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ELIMINATION OF SUBSTANCES WITH ANTIBIOTIC OR ESTROGENIC ACTIVITY FROM DEWATERED SEWAGE SLUDGE

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SUMMARY: Many different pharmaceutical chemicals are found in sewage sludge as a result of the sorption mechanism associated with the process of wastewater treatment. In our study, we focused on substances with inhibitory (antibiotic) and estrogenic (endocrine disruptors) activity to assess the unpredictable risk to the soil environment associated with the usage of sewage sludge as a fertilizer in agriculture.

We evaluated the presence of bacterial pathogens, residual antibiotic activity and four detected xenoestrogenes (4-nonylphenol, 17α-ethynyl estradiol, bisphenol-A, irgasan) after bio-drying process of dewatered anaerobically stabilized sewage sludge (WWTP for 30 000 p.e.). Till now we have completed three bio-drying cycles in two reactors (R1, R2) working under different modes of aeration. Results from the third bio-drying cycle are presented. The courses of two remaining cycles were very similar and fully support conclusions published in this contribution. While 360 h bio-drying cycle led to the removal of all pathogens that were monitored (total coliforms, *E. coli* and enterococci bacteria) along with the residual antibiotic activity; the concentration of endocrine disruptors decreased by 71% in R1 (maximum temperature in the reactor 48,1°C) and 43% in R2 (max. temperature 62,6°C).

Thereafter, we pyrolysed the output samples from the bio-drying experiments. For the purpose of comparison, we analysed the sludge collected from substantially larger WWTP (>250 000 p.e.). before and after pyrolysis (without a bio-drying step). The efficiency of antibiotic activity and xenoestrogenes removal by pyrolysis was high and varied between 93.1 and 100%. Using dewatered sewage sludge as a fertilizer in the agriculture may pose a serious risk due to the significant concentration of substances with antibiotic activity and/or endocrine disrupting properties. Nonetheless, using sewage sludge-derived biochar as a fertilizer seems to be an acceptable way as to how the potential of the sludge can be put to use in agriculture. For certain types of sludge, the bio-drying process itself can lead to the production of hygienic, odour-free and stabilized material, well suited for the use as fertilizer. The incineration of sludge should be considered only if the concentrations of heavy metals exceed acceptable limits..

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1. INTRODUCTION

Recently, many research teams have started focusing on the problems of incomplete elimination of organic compounds such as antibiotic (Batt et al., 2006, Barber et al., 2009, Dong et al., 2016, Tran et al., 2016) and (potentially) estrogenic or other activities (Muller et al., 2010, Prusaliewicz et al., 2007, Camino Sanchez et al., 2016) in the wastewater treatment plants (WWTPs). The compounds such as antibiotics, estrogenes, parabenes, UV filters, e.g. benzophenones, or biocides, e.g. triclosan, can be degraded completely, but more often they are degraded only partially. Furthermore, degradation products of these compounds are adsorbed in the sewage sludge (Dorival-Garcia et al., 2013, Li et al., 2013). The sewage sludge is an inevitable by-product of wastewater treatment process, which can be landfilled (after dewatering) or recycled as an agricultural soil amendment that leads to decreased landfilling (Camino-Sanchez et al., 2016). The sludge contains organic compounds, macro and micronutrients, trace elements and microorganisms useful for plants. Unfortunately, it is also enriched with heavy metals, pathogens and low concentrations of hazardous compounds, which represent factors limiting its use as fertilizer (Hossain et al., 2011). According to the sludge composition, efficient ways to decrease such risks are lime stabilization, incineration, composting (Camino-Sanchez et al., 2016, Jin et al., 2016,) or bio-drying (Zhang et al., 2016). These processes eliminate most of the pathogens but do not solve the problem of inorganic pollution.

