BONUS PROMISE PROJECT (1.4.2014 – 31.3.2017)
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1 Project outline of goals and results envisaged at the beginning of the project cycle

Global phosphorus (P) reserves are finite and sustainable use and recycling of P is imperative to prevent scarcity in future as well as reduce diffuse P losses from agriculture that contributes significantly to the eutrophication of the Baltic Sea. At present, organic P sources such as animal manures and sewage sludge are increasingly digested in biogas plants to produce energy and the digestates are further used as fertilisers. There is only limited knowledge on possible contamination of P-rich digestates with heavy metals, antibiotics and pathogens, and concern on their contamination may hamper efficient recycling of P.

The prime target of the **BONUS PROMISE** project was to evaluate methods to close the agricultural P cycle with special view to contamination risks when recycling the organic waste materials. In the framework of the project, input and output materials of biogas plants of different origin (animal manure and sewage sludge) were collected in Finland, Sweden and Germany, organic and inorganic contaminants were analysed, and the suitability of digestates as P fertilizers was assessed. As an example of an alternative processing method for digestion, thermo-chemical treatment of wastes for removing environmentally relevant contaminants was appraised.

2 Work carried out in the project

Samples were taken from 29 biogas plants in Sweden, Finland and Germany. Sampled materials comprised different kinds of manure, plant material and sewage sludge in varying ratios. Antibiotics were determined in all sampled input materials and digestates, whereas pathogens (from 24 plants) were also determined in samples taken from the digestor. P availability and heavy metals were determined in materials suitable for field application. The sampling was designed to study general trends in contaminant levels of digested manure and sewage sludge with varying treatment parameters and substrates. Since each biogas plant was sampled only once the samples should be viewed as test samples and not representative for a specific plant.

Sophisticated methodological work and quality assurance of the analytical procedures proved to be necessary because of the complex matrices. Additional toxicological studies with antibiotics were performed for improved risk assessment. Also, a study was performed to conclude the regrowth potential of pathogen and indicator organisms in a post-pasteurized compared to a pre-pasteurized digested residue.

3 Main results achieved during the project

Contamination of organic materials by antibiotics

Eight antibiotics representing three different antibiotic classes were analysed in the organic waste materials: From the group of sulfonamides sulfadiazine (SFD) and sulfamethazine (SMZ), from the fluoroquinolones enrofloxacin (EF), ciprofloxacin (CF) and difloxacin (DF) and from the class of the tetracyclines chlortetracycline (CTC), oxytetracycline (OTC) and tetracycline (TC). Input and output materials of biogas plants were sampled at the same day. Hence, results cannot be set directly into

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relation as the materials originate from different batches, and the results are presented separately (Table 1).

All sewage sludge samples proved to be contaminated with antibiotics (Table 1) as well as 88% of the pig slurry, 67% of the poultry dung and 50% of the cattle manure samples. Poultry dung contained distinctly more antibiotics than cattle manure or pig slurry. In poultry dung the dominating antibiotics were EF, CF and TC. The highest antibiotic contamination with values > 8600 µg EF/kg dry matter (DM) was detected in dry chicken manure. The highest concentration of 8 626 µg EF/kg DM and 8 180 µg TC/kg DM was found in poultry dung followed by 7 781 µg OTC/kg DM in pig slurry. In cattle manure OTC, TC and EF prevailed in higher concentrations while in pig slurry SFD was also often found.

Table 1. Contamination of organic waste materials with antibiotics prior to digestion in biogas plants.

Substrate	Number of samples (n)	Antibiotic contamination (n)	Proportion of contamination
Cattle manure	10	5	50%
Pig slurry	8	7	88%
Poultry dung	9	6	67%
Mixed substrates from more than 1 animal*	4	3	75%
Sewage Sludge	12	12	100%

*Two samples were mixtures from cattle and pig slurry and the other two from cattle, chicken and pig slurry.

The composition of antibiotics in sewage sludge differed from that in animal manures: CF and TC were detected in all sewage sludge samples and very often in high concentrations. EF and SFD were detected frequently. In all sewage sludge samples TCs were found, while in manures and slurries OTC was the dominating tetracycline.

Comparable to the input materials the digestates contained also significant amounts of antibiotics (Table 2). Again all digestates derived from sewage sludge were contaminated with antibiotics; a maximum value of 2 440 µg TC/kg DM was determined. In one digestate based on poultry dung maximum values of 29 758 µg TC/kg DM and 2 406 µg EF/kg DM were detected. Out of all substrates and digestates (animal manures + sewage sludge), 79% and 86% contained antibiotics, respectively.

Table 2. Contamination of digestates with antibiotics.

