WINERY SANITATION

Section 2.

Acidulated Sulfur Dioxide

Acidulated SO_2 is an effective sanitizing agent especially for hoses and other enclosed systems.

- The antimicrobial activity of SO_2 is pH-dependent.
- Sulfur dioxide is usually made up as 100 mg/L SO_2 (or 200 mg/L potassium metabisulfite) in cold water acidulated with citric acid at 3 g/L.

Due to its volatility and corrosive properties, as well as employee health concerns, acidulated SO_2 solutions should only be used in a well-ventilated area away from metal surfaces. Employees should also be cautioned to avoid direct contact or inhalation of SO_2. Although wineries commonly prepare this sanitizer in acidulated hot water, this practice serves only to increase the volatility of SO_2 (and, hence, reduce its concentration), as well as increasing safety risks.
When not in use, SO_2 solutions should be stored in clearly identified, sealed containers to minimize volatilization.

Peroxides

Peroxides or “proxy” compounds are characterized by having at least one pair of highly reactive covalently bonded oxygen atoms (–O–O–) that break down to generate toxic singlet or superoxide (O_2^-) oxygen. Hydrogen peroxide (H_2O_2), as commercially available, ranges from 3 to 30% v/v.

- Unless stored in a sealed container, H_2O_2 rapidly breaks down.
- Even when stored properly, chemical decomposition occurs and, thus, it is best to replace laboratory peroxide (30% v/v) on a regular basis.
- At concentrations >5% v/v, hydrogen peroxide becomes a strong irritant that can cause burns and blisters on exposed skin.

Sodium percarbonate is a stabilized powder containing hydrogen peroxide. The product is widely used as the active component in laundry detergent and all fabric bleach as well as denture cleaners, pulp and paper bleaching and wine barrel treatment. Sodium percarbonate has an available oxygen equivalent to 27.5% H_2O_2 and, like peroxide, breaks down to the reactive form (oxygen) as well as water and, additionally, sodium carbonate upon full reaction.

Sodium percarbonate is sold under the trade name Proxycarb™ and is widely used to treat .barrels. However, given the porous nature of wood, treatments do not result in 100% kill. There is a high probability that viable populations can be sequestered in areas where the active agent can not reach.

Peroxyacetic acid (PAA), sometimes referred to as “peracetic acid,” is a highly reactive oxidant with antimicrobial properties similar to hydrogen peroxide. In diluted form, its best applications include barrel and bottling line sanitation and sterilization. As a sanitizer and sterilant, PAA has several desirable characteristics over H_2O_2 including the following:

- better stability at application concentrations (100 to 200 mg/L)
- improved compatibility with hard water
- reduced foaming
- exhibits reduced corrosive properties and is biodegradable

Ozone

Ozone (O_3) is one of the most potent sanitizers available. As a strong oxidant, ozone is unstable, with a half–life of only 20 to 30 minutes, depending on conditions (Khadre et al. 2001).

- Ozone is most commonly dissolved in water rather than applied as a gas. Its ability as a sanitizer is a function of time and concentration, among other factors.
- Because O_3 rapidly degrades to O_2, it cannot be stored and must be generated on demand. This is accomplished by use of equipment that exposes a stream of dry air to either ultraviolet light (185 nm) or electrical discharge (common winery ozonators).
- O_3 is used in clean-in-place (CIP) operations such as the bottling line or for treating in–house water for off–odors or discoloration.
- Ozone degrades rapidly in water with a high mineral content.
- Ozone degrades rapidly in warm (>35^{o}C/95^{o}F) water. Therefore, its primary application is cold water, including in recirculation systems.

Ozone is effective against bacteria, fungi, as well as bacterial and fungal spores (Khadre et al., 2001). Ozone is less corrosive against stainless steel (316L) than chlorine Hampson (2000). It generally does not break down gaskets. Greene et al. (1994) noted only slight differences between several gasket types (Buna N, white Buna N, EPDM or ethylene propylene diene monomer, polyethylene, silicone rubber, Teflon, and Viton) treated with ozone.

Ozone is frequently used in barrels as follows:

- Preliminary removal of debris using high-pressure water wash followed by thorough blast with stream or hot water.
- Cool water rinse for 2-3 minutes prior to ozonation.
- Treat with filtered and deionized ozonated water (minerals can significantly limit the “holding power” of ozonated water).
- Ozