Investigation of Appropriate Cleaning Solutions for Removal of Denatonium Benzoate from Distillery Equipment

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During the COVID-19 pandemic, alcohol distilleries pivoted their production lines to manufacture hand sanitizer. Denatonium benzoate is a bittering agent and denaturant in hand sanitizer and is detectable in trace amounts. As a result, transitioning between hand sanitizer back to distilled spirits creates products with bitter flavors. Several cleaning methods were studied to determine their effectiveness in removing denatonium benzoate from materials in distillery equipment. Hydrogen peroxide and activated carbon were most effective in removing denatonium benzoate in the solution phase, with more than 40% removed compared to the original solution concentration. Strong acidic and basic cleaners were ineffective, with less than 10% of the original compound removed. When tested as cleaners on the distillery materials, hydrogen peroxide and activated carbon methods were no more effective than other rinsing (water, glycerol) or extraction (pure ethanol) cleaners for removing denatonium benzoate. Chemical compatibility, specifically with concentrated ethanol, plays a large role in the permeation of denatonium benzoate into and out of some materials. Hard materials, such as metals and rigid polymers, have good compatibility with ethanol, resulting in little swelling and denatonium benzoate penetration when soaked with sanitizer. Since they retained little denatonium benzoate, they are cleaned by simple rising. However, elastomeric materials vary greatly in their compatibility with high proof ethanol, leading to swelling or breakdown in the presence of hand sanitizer and a greater amount of denatonium benzoate leaching into the material. While ethanol effectively extracts denatonium benzoate out of the elastomers, it damages the material, requiring more frequent replacement.

KEYWORDS
COVID-19
hand sanitizer
denatonium benzoate
distillery cleaning
compatibility

RECEIVED: April 22, 2021
ACCEPTED: June 14, 2021

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INTRODUCTION

The Coronavirus Disease 2019 (COVID-19) created a global pandemic resulting in unprecedented challenges to businesses and personal lifestyles. While this was first and foremost a public health crisis, it also brought about many changes in how people functioned in their daily lives. Businesses shut down, and the majority of people were forced to work from home, leading to a greater demand on many common household goods [1]. As government health agencies repeatedly marketed the message of frequent hand washing and sanitization as the universal strategy to help stay healthy, hand sanitizer became a key target of consumer demand. The beverage industry, particularly distilleries, were granted U.S. Food and Drug Administration (FDA) allowances for the production of hand sanitizer to increase supply [2]. These distilleries were already accustomed to distilling and blending high proof ethanol for human consumption. In pivoting to hand sanitizer, distilleries needed only to blend ethanol with a few additives to produce the sanitizer product. Their facilities were already equipped for ethanol distillation, blending, and bottling, not only combating the supply shortage but also helping to speed up the time to market.

For distilleries to bottle their product as hand sanitizer, a few additives had to be mixed with the ethanol prior to bottling in accordance with FDA and Alcohol and Tobacco Tax and Trade Bureau (TTB) guidelines [3,4]. These included hydrogen peroxide (antiseptic), glycerol (gentle on hands and reduces evaporation), denatonium benzoate (bittering agent), and water (dilutant) [2]. Denatonium benzoate, sold commercially as Bitrex, is a compound that has been used for many years as a denaturing agent in many commercial products, including but not limited to alcohol, cleaning fluids, cosmetics, pesticides, and other household items [5]. It is known for being an extremely bitter tasting compound and is added to these often toxic products to deter consumption and prevent bodily harm [6]. Uniquely, denatonium benzoate can be detected by taste at ppb levels [7].

Due to the potency of denatonium benzoate at such low concentrations, it is extremely difficult to remove from distillery production lines. As distilleries transitioned from manufacturing hand sanitizer back to spirit production, many found that their products were tainted by the characteristic bitter taste of denatonium benzoate. This became an unintended consequence for distilleries producing hand sanitizer, and there was no scientific research available to offer guidance on appropriate cleaning methods for the many materials of construction used in a modern distillery. Now that the role of a distillery firmly includes public health response, it is crucial to develop successful cleaning methods for removing denatonium benzoate from manufacturing equipment. Hydrogen peroxide cleaning is suggested by manufacturers of denatonium benzoate, but some materials that are common in a distillery are not compatible with hydrogen peroxide [8-14].

Distilleries may be called upon once again to assist with hand sanitizer production for public health emergencies, and we seek to better support a transition between sanitizer and premium spirits with an essential flavor profile. Without appropriate and proven cleaning methods in place, the distiller takes significant risks in transitioning back to beverage production. To help provide guidance to these distilleries, we investigated the efficacy of conventional cleaning techniques on the removal of denatonium benzoate from materials used in distillery equipment. We focused on cleaning techniques and materials common in the distillery setting, including solutions of caustic soda (sodium hydroxide), citric acid, hot water, and ethanol. We also looked at cleaning with the glycerol and hydrogen peroxide used in sanitizer production. Finally, distillers frequently use activated carbon before bottling, and we determined the effectiveness of activated carbon as an adsorbent to remove denatonium benzoate from solution.

Our approach to studying the cleaning methods in a distillery first focused on the materials compatibility between the proposed cleaning solutions and a broad cross section of materials used in a distillery (copper, stainless steel, elastomer gaskets, IBC totes, etc.). We then looked at the potential for cleaning solutions to capture or react with denatonium benzoate in solution, which describes the effectiveness at denatonium benzoate reduction within pooled solutions in tanks and piping dead legs. To determine the efficacy of removing residual denatonium benzoate from the surface of process equipment, we tested a range of solutions on materials that have been submerged in sanitizer for prolonged periods. In all, these cleaning studies provide guidance to the distiller on the removal of denatonium benzoate from their facilities during the transition back to flavor sensitive products.