Bio-drying was invented as an innovative method for the treatment of sludge with high moisture content, which is faster than common composting (Zhang et al., 2016). The sludge is dried with the heat that is generated during aerobic degradation of organic substances. It has been demonstrated that bio-drying is a prospective method for the reduction of sludge volume and pre-stabilization that favours short-term storage, low transport requirements and facilitate the possibility of sludge incineration (Zhao et al., 2010, Zhao et. al., 2011).

In our work, we compared two different modes of aeration control during bio-drying of the anaerobically stabilized sewage sludge mixed with wood chips. We tried to evaluate bio-drying as a key process of the sludge management which hygienize the sludge, according to the Czech legislation. Due to its potential as fertilizer, we prefer stabilized, hygienic and odor-free output material usable in agriculture to production of solid fuel and its subsequent incineration. We also followed the fate of selected residual pharmaceutics in the sludge, although the Czech environmental authorities do not yet require their elimination. The objectives of this study were thus as follows: 1) to prove the presence or absence of substances with antibiotic or estrogenic activity in dewatered sewage sludge samples in different stages of the process, 2) to find a proper technology for dewatered sludge treatment to make it a useful and harmless fertiliser.

2. MATERIALS AND METHODS

Dewatered anaerobically stabilized sewage sludge was collected from two WWTPs (30 0000 and >250 000 p.e.). in the Czech Republic.

Experiments were performed in two laboratory scale bio-drying reactors. The cylindrical bodies of both the reactors were made of polypropylene. Polypropylene mesh was used to divide each cylinder in order to create a reactor chamber of volume 100 dm^3. Such arrangement enabled sufficient air inlet and distribution. The air inlet port and the leachate drain-off were placed in the bottom of the reactor bodies. The air outlet port, the loading port and the exhaust port for gas sampling were placed in the ceiling of the reactors. Three other ports for temperature measurement were placed on the side of each reactor. A bag with crushed paper was placed under the ceiling of each rector to prevent condensation of water. Outer walls of the

reactor were insulated by 10 cm thick PUR foam. Two Secoh SLL50 blowers were used as air sources, (see Figure 1).

Temperature in the lower, middle and upper layers (Papouch THT2 temperature meter), oxygen concentration in the middle of dried sludge body (ASEKO ASIN GTE and GREISINGER OXY 3690 MP), air consumption and airflow rate (Membrane gas flow meter ELSTER BK G4 and electronic pulse counter INZ 61) were measured in each reactor. Additionally, the temperature and relative humidity of the air (Papouch THT2) were measured in the laboratory. All the above mentioned parameters were recorded in 5s intervals.

Roughly 30 kg of the sludge, mixed with wood chips in the ratio of 5:2 (W/W), were loaded into both of the reactors. The sewage sludge for the bio-drying process was collected from a middle size WWTP for 30 000 p.e.

The aeration was automatically controlled by PC. Two different aeration regimes were used. In the first reactor (R1), the aeration was controlled by the oxygen concentration (maintaining the oxygen concentration between 16 and 20% by aeration pulses) until a temperature of 40°C was reached. After that, a PID control device (PIXSYS DRR245 and TRIAC regulator) maintained the temperature constantly at 40°C in the middle layer by decreasing and increasing the blower performance. In the second reactor (R2), constant aeration intervals were set. The blower was set on for 42 seconds every 15 minutes. Three bio-drying runs were performed and each of the runs lasted 10 to 15 days.

Samples for the determination of dry mass, contents of antibiotic residue and endocrine disruptors and microbiology analysis were collected at the beginning and at the end of each run.

