Anaerobic stabilisation of pig slurry as a tool for animal waste sanitation

Papajová Ingrid[1*], Papaj Ján[2], Pipiková Jana[1], Juriš Peter[1], Venglovský Ján[3]
(1) Institute of Parasitology SAS, Hlinkova 3, 040 01 Košice, Slovak Republic
(2) FEI TU of Košice, Letná 9, 042 00 Košice, Slovak Republic
(3) University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovak Republic
*Corresponding author: papaj@saske.sk

Abstract
The effect of anaerobically digested slurry stores in ground lagoons on the survival of parasitic germs were studied. A high percentage of devitalized unembryonated *A. suum* eggs (47.46 ± 0.78 %) stored 11 months (from May to March) in a ground slurry lagoon points to the impact of high concentration of NH_4^+ cations, which have been releasing during a period of time from an open area of the ground lagoon, and nitrogen on devitalization of developmental stages of endoparasites. The number of devitalized *A. suum* eggs has increased towards to the bottom of lagoon. This way of treatment is thus not associated with a risk of dissemination, survival and potential spread of developmental stages of endoparasites to the environment via stabilized organic wastes.

Introduction
Animal production pose potential hazards of environmental contamination with pathogenic microorganisms. These are particularly related to a subsequent storage processing and utilization of animal organic wastes. A major source of pathogenic microorganisms in the environment are excrements from clinically and subclinically infected farm animals. Particularly in pig faeces can be parasitic pathogens frequently encountered, among which bacteria of the family Enterobacteriaceae (some of them are zoonotic), protozoa and eggs or larvae of enteronematodes are commonly found.
The parasitic propagative stages, mainly endoparasitic protozoa and helminths, develop mostly outside their host´s organism. *A. suum* eggs are hygienically the most problematic ones. They are amongst the helminth eggs most resistant to environmental factors and may survive in the nature for many years, therefore, they tend to accumulate in the environment (soil, water) and serve as an infectious entity for both man and animals [1]. *A. suum* infects pigs and is of major economical significance due to production losses linked to reduced feed conversion efficiency and losses to the mean industry associated with the condemnation of "milk-spot" livers. *Ascaris* infects over a quarter of the world´s human population (1.47 billion people worldwide) and clinically affects ~335 million people [2].
Therefore, to prevent health risks (for human as well as for animals) and odour nuisance from animal wastes, different methods for a satisfactory utilisation and sanitation have been researched [1], [3]. There are big variations in the treatment of animal wastes (aerobic and anaerobic stabilization, composting etc.).
Our study was concentrated on protection against spreading parasitic germs through slurry. The physical-chemical changes in pig slurry treated by ecologically acceptable and energetically beneficial anaerobic stabilization and the effect of anaerobically digested slurry stores in ground lagoons on the survival of parasitic germs were studied.

Material and Methods
To determine helminth eggs count in slurry (input and output samples from bioreactor and in lagoon samples – supernatant and sediment), 50 ml from each of the 1 l sample was taken and examined by a sedimentation-flotation method.
We used the ''artificial contamination of lagoon and organic wastes'' approach to make sure that there is s sufficient number of positive samples in our observations. *A. suum* eggs were isolated by dissection of a distal uterine part of female pig ascaris. The distal uterine ends were then removed to a glass homogenizer and processed. The water suspension of eggs was stored in an Erlenmayer flask in a refrigerator at 4°C.
Model eggs were inoculated by a micropipette into polyurethane carriers, prepared according to Plachý and Juriš [4], at a dose of 1 000 eggs per one carrier. A porous cellular plastic-soft expanded

