Inactivation of microbial indicators of fecal contamination in struvite produced from source-separated human urine

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Highlights:

- Inactivation increased as temperature increased for *E. faecalis* and *E. coli*.
- It was possible to determine the time for inactivation of pathogens in struvite.
- *E. coli* was an excellent indicator of inactivation since it was the most resistant.

Keywords: Ecological sanitation; Phosphorus recovery; Pathogens.

INTRODUCTION

Natural resources are essential for human survival and development. However, these resources are being depleted at an alarming rate, due to population growth and unsustainable consumption patterns (HUO; PENG, 2023). Phosphorus is an essential and irreplaceable mineral for food production, mainly from phosphate rocks, a non-renewable resource (ZAHED et al., 2022). Issues involving phosphorus depletion have become a major worldwide concern in recent years. As a result, different ways to recover phosphorus from wastewater, sewage sludge, and human urine have been investigated. Phosphorus can be recovered from urine through precipitation with magnesium and ammonium, such as struvite, a slow-release fertilizer (SUN; MOHAMMED; LIU, 2020). Precipitation of struvite from urine results in high recovery efficiencies (> 90%); it is a simple and low-cost technology (PATEL; MUNGRAY; MUNGRAY, 2020). However, little is known about the behavior of pathogenic microorganisms found in urine during this process. Therefore, in this study, we investigated the behavior of the microorganisms that indicate fecal contamination *Escherichia coli (E. coli)*, *Salmonella enterica (S. enterica)*, and *Enterococcus faecalis (E. faecalis)* during the drying of struvite at room temperature and controlled temperature. The effect of drying temperature and moisture content of the samples on the inactivation of microorganisms was also investigated.

METHODOLOGY

Urine was obtained in an intensive collection campaign conducted in the toilets of the Department of Sanitary and Environmental Engineering of the Federal University of Santa Catarina in Florianópolis (Brazil). The campaign lasted about 60 days, from August to September 2023, and collected over 90 liters of urine. The collected urine was stored in polyethylene reservoirs until the complete hydrolysis of urea, and the pH was increased to a value of approximately 9.0, which is ideal for struvite precipitation.

The inoculum was produced by adding an aliquot of 2 ml of the bacteria to 50 ml of Modified Scholten's Broth (MSB) culture medium for *E. coli*, Tryptone Yeast Extract Glucose Broth (TYGB) for *S. enterica*,

and Trypticase Soy Broth (TSB) for *E. faecalis*, with subsequent incubation at 37 ± 2 ºC (MSB and TYGB) and 41 ± 2ºC (TSB) for 18 hours. The inoculation of the bacteria was conducted at a concentration of 1% of each bacteria in terms of inoculum:urine. The inoculum had average concentrations of *E. coli, S.enterica,* and *E. faecalis* equal to 3.98×10^7, 3.70×10^7, and 4.15×10^7 CFU.ml^{-1}, respectively. Six tests were conducted on a struvite precipitation reactor with 15 liters of bacteria-contaminated urine and $MgCl_2$.

The reactor operated with a rotational speed of 130 rpm, stirring time of 15 minutes, and settling time of 60 minutes. The struvite was collected at the end of the test by emptying 1/3 of the reactor. A part of the recovered struvite was dried at room temperature (21 ± 2 ºC) and another under controlled temperature (in an oven at 35 ºC). Samples were collected on days 0 (after the end of processes), 1, 2, 3, and 4 of drying to evaluate the inactivation.

The concentration of bacteria was determined by serial dilutions of 1 g of sample in 9 ml of sterile peptone saline, followed by plating of 0.1 ml on plates of MacConkey for *E. coli*, XLD (Xylone Lysine Deoxycholate) for *S. enterica*, and SlaBa (Slanetz and Bartley Agar) for *E. faecalis*. The MacConkey and XLD plates were incubated at 37 ± 2 ºC for 24 hours. The SlaBa plates were incubated at 41 ± 2 ºC for 48 hours. The results were expressed in colony-forming units per gram (CFU.g^{-1}). The microbiological data obtained were transformed into $\log_{10}$ and tested with a linear decay model, according to the following equation [$\log_{10}A = -kt + constant$], where A is the cell concentration, in CFU.g^{-1}, k is the inactivation coefficient and t is the time, in days.

RESULTS AND CONCLUSIONS

The average phosphorus recovery was 83.7%, resulting in an average production of 1.5 grams of struvite per liter of urine. The bacteria were found in struvite analyzed immediately after precipitation, in average concentrations of 6.0×10^5 CFU.g^{-1} for *E. coli*, 1.0×10^5 CFU.g^{-1} for *S. enterica*, and 1.1×10^7 CFU.g^{-1} for *E. faecalis*. As the effluent from the struvite precipitation had very similar concentrations of the three bacteria, these results can be attributed to residual urine, confirmed by the moisture content of the samples above 80% after precipitation. The relationship between moisture content and the presence of microorganisms indicating fecal contamination in struvite was also observed by Decrey et al. (2011).