MATERIALS AND METHODS

Cleaning methods chosen for testing were categorized as dilution, extraction, reactive, or adsorption methods. Cleaning reagents were obtained from VWR International (Radnor, PA) and Northern Brewer (Roseville, MN). The distillery materials tested in these studies mimic those present in actual production lines and were requested for testing by local distillers. All materials for these studies were obtained from Grainger (Lake Forest, IL).

MEASURING DENATONIUM BENZOATE CONCENTRATION

The concentration of denatonium benzoate in solution for all studies was determined using LC-ESI-MS analysis. Separations were performed using a Shimadzu Nexera X2 modular UHPLC system (Torrance, CA) consisting of the following modules: SIL-30AC, LC-30AD, CTO-20A, CBM-20A, and DGU-20A equipped with a Kromasil ExternityXT C18 UHPLC column (2.1 x 50 mm, 2.5 µm particles (Supelco, Bellefonte, PA)). The mobile phase gradient used for the separation was initialized at 85% water:15% acetonitrile, held for two minutes, then increased to 15% water:85% acetonitrile at 6.5 minutes, held at this percentage for two minutes and returned to starting conditions at nine minutes. The UHPLC effluent was coupled to a QExactive orbitrap mass spectrometer (ThermoScientific, Waltham, MA) equipped with a HESI source. Data was acquired in the full scan mode at 140,000 mass resolution. Reconstructed high-resolution accurate mass ion chromatograms were used to quantify the denatonium cation. Initial standards of denatonium benzoate diluted in deionized (DI) water were prepared and a linear dynamic range of ~200 fg – 50 ng was determined using LC-MS.

DISTILLERY MATERIALS MASS CHANGE IN ETHANOL

Metals (304 stainless steel and 122 copper), elastomers

(EPDM, Nitrile, 70 Shore A Silicone, Viton FKM), and rigid polymers (polypropylene (PP) and ultra high molecular weight polyethylene (UHMWPE)) were tested in various ethanol solutions to determine any changes in structure and mass. The surface area and volume of each sample is provided in start Table 1. The initial mass of each material was first measured and recorded. The materials were then submerged in 140 proof ethanol, 200 proof ethanol, or hand sanitizer for three days. The hand sanitizer was a liquid solution prepared with 77% v/v of 200 proof ethanol, 1.4% v/v of pure glycerol, 0.38% v/v of 30% hydrogen peroxide, 0.00037% w/v of denatonium benzoate, and 17% v/v of deionized (DI) water according to FDA and TTB guidance. After three days, the materials were removed from their respective solution, dried completely, and the final mass was recorded. The percent change in mass was calculated by comparing the final mass to the initial mass of the material. Three replicates were prepared for each of the materials in each of the ethanol solutions.

SOLUTION PHASE INTERACTIONS WITH DENATONIUM BENZOATE

Initial studies tested the solution phase activity of various cleaning approaches to consuming denatonium benzoate. Six cleaning methods were chosen for testing based on cleaning products readily available or easily attainable in distilleries that are producing hand sanitizer. Cleaning solutions were prepared in DI water according to literature to an appropriate concentration used in the industry.

Solutions of 0.5 mg/mL denatonium benzoate in DI water were prepared. Each solution was mixed with a respective cleaning solution to achieve a final denatonium benzoate concentration of 0.05 mg/mL. The solutions were stirred continuously, and the treatment proceeded for 15 minutes. An appropriate neutralization strategy was used for each solution to halt the activity and adjust the pH to 7. Table 1 details the cleaning methods investigated, appropriate concentrations, and neutralization strategies used in this study.

LC-MS analysis was used to determine the remaining concentration of denatonium benzoate in solution. Three replicates were performed per cleaning method. The remaining denatonium benzoate concentration in each solution was compared to controls of 0.05 mg/mL denatonium benzoate in DI water to determine the ratio of denatonium benzoate remaining in solution relative to the initial amount.

DENATONIUM BENZOATE REMOVAL FROM DISTILLERY MATERIALS USING CLEANING METHODS

Results from the solution phase reaction studies were used in determining appropriate cleaning solutions for extracting denatonium benzoate from the distillery materials. Only cleaning solutions that were promising for denatonium benzoate removal were continued for further testing. Table 2 lists the cleaning methods that were used for testing with distillery materials. Hot water was prepared by heating DI water to 70˚C. All other solutions were prepared with room temperature DI water at 24˚C.

The same distillery materials were used as in the previous mass change experiments. Materials were submerged in hand sanitizer solution for three days. The materials were

TABLE 1 Cleaning methods tested for the removal of denatonium benzoate in solution with concentrations used in distilling applications. Neutralization strategies were specific to each cleaning method to adjust the pH. Activated carbon NORIT GAC 12-40 Mesh corresponds to when 90% of granules are captured with a sieve size between 0.42 to 1.70 mm.

