Organic Compounds of Natural Origin in Sludge from Waste-Water Treatment Plant

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Summary

Organic compounds of natural origin were analyzed in three types of sludge produced by waste water treatment plant – limed sludge with 12 % of CaO, limed sludge with 6% of CaO and sludge without processing. Analysis was performed by pyrolytic gas chromatography with mass spectrometry detection (Py-GC/MS). The main component pf natural origin in waste-water treatment sludge were determined. They comprise compounds form the following groups: fatty acids and their methylesters, steroids, substances derived from polysaccharides, substances derived from lignin, compounds with nitrogen (both heterocyclic and aliphatic), substances with so called non-specific origin (toluene, styrene, acetic acid, methylphenols and phenols). Interpretation was attempted for identification of organic compounds sources as well as determination of humification and mineralization degree. The ratio benzene/toluene was used as the criterion for humification in waste-water treatment sludge. Benzene is derived from degradation of condensed aromatic structure, toluene is derived from both condensed aromatic structures and pseudostable organic matter. The ratio furfural/pyrolle was used of expression of mineralization degree of organic matter. Furfural is a product of labile organic matter (saccharides) while pyrolle originates from stable organic matter (humified components containing nitrogen). Compared with non-limed sludges, limed sludges have higher rate of decomposition of organic matter as well as subsequent mineralization. Limed sludges accumulate more compounds derived from lignine which is caused by more intensive decomposition of polysaccharides. From the point of view of utilization in agriculture, limed sludge is the most suitable sludge produced by waste-water treatment.

Keywords: waste–water treatment sludge; organic compounds; humification; mineralization; Py-GC/MS

Introduction

Sludges formed as a side product by waste–water treatment represent heterogenous mixtures of organic and inorganic substances. Typical organic matter in sludges [1] includes microoganisms, extracellular material and residues from waste-water (paper, plant residues, oils and fats, faecal material, organic pollutants etc.). Waste-water treatment sludges contain highly complex mixtures of organic molecules [2] which include naturally formed compounds – proteins and peptides, cellulose, hemicellulose, lipids and plant macromolecules with phenolic structure (fragments of lignin and lignans) or aliphatic structure (cutines, suberines). Waste-water treatment sludges also contain typical pollutants derived from human anthropic activity, e.g. synthetic polymers, organic micropollutants (PAHs, PCBs, phthalates, chlorbenzenes, pesticides) and residues from medicaments. A successful utilization of waste-water treatment sludges for gardening and agricultural applications depends on concentrations of organic pollutants and substances of natural origin, The aim of this article is to evaluate an occurrence and amount of organic substances of natural origin in the three types waste-water treatment sludges prepared by various processing and to determine their humification and mineralization parameters by means of Py-GC/MS.

Material and methods

For purposes of determination the changes in concentrations of natural organic compounds there were sampled three types of sludges from waste water treatment plant which processes water from 320 000 of equivalent inhabitants. Sludge is hygienized by liming. 3 samples of sludge were compared: limed sludge containing 12% of free CaO, limed sludge containing 6% of free CaO and non-limed sludge. Samples of limed sludge were collected 24 hours after the end of hygienization process.

The analysis was performed by pyrolytic gas chromatography with mass spectrometry detector (Py-GC/MS). The sample of maximum mass of 100µg was inserted into quartz-glass pyrolytical tube. Both ends of tube were closed by quartz-glass wool. The sample was then introduced into the pyrolytic unit where it was pyrolyzed at the following conditions: temperature 650°C, time 10 s, rate of temperature increase 20°C/ms. The released products were transferred by interconnection Py-GC (285°C) to the input (250°C) of gas chromatograph HP Agilent 7820 A, where they were separated at the column HP 5 (30m x 0.25mm x 250µm). The temperature conditions of separation: 50 °C (2 min. delay) to 100°C (2min. delay), temperature rate 30°C/min, from 100 °C to 280 °C (3 min. delay) the temperature rate 6°C. The conditions of MS detector: temperature of quadrupole 150 °C, ion source 230 °C. The analysis was performed in the scan mode from 28 to 650 Da and SIM mode for determination of steroid compounds. The substances were identified by comparison with the NIST Library of spectra and with standards.

