007/04/25) the containers were dug out, visually controlled and cleaned. Soil adhering to the adipocere was quantified as ash content, after combustion of the samples in a furnace at 550 °C. The adipocere weight was than calculated as ash-free dry matter.

The chemical composition was analyzed in separate subsamples prepared from the stored adipocere of types A–D. There was insufficient material of adipocere type E for a chemical analysis to be performed. The N content of the samples was determined using the Kjeldahl method. Fatty acids in the samples were analyzed by Dr. Reinhard Bierl, Trier University, Germany after extraction with dichloromethane/methanol [18].

The rate constant of adipocere decomposition was calculated according to a simple first-order kinetic.

$$\text{rate constant}, k = -\frac{\ln(A_t/A_0)}{t} \quad (1)$$

$$\text{half-life}, t_{1/2} = \frac{\ln(2)}{k} \quad (2)$$

where A_t is the dry weight of specimen at end of incubation, A_0 the dry weight of specimen at start of incubation, and t is the incubation time.

Statistical significance was tested using the Kruskal–Wallis test.

3. Results

3.1. Time course of adipocere disappearance

In Table 2, the weight loss of the adipocere samples in the experimental treatments is expressed as adipocere half-life in years.

Without air contact, adipocere disappeared very slowly, with a half-life of between 11 and 82 years. In contact with air, disappearance was accelerated about tenfold. Comparing treatments (b) and (c), it can be concluded that the osmotic potential has an influence on the rate of adipocere disappearance, too. Adipocere incubated on sand or soil in vitro (treatments (d) and (e)) disappeared more slowly than that incubated in contact with physiological saline. The reason for this phenomenon could be that less water was available to decomposers in the sand and soil incubations, which were only wetted every 2–4 weeks. In contrast to the fauna free incubation on sand, mites (Acari), springtails (Collembola), woodlice (Oniscoidea), myriapods (Diplopoda and Chilopoda), snails (Gastropoda) and various insect larvae were present in the incubations with living soil. While dense hyphal mats had developed on the adipocere incubated on fauna free sand within 4 weeks, the development of mycelia in the incubations with soil fauna was less intense during the first 3 months (photographs in supplementary data). Using a binocular lens (40× magnification), no signs of direct adipocere uptake by the animals were found. There was a tendency of faster adipocere disappearance in incubations with soil animals. Outliers with weight increase during incubation were excluded from the analysis. Inclusion of these outliers leads to half-lives of 14.2 years in sample A and 9.9 years in sample D, respectively, with a general mean of 7.1 years in treatment (e).

Incubation in the field (treatment (f)) revealed the fastest disappearance in all types of adipocere (Table 2; $p < 0.01$). In all treatments, adipocere sample B had the fastest rate of disap-

Table 1
Origin of the adipocere samples studied.

Sample	Burial period	State of corpse	State of grave
A	34 years	Female, body largely preserved. Adipocere taken from thigh	Coffin completely preserved, approx. 1 m coverage with soil, very wet loamy soil but no stagnant water
B	42 years	Male, age 83 years, body largely preserved. Adipocere taken from chest	Coffin completely preserved, upper half lying in sand, lower half embedded in silt–clay soil
C	21 years	Female. Adipocere taken from chest	Wood of coffin well preserved, silty, very wet soil but no stagnant water
D	>35 years	Body well preserved, lying in water. Adipocere taken from thigh	Coffin completely preserved with a plastic lining. Soil around coffin very compact wet loam colored brown and blackish-blue
E	>35 years	Upper part of body preserved up to thighs. Adipocere taken from abdominal region	Soil around coffin very compact wet loam colored brown and blue

Table 2
Time (years) for 50% disappearance of adipocere in the studied samples under various experimental treatments.

Sample	Treatment					
	(a) Aq. dem. anoxic	(b) Aq. dem. oxic	(c) Physiol. saline, oxic	(d) On sand (laboratory)	(e) On soil (laboratory)	(f) In the field
A	36.9	8.1	3.1	4.1	3.4	1.5
B	11.5	1.5	1.4	2.4	0.7	1.2
C	20.2	9.3	3.8	10.3	3.5	1.4
D	81.5	4.2	2.9	8.6	5.2	1.3
E	33.8	5.6	2.5	8.5	7.4	2.1
Mean	36.8	5.7	2.8	6.8	4.0	1.5
Std. dev.	±27.0	±3.1	±0.9	±3.3	±2.5	±0.3

pearance ($p < 0.05$). In one of the field incubated specimens of sample B, traces of feeding activity were found at 40× magnification on the adipocere surface. The lower half-life of sample B was least pronounced in the field incubation (Table 2).

