Development of Low-Toxicity Wastewater Stabilization for Spacecraft Water Recovery Systems

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Wastewater stabilization was an essential component of the spacecraft water cycle. The purpose of stabilizing wastewater was two-fold. First, stabilization prevents the breakdown of urea into ammonia, a toxic gas at high concentrations. Second, it prevents the growth of microorganisms, thereby mitigating hardware and water quality issues due to due biofilm and planktonic growth. Current stabilization techniques involve oxidizers and strong acids (pH=2) such as chromic and sulfuric acid, which are highly toxic and pose a risk to crew health. The purpose of this effort was to explore less toxic stabilization techniques, such as food-grade and commercial care preservatives. Additionally, certain preservatives were tested in the presence of a low-toxicity organic acid. Triplicate 300-mL volumes of urine were dosed with a predetermined quantity of stabilizer and stored for two weeks. During that time, pH, total organic carbon (TOC), ammonia, and turbidity were monitored. Those preservatives that showed the lowest visible microbial growth and stable pH were further tested in a six-month stability study. The results of the six-month study are also included in this paper.

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I. INTRODUCTION

Wastewater stabilization was an essential component of the spacecraft water cycle. The purpose of stabilizing wastewater was two-fold. First, stabilization prevents the conversion of urea into ammonia, a toxic gas at high concentrations, via urea hydrolysis. Second, it prevents the growth of microorganisms, thereby mitigating hardware and water quality issues due to fouling by planktonic bacteria and/or biofilms. Current stabilization techniques involve oxidizers and strong acids such as chromic and sulfuric acids, which are highly toxic and pose a risk to crew health. The purpose of this task was to evaluate alternative chemicals to identify formulations and/or protocols that reduce the toxicity while maintaining wastewater stability, such as food-grade and cosmetic preservatives.

II. BACKGROUND

Previous studies have evaluated the efficacy of organic acids for urine stabilization. Verostko et al (2007) compared the performance of three organic acids (citric, malonic, and maleic acid), to sulfuric acid. Test results revealed that maleic acid maintained urine stability longer than the other organic acids. Because of its low toxicity level and demonstrated high performance, maleic acid has been selected as the acid of choice for this study.

While state-of-the art (SOA) spacecraft urine pretreatment targets a pH of 2, this by definition increases the toxicity level of the fluid. In general, the more neutral the pH, the less toxic the solution; in order to decrease from a Tox Level 2 (SOA) to a Tox Level 1, a higher pH must be explored.

Low pH stabilization solutions are currently used on-orbit because they offer the benefits of microbial control and a higher product water quality from a distillation system. As the pH increases, ammonium ion (NH4+) converts to ammonia gas (NH3), which easily passes through a distillation system and lowers the quality of the distillate. In addition, near-neutral pH values of 5-7 offer virtually no microbial control. To balance the toxicity of low pH and the microbial/distillate quality concerns with a high pH, a pH of 4.0 ± 0.1 was selected as the target for this study.

Previous evaluations of urine solutions adjusted to a pH between 2 and 4 still demonstrated microbial activity; therefore, acidification alone cannot be counted on to prevent microbial growth. To complement the maleic acid, various chemicals was used to either eliminate microorganisms (biocides) or prevent their growth (biostatics). These chemicals are typically used as preservatives in foods, cosmetics, portable and airline toilets, and other low-toxicity commercial products.

III. MATERIALS AND METHODS

Initial testing down-selected the most effective formulation from a suite of preservatives by conducting a series of two-week screening tests. The top candidates from the two-week testing were carried forward for long-term (six-month) testing. Once a final candidate was determined from the six-month testing, the dose quantity of that preservative was refined through a series of dose optimization studies. This section summarizes the procedures for each of those series of tests and any deviations from the test plan.

The urine for each test was composed of 25% female and 20% morning void contributions to approximate a flight-based urine composition. Flush water was added to the urine at a ratio of 265 mL flush water per liter of urine. Each formulation and dose quantity was tested in triplicate. Controls for testing included urine only and urine containing maleic acid titrated to a pH of 4.0 ± 0.1. The urine formulation used for these evaluations was augmented with a fixed quantity of organic and inorganic compounds in order to mimic the composition of on-orbit/crew urine at the 95th percentile calcium concentration (Table 1). The

temperature for all tests was 25±2 °C, regulated in an incubator or plant growth chamber. Specific preservatives that were tested and analyses that were performed are shown in the following subsections for each test.

Table 1. Approximate 95th-percentile major ion concentrations in crew urine.

	Cl-	PO43-	SO42-	Na+	NH4+	K+	Ca2+	Mg2+	TOC
Concentration (mg/L)	5569	2347	3009	2971	13202	2000	280	84	8456

A. Two-week Preservative Screenings

Each preservative was tested at a 5 g/L working concentration or the solubility limit of the preservative (Table 2). The initial preservative test was two weeks in duration. Solution volumes were 300 mL in BOD bottles and were not stirred, since stirring is unlikely for future on-orbit operations. As shown in Figure 1, the flush water was the mechanism by which the preservative was added to the urine. Maleic acid was added in powder form directly to the urine/flush water mix to titrate to a pH of 4.

Table 2. Summary of preservatives considered for evaluation and test concentrations. The final three preservatives, polyaminopropyl biguanide, MIT, and CMIT (shaded in grey) were not included in the two-week evaluation due to lack of availability. However, they were originally considered for testing and as such are included in the preservative list.

Compound	Density (g/mL)	Maximum Concentration (g/L)	Test Dates	
			Without Acid	With acid
Methyl paraben	1.21	2.5	1/26/12-2/10/12	N/A
Methyl paraben with 2-phenoxyethanol	1.21 (methyl paraben), 1.103 (2-phenoxyethanol)	2.5	1/6/12-1/20/12	N/A
DMDM hydantoin (55% pure)	1.04	5	12/1/11-12/15/11	1/6/12-1/20/12
Polyhexamethylene biguanide hydrochloride (Vantocil™ IB, 20% pure)	1.02	25	1/26/12-2/10/12	1/26/12-2/10/12
2-bromo-2-nitropropane-1,3-diol (Bronopol)	1.91	5	12/1/11-12/15/11, 1/6/12-1/20/12	1/6/12-1/20/12
Imidazolidinyl urea	1.85	5	1/6/12-1/20/12	1/6/12-1/20/12
Diazolidinyl urea	1.309	5	1/6/12-1/20/12	1/6/12-1/20/12
Polyaminopropyl biguanide				
2-Methyl-4-isothiazolin-3-one (MIT)				
5-chloro-2-methyl-4-isothiazolin-3-one (CMIT)				

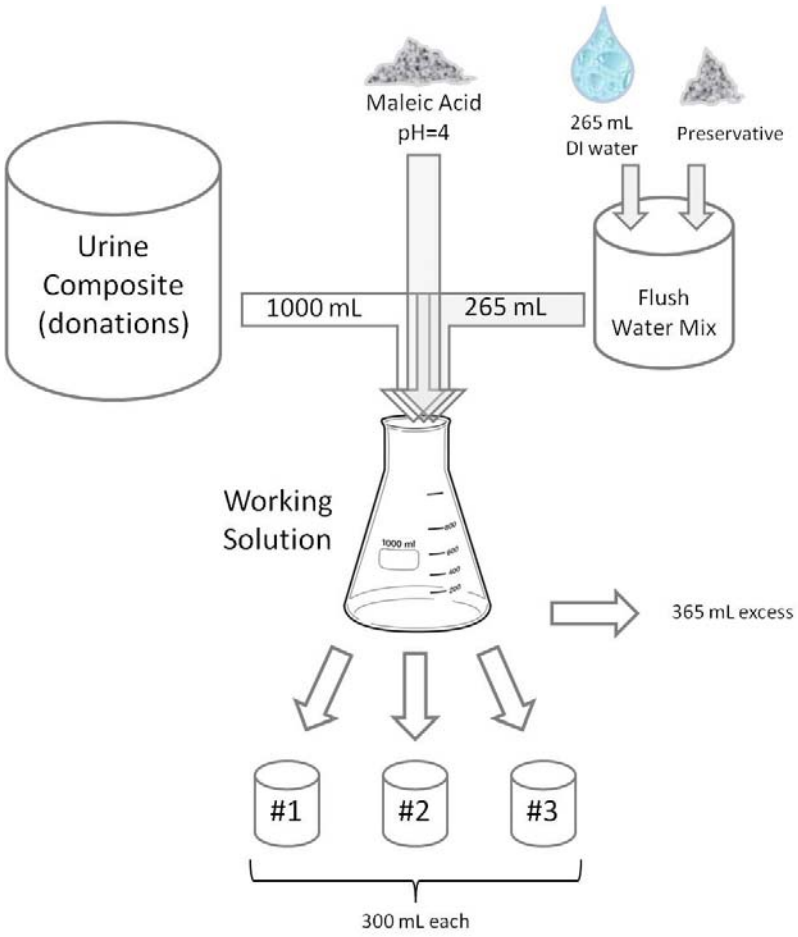

Figure 1. Two-week preservative evaluation process diagram.

Table 3. Summary of analyses to be performed during two-week preservative evaluation. Heterotrophic plate count (HPC) was not conducted for the two-week evaluation.

Property	Pass/Fail Value	Frequency
pH	8	Day 0, 1, 3, 7, 14 (or last day)
NH_3 (mg/L)	<350 mg/L	Weekly (or at end of test)
TOC (mg/L)	<10% increase	Weekly (or at end of test)
Turbidity	Monitor only	Day 0, 1, 3, 7, 14 (or last day)
HPC (CFU/mL)	10^2 increase	Weekly (or at end of test)

B. Six-month Preservative Screening

Based on the results of the two-week preservative evaluation, three formulations were tested in the six-month evaluation: DMDM hydantoin and 2-bromo-2-nitropropane-1,3-diol (Bronopol) with and without acid. The DMDM hydantoin was tested without acid because it was only effective for microbial control between pH values of 4.5 and 9.5. The final preservative concentrations were identical to those of the two

week evaluation. Each test was performed in triplicate and monitored for the properties listed below. The test was begun on June 21, 2012 and completed on December 18, 2012.