polyurethane, commercially known as a plastic foam, was used as a material for the carriers. It is an additive product of polyisocyanates and compounds with a high content of hydroxylic groups. It consists of a network of interconnected cells, resembling a honeycomb. Its polyurethane structure allows for a sufficient contact of helminth eggs with the environment, preventing them from release and consequently improving their recovery. For mechanical protection the carriers were placed to perforated plastic net before introducing them into the organic wastes. Three samples were taken and analysed at each sampling interval. The eggs were re-isolated from the inoculated carriers as follows: the carriers were cut into small pieces and washed in a mortar with 3-5 ml portions of saline, thoroughly stirred and filtered through a sieve into test tubes. After centrifugation, sediments were transferred to Petri dishes.
The viability of exposed unembryonated *A. suum* eggs was determined by incubation up to the embryonated stage in a thermostat at 26°C for 21 days. Petri dishes with *A. suum* eggs were aerated daily with micropipette. The developmental ability of *A. suum* eggs was compared with that of the control eggs which were kept in distilled water under aerobic conditions.
The samples were examined for the pH using a pH electrode (HACH Company, Loveland, Colorado, USA). Dry matter (drying at 105°C to a constant weight), residum-on-ignition (550°C for 4 h), and water soluble ammonium nitrogen (NH_4^+) by titration. Chemical oxygen demand (COD) was determined on the basis of organic substances oxidation in sample by potassium dichromate in sulfuric acid medium during 2-hour boiling in a COD reactor (HACH Company, Loveland, Colorado, USA). Portion of samples for total nitrogen (N_t) determinations were digested using a HACH-Digesdahl apparatus (HACH Company, Loveland, Colorado, USA). N_t was distilled with NaOH (40 %).
The physical and chemical properties of slurry, as well as the number of demaged eggs were expressed as mean values ± standard deviation ($\overline{X}$ ±SD).

Results and Discussion

Investigations were carried out under operating conditions of the large-capacity pig farm in Slovak Republic. Technological equipment for anaerobic treatment of pig slurry on the principle of methanogenesis with the production of biogas was built up on the farm. Pig slurry was treated in the bioreactor (2 500 m^3). The stirring of the substrate in this reactor was done at the expense of energy of the generated biogas. Mean daily input of raw pig slurry in bioreactor of biogas plant varied between 78 and 144 m^3. The volume of digested slurry after methanogenesis was equal to that of the input. Two lagoons were the part of the biogas plant. The volume of larger lagoon is 20 000 m^3 and that of smaller lagoon is 5 000 m^3. Both lagoons serve as reservoirs of digested slurry. Liquid fraction from the smaller lagoon was carried away and spread on fields. The presence and survival of parasite eggs were studied in the larger lagoon. Samples were taken from raw slurry collecting basin before the inlet in to bioreactor (input samples), from outlet of digested slurry after methanogenesis in bioreactor (output samples), from supernatant (liquid fraction) and from lagoon sludge (sediment). The slurry samples for parasitological and physical and chemical examination were collected monthly during 29 month.
Slurry from the pig farm stored in the collecting basin showed a considerable variability during the period of study (Table 1). Like raw pig slurry also slurry stabilised by anaerobic process showed variability of its physical-chemical parameters on its out flow from bioreactor (Table 2). Anaerobically stabilized slurry was pumped from bioreactors into slurry ground lagoon for further storage. Results of the chemical analysis of liquid fraction (supernatant) are presented in Table 3 and those of solid fraction (sediment) of lagoon in Table 5.
A. suum eggs and *Oesophagostomum* sp. eggs were rarely detected in slurry on the input and also on the output of bioreactor. Similar results of helminths eggs occurrence in anaerobic slurry treatment were also presented by Juriš et al. [5]. No helminth eggs were found in the supernatant of digested slurry from the lagoon. *A. suum* eggs were found in sediment. High percentage of devitalised unembryonated *A. suum* eggs (47.46 ± 0.78 %) stored 11 months (from month 13 to month 23) in a ground slurry lagoon points to the impact of high concentration of NH_4^+ (max. 5 358 $mg.l^{-1}$ in sediment compared to 1 863 $mg.l^{-1}$ in supernatant), which are releasing during a period of time from an open area of the ground lagoon, and nitrogen (max. 9 854 $mg.l^{-1}$ in sediment compared to 2 283 $mg.l^{-1}$ in supernatant) on devitalization of developmental stages of endoparasites. The number of devitalised

A. suum eggs increased towards to the bottom of lagoon. In the control groups, only 19.60 ± 1.80 % of *A. suum* eggs were devitalized (Table 4).