Results

The main components of natural origin in waste-water treatment sludge comprise fatty acids, compounds derived from decomposition of several organic substances (lignin, polysaccharides, proteins, peptides) and steroides.

Fatty acids and their methylesters belong to the main components of waste-water treatment sludges. The most important representants are hexadecanoids acids (C16:0, palmitic), octadecanoic (C18:0, stearic) acids. These fatty acids form typical component of sludges originated in household and enterprises for preparation of food [3]. The highest concentration of above mentioned fatty acids was determined in the sample of limed sludge with 6% of free CaO – 29.5 μg/mg, next is non-limed sludge with 20.3 μg/mg. The limed sludge contains the lowest amount of fatty acids (14.6 μg/mg). The source of fatty acids in waste-water treatment sludges are bacteria, fragments of plants - thallophytes and products of animal character. Fatty acids derived from animals and thallophytes are characterized by prevalence of even carbons over add ones with dominant homologues C16 and C18 [4]. The usual range for these fatty acids is from C12 to C32. Fatty acids derived from bacteria are characterized by the prevalence of odd carbons. Among typical representants there are hexadecanoic acid, hexadecane and octadecane acid. Fatty acids derived from plants and animals and present in the analysed sludges, are characterized by the presence of lauric acid, myrist acid and pentadecanoic acid in the range 1.8 – 3.6μg/mg. The maximum value has the sample limed sludge with 6% of free CaO (3.6μg/mg), minimum values has the sample of limed sludge.

Steroids form the important component of analysed samples. From the group of stereoids there were identified 5β-cholestan-3β-ol (coprostanol) and its α-epimer epicoprostanol, 5β-stanol and cholesterol (cholest-5-en-3β-ol). 5β-cholestan-3β-ol (coprostanol) represents marker that identifies human faeces in environment [5]. This marker has a long persistence [6] and is typical for waste waters and sludges from households [3]. Coprostanol is produced in digestive tract of organisms by anaerobic microorganisms from cholesterol. The concentration of of coprostanol ranges from 100 to 200ng/mg. The amount of coprostanol also depends on the procedure for sludge processing as well as its amount in input material. Epicoprostanol is only produced by anaerobic bacteria [7] in human organism, partly it can also be produced by animals, but in substantially lower concentrations. Epicoprostanol is present in analysed samples in concentrations between 70 – 80ng/mg. Both the limed sludge (80ng/mg) and limed sludge with 6% of free CaO (75ng/mg) have slightly higher concentrations than non-limed sludge (70ng/mg). The distribution of sterols [8] makes it possible to distinguish an origin of sludge (sludges from households, sludges from food-processing, sludges from paper production). The ratio coprostanol/Σsterols is used for identification. Sludges form households have the value of this ratio in the range from 0.1 to 0.3. Cholesterol was in waste-water treatment sludges determined in similar concentrations like above mentioned sterols (85 – 125ng/mg). The concentrations of 5β-stanol ranges from 52 to 98ng/mg.

