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Full Length Research Paper

Lactic acid from food waste enhances pathogen inactivation and urea stabilization in human urine

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In areas where conventional sewer systems are not possible, innovative and low-cost methods are needed to get rid of pathogens in human urine. Treatment through acidification is a reliable method. In this study, the effect of fresh cabbage waste, potato peel, and teff flour water as substrate for lactic acid treatment of source-separated urine was investigated. Laboratory scale batch experiments were conducted to compare the substrates for pH 4 and high distribution of lactic acid bacteria (LAB), important for urine hygienization and urea stabilization. It was found that with the addition of 10% molasses as a sugar supplement, fermentation of fresh cabbage waste could establish the desired effect for lactic acid treatment of source-separated urine. The final pH in the urine mixed with lactic acid in the ratio of 1:1 and 1:2 (lactic acid: urine) was 4.12 and 4.26, respectively. While the final pH in the 1:4 and control reactors was 8.3 and 9.2, respectively. Escherichia coli count was below the detection limit in both 1:1 and 1:2 reactors after 5 days, whereas the number of *E. coli* in the samples collected from 1:4 and control reactors showed only slight reduction until the final day of the treatment process. Urea decomposition improved in 1:1 and 1:2 reactors, while it kept increasing in 1:4 and control reactors. The results revealed that food waste produced lactic acid enhances pathogen inactivation, urea stabilization and reduce odors in human urine.

Key words: Lactic acid, odor control, pathogen inactivation, urea stabilization

INTRODUCTION

In urban areas of developing countries, availability and safety of sanitation system is still a major problem. The sanitation coverage improvement in these countries is challenged by rapid increases in urban populations. According to the WHO/UNICEF (2019) progress report (2000 - 2017), though there is some improvement toward

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Sustainable Development Goal (SDG) 6.1 for universal and equitable access to safe and affordable drinking water for all, progress toward SDG 6.2 pertaining to sanitation access is lagging. The problem arises not only from the lack of sanitation facilities but also from challenges of excreta management after collection. As reported by WHO/UNICEF (2017), of the 2.8 billion people using sewer connections, only 1.9 billion people have safely managed sanitation where human excreta is treated. In most cases, sewer systems are not practical due to high costs and challenging topography (World Bank, 2019). Innovative solutions are needed that provide on-site sanitation options. Here, we discuss one developing sanitation option that couples responsible food waste management to produce lactic acid with treatment of urine for pathogen inactivation, urea stabilization and odor control.

Collection and processing of human urine for agricultural purposes offers multiple benefits. In agrarian countries like Ethiopia, extensive agricultural practices can deplete soils and extract nutrient rich humus. Furthermore, conventional sanitation and fecal sludge management systems are not equipped to recycle precious nutrients that increase the productivity of soils (Strande et al., 2014; Munoz et al., 2018). Urine contains valuable nutrients for plant growth (Anderson, 2015a), and several technologies at the laboratory and industrial scales have been shown to recover nitrogen, phosphorus and potassium from urine for agricultural purposes (Pronk and Kone, 2009; Yang et al., 2015; Shepherd et al., 2016; Simha et al., 2018). These technologies include struvite recovery via precipitation (Wilsenach et al., 2007), microbial fuel cells (Chouler et al., 2016), solar evaporation processes (Antonini et al., 2011), nitrification and distillation (Udert et al., 2003), electrochemical stripping (Tarpeh et al., 2018), and electrolysis for sanitization and removal of nitrogen and organic compounds (Zollig et al., 2015). Although these methods all show promise, they are hindered by several drawbacks. For example, struvite precipitation requires pretreatment to inactivate pathogens that can otherwise survive in the process and be retained in the cake and can contaminate the soil and crops (Decrey et al., 2011). As a result, the sanitization of struvite is incomplete without a long drying phase. Nitrification/distillation is more complex and technically challenging (Udert and Wachter, 2012). Nutrient recovery by electrochemical methods is sometimes limited and might pose a health risk due to the production of chlorinated by-products (Udert et al., 2014; Tarpeh et al., 2018).