The parameters of measure in Table 4 include TAN/TN and ions via ion chromatography (IC), in addition to the parameters from the two-week evaluation. This allowed for an understanding of the impact of the pretreatment to the full solution chemistry. Solution volumes were prepared in 2-L urine batches (2.53 L including flush water) and distributed into sterile syringes as shown in Figure 2. Sample volumes needed for each analysis can be found in Table 4 below. Quantities of materials needed for the study can be found in Table 5.

If gas generation was noted in a syringe, a luer lock valve was attached to that syringe for gas sampling (see **Error! Reference source not found.**). Because each 60-mL syringe was filled with only 40-mL of solution, there was 20-mL of room for gas expansion. The gas sample volume was planned to be injected into the AWRSDF micro-gas chromatograph for analysis; however, since no substantial gas generation was observed for any of the samples, gas analysis was not performed.

Table 4. Summary of analyses to be performed during six month preservative evaluation.

Property	Pass/Fail Value	Frequency	Volume Needed (mL)	Stop test if property exceeds pass/fail value?	Analysis Location
pH	8	Day 0, 1, 3, 7, 14, 28, 42, 56, 70, 84, 98, 112, 126, 140, 154, 168, 182	10	Y	AWRSDF
TAN/TN (mg/L)	<10% increase	Day 0, 1, 3, 7, 14, 28, 42, 56, 70, 84, 98, 112, 126, 140, 154, 168, 182	5	Y	7/2010
Ions (mg/L)	Monitor only	Day 0, 1, 3, 7, 14, 28, 42, 56, 70, 84, 98, 112, 126, 140, 154, 168, 182	5	N	7/2010
TOC (mg/L)	<10% increase	Day 0, 1, 3, 7, 14, 28, 42, 56, 70, 84, 98, 112, 126, 140, 154, 168, 182	5	N	7/2010
Turbidity	Monitor only	Day 0, 1, 3, 7, 14, 28, 42, 56, 70, 84, 98, 112, 126, 140, 154, 168, 182	20	N	7B/1300B
HPC (CFU/mL)	$<10^2$ increase	Day 0, 1, 3, 7, 14, 28, 42, 56, 70, 84, 98, 112, 126, 140, 154, 168, 182	5	N	7B/1300B
Yeast/mold (CFU/100 mL)	$<10^2$ increase	Day 0, 1, 3, 7, 14, 28, 42, 56, 70, 84, 98, 112, 126, 140, 154, 168, 182	5	N	7B/1300B

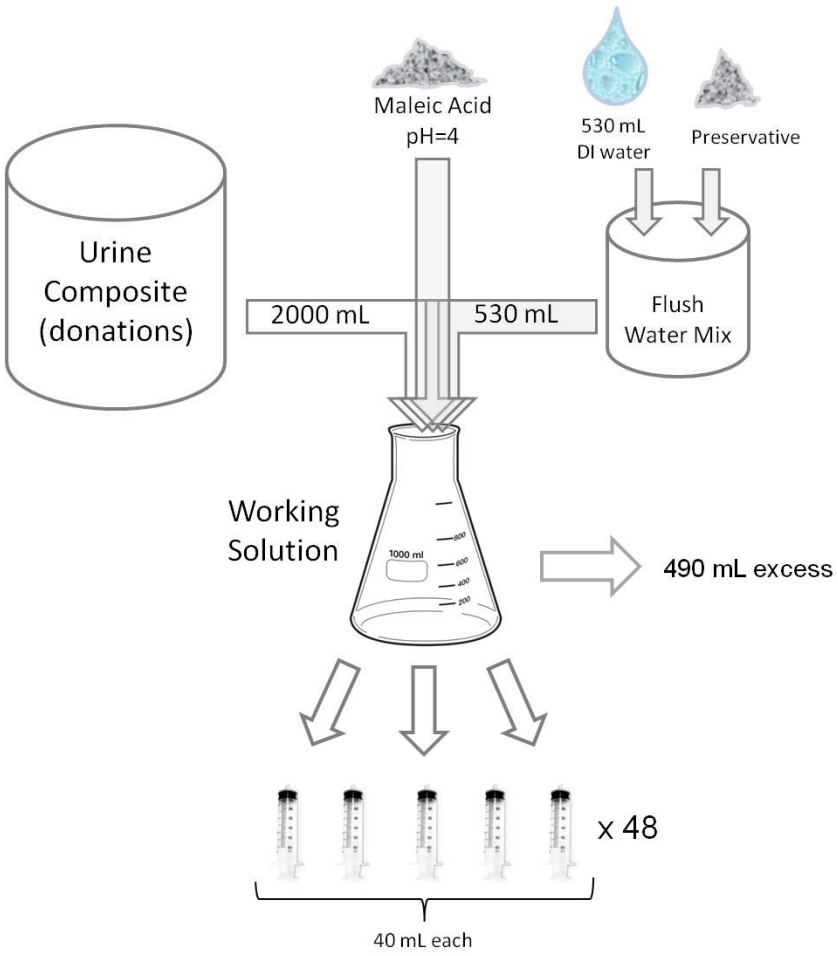

Figure 2. Process diagram for the six-month study.

Table 5. Materials needed for six-month study.

Material	Quantity Needed
60 mL syringes	240
Fixed, augmented urine	10 L
Petri dishes (total)	510
Bronopol	25.3 g
DMDM hydantoin	18.18 mL
Maleic acid (total)	7.48 g (est.)

C. Dose Optimization Studies: Test 1

In order to compare the AES LTS options to flight pretreatments, the initial dose quantity was selected to mimic the mass concentration used in flight (5 g/L). However, this may be in excess of what was required for the LTS to be effective. Based on the results of the six-month preservative evaluation, the dose quantity of the top candidate was tested for a 1-month period. Pretreatment solutions containing 2-bromo-2-nitropropane-1,3-diol (Bronopol) were evaluated using the same protocol as the six-month study. The test was begun on February 13, 2013 and completed on March 13, 2013. The following LTS dose concentrations were tested:

- 0 g/L (control, with and without acid)
- 1 g/L
- 2.5 g/L
- 3.5 g/L
- 5 g/L (replicate of six-month testing)

The parameters of measure in Table 6 were the same as those of the six-month study. Solutions required 800-mL urine (1.01 L including flush water). Solutions were distributed into sterile syringes with no mechanical agitation. The temperature for testing was 25 ± 2 °C, regulated in a plant growth chamber. Quantities of materials used for the study can be found in Table 7.

Table 6. Summary of analyses to be performed during dose optimization evaluations.

Property	Pass/Fail Value	Frequency	Volume Needed (mL)	Stop test if property exceeds pass/fail value?	Analysis Location
pH	8	Day 0, 1, 3, 7, 14, 21, 28	10	Y	AWRSDF
TAN/TN (mg/L)	<10% increase	Day 0, 1, 3, 7, 14, 21, 28	5	Y	7/2010
Ions (mg/L)	Monitor only	Day 0, 1, 3, 7, 14, 21, 28	5	N	7/2010
TOC (mg/L)	<10% increase	Day 0, 1, 3, 7, 14, 21, 28	5	N	7/2010
Turbidity	Monitor only	Day 0, 1, 3, 7, 14, 21, 28	20	N	7B/1300B
HPC (CFU/mL)	$<10^2$ increase	Day 0, 1, 3, 7, 14, 21, 28	5	N	7B/1300B
Yeast/mold (CFU/100 mL)	$<10^2$ increase	Day 0, 1, 3, 7, 14, 21, 28	5	N	7B/1300B

Table 7. Materials needed for dose optimization study.

Material	Quantity Needed
60 mL syringes	180
Fixed, augmented urine	8 L
Petri dishes (total)	360
Bronopol	24.3 g
Maleic acid (total)	15 g (est.)

D. Dose Optimization Studies: Test 2

Due to the high effectiveness of the dosing profile used in the previous dose optimization evaluation, a further range of dose quantities was evaluated. Based on the results of the previous test, 2-bromo-2-nitropropane-1,3-diol (Bronopol) was evaluated both with and without acidification.

As with the previous evaluations, the urine for this screening was 95% fixed augmented urine, with 25% female and 20% morning void contributions. Flush water was added to the urine at a ratio of 265 mL flush water per L of urine. Each test was performed in triplicate and monitored for the properties listed below.

Controls for testing will include urine + acid and urine only; as stated above, the acid of choice was maleic acid. The target pH with maleic acid was pH = 4. The test was begun on April 24, 2013 and completed on May 22, 2013 and evaluated the following LTS dose concentrations:

- 0 mg/L (control, with and without acid)
- 50 mg/L
- 100 mg/L
- 250 mg/L
- 500 mg/L
- 750 mg/L
- 1000 mg/L (replicate of previous test)

The parameters of measure in Table 8 are the same as those for the previous test, with the addition of formate and nitrite to the ion analyses. Solutions will consist of 2.51 mL urine (3.17 L including flush water); this includes a 10% margin beyond the minimum volume needed. Solutions was distributed into sterile syringes; they will not be stirred or agitated. Temperature for testing was 25 ± 2 °C, regulated in an incubator or plant growth chamber. Sample volumes needed for each analysis can be found in Table 8 below. Quantities of materials needed for the study can be found in Table 9. A sterile luer lock cap was attached to each syringe to prevent exposure to laboratory air.

Table 8. Summary of analyses to be performed during dose optimization evaluations.

Property	Pass/Fail Value	Frequency	Volume Needed (mL)	Stop test if property exceeds pass/fail value?	Analysis Location
pH	8	Day 0, 1, 3, 7, 14, 21, 28	10	Y	AWRSDF
TAN/TN (mg/L)	<10% increase	Day 0, 1, 3, 7, 14, 21, 28	5	Y	7/2010
Ions (mg/L), including formate and nitrite	Monitor only	Day 0, 1, 3, 7, 14, 21, 28	5	N	7/2010
TOC (mg/L)	<10% increase	Day 0, 1, 3, 7, 14, 21, 28	5	N	7/2010
Turbidity	Monitor only	Day 0, 1, 3, 7, 14, 21, 28	20	N	7B/1300B
HPC (CFU/mL)	$<10^2$ increase	Day 0, 1, 3, 7, 14, 21, 28	5	N	7B/1300B
Yeast/mold (CFU/100 mL)	$<10^2$ increase	Day 0, 1, 3, 7, 14, 21, 28	5	N	7B/1300B

Table 9. Materials needed for dose optimization study.

Material	Quantity Needed
60 mL syringes	252
Fixed, augmented urine	18.0 L
Petri dishes (total)	504
Bronopol	8.41 g
Maleic acid (total)	17.56 g (est.)