CLEANING METHOD	CONCENTRATION FROM LITERATURE	CONCENTRATION USED IN SOLUTION PREPARATION	NEUTRALIZATION STRATEGY
Citric Acid	1 tbsp/gal [15]	3.78×10^{-3} g/mL	Addition of 1N Sodium Hydroxide
Hydrogen Peroxide	3% v/v [16]	5.00 mL of 30% H_2O_2 diluted in 45.00 mL DI H_2O	Addition of 1M Sodium Metabisulfite, then 1N Sodium Hydroxide
Bleach	200 µL/L [5]	0.200 µL/mL	Addition of 1M Sodium Metabisulfite, then 1N Sodium Hydroxide
Sodium Hydroxide	1% w/v [17]	1.00 g/mL	Addition of 37% Hydrochloric Acid
Powdered Brewery Wash	1 oz/gal [18]	7.49×10^{-3} g/mL	Addition of 37% Hydrochloric Acid
Activated Carbon	150 mg/L (NORIT GAC 12-40 Mesh)[19]	1.50×10^{-4} g/mL	Filter 12-40 Mesh Activated Carbon Particles from Solution

then removed from solution and submerged in a respective cleaning solution, which was continuously stirred for 15 minutes. The materials were removed from the cleaner and submerged into 200 proof ethanol for 24 hours to extract the remaining denatonium benzoate from the material. The liquid extracts were then analyzed using LC-MS analysis to determine the relative amount of denatonium benzoate remaining. Three replicates were prepared for each of the materials using each cleaning solution. Control samples of 0.05 mg/mL denatonium benzoate in DI water were prepared for each batch of samples and LC-MS analysis was used to determine the original concentration of denatonium benzoate in solution before treatment. The concentration of denatonium benzoate in each of the sample extracts after cleaning was compared to the concentration of denatonium benzoate in the control samples to determine the percentage of denatonium benzoate remaining after treatment.

TABLE 2 Cleaning solutions tested and their method of removing denatonium benzoate from various distillery materials.

CLEANING SOLUTION	METHOD OF CLEANING
Hot Water	Dilution
Pure Ethanol	Extraction/Dilution
25% v/v Glycerol	Extraction/Dilution
3% v/v Hydrogen Peroxide	Reactive & Dilution
Activated Carbon	Adsorption & Dilution

OUTLIER TESTING

Materials extraction studies were repeated for polypropylene (PP) in glycerol and ethanol rinsing methods, as they produced large standard deviations with three replicates. Once replicates were analyzed, the 6 data points were combined for outliers to be determined using the method based on the interquartile range. Upper and lower limits for outlier determination were calculated using the 25th (Q1) and 75th (Q3) percentiles and the interquartile range (IQR = Q3 – Q1) of the data set. Any outliers detected in the data set were noted, but not included when graphing.

$$Upper\ Outlier\ Limit = Q3 + 1.5 * IQR \quad \text{[Equation 1]}$$

$$Lower\ Outlier\ Limit = Q1 - 1.5 * IQR \quad \text{[Equation 2]}$$

SIGNIFICANCE TESTING

For statistical analysis, two-group t-testing was used to compare the percent change in mass between materials when placed in various ethanol solutions. Two-group t-testing was also used to relate the relative amount of denatonium benzoate remaining in solution from various cleaning methods in the initial reaction and materials extraction studies. Differences in the samples were considered significant if $p<0.05$.

TABLE 3 Chemical compatibility of common distillery materials with various cleaning methods tested for denatonium benzoate removal. Each pairing was rated from A-D for resistance to chemical attack, and 1 for thermal dependence if applicable [8-13]. PBW is a powdered brewery wash with main ingredients sodium metasilicate and sodium percarbonate. EPDM is ethylene propylene diene monomer and UHMWPE is ultra-high-molecular-weight polyethylene.

MATERIAL	HOT WATER	ETHANOL	GLYCEROL	CITRIC ACID	HYDROGEN PEROXIDE	BLEACH	SODIUM HYDROXIDE	PBW	ACTIVATED CARBON
EPDM	A	A	A	A	B1	A1	A1	A	A
Nitrile	A	D	A	A	B1	C1	B1	D	A
Viton (FKM)	A	D	A	A	A	A	B	D	A
Silicone	B	B	A1	A1	B1	B1	A1	C	A
UHMWPE	A	A	A	A	A	A	A	A	A
Polypropylene	A	A	A	A	A	A1	A	A	A
304 Stainless Steel	A	A	A	A	B	D	A	A	A
122 Copper	A	A	A	C	D	D	D	B	A

A	Excellent – Resistance to chemical attack. No change to material structure.
B	Good – Slight chemical attack occurs and only minor effects to material structure, including slight corrosion or discoloration.
C	Fair – Some chemical attack occurs and moderate effects to material structure, including loss of strength and swelling. Not recommended for continuous use.
D	Poor – No resistance to chemical attack and severe effects to material structure. Immediate damage may occur. Not recommended for any use.
1	Compatible to 72˚F (22˚C)

RESULTS AND DISCUSSION

Manufacturing facilities specifically choose equipment materials that will be compatible with the liquids and solids that they will be in contact with during production. For example, distillery lines are designed to transport the liquids to and from the distillation columns; additionally, the columns themselves are designed to withstand high heat and the transport of concentrated ethanol. However, when these distilleries transitioned their systems to hand sanitizer production during the COVID-19 pandemic, these materials were in contact with many reagents that were outside of their original purpose, including the bittering agent denatonium benzoate.