Table 1. Physico-chemical properties of raw pig slurry

Month	pH	COD ($mg.l^{-1}$)	DM (%)	IM (%)	OM (%)	NH_4^+ ($mg.l^{-1}$)	N_t ($mg.l^{-1}$)
0.	7.44	14 833	2.75	31.87	68.13	1 774	2 419
1.	7.34	2 000	0.84	51.42	48.58	1 186	1 401
2.	7.17	9 297	0.95	43.04	56.96	821	1 195
3.	7.03	13 500	1.14	57.71	42.29	1 202	1 485
4.	7.00	20 900	1.57	38.74	61.26	1 078	1 363
5.	7.35	14 824	0.81	45.71	54.29	1 037	1 191
6.	7.36	13 333	2.52	17.36	52.64	1 247	1 429
11.	6.61	21 795	5.30	33.02	66.98	1 695	1 089
17.	6.95	12 750	0.95	30.53	69.47	1 478	1 010
21.	6.95	22 530	2.80	19.97	80.03	1 358	1 872

Table 2. Physico-chemical properties of digested pig slurry

Month	pH	COD ($mg.l^{-1}$)	DM (%)	IM (%)	OM (%)	NH_4^+ ($mg.l^{-1}$)	N_t ($mg.l^{-1}$)
0.	8.50	36 333	-	-	-	2 633	6 320
1.	7.74	10 500	0.81	56.54	43.46	2 204	2 605
2.	7.63	17 820	1.24	48.50	51.50	2 157	2 699
3.	7.80	8 500	1.96	59.69	40.31	2 045	2 549
4.	7.69	17 100	3.16	41.81	58.19	1 933	3 138
5.	7.77	6 092	4.48	42.06	57.94	1 898	1 982
6.	7.92	2 186	2.91	42.87	57.13	2 437	3 516
11.	7.88	4 872	0.50	70.00	30.00	2 171	1 530
17.	7.37	7 750	6.45	39.84	60.16	2 248	1 936
21.	7.66	42 169	7.85	33.81	66.19	2 655	3 399

Table 3. Physico-chemical properties of supernatant from stabilized pig slurry stored in lagoon

Month	pH	COD ($mg.l^{-1}$)	DM (%)	IM (%)	OM (%)	NH_4^+ ($mg.l^{-1}$)	N_t ($mg.l^{-1}$)
0.	8.30	4 500	0.50	61.06	38.94	1 737	1 910
1.	8.20	4 000	0.50	70.39	29.61	1 307	1 428
2.	8.17	2 002	0.68	57.58	42.42	1 345	1 569
3.	8.34	3 500	0.66	57.75	42.25	1 111	1 214
4.	8.10	7 600	0.93	56.04	43.96	1 408	1 662
5.	8.08	6 552	0.87	52.76	47.24	1 135	1 172
6.	8.29	1 530	0.71	57.59	42.41	1 107	1 223
13.	8.21	7 059	0.70	55.53	44.47	1 863	2 283
14.	8.07	818	1.68	46.60	53.40	1 569	1 569
15.	8.28	1 904	0.66	54.83	45.17	1 331	1 317
16.	8.21	5 385	0.63	56.38	43.62	896	882
17.	8.29	8 605	0.60	54.27	45.73	616	1 415
23.	8.32	3 333	0.35	71.43	28.57	672	1 016
29.	7.95	5 000	0.75	45.33	54.67	862	1 031

Table 4. Damage of *A. suum* eggs during long term storage of anaerobic stabilized pig slurry in lagoon