IV. DATA AND RESULTS

A. Two-week Preservative Screenings

The pH values for the two-week evaluations are shown below. Due to the large number of samples and wide range of pH values, the data is split into unacidified and acidified graphs. Because the evaluations were conducted in three separate tests (with dates shown in Table 2), the controls and unacidified Bronopol samples are numbered in the order in which the sequence of tests were conducted. For example, the Urine Only – 1 served as the control for the first preservative evaluation, which was conducted from 12/1/11-12/15/11; Urine Only – 2 was the control for the second evaluation, from 1/6/12-1/20/12; and Urine Only – 3 was the control for the third evaluation, from 1/26/12-2/10/12.

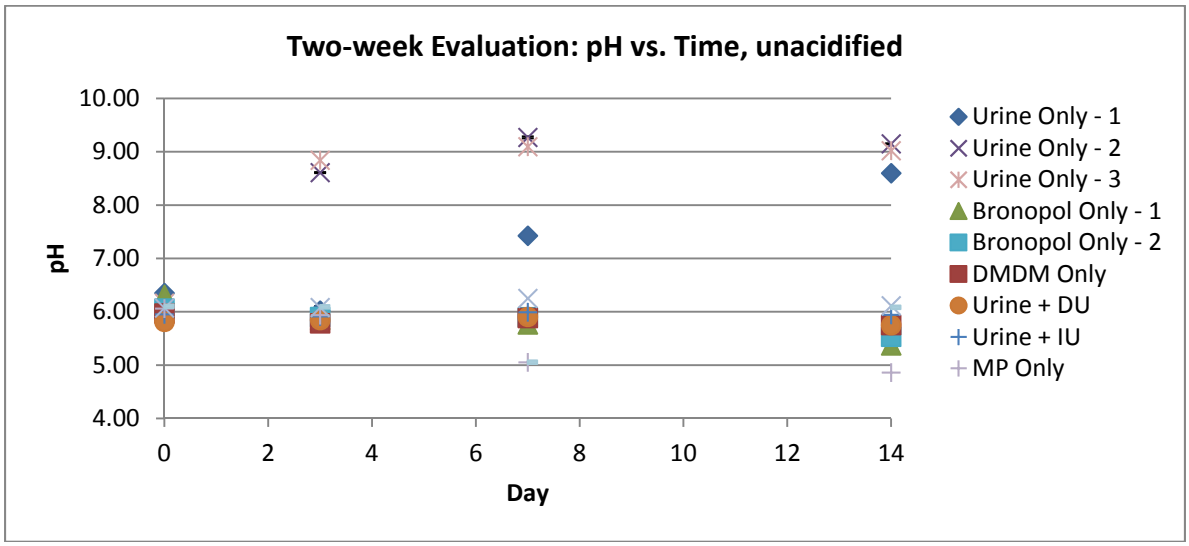

Figure 3. Unacidified sample pH data for the two-week preservative evaluation. The displayed values are averages of triplicate sample points.

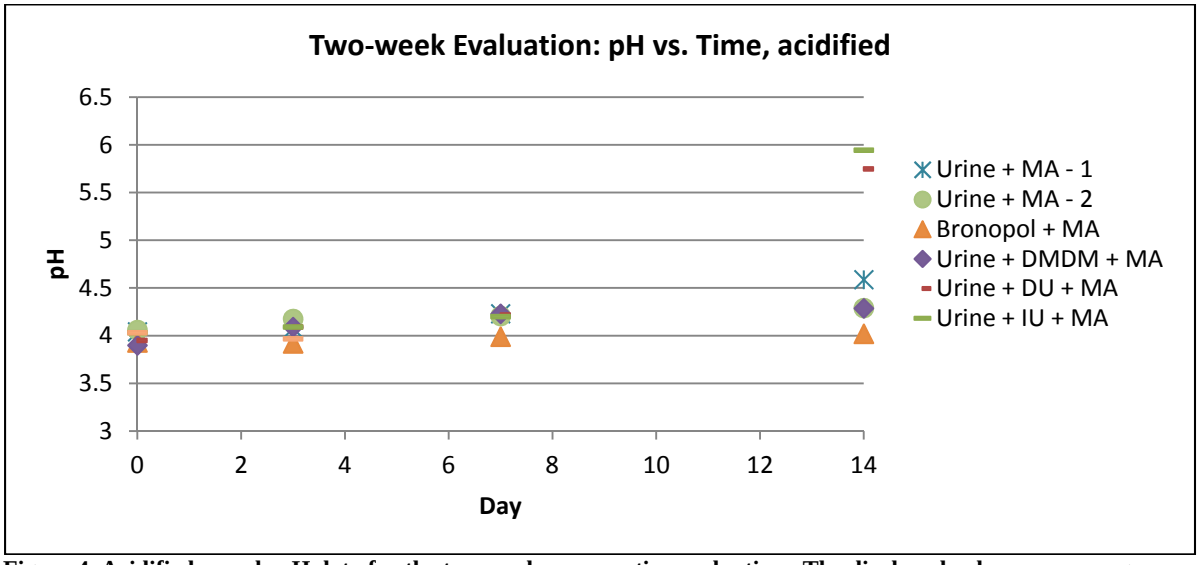

Figure 4. Acidified sample pH data for the two-week preservative evaluation. The displayed values are averages of triplicate sample points.

Increases in pH are often an indicator of microbial growth and/or chemical degradation of the urine. When urea is decomposed and ammonia/ammonium ion are produced, the pH of the bulk fluid will increase. As expected, the unacidified controls quickly degraded because they lacked any pretreatment, which was demonstrated by a rapid increase in pH. The acidified controls also showed an increase in pH, though much more slowly due to the minor stabilizing effect of the maleic acid. Several preservatives showed increases in pH, which were indications of failure of stabilization. These pH increases, coupled with substantial visual growth in many of the samples, prompted removal of said preservatives from further testing; examples of such growth can be seen in Figure 5. The unacidified Bronopol samples all showed decreases in pH, which is thought to be a result of the Bronopol degradation process. Because low pH inhibits bacterial growth, this property would be of benefit to the urine stabilization application.

Figure 5. Examples of visible growth in two failed preservatives and one successful preservative. Visible growth can be seen in acidified diazolidinyl urea (left) and acidified imidazolidinyl urea (middle). No visible growth was seen in Bronopol (right).

Increases in NH_4^+ concentration indicate chemical and microbiologically-induced degradation of the urine. Again, this is most apparent with the unacidified urine control samples (Figure 6). A summary of all data for the two-week evaluation can be seen in Figure 8. While some increases in pH, turbidity, and NH_4^+ were seen for several preservatives, those without visible growth (Bronopol and DMDM hydantoin) were selected for six-month testing.

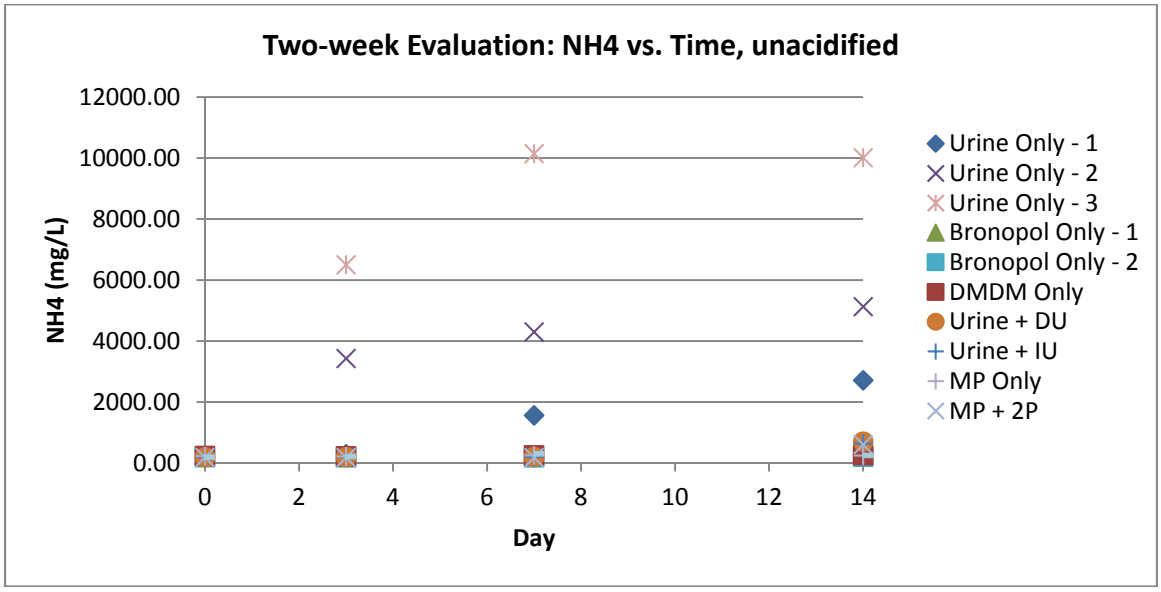

Figure 6. Ammonium ion concentrations versus time for unacidified preservative samples. The displayed values are averages of triplicate sample points.

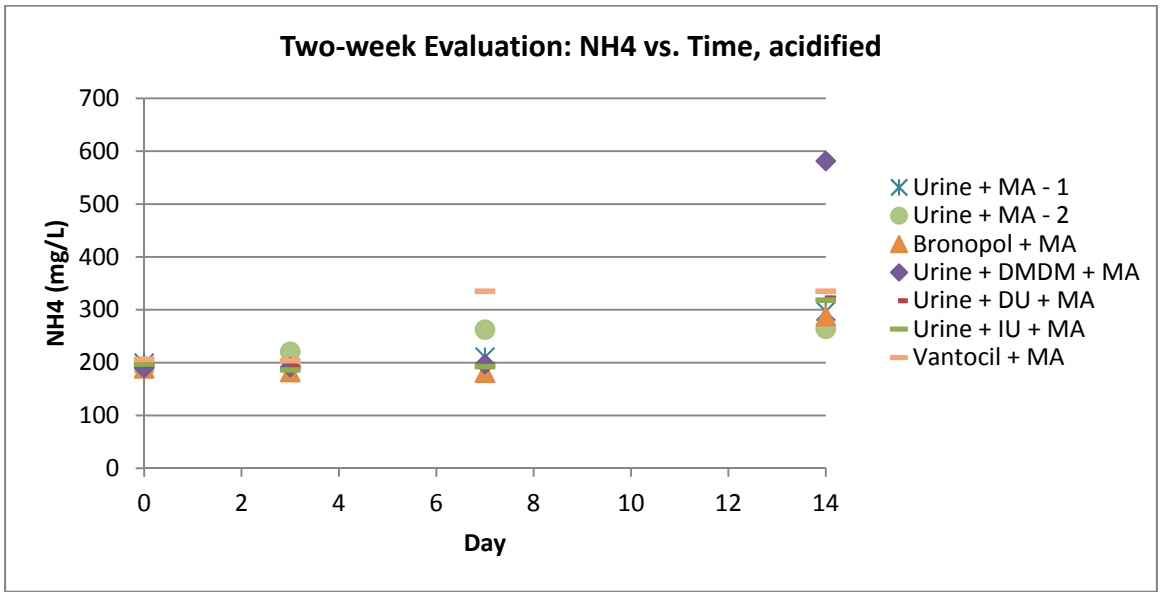

Figure 7. Ammonium ion concentrations versus time for acidified preservative samples. The displayed values are averages of triplicate sample points.

