PATHOGENS INACTIVATION KINETICS IN CO-DIGESTIONOF SWINE MANURE AND SWINE CARCASS

Tápparo, D. C.*[1]; Viancelli, A.[2]; Amaral, A. C.[1]; Fongaro, G.[3]; Steinmetz, R. L. R.[4]; Kunz, A.[1.4]

[1]Universidade do Oeste do Paraná – UNIOESTE/CCET/PGEAGRI Cascavel, PR, Brazil
[2]Complexo de Desenvolvimento Científico - Universidade do Contestado, Concórdia, SC, Brazil
[3]Laboratório de Virologia Aplicada, UFSC, Florianópolis, Brazil
[4]Embrapa Suínos e Aves, Concórdia, SC, Brazil

ABSTRACT: Swine intensive production increases the necessity of efficient manure management and treatment. Additionally, animal carcasses disposal inside or outside animal rearing farms is under concern and object of discussion because biosecurity protocols. Anaerobic digestion has a potential to convert biodegradable organic carbon into biogas. Swine manure and swine carcass co-digestion could be a feasible alternative to treat these residues and generate a renovable energy source. However, biodigestion has limited capacity to remove pathogenic micro-organisms and this must be studied to minimize the biologic risks and assure safe disposal and use of digestate. Considering this, the objective of this study was to evaluate the *Escherichia coli* (*E. coli*) and *Salmonella enterica* – serovar Senftenberg (*S.* Senftenberg) inactivation kinetics during swine manure and swine carcass co-digestion. The inactivation experiments were conducted in triplicates, where different inactivation strategies were performed at two temperatures (24ºC and 37ºC).Two swine carcass/swine manure ratios were studied(3$kg_{carcass}.m^{-3}_{manure}$and 15 $kg_{carcass}.m^{-3}_{manure}$). *S.* Senftenberg was total inactivated after 10 days for both temperature and both ratios of swine carcass/swine manure. At 37ºC and ratio of 3$kg_{carcass}.m^{-3}_{manure}$*E.coli* was total inactivated after 10 days and at ratio 15 $kg_{carcass}.m^{-3}_{manure}$after 8 days. At 24ºC it were necessary 25 days and 31 days for relations 15$kg.m^{-3}$ and 3$kg.m^{-3}$ respectively. Digestion temperature influenced the inactivation process being more pronounced for *E. coli*. In batch reactors operating at 37ºC it is suggested hydraulic retention time (HRT) greater than 10 days for total elimination of *E.coli* and *S*. Senftenberg, while at 24ºC for at least 30 days.

Keywords: Animal residues**,** *E.coli*, *S.*Senftenberg.

INTRODUCTION

Swine intensive production increases the necessity of efficient manure management and treatment, because the amount of waste produced (Kunz et al., 2012). Additionally, animal carcasses management and disposal are other big challenges to be dealt in modern swine production systems. Recent studies have demonstrated the possible co-digestion of swine manure and swine carcasses (Massé et al., 2008; Rajagopal et al., 2014;Tápparo et al., 2016).

However, as well as swine carcass, swine manure presents numerous pathogenic micro-organisms, including, *Salmonella* sp., PCV2 and others (Viancelli et al, 2012). Studies have identified these microorganisms in swine wastewater after anaerobic digestion at room temperature (Viancelli et al., 2013; Fongaro et al, 2014). Biodigestion has limited capacity to remove pathogenic micro-organisms and this must be studied to minimize the biologic risks and assure safe disposal and use of digestate. Pathogens present in animal manure are a serious issue, once can pose a risk to public and environmental health (Top et al., 2009).

Considering this, the present study aimed to evaluate *E.coli* and *S.* Senftenberg inactivation kinetics during co-digestion of swine manure and swine carcasses at 24ºC and 37ºC temperatures, and with different proportions of carcass/manure.

MATERIAL AND METHODS

Manure and Carcass sampling: representative manure samples from a gestation sow house were collected on a swine farm in Concórdia, Santa Catarina, Brazil. For swine carcasses, samples were prepared using a representative portion of swine carcass (composted for meat, fat and skin) after grinding < 4mm.

