

Inactivation Effect According to the Thermophilic Aerobic Oxidation Process of Encephalomyocarditis Virus in Swine Manure

Kim, Soo-Ryang
Faculty of Animal Life Resources, Sangji University

Kim, Ha-Je
Department of Environmental Engineering, Sangji University

Hong, In-Gi
Department of Environmental Engineering, Sangji University

Ahn, Hee-Kwon
Department of Animal Biosystems Science, Chungnam National University

他

<https://doi.org/10.5109/1543413>

出版情報：九州大学大学院農学研究院紀要. 60 (2), pp.485-492, 2015-09-18. Faculty of
Agriculture, Kyushu University

バージョン：

権利関係：



Inactivation Effect According to the Thermophilic Aerobic Oxidation Process of Encephalomyocarditis Virus in Swine Manure

Soo-Ryang KIM¹, Ha-Je KIM², In-Gi HONG², Hee-Kwon AHN³, Shaffiqur RAHMAN⁴,
Kwang-Hwa JEONG⁵, Joong-Bok LEE⁶, Dong-Kyun KIM¹,
Mitsuyasu YABE and Myung-Gyu LEE^{2*}

Laboratory of Environmental Economics, Department of Agricultural and Resource Economics,
Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan
(Received April 10, 2015 and accepted May 19, 2015)

Livestock manure is a major source of pathogens (e.g., foot and mouth disease–FMD) and pollutants, in addition to odors from microbial degradation of organic matter. In this study, the feasibility of a Thermophilic Aerobic Oxidation (TAO) method for the treatment of Encephalomyocarditis virus (EMCV) in swine manure was investigated in reference to a classical alkaline method. In the course of this study, relative performance of two treatments was also tested against chemical oxygen demand (COD) and nutrient reduction. The TAO and alkaline method reduced total kjeldahl nitrogen (TKN) concentration by 52% and alkaline method 23%, respectively. In the case of $\text{NH}_4\text{-N}$, they reduced 67 and 11%, respectively. Similarly, the former also reduced the EC significantly compared to the latter. Based on this study, deactivation of the EMCV by the alkaline treatment system reduced EMCV by the latter approached 99.97% within an hour, whereas the former did the same a little slowly (by 3 hours). The overall results of our study suggest that TAO method is equally good or better than the alkaline method in treating infected manure to reduce microorganisms to an acceptable level. Thus, TAO system is considered an appropriate alternative to the alkaline treatment method for hygienic management of livestock manure.

Key words: Swine manure, Pathogens, Encephalomyocarditis virus (EMCV), Foot and mouth disease (FMD), Alkaline treatment, Thermophilic Aerobic Oxidation (TAO)

INTRODUCTION

Concentrated animal feeding operations (CAFOs) are deemed to generate significant amounts of manure and wastewater that contain nutrients and pathogens. If manure and wastewater from livestock production facilities are not managed and treated properly, they may pose both environmental and public health concerns (Burkholder *et al.*, 2007). In particular, if manure contaminates food crops (e.g., leafy vegetables consumed as food), contaminants and pathogens may easily penetrate into the food chain and water system (Unc and Goss, 2004). Thus, manure may act as a medium to transfer a variety of manure borne diseases to humans.

Manure harbors many microorganisms, but the most common human pathogens are *Campylobacter* spp., *Salmonella* spp., verotoxigenic *Escherichia coli* O157, *Listeria monocytogenes*, and *Cryptosporidium parvum* (Plaut, 2000; Adak *et al.*, 2002). Survival of these microorganisms depends on the medium they grow. For

example, *E. coli* O157, *Salmonella* and *Campylobacter* may survive in stored slurries and wastewater for up to three months depending on the storage temperature, whereas *Listeria* may survive up to six months in slurry or wastewater (Nicholson *et al.*, 2005). Similarly, others also reported that non-toxigenic *E. coli* may survive in soil for 7–8 days (Taylor and Burrows, 1971) to a few weeks (Linton and Hinton, 1984). *Salmonella* is likely to survive for a longer period of time (up to 968 days) in soils (Jones, 1986). *Campylobacter*, (naturally present in dairy cattle slurry) applied to cropland in the spring may be detected 20 days after land application of manure (Linton and Hinton, 1984). Therefore, it is important to treat manure, especially infected manure, before disposing of or applying it to cropland to reduce environmental and public health concerns.

There are many treatment options (for livestock manure), the most common of which are physical, chemical and biological methods. Physical treatments use temperature and/or pressure, and chemical treatments typically use acidic or basic materials to adjust the pH below 4 or above 10. Biological treatments include composting (solid) and/or anaerobic/aerobic methods (liquid) (Heinonen-Tanski *et al.*, 2006). Common treatment methods include pasteurization, chemical disinfection, heap composting, aeration, and biogas production of livestock manure. Some of the characteristics of these treatment methods are shown in Table 1.