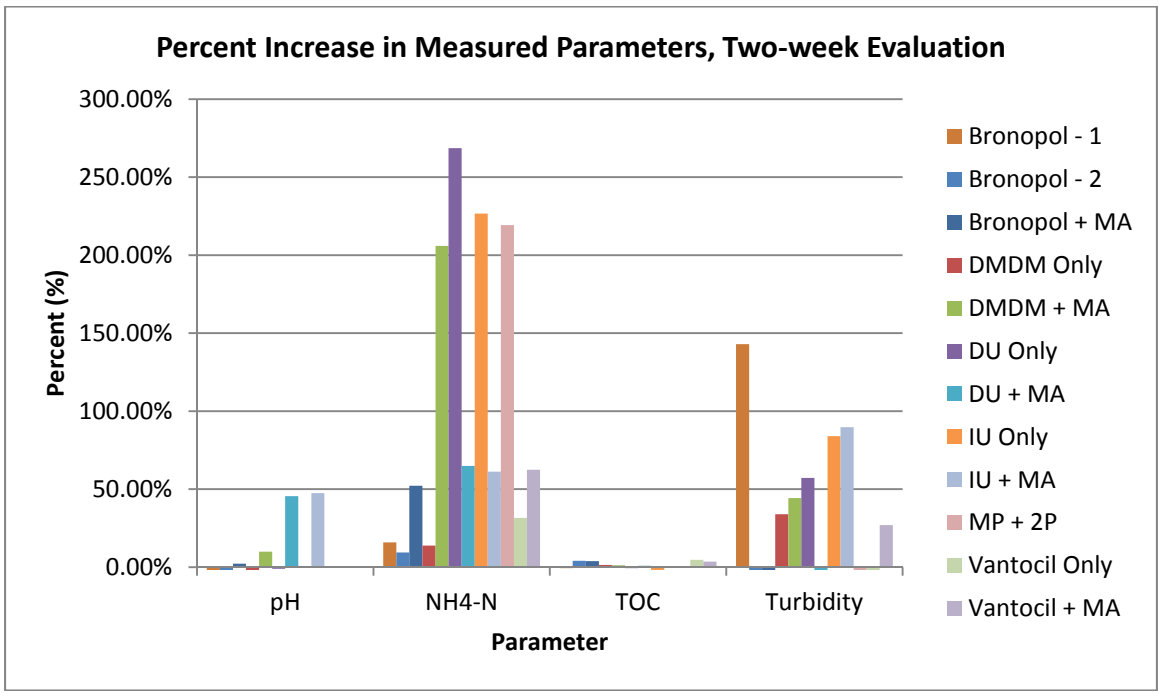

Figure 8. Percent increase in parameters (pH, NH_4, TOC, and turbidity) for the two-week preservative evaluation. Percent increase is measured as the final (day 14) parameter value minus the initial parameter value, as a percentage of the initial value.

B. Six-month Preservative Screening

The six-month preservative evaluation included a urine-only control, urine with maleic acid, DMDM hydantoin without acid, and Bronopol both with and without acid. In contrast to the two-week evaluation, the six-month evaluation included sterile syringes to store the pretreated urine (Figure 9, left). The samples were stored in a temperature-controlled plant growth chamber in the Advanced Water Recovery Systems Development Facility (building 7, room 1300) as shown in Figure 9 (right).

Figure 9. Samples were stored in sterile syringes for the six-month pretreatment evaluation (left). The syringes were stored in a temperature-controlled plant growth chamber (right).

Both acidified and unacidified urine showed increases in pH throughout the 180-day period (Figure 10). As expected, the unacidified urine control showed a steep increase in pH initially and maintained a high pH throughout the course of the evaluation. Similar to the two-week evaluations, the acidified urine control showed a slower but notable increase in pH. Again, this is a result of the inhibition of bacterial growth due to the presence of maleic acid. Both unacidified and acidified Bronopol sample sets showed no increases in pH. In fact, the unacidified Bronopol samples showed the same steady decrease in pH as with the two-week evaluation, though more pronounced due to the longer duration of testing. As a result of this pronounced drop in pH, it was decided that further exploration into the mechanism of Bronopol degradation was warranted (discussed later in this paper). Additionally, no statistically significant growth was observed for either suite of Bronopol samples throughout the course of the evaluation.

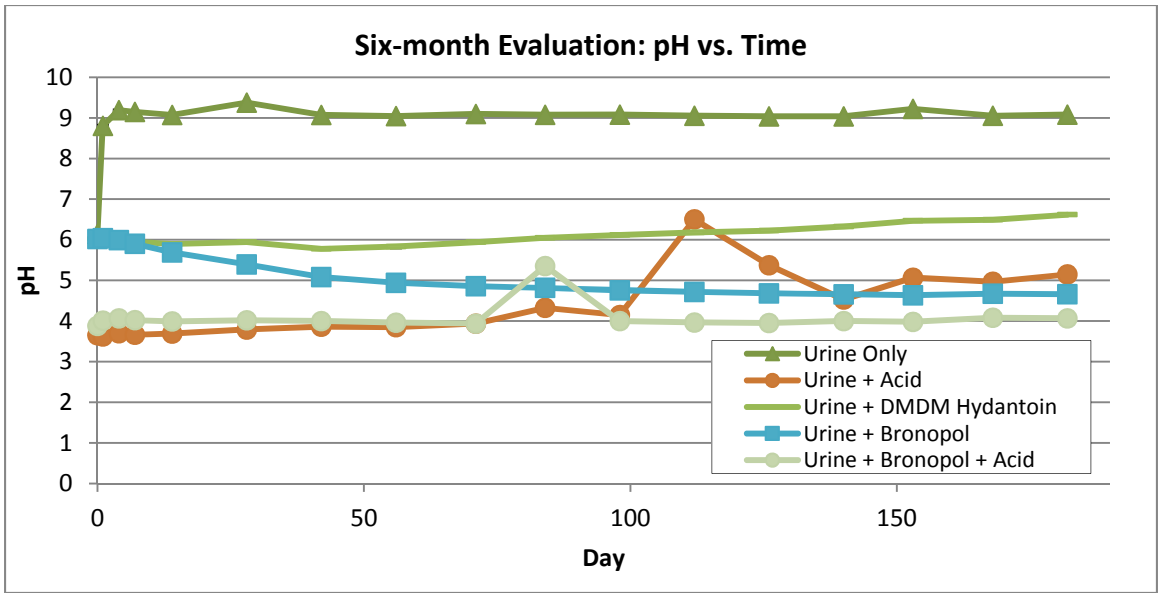

Figure 10. pH values for the six-month preservative evaluation. All values are averages of triplicate samples.

The DMDM hydantoin maintained its initial pH (or slightly less than) through day 84. After that time, however, the pH began to increase, indicating that the stabilization threshold of the DMDM hydantoin had been reached. This was supported by microbiological data (Figure 11 and Figure 12), which showed that the DMDM hydantoin had in fact failed to prevent growth within the first week of testing. Visible growth was seen in the DMDM hydantoin samples by day 84; shows the contrast between the DMDM hydantoin sample with growth and a Bronopol sample with no growth.

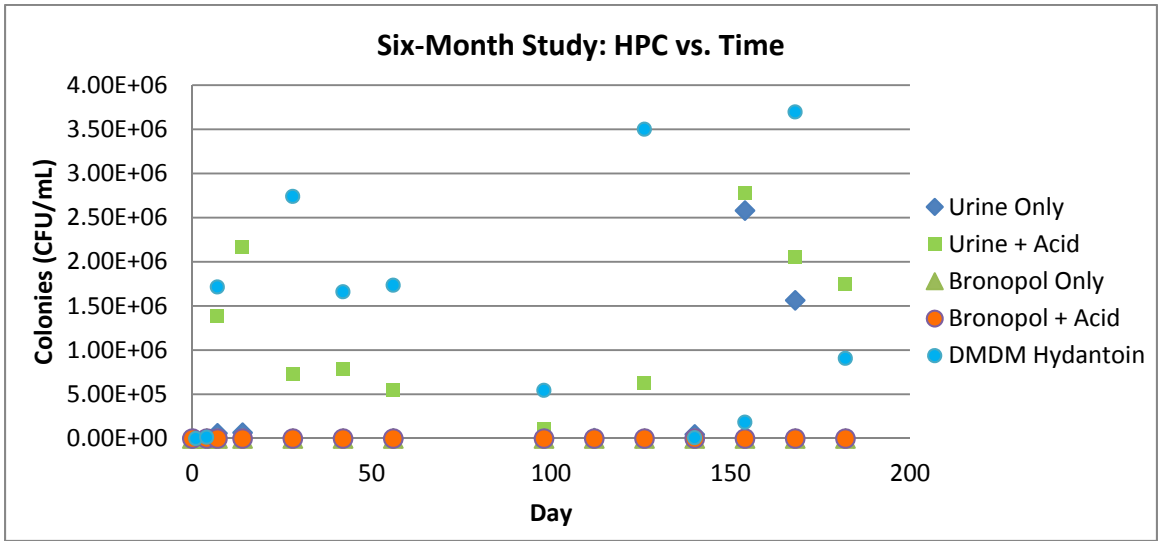

Figure 11. Heterotrophic plate count (HPC) bacterial data versus time for the six-month preservative evaluation. The values shown are averages of triplicate samples.