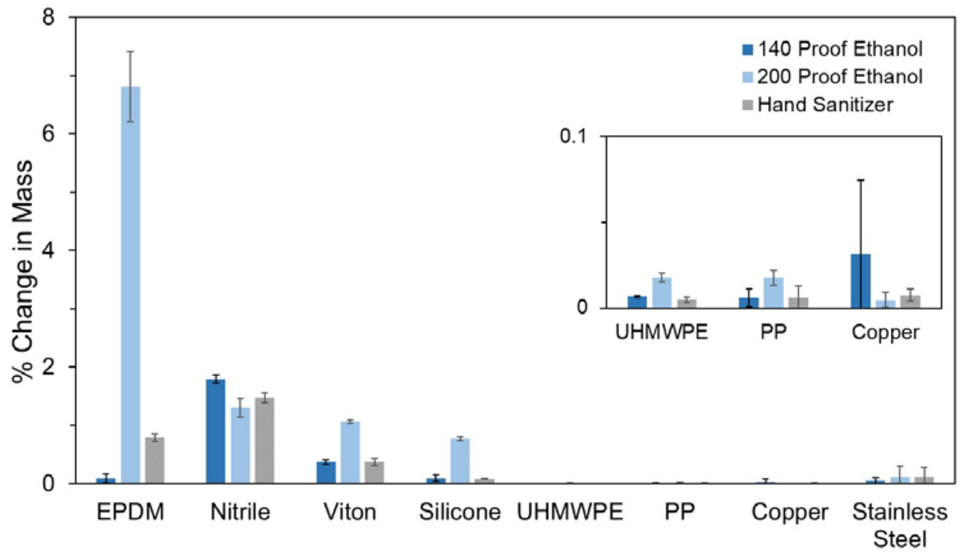

FIGURE 1 Percent change in mass of each distillery material after being soaked in 140 proof ethanol, 200 proof ethanol, and hand sanitizer solutions for three days. EPDM is ethylene propylene diene monomer, UHMWPE is ultra-high-molecular-weight polyethylene, and PP is polypropylene. Error bars represent the standard deviation of n=3 sample replicates.

When deciding on cleaning approaches to remove trace amounts of denatonium benzoate within the production lines, it was important to determine if these cleaning chemicals would be compatible with each of the equipment materials. The materials were categorized into three groups – metals, elastomers, and rigid polymers. All materials were chemically compatible with hot water, glycerol, and activated carbon. The metals and rigid polymeric materials were also compatible with ethanol, although some elastomers were prone to chemical damage (Table 3).

The chemical compatibility of each material with ethanol played a key role in whether denatonium benzoate could permeate into or out of the material. The metal and rigid polymeric materials all have excellent compatibility with ethanol, and as expected, had little changes in their original mass when placed in any ethanol solution for prolonged periods of time (Figure 1, SI Table 2).

In comparison, there was more variability in the mass change of the elastomeric materials relative to their original mass, corresponding to differences in the compatibility of these materials with ethanol (Table 3). This was especially evident when the elastomers were placed in hand sanitizer and 200 proof ethanol, as their percent change in mass was significantly different than all the metal and rigid polymers, indicating that at high proof, these materials were more prone to either swelling or structural break down (SI Tables 3-4). As a result of having greater percent changes in their mass, the elastomeric materials are more likely to allow permeation of denatonium benzoate than the other tested materials. Additionally, it is expected that 200 proof ethanol will be able to permeate into these elastomers to extract out any denatonium benzoate that penetrates the original material.

The cleaning methods studied were liquid solutions except for activated carbon, which contains solid particles that are free-flowing in DI water. Activated carbon is a powdered derivative of charcoal that has been oxidized and contains a large number of micropores, increasing the surface area available to adsorb various chemicals or pollutants in solution, in this case denatonium benzoate [20]. Activated carbon with 12-40 mesh was used in these studies, as it is one of the most common sizes used in industrial systems [21]. This 12-40 mesh relates to a sieve size in which most of the activated carbon granules are retained, and in this case corresponds to 90% of granules being retained with sieve sizes of 0.42 – 1.70 mm. Because it is considered an inert solid material, activated carbon is not chemically reactive and will not damage the chemical nature or cause swelling of any of the distillery materials. However, due to its nature as a granular solid, there are concerns with abrasion as well as becoming trapped in tubing and pump systems [22]. This makes the long-term uses of activated carbon cleaning less desirable, as it could erode the materials over time. To combat these issues, it is recommended to install an appropriate filter system to remove the solid particles once used or to create a mesh/membrane setup within the equipment to contain the activated carbon, so liquids that need denatonium benzoate removal could flow through the mesh instead of allowing free-floating particles in solution. Since all other cleaners are liquid solutions, they are not a concern for abrasion.

All metals and most rubbers were poorly compatible with bleach. It is known that bleach is corrosive to stainless steel and copper alloys, as the hypochlorite ions undergo a redox reaction to form free chloride ions, which adsorbs on the metal's surface and initiates local pitting corrosion [23-25]. Bleach is concerning to many elastomer seals and gaskets for the same reason, as it is incompatible with three of the four elastomer materials used in these studies (Table 3). Additionally, chlorine cleaners, such as bleach, are not recommended in distillery settings, as they can form TCA (2,4,6-trichloroanisole). TCA is concerning because it creates mold within production lines and aging barrels which is very difficult to remove and can change the flavors of the alcohol products [26]. Therefore, bleach should be avoided and only be used as a last resort for cleaning distillery equipment. Instead, many distilleries use dilute citric acid to clean copper stills, as it is a chelating agent that binds to ions to prevent precipitate deposits on the surface of the copper [27,28]. However, increasing the citric acid concentration or using it excessively is known to shorten the lifespan of the still, and therefore should only be used sparingly at or below 1 tbsp/gal [15].