Inactivation experiments: conducted using 500 mL glass flasks, with triplicates for each condition. Swine carcass/manure mixture was inoculated with 10^5 colonies forming units (CFUs) of *S.* Senftenberg and *E.coli*. Four inactivation strategies were performed using two temperatures (24ºC and 37ºC) and two swine carcass/swine manure ratios (3 $kg_{carcass}.m^{-3}_{manure}$ and 15 $kg_{carcass}.m^{-3}_{manure}$).These ratios represent one and five times the swine mortality (7%) and manure production (16.2 $L._{animal}.d^{-1}$) in gestation sows house from Brazilian farms (FATMA 2014; MACHADO, 2014).

***E.coli* analysis:** during the experiment 6 mL (liquid and solid fraction) was collected and submitted to tenfold serial dilution in 0.9% saline. Subsequently, *E.coli* cells present in 1 mL of each dilution were quantified using Chromocult® Coliform Agar (Merck, Germany) following the manufacturer's instructions. The results were expressed as colony-forming units (CFUs).

***S.* Senftenberg analysis:** during the experiment 6 mL (liquid and solid fraction) was collected and submitted to tenfold serial dilution in 0.9% saline. Subsequently, *S.* Senftenberg cells were quantified in xylose-lysine-deoxycholate agar(Merck, Germany)as described by Magri et al., 2013. The results were expressed as colony-forming units (CFUs).

Statistical analysis: Decimal decay rates (T90 values) and inactivation rate (*k*) were derived from the slopes of the statistical relationships between microbial numbers and time obtained by linear regression analysis (Microsoft's Excel 2010).

RESULTS AND DISCUSSION

Results are based on the batch digesters (Tápparo et al, 2016). *S.*Senftenberg was totally inactivated after 10 days in both temperatures and both ratios of swine carcass/swine manure.

At 37ºC and ratio of $3kg_{carcass}.m^{-3}_{manure}$, *E.coli* was totally inactivated after 10 days, and at $15kg_{carcass}.m^{-3}_{manure}$ratio after 8 days. At 24ºC were necessary 25 days and 31 days for relations $15kg.m^{-3}$ and $3kg.m^{-3}$ respectively (Figure 1a). According to Pandey et al., (2011) and Franke-Whittle and Insam (2013), temperature is the most important factor which influences the pathogen inactivation during anaerobic digestion. Results from the present study showed that *E. coli* was more resistant than *S*. Senftenberg.

E.coli and *S.* Typhimurium inactivation studies on mesophilic anaerobic digestion treating dairy manure, demonstrated that *E.coli* could take about 80 days for total inactivation, while *S.* Typhimurium can be achieved between 30 and 35 days (PANDEY et al., 2016).

Table 1 presents linear regression equation results , with R^2, inactivation rate (*-k*) and T90 (time necessary for 90% inactivation).The values of T90% for *E.coli* and *S*. Senftenberg did not vary according to the carcass amount for both temperatures, suggesting that the amount of carcass added per m^3 of manure does not influence the time required for inactivation of 1 log. Comparing the *k* values between model microorganisms, *E. coli* at 24 ° C were significantly more stable than *S*. Senftenberg and *E. coli* at 37 ° C.

CONCLUSION

Digestion temperature influenced the inactivation process being more pronounced for *E. coli*. This suggested that mesophilic temperature is more indicated than room temperature for pathogen inactivation in swine carcass and swine manure co-digestion, specially for *E. coli*. In batch reactors operating in the 37ºC it is suggested hydraulic retention time (HRT) greater than 10 days for total elimination of *E.coli* and *S.* Senftenberg , while at 24ºC for at least 30 days.

ACKNOWLEDGMENTS

Embrapa Swine and Poultry (TEC-DAM Project), Universidade do Contestado and Capes.

REFERENCES

FATMA, 2014. Instrução Normativa IN-11.Portaria Inter setorial n01/04, de 14.11.2014. FATMA, Florianópolis [online] Disponível em: http://www.fatma.sc. gov.br/conteudo/ instrucoes-normativas.