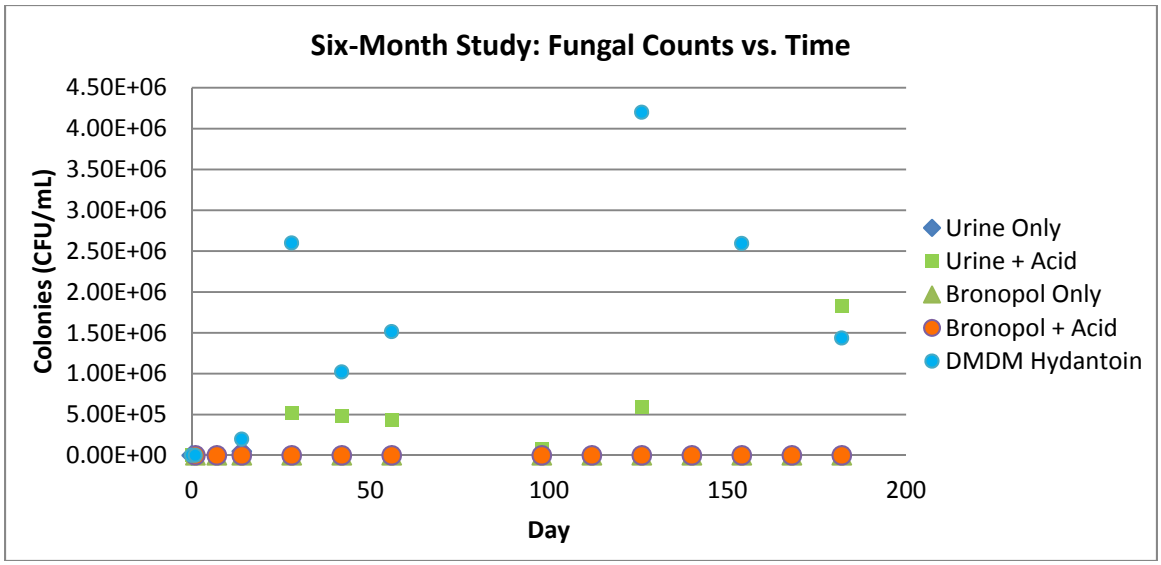

Figure 12. Fungal (yeast/mold) plate count data versus time for the six-month preservative evaluation. The values shown are averages of triplicate samples.

Figure 13. Six-month evaluation sample syringes contrasting visible growth (DMDM hydantoin, top) with no growth (Bronopol, bottom).

Further information can be gathered on the chemical degradation processes by analyzing the solution chemistry. A decrease in total nitrogen for all solutions can be seen in Figure 14. Typically, a decrease in total nitrogen in solution is an indirect indication of the generation of nitrogen, nitrogen oxide, or other nitrogen-containing gas. Concurrent to this decrease in total nitrogen, a rapid increase in ammonium ion (NH_4^+) in solution can be seen for the unacidified control, and in a delayed manner for the acidified and/or pretreated samples (Figure 15). The increase in ammonium ion can be attributed to the breakdown of urea; the equilibrium chemistry of ammonium ion will result in the generation of ammonium gas. Because the syringes used in the six-month study were not capped, it is possible that any nitrogen-containing gas that was generated was lost to the atmosphere, resulting in the general decline in total nitrogen over time. Figure 16 shows the concentration variations of the Bronopol-pretreated solutions over time for total nitrogen and ammonium ion. The presence of the maleic acid had no discernable impact on the nitrogen chemistry or performance of the Bronopol over the course of the six-month study. Both unacidified and acidified Bronopol-pretreated solutions show a general decline over time in total nitrogen, and an increase in ammonium ion, especially starting at day 126. The rapid increase in ammonium ion starting at Day 126 is likely due to the onset of urea hydrolysis as a result of the loss of Bronopol. It can therefore be concluded that the Bronopol failed to prevent chemical degradation of the urine at the 126-day mark.

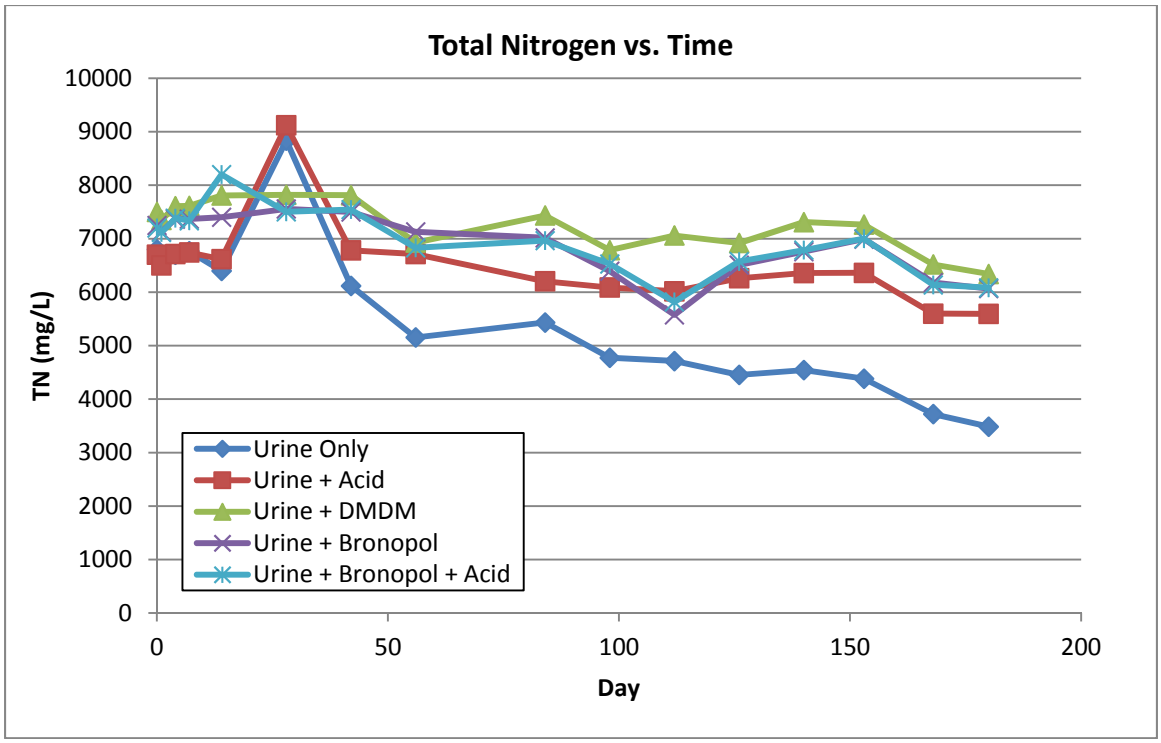

Figure 14. Total nitrogen trends versus time for six-month study. All values are averages of triplicate samples.

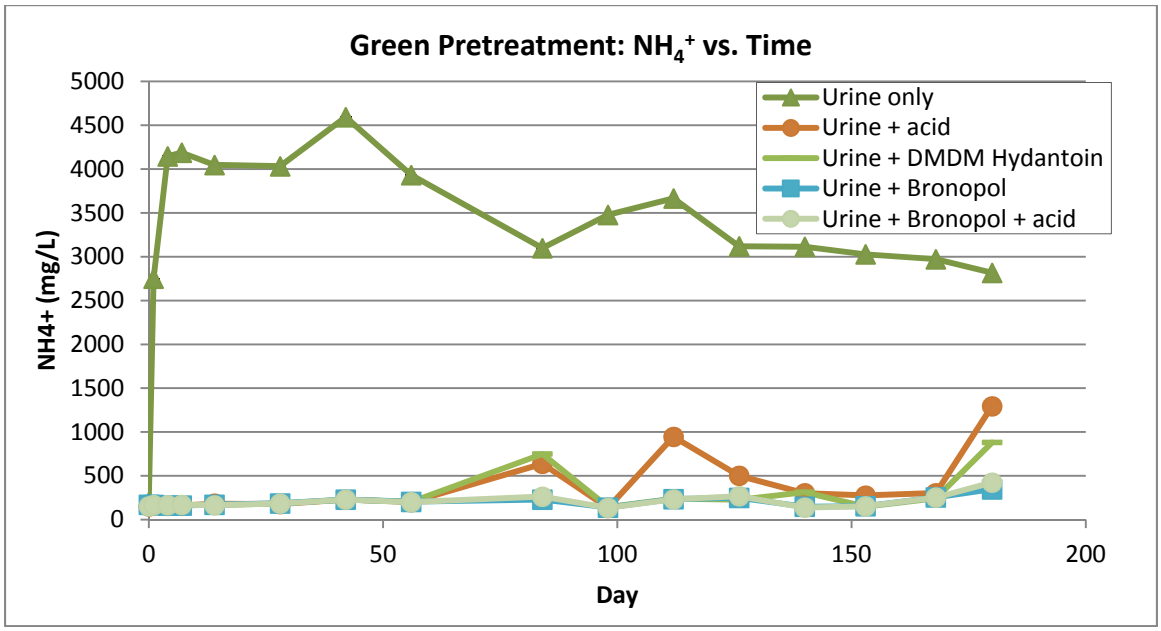

Figure 15. Ammonium ion concentration versus time for the six-month study. All values are averages of triplicate samples.