After confirming the presence of bacteria in the struvite, drying at room temperature (21 ± 2 ºC) and controlled temperature (35 ºC) was evaluated for the potential for inactivating the microorganisms (Figure 1). The concentration of *E. coli* and *E. faecalis* decreased over the days, allowing us to estimate the inactivation coefficient (k) and the time needed to inactivate 1 logarithmic unit (T_{90}). The concentrations of *S. enterica* did not change from day 1, remaining at 1.0×10^1 CFU.g^{-1}. Therefore, testing the linear decay model for this bacteria was impossible.

The k and T90 values obtained for room temperature were -0.6781 and 35.3 hours for *E. coli* and -1.347 and 17.8 hours for *E. faecalis*. For controlled temperature, the values of k and T_{90} were -1.079 and 22.3 hours for *E. coli* and -1.440 and 16.5 hours for *E. faecalis*. Based on these results, it can be inferred that the temperature higher than the ambient temperature accelerated the drying of struvite, which mainly

contributed to the inactivation of *E. coli* in a shorter time, which is supported by Xu et al. (2022). A correlation between increasing inactivation coefficient and increasing temperature was also observed for *E. coli* in urine storage (AHMED et al., 2017).

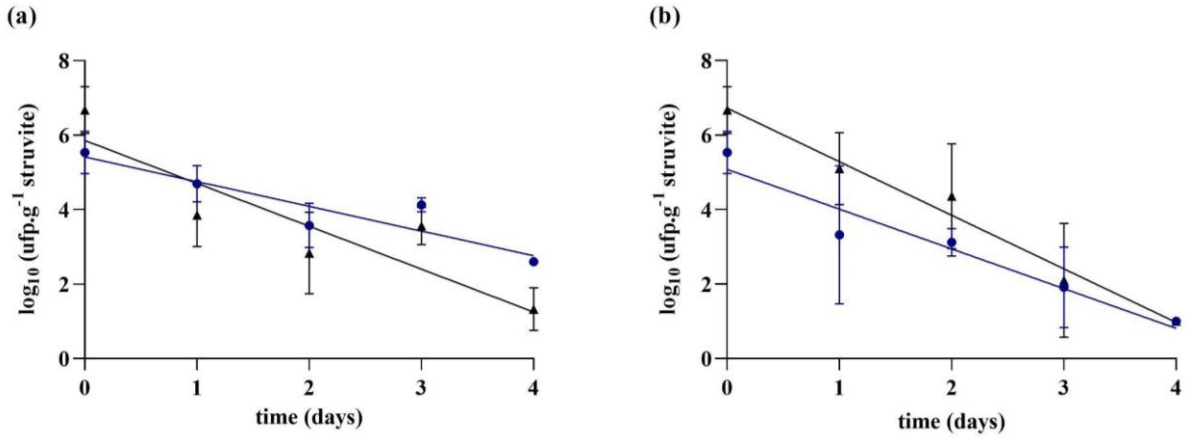

Figure 1. Inactivation of *Escherichia coli* (•) and *Enterococcus faecalis* (▲) during struvite drying at (a) room temperature (21 ± 2ºC) and (b) controlled temperature (35 ºC).

Inactivation of *E. faecalis* also increased with increasing drying temperature; however, the increase was less prominent than in relation to *E. coli*. In both cases, *S. enterica* was the least resistant indicator, as Bichel et al. (2016) observed. In our study, the moisture content of the samples decreased to approximately 40% at the end of the drying process. It is known that reduced water availability can compromise microbial metabolism and reproduction, leading to increased inactivation. We concluded, therefore, that the combination of increased temperature and decreased moisture content influenced the inactivation of the bacteria.

Drying at room temperature and controlled temperature proved effective in inactivating *E. coli*, *S. enterica*, and *E. faecalis*. The results of the study made it possible to estimate the time needed to sanitize the fertilizer produced and, consequently, the time required to reduce the risks of contamination of crops. The combination of increased temperature and decreased moisture content was crucial for the inactivation of the bacteria, corroborating other findings in the literature. Thus, it is recommended that further studies consider these factors to ensure the microbiological safety of struvite. The results also suggest that *E. coli* is a good indicator since it showed greater resistance in struvite. However, additional studies on the inactivation of other microorganisms, such as human enteric viruses and viral indicators, during drying of struvite are needed.

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