Treatment techniques have been developed to create hygienic end products from human excreta for re-use or disposal (Factura et al., 2010; Anderson et al., 2015b; Magri et al., 2015). According to Anderson et al. (2015b), lactic acid fermentation (LAF) successfully inactivates pathogens in feces and urine, and preserves organic

material that is valuable during reuse. With LAF, lactic acid bacteria (LAB) easily metabolize degradable carbohydrates to lactic acid. The genera *Leuconostoc*, *Lactobacillus*, and *Streptococcus* are used for food preservation by fermentation industries (Hofvendahl and Hahn-Hagerdal, 2000) and are found among some foodbased silage materials such as cabbage (Yang et al., 2010). LAF is an effective natural process because it reduces pH and produces antimicrobial compounds, such as lactic acid, that effectively eliminate non-desirable microorganisms and pathogens, creating a more hygienic product (Noike et al., 2002).

Different methods have been proposed to reduce ammonia volatilization and inhibit urea decomposition in the urine. Biological nitrification with use of ammonia and nitrite oxidizing bacteria also stabilizes nitrogen, though maintaining bacterial activity in high strength ammonia solutions as urine is challenging and requires skilled handling (Udert and Wachter, 2012). Temperature affects urine storage and benefits pathogen elimination (Vinnerås et al., 2008; Spinks et al., 2006; Dobrowsky et al., 2015). The limitation is its practical application as it is energy intensive. Maintaining a pH < 4 by adding strong acetic and sulphuric acids (2.9 g/L) inhibited urea decomposition and inactivates pathogen (Hellstrom et al., 1999); however, it encores high cost and may create heath risk during handling of the acids (Maurer et al., 2006). Hence, it is necessary to develop simple and cost effective method that minimizes urea decomposition in order to reduce nitrogen loss.

LAF is a cheap and simple method that can be achieved through acidification at pH < 4 (Anderson et al., 2015b; Andreev et al., 2017). However, not all LAB species are able to produce lactic acid in feces and urine, making them ineffective for pathogen inactivation (Odey et al., 2018). In this study, we set out to identify effective, cheap and locally available waste sources likely to contain LAB that could be fermented to produce lactic acid as a sanitizing agent for human urine prior to being used as a fertilizer. We specifically evaluated different organic sources for their ability to create LAB inocula that produced enough lactic acid to eliminate fecal indicator bacteria. We also evaluated the best performing organic sources for its ability to inactivate pathogen, preserve the nutrient value of the urine and reduce odor.

MATERIALS AND METHODS

Preparing organic waste sources for fermentation

Fresh cabbage waste, potato peel, and teff flour extract served as organic waste sources (heretofore called "substrates"); these substrates were all collected in Addis Ababa, Ethiopia and were selected based on availability. Fresh cabbage waste was collected from the big vegetable market; potato peel was collected from street food makers; and teff (*Eragrostis tef*) flour was collected from

Table 1. Initial urine and lactic acid characteristics.

Parameter	Fresh urine	Extracted Lactic acid
рН	6.83 ± 0.03	3.90 ± 0.01
E. coli (CFU/100 mL)	$2.12 \pm 0.12 \times 10^4$	-
NH ₄ ⁺ (mg/L)	485.52 ± 120.5	-

a millhouse. The fresh cabbage waste and potato peel were both pulverized with a heavy-duty blender in the laboratory at Addis Ababa Institute of Technology, Addis Ababa University (AAU). Teff flour was used directly as collected. Fifty grams of each substrate was mixed with 50 ml of distilled water in its own vessel, and sealed to make it air-tight and incubated at 37°C for seven days based on the method of Omar et al. (2009). The concentration of LAB and *E.coli* was enumerated for each source using the method described below. We used the same protocol with fresh cabbage substrate experiments that were supplemented with an additional 50 ml of 10% molasses to the fermentation reactors (subsequently deemed cabbage + sugar fermentation) to enhance lactic acid formation.

Preparation of lactic acid stock

Lactic acid was recovered for use in urine sanitization using the methods of Mumtaz et al. (2008), Omar et al. (2009) and Phang et al. (2002). Specifically, the cabbage + sugar fermentation vessels were incubated at 37°C for seven days, then frozen at - 30°C overnight followed by thawing in a drying oven at 60°C for 2-3 h. The solution was centrifuged and filtered with 0.8 μ m cellulose acetate filter paper and a vacuum pump (KNF Neuberger, Germany). Finally, the water was evaporated at 50°C under vacuum for eight hours using a rotary evaporator (ROV 400, Czech Republic). After most of the liquid was evaporated, a clear brownish solution containing lactic acid was recovered (27.64 \pm 3.03 ml). The solution pH was measured for each batch and is presented in Table 1.