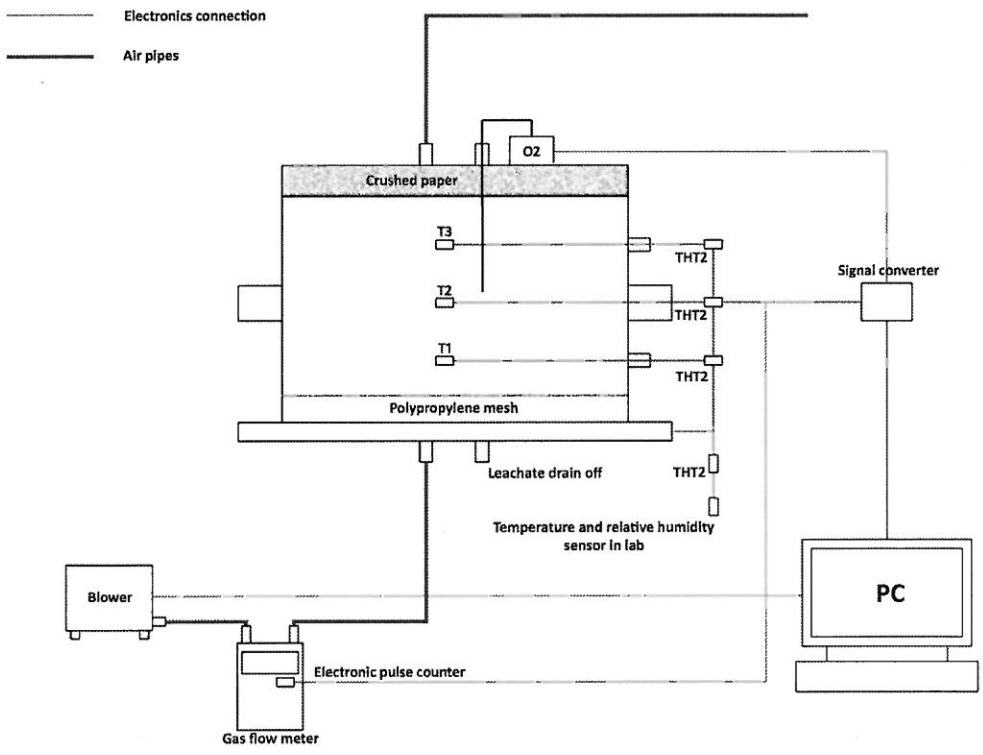

Figure 1: Reactor scheme

The samples were pyrolysed after bio-drying in a bench scale batch reactor made of

stainless steel. The reactor was equipped with a gas distributor in the bottom part and was heated up in an electrical oven. The inside temperature of the reactor was measured by a thermocouple (type K) placed just above the top layer of the feedstock. In order to keep an inert atmosphere and remove the gases that were generated, nitrogen was introduced through the bottom of the reactor at a flow rate of 1 m^3/h. The experiments were started by putting the reactor into the cold oven and switching the heating on. The temperature in the reactor increased continuously up to a maximum (500 °C). After one hour at the maximum temperature, the heating was switched off and the reactor was cooled down under the nitrogen flow.

A modified method was developed for the determination of antibiotic activity. The method is typically used for the determination of residual antibiotic activity in food (meet, milk) and the principle involves the diffusion of antibiotic compounds from the sludge or biochar samples into a solid culture medium on a Petri dish (DEV Agar, Merck), which contains spores of a sensitive bacterial strain (*Bacillus subtilis*). In our experiment, the inactivated sample (85°C for 2 minutes and cooled to room temperature) was mixed with distilled water 1:1 (w/w) and applied into a hole made in the solid medium and cultivated for 18 hours at 30°C. The negative control was a piece of cotton wool mixed with distilled water 1:1 (w/w). After 18 hours, the culture medium became cloudy (*Bacillus subtilis* colonies) except for the places around the tested samples where a clear zone could be seen in the cases when inhibitory compounds were present in the sample (see Figure 2).

