

# SEWAGE SLUDGE HYGIENIZATION BY PRE-TREATMENT AND TWO STEPS ANAEROBIC DIGESTION FROM THE ASPECT OF PATHOGENS REDUCTION IN AGRICULTURAL UTILIZATION OF SLUDGE

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## INTRODUCTION

Regarding pathogens, the regulations for use of sewage sludge for agricultural purposes are based on the following principles: a requirement treatment (conditioning) sewage, including thermal treatment (to reduce the amount of pathogens) and assurance of the microbiological quality of the sludge's. The assessment of a sanitary condition of sludge is based on the indirect conclusion on the content of pathogenic bacteria and eggs of intestinal parasites and is based on determining the so-called sanitary indicator. Until recently, the species used as a sanitary indicator of bacteria was a lactose-positive rods *Escherichia coli*. Currently, under the Regulation of the Minister of the Environment, a new bacteria indicator *Salmonella sp.* is being used in sanitary assessment.

The poster presents results from hygienization of sewage sludge by pre-treatment and two steps anaerobic digestion (mesophilic/thermophilic fermentation).

## MATERIAL AND METHODS

Surplus activated sludge samples, from the secondary settling tanks, concentration of suspended solids in average 11,81 g/dm<sup>3</sup>, were taken from municipal wastewater treatment plant in the Silesian voivode ship in Poland, operating according to the Enhanced Biological Nutrient Removal (EBNR) processes.

### Hydrodynamic hygienization/disintegration

Hydrodynamic disintegration was executed by the application of a pressure pump (12 bar), which re-circulated sewage sludge from a 25-litre container through a constructed cavitation nozzle. The process was carried out for 15, 30, 45 and 60 minutes, which corresponded to 3, 6, 9, and 12 multiplicity flow by the cavitation nozzle.

### Hygienization/disintegration by freezing/thawing

For hygienization of surplus activated sludge, the following volume ratios of the surplus sludge to dry ice were used, i.e. 1 : 0.25; 1 : 0.5; 1 : 0.75; 1 : 1.

### The fermentation experiments

The anaerobic digestion experiments were performed in a glass fermenter (2.5 L volume). The reactor has been located in thermostatic conditions, with constant temperatures 35±1°C and 55±1°C under mesophilic and thermophilic conditions respectively. Holding time of 12 days for mesophilic conditions and 13 days for thermophilic conditions were used. The hygienization process was determined after the mesophilic process and after 25 days.

### Bacteria and coliphages

The studies related also to the total number of bacteria contained in the surplus sludge before and after the destruction (disintegration by hydrodynamic cavitation and dry ice) and two steps anaerobic digestion (mesophilic/thermophilic fermentation). The analyses on surplus sludge were performed before and after the hygienization process, and they were aimed at detection of *Salmonella sp.*, *E. coli*, *Clostridium perfringens* and coliphages. The determinations of microorganisms - *E. coli*, *Clostridium perfringens* - were made in accordance with Project Routes (2011-2014). Grant agreement n°265156.

Detection and enumeration of *Salmonella sp.* were investigated according to Bergey's Manual Trust. In order to verify the taxonomic classification of *Salmonella sp.* and *E. coli* the API 20E biochemical tests were used. Detection and enumeration of bacteriophages were investigated according to ISO 10705-2:2000.

### Helminths parasites

The studies concerned also to the total number of parasites eggs contained in the surplus sludge before and after the hygienization by hydrodynamic cavitation, dry ice and two steps fermentation. Detection and enumeration of *Ascaris sp.* and *Trichuris sp.* were investigated according to PN-Z-19000-4:2001. Eggs of intestinal parasites were isolated from the tested sample by shaking, centrifugation, filtration of flotation suspension and microscopic observation.

Eggs of *Toxocara sp.* were isolated from an investigated sample by diethyl ether, shaking, centrifugation, filtration of flotation suspension processes and microscopic observation. Eggs of *Toxocara sp.* were enumerated according to the McMaster chamber.

The microscope used – Nikon Alphaphot – 2 YS coupled with a camera Panasonic GP – KR 222 allowed also for size measurements by a program Lucia – ScMeans Verion 4.51.

