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Organic geochemistry of sewage sludge. I. Lipid fractionation by thin layer chromatography

C. Payet, C. Bryselbout, J.L. Morel and E. Lichtfouse

Laboratoire Sols et Environnement, INRA/ENSAIA-INPL, BP. 172, 54505 Vandœuvre-Lès-Nancy, France

This article reports detailed procedures: 1) to decrease organic contamination of samples in the laboratory; 2) to extract sewage sludge with methylene chloride; and 3) to fractionate extracts by silica-gel thin layer chromatography (TLC) into fractions enriched in alkanes, aromatic hydrocarbons, ketones, alcohols and fatty acids, eluting either hexane or methylene chloride.

Introduction

Sewage sludge contain various organic substances inherited from the decay of urban waste waters. Molecular studies of sewage sludge have been mainly focused on xenobiotics because sludge can be recycled as fertilisers for agricultural production [1]. Nonetheless, knowledge on the source, transformation and fate of sludge molecules is scarce [2]. Here,

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we report the first step of a molecular study aiming at the systematic identification of sewage sludge lipids.

Decontamination

Solvents were distillated in glass. Glass funnels, 250 mL Pyrex round-bottom flasks, 600 mL Pyrex beakers, steel mortar and rotary evaporator were vigorously washed with dish detergent, hot water (7 ×), distilled water (3 ×), allowed to dry at room temperature, then washed with distilled methylene chloride. Cellulose acetate filters, 10 mL glass vials, TLC glass jam-pots, aluminium foil, 2 mL screw-top vials, steel spatulas, cotton wool, pipette Pasteur and teflon-plugged glass syringes were washed with distilled methylene chloride prior use. 10 × 5 cm silica-gel 60 pre-coated F-254 glass TLC Merck plates were used. After labelling the top of the plates with a lead pencil, the plates were washed by elution of distilled ethyl acetate, allowed to dry on aluminium foil under a ventilated hood, reactivated 2 h at 50 °C, 2 h at 80 °C, 2 h at 110 °C in a ventilated oven, then allowed to cool at room temperature. The aim of this step is to concentrate the contaminants at the top 0.5 cm of the plates. The plates were handled with fingers touching only the top 0.5 cm.

Extraction

Sludge (~ 300 g) were freeze-dried then finely ground 1 min using a steel-ball shaking mortar. ~ 60 g of dried sludge were extracted 3 times with methylene chloride (120 mL, 60 mL, 60 mL) in a 600 mL Pyrex beaker dipped 15 min into a sonication water-bath. The liquid phase was transferred onto a cellulose acetate filter fitted on a glass funnel, using a spatula to guide the liquid flow. The filtrates were collected in a 250 mL round-bottom flask, concentrated at nearly 1 mL at 30 °C under vacuum, transferred into a 10 mL glass vial with a 1 mL teflon-plugged syringe, allowed to dry under a ventilated hood, then kept at –20 °C.

Fractionation

A sub-sample of the extract (~ 0.5 mg) was prepared by dilution with methylene chloride, transferred into a 2 mL glass vial, then concentrated under a ventilated hood. Vertical lines were drawn with a lead pencil at 1.5 cm (migration start) and 9 cm (end) on the TLC plates. A simple rising device allowed the absence of contact between the ruler and the silica-gel. The extract in CH_2Cl_2 was spot-transferred onto the 1.5 cm line of the plate with a glass capillary, allowed to dry 3 min, then eluted either with hexane or CH_2Cl_2. Pure standards of increasing polarity (*n*-octacosane, 2-methylphenanthrene, 1,2:3,4-dibenzanthracene, friedelin, cholesterol) were eluted on a separate TLC plate. Standards were revealed by UV (254 nm), then by short dipping into a 5% v/v EtOH-H_2SO_4 solution followed by hot-plate heating (~ 250 °C) under a ventilated hood. Rf values refers as ratios of migration length substance versus solvent.

TLC elution of extracts with hexane allowed to fractionate alkane- and aromatic hydrocarbon enriched fractions

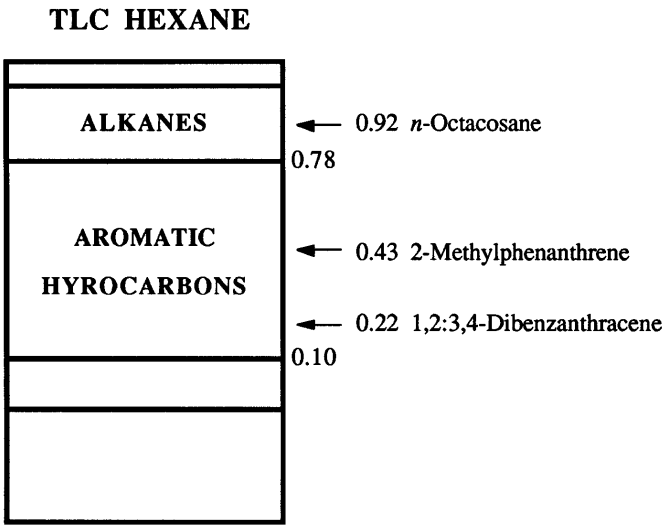

Figure 1. Fractionation of sewage sludge CH_2Cl_2 extracts into alkane- and aromatic-enriched fractions by thin layer chromatography (TLC) eluting hexane.

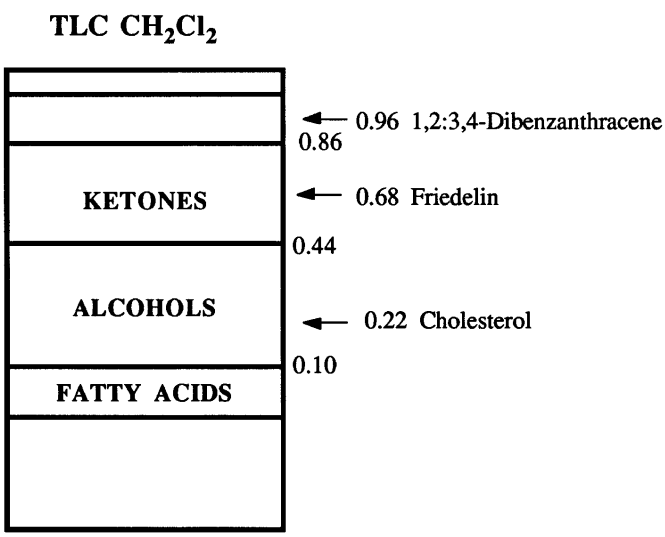

Figure 2. Fractionation of sewage sludge CH_2Cl_2 extracts into ketone-, alcohol- and fatty acid-enriched fractions by thin layer chromatography (TLC) eluting CH_2Cl_2.

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(Fig. 1). TLC elution of extracts with CH_2Cl_2 allowed to fractionate ketone-, alcohol- and fatty acid enriched fractions (Fig. 2). The fractions were recovered from the plates by scratching the silica gel toward aluminium foil with a spatula, by transferring the silica gel via a glass funnel (fitted with a teflon tube) on a pipette Pasteur plugged with cotton wool [3], then by elution of a solvent (~ 2 mL) into a 2 mL vial using CH_2Cl_2 except for the fatty acid fraction (ethyl acetate). Analyses of the fractions by gas chromatography coupled to mass spectrometry will be reported elsewhere.

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