Biochemical Engineering Journal 105 (2016) 497–504

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Regular article

Occurrence of plant and fecal steroid and their evolution during co-composting of sewage sludge and lignocellulosic waste

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ARTICLE INFO

Article history:
Received 31 July 2015
Received in revised form 26 October 2015
Accepted 27 October 2015
Available online 6 November 2015

Keywords:
Lignin
Waste treatment
Fecal and plant steroids
Analytical pyrolysis (Py-GC/MS)
Biodesulfurization
Biotransformations

ABSTRACT

Steroid composition change during six months of co-composting of sewage sludge and palm waste was studied using Py-GC/MS. The main steroids identified were C_{27}–C_{29} sterenes and stanols, 5β-cholesta-3-one, cholesta-3,5-diene and 2 thiosteranes. Except for thiosteranes and some of the C_{27}–C_{29} cholestenes, the relative concentrations decreased during co-composting due to microbial degradation. Plant 5α-cholestanol, 24-methyl- and ethyl-5α-cholestanols were accompanied by fecal 5β-cholestanol and 5β-cholestanone. Some of the sterenes and cholesta-3,5-diene were formed by pyrolysis of bound forms of stanols and cholesterol, respectively. Sulfurization occurred during co-composting and increased with time. The process affects cholestanol and campestanol of plant origin leading as pyrolysis products to thiocholestane and thiocampestane (with the 5α (H) configuration), respectively, and to the corresponding sterenes. The change in steroids during co-composting is positively correlated with the physico-chemical parameters of mature compost, especially C/N and NH_4^+ / NO_3^- ratios.

1. Introduction

Humans contribute significantly to steroids in the environment, the main source of steroids in freshwater is considered to be of human origin [17]. Algae and higher plants are also an important source of phytosterols such as sitosterol and stigmasterol [42]. Similarly, fungi and yeasts contribute to the synthesis of the steroid ergosterol [29,10]. These compounds are generally excreted in the urine and feces, especially cholesterol and its derivatives (coprostanol and cholestanol). Steroids are considered to possess a resistant skeleton. From an environmental point of view, the abatement rate of steroids during wastewater treatment is limited [19], which can explain their accumulation in the solid phase (sludge). Although desorption and biotransformation of steroid compounds are considered low, several studies have shown that sediment and sewage sludge act like as steroid traps [23,24]. The same authors detected steroids in the effluents of sewage sludge and surface water. However steroid behavior depends on their chemical composition and the receiving environment. Other authors [25,15] have shown that steroids can undergo biotransformation despite their potential bio-concentration which can lead to serious environmental problems [8].

The parameters involved in their degradation need to be well known and their action well understood. Indeed, the major studies have so far focused only on the presence and the abundance of steroids in liquid effluents [30]. Some steroids or steroid derivatives are considered as biomarkers that provide a method for detecting evidence of pollution in both wastewater and sediment [36,27]. Similarly, Tyagi et al. and Nash et al. [39,30] showed that some sterols are specific to fecal matter and can be used as biomarkers of pollution of human origin in water.

Because of the potential value of steroids to indicate a certain level of pollution, the quantitative and qualitative analysis of compost in terms of these compounds should bring interesting information on compost quality. However, steroids are often poorly known and usually remain unidentified as there is no comprehensive study on the monitoring these compounds during composting. To overcome this shortcoming, we present a study of the identification of steroids and their behavior during co-composting of sewage sludge-palm tree waste, using the technique of analytical pyrolysis coupled with gas chromatography-mass spectrometry (Py-GC/MS).

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http://dx.doi.org/10.1016/j.bej.2015.10.025
1369-703X/

2. Material and methods

2.1. Co-composting assay and physico-chemical analysis

The co-composting experiment was conducted on a composting platform located in Marrakech municipal plant nursery. A mixture of 1/3 sewage sludge and 2/3 date palm waste (v/v) with a total volume of 4 m^3 was composted.

The date palm debris had been previously stored for 3 months (June–August) at ambient temperature. The mean features of the co-composted substrates are a C/N ratio of about 37.13 and 20.8, total Kjeldahl nitrogen (% TKN) of about 1.36 ± 0.24 and 1.50 ± 0.2, total organic carbon (% TOC) of about 50.5 ± 0.61 and 31.20 ± 0.11 respectively for palm tree waste and sewage sludge [12].

The mixture was carefully homogenized, moisture was adjusted to 60% (optimal value for composting), and the mixture was windrowed. The windrow was turned over manually with weekly frequency to enable mixture ventilation. Homogeneous samples (1 kg) were obtained by careful mixing of several sub samples taken at different points (height and length) of the windrow followed by quartering. The samples were kept at −20 °C until analysis. The temperature of the windrow was measured every day at different heights in the compost using sensors with data memory (PH0700115 model 1.20, Ector-Traçability software, ECTOR France). Moisture contents were determined by drying 100 g of co-compost at 105 °C for 48 h. The pH was measured in an aqueous extract of the compost at room temperature (1 g/10 ml of distilled water). Total organic carbon and ash content of dried samples, were calculated after calcination in a muffle furnace at 600 °C for 6 h. TKN was assayed by 0.5 g samples by using the classical Kjeldahl procedure, by steam distillation according to AFNOR T90-1110 standard. Likewise, ammonium ion content was assayed by alkaline distillation and nitrates after reduction by Dewarda alloying [12].