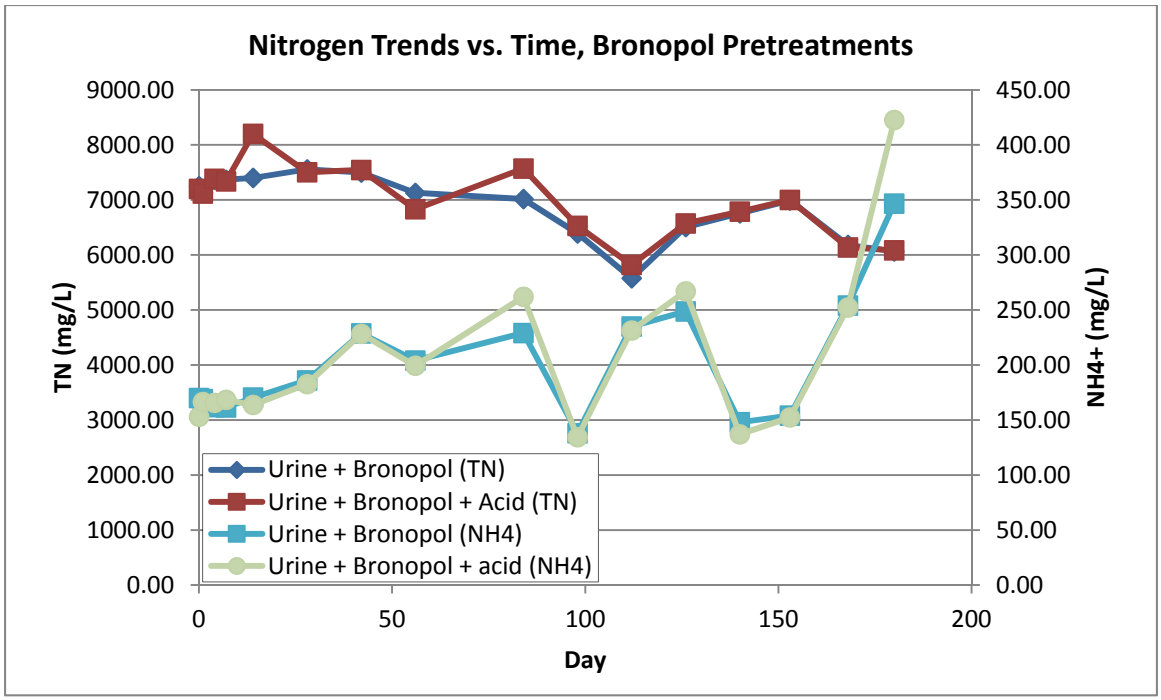

Figure 16. Total nitrogen and ammonium ion concentrations versus time for both acidified and unacidified Bronopol pretreatments. All values are averages of triplicate samples.

C. Dose Optimization Studies: Test 1

The purpose of the dose optimization testing was to determine the lowest dose of Bronopol that would be effective at pretreating urine. Each dose optimization test was 28 days long. No increases in pH were observed for the duration of the testing, and the expected decrease in pH of the unacidified Bronopol samples was apparent. Additionally, there was no statistically significant growth of bacteria or fungus for the duration of the test across all dose quantities. While there were two outlying data points, those are believed to be artifacts of the plating process or sample contamination. Finally, all Bronopol-dosed samples experienced no statistically-significant changes in ammonium ion concentration (Figure 20); the same is true of all major ions except bromide.

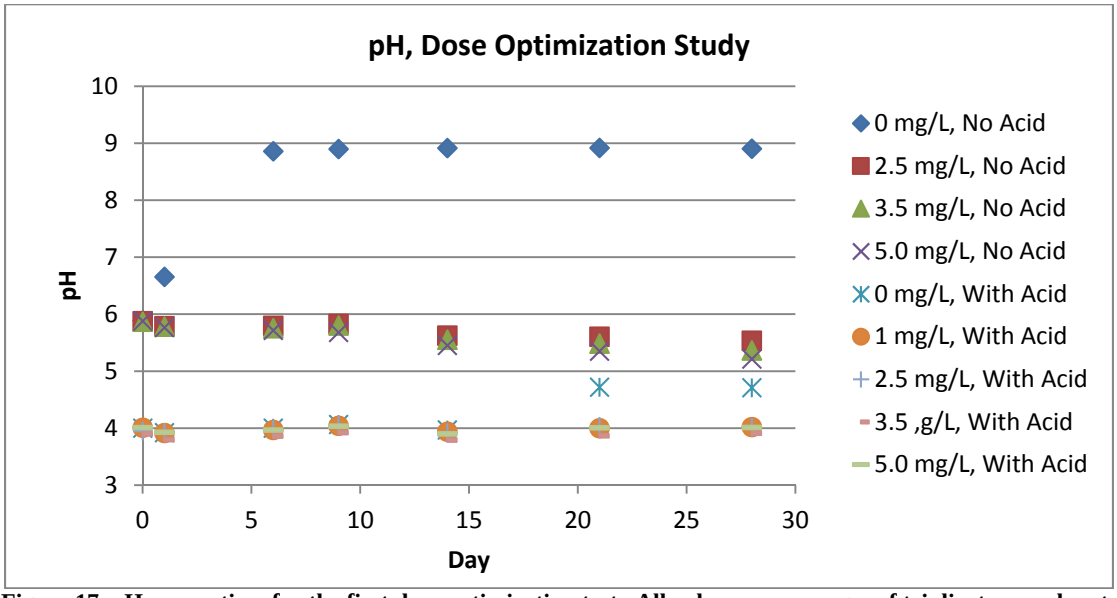

Figure 17. pH versus time for the first dose optimization test. All values are averages of triplicate sample sets.

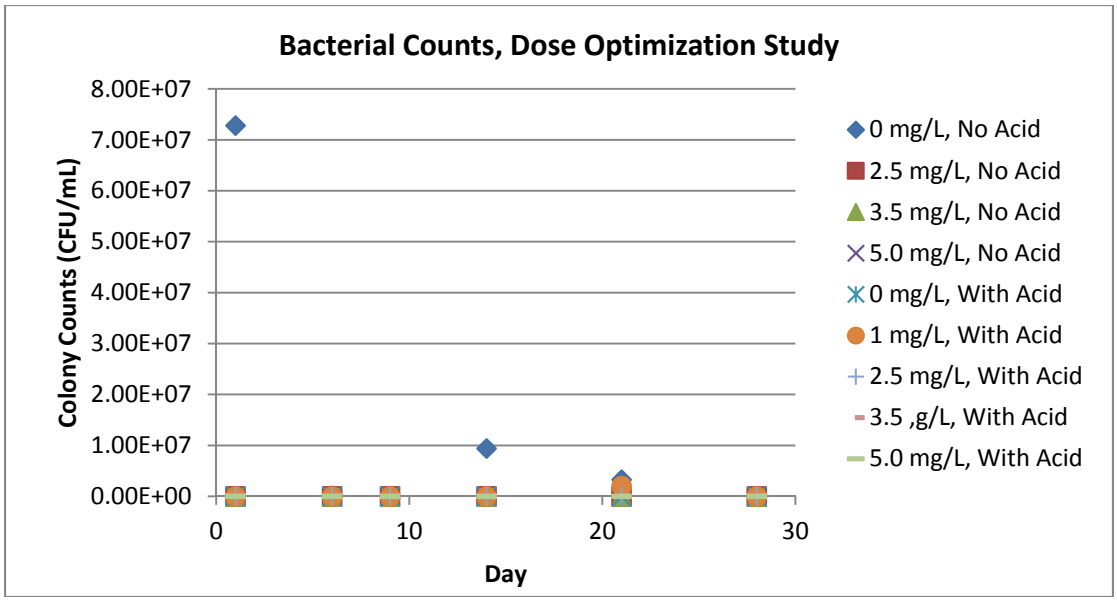

Figure 18. Heterotrophic plate count versus time for the first dose optimization test. All values are averages of triplicate sample sets.

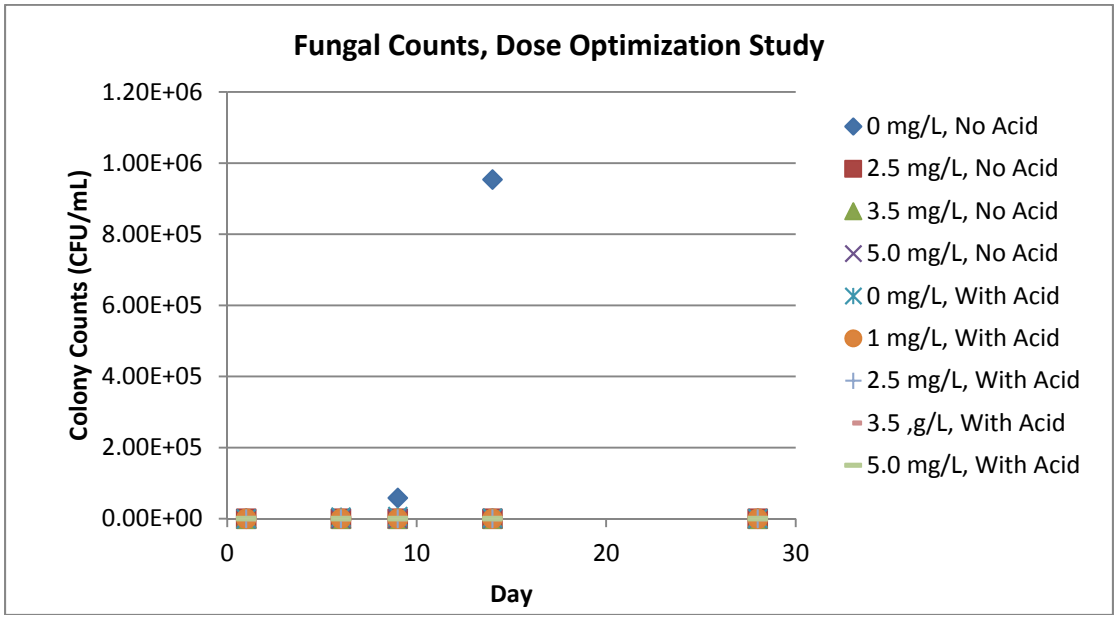

Figure 19. Fungal counts versus time for the first dose optimization test. All values are averages of triplicate sample sets.