Digestate	Number of samples (n)	Samples with antibiotic contamination (n)	Proportion of contamination
Containing cattle manure	13	7	54%
Containing pig slurry	14	7	50%
Containing poultry dung	17	14	82%
Containing sewage sludge	18	18	100%

The results showed that only very little, if any, of degradation of antibiotics occurred within the fermentation process in the biogas plants, and high concentrations of EF and TC were found in the digestates. TCs seemed to be even enriched as higher concentrations (maximum 3 282 µg TC/kg DM) were found in the digestates from sewage sludge than in the original substrate (maximum 2 240 µg TC/kg DM).

The results revealed that the direct use of farmyard manure and sewage sludge will yield a contamination with antibiotics which is comparable to that of digestates, while tetracyclines and fluoroquinolones were even found in elevated concentrations. Digestion of organic wastes in biogas

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plants is thus no measure to reduce antibiotic contaminations and also the digestates need to be evaluated critically with respect to the possible formation of antimicrobial resistance in soil. Furthermore, additional toxicological experiments were also carried out. For example, these revealed that presence of different antibiotics as opposed to a single compound alone, resulted in even higher toxicity in the case of seed germination of Sinapis alba.

Contamination of organic materials with heavy metals

The most abundant heavy metals among the studied P sources were zinc (Zn), copper (Cu), chromium (Cr) and nickel (Ni) while the rarest were mercury (Hg) and cadmium (Cd). Among manures, Zn and Cu concentrations were highest in pig manures and lowest in cattle manures. Manures contained more heavy metals per kilogram of P than superphosphate with the exceptions of Cd and uranium (U), but mostly less than the sewage sludges. In sewage sludges the most abundant heavy metals were Zn, Cu, Cr, Ni, U and lead (Pb) and the concentrations were higher than in superphosphate. However, the concentrations were under the limits set by the sewage sludge directive (86/278/EEC) and only occasionally exceeded the stricter national limit values. Uranium concentrations were higher in Swedish and Finnish sewage sludge compared to Germany probably due to geological reasons.

Struvite (magnesium ammonium phosphate), superphosphate and the liquid fraction of sewage sludge (after struvite precipitation) had most often the lowest heavy metal concentrations. AshDec – process (thermo-chemical ash treatment) for the sewage sludge ash reduced Cd and Hg concentrations compared to the sewage sludge used as a feeding material. High Cr and Ni contents detected in AshDec –product resulted from corrosion of the unprotected steel reactor walls of the test equipment in contrast to refractory lined reactor walls of industrial-size equipment.

Contamination of organic materials by pathogen microorganisms

The study showed a variation in the occurrence of pathogens between different substrates. A major difference was observed in the presence of salmonella between samples of animal origin (manure) compared to samples of human origin (sewage).

Reduction of pathogens during anaerobic digestion depends on the treatment temperature. However, as the heat tolerance of organisms may vary widely the effect of anaerobic treatment will not only depend on treatment temperature but also on the ability of an organism to withstand increased temperatures. In general, thermophilic digestion is required to achieve a significant pathogen reduction, while mesophilic digestion will leave majority of the pathogens present and more or less unaffected. Due to the relatively low number of samples the data analysis includes all digestion plants independent of the treatment temperature and therefore differences in treatment temperature cannot be distinguished. Screening of samples in this study showed that *Salmonella* spp., *Cryptosporidium* spp. and *Giardia* could still be detected in the end-products from the biogas plants (Table 3).

Table 3. Percentage of organic waste (raw) materials, process samples and end-products of digestion positive for pathogen microorganisms.

Samples point	*Salmonella*	*Campylobacter*	*E. coli* O157	*Cryptosporidium*	*Giardia*
Raw material	64% (18/28)	14% (4/28)	0 % (0/28)	4% (1/28)	25% (7/28)
Process sample	48% (13/27)	7% (2/27)	0 % (0/27)	7% (2/27)	15% (4/27)
End-product	39% (11/28)	0% (0/28)	0% (0/28)	4% (1/28)	18% (5/28)

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Presence of pathogens in the end-products of digestion suggests that pathogens may survive the digestion process or there is a possibility of re-contamination of the end-product (Table 3). The risk associated to recontamination of the end-product will depend on the level of contamination and the pathogen in question, e.g. in the presence of favorable conditions salmonella may multiply in the end-product. Thus, routines for handling and storage are extremely important to minimize the risk for recontamination of the end-products of digestion.

There are several benefits of post-pasteurization in comparison to pre-pasteurization in a digestion plant. However, post-pasteurization is associated with an increased risk of regrowth of pathogenic bacteria in case of recontamination of the material following the pasteurization. Within the present project the potential differences in survival and regrowth of bacterial indicator organisms and pathogens in digested residues was studied. Here pasteurization refers to heat treatment at 70 °C for 60 minutes. It was demonstrated that the regrowth potential for indicator organisms tends to increase in post-pasteurized compared to pre-pasteurized material while no significant difference in re-growth potential could be observed for salmonella.