Urine collection and experimental setup

Fresh urine was collected from a group of 10 male volunteers (aged 28 to 47 years old) in the School of Civil and Environmental Engineering at AAU. The collection was done within one day in a disinfected 10-L plastic jerry can. The collected urine was mixed, the initial pH was measured using a pH meter (Jenway 3505, UK), and subsamples were taken to quantify *Escherichia coli* using Compact Dry ECO plates (HyServe, Germany). Since measuring different pathogens is labor-intensive and costly, an indicator organism was chosen to approximate the amount of pathogen inactivation achieved during treatment. *E. coli* was used in this study as an indicator organism to assess the effectiveness of lactic acid for bacterial inactivation in human urine, which is frequently cross-contaminated with enteric pathogens.

The ammonium content was analyzed using the Spectroquant reagent kit 1.14752.0002 (Merck, 2019). Using a spectrophotometer (Spectro UV-VIS Double Beam PC (UVD-3200), Labomed INC) and 10 mm cells, the absorbance of wavelength 690 nm was measured. For the conversion from absorbance to concentrations a standard curve was prepared with concentrations 0.1, 0.5, 1, and 3 mg NH₄/L prepared from standard solution (1000 mg NH₄/L).

Four 500 ml bottles, autoclaved at 119°C for 15 min, were filled with 125 ml urine. Each of the bottles was designated as reactors 1 - 4. Lactic acid was added in the proportions of 1:1, 1:2, 1:4 (lactic acid to urine, v/v) into the first three reactors. The fourth reactor contained only urine and was used as the control. All experiments were carried out in duplicate, and results (pH, NH₄⁺, and *E. coli*) that were monitored nine times from each reactor over 33 days are reported as average values.

Odor evaluation

The odor strength of the urine during treatment with lactic acid was evaluated by eight people. The potency of the perceived odor was evaluated by using a scale rank that has been used previously (Andreev et al., 2017). The scale rank categories are: 0 (no odor), 1 (very faint odor), 2 (faint odor), 3 (distinct odor), 4 (strong odor), 5 (very strong odor), and 6 (extremely strong odor).

Culture enumeration

Bacterial culturing was conducted in duplicate by applying 0.1 mL of serially diluted (1/10) sample in sterile distilled water. Lactic acid bacteria were cultured at 37°C for 24 h on MRS agar (Standard Method 9215), a *Lactobacillus* selective culture medium. *E. coli* was enumerated at 37°C for 24 h with Compact Dry ECO plates (HyServe, Germany) that use a dry chromogenic medium that is reconstituted when the liquid sample is applied; *E. coli* colonies present as blue. The method has an accuracy of +/- 0.5 log₁₀ and a detection limit of 1 CFU per ml.

Statistical analysis

The experimental data were statistically processed via a Tukey test of multiple comparisons within ANOVA one-way analysis of variance and using Minitab 17 statistical software. The means of LAB and pH between substrates; and pH and ammonium concentration in all treatment reactors were compared for significance differences (at 95% significance, based on p values and confidence interval CI).

RESULTS AND DISCUSSION

All three waste sources produced LAB and inactivated *E. coli*, but to different degrees and at different rates

The waste sources produced different pH responses to fermentation (Figure 1A). The initial pH of each substrate

