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CASE REPORT

Gas analysis of exhumed cadavers buried for 30 years: a case report about long time alteration

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Abstract Due to important alteration caused by long time decomposition, the gases in human bodies buried for more than a year have not been investigated. For the first time, the results of gas analysis sampled from bodies recently exhumed after 30 years are presented. Adipocere formation has prevented the bodies from too important alteration, and gaseous areas were identified. The sampling was performed with airtight syringes assisted by multi-detector computed tomography (MDCT) in those specific areas. The important amount of methane (CH_4), coupled to weak amounts of hydrogen (H_2) and carbon dioxide (CO_2), usual gaseous alteration indicators, have permitted to confirm methanogenesis mechanism for long period of alteration. H_2 and CO_2 produced during the first stages of the alteration process were consumed through anaerobic oxidation by methanogenic bacteria, generating CH_4.

Keywords Alteration · Gas analysis · HS-GC-MS/TCD · MDCT

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Introduction

Gas analysis is a very complex investigation in analytical chemistry. The most challenging task concerns the sampling step, because of leaks that need to be avoided. Today, sampling strategies have been developed to fulfil this drawback [1–6]. However, postmortem alteration gas investigation presents another difficulty. Several studies showed that multi-detector computed tomography (MDCT) is helpful to detect gas presence in bodies [7–9]. However, depending on the time and environmental conditions of alteration, the body can be differently altered and a decompartmentalization can lead to an open system whose gas composition is similar to ambient air. This may be the main reason why the gaseous composition of bodies with an alteration time of more than several weeks has not been reported yet.

The aim of this case report is to validate the hypothesis of CH_4 generation during human body alteration. After 30 years of burial, high amounts of CH_4 (>10 %) are expected, generated by methanogenic bacteria from anaerobic oxidation of H_2 with CO_2. Therefore, weak percentages of H_2 and CO_2 could be also expected. To our knowledge, gaseous compositions from 30 years buried bodies are reported for the first time.

Case history

Twelve bodies buried in the mid-1980s of the last century have been recently exhumed for the reaffectation of a cemetery. Because of the soil quality in the burial ground (clay soil), the bodies underwent a specific alteration. Indeed, due to oxygen deficiency conditions, most of decomposers (bacteria, fungi and scavengers) could not alter the bodies as in usual conditions. Then, after the autolysis, oxygen present in the body is quickly depleted by the aerobic organisms which creates an optimal environment for the growth of anaerobic

organisms. However, due to the very stable burial conditions (cold temperature) and specific moisture, most of the exhumed bodies showed good preservation and adipocere. The bodies were partially very well preserved because the decomposition has not reached the main alteration stage.

Materials and methods

Subjects

Two bodies (SZ_13006 and SZ_13004) among the twelve exhumed ones (cemetery of Einsiedeln, canton of Schwyz, Switzerland) underwent a full CT scan examination following an autopsy (Fig. 1). Decomposition has led to an alteration of organs and tissues but relative airtight gaseous volumes can be clearly identified, well protected by adipoceres. SZ_13004 body was stored in a sealed zinc coffin, embalmed and wrapped into a shroud (deceased probably abroad) and buried in a dense clay soil. The body was exhumed on June 5th, 2013, wrapped into a plastic sheet, stored under soil at the cemetery until June 27th, 2013 when the body was transported to CURML (University Center of Legal Medicine, Lausanne, Switzerland) and autopsied the same day.

SZ_13006 body was preserved in a wooden coffin in dense clay soil, and the body was covered by a kind of plastic sheet which was an inlay of the coffin. The body was exhumed on June 5th, 2013, wrapped into a plastic sheet, stored under soil at the cemetery until June 27th, 2013 when the body was transported to CURML (University Center of Legal Medicine, Lausanne, Switzerland) and autopsied on July 2nd, 2013.

Collection of gas samples from the bodies

Gas sampling was performed under MDCT guiding following a standardized protocol recently developed in our centre [6]. Gas sampling is the critical step in this work. The sampling protocol must guarantee the absence of leaks from the bodies, and the sampling material must guarantee the absence of air contamination in the bodies and/or gas sampled (Fig. 2). Gas bubbles were detected by multi-detector computed tomography (MDCT) on a native CT scan [10]. Using the biopsy mode which corresponds to a radiological guidance, it was possible to target the aeric region to be punctured. A three-way tap in closed position is mounted on a needle and introduced through the body thanks to three-dimensional coordinates. A second CT scan is performed to check the position of the needles in the body. Luer-lock PTFE syringes were then mounted on the tap which was slowly opened. Therefore, gas volumes were sampled from the sampling sites of interest. The three-way taps were closed, and the system (syringe+tap+needle) was removed from the body. The gas samples were then individually transferred in headspace vial. These vials were preliminary filled with stabilized water (Millipore) heated during 3 h at 60 °C, then kept in fridge until use. Two needles were installed through the septum: the needle of the system (syringe+tap+needle) and another simple needle. The transferred gas takes the place of the water which can be evacuated through the second inserted needle. A residual water quantity must be left to permit a complete airtightness. Vials were stored upside down in fridge until analyses. Preliminary studies have already been led and have concluded for sample stability over at least 2 months.