The adipocere samples contained a small amount of water at the end of the incubation period. The water content was between 20% and 60% of dry weight in the sand incubated specimens, 10–50% in the soil incubations and 17–40% in the field incubations.

3.2. Chemical composition of adipocere samples

The content of nitrogen and fatty acids in samples A–D is shown in Table 3. The data represent the situation at the start of the experiment.

Among the four adipocere samples analyzed, type B stand out due to its higher nitrogen content and lack of hydroxystearic acid. Adipocere type B also had a significantly faster disappearance time than the other samples studied. The general fatty acid concentration seems to be lower in sample C than in the others. There is a high dominance of saturated fatty acids, mainly palmitic acid (16:0), myristic acid (14:0), and (except sample B) 10 hydroxystearic acid (18:0 10 OH).

A closer look at the fatty acid distribution in the four samples shows that sample B deviates from the other three with a higher percentage of unsaturated fatty acids and myristic acid.

4. Discussion

4.1. Disappearance/degradation of adipocere

We prefer to use the term 'disappearance' rather than 'degradation' because we did not analyze the chemical alteration of the observed samples. The weight loss measured may be the result of mineralization. However, particularly in the field exposed samples, disappearance could also be caused by the displacement of small fragments resulting from the mechanical impact of soil macrofauna. The weight loss in treatments (a)–(c) can be attributed solely to mineralization since the whole Petri dish with all remaining residuals was dried and weighed at the end of the experiment.

Table 3
Content of nitrogen and fatty acids in the adipocere samples (related to wet weight).

	Adipocere sample			
	A	B	C	D
Total nitrogen (mg g^{-1})	1.9	6.7	2.3	1.9
Fatty acids (μg kg^{-1})				
C14:0 myristic acid	1088	1388	481	1032
C14:1 myristoleic acid	68	40	n.d.	2
C16:0 palmitic acid	2667	2125	1329	2491
C16:1 palmitoleic acid	129	278	18	65
C18:0 stearic acid	318	183	186	336
C18:1 oleic acid	224	432	84	212
C20:0 arachidic acid	35	92	15	44
C18:0 10 OH hydroxystearic acid	1731	n.d.	523	2117

n.d., not detectable.

The time for 50% disappearance of adipocere under anaerobic conditions ranged from 11 to 82 years. These results correspond to the generally observed persistence of adipocere in situations with restricted aeration. A massive block of adipocere persisted even after 120 years in an interment where the coffin was situated below the water table [15]. If the environment of the adipocere is changed to optimal aeration and moisture (treatment (c)), degradation is accelerated more than tenfold. Similar results were obtained by Schoenen [16] with a different set of adipocere samples. The adoption of first-order kinetics to the percentage loss figures given in [16] reveals half-lives of between 1 and 7.6 years at an incubation regime corresponding to treatment (b) in this study. Sun et al. [19], studying sediments, found degradation rate constants of palmitic acid (a main constituent of adipocere) that correspond to half-lives of 3 days in oxic and 11 days in anoxic conditions. The difference to the adipocere samples is nearly an order of magnitude. In the sediments the fatty acid was finely suspended, while in the form of adipocere it is aggregated into a compact block and is therefore much less accessible to the microbiota.

It was established that the disappearance of adipocere was slower in those treatments where the material was placed in open plastic tubes on the soil/sand surface (treatments (d) and (e)) compared to being placed in Petri dishes with a shallow layer of water. This fact points to the importance of accessible water for the degradation of adipocere. In the adipocere incubations on living soil (treatment (e)), oribatid mites, collembola, small isopods, small gastropods, diplopods and lithobiomorph chilopods were observed near the adipocere specimens. In the presence of soil microfauna and mesofauna, however, the general mean of adipocere disappearance was only slightly faster than on sterilized quartz sand. The exposition of adipocere in the Ah-horizon of a well-aerated and biologically active field soil (treatment (f)) resulted in the shortest half-life of adipocere disappearance, which was usually below 2 years. This fact points to the mixing effect of the soil macrofauna (earthworms, etc.) bringing soil, feces and the associated microbiota into contact with the adipocere.