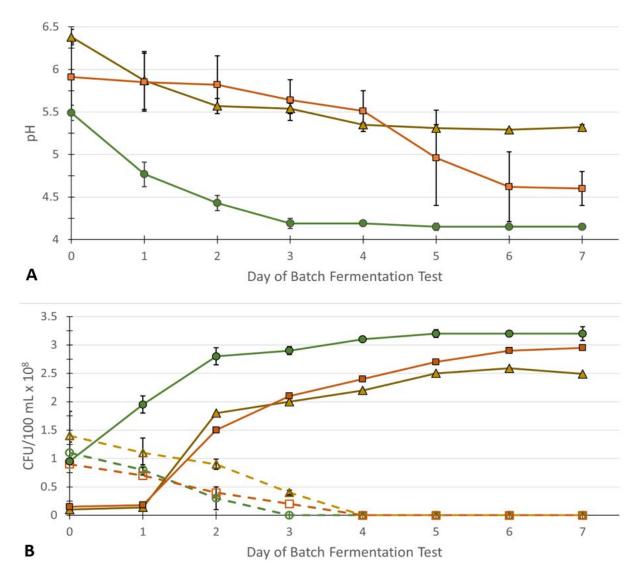


Figure 1. Change in pH, lactic acid bacteria counts, and *E. coli* counts during batch fermentation tests conducted with three waste sources. Cabbage = ● (green); potato peel = ▲ (brown); teff flour = ■ (orange). Figure 1A shows changes in pH during batch tests. Figure 1B shows changes in lactic acid bacteria (solid lines) and *E. coli* (dashed lines). Zero data points on the x-axis reflect samples where *E. coli* was not detected. Symbols reflect the average of duplicate samples that are given by the extremes of the error bars.

ranged between 5.5 (cabbage) and 6.4 (potato peel). Although pH decreased due to fermentation, it did so at different rates and to different degrees for each waste source. The pH of the fresh cabbage and potato peel wastes stabilized at average values of 4.2 and 5.3 by day three and five, respectively, and remained there until day seven. The pH for the teff flour continued to decrease through day six where it reached an average pH of 4.6. These results show that cabbage waste produced the lowest sustained pH during the experiment, and did so more rapidly than the other two wastes.

The pH results corroborate changes in lactic acid bacteria (LAB) and *E. coli* counts shown in Figure 2B.

Throughout the experiment, all three wastes resulted in a multi-fold increase in LAB counts: 3.4x for cabbage (0.95x10⁸ to 3.2x10⁸ CFU/100 mL); 20x for teff flour (0.15x10⁸ to 2.95x10⁸ CFU/100 mL); and 25x for potato peel (0.10x10⁸ to 2.49x10⁸ CFU/100 mL). However, because the cabbage waste started out with more LAB at time zero, its average final LAB concentration (3.2±0.12x10⁸ CFU/100 mL) exceeded all other fermentation batch tests. Furthermore, the fermented cabbage waste eliminated detectable *E. coli* counts by day three, and both teff flour and potato peel did so by day four, suggesting that acidic pH values lead to *E. coli* inactivation under all test conditions.

The fermentation test results indicate that all three waste types can produce conditions favorable for inactivating pathogens and stabilizing urine. All three waste forms create acidic conditions although cabbage did so more quickly. Furthermore, all three wastes rapidly inactivated E. coli, an enteric pathogen indicator, with only moderate differences in the rate of inactivation. While the complete mechanism of inactivation was not determined, acidic pH's are outside of the ideal range for optimum growth (Desmarchelier and Fegan, 2003) and likely contributed to its inactivation. Finally, all three wastes showed the potential to generate lactic acid given the proliferation of LAB and decrease in pH under all conditions. Consequently, fresh cabbage, potato peels or teff flour can be used to generate lactic acid for urine processing and pathogen inactivation. However, differences in the rate and extent of LAB generation are notable. Cabbage is naturally rich in LAB and support rapid fermentation (Yang et al., 2010), which was seen in this study. Despite having an elevated LAB starting point and maintaining the highest LAB count throughout the experiment, cabbage produced LAB less rapidly than the other two waste types over the seven day trial. Despite its limitations, fresh cabbage was selected for the next experimental step to grow LAB for application to process urine given that it produced the highest LAB concentrations, lowest pH and allowed for E. coli inactivation. To enhance lactic acid production, simple carbohydrate supplementation was evaluated. We predict that co-fermentation of cabbage with other starch- or carbohydrate-rich materials will enhance LAB production, and future work should focus on identifying appropriate mixtures.

Molasses supplementation with fresh cabbage had a small positive effect on enhancing LAB growth and the rate of *E. coli* inactivation. First, pH decreased until day 6 when it reached just below 4 (3.87). Molasses supports the formation of more lactic acid bacteria than is achieved with cabbage alone, and would also support further pH suppression.