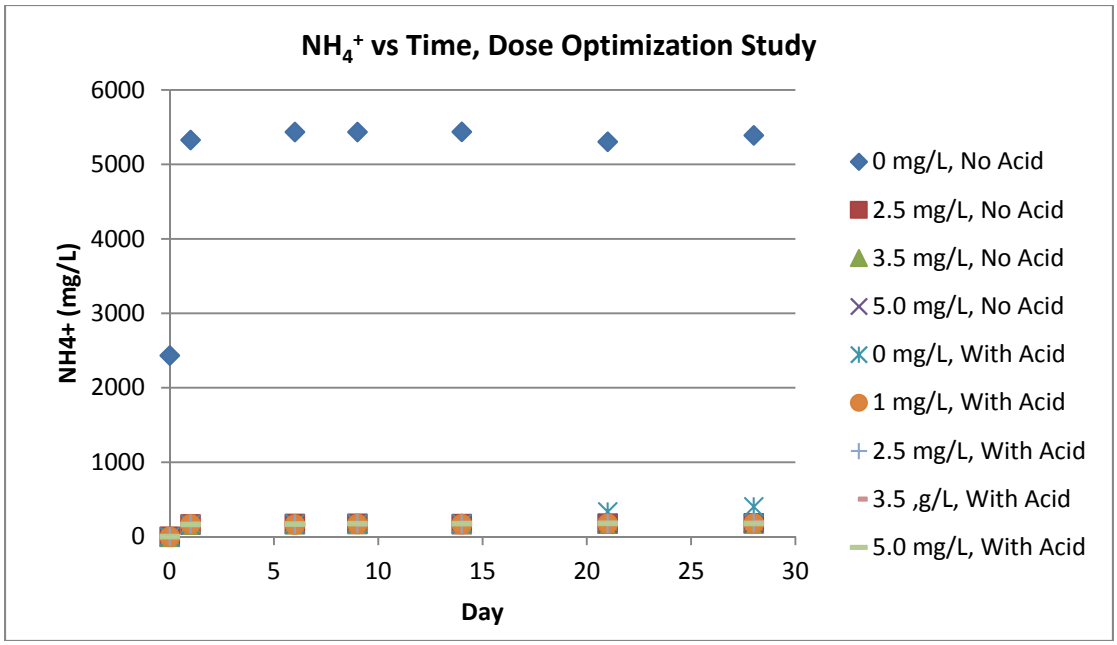

Figure 20. Ammonium ion concentration versus time for the first dose optimization test. All values are averages of triplicate sample sets.

The unacidified sample sets have provided additional insight into the Bronopol decomposition mechanism in the presence of urine. As described in the previous two tests, a steady decline in pH can be observed for unacidified, Bronopol-pretreated urine. Prior to conducting the first dose optimization test, it was believed that the decline in pH was a result of the decomposition of Bronopol; without correlating the degree of pH drop to the amount of Bronopol, however, this link could not be confirmed. Based on the results of this dose optimization test, a strong correlation can be seen between the decline in pH and the production of bromide ions for unacidified, pretreated urine (Figure 21). Additionally, the presence of acid correlates to a lack of bromide ion production; this indicates that the presence of acid either inhibits the degradation of Bronopol or the acid reacts with the bromide ion in such a way as to prevent it from being detected via ion chromatography.

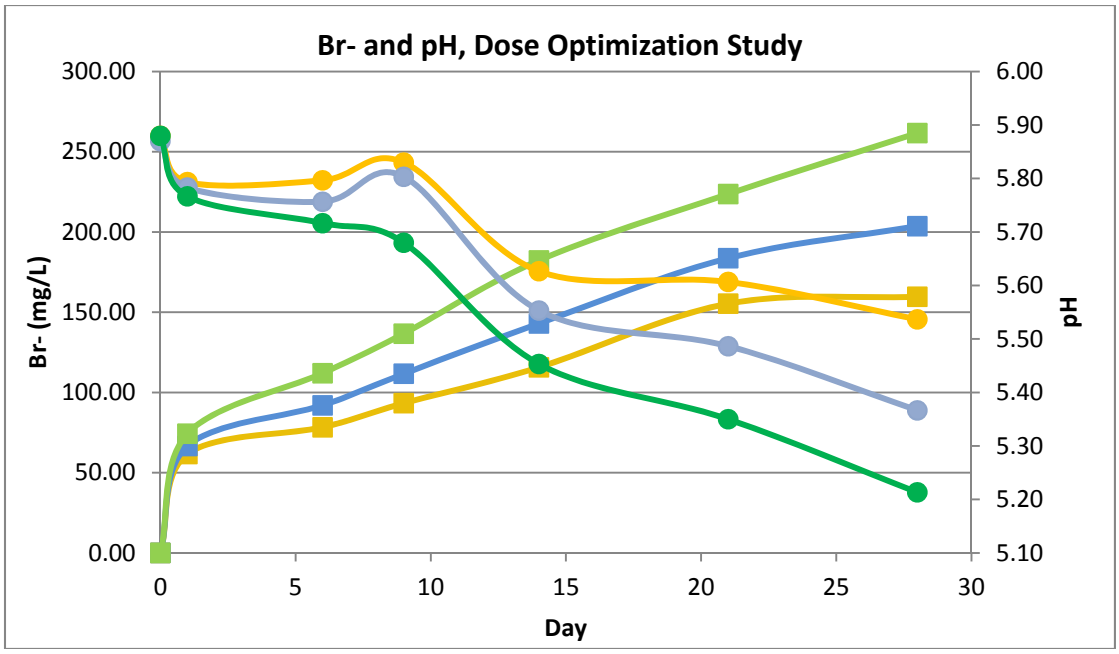

Figure 21. pH and bromide ion concentrations as a function of time for unacidified, pretreated urine samples. Orange lines = 2.5 g/L Bronopol; blue lines = 3.5 g/L Bronopol, green lines = 5.0 g/L Bronopol. Circles represent pH values while squares represent bromide concentrations. All data points listed are averaged of triplicate sample sets.

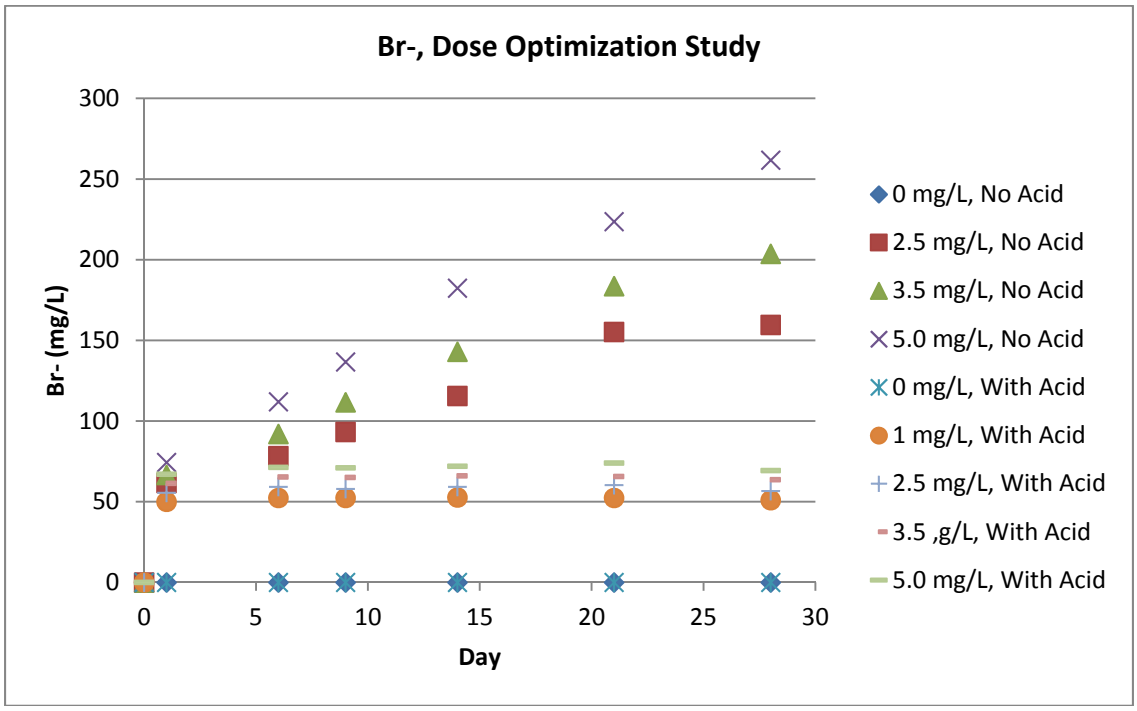

Figure 22. Bromide ion concentration as a function of time for acidified and unacidified urine.

D. Dose Optimization Studies: Test 2

Substantial bacterial control was accomplished for unacidified dose quantities up to and including 250 mg/L of Bronopol. However, colony counts up to 40 CFU/mL were observed for the lower doses (250 and

500 mg/L) at the 28-day mark, indicating that the pretreatment would likely not have mitigated substantial growth beyond one month (Figure 23). For Bronopol doses up to 750 mg/L with acid, bacterial growth was substantial; higher doses showed low levels of bacterial growth (Figure 24).

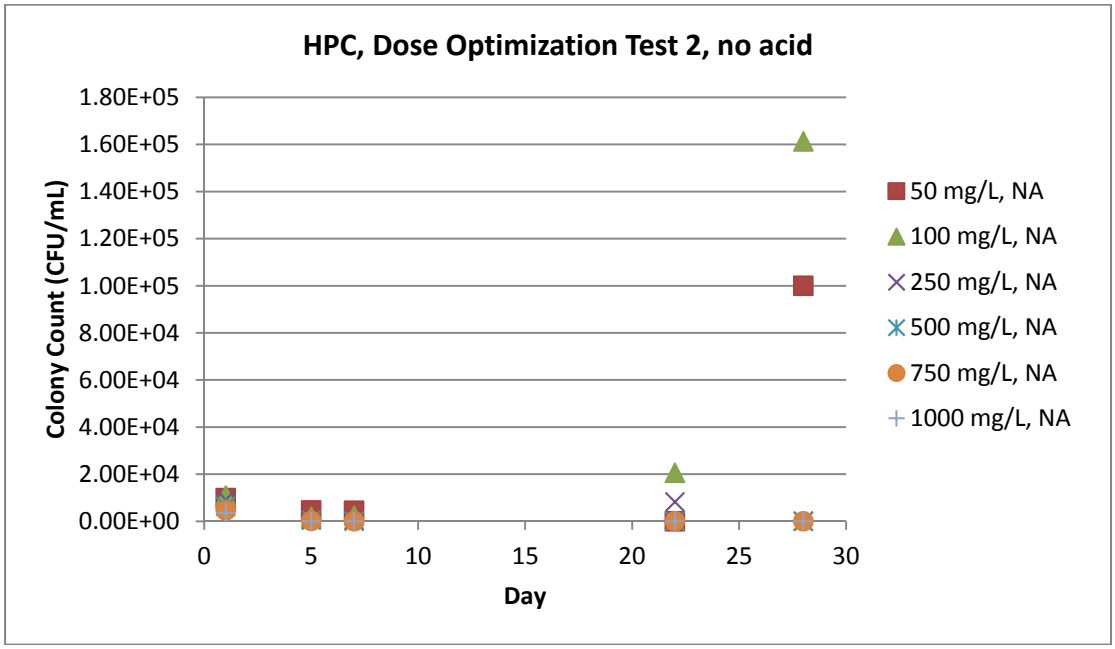

Figure 23. Heterotrophic plate count for the dose optimization test, unacidified samples only.