Most of these methods destroy microorganisms to an acceptable level of hygienic risk, while requiring longer treatment time. There is need for a robust treatment that reduces microorganisms in contaminated manure to an

¹ Faculty of Animal Life Resources, Sangji University, Wonju 220-702, South Korea

² Department of Environmental Engineering, Sangji University, Wonju 220-702, South Korea

³ Department of Animal Biosystems Science, Chungnam National University, Daejeon 305-764, South Korea

⁴ Department of Agricultural and Biosystems Engineering, North Dakota State University

⁵ Animal Environment Division, National Institute of Animal Science, RDA, Suwon, South Korea

⁶ Laboratory of infectious disease, College of Veterinary Medicine, Konkuk University, Seoul 143-701, South Korea

* Corresponding author (E-mail: mglee@sangji.ac.kr)

Table 1. A summary of advantages and disadvantages of different manure treatment methods^{a)}

Treatment method	Hygienisation limitations	Costs	Technical limitations
Pasteurization	Good at 70°C	Capital costs high, can be divided into heating and electricity needed	Mainly for slurry
Chemical disinfection	Good	Capital cost low, operation costs higher	Both of solid manure and slurry
Heap composting	Better if temperature increases up to 60°C	Capital costs low, Labor costs rather high	For solid manure, no heating is required
Aeration	Temperature of 30–40°C should be reached. The 60–70°C may be possible	Capital costs rather low, electricity needed	For slurry and food industry by-products
Biogas production	Temperature of 50–60°C should be reached	Capital costs very high, heating and electricity needed, but can be extracted from process	Suitable for slurry and partly for solid manure and food industry by-product

a) Data extracted from Heinonen–Tanski *et al.* (2006)

acceptable limit within a short period of treatment time. Auto-thermal thermophilic aerobic digestion (ATAD) is a well-recognized technology for treating municipal sludge and wastewater to reduce microorganisms. In this type of bioreactor, the temperature rises to over 50°C due to the conservation of heat produced by the aerobic metabolism of the microorganisms that consume the organic material present in the sludge (Tchobanoglous *et al.*, 2003). In this system, a well-designed bioreactor can self-heat up to 75°C (but preferably in the range of 55–65°C), thus causing pathogen destruction while conserving nitrogen (Juteau, 2006). Thermophilic Aerobic Oxidation (TAO) is a system that treats liquid manure in a shorter time compared to ATAD, destroying pathogens and converting swine manure into high-quality liquid fertilizer (Lee *et al.*, 2004). Additionally, the TAO system also reduces odor during the process due to use of a proprietary microbe (Lee *et al.*, 1999; Lee and Lee, 2000a).

In Korea, foot and mouth disease (FMD) that took place from November, 2010 to April, 2011 caused loss of approximately 2.1 billion dollars in 144 days (Shin *et al.*, 2012). Due to FMD, 151,000 cows and 3,285,000 pigs

were incinerated and buried (Kim and Kim, 2011). The FMD virus is a class A disease specified by the Office International des Epizooties (OIE) (Kim *et al.*, 2012). The FMD virus may be infected quickly to other healthy animals through saliva, sputum, nasal mucus, and feces. Typically, large quantities of FMD virus are present in the saliva of an infected animal. However, a small amount of virus may also be present in feces and urine, which raises questions regarding how barn manure or wastes can be safely handled during an outbreak of FMD, particularly regarding safe disposal of manure onto pastures as fertilizer (Sellers, 1971). The FMD virus is most stable at near-neutral pH and is sensitive to even mild acidity. Manure pH is neutral to slightly alkaline. The combination of rapidly increased temperature above 50°C and pH above or below 7 will kill 90% of the virus (Pharo, 2002). Inactivation parameters are listed in Table 2.

In Korea the standard protocol is to subject the FMD virus-infected manure to chemical treatment following the FMD Standing Operating Procedure (SOP). Manure is treated with alkaline material (normally sodium hydroxide–NaOH, 97.0% with total nitrogen 0.002%) at pH >10

Table 2. The effect of temperature and pH on time for 90% foot-and-mouth virus inactivation^{a)}

Effect of temperature (at pH 7.5)		Effect of pH (at 4°C)	
Temperature	Inactivation time (90%)	pH	Inactivation time (90%)
61°C	30 seconds	10.0	14 hours
55°C	2 minutes	9.0	1 week
43°C	1 hour	8.0	3 weeks
39°C	7 hour	7.0–7.5	> 5 weeks
37°C	21 hour	6.5	14 hours
20°C	11 days	6.0	1 minute
4°C	18 weeks	5.0	1 second

a) Data extracted from Pharo (2002)

for approximately 2–3 days. Treated manure is neutralized with acidic agent (pH 6–8) in accordance with the treatment protocols for livestock manure. However, if a large-scale FMD outbreak occurs, such approach is no longer valid due to the difficulties in management (e.g. treatment method, inactivation effect, after-treatment, cost, operation time etc.) of pathogen-infected manure. The application of the TAO system might shed light on treating infected manure in a short period, while also producing high-quality fertilizer as an end product. Therefore, contaminated livestock manure treatment is an urgent solution that can reduce contaminants quickly while reducing the risk of pathogenic viruses when manure is applied to cropland or pastureland.

This study was conducted to compare the high-temperature liquid fermentation method (TAO system) to the standard alkaline FMD manure treatment. Effectiveness in mitigating microorganisms and other contaminants from manure in a relatively short period of time in order to respond to disease outbreak under Korean manure management practices was studied.

As mentioned before, the TAO system can rapidly decompose organic liquid materials into a safe fermented liquid fertilizer rich in nutrients. This system can also reduce odor-causing substances such as Volatile Fatty Acids (VFA) and contaminants, organic substances, nitrogen and phosphorus. Additionally, this process can kill pathogenic microorganisms due to self-sustaining operating temperature at $>50^{\circ}\text{C}$ regardless of seasonal temperature changes (Lee and Lee, 2000b; Lee and Lee 2000c).

In this study, an Encephalomyocarditis virus (EMCV) similar to the FMD virus was used. The inactivation of EMCV virus followed the same FMD chemical treatment SOP. The objective of this study was to compare the effectiveness of the TAO system with the standard alkaline treatment of manure processing in mitigating pathogens and other contaminants from pig manure.