Consistent with the suppression in pH is a slight but measurable increase in the change in LAB compared with the case when molasses was not added (3.2 x 10^8 CFU/100 ml). The count reached the maximum value (5.0 x 10^8 CFU/100 ml) on the sixth day. The LAB concentrations of fermented fresh cabbage waste with and without molasses addition significantly differed from each other (p < 0.05). From Tukey's multiple comparison tests, the LAB level of fermented fresh cabbage waste with molasses was significantly greater than the fermented fresh cabbage without molasses.

Several studies have shown the effect of molasses addition as a sugar supplement for the production of lactic acid. Bautista-Trujjilo et al. (2009) observed, on the study conducted to evaluate the potential of molasses and Whey addition to ensile maize, a higher and faster

production of lactic acid with significant decrease to pH 3.9. In this study and supported by Weinberg et al. (1988) high lactic acid bacteria distribution was observed when molasses was added which might be due to the rapid degradation of the water soluble carbohydrate in the molasses. Thus, reduction in pH was observed due to consumption of water soluble carbohydrate by lactic acid bacterium forming lactic acid thereby creating favorable conditions to hydrolyze polysaccharides in substrates so that they become available for LAB (Zhang et al., 2000; Bautista-Trujjilo et al., 2009). The disappearance of E. coli during fermentation of the substrates was also observed in this study. This may be due to the influence of the low pH and excretion of inhibitory substances, such as bacteriocins, lactic acid, hydrogen peroxide, glucose oxidase and other compounds which are responsible for inactivation and death of undesirable microorganism that might inhibit fermentation (Von Boberfeld, 2001; Saranraj et al., 2013).

Fermented waste-generated lactic acid stabilized urea and inactivated *E. coli* in urine

Addition of fermented waste-generated lactic acid solution (pH 3.9) stabilized the pH in human urine when present at a high enough volume. Varying initial pH conditions were created in the fresh urine samples stored with lactic acid at different dilutions: pH of 4.0, 4.9 and 6.5 for 1:1, 1:2 and 1:4, respectively, from an initial urine pH value of 6.8. The acid was not able to prevent urea hydrolysis for the 1:4 dilution treatment, where pH increased to 8.0 by the 5th day and was similar to the control pH of 8.3 (Figure 2A). The pH of these two treatments reached a maximum value of 8.3 (1:4 dilution) and 9.2 (control) at the end of the treatment, which is consistent with what happens when urea in the urine is hydrolyzed to ammonia (Chang et al., 2015). In contrast, the 1:1 and 1:2 dilution treatments maintained a stable acidic pH throughout the treatment and ended at pH 4.12 and 4.26, respectively.

Meanwhile, ammonium concentration of the control and 1:4 reactors increased from 485.52 \pm 120.5 mg/L to 2480 \pm 130.0 mg/L and 2340 \pm 160.2 mg/L respectively on the 5th day of treatment before reaching their maximum value of 4000 \pm 228.5 mg/L and 3862 \pm 128.5 mg/L as shown in Figure 2B. On the other hand, ammonium concentration for 1:1 and 1:2 reactors increased to 690 \pm 18.5 mg/L and 752.47 \pm 45.2 mg/L respectively from the initial value of 485.52 \pm 120.5 on 5th day of the treatment before stabilizing. The final urine characteristics at the end of the treatment process are described in Table 2.

The waste-generated lactic acid effectively inactivated *E. coli* in treatments where pH remained around 4. The initial concentration of *E. coli* in the field-collected urine before treatment was $2.21 \pm 0.13 \times 10^4$ CFU/100 ml. In