Dense mats of fungal hyphae covered the samples during incubation, indicating that fungi play a major role in the decomposition of adipocere. The amount of fungal hyphae was much less on the specimens incubated on living soil than those on sterile quartz sand (photographs in online supplementary data). This may be attributed to the grazing of fungi by the soil mesofauna [20]. At the end of the incubation period, the hyphal cover of the adipocere on sand was more similar to that on soil than at day 58. The faunal impact in the soil incubations may only have been in effect for a limited amount of time, since not all of the fauna survived the whole duration of the experiment.

Table 4
Fatty acid composition of adipocere-like substances (%; n.d., not detected).

Source of substrate	Fatty acid						Triacyl-glycerol	Reference
	C14:0	C16:0	C16:1	C18:0	C18:1	C18:0 10 OH		
Woodland/30 cm	10	51	6	7	22	3	21	[21] Buried pig, 12 months
Woodland/60 cm	16	29	9	2	32	13	43	[21] Buried pig, 12 months
Anaerobic cage	5	59	n.d.	29	6	1		[1] Control burial, pig, 12 months
Soil 50–70 cm	11	69	4	11	5	n.d.		[8] Foot-and-mouth burial pit, cows and pigs, burial time ~35 years
Fresh corpse	<1–6	17–32	3–10	2–10	27–46	n.d.		[9] I–K: two females, one male, various tissue specimens
Glacier mummy	3–9	18–36	n.d.	3–19	1–4	21–49		[9] B: female, 29 years' burial time, in ice-in water-exposed
Glacier mummy	15–21	35–46	n.d.	3–7	3–4	10–34		[9] D: female, 50 years' burial time, 50 m depth in mountain lake
Samples A and C	17–18	43–50	1–2	5–7	3–4	20–28		This study, females, burial time 21–34 years; stored samples
Sample B	31	47	6	4	10	n.d.		This study, male, burial time 42 years, stored sample

4.2. Chemical composition of adipocere samples

The studied adipocere materials differed in their nitrogen content, with a significantly faster disappearance of the adipocere being ascertained with the highest nitrogen content. Nitrogen has not yet been explored as a constituent of adipocere, which is ideally thought of as an accumulation of fatty acid molecules composed only of carbon, hydrogen and oxygen. The presence of nitrogen in all studied adipocere samples suggests that cell and tissue residues may be present between the agglomerated fatty acids in various amounts. It cannot be ruled out that microbiota (particularly fungi) invading the adipocere mass also contributed to the nitrogen measured.

Literature data on the fatty acid composition of decay products are collected in Table 4.

The low percentage of oleic acid (C18:1) in samples A, C, and D of this study indicates their advanced state of decay and adipocere formation [12,22]. Of the cases in Table 4, the fatty acid composition of the 50 years old glacier mummy from a mountain lake (sample D in [9]) is closest to that in our samples A, C, and D. The relatively high percentage of myristic acid (14:0) as well as of oleic acid (18:1) in sample B of the present study is a specific feature that does not match the other adipocere samples in Table 4. Table 4 shows that the fatty acid composition of adipocere varies considerably in relation to burial time and specific site conditions. It can be concluded that the fatty acid analysis represents an actual steady state in a concurring pattern of adipocere-forming and adipocere-degrading processes. The availability of nitrogen stimulates the microbial transformation, since nitrogen is essential for the build-up of microbial biomass.

The formation of adipocere must be seen as the result of complex and site-specific processes. There will always be an oxygen deficit, enforcing anaerobic processes of decomposition, in a buried corpse because there are no aerating scavengers and insect larvae. The fatty acids and other anaerobic decay products will diffuse to the outside of the corpse. The fatty acids may accumulate there, forming an adipocere shield in an anaerobic situation, or they are degraded by fungi and gram positive bacteria [15] under aerobic conditions. The meso- and microfauna of the soil can influence this by stimulating microbial activity. Once formed, an adipocere layer may act as an exchange barrier [6] inhibiting subsequent decomposition. On the other hand, this study has shown that even compact adipocere aggregates are degraded in an environment with sufficient water and oxygen.

5. Conclusion

In conclusion, the complete decomposition of an adipocere-laden corpse can be expected after aeration within a time span of less than 10 years. Decomposition is mainly driven by microbiota. The presence of soil meso- and macrofauna has a stimulating effect on the disappearance of adipocere.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.forsciint.2009.03.005.

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