From a general chemical compatibility standpoint, the safest universal cleaners for distillery equipment would be hot water, glycerol, and activated carbon. All listed materials are good at resisting chemical attack from these solvents. This is because these three cleaning methods are better for binding to or rinsing denatonium benzoate away from a material rather than chemically reacting with it. These methods are low risk for systems with unknown or highly susceptible materials of construction. However, the other cleaning methods should not be overlooked, as some may be better depending on the classification of the material (metal, elastomer, rigid polymer) and would still be appropriate for use if certain materials could be isolated from others through system disassembly.

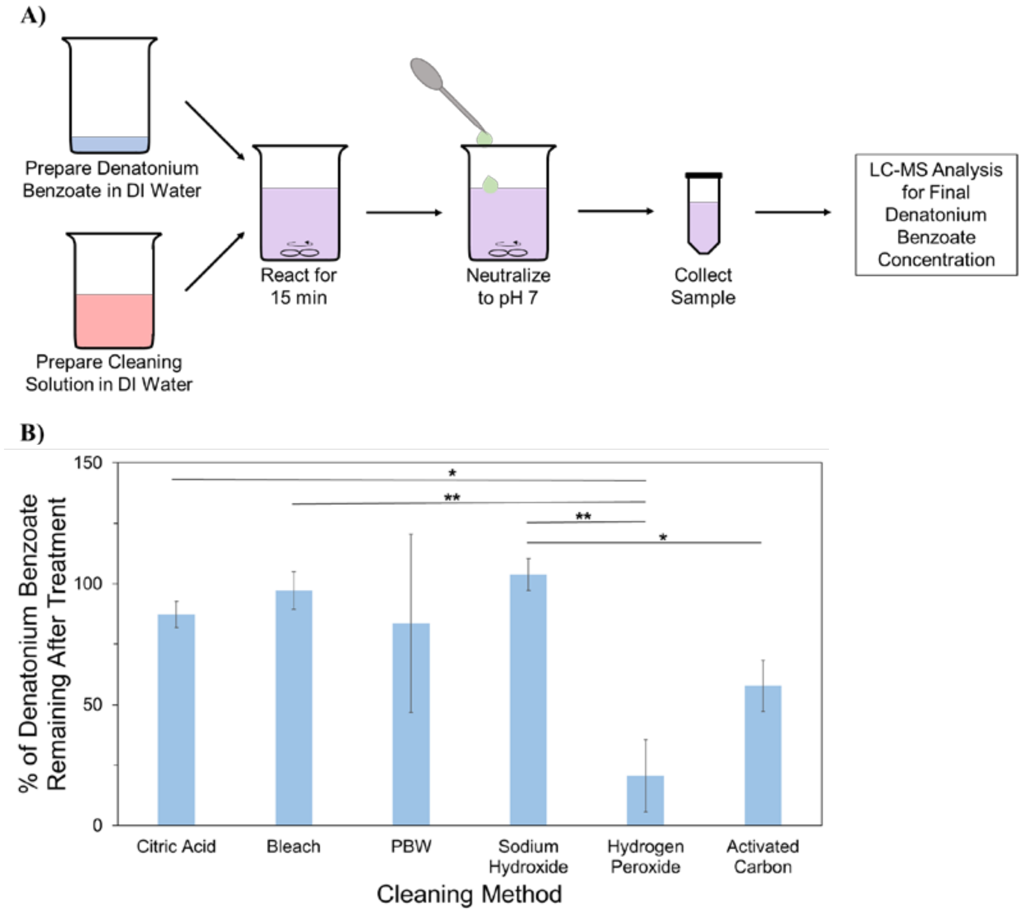

FIGURE 2 A) Schematic representation of solution phase interactions of various cleaning methods with denatonium benzoate. Cleaning solutions were prepared according to Table 1. B) Percentage of denatonium benzoate remaining in solution compared to the original concentration after treatment with various cleaning methods. PBW (powdered brewery wash) is a non-caustic oxidizing alkaline cleaner. Significance was determined using 2-group t-testing with $p<0.05$(*) and $p<0.01$(**). Error bars represent the standard deviation of n=3 sample replicates.

ELIMINATION OF DENATONIUM BENZOATE VIA SOLUTION PHASE INTERACTIONS

We first determined if there were any common distillery cleaning methods that would be effective in reducing the concentration of denatonium benzoate in solution prior to testing on the distillery materials. Cleaning solutions (Table 1) were reacted with a solution of denatonium benzoate in DI water for 15 minutes, then neutralized to pH 7. Using LC-MS, the percentage of denatonium benzoate remaining in solution was determined, which gave insight into which cleaning methods performed best (Figure 2A).