Table 2. Final urine characteristics	from the treatment process.
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Parameter	Final urine characteristics (Mean ±SD)			
Parameter	1:1 reactor	1:2 reactor	1:4 reactor	Control
рН	4.12±0.04	4.26±0.06	8.3±0.26	9.2±0.08
NH ₄ ⁺ (mg/L)	696.86±16.45	788.8±9.28	3862±15.25	4000±42.02

the control and 1:4 dilution treatment, slight E. coli inactivation was observed in urine that underwent urea hydrolysis to ammonia/um and where the pH was basic. By the end of the experiment, the culturable E. coli concentration declined to 0.079 x 10⁴ CFU/100 mL in the 1:4 dilution treatment and 0.1 x 10⁴ CFU/100 ml in the control, indicating that most of the inactivation was inherent to the control and was not affected by the lactic acid addition for this treatment. The slight inactivation observed is consistent with the work of others that shows a need for acidic pH to achieve significant E. coli inactivation (Zhou et al., 2017). In contrast, in the 1:1 and 1:2 dilution treatments where more lactic acid was added and a stable acidic pH was maintained, complete E. coli inactivation occurred within 9 days. The results are converted to log10 CFU/100 mL and are presented in Figure 2C. These results are in line with previous studies that were conducted with the lactic acid created by fermentation in situ during fecal sludge treatment. Anderson et al. (2015b) observed the sanitizing effect of lactic acid production by fermentation during fecal sludge treatment when E. coli was reduced to below detection at pH 4.2. Similarly, other studies have shown E. coli inactivation at pH values of 3.5 (Zhu et al., 2006) and 4.5 (ICMSF, 1996). Future studies should focus on evaluating the low pH stability of other bacteria or viruses that are known to be present in human urine.

Odor reduced during urine lactic acid treatment

Bacterial metabolism or thermal reactions produced offensive odor in urine (Troccaz et al., 2013). Ammonia is a major malodourous compound that is volatized during storage and urea hydrolysis (Zhang et al., 2013). In addition, organic compounds with low odor threshold values are found in urine after storage (Troccaz et al., 2013). The challenge of odor makes collection of urine in a source separation system and its reuse as a fertilizer difficult to recruit dedicated users. In this study, we focused on strategies for keeping urine at a low pH to-in part- address these concerns over odor.

The perceptions of eight individuals on the odor of treated urine was collected and analyzed. All responders stated that the 1:1 and 1:2 dilution treatments smelled like lactic acid or alcohol, and half the respondents considered the odor to be very faint while the others

considered it to have a distinct odor. In the 1:4 diluted treatments, all respondents found the odor to be very strong. The odor in the control reactor was found to be extremely offensive and was reported by all respondents as the strongest odor. The reduction in smell with more lactic acid may be associated with the destruction of microorganisms responsible for decomposing compounds that produce offensive odors (Yemane et al., 2014). Also, urea hydrolysis, which constitutes most of the objectionable odor in urine through ammonia production, is inhibited by lactic acid treatment. As reported by Zhu (2000) and Wang et al. (2001), lactic acid can deodorize the offensive odor of excreta by inhibiting microorganisms that produce malodourous ingredients.

Thus, in this study, the reduction of offensive odor could make sanitized urine more acceptable for use in agriculture as a soil amendment. Less odor also brings urine-derived fertilizers in-line with odorless organic matter used by farmers as a soil amendment. Other studies on urine odor perception are rare; however, useful information can be garnered from fecal sludge and food waste experiences. For instance, studies reported the effects of lactic acid on the removal of odor in fecal sludge and organic wastes. Odey et al. (2018) reported that odor was suppressed during the lactic acid fermentation of fecal sludge. Wang et al. (2001) reported that odor was suppressed during the lactic acid fermentation of kitchen biowaste and fish waste, possibly due in part to the presence of lactic acid bacteria (Huang et al., 2006). Yemane et al. (2014) reported that objectionable odor changed to a more pleasant, citric odor during the fermentation of human fecal matter with kitchen waste and molasses.

Thus, considering the potential of lactic acid for hygienization human urine, lactic acid produced from food waste in this study can be used as a low cost treatment technique for source separated urine.

Replication tests

Based on the above experimental results, food waste produced lactic acid was applied to human urine for 9 days in two duplicate tests to test the reproducibility of the selected operational conditions (pH, NH_4^+ and *E. coli*). The experimental results for the lactic acid treatment of the two urine samples are presented in Figure 3.