Substances derived from polysaccharides are represented by furanes, furfural, 2-furancarboxylaldehyde, 2-cyclopentenon, 2,3-dihydro-benzofuran, 2-hydroxy-3-methylcyclopentenon, 2-methylcyclopenthenon, 3-methyl-2cyclopenthenon, 2,3-dimethylcyclopentenon, 5-methyl-2-furancarboxylaldehyde and 2-furanmethanol. The source of polysaccharides detected in sludges can be from cellulose paper but also from cell walls of bacteria (petidoglycanes), from bacterial exopolysaccharides and from fragments of plants [9]. The highest amount of derivates related to polysaccharides was found in the sample on non-limed sludge (35.8μg/mg). Both limed and limed with 6% of free CaO have concentrations of substances derived from polysaccharides in the range from 16.8 to 18.8μg/mg. Substances derived from lignin are present in all samples of sludges. In the samples there were identified quajacol, eugenol and ethylguaiacol, all of which can be considered to be unequivocal markers for lignin [10]. An occurrence of these substances (guaiacyl type) is connected with biomass originated prevalently from conifers [11]. The concentration of substances derived from lignin is the highest in the sample of limed sludge (1.78μg/mg). The non-limed sludge has the lowest concentration (0.47μg/mg) of derivatives related to lignin. The hygienized sludge with 6% of free CaO contains 0.98μg/mg of lignin derivatives. In addition, methylphenols (2-,3-,4-methylphenol), ethylphenols (3-,4-,ethylphenol) and phenol are present. The origin of these substances is not unequivocal, they can originate from peptides and proteins with content of tyrosine [12], but also from lignins and tannins [11]. Concentrations of phenol in the samples ranges from 1 to 5.8μg/mg. The highest concentration of phenol is in the sample of limed sludge (5.5μg/mg) and hygienized sludge with 6% of free CaO (5.8μg/mg). The lowest concentration of phenol is in the non-limed sludge (1μg/mg). Methylphenols have the same trends as phenol. The lowest concentrations are connected with non-limed sludge.

Compounds with nitrogen belong to the substances with the highest concentrations. Both heterocyclic and aliphatic compounds with nitrogen can be found in pyrolysates of samples. Dominant heterocyclic compounds are indol and its methylated heterocyclic compounds, pyrrole and its methylated forms, pyridine and its methylated forms and heterocyclic amines (piperidines, piperazines and their methylated forms). The main sources of nitrogen compounds in waste-water treatment sludges are peptides and proteins which contain various aminoacids. Proteins and peptides are formed prevalently from bacteria and their exopolymers [13]. Another source is formed by faecal products and plant fragments. From aminoacids there were identified alanine, cysteine and arginine. The highest concentration of proteins is in the hygienized sludge with the content of 6% free lime (0.7μg/mg). Aliphatic nitrogen is represented mostly by amines, amides and nitriles. Acetonitrile together with nitriles is very often related to the low-ripe organic matter. Acetonitrile is derived from peptides and proteins [14]. Acetonitrile is found in sludges in the range from 2.1 to 2.5μg/mg. Pyrrole and its derivates originate from proteins like proline, hydroxyproline, glycine and glutamic acid [14].They can partly originate from

plant pigments [15]. The lowest concentration of pyrrole was determined in the sample of non-limed sludge (1.6µg/mg), maximum concentration was found for limed sludge with 6% of free CaO (13.5µg/mg). Indol and its methylated forms are indicators of peptides containing tryptophane. The concentration range of indol is from 0.5 (limed sludge with 6% of free lime) to 11.4µg/mg (limed sludge). Pyridine and its derivatives originate from proteins and peptides [12]. Pyridine is present in sludges in very low concentrations of approximately 0.2 to 1.5µg/mg. Benzenpropanenitril is formed by pyrolysis of peptides containing phenylalanine, in waste-water treatment sludge is contained in the range from 2.5 (limed with 6% of free CaO) to 3.4µg/mg (limed sludge). Acetamide can originate from proteins containing glycine or it can be derived from aminosaccharides [16]. Acetamide is present in all analysed samples.

The highest concentration of acetamine is in limed sludge with 6% of free CaO (7.8µg/mg), the lowest concentration was found in non-limed sludge (0.9µg/mg). Both limed sludges have higher concentrations of heterocyclic nitrogen than aliphatic nitrogen. The concentration of heterocyclic and aliphatic nitrogen for different sludge are listed in Table 1.

Non-limed sludge has very similar content of aliphatic and heterocyclic nitrogen. From the comparison of non-limed and limed sludge it is apparent that concentration of heterocyclic nitrogen is probably increased by addition of lime. It causes the change of physical and chemical parameters of environment (pH value) which apparently support the origin of relatively more stable forms of nitrogen that are important for gardening and agricultural applications.