Initial testing determined that hydrogen peroxide and activated carbon were promising in lowering the denatonium benzoate concentration in solution, as they had the lowest average percent of denatonium benzoate remaining compared to the starting concentration. Hydrogen peroxide was effective in removing 79% of the denatonium benzoate present in the original solution, outperforming all other tested cleaners. It is used in many cleaning products as an antiseptic and can also be used individually as a stand-alone cleaner as well. Hydrogen peroxide is a strong oxidizing agent. In solution, hydrogen peroxide can dissociate to produce hydroxyl and hydroperoxyl free radicals that react with organic compounds and convert these organic compounds into more soluble materials that are easily removed in water [29, 30]. Therefore, it is successful at cleaning many larger organic compounds, such as denatonium benzoate. Activated carbon, which uses adsorption, was the second-best cleaning method, removing 42% of the denatonium benzoate from the original solution. As activated carbon is inert, it did not require neutralization, only filtering out the solid particles from solution. Statistical analysis revealed that these two cleaning methods were the only ones with significant differences in the percentage of denatonium benzoate remaining after treatment when compared to at least one other method (Figure 2B).

Denatonium benzoate is an effective bittering agent in a wide pH range, supporting its effectiveness at rendering the alcohol unpalatable even when mixed with a variety of beverages [31]. There are a variety of cleaning methods used for removal of denatonium benzoate based on acid or base interactions. Acidic reaction systems are used to clean mineral deposits, rust, oxides, and tarnish from surfaces [32]. They usually contain chelants, which bind to metal ions and prevent them from forming precipitates on their surface. Citric acid is an excellent chelating agent, and is widely known to bind with copper ions, making it a popular cleaner for stills and other equipment. Basic (high pH) reaction systems are more commonly used to remove oils and fats from surfaces [32,33]. When using a basic cleaner, a chemical reaction called saponification occurs, converting organic fats and oils into a soap that is water soluble and can easily be removed from the surface [34]. However, high pH cleaners are generally more corrosive to metals and milder alkaline cleaners are recommended to avoid surface damage.

The remaining cleaning methods tested were extremely acidic (citric acid) or basic (bleach, powdered brewery wash (PBW), sodium hydroxide), but were not as effective in removing denatonium benzoate. Each method had on average greater than 80% denatonium benzoate remaining in solution after treatment. In addition to bleach having poor chemical compatibility with the materials used in distillery equipment (Table 3), bleach performed poorly in the reaction studies, with the final solution retaining 97% of the denatonium benzoate from the original solution.

PBW had large variability in the sample data, making it difficult to determine its efficacy in removing denatonium benzoate from solution. Since PBW is an alkaline cleaner, with primary ingredients sodium percarbonate and sodium metasilicate, it was expected to perform similarly to other alkaline cleaners.

Overall, hydrogen peroxide and activated carbon were the only compounds that effectively reduced the level of denatonium benzoate through solution phase neutralization. The pH stability noted in the literature is supported here, where reactions with acids and bases failed to reduce the solution level of denatonium benzoate.

REMOVAL OF DENATONIUM BENZOATE FROM DISTILLERY MATERIALS

Since residual amounts of denatonium benzoate left on distillery equipment can cause extremely bitter flavors in bourbon products or other spirits, it is important to determine appropriate cleaning methods that will not only be compatible with the equipment materials, but also be successful at denatonium benzoate removal. Here, we simulate the cleaning of several distillery materials that have been exposed to a controlled dose of a denatonium benzoate through hand sanitizer. Effective cleaning methods determined from the solution phase studies (Figure 2B), as well as a few additional rinsing approaches, were tested for removing denatonium benzoate from distillery materials. These materials were soaked in hand sanitizer solution containing denatonium benzoate for three days, transitioned to an appropriate cleaning method for 15 minutes, then placed in pure ethanol for 24 hours to extract any remaining denatonium benzoate from the materials (Figure 3A).

METALS

Metals are generally impermeable to water and other chemicals [35]. As a result, copper and stainless steel are two common metals used in manufacturing equipment, such as distillation columns, stills, and storage vessels [36]. We expected that when soaked in hand sanitizer, denatonium benzoate would not absorb into copper or stainless steel and would instead remain in the surrounding solution; as a result, only remnants of the denatonium benzoate would remain when the hand sanitizer was removed and replaced with a cleaning method. The results in Figure 3B support this hypothesis, as the two metals tested had the lowest percent of denatonium benzoate remaining after treatment when compared to the other elastomers and rigid polymeric materials. Additionally, there is no significant difference between the effectiveness of any of the cleaning solutions with these two metals, so these systems are effectively cleaned with any form of rinsing.

ELASTOMERS

The elastomer materials tested (EPDM, nitrile, Viton, and silicone) are commonly found in pipe fittings, such as gaskets and O-rings, that can withstand high pressure and prevent liquid or gas permeation into important downstream equipment [37]. These materials had the highest variability in the removal of denatonium benzoate after exposure to the hand sanitizer solution and cleaning treatment.

Nitrile and Viton in particular, had the lowest percentage of denatonium benzoate remaining when rinsed with 200 proof ethanol, likely due to their high swelling in ethanol (Figure 1) and poor chemical compatibility with polar solvents [38]. Since ethanol is polar due to its hydroxyl group, it can undergo a substitution reaction with the polar groups in the elastomer backbone; as a result, it is prone to an increase in swelling and permeation of ethanol into the material, compromising the structural integrity of the elastomer with long-term exposure [38].