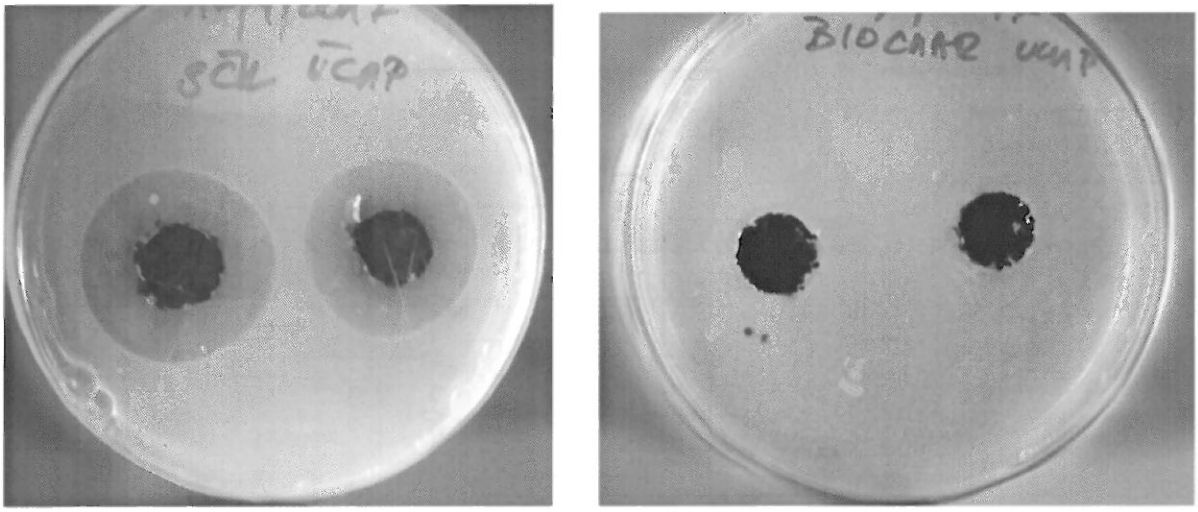

Figure 2: Left – solid samples containing compounds with antibiotic activity, the clear zones around each sample are seen. Right – a solid sample without antibiotic activity.

The endocrine disruptor 4-nonylphenol, 17α-ethynyl estradiol, bisphenol-A and irgasan were analysed using GC-MS according to Křesinová et. al (2017).

The total coliforms and *E.coli* and enterococci were determined according to the Czech national standards ČSN EN ISO 9308-1 and ČSN EN ISO 7899-2.

3. RESULTS AND DISCUSSION

Three bio-drying cycles in two reactors R1 and R2 had been completed till now. In this paper, we are presenting the results of the third cycle. A typical course of the evolution of temperature which took place in both of the previously described aeration programmes are displayed in Figures 3 and 5. Maximum temperatures that the reactors R1 and R2 reached were 48.1°C and 62.6°C, respectively. Figures 4 and 6 document the activities of blowers during the course of the experiment.

