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Composting of oiled bleaching earth: Fatty acids degradation, phytotoxicity and mutagenicity changes

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ABSTRACT

Due to high fat content the treatment of oiled bleaching earth is very challenging. The neutralization of such waste may be carried out by employing the composting process. In this study, the efficiency of the composting process conducted with 19% addition of commercial oiled bleaching earth to waste sludge was evaluated. Maize straw was used as a structural material. The activity of lipases and dehydrogenases was measured during the biodegradation process. As a result of composting, changes in C/N ratio occurred (from 31 to 15) and the efficiency of fatty acids biodegradation reached 95%. The composting process also resulted in the removal of the initial phytotoxicity of composts. The mature compost did not exhibit any form of phytotoxicity during assays with Phytotoxkit. The Ames test excluded mutagenicity of any components in the compost. These results suggest that the proposed composting method may potentially be employed for rapid and efficient remediation of industrial waste containing oiled bleaching earth and waste sludge.

1. Introduction

The production and processing of edible oils and fats is among many branches of food industry, which produce challenging wastes in terms of biodegradability (Arvanitoyannis and Kassaveti, 2007; Chandra and Sathiavelu, 2009). The waste fats are collected after cleaning refining devices, hardening of fats, and other technological operations. Such wastes commonly consist of fatty acids after refining, oil slime, autoclave oil, and oiled bleaching earth (which contains 10–60% fatty substances, depending on the technology used). About 40000 tons of oiled bleaching earth is generated by fat production plants each year in Poland alone and it is expected that the quantity may increase in the following years (Piotrowska-Cyplik et al., 2012). Land disposal of oiled bleaching earth is usually not preferred in agriculture. Generally, a negative influence on the physico-chemical properties of soil and, in consequence, on the growth and development of plants can be observed if the content of oiled bleaching earth in the soil mass exceeds 3% (Wierzba et al., 2000). The application of the microbiological composting process is among the most rational utilization methods for the residues rich in oils and fats (Michailides et al., 2011; Tortosa et al., 2012). Recent experiments confirmed the usefulness of composting for the utilization of residues rich in oils and fats, fat sludges or post-filtration fatty acids (Altieri et al., 2011; Maliki and Lai, 2011). However, this strategy requires improving the unfavorable conditions, which are inevitably associated with fatty substances, as well as determining the crucial factors associated with the efficiency of fat degrading microorganisms (Toledo et al., 2006). The addition of a structural material may enhance the physical properties of the waste. This contributes to better maintenance of aerobic conditions, which result in increased removal efficiency (Klauss and Papadimitrou, 2002; Kulcu and Yaldiz, 2007).

The objective of the presented studies was to investigate the course of biodegradation of fatty acids in waste products (oiled bleaching earth and sewage sludge) obtained after refining of plant oils and sewage sludge treatment, during the composting process.

In order to achieve the desirable process efficiency, it is essential to improve the physical properties of the sludge by the inclusion of

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structural materials. Such materials are helpful for creating aerobic conditions, which are necessary for proper functioning of microorganisms associated with the degradation of fat substances. Therefore, the method proposed in this study is focused on composting of the oiled bleaching earth together with sewage sludge and wheat straw, which makes it possible to utilize the produced compost in agriculture.

2. Materials and methods

2.1. Materials and experimental set-up

The oiled bleaching earth used in the study was obtained from the largest processor of oil seeds and manufacturer of vegetable fats in Poland and one of the largest in Central Europe – (Zakłady Tłuszczowe Kruszwica, Poland) and contained 55% of fatty substances. Dewatered sewage sludge (20% d.m.) was obtained from the Municipal Waste Treatment Plant in Koziegłowy near Poznań (Poland).

Composting was performed in an two-chamber bioreactor (chamber volume of 125 dm^3) and each bioreactor chamber was in triplicate The precise description of the bioreactor and a section on assessment of gas emissions (CO_2, NH_3, CH_4), O_2% and oxygen uptake is given in manuscript (Piotrowska-Cyplik et al., 2009).

The composting process was carried out in two variants:

Bioreactor A: 8.75 kg oiled bleaching earth [19% (w/w)], 35 kg of dewatered sewage sludge [77% (w/w)], 1.6 kg of maize straw [4% (w/w)],

Bioreactor B: 43 kg of dewatered sewage sludge [96% (w/w)], 1.6 kg of maize straw [4% (w/w)].

Composting of sole oiled bleaching earth was not possible due to material structure and high content of fatty substances which inhibited aerobic microbial processes.

2.2. Studies on basic physicochemical parameters of composts

In order to perform physicochemical analyses, a 180 g sample was collected from each bioreactor. The samples were prepared by mixing six smaller samples (30 g) taken from six different points of the bioreactor. The samples were collected seven times: first time: 0 day (B, $t = 0$ – starting of composting process), in the first mesophilic phase: 5 day (M1) three times in thermophilic phase:14 day (T1), 22 day (T2), 32 day (T3), in the second mesophilic phase: 40 day (M2), and in the maturation phase: 44 day (E).