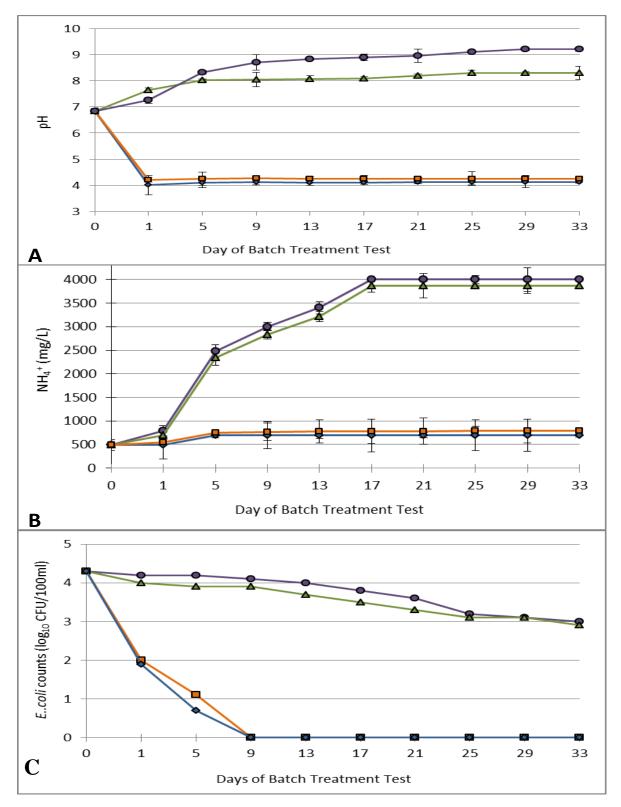


Figure 2. Change in pH, ammonium concentration, and *E. coli* counts during batch treatment tests. Control = • (purple); 1:4 = ▲ (green); 1:2 = ■ (orange); 1:1 = • (Blue). Figure 2A shows changes in pH during batch tests. Figure 2B shows changes in ammonium concentration during batch test. Figure 2C shows *E. coli* distribution during batch test. Zero data points on the x-axis reflect samples where *E. coli* was not detected. Symbols reflect the average of duplicate samples that are given by the extremes of the error bars.

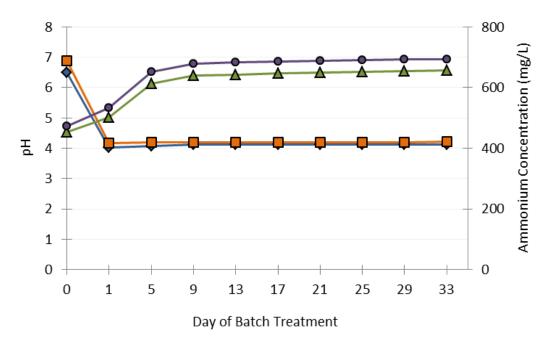


Figure 3. pH value and ammonium concentrations in the two urine samples. pH of sample test-1 (■), pH of sample test-2 (♦), ammonium concentration of sample test-1 (•), and ammonium concentration of sample test-2 (▲).

No variation in rate of *E. coli* inactivation was observed between the two test samples. *E. coli* in the two urine samples was completely inactivated on the 9th day of the treatment process. This result is consistent with the previous experimental results. For urea hydrolysis slight variation was found in the pH and ammonium concentration of the two urine samples until the 5th day and no variation was observed onwards between the two test samples during 9 days of the treatment process.

Both pH and ammonium content stabilized starting from the 9th day of the treatment process, which is consistent with the previous experimental result. The pH value of the two urine samples on the 9th day of the treatment were 4.13 and 4.19, whereas the ammonium concentrations were 639 and 679 mg/L. These results further supported the efficiency and reproducibility of the previous experimental results.

Conclusion

In summary, this experimental study focused on evaluating a sustainable waste management approach. The study showed that producing pH value [4 through the introduction of lactic acid realized urea stabilization and reduction in both indicator bacteria and odors in nine days of the treatment. Readily available food waste was used as a low cost substrate to grow lactic acid bacteria and produce lactic acid. When sufficient lactic acid was added to fresh human urine, the urine was held stable at

acidic pH over 33 days. *E. coli* was inactivated at or below pH 4. The results suggest that further work is warranted to further develop methods for using food waste to support the development of urine-derived fertilizers. In future work, alternative urine-relevant indicator organisms should be included in the study.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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