FONGARO, G., VIANCELLI, A.; MAGRI, M. E.; ELMAHDY, E.M.; BIESUS, L. L.; KICH, J. D.; KUNZ, A.; BARARDI, C.R. Utility of specific biomarkers to assess safety of swine manure for biofertilizing purposes. Science of the Total Environment, 479, 277–283. 2014.

FRANKE-WHITTLE, I.; INSAM, H. Treatment alternatives of slaughterhouse wastes, and their effect on the inactivation of different pathogens: A review. Critical review in Microbiology, v. 39, p. 139-151, 2013.

KUNZ, A., STEINMETZ, R. L. R., DAMASCENO, S., AND COLDEBELA, A. Nitrogen removal from swine wastewater by combining treated effluent with raw manure. Scientia Agrícola, 69, 352–356. 2012.

MACHADO, G. Estratégias de descarte de matrizes e reposição de plantéis. In: Produção de suínos: teoria e prática. Associação Brasileira de Criadores de Suínos. Brasília, DF. 2014.

MAGRI, M. E, PHILIPPI, L.S; VINNERÅS, B. Inactivation of pathogens in feces by desiccation and urea treatment for application in urine-diverting dry toilets. Appliedand Environmental Microbiology.79(7), p. 2156–2163, 2013.

MASSÉ, D. I.; MASSE, L. HINCE, J. F.; POMAR, C. Psychrophilic anaerobic digestion biotechnology for swine mortality disposal.Bioresource Technology, 99, p. 7307-7311.2008.

PANDEY, P. K.; SOUPIR, M. L. Escherichia coli inactivation kinetics in anaerobic digestion of dairy manure under moderate, mesophilic and thermophilic temperatures. AMB express. 2011.

PANDEY, P.K.; CAO, W.; WANG, Y.; VADDELLA, V.; CASTILLO, A. R.; SOUZA, A.; DEL RIO, N. S. Simulating the effects of mesophilic anaerobic and aerobic digestions, lagoon system, and composting on pathogen inactivation. Ecological Engineering, v. 97, p. 633-641, 2016.

RAJAGOPAL, R.; SAADY, N. M. C. Low-Temperature Anaerobic Co-Digestion of Swine Carcass and Swine Manure: Impact of High Swine Carcass Loading Rate. Transactionsofthe ASABE, v. 57, n. 6, p. 1811–1816, 2014.

TÁPPARO, D. C.; KUNZ, A.; AMARAL, A. C.; STEINMETZ, R. L. R.; GASPARETO, T. C. Determinação da capacidade de produção de biogás a partir da digestão de carcaça suína e sua codigestão com dejeto suíno. In: XLV Congresso Brasileiro de Engenharia Agrícola-CONBEA. Florianópolis, SC. 2016.

TOP, E.; SCOTT, A.; LAPEN, D. R.; LYAUTEC, E.; DURIEZ, P. Livestock waste treatment systems dor reducing environmental exposure to hazardous enteric pathogens: some considerations. Bioresource technology, v.100, p. 5395-5398. 2009.

VIANCELLI, A., GARCIA, L. A.; KUNZ, A.; STEINMETZ, R.; ESTEVES, P. A.; BARARDI, C. R. Detection of circoviruses and porcine adenoviruses in water samples collected from swine manure treatment systems. Research in Veterinary Science, 93, 538–543. 2012.

VIANCELLI, A.; KUNZ, A.; STEINMETZ, R. L. R.; KICH, J. D.; SOUZA, C. K.; CANAL, C. W.; COLDEBELLA, A.; ESTEVES, P. A.; BARARDI, C. R. M. Performance of two swine manure treatment systems on chemical composition and on the reduction of pathogens. Chemosphere, 90, 1539-1544. 2013.

Table 1. *S.* Senftenberg and *E.coli* median T90, inactivation rate and R^2 for four different studied strategies.