Substances with so called non-specific origin [17] were identified in pyrochromatograms of all samples. Among these substances belong toluene, styrene, acetic acid and above mentioned methylphenols and phenols (Fig.1). These substances can originate from more sources. Toluene and alkylbenzenes can be formed by decomposition of peptides and proteins containing tyrosine, but also from lignin and tannins [11]. The diagnostic ratio for distinguishing the source was not defined. Styrene can originate by degradation of proteins and peptides containing phenylalanine [14], lignin and tannins [18]. Styrene can also be derived from synthetic polymers (plasts and polystyrene). For styrene it is only possible to distinguish the synthetic and natural origin on the basis of ratio E3/S (toluene/styrene), but it is not possible to determine exactly the natural source (tannins, lignins, proteins). The analysed samples had the value of ratio E3/S less than 1 which indicates the synthetic origin of styrene.

Humification and mineralization criteria in waste-water treatment sludge (Ceccanti et al., 1986) were evaluated on the basis of ratios B/E3 (benzene/toluene) and N/O (furfural/pyrrole). The ratio B/E3 expresses humification of certain substrate. Benzene is derived from degradation of condensed aromatic structures [19], toluene is derived from both condensed aromatic structures and pseudostable organic matter. The highest degree of humification was determined for limed sludge (B/E3 = 1.91), the lowest degree was determined for non-limed sludge (B/E3 = 0.21). The mineralization of organic matter [20] can be expressed by the ratio N/O (furfural/pyrrole). Furfural is a product of labile organic matter (saccharides), pyrrole originates

Tab. 1 Aliphatic and heterocyclic nitrogen in sludges

Tab. 1 Alifatyczne i heterocykliczne związki azotu w osadzie

	Limed sludge	Non-limed sludge	Sludge with 6% of free lime
Aliphatic nitrogen (µg/mg)	33.44	21.81	46.40
Heterocyclic nitrogen (µg/mg)	63.18	23.15	66.55