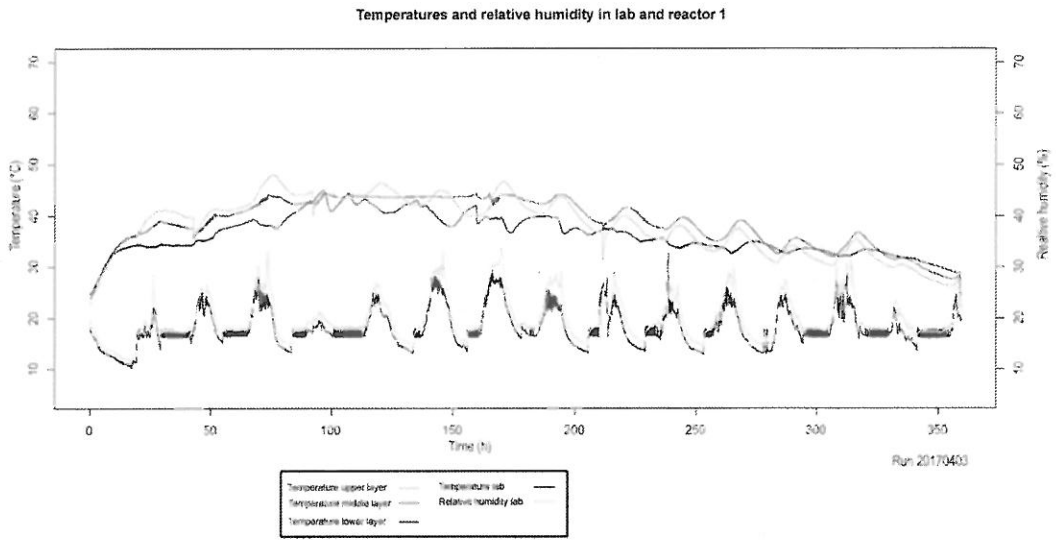

Figure 3: Temperature course during biodrying experiment in reactor R1

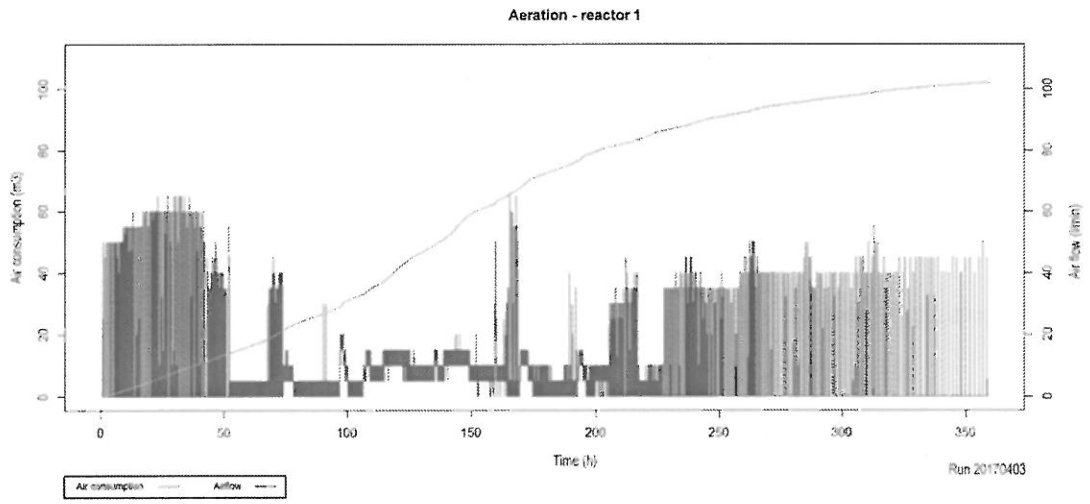

Figure 4: Blower activity in reactor R1

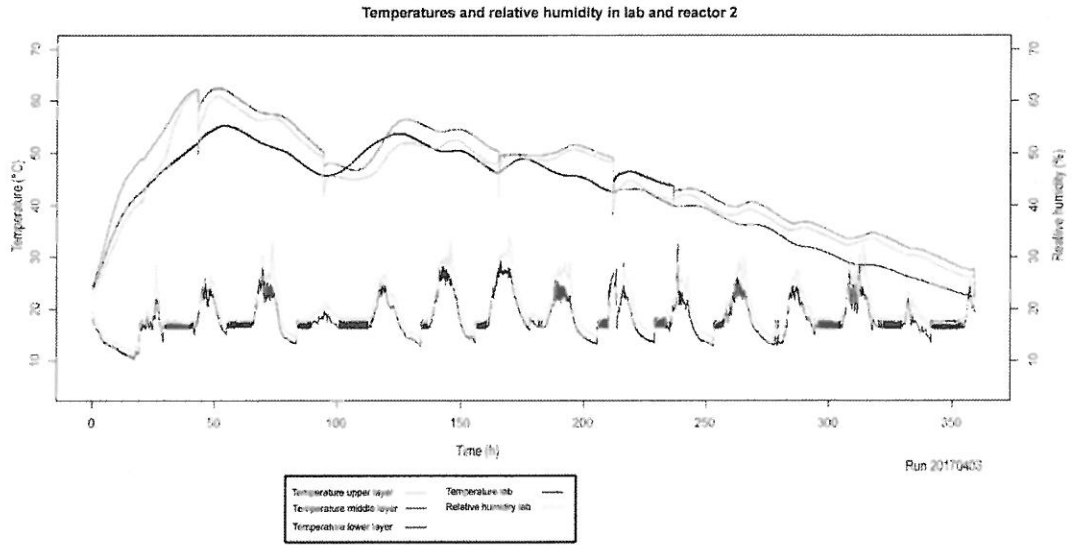

Figure 5: Temperature course during biodrying experiment in reactor R2

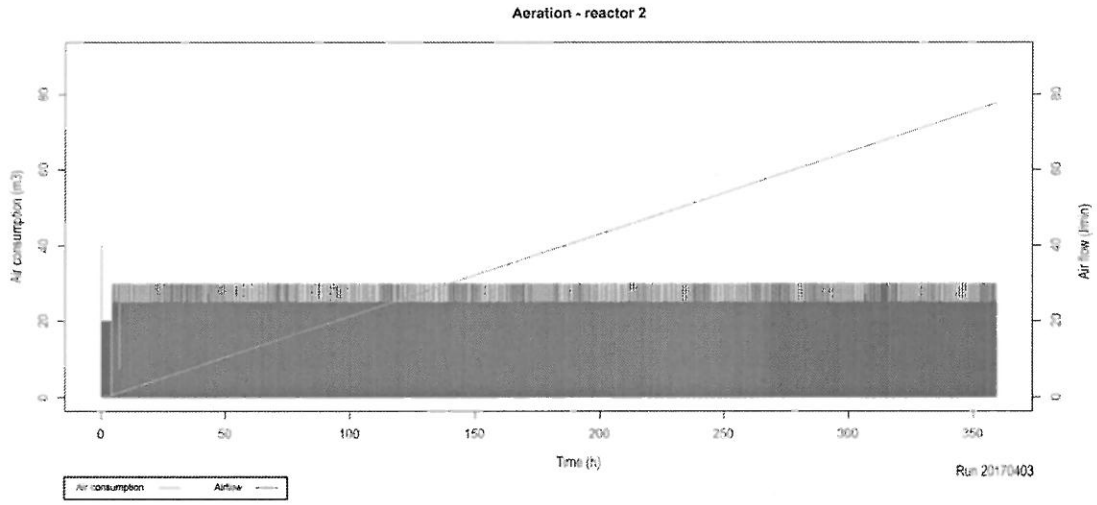

Figure 4: Blower activity in reactor R2

The dry matter content of the final product increased from initial 45.0±4.9 % to 60.0±8.0% in R1 and 59.4±4.3% in R2 (means of 6 samples). The initial concentrations of pathogens, which ranged between 5.10^3 and $8.3.10^3$ CFU/kg (see Table 1) dropped to zero. We obtained odor-free, hygienic and bulk material, suitable for the use in agriculture in terms with the Czech legislation system (heavy metals concentrations fit the limits for the use of sludge as fertilizer after this technological step – data not shown).

Changes in the antibiotic activity (ATB) and the total contents of the xenoestrogenes detected after the third bio-drying cycle are summarized in Table 1. While ATB-activity was not identified in the output sample, the decreases in the endocrine disruptors were about 71% and 43% in R1 and R2 respectively. Probably, the higher maximum temperature in R2 negatively influenced the microbial diversity in the treated material, which led to the decreased efficiency in biodegrading the monitored xenobiotics.

Table 1: Presence of pathogens, antibiotic activity and endocrine disruptors in the input and output samples (ATB: antibiotic, ED: endocrine disruptors). The values in the brackets stand for the standard deviations.