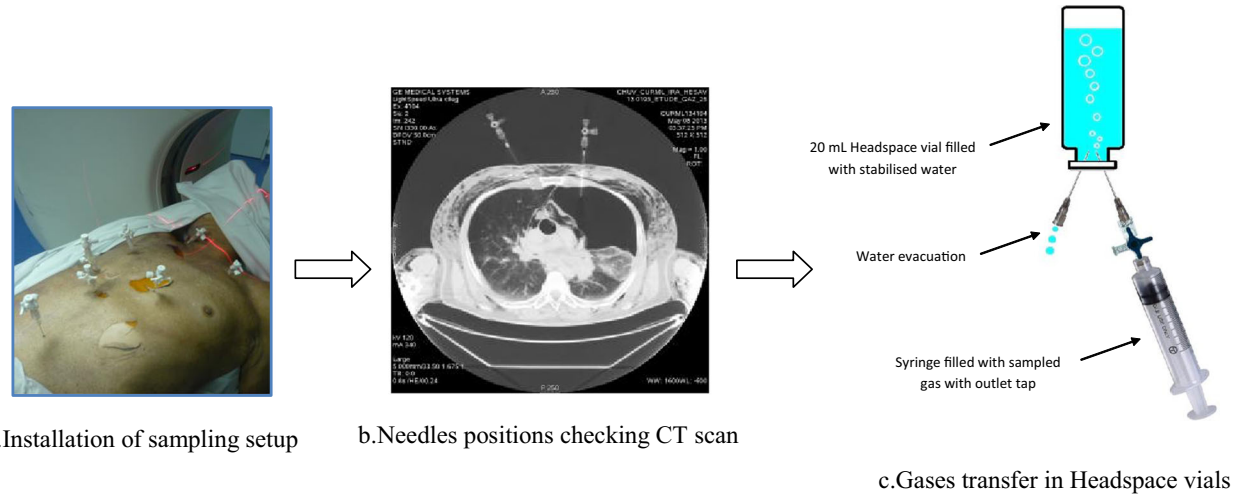
Fig. 1 Photos of the two cadavers analysed in the study with gas compositions in Figs. 3 and 4, respectively

Reagents

All the analytical gases used were from Carbagas (Lausanne, Switzerland): certified CO_2 (purity of 99.998 %) and certified methane (99.995 % purity). Atmospheric air was used as source of O_2 and N_2.

GC-TCD/MS analysis

An Agilent 6890N GC (Agilent Technologies, Palo Alto, CA) combined with a headspace gas autosampler and equipped with an Agilent select permanent gases column arrangement was used. This column arrangement is specially designed for gas analysis and contains two capillary columns in parallel: a molecular sieve 5 Å PLOT capillary column (10 m×0.32 mm i.d.) and a Porabond Q (50 m×0.53 mm i.d.), allowing the separation of carbon dioxide. The end of the capillary column was installed on a three-way valve mounted into the gas chromatograph. This valve enables the analytes to be directed, by a manual commutation, to a thermal conductivity detector (TCD) or to an Agilent 5973 mass spectrometer (MS) (Agilent Technologies, Palo Alto, CA). The temperature was set to an isothermal (45 °C) held for 8 min, and the injector (splitless mode) was set at 100 °C. Helium was employed as the carrier gas (8 ml min^{-1}, constant flow). The gas identification was performed by the injection of gas standards and mass spectra. The MS was operating in the electron ionization (EI) mode at



a.Installation of sampling setup b.Needles positions checking CT scan c.Gases transfer in Headspace vials

Fig. 2 Gas sampling protocol of alteration gases. **a** Installation of needles and outlet taps in the sampling site of interest, **b** CT-guided biopsy and **c** experimental setup for filling HS vials with gases from biological matrices—illustrative photos and CT scan only and not cases included in the study

70 eV with an ion source temperature of 230 °C. The analyser temperature was set at 150 °C and the interface MS temperature at 250 °C. Acquisition of signals was in full scan mode (2–100 amu). The gas quantification was performed with the TCD at 150 °C, prior calibrated for each gas with standard gases. The use of TCD was mandatory to put in evidence the eventual presence of H_2. With this capillary column system, all the gases could be detected in the same run. As the TCD H_2 calibration is complex, the amount of H2 in the gas mixture was calculated to be the difference between the sum of all the other components up to 100 % [11, 12].