Viton is a brand of fluorocarbon elastomer (FKM) used in many sealing applications. There are various specialty types of Viton products, varying between 60-70% fluorine content, which in turn varies the chemical and temperature resistance of the material [38,39]. Viton products have improved performance and resistance to ethanol and other reagents by increasing the fluorine content because the bulky fluorine atoms can help shield the polymer backbone from attack [40]. Conversely, a lower fluorine content reduces this shielding and increases attack to the polymer backbone, resulting in increased permeation of ethanol and swelling of the elastomer (Figure 1) [41]. A type of Viton FKM, the form of Viton with a lower fluorine content, was used in these studies rather than Viton ETP, which contains a higher fluorine content. This explains why Viton in these studies enabled swelling in ethanol (Figure 1) and most of the denatonium benzoate was removed from the material during the cleaning step (Figure 3).

Because both nitrile and Viton have poor chemical resistance to ethanol, the denatonium benzoate likely leaches into these materials with the hand sanitizer soak [12,13]. Then when cleaned with 200 proof ethanol, the ethanol facilitates an improved removal of denatonium benzoate compared to other cleaning methods in Figure 3B. However, both materials have good resistance to the permeation of water, hydrogen peroxide, and glycerol solutions [12,13]. This indicates that none of these methods will work well in extracting out the denatonium benzoate once it is leached into the material. A two-group t-test revealed that there is a significant difference for nitrile and Viton cleaned with hydrogen peroxide compared to all other materials tested

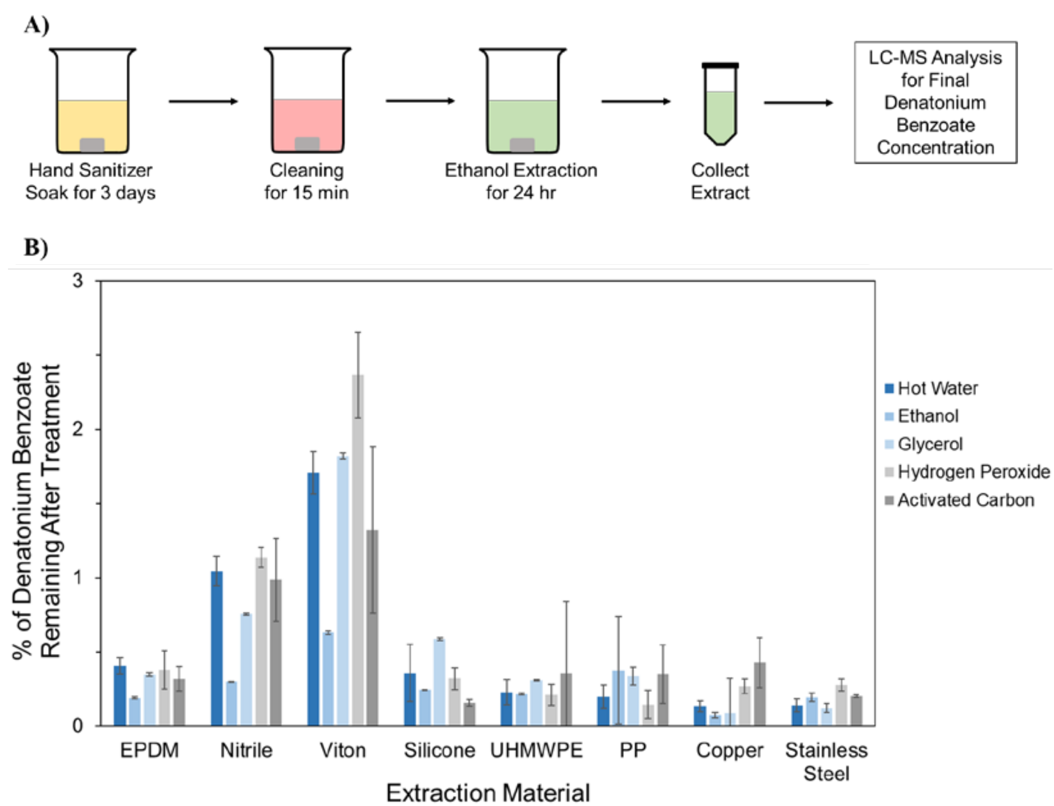

FIGURE 3 A) Schematic representation of the interaction and removal of denatonium benzoate with various distillery materials. B) Percent of denatonium benzoate remaining in solution following a soak in hand sanitizer solution, cleaning, and extraction with ethanol compared to the original concentration (control). EPDM is ethylene propylene diene monomer, UHMWPE is ultra-high-molecular-weight polyethylene, and PP is polypropylene.

TABLE 4 Hydrogen peroxide cleaning effectiveness across material types. A) P-values obtained from significance testing using a two-group t-test comparing hydrogen peroxide cleaning of Nitrile and Viton to all other materials with this same cleaner. B) P-values comparing ethanol and hydrogen peroxide cleaning methods for Nitrile and Viton materials. Comparisons were considered significant if p<0.05. EPDM is ethylene propylene diene monomer, UHMWPE is ultra-high-molecular-weight polyethylene, and PP is polypropylene.

A)	VITON	EPDM	SILICONE	PP	UHMWPE	304 STAINLESS STEEL	122 COPPER
Nitrile	1.63E-03	6.60E-04	1.15E-04	9.45E-05	6.60E-05	5.70E-05	7.86E-05
Viton		3.27E-04	2.32E-04	1.81E-04	1.88E-04	2.04E-04	2.07E-04

B)	ETHANOL vs HYDROGEN PEROXIDE
Nitrile	2.52E-05
Viton	5.17E-04

with this cleaner (Table 4A), where the peroxide is particularly poor at removing the large amounts of denatonium benzoate imbibed in these materials. In sharp contrast to solution phase experiments (Figure 2), the hydrogen peroxide is significantly less effective at removing denatonium benzoate from the nitrile and Viton than ethanol rinsing (Table 4B). This is likely due to these elastomers having high swelling and poor chemical resistance to ethanol, while having good compatibility with hydrogen peroxide (Table 1).