MATERIALS AND METHODS

Materials

Raw pig manure was collected from a pig farm located in Wonju city of Gangwon-do, Korea and samples were

stored at 4°C before analysis. Manure physicochemical analysis such as pH, electrical conductivity (EC), chemical oxygen demands (CODcr), total phosphorus (TP), total kjeldahl nitrogen (TKN), and ammonium-nitrogen ($\text{NH}_4\text{-N}$) were determined using standard methods as described in the physicochemical properties of treated and untreated manure samples.

Experimental methods

Two treatment processes, alkaline and a TAO manure treatment, were evaluated and compared.

Alkaline manure treatment method

The Standing Operating Procedure (SOP) for inactivating the FMD virus was based on recommendations provided by the Korean Government (Table 3) (MAFRA, 2012). Sodium hydroxide (NaOH, 97.0%, total nitrogen, 0.002%) was added to manure at 10 g/L in a 200-L tank (540 mm (D) \times 900 mm (H)) (Fig. 1A) and stirred for 84 hours. Samples were collected at a predetermined times. Samples were stored in a refrigerator and kept at 4°C before analysis.

Thermophilic Aerobic Oxidation (TAO) manure treatment method

The TAO reactor (200 L) used in this study consisted of an inlet, outlet, aeration motor, parcel device and control device measuring 540 mm (D) \times 900 mm (H) (Fig. 1B). A viewing port was installed to visualize internal sensors, operating levels and internal changes (of temperature) during operation. The reactor had a concave bottom for ease of removing digestate and cleaning. A lot of 120 L of pig slurry was placed in the reactor and stirred for 84 hours. The physicochemical properties of liquid within the reactor and the inactivation effects of EMCV were monitored during the study period.

EMCV inactivation test method

The Dialysis Cassettes (Slide-A-LyzerTM, Thermo Scientific, USA) were used for handling viruses and extracting samples in this study. The specification of regenerated-cellulose dialysis membrane and the Dialysis Cassettes are shown in Table 4. In this study, dialysis cassettes were used due to their excellent capacity for pH

Table 3. Alkaline treatment method of manure according to the Standing Operating Procedure (SOP) for FMD

Item	Disinfection and Neutralization Agents	Disinfectant Application Amount per 1 ton of Manure ^{a)}	pH Range	Treatment Period
Alkaline Treatment	NaOH 98%	5 kg/ton	pH \geq 10	2~3 days
	CaO 85%	11 kg/ton	pH \geq 10	2~3 days
Neutralization Treatment ^{b)}	Citric acid 94%	5 kg/ton	pH 6~8	–

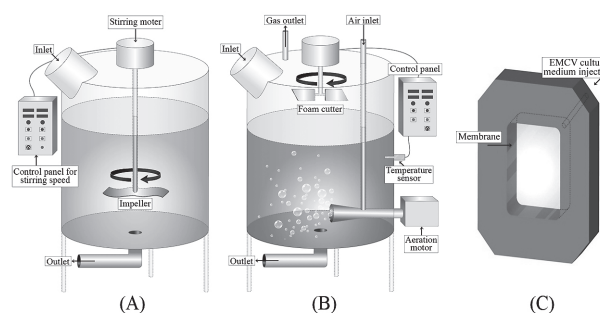
a) The disinfectant application amount should be adjusted according to the characteristics of manure.

b) The neutralized liquid or solid manure may be processed for liquid fertilizers, composts or others consignments. However, the process criteria according to 'Act on the management and use of livestock excreta' should be adhered to during the process.

Table 4. Dialysis Membrane Specifications used in the experiment

Composition	Regenerated cellulose synthesized by the Viscose method
Thickness	0.9 to 1.2 mil (22.5 to 30 μm)
Sample volume	5–3mL
Membrane	10,000 MWCO ^{a)}

a) Molecular weight cut-off

**Fig. 1.** Equipment and materials used in this study. (A) Alkaline treatment; (B) TAO treatment; (C) Dialysis cassette.

and gas exchange internally and externally in addition to their robust preservation of viruses (Khan *et al.*, 2013).

The FMD virus is a high-risk pathogen and it is difficult to handle. Thus, instead of the FMD virus, actual tests were conducted with EMCV, which is similar in nature. The Vero cell (Vero ATCC[®] CCL-81[™] *Cercopithecus aethiops* kidney) was used for the cell line of viral cell culture. A total of 3 mL of cell culture medium was placed in the Dialysis Cassette as show in Fig. 1C. The initial titer of EMCV was 7.75 log₁₀ TCID₅₀/mL and the Dialysis Cassette was placed on the Dialysis Cassette rack. The initial titer of EMCV was measured using the Reed–Muench method (Reed and Muench, 1938).

The inactivation assessment test of EMCV was carried out by four treatments such as a. control, b. fixed room temperature, c. alkaline treatment, d. TAO treatment. Like other treatments, in the control treatment, the same standard storage tank was used, but no treatment was added after adding 100 L of pig manure slurry. In the case of fixed room temperature, the Dialysis Cassette was injected with virus culture medium, and placed in the same room under the same environmental conditions.

For each treatment, 18 Dialysis Cassettes were injected with virus culture medium and placed on the Dialysis Cassette rack. The control, alkaline and TAO treatment, and the Dialysis Cassette rack were located approximately 20 cm away from the bottom of each reactor and retaining tank. During each sampling, nine samples were taken for each treatment. However, two Dialysis Cassettes per treatment were used for EMCV analysis.