By ensuring that precautions to limit the risk of recontamination after sanitization are in place the increased probability of growth in a post-pasteurized material does not necessarily increase the probability of disease transmission. However, survival and growth is a complex process that is affected by many parameters and therefore might be hard to predict.

The risk for presence of pathogens in the end-products of digestion plants is evident. However, land-application of organic material does not inevitably pose a risk for disease transmission to humans and animals. Health risks that pathogens pose are highly dependent on the concentration of their occurrence in the materials, transport of the pathogens in the environment and survival in the agricultural settings. Also, the infectious dose varies widely between pathogens and depends on the health status of the host. A large spectrum of parameters must be considered and the choice of sanitizing treatments must include the risk for disease transmission as well as the cost of additional treatments. Treatments to ensure safe end-products, commonly in practice at digestion plants, include pasteurization at 70°C for 60 minutes and the thermophilic digestion.

Solubility and bioavailability of P in organic materials

Digestion had no effect on P solubility in organic waste materials. The shares of water-soluble P in cattle, pig and poultry manure were 60%, 36% and 55%, respectively. According to chemical extraction (Hedley fractionation), share of labile P content in cattle, pig and poultry manures were 83%, 58% and 67%, respectively. Solubility of P decreased if the digestates contained also industrial side streams or sewage sludge. In sewage sludges, water-soluble and labile P contents were only 0.6% and 4%, respectively. The main fraction in sewage sludges was NaOH-extractable P due to the use of Fe-containing precipitation chemicals at the waste water treatment plants for removing P from the liquid phase. Water-soluble and labile P content in manures and sewage sludges were depressed along with increasing molar ratio of Fe+Al/P and there the Fe content had the dominant role. In the liquid fractions of digestates P was mostly in easily soluble form, regardless of the used substrates. Thermal treatment of cattle and pig manures and sewage sludges decreased P solubility. The main P fraction after thermal treatment as well as in the AshDec-product and in struvite was the least soluble one, acid soluble (1 M HCl) P.

Gasification of sewage sludge increased the acid-soluble P share from 50% to 94% and further to 97% after treating the ash with the AshDec -method indicating a further decrease in P solubility. Also struvite had low water-soluble and labile P contents and the less soluble P fractions (0.1 M NaOH and 1 M HCl extractable P) were the main P fractions (93%).

For a more realistic assessment of bioavailability of P compared to the solubility tests, DGT-method was used after incubating the organic waste materials in a P deficient sandy soil. Bioavailability of P

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in manure by DGT was comparable to that of superphosphate, whereas in sewage sludges it was on average 35% depending on the Fe and Al content of the sludges. Gasification decreased P bioavailability, however, the AshDec –process increased it dramatically above that of superphosphate. Although struvite had a low labile P content according to Hedley fractionation, DGT-method predicted comparable bioavailability to mineral P fertilizer (superphosphate).

These results clearly indicate that the commonly used chemical solubility tests do not always give a reliable estimation of P bioavailability, especially for such materials as struvite or the AshDec -product. While P fertilization recommendations are based on solubility the results call for critical evaluation of the methods for better knowledge on sustainable use of P and thereby the actions needed to reduce the P leaching potential to surface waters.

Conclusions

Positive attitude of consumers towards recycled fertilizers is an utmost importance for efficient P recycling. Especially the contamination by pathogens, heavy metals and harmful organic substances such as antibiotics, poses an important role for the acceptance of food produced by using recycled fertilizers. This study provided knowledge on how thermophilic anaerobic digestion and pasteurization may reduce the contamination risk of some pathogens but not those of heavy metals or antibiotics. The study also demonstrated ways to eliminate most of the risks by gasification and further treatment of the ash. These results are available for the decision makers to promote better quality of recycled fertilizers and to enhance the circular economy of valuable P resources.

4 The continuity plan of the project

- Collected data will be used as a reference and a base for the future research needs.
- Sample bank of organic waste materials will be preserved for 2 years for the future research needs.
- Peer-reviewed articles will be prepared from the project results.
- The analytical methods for the determination of antibiotics in soils, plants and fertiliser materials need to be harmonised in order to enable a bias-free comparison of data.
- Studies on the uptake of antibiotics by plants, behaviour in soils and mixture toxicology need to be intensified.
- Struvite is a mineral fertilizer produced from organic waste materials, regularly characterised as being almost free from organic contaminants. However, first analyses revealed that struvite was contaminated with antibiotics but a comprehensive survey is missing.
- The choice of sanitizing treatments during processing of organic waste materials must be taken into consideration when evaluating the risk for disease transmission as well as the cost of additional treatment.
- Analytical methods for reliable determination of phosphorus bioavailability in various materials (before and after processing) that are potential fertilizers need to be verified.

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