Scan parameters

Non-enhanced MDCT scans were carried out after the reception of the bodies using an eight-row MDCT unit (CT LightSpeed 8; GE HealthCare, Milwaukee, WI, USA) with the following scan parameters: scan field of view (large) and display field of view, 50 cm; slice-thickness, 1.25 mm; 120 KV; 100–350 mA (modulated); rotation tube, 1 s. Standard and bone filter reconstructions were acquired.

Results and discussion

The main part of publications in legal medicine done on gas analysis concerns the possibility to distinguish alteration gases from vital embolism. Studies have been performed on rabbits [13–15], rats [15] and cetaceans [16]. Generally, the alteration time did not overcome 4 weeks. Alteration is diagnosed if hydrogen (H_2), methane (CH_4) or hydrogen sulphide (H_2S) is detected. Moreover, with this kind of alteration time, oxygen (O_2) concentration, although variable, should not overcome 10 %, nitrogen (N_2) concentration should be lower than 70 %, carbon dioxide (CO_2) should be higher than 15 % and the ratio CO_2/N_2 should be higher than 0.2 [17]. It is preferable to sample gas in areas independent from the endogenous formation of gases such as stomach or intestines because the origin of CH_4 or H_2 could be difficult to identify (endogenous generation vs. alteration). In these cases, as the microflora and the food of rabbits, rats or cetaceans are really different from this of human, an extrapolation of these works to human postmortem physiology is not relevant. However, results obtained in heart cavities provide useful information to understand human body alteration.

Presence of methane and hydrogen in the rabbit's intestines and heart was found random and did not seem to have any relationships to postmortem time or decomposition (67 h of postmortem delay, heart gas composition about 30 % of CO_2, 30 % of N_2, 30 % of H_2, 8 % of O_2 and 2 % of CH_4) [14]. Twenty days after death, the gas composition in rabbit abdominal cavity (similar to this in rabbit stomach containing some food and left at 15 °C) approximated 50 % of CO_2, 15 % of N_2 and 35 % of H_2, and CH_4 was not reported [15]. In other studies also performed on rabbits, 13 days after death, the cardiac gas composition was about 20 % of CO_2, 20 % of N_2, 50 % of H_2, 2 % of O_2 and 8 % of CH_4, and the abdominal gas composition was about 47 % of CO_2, 7 % of N_2, 30 % of H_2, 1.5 % of O_2 and 14.5 % of CH_4. Environmental temperature, food, sampling sites and sampling protocols can easily explain those differences.

Concerning gas composition in altered human bodies, only few data are available. Gas composition in decomposing bodies have been reported close to 23 to 46 % of N_2, 5 to 8 % of O_2, 3 to 45 % of CO_2 and 14 to 27 % of H_2, without any information concerning the postmortem delay or sampling sites [18]. In one case, CH_4 was detected (0.1 %). Several other cases were reported in Germany. The cardiac gas composition was close to, after 1 week of alteration, 30 % of CO_2, 30 % of N_2, 30 % of H_2, <5 % of O_2 and <5 % of CH_4; after 2 weeks of alteration, 50 % of CO_2, 20 % of N_2, 25 % of