Storage (month)	Damaged *A. suum* eggs ($\overline{X}$ %±SD)	
	Lagoon	**Control**
May (13)	16.23 ± 3.22	14.80 ± 2.43
June (14)	38.27 ± 2.51	15.79 ± 2.44
September (17)	40.37 ± 2.94	18.23 ± 1.22
March (23)	47.46 ± 0.78	19.60 ± 1.80

Table 5. Physico-chemical properties of sediment from stabilized pig slurry stored in lagoon

Month	pH	COD ($mg.l^{-1}$)	DM (%)	IM (%)	OM (%)	NH_4^+ ($mg.l^{-1}$)	N_t ($mg.l^{-1}$)
0.	8.37	9333	1.17	49.21	50.79	5 778	5 963
1.	8.13	11000	1.17	48.26	51.74	5 635	6 041
2.	8.07	6170	1,70	43.21	56.79	7 344	9 652
3.	8.09	4500	1.28	31.52	52.88	4 042	6 782
4.	7.90	55100	1.12	34.90	65.10	3 913	7 298
5.	8.08	8965	-	-	-	-	-
6.	7.87	7322	-	-	-	-	-
13.	-	-	-	-	-	-	-
14.	7.73	6367	-	-	-	-	-
15.	8.24	2494	1.72	32.11	67.89	5513	11658
16.	8.17	5897	0.73	51.16	48.84	3740	3571
17.	8.12	27186	1.19	45.41	54.59	5402	6493
23.	8.11	-	13.01	55.56	44.44	72289	57831
29.	-	-	-	-	-	-	-

Conclusion and perspectives

Processes of slurry anaerobic stabilization represent an effective method in terms of energy, since the substantial portion of energy present in easily decomposable organic constituents of the substrate is acquired in the form of biogas. Non-decomposed organic matter is well stabilized from hygienic point of view. Anaerobic stabilization increases the proportion of biogenic element (especially nitrogen) converting stabilized excrements into quality fertilizer. Anaerobically stabilized pig slurry stored in lagoon significantly influence the quality and quantity of grasses, depending on the dose of slurry used and on weather conditions. From the nutritional point of view, the sludge (sediment) from ground lagoon is also important for plants. The high amount of nitrogen is apparently the result of the decomposition process going on in lagoon.

Hazard of contamination of field fertilized with the lagoon effluent increases when raw slurry is used for fertilization of soil or pastures. When slurry is processed in a wastewater treatment plant, parasitic eggs concentrated in solid fraction. It is therefore necessary to pay a proper attention to slurry processing.

The anaerobic stabilization was demonstrated to be very suitable for the sanitation of pig slurry. This way of treatment is thus not associated with a risk of dissemination, survival and potential spread of developmental stages of endoparasites to the environment via stabilized organic wastes

Acknowledgements

This study was financially supported by the project VEGA No. 2/0140/13.

References

[1] Papajová I, Juriš P, 2009. The effect of composting on the survival of parasitic germs. In: Pereira JC, Bolin JL (Ed.); Composting: Processing, Materials and Approaches, 124-171, Nova Science Publishers, New York, USA

[2] Crompton DW, 1999. How much human helminthiasis is there in the world? Journal of Parasitology 85, 397-403.

[3] Sasáková N, Juriš P, Papajová I, Vargová M, Ondrašovičová O, Ondrašovič M, Kašková A, Szabová E, 2005. Parasitological and bacteriological risks to animal and human health arising from waste-water treatment plant. Helminthologia 42, 137-142.

[4] Plachý P, Juriš P, 1995. Use of polyurethane carrier for assessing the survival of helminth eggs in liquid biological sludges. Vet. Med. 40, 323-326.

[5] Juriš P, Vasilková Z, Krupicer I, Plachý P, Sasáková N, 1996. Hygienic and ecological aspects of mesophilic stabilization of swine liquid excrements under the operating conditions. In: Hygienic and ecological problems in relation to veterinary medicine, 119-128, Košice, Slovak Republic (In Slovak).