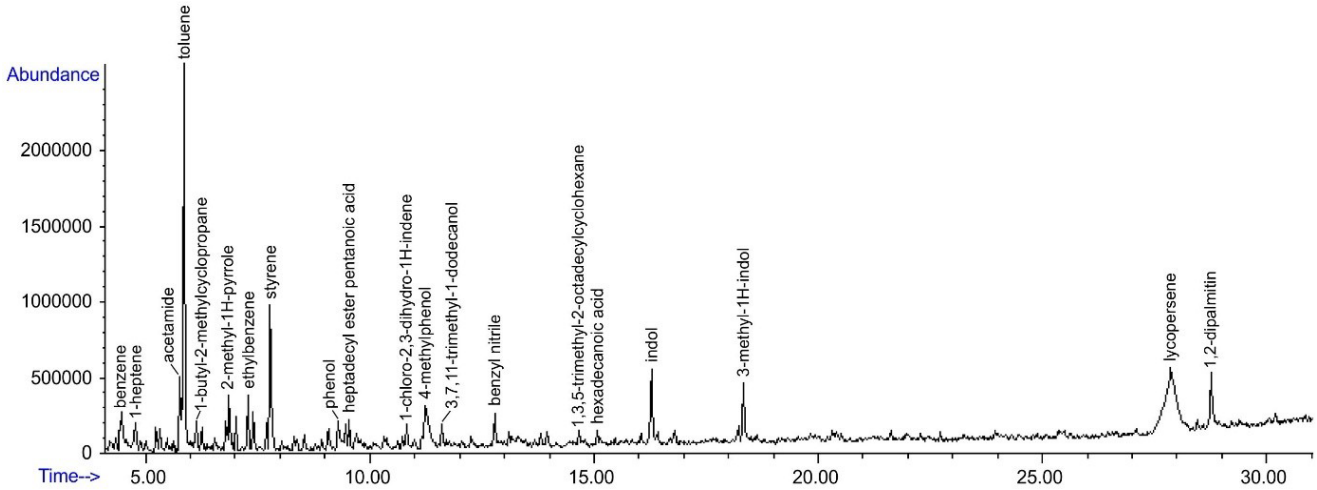

Fig. 1 The pyrochromatogram of limed sludge with content of 6 % of free lime

Rys. 1 Pirochromatogram wapnowanych osadów o zawartości 6% czystego wapna

from stable organic matter (humified components containing nitrogen). The lowest degree of mineralization was determined for sample of non-limed sludge (N/O = 0.19). The highest degree of mineralization was found for limed sludge (N/O = 0.08), and after it, limed sludge with 6 % of free lime follows (N/O = 0.10).

Conclusions

Limed sludges have similar behaviour in accumulation of substances derived from polysaccharides, proteins and lignin. Both limed sludges contain less derivatives originated from polysaccharides in comparison with non-limed sludge. It is probably caused by higher rate of decomposition of organic matter and subsequent mineralization of both substrates. This fact was also confirmed by value of mineralization index (N/O) which indicates that limed sludges have higher mineralization than non-limed sludge. Limed sludges accumulate more derivatives originated from lignin which is caused by more intensive decomposition of polysaccharides. Lignin degradable with difficulties and fragments of lignincellulose remain in substrate. Both substances participate in building of stable structure of organic matter. This fact was confirmed by calculation of humification index (B/E3). The highest degree of humification was determined for limed sludges. From the point of view of organic matter content, degree of mineralization and humification, limed sludge are the most suitable waste-water treatment sludge for possible gardening and agricultural application.

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Związki organiczne pochodzenia naturalnego w osadach z oczyszczalni ścieków

Przeanalizowano związki organiczne pochodzenia naturalnego w trzech typach osadów wytwarzanych przez oczyszczalnie ścieków - szlam wapnowany zawierający 12% CaO, szlam wapnowany z 6% CaO i osady nieprzetworzone. Analizę przeprowadzono za pomocą pirolitycznej chromatografii gazowej ze spektrometrią masową (Py-GC / MS). Określono główne składniki pochodzenia naturalnego w osadach z oczyszczalni ścieków. Stanowią one związki z następujących grup: kwasy tłuszczowe oraz ich estry metylowe, steroidy, substancje pochodzących z polisacharydów, substancje pochodzące z ligniny, związki z azotem (zarówno heterocykliczе i alifatyczne), substancje o tzw. niespecyficznym pochodzeniu (toluen, styren, kwas octowy, metylofenole i fenole). Przedstawiono próbę identyfikacji źródeł związków organicznych, a także określenia stopnia humifikacji i mineralizacji. Stosunek benzenu do toluenu stosuje się w osadach z oczyszczalni ścieków jako kryterium humifikacji. Benzen jest pochodną z degradacji skondensowanej struktury aromatycznej, toluen pochodzi zarówno ze skondensowanych struktur aromatycznych jak i z pseudostabilnej materii organicznej. Stosunek furfural/pyrol użyto w celu ekspresji stopnia mineralizacji materii organicznej. Furfural jest produktem nietrwałej substancji organicznej (sacharydy), a pyrol pochodzi ze stabilnej materii organicznej (nawilżonych składników zawierających azot). W porównaniu z niewapnowanymi osadami, osady wapnowane mają wyższy wskaźnik rozkładu materii organicznej, jak i późniejszej mineralizacji. Wapnowane osady gromadzą więcej związków pochodzących z ligniny co jest spowodowane bardziej intensywnym rozkładem polisacharydów. Z punktu widzenia wykorzystania w rolnictwie, szlam wapnowany jest najbardziej odpowiednim osadem wytwarzanym przez oczyszczalnie ścieków.

Słowa kluczowe: przeróbka ścieków i osadów, związki organiczne, humifikacja, mineralizacja, Py-GC/MS