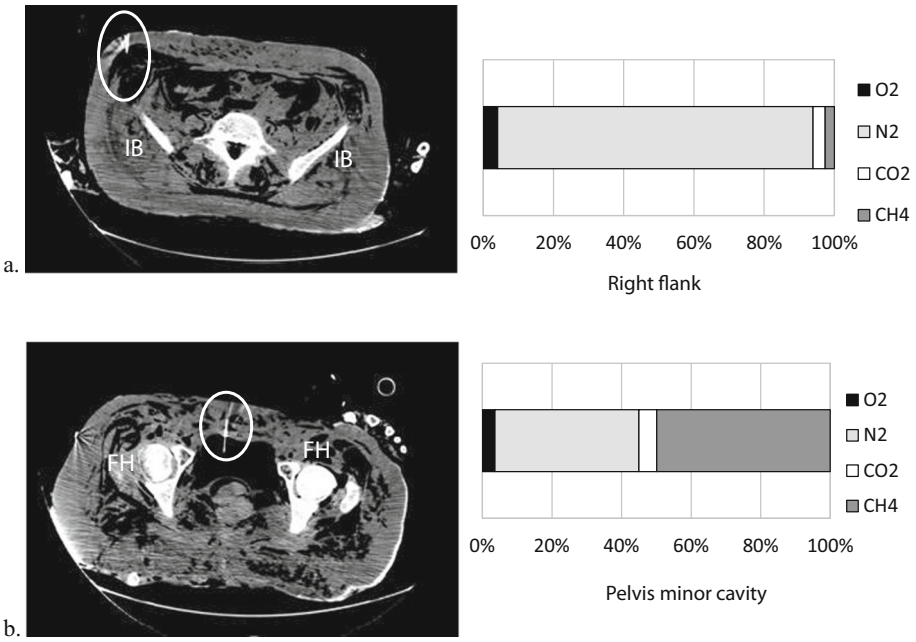


Fig. 3 CT scan images and gas composition of the right flank and pelvic minor cavities from a 30-year buried human body (sampling needles in the *white circles* on CT scan images; *IB* iliac bone, *FH* femoral head). **a** Right flank. **b** Pelvis minor cavity

H_2, <5 % of O_2 and <5 % of CH_4 and after three weeks of alteration, 25 % of CO_2, 45 % of N_2, 25 % of H_2, <5 % of O_2 and 5 % of CH_4 [19]. In 1980, the case of a baby found dead after 1~2 weeks revealed a gastric gas composition about 10 % of CO_2, 34 % of N_2, 51 % of H_2, 3 % of O_2 and no CH_4. The same work reported the case of a woman found dead after 24 days showing a gastric content gas composition close to 55 % of CO_2, 10 % of N_2, 33 % of H_2, 0.8 % of O_2 and 2 % of CH_4 and a connective tissue of thymus gas composition close to 36 % of CO_2, 29 % of N_2, 32 % of H_2, 3 % of O_2 and 0.7 % of CH_4 [15]. Another study reported the cardiac gas composition in bodies exhumed after 10 to 64 weeks [11, 12]. The composition is very random but a global trend can be noticed. In bodies with a postmortem delay of less than about 30~35 weeks, the concentrations of alteration gases CO_2 and H_2 are close to or superior to 20 %. If the postmortem delay overcomes 35 weeks, CO_2 and H_2 concentrations tend to decrease and CH_4 is generated. Therefore, at the first steps of alteration, O_2 and N_2 seem to decrease, consumed by the microorganisms which produce H_2 and CO_2 as fermentation

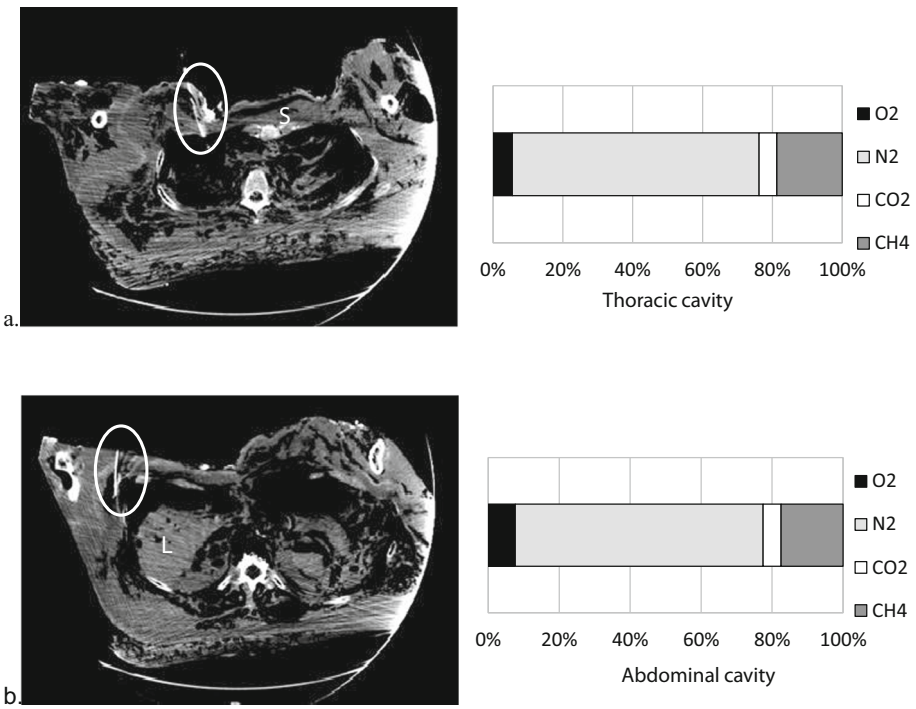


Fig. 4 CT scan images and gas composition of thoracic and abdominal cavities from a 30-year buried human body (sampling needles in the *white circles* on CT scan images; *S* sternum, *L* liver). **a** Thoracic cavity. **b** Abdominal cavity

products. After a long time of alteration (several weeks to 1 year), H_2 and CO_2 decrease and CH_4 appears. Indeed, all CH_4 bacteria can obtain the energy required for growth by the anaerobic oxidation of molecular H_2 with CO_2 [15]. However, even if after this kind of postmortem delay (several weeks to 1 year), CH_4 concentration reaches a maximum <10 %.