While ethanol can effectively remove denatonium benzoate from these two materials, it is only recommended as a short-cycle cleaner and not as a long-term cleaning solution, as it can cause swelling and damage the material backbone, weakening the elastomeric structure with time (Figure 1). If 200 proof ethanol is used routinely as a cleaning method for denatonium benzoate, these elastomers will need replacement more frequently.

As EPDM and silicone rubbers have a fair chemical resistance to ethanol, it is likely that the denatonium benzoate in the hand sanitizer solution was not able to diffuse into these materials as easily as nitrile and Viton, therefore, not as much was retained within the material to be removed by a cleaner [11,13]. The percentage of denatonium benzoate extracted out of the EPDM and silicone materials was much lower than that of nitrile and Viton and was much more comparable to the rigid polymer and metal materials. EPDM rubber also has a low absorption of water, acting as a barrier to not absorb water-based liquids, which often leads to its use in outdoor applications [42,43]. Both EPDM and silicone are more resistant to chemical attack by polar solvents [44,45]. This suggests why the hand sanitizer containing ethanol and denatonium benzoate did not diffuse into these materials as much as the other elastomers (Figure 1). Consequently, there was less denatonium benzoate to be extracted out of the material and an overall lower percentage of denatonium benzoate remaining in the final extracts (Figure 3B). However, both materials are still prone to swelling and structural changes in the presence of 200 proof ethanol, used in extracting out any denatonium benzoate after the hand sanitizer soak, so they will still need constant monitoring and replacement if cleaned frequently with high proof ethanol (Figure 1).

RIGID POLYMERS

More rigid polymeric materials, such as plastics (UHMWPE and PP), are found in distilleries in totes, storage containers, and components in pumps and valves. They are considered porous plastics and are similar in terms of mechanical strength and chemical resistance [46,47]. Both plastics offer good chemical resistance to the components in the hand sanitizer solution and minimal swelling (Figure 1), so we expect that little denatonium benzoate would permeate into the material when placed in hand sanitizer solution [8,9,48,49]. From the findings in Figure 3B, this hypothesis is supported, as both had low levels of denatonium benzoate extracted from the materials. See SI Table 5 for outlier sample analysis. The low magnitude of extracted denatonium benzoate was comparable to the impermeable copper and stainless steel samples.

CONCLUSION

Material and chemical compatibility plays a large role in selection of a cleaning approach in a given processing facility. From the initial solution phase interactions, many cleaners, particularly those extremely acidic or basic, were ruled out for being ineffective in removing the denatonium benzoate or for having poor compatibility with several of the materials tested. Of the cleaners studied in the solution interactions for pooled liquids, only hydrogen peroxide and activated carbon were effective in eliminating a significant amount of denatonium benzoate while still being compatible with most common distillery materials. Therefore, it recommended to use hydrogen peroxide or activated carbon in a distillery setting where dead legs or pooled liquids are present.

We also observed a significant role of material of construction in the persistence of denatonium benzoate. All cleaners performed similarly in removing denatonium benzoate from the surface of metals and rigid polymers. For these cases where little pooled liquid is expected, it would be recommended to use cleaners categorized as rinsing approaches with relatively neutral pH, such as water, glycerol, or ethanol, as they are readily available and would not cause issues when transitioning back to spirits production. Activated carbon performed similarly to the rinsing approaches. Activated carbon is unique in that it is an inert adsorption method that will not cause a chemical reaction with any of the materials or species. However, due to being a solid material, it is more likely to cause abrasive damages and cause clogging in equipment with use.

Elastomer materials used in seals and gaskets are the most challenging for the elimination of denatonium benzoate that has been in contact with the materials for 24 hours. While very little denatonium benzoate in the hand sanitizer solution is being leached into the metal or rigid polymer materials, there was a large percentage of denatonium benzoate remaining after cleaning in some elastomer materials. All elastomers had noticeable mass changes in the presence of hand sanitizer and 200 proof ethanol solutions, indicating that denatonium benzoate can permeate into these swollen materials. Only 200 proof ethanol worked well in removing denatonium benzoate from the elastomeric materials, likely due to these materials having high swelling and poor compatibility with high proof ethanol. There is a tradeoff between removing the denatonium benzoate from the elastomers and maintaining the structural integrity of the material. Because of this poor chemical resistance, ethanol will cause these materials to swell or break down, so it is only recommended for removing the denatonium benzoate if this degradation can be tolerated or the elastomer components may be replaced. If larger quantities of ethanol are to be run through the system, it is recommended to use another cleaning method on these materials that would not cause swelling and damage its structural integrity. If 200 proof ethanol is used often for cleaning, it is likely that all of the elastomers will need more frequent replacement.

FUNDING STATEMENT

This publication was supported in part by the University of Kentucky's COVID-19 Unified Research Experts (CURE) Alliance. This publication was supported in part by the National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health, through Grant UL1TR001998. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. This material is based upon work supported by the National Science Foundation Graduate Research Fellowship under Grant No. 1839289. Any opinion, findings, and conclusions or recommendations expressed in this material are those of the authors(s) and do not necessarily reflect the views of the National Science Foundation.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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