

PATHOGEN INACTIVATION BY HYDRATED LIME ADDITION IN SWINE WASTEWATER FOR REUSE PURPOSES

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ABSTRACT: The present study aimed to evaluate pathogens inactivation (*Escherichia coli*, *Salmonella enterica* serovar *typhimurium* and *Porcine circovirus* type 2), in the liquid fraction generated after Ca(OH)₂ addition in swine wastewater, exposed for 3 and 24 hours at different pH conditions: 9.0, 9.5 and 10.0. The results showed the total inactivation of *E. coli*, *Salmonella* and PCV2 at pH 10.0 after 24 h exposition. The liquid fraction could be safely reused for the irrigation of crops, or cleaning the swine production facilities.

Keywords: *Salmonella*, *E. coli*, *Porcine circovirus*.

INTRODUCTION

The presence of pathogenic micro-organisms in the wastewater is a sanitation concern. Multiple studies have identified *Salmonella*, *Escherichia coli* (*E. coli*), *Porcine circovirus* type 2 (PCV2) and many other micro-organisms in swine wastewater even after treatment (Fongaro et al., 2014; Viancelli et al., 2013; Vanotti et al., 2005).

The necessity of inactivating pathogens present in manure prior to land application has justified the use of advanced technologies (Macauley et al., 2006; Wong and Sevan 2009). However, some alternatives, such as ozone, are expensive, and others, such as UV light, are not effective against manure due to the organic material and suspended solids present in the effluent that can inhibit the ability of the UV light to penetrate the liquid (Billota and Daniel, 2012).

The use of hydrated lime (calcium hydroxide, Ca(OH)₂) is an attractive wastewater treatment option due to the ability of lime to kill pathogens (Wong and Selvam 2009). However, it is important to determine the pH value needed to remove pathogens from the wastewater as well.

The present study aimed to evaluate the pathogen inactivation (*E. coli*, *Salmonella enterica* serovar *typhimurium* and PCV2) in the liquid fraction generated after the addition of Ca(OH)₂ to swine wastewater.

MATERIAL AND METHODS

Pathogen inactivation experiments: Wastewater samples were collected from a secondary settling tank in swine manure treatment system located at Embrapa Swine and Poultry (Kunz et al., 2009). Inactivation experiments were conducted using 1 L of wastewater (with six replicates). Ca(OH)₂ (10 %, w v⁻¹) was added to the samples under stirring (300 rpm) until the desired pH was reached. The wastewater was inoculated with 10³ NMP of *Salmonella enterica* serovar Typhimurium (ATCC 15631). *E. coli* and PCV2 were not inoculated because they were already present in the wastewater. Three inactivation strategies were performed at pH values of 9.0, 9.5 and 10.0. Liquid fraction samples were collected at t₀, t_{3h} and t_{24h}.

Micro-organisms analysis: *E. coli* quantification was performed using Chromocult® Coliform agar following the manufacturer's instructions. *Salmonella* spp. was quantified as described by Andrews et al. (2011). PCV2 was quantified by qPCR following the protocols described by Viancelli et al. (2012).

RESULTS AND DISCUSSION

An overview of pathogen inactivation is presented in Table 1. Exposure to a pH of 9.0 for three hours resulted in a $3 \log_{10}$ inactivation of PCV2, but no reduction in the levels of *E. coli* and *Salmonella* was observed. *E. coli* inactivation was first observed after 24 h. The treatment at pH 10.0 was the most effective at inactivating the studied pathogens. All of the bacteria and viruses were killed after 24 h of exposure.

Elevating the pH up to 8.5 results in the formation of carbonate that subsequently forms insoluble metal salt complexes with divalent cations (Mg^{2+} , Ca^{2+} , Zn^{2+} , Fe^{2+}). This renders the cations unavailable to bacterial enzymatic activities and consequently kills the bacteria. The divalent cations also help to stabilize the lipopolysaccharide (LPS) in the outer membrane of Gram-negative bacteria such as *E. coli* and *Salmonella*. Therefore, at an elevated pH, the LPS loses structural integrity, and the periplasmic proteins can be damaged (Jarvis, 2001).

The proposed inactivation mechanism for some viruses is the cleavage of nucleic acids by ammonia (Ward, 1978). Koch and Euler (1984) hypothesized that high pH itself was not the cause of viral inactivation; they instead posited that the ammonia released due to the increased pH was responsible for the viral inactivation.

The inactivation of pathogens is an important concern when the goal of the treatment is water reuse. The advantages of reusing water is evident when considering that the amount of water used in agriculture is much higher than the amount used for any other purpose (i.e., for domestic and industrial uses combined) (WHO, 2006). The WHO legislation recommends the reuse of water in drip irrigation when the water contains less than 10^4 / 100 mL of *E. coli* (as in the present study) (WHO, 2006). However, other regulations recommend that the *E. coli* amount be below 10^2 / 100 mL in the reused water (EPA, 2012).

In general, the pathogen reduction obtained after 24 h of exposure to a pH of 9.5 is sufficient to allow the liquid fraction to be reused in activities such as root and leaf crop irrigation and cleaning the swine production facilities (WHO, 2006).

CONCLUSION

The results presented in this study show the efficiency of pH elevation with $Ca(OH)_2$ in the inactivation of *E. coli*, *Salmonella* and PCV2. If wastewater is treated at a pH of 10.0 for 24 h, the pathogenic micro-organisms can be inactivated, and this water could be safely used for the irrigation of crops, cleaning the swine production facilities.

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Table 1. Overview from the pathogens reduction (\log_{10}) in the reuse water after exposition to different pH and time.

| pH | 9.0 | | 9.0 | | 10.0 | |
|---|-----------------|------------------|-----------------|------------------|-----------------|------------------|
| | T _{3H} | T _{24H} | T _{3H} | T _{24H} | T _{3H} | T _{24H} |
| <i>E. coli</i> (T _{0h} = 10 ³ CFU) | 0 | 1 | 1 | ND | 1 | ND |
| <i>Salmonella</i> (T _{0h} = 10 ⁴ MPN) | 0 | 0 | 0 | 1 | 1 | ND |
| PCV2 (T _{0h} = 10 ⁷ gc) | 3 | 4 | 3 | 5 | 4 | ND |

ND = not detected.