For assaying of dry substance the material was dried at 60 and 105 °C until solid mass was obtained. The organic matter content was assayed by weight determination after roasting at 500 °C. Organic carbon content was examined using an Elementar Analyser Vario EL III (Germany). Total nitrogen content was measured by Kjeldahl method (Kjeltec System 1026 Tecator, Denmark).

2.3. Activity of enzymes

2.3.1. Determination of lipolytic activity

The method described by Cyplik et al. (2012) was used for the investigations of lipolytic activity. The activity was determined on the basis of hydrolysis of para-nitrophenyl palmitate (p-NPP), which is caused by enzymes excreted by bacteria during composting. The lipolytic activity was expressed as the micromolar amount of p-NPP released during 1 h at 37 °C by 1 g of compost. The reaction mixture consisted of 540 μl of solution A (2 mM p-NPP dissolved in 2-propanol) and 4860 μl of solution B (100 ml of phosphate buffer (pH 6.5 or 8.0) adjusted to pH of the compost, 0.1 g Arabic gum and 4 ml Triton X-100). The obtained mixture was emulsified for 10 min in an ultrasonic bath, and then heated for 5 min in a water bath at 37 °C. After the initial heating, 1 g of compost was added to the mixture. The sample was then incubated for 15 min at 37 °C. Content of the probe was centrifuged at 3600 g, for 15 min, at 25 °C. Absorbance was measured at 410 nm using a spectrophotometer (Thermo Scientific, USA).

2.3.2. Determination of dehydrogenase activity

As much as 15 ml of 2 g l^{-1} TTC (triphenyltetrazolium chloride) and 20 mL of phosphate buffer were added to 3 g of sample, and the mixture was incubated for 30 min at room temperature. The samples were then centrifuged (HAERAUS, Germany) for 15 min at 3000 g. The centrifuged sample was extracted with 20 mL of ethanol and centrifuged again for 15 min at 3000 g. Finally, extinction was measured at 490 nm. Dehydrogenase activity was expressed as μM TF g^{-1} h^{-1} (TF – triphenylformazan).

2.4. Assaying of fatty acids

Quantitative and qualitative characterization of fatty acids in oiled bleaching earth and in composts with sewage sludge was performed with the use of Folch method (Folch et al., 1957). Analysis of methyl esters was performed on a gas chromatograph (Hewlett Packard 5890) equipped with a flame ionization detector (FID) at 260 °C and split (1:50) injection system. A capillary column (30 m × 0.25 mm × 0.25 μm) (Innowax) was used with helium as carrier gas (1.5 ml min^{-1}). The analysis proceeded at temperature programmed from 60 to 200 °C (at rate of 12 °C min^{-1}), with the concluding temperature lasting for 20 min. Injector temperature was at 240 °C. Identification of fatty acids was performed based on retention times of standards (Supelco 37 Component Fame Mix). Quantitative interpretation was performed based on peak surface in comparison to an internal standard – heptadecanoic acid methyl ester.

2.5. Determination of mutagenicity and phytotoxicity of composts

Mutagenic properties of investigated samples were determined based on the Ames bacterial test with the use of four genetically modified strains of *Salmonella typhimurium*: TA98, TA100, TA1535, and TA1537 originating from the Polish Collection of Microorganisms of the Institute of Immunology and Experimental Therapy PAN in Wrocław. The test relies on the reversion of mutation of genetically altered *S. typhimurium* strain, incapable of histidine synthesis. Recognized mutagenic factors influence bacterial genome by reversing its original mutation and restoring the functional gene coding for histidine, thus facilitating growth and colony formation on a medium with slight addition of this amino-acid.

Investigations on mutagenicity were performed in consistence with the procedure described by Maron and Ames (1983). Reaction mixtures comprised the following components: 100 μl of bacterial suspension after 16 h of culturing in liquid medium, standard mutagen or tested extract (the centrifuged sample was extracted with 20 ml of distilled water and centrifuged for a further 15 min at 3000 g^{-1}), and phosphate buffer (PBS) in the amount filling up to the total of 1 ml. Prepared reaction mixtures were pre-incubated at 37 °C for 20 min. Next, they were introduced into top agar containing trace amounts of histidine and biotin. Then, they were poured on the surface of plates with water agar supplemented with mineral salts and 10% glucose. The plates were incubated at 37 °C. After 48 h culturing, grown bacterial colonies were counted, and the results were compared with control samples: 1) negative, with no addition of mutagenic compound, and 2) positive, with a reference mutagen (2-aminofluorene for TA98 strain; sodium azide for TA100 and TA1535, 2-aminoacridine for TA1537). Based on the results, a mutagenic activity (AM) of the tested extracts was