	T	*S.* Senftenberg			*E.coli*		
	(˚C)	-k (days)	T90 (days)	R^2	-k (days)	T90 (days)	R^2
$3kg_{carcass}m^{-3}_{manure}$	24	0.4851	2.0	0.8856	0.3310	3.0	0.8452
	37	0.4092	2.5	0.7652	0.6200	1.6	0.9761
$15kg_{carcass}\ m^{-3}_{manure}$	24	0.5090	2.0	0.8893	0.3184	3.1	0.9407
	37	0.3902	2.5	0.8616	0.5692	1.6	0.1562

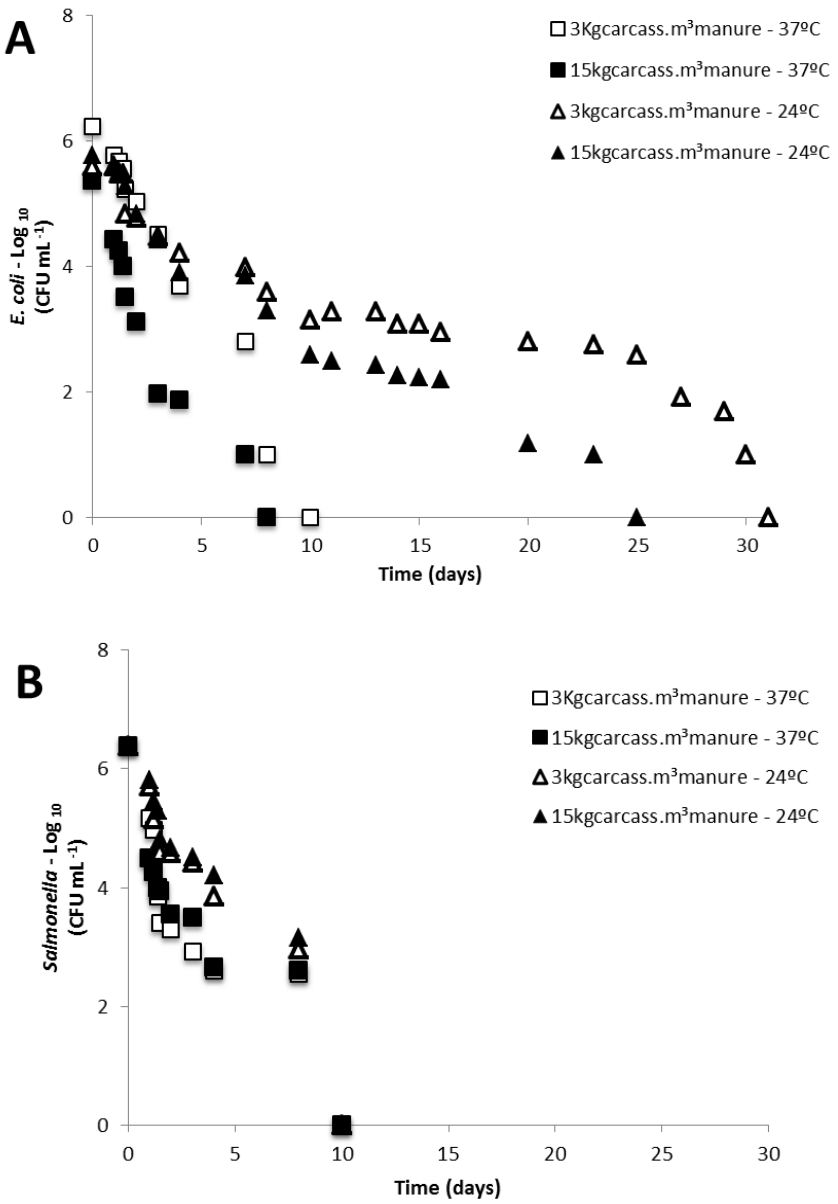

Figure 1. a)*E.coli* (CFU mL^{-1}); b) *S.* Senftenberg(CFU mL^{-1}) inactivation during the swine carcasses and swine manure anaerobic co-digestion at 37ºC and 24ºC.