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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