2.2. Pyrolysis (Py-GC/MS)

After freeze-drying, the ground sample was placed in a stainless steel cup. Pyrolysis took place at 600 °C for one minute in a Frontier Lab (EGA PY 3030D) pyrolyzer equipped with an AS-1020E auto sampler and directly coupled to a quadrupolar Shimadzu QP2010 Ultra GC/MS. The pyrolysis products were sent directly to the GC/MS in a stream of helium. Direct coupling prevents the loss of volatile compounds or possible degradation of the pyrolysis products [13]. The GC separations were conducted in a fused silica capillary column (BPX 5 (SGE), 5% Phenyl Polysilylphenyl-siloxane, 30 m length, 0.25 mm i.d., 0.25 μm film thickness) and helium 5.5 (Messer), 999,995% purity as carrier gas. The injector was set to 250 °C with a split of 100/1. The column temperature was programmed from 60 to 300 °C at 5 °C.min^{-1} and held at 300 °C for 15 min. The ionization mode was electron impact (70 eV), the data were recorded in full scan mode, the source temperature was 220 °C and the transfer line was set to 280 °C.

The compounds were identified on the basis of their GC retention times and by comparison of their mass spectra with analytical standards and data from the literature. The main standards used were 5α-cholestan-3β-ol (Chiron), 5β-cholestan-3β-ol (coprostanol) (Sigma), 24-ethyl-5α-cholestan-3β-ol (Chiron), 5α-cholestan-3-one (Sigma), 5β-cholestan-3-one (Chiron) and cholest-5-ene (Sigma). Authentic 3β-thio-5α-cholestane was provided by Dr P. Adam (University of Strasbourg).

The relative abundance (P_i) of each pyrolysis product was calculated on the basis of the area a_i of the considered component compared to the total area of the pyrogram [13].

$$P_i = \frac{a_i}{\Sigma_1^n a_i}$$

Peak integration was performed on the total ion chromatogram (TIC) using Shimadzu software GC/MS Solution.

3. Results and discussion

3.1. Changes in steroids

The pyrogram obtained for sewage sludge-palm tree waste samples at different stages of co-composting showed a variety of steroids (Fig. 1). The main families of tetracyclic triterpenes are 3 sterenes, 1 steradiene, 4 stanols, 1 stanone, 2 thiosteranes (Table 1). Four isomers of cholestenes (named 1–4) and 24-ethylcholestenes (1–4), two isomers (1, 2) of 24-methylcholestenes were identified. The double bond cannot be localized in the skeleton on the basis of the mass spectra so it was not possible to identify the individual isomers. The diene identified was cholesta-3,5-diene ($\Delta^{3,5}$cholestadiene). The 4 stanols, the cholestanone, the thiocholestane and the thiocampestane were functionalized on the carbon 3 (A ring). The ketone was found to be similar to the authentic 5β-cholestan-3-one (and differed from the authentic 5α-isomer) on the basis of GC retention times and mass spectra. The stanols were fully identified (comparison with authentic standards) as 5α- and 5β-cholestan-3β-ols, 24-methyl- and 24-ethyl-5α-cholestan-3β-ols and thiocholestane as 3β-thio-5α-cholestane (Table 1). No epi-stanols resulting from the microbially mediated epimerisation of 5β-stanols [7,6] were detected in the pyrolysates.

Considering the changes in relative amounts of steroid during co-composting, the different steroid components can be separated into three groups (Table 2). The first group, with decreasing amounts during co-composting included: cholestenes 1, 2, cholesta-3,5-diene, 24-ethylcholestenes 1 and 2, 5β-cholestan-3β-ol (coprostanol), 5α-cholestan-3β-ol, 5β-cholestan-3-one and 24-ethyl-5α-cholestan-3β-ol (stigmastanol). The second group was made up of isomers 3 and 4 of cholestenes, isomer 3 of 24-ethylcholestene, 3β-thio-5α-cholestane (thiocholestane) and 24-methyl-3β-thio-5α-cholestane (thiocampestane) whose levels increased during co-composting (Table 2). It can be observed that the changes of the relative concentrations with time for the two groups occurred progressively (Table 2). The third group contained only 3 steroid components with fluctuating concentrations presenting no apparent trends: the two 24-methylcholestenes and isomer 4 of 24-ethylcholestene (Table 2).

The initial steroid content was 30% of the total identified compounds present in the pyrogram (Table 3). Their relative amounts decreased during co-composting to reach about 23% in the stabilization phase (first month). The degradation of steroids continued and reached a turnover rate of about 68 % at the end of the maturation phase (Table 3). Steroids appeared less resistant than lignin components during co-composting (Table 3). Indeed, there was almost total disappearance of stanols i.e., 5β-cholestan-3β-ol (coprostanol), 5α-cholestan-3β-ol, 24-methyl-5α-cholestan-3β-ol (campestanol), and 24-ethyl-5α-cholestan-3β-ol (stigmastanol) (Table 2). The same trend was also observed for isomers 1 and 2 of both cholestenes and 24-ethylcholestenes (Table 2).

All the steroid compounds identified in the pyrolysates originated from C_{27} to C_{29} sterols, mainly cholesterol (cholest-5-en-3β-ol) which is ubiquitous (animals, zooplankton, algae) [37] and phytosterols assitosterol (β-sitosterol) 24-ethylcholest-5-en-3β-ol, 24-ethylcholest-5, 22-dien-3β-ol (stigmasterol), 24-methylcholest-5-en-3β-ol (campesterol). However no sterols were detected in the pyrolysates, even for the T0 sample despite the presence of plant waste, which was unexpected. Despite the presence of cholesta-3,5-diene, the occurrence of dehydration of free, uncombined sterols during pyrolysis is rather unlikely considering