## RESULTS AND DISCUSSIONS

National and international regulations require that sludge have to be stabilized and hygienized before applying it on the land. Hygienization is the destruction of pathogenic microorganisms. The removal of pathogenic bacteria and helminths from sewage sludge by hydrodynamic destruction and freezing/thawing may considerably reduce the transfer operation of illnesses. The mechanical process and freezing/thawing caused the destruction of microorganisms, and thus contributed to a partial hygienization of surplus sludge, which is confirmed by the results of the microbiological and parasitological analyzes (Tab.1, 2).

Hydrodynamic disintegration/hygenization					
Bacteria, Phages [cfu/g <sub>d.w.</sub> ], [pfu/g <sub>d.w.</sub> ]	Without disintegration	Time			
		15 min.	30 min.	45 min.	60 min.
<i>Salmonella sp.</i>	4.1·10 <sup>3</sup>	3.7·10 <sup>3</sup>	1.7·10 <sup>3</sup>	0.3·10 <sup>3</sup>	0
<i>Escherichia coli</i>	6.4·10 <sup>5</sup>	5.9·10 <sup>4</sup>	4.4·10 <sup>4</sup>	3.7·10 <sup>4</sup>	3.3·10 <sup>4</sup>
<i>Clostridium perfringens</i>	6.1·10 <sup>4</sup>	5.6·10 <sup>4</sup>	4.5·10 <sup>4</sup>	4.2·10 <sup>4</sup>	2.3·10 <sup>4</sup>
Coliphages	5.9·10 <sup>5</sup>	5.6·10 <sup>5</sup>	5.2·10 <sup>5</sup>	4.6·10 <sup>5</sup>	4.1·10 <sup>5</sup>
Helminths [eggs/kg <sub>d.w.</sub> ]					
	Without disintegration	Time			
		15 min.	30 min.	45 min.	60 min.
<i>Ascaris sp.</i>	1.7·10 <sup>3</sup>	1.3·10 <sup>3</sup>	6.3·10 <sup>2</sup>	0	0
<i>Trichuris sp.</i>	9.6·10 <sup>2</sup>	6.8·10 <sup>2</sup>	3.2·10 <sup>2</sup>	0	0
<i>Toxocara sp.</i>	3.8·10 <sup>2</sup>	1.5·10 <sup>2</sup>	0.1·10 <sup>2</sup>	0	0

Tab. 1. Bacteria and helminths eggs elimination as a result of the hydrodynamic disintegration/hygenization.

Disintegration/hygenization by freezing/thawing					
Bacteria, Phages [cfu/g <sub>d.w.</sub> ], [pfu/g <sub>d.w.</sub> ]	Without disintegration	Volume ratio of the surplus sludge to dry ice			
		1 : 0.25	1 : 0.5	1 : 0.75	1 : 1
<i>Salmonella sp.</i>	4.1·10 <sup>3</sup>	4.0·10 <sup>3</sup>	3.6·10 <sup>3</sup>	2.9·10 <sup>3</sup>	1.3·10 <sup>3</sup>
<i>Escherichia coli</i>	6.4·10 <sup>5</sup>	5.9·10 <sup>5</sup>	5.2·10 <sup>5</sup>	4.1·10 <sup>5</sup>	2.1·10 <sup>5</sup>
<i>Clostridium perfringens</i>	6.1·10 <sup>4</sup>	5.8·10 <sup>4</sup>	5.3·10 <sup>4</sup>	4.7·10 <sup>4</sup>	3.6·10 <sup>4</sup>
Coliphages	5.9·10 <sup>5</sup>	5.8·10 <sup>5</sup>	5.5·10 <sup>5</sup>	5.0·10 <sup>5</sup>	4.8·10 <sup>5</sup>
Helminths [eggs/kg <sub>d.w.</sub> ]					
	Without disintegration	Volume ratio of the surplus sludge to dry ice			
		1 : 0.25	1 : 0.5	1 : 0.75	1 : 1
<i>Ascaris sp.</i>	1.7·10 <sup>3</sup>	1.4·10 <sup>3</sup>	6.4·10 <sup>2</sup>	4.2·10 <sup>2</sup>	2.5·10 <sup>2</sup>
<i>Trichuris sp.</i>	9.6·10 <sup>2</sup>	7.2·10 <sup>2</sup>	4.9·10 <sup>2</sup>	2.5·10 <sup>2</sup>	1.1·10 <sup>2</sup>
<i>Toxocara sp.</i>	3.8·10 <sup>2</sup>	3.1·10 <sup>2</sup>	2.8·10 <sup>2</sup>	1.1·10 <sup>2</sup>	0.2·10 <sup>2</sup>

Tab. 2. Bacteria and helminths eggs elimination as a result of the disintegration/hygenization by freezing/thawing processes.