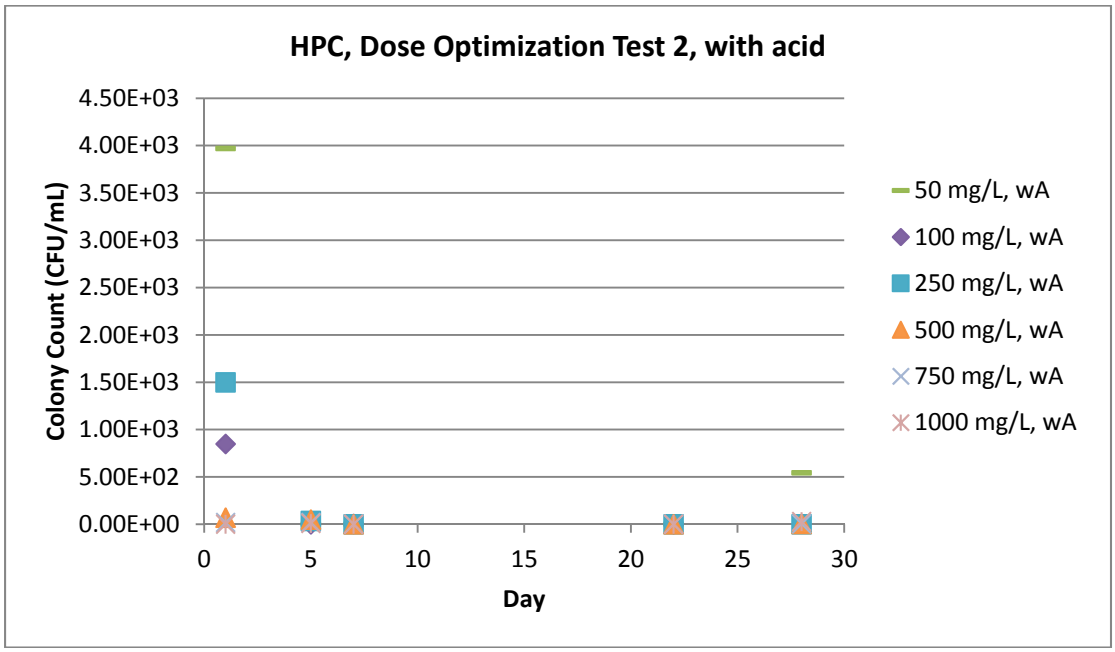

Figure 24. Heterotrophic plate count for the dose optimization test, acidified samples only.

For doses up to and including 250 mg/L without acid, the pretreatment was inadequate in preventing substantial fungal growth (Figure 25). All doses (up to/including 1000 mg/L) with acid failed to prevent fungal growth for the duration of the test (Figure 26).

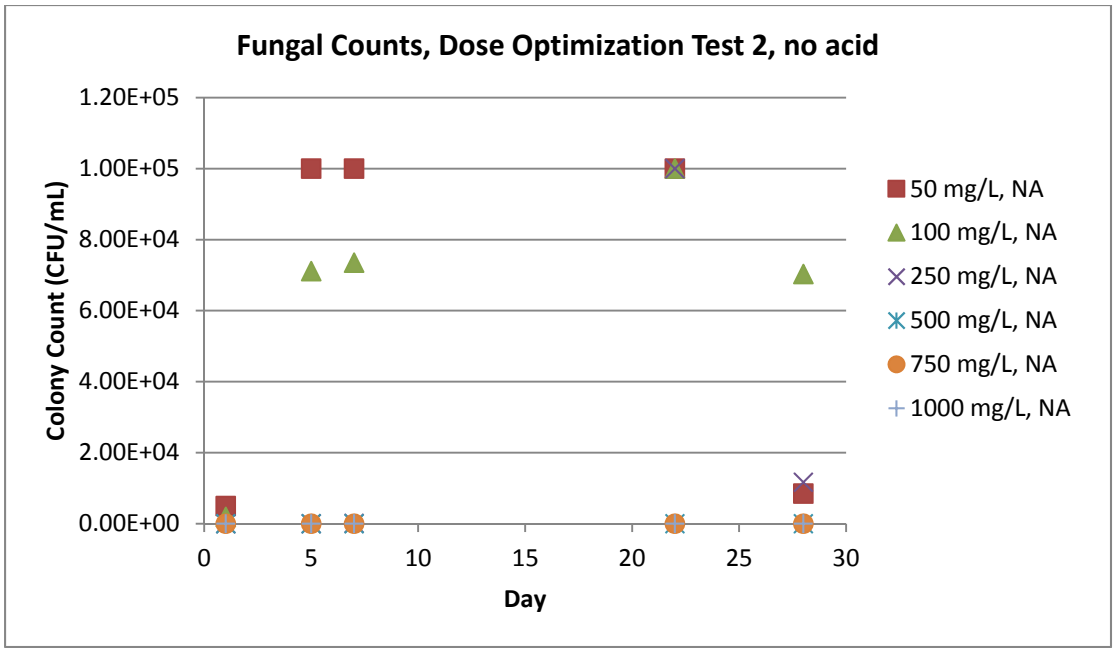

Figure 25. Fungal counts for the dose optimization test, unacidified samples only.

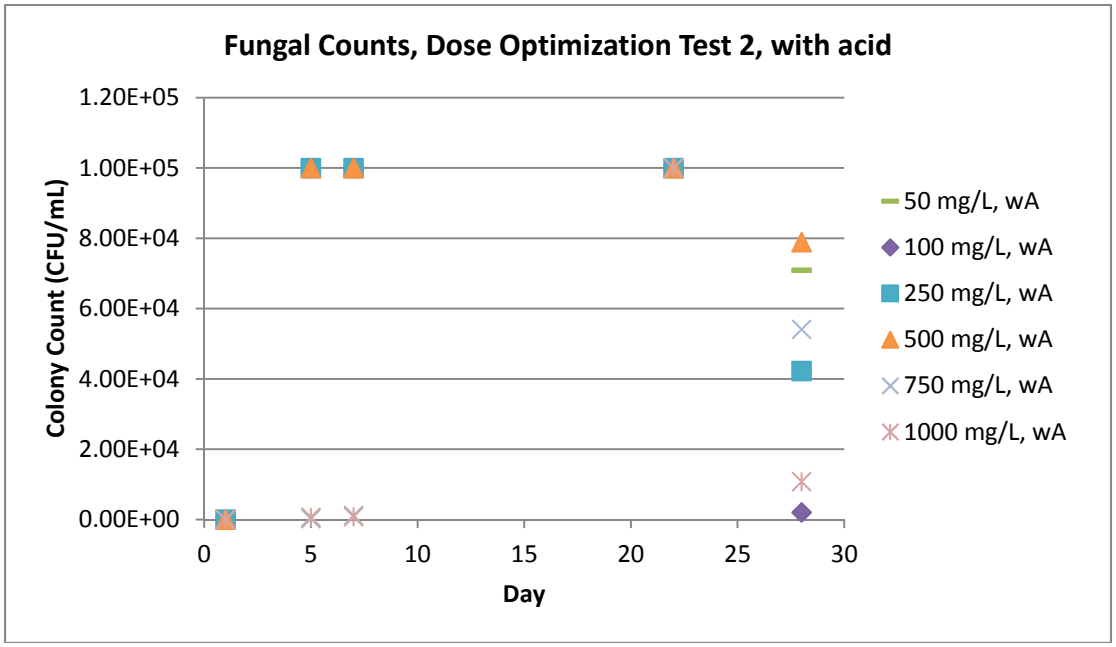

Figure 26. Fungal counts for the dose optimization test, acidified samples only.

While none of the low doses of Bronopol succeeded in preventing microbial growth, the presence or absence of acid plays a role in the Bronopol antimicrobial capabilities. The tradeoff between bacterial inhibition and fungal inhibition correlates to the presence/absence of acid, with acidification inhibiting bacterial growth and the lack of acid permitting the Bronopol to inhibit fungal growth. The prevention of fungal growth correlates to the generation of bromide ion for the higher doses.

V. Conclusion

Because Bronopol did not prevent microbial growth during the final study, it was determined to use the dose of 5 g of Bronopol for continued testing since it had shown to prevent microbial growth with and without acid in the 6 month test. In order to determine whether the urine pretreatment formula could be used in a distillation system, a test using 5 g/L of Bronopol was tested with and without acid using a bench top rotary evaporator that simulated the distillation of urine to recover water from waste. The rotary evaporator was used to mimic the conditions of the urine distiller on the International Space Station to simulate the distillation of pretreated urine for water recovery. The rotary evaporator uses a hot water bath at 60° C, a vacuum pressure of 65 mbar, a chilling coil which condenses the evaporated liquid at 10° C, and a rotation speed of 50 rpm. The urine is distilled under those conditions until 85% of the water is removed. Unfortunately, the distillation of urine, pretreated with 5 g of Bronopol, with and without acid, to 85% water recovery led to substantial precipitate in the brine. Three attempts at rotary evaporation were attempted with three different batches of urine to verify, but in every circumstance, large visible pieces of precipitate formed which are not acceptable for using this pretreatment in a distillation system. The precipitate would prevent the distiller from working properly as it would block tubing. The testing of green pretreatment formulation was to provide microbial control in urine for storage. However, after the failure of the pretreated urine to be distilled without precipitation formation, the continued work was to find a formulation that prevented precipitation after distillation.

VI. Further Work

To help with the solubility of precipitation, different acids were tested with 5 g/L of Bronopol in urine. Citric acid can chelate calcium, which was presumed to be part of the precipitate, possibly calcium sulfate. Five grams of Bronopol was added to a liter of urine and then titrated to a pH of 2.5 using citric acid. Unfortunately, precipitates formed using with this formulation as well. The precipitates were determined to be uric acid using FTIR.

Due to the lack of an oxidizer in the formulation, uric acid crystals would form in the resulting brine. Further work is ongoing to oxidize the uric acid crystals in order to eliminate precipitates. Alternative oxidizers are showing promise at 85% water recovery in the brine. Also, concurrently, the uric acid specific enzyme, is being investigated.