Parameters	Total coliforms (CFU/g)	*E.coli* (CFU/g)	Enterococci (CFU/g)	ATB-activity (mm)*	ΣED (µg/kg)**
Input	5.10^3 (±0,2)	$8.3.10^3$(±0.8)	$6.5.10^3$ (±1.4)	3.3 (±0.6)	2 506.2 (±360.2)
Output R1	0	0	0	0	730.8 (±393.8)
Output R2	0	0	0	0	1 437 (±21.4)

* mm of clear zone on the agar plate

** Sum of concentrations of bisphenol-A and irgasan (4-nonylphenol, 17α-ethinylestradiol were not detected)

In order to get a better overview of the relevant compounds, an additional sludge sample from another WWTP for more than 250 000 p. e. was collected and analysed for ATB-activity and the concentration of four detected xenoestrogenes. The new sample and the output samples from the above mentioned bio-drying experiments (sludge collected from WWTP for 30 000 p.e.) were pyrolysed. We were interested in the contents of the monitored substances that remained in the pyrolysis solid residue – biochar.

Table 2: The presence of antibiotic activity and endocrine disruptors in the sludge from WWTP for 30 000 p.e.and >250 000 p.e.before and after pyrolysis (ATB: antibiotic, BPA: bisphenol-A, IRG: irgasan, 4-nph: 4-nonylphenol, EE2: 17α-ethynyl estradiol)

Parameters	ATB-activity (mm)*	BPA (µg/kg)	IRG (µg/kg)	4-nph (µg/kg)	EE2 (µg/kg)
Sludge $WWTP_{30\,000}$	4	3 456 (±463.4)	2 543.4 (±307.5)	ND	ND
Biochar $WWTP_{30\,000}$	0	35.6 (±13)	2.4 (±0.3)	ND	ND
Sludge $WWTP_{>250\,000}$	11	4 604.4 (±827.6)	9 169.5 (±1 435.9)	151.5 (±19)	87.7 (±27.4)
Biochar $WWTP_{>250\,000}$	0	99.8 (±89.6)	ND	10.5 (±1.7)	ND

* mm of clear zone on the agar plate

ND – not detected

Bisphenol-A and irgasan were removed with 99% and 99.9% efficiency from the sludge of $WWTP_{30\,000}$ (4-nonylphenol and 17α-ethynyl estradiol were not detected). In the sludge that was collected from $WWTP_{>250\,000,}$ 97,8%, 100%, 93,1% and 100% removal was observed in case of bisphenol-A, irgasan, 4-nonylphenol and 17α-ethynilestradiol respectively. The results (see Table 2) show that pyrolysis represents an alternative way to remove these problematic substances with a satisfactory efficiency.

CONCLUSIONS

We focused on substances which could pose a significant threat to the environment but the monitoring of which is not required in the Czech Republic. According to the results of this study, we observed a certain influence of bio-drying (which actually represents the thermophilic phase of the composting process) on the antibiotic activity and the concentrations of endocrine disruptors. It is in agreement with the results of previously published studies describing a significant decrease of antibiotics content (Mitchell et. al., 2015) and other organic micro pollutants (Poulsen and Bester, 2010) during the thermophilic phase of composting. The efficiency of the process is probably connected to the operating temperature in the reactor. (In the present, the influence of the maximum temperatures in both reactors on the microbial diversity of the treated material is evaluated using PLFA analysis).
Nevertheless, the most reliable solution for the potential problem with pharmaceutics remaining in the sludge seems to be pyrolysis, especially if they occur in elevated concentrations. In contrast to incineration, the use of pyrolysis can ensure desirable preservation of the fertilizing potential of sewage sludge.
In conclusion, we state that bio-drying could be considered a key method in the dewatered sludge management because 1) it decreases the content of harmful substances and is useful if the use as fertilizer is preferred or 2) enables pre-processing of sludge for thermal treatment in case its direct application in agriculture is not possible.

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