Sampling and analysis of EMCV samples

The Dialysis Cassette injected with virus culture medium was collected at 1, 3, 6, 12, 24, 48 and 84 hours following each treatment. After sampling, 2 mL of virus culture medium contents from the Dialysis Cassette was placed into the conical tube, rapidly cooled using dry ice and ethanol, and stored at -70°C for 2 weeks and the inactivation effects of EMCV were analyzed and assessed. The collected samples were used for the assessment of the activation of EMCV, and the Vero cell was used as the test cell. Each undiluted solution samples were treated with gentamycin. The samples treated with gentamycin were diluted with the medium by tenfold, and 25- μL diluted samples were immunized into the cell and dispensed into the medium after 40 minutes. A lot of 175 μL of each was cultured for 3 days for observation of the cytopathic effect (CPE). The cytopathic effect occurs as a result of the virus being infected with tissue culture cells and the quantification of virus using such possible modification effects.

Physicochemical properties of treated and untreated manure

Each treatment operation was repeated five times. The liquid manure sample of the control, alkaline treatment and TAO system treatment were collected at 1, 3, 6, 12, 24, 48 and 84 hours. The liquid manure samples of each treatment were analyzed for pH, EC, COD_{Cr}, TP, TKN and NH₄-N. pH and EC were measured with a handheld meter (YSI-556MPS, USA) immediately after collection. COD_{Cr} was determined following the standard methods (APHA, 2005). TP was determined by the ascorbic acid method (APHA, 2005) using a spectrophotometer (Optizen, Mecasys, Korea) at a wavelength of 880 nm. TKN and NH₄-N were analyzed by the kjeldahl method in accordance with standard sewage analysis methods (JSWA, 1984).

RESULTS AND DISCUSSION

Some of the initial physicochemical properties of manure have been listed in Table 5. Following treatments, these changes in properties are described in the following sections.

Changes in internal temperature of the reactor during TAO treatment

During the TAO treatment process, the outside and inside temperature of the reactor was monitored and presented in Fig. 2. During the TAO system treatment,

Table 5. The physicochemical characteristics of swine manure used in the experiment

Parameter	Average	Parameter	Average
pH	7.55 \pm 0.2	COD _{Cr} (mg/L)	54,750 \pm 5,000
EC (mS/cm)	23.5 \pm 1.33	TKN (mg/L)	4,060 \pm 328
TS (mg/L)	29,933 \pm 1,107	NH ₄ -N (mg/L)	2,660 \pm 165
TP (mg/L)	983 \pm 20		

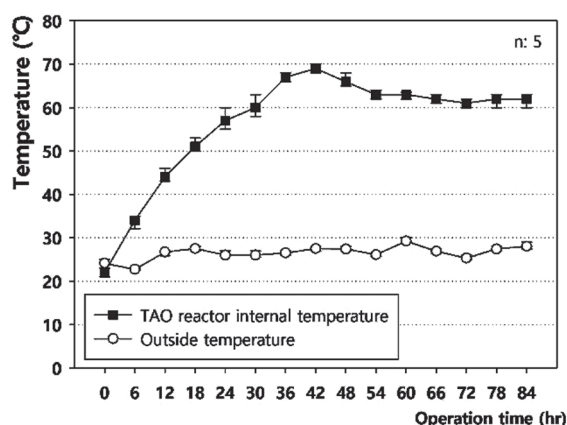


Fig. 2. Change in temperature during TAO treatment.

the outside temperature remained constant at 20°C, but TAO system temperature started to increase from 22°C to 50°C within 18 hours of treatment and maintained an average temperature of 62°C during the remainder of the study period (84 hours).

At 60°C, a significant reduction in COD and ammonia and a complete removal of fecal coliforms, such as *Campylobacter spp.*, are likely to happen (Juteau *et al.*, 2004). Similarly, fecal streptococci and Bovine parvovirus concentration are likely to decrease in a full-scale AT process operated at 55°C, but they are not eliminated completely (Martens *et al.*, 1998). Other researchers (Monteith *et al.*, 1986) have also found that bovine enterovirus can be inactivated to below detectable levels in digested liquid manure heated to 70°C, though some bovine parvovirus was detected after this treatment. Bøtner (1991) looked at the temperature-dependent inactivation of Aujeszky's Disease Virus (ADV) during aerobic storage at 5 and 55°C. He noticed that the virus was only inactivated after 15 weeks at 5°C, whereas no virus was detected after 10 min at 55°C.

Herniman *et al.* (1973) looked at Swine vesicular disease virus (SVDV) and studied thermal activation in both milk and pig slurry. They found that virus in milk can be inactivated by heating to 60°C within 2 min, whereas virus in slurry required a slightly higher temperature (64°C) for inactivation for the same treatment time. Böhm *et al.* (1984) studied the inactivation from 10^7 to 10^8 IU to below detectable levels of several animal viruses in a rotating aeration system. They found that the FMD virus was undetectable after 48 hours of aeration at a pH of 8 at 50°C; ADV needed 5 hours of aeration at 40°C for inactivation, and SVDV required 48 hours of aeration at the same temperature. In this study, the TAO system was able to maintain sustainable temperature >60°C required for destroying most of the virus. Thus, this system may be used for treating infected manure as an alternative to the alkaline treatment system.

Changes in physicochemical properties of manure during SOP alkaline treatment and TAO treatment

A pH of 10.8 was reached in 60 minutes with a pH of

10.5 maintained until the end of the 84-hour experiment (Fig. 3A). A pH value >10 above 10°C is sufficient for inactivation of bacteria (Allievi *et al.*, 1994; Bachrach *et al.*, 1957). In this study, the pH of alkaline treatment rose above 10 after 60 minutes of treatment initiation and maintained pH >10 throughout the study. Alkaline treatment is well-known for its ability to destroy pathogens, and this might be the case in this study (Sobsey *et al.*, 2006; Hrazdira *et al.*, 2002; Callis *et al.*, 1986). However, for the control, the pH remained on an average 7.7 until the completion of the test. Thus, it is unlikely that there will be any changes of microorganisms in the control based on pH. In the case of TAO treatment, pH gradually increased from pH 7.7 to 9.5 over time, and this trend was similar to other studies involving pig manure (Zhang and Zhu, 2005). The drastic increase in pH by aeration may be due to the conversion of ammonium into ammonia (Luo *et al.*, 2001).