For both cases of this study, only four gases are noticeable: O_2, N_2, CO_2 and CH_4. Among all the gas samples, O_2 concentration does not overcome 5 %. Body 1 presents a high amount of N_2 in the right flank (about 90 %) and small amounts of CO_2 and CH_4 (both <5 %) (Fig. 3a). The important N_2 amount could be due to gas diffusion inside the body or air contamination due to the difficulty of sampling (but O_2 concentration does not seem to be influenced). However, for the same body, N_2 concentration in the pelvic cavity is close to 40 %. CO_2 concentration remains similar to this of the right flank (<5 %) but CH_4 concentration represents almost 50 % (Fig. 3b). Gas compositions in the abdominal (Fig. 4a) and thoracic cavities (Fig. 4b) of the body 2 are nearly similar: O_2 between 5 and 10 %, N_2 close to 70 %, CO_2 <5 % and CH_4 close to 20 %.

H_2 was surprisingly not present whereas it constitutes a major alteration gas. After 30 years, H_2, which is a very volatile gas, could have been released in the ground but the most credible explanation is its anaerobic oxidation with CO_2. As for H_2, it is surprising to notice the weak amount of CO_2. The absence of H_2 and the weak amounts of CO_2 (<5 % whatever the body and the sampling site) strengthen the hypothesis of CH_4 generation from H_2 oxidation by methanogenic bacteria.

In microbiology, H_2 and CO_2 are major end products of mixed acid fermentation performed by many enteric bacteria including *Escherichia*, *Shigella*, *Proteus* and *Yersinia*. Sugars can be rapidly transformed in pyruvic acid, latter converted in formic acid produced by pyruvate cleavage. Finally, formic acid is converted in H_2 and CO_2. Other microorganisms such as anaerobic spore-forming bacteria *Clostridium* are implied in a fermentation leading to acetic and butyric acids, H_2 and CO_2. Cleavage of pyruvate yields acetyl-S-CoA, H_2 and CO_2 directly [15]. As result, CO_2 and H_2 appear as predominant alteration gases and reliable alteration indicators. However, in the cases herein, CO_2 is at weak level and H_2 is absent. This observation is explained by the anaerobic oxidation of H_2 with CO_2: $4\ H_2+CO_2 \rightarrow CH_4+2\ H_2O$. Taking into account the stoichiometry, H_2 amount should decrease in larger quantity than CO_2. Therefore, it can be hypothesized that all H_2 produced during decomposition was converted in CH_4, H_2 being the constraining factor. Adipoceres and cold environmental temperatures induce an acidic environment and should limit the growth of anaerobic microflora of normal alteration. Conversely, methanogenic bacteria can growth in various pH environments and from temperature as cold as 4 °C (optimum at 22 °C) [20]. With high concentration of CO_2 and H_2, cold temperature and acidified pH, methanogenic activity is ideal.

As a result, the detection of methane in gas sample is a reliable indicator of advanced stage of alteration. The magnitude of the alteration could be directly linked to high concentrations of methane and weak concentrations of CO_2 and H_2. However, the moisture and environmental temperature seem to be key elements to rule the anaerobic oxidation of H_2 in presence of CO_2.

The cases presented in this study constitute a specific decomposition because the alteration conditions are optimal for methanogenic microorganisms. For bodies stored in water or in dry atmosphere, the gas composition could change, even in case of long alteration. Low methane amounts could be expected because of the predation which can alter the integrity of the sampling sites leading to leaks of gaseous substrates and air contamination. The use of MDCT guarantees sampling precision even in small gaseous volumes and to find out precise diagnoses when correlated with gas analyses. Additionally, to parameters such as the species, environmental temperature, sampling sites etc., the main source of variations between the studies available originates from the sampling. Underwater direct sampling, airtight syringes, during autopsy (risk of leaks or air contamination), use of aspirometer, CT scan assistance etc., constitute important criteria that need to be harmonized. The body gas sampling protocol developed in our medicolegal centre increases the precision of the sampling and could be extended to other medicolegal centres to compare obtained results.

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