After hygienization by hydrodynamic cavitation and dry ice, a gradual decrease in the population of bacteria was observed (tab. 1). After 60 minutes of mechanical sludge microorganisms disintegration resulted in total elimination of *Salmonella sp.* (tab. 1). The number of *Salmonella sp.* in 1 g<sub>d.w.</sub> of the volume ratio of the sludge to dry ice 1 : 1 was reduced by about 68% (tab. 2).

Hygienisation by two steps anaerobic digestion			
Bacteria, Phages [cfu/g <sub>d.w.</sub> ], [pfu/g <sub>d.w.</sub> ]	Untreated	Mesophilic process 35±1°C 12 days	Thermophilic process 55±1°C 13 days
		<i>Salmonella sp.</i>	1.3·10 <sup>4</sup>
<i>Escherichia coli</i>	1.9·10 <sup>5</sup>	1.1·10 <sup>5</sup>	0
<i>Clostridium perfringens</i>	7.2·10 <sup>4</sup>	5.5·10 <sup>4</sup>	1.3·10 <sup>4</sup>
Coliphages	1.2·10 <sup>5</sup>	1.1·10 <sup>5</sup>	0
Helminths [eggs/kg <sub>d.w.</sub> ]			
	Untreated	Mesophilic process 35±1°C 12 days	Thermophilic process 55±1°C 13 days
		<i>Ascaris sp.</i>	1.6·10 <sup>3</sup>
<i>Trichuris sp.</i>	5.1·10 <sup>2</sup>	1.3·10 <sup>2</sup>	0
<i>Toxocara sp.</i>	4.8·10 <sup>2</sup>	1.4·10 <sup>2</sup>	0

Tab.3. Reduction of indicators organisms in two steps anaerobic digestion process.

Depending on the disintegration by hydrodynamic disintegration and freezing/thawing (the volume ratio of the sludge to dry ice 1 : 1) a reduction number of *E. coli* in sewage sludge about 95% and 67% - respectively was observed (tab.1, 2). On the basis of microbiological tests, a gradual elimination of the *Clostridium perfringens* bacteria caused by the growth of the volume ratio of dry ice to the sludge and cavitation time was observed. The number of *Clostridium perfringens* rods in the sludge were reduced by 62% (after 60 minutes of disintegration by hydrodynamic cavitation), and 41% (dry ice), (tab.1, 2).

The prolonged time of hygienization by hydrodynamic disintegration results in a decrease of the overall number of coliphages. After 60 minutes of the processes, the quantity decreases by about 31% (tab. 1). The dry ice had a destructive effect on a number of coliphages too. After the process hygienization, this number lowered (for the volume ratio of sludge to dry ice 1 : 1) by 19% (tab. 2).

The hydrodynamic disintegration and dry ice destroy the eggs of helminths. The disintegration of the surplus activated sludge by hydrodynamic cavitation (60 min.) resulted in the total reduced number of helminths eggs (tab. 1). As a result of disintegration by dry ice, overall number of eggs was decreased (tab. 1). *Ascaris sp.* by about 85%, *Trichuris sp.* by about 89% and *Toxocara sp.* by 95% (tab. 2). The obtained results confirm that mesophilic anaerobic digestion shows the ability of helminths ova reduction. Analyzed indicators, *Ascaris sp.*, *Trichuris sp.* and *Toxocara sp.*, were reduced approximately by 76%, 75% and 71% - respectively (tab. 3).

## CONCLUSIONS

1. Pre-treatment processes (hydrodynamic disintegration, freezing/thawing) and two steps anaerobic digestion are a method to destroy of bacteria and helminths parasites in sewage sludge.
2. Hydrodynamic disintegration and thermophilic anaerobic digestion reduce sanitary indicators of sewage sludge - *Salmonella sp.*, *Ascaris sp.*, *Trichuris sp.* and *Toxocara sp.* - to undetectable levels.
3. Application of hydrodynamic disintegration and thermophilic anaerobic digestion for sewage sludge hygienization on a wide scale can reduce transmission of sanitary indicators in agricultural utilization of sludge.