Changes in EC of different treatments is shown in Fig. 3B. TAO treatment reduced EC from 22.04 mS/cm to 10 mS/cm within 84 hours of treatment. The EC concentration of alkaline-treated manure increased slightly. EC remained at 22 mS/cm for the control treatment. Generally when pig manure is aerated, it is known to reduce the concentration of EC (Choi *et al.*, 2010). The TAO system reduced EC significantly. Thus, if TAO-treated manure is applied to crop land it will reduce salt accumulation in soil. Manure treated with other methods may lead to accumulation of salt in cropland, so the EC of soil must be measured before applying manure to cropland (Kim *et al.*, 2012).

In the case of TAO treatment, the COD_{cr} decreased from 53,750 mg/L to 26,000 mg/L after 84 hours of treatment, thus showing effectiveness (52% reduction) of the TAO system over the alkaline treatment and control, where no COD reduction was noticed (Fig. 3C). Generally, land application of manure across the farmland may increase COD flow into the water, thus potentially increasing COD load downstream (Hong and Lee, 2011). So, in order to ensure safe dissemination of livestock manure on to the farmland and to reduce secondary environmental pollution, COD reduction should be a priority of those responsible for applying manure. The TAO treatment system may be adapted to reduce COD before applying manure to cropland, thus minimizing environmental concerns.

In the case of alkaline treatment, the average concentration of TP was 927 mg/L, and in the control treatment the average TP concentration was 962 mg/L. Thus, there were no noticeable changes in TP for both treatments. In the case of TAO treatment, TP concentrations decreased by 39% (from 983 mg/L to 597 mg/L) (Fig. 3D). The reduction of TP by the TAO system is likely due to precipitation of biological and chemical phosphorus. The reason for this is that even at low aeration of swine liquid manure, there is the formation of insoluble hydroxyl apatite and struvite (Suzuki *et al.*, 2002; Ndegwa *et al.*, 2003). This crystallization seems to be due to the increase in pH induced by aeration (Juteau, 2006). The phenomenon is temperature-dependent in

both psychrophilic (Ndegwa *et al.*, 2003) and thermophilic (Juteau *et al.*, 2004) ranges. Thus, the TAO system is also able to reduce the TP concentration from liquid swine manure; therefore, this system will reduce TP from runoff and minimize environmental concern from disposal of manure.

The TKN concentration in the control treatment changed slightly towards the end (from 4,060 mg/L to 3,830 mg/L during 84-hour treatment time) (Fig. 3E). In case of alkaline treatment, TKN concentration decreased by 23% (from 4,060 mg/L to 3,150 mg/L), whereas the TAO treatment reduces TKN concentration by 52% (from 4,060 mg/L to 1,960 mg/L) within the same treatment time. Similar to TKN, $\text{NH}_4\text{-N}$ concentration in the control treatment remained pretty much constant during the 84-hour treatment time (Fig. 3F). Like TKN, alkaline treatment was also not very effective in reducing $\text{NH}_4\text{-N}$ concentration.

Alkaline treatment reduced $\text{NH}_4\text{-N}$ concentration by

only 11% from 2,660 mg/L to 2,380 mg/L. In contrast, TAO treatment reduced $\text{NH}_4\text{-N}$ concentration by 67% from 2,660 mg/L to 875 mg/L. The reduction of $\text{NH}_4\text{-N}$ is likely due to degassing of $\text{NH}_4\text{-N}$ during the aeration, as the proportion of free ammonia becomes higher with higher pH in the solution (An *et al.*, 2011). According to Liao *et al.* (1995), degassing of 80% ammonia occur within 20 hours of treatment at 20°C, but it took 30 hours at 13°C at the same pH. Thus, the TAO system outperformed alkaline treatment in reducing $\text{NH}_4\text{-N}$ in manure, providing additional benefits along with microorganism reduction.

Deactivation effect of EMCV in alkaline treatment and TAO treatment

In order to examine the changes of EMCV concentration, the potency of the virus was examined. The initial titer level of the virus at the control room temperature was 10^8 TCID₅₀/mL, but after 84 hours its concentration

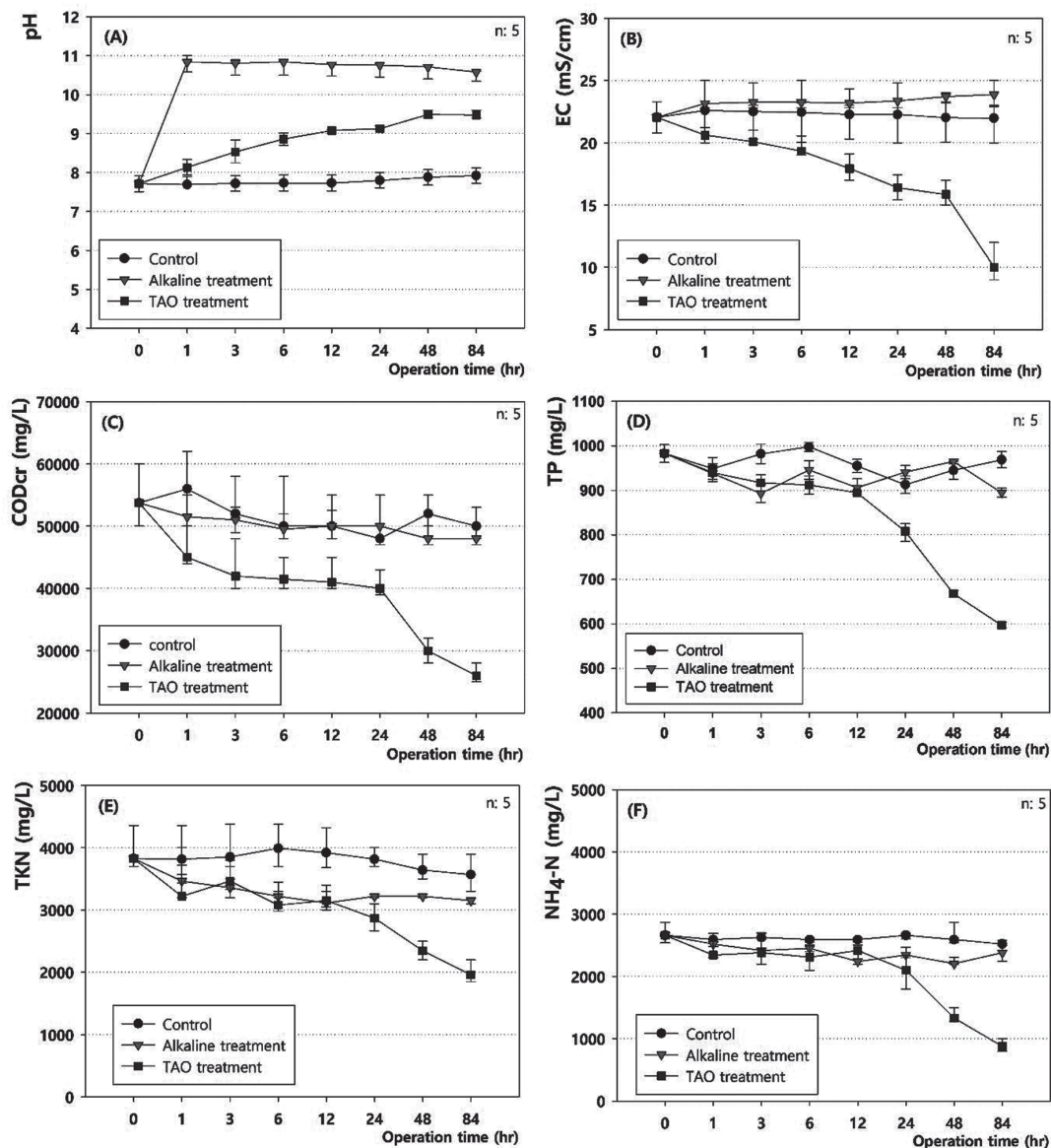


Fig. 3. The changes in physicochemical properties with respect to treatment plot of liquid samples. (A) pH; (B) EC; (C) COD_{cr}; (D) TP; (E) TKN; (F) $\text{NH}_4\text{-N}$.

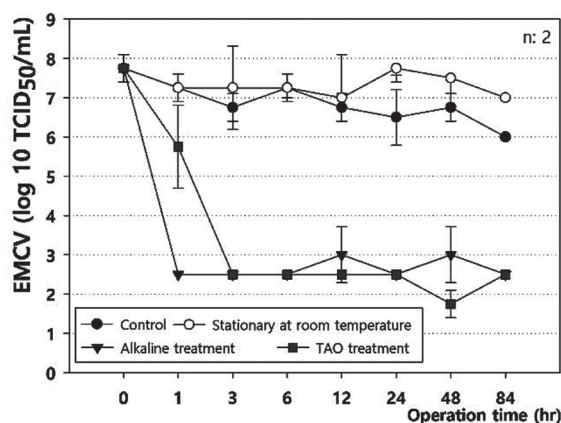


Fig. 4. EMCV changes with respect to treatment plot of liquid samples.

dropped to 10^7 TCID₅₀/mL (Fig. 4). Similarly, after completing the control treatment, the titer of the virus in the control was 10^6 TCID₅₀/mL, which confirmed that the titer of the virus was maintained at a higher level. In the case of alkaline treatment, the titer of EMCV dropped to $10^{2.5}$ TCID₅₀/mL within one hour of treatment, thus reducing EMCV's concentration by 99.97%. However, it took three hours for the TAO system to reduce the titer of EMCV at the same reduction level (99.97%); i.e., $10^{2.5}$ TCID₅₀/mL. The EMCV used in the study has stronger survivability than FMDV in the natural ecosystems, and the test conditions represent 1,000 times higher concentration (10^7) than the expression levels of the pathogenic viruses (10^4), thus confirming that EMCV was actually deactivated.

CONCLUSIONS

Livestock manure harbors pathogens and pollutants like FMD, and it is also a source of mal-odorous compounds due to the microbial degradation of organic matter. Due to the FMD virus outbreak in Korea, a chemical treatment method with a strict SOP has been implemented to reduce pathogenic microorganisms. At the same time, the Korean Government is exploring other alternative robust treatment options to manage and mitigate any future disease outbreak and, consequently environmental problem and health-related concern.

Although chemical treatment methods are effective for controlling the pathogenic microorganisms, this treatment method has issues with the post-treatment management of treated liquid manure. Thus, the TAO system is an alternative treatment option, which is very effective in reducing pathogenic microorganisms and other pollutants. Compared to alkaline treatment, TAO treatment reduces COD significantly from pig manure by 52%, but there is no COD reduction following alkaline treatment.

Similarly, the TAO system reduced TKN concentration by 52%, but alkaline treatment reduced TKN by 23%. This means that treating manure with the TAO system would reduce contaminant runoff compared with the alkaline treatment. Similarly, based on the deactivation

of EMCV, TAO treatment is equally good or better than the alkaline chemical treatment in treating infected manure to reduce microorganisms to an acceptable level. Additionally, the TAO system also reduce odor and produce high-quality liquid fertilizer. Therefore, the TAO system may be considered as an alternative to alkaline treatment to treat infected manure or manure-borne disease.

ACKNOWLEDGEMENTS

This research was funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA), Republic of Korea (project No. 314010-4) and partly supported by the 2014 sabbatical project from Sangji University, Republic of Korea. Particularly, thank to Prof. Yabe, Kyushu University in Japan for his collaboration in experimental design, interpretation of results and help to write research paper in this research.

REFERENCES

- Adak, G. K., S. M. Long, and S. J. O'Brien 2002 Trends in indigenous foodborne disease and deaths. England and Wales: (1992 to 2000). *Gut*, **51**: 832–841
- Allievi, L., A. Colombi, E. Calcaterra, and A. Ferrari 1994 Inactivation of fecal bacteria in sewage sludge by alkaline treatment. *Bioresource Technology*, **49**: 25–30
- An, J. K., J. H. Lim, Y. J. Back, T. Y. Chung, and H. K. Chung 2011 Effect of the physical parameters and alkalinity in the ammonia stripping. *Journal of Korean Society of Environmental Engineers*, **33**: 583–590
- APHA 2005 *Standard Methods for the examination of water and wastewater*. American Public Health Association/American Water Works Association/Water Environment Federation. Washington
- Bachrach, H. L., S. S. Breese, J. J. Callis, W. R. Hess, and R. E. Patty 1957 Inactivation of foot-and-mouth disease virus by pH and temperature changes and by formaldehyde. *Proceedings of the Society for Experimental Biology and Medicine*, **95**: 147–152
- Böhm, R., R. H. Kuhlmann, and D. Strauch 1984 The effect of microwave treatment on viruses in liquid manure. *Agricultural Wastes*, **9**: 147–154
- Bøtner, A. 1991 Survival of Aujeszky's disease virus in slurry at various temperatures. *Veterinary Microbiology*, **29**: 225–235
- Burkholder, J., B. Libra, P. Weyer, S. Heathcote, D. Kolpin, P. S. Thorne, and M. Wichman 2007 Impacts of waste from concentrated animal feeding operations on water quality. *Environmental Health Perspectives*, **115**: 308–312
- Callis, J., and D. Gregg 1986 Foot-and-mouth disease in cattle. In *Current Veterinary Therap* (Vol. 3, ed. by J. L. Howard, W. B. Saunders Company, Philadelphia, PA. pp. 437–439
- Choi, D. Y., J. H. Kwag, K. H. Park, J. I. Song, J. H. Kim, H. S. Kang, C. B. Han, S. W. Choi, and C. S. Lee 2010 Study on the development of measuring system for fermentation degree of liquid swine manure using visible ray. *Journal of Animal Environmental Science*, **16**: 227–236
- Heinonen-Tanski, H., M. Mohaibes, P. Karinen, and J. Koivunen 2006 Methods to reduce pathogen microorganisms in manure. *Livestock Science*, **102**: 248–255
- Herniman, K. A. J., P. M. Medhurst, J. N. Wilson, and R. F. Sellers 1973 The action of heat, chemicals and disinfectants on swine vesicular disease virus. *Veterinary Record*, **90**: 620–624
- Hong, S. G., and N. H. Lee 2001 Assessment of pollutant loading potential during land application of animal waste. *Korean society of agricultural engineers*, **43**: 66–74
- Hrazdira, J., H. Kusa, and J. Kadlcik 2002 Hygienization of sew-

- age sludges by liming. In "(Ramiran 2002 Proceedings of 10th International Conference Hygiene Safety)", ed. by J. Venglovsky, G. Greserova. University of Veterinary Medicine, Kosice, Slovak Republic. pp. 67–69
- Jones, P. W. 1986 Sewage sludge as a vector of Salmonellosis. In "Epidemiological Studies of Risks Associated with the Agricultural use of Sewage Sludge" (ed. by J. C. Block, A. H. Haieelaar, and P. L'Hermite, Elsevier, London. pp. 21–23.
- Juteau, P. 2006 Review of the use of aerobic thermophilic bioprocesses for the treatment of swine waste. *Livestock Science*, **102**: 187–196
- Juteau, P., D. Tremblay, C. B. Ould-Moulaye, J. G. Bisaillon, and R. Beaudet 2004 Swine waste treatment by self-heating aerobic thermophilic bioreactors. *Water Research*, **38**: 539–546
- Khan, M. A., K. H. Jeong, H. K. Ahn, Y. J. Lee, E. J. Kim, T. D. Glanville, and D. Y. Choi 2013 Development of an avian influenza virus inactivation evaluation method using dialysis cassette in animal manure and mortality disposal systems. *Biosystems Engineering*, **114**: 60–64
- Kim, C. L., S. R. Kim, H. J. Kim, S. J. Jeon, H. Han, D. K. Kim, and M. G. Lee 2012 Physicochemical changes of swine manure by the treatment of acid and alkali for inactivation of pathogenic microorganisms. *Journal of Animal Environmental Science*, **18**: 229–234
- Kim, D. R., and H. M. Kim 2011 Legal study on livestock burial sites resulting from the epidemic. *Public Land Law Review*, **55**: 269–288
- Lee, M. G. and W. I. Lee 2000a Operation condition and characteristics for treatment of piggery slurry using Thermophilic Aerobic Oxidation (TAO) system. *Journal of Animal Environmental Science*, **6**: 161–168
- Lee, M. G., J. S. Her, M. H. Tae, J. Y. Joung, and O. J. Kwon 1999 Basic studies on deodorization management of the efflux from swine slurry treated by the thermophilic aerobic oxidation (TAO) reactor. *Journal of Animal Environmental Science*, **5**: 123–132
- Lee, W. I. and M. G. Lee 2000b Continuous treatment of piggery slurry using the Thermophilic Aerobic Oxidation (TAO) system. *Journal of Animal Environmental Science*, **6**: 169–174
- Lee, W. I. and M. G. Lee 2000c Reducing technique for nitrogen and phosphorus in piggery slurry by the Thermophilic Aerobic Oxidation (TAO) system. *Journal of Animal Environmental Science*, **6**: 185–190
- Lee, W. I., H. Tsujii, T. Maki, and M. G. Lee 2004 Inactivation of pathogenic bacteria by addition of thermophilic bacteria in the thermophilic aerobic oxidation (TAO) system. *Journal of Animal Environmental Science*, **10**: 111–118
- Liao, P. H., A. Chen, and K. V. Lo 1995 Removal of nitrogen from swine manure wastewaters by ammonia stripping. *Bioresource Technology*, **54**: 17–20
- Linton, A. H. and M. H. Hinton 1984 The ecology of antibiotic resistant bacteria in animals and the environment. In "Antimicrobials and Agriculture" (ed. by M. Woodbine, Butterworths, London. pp. 533–549
- Luo, A., J. Zhu, and P. M. Ndegwa 2001 Phosphorus transformations in swine manure during continuous and intermittent aeration processes. *Transactions of the ASAE*, **44**: 967–972.
- MAFRA. 2012. *Standing operating procedure (SOP) for Foot-and-Mouth Disease*. Ministry of Agriculture, Food and Rural Affairs, Korea
- Martens, W., A. Fink, W. Philip, W. Weber, D. Winter, and R. Böhm 1998 Inactivation of viral and bacterial pathogens in large scale slurry treatment plants. In "(Proceeding of RAMIRAN 98)" ed. by J. Martinez *et al.* (Eds.), 8th International Conference on Management Strategies for Organic Waste Use in Agriculture. pp. 529–539
- Monteith, H. D., E. E. Shannon, and J. B. Derbyshire 1986 The inactivation of a bovine enterovirus and a bovine parvovirus in cattle manure by anaerobic digestion, heat treatment, gamma irradiation, ensilage and composting. *Journal of hygiene*, **97**: 175–184
- Ndegwa, P. M., J. Zhu, and A. Luo 2003 Influence of temperature and time on phosphorus removal in swine manure during batch aeration. *Journal of Environmental Science and Health*, **38**: 73–87
- Nicholson, F. A., S. J. Groves, and B. J. Chambers 2005 Pathogen survival during livestock manure storage and following land application. *Bioresource Technology*, **96**: 135–143
- Pharo, H. J. 2002 Foot-and-mouth disease: an assessment of the risks facing New Zealand. *New Zealand Veterinary Journal*, **50**: 46–55
- Plaut, A. G. 2000 Clinical pathology of foodborne diseases: notes on the patient with foodborne gastrointestinal illness. *Journal of Food Protection*, **63**: 822–826.
- Reed, L. J. and H. Muench 1938 A simple method of estimating fifty percent endpoints. *The American Journal of Hygiene*, **27**: 493–497
- Sellers, R. F. 1971 Quantitative aspects of the spread of foot and mouth disease. *Vet. Bull.*, **41**: 431–439
- Shin, H. S., S.H. Roh, J. S. Choi, H. M. Phoung, H. J. Yang, and J. I. Lee 2012 Impact analysis on consumer's consumption patterns for FMD in domestic livestock products: Case on Chuncheon city. *Annals of Animal Resource Sciences*, **23**: 39–49
- Sobsey, M. D., L. A. Khatib, V. R. Hill, E. Alolija, and S. Pillai 2006 *Pathogens in animal wastes and the impacts of waste management practices on their survival, transport and fate*. Animal Agriculture and the Environment National Center for Manure and Waste Management, White Papers. pp. 609–666
- Stanley, K. N., J. S. Wallace, and K. Jones 1998 Note: Thermophilic campylobacters in dairy slurries on Lancashire farms: seasonal effects of storage and land application. *Journal of Applied Microbiology*, **85**: 405–409
- Suzuki, K., Y. Tanaka, T. Osada, and M. Waki 2002 Removal of phosphate, magnesium and calcium from swine wastewater through crystallization enhanced by aeration. *Water Research*, **36**: 2991–2998
- Taylor, R. J. and M. R. Burrows 1971 The survival of *Escherichia coli* and *Salmonella dublin* in slurry on pasture and the infectivity of *Salmonella dublin* for grazing calves. *British Veterinary Journal*, **127**: 536–543
- Tchobanoglous, G., F. L. Burton, and H. D. Stensel 2003 *Treatment, Reuse and Disposal of solids and biosolids*. In "Wastewater engineering, Treatment and reuse, 4th Ed., Metcalf and Eddy Inc., McGraw-Hill, New York. pp. 1541–1545
- Unc, A. and M. J. Goss 2004 Transport of bacteria from manure and protection of water resources. *Applied Soil Ecology*, **25**: 1–18
- Zhang, Z. and J. Zhu 2005 Effectiveness of short-term aeration in treating swine finishing manure to reduce odour generation potential. *Agriculture. Ecosystems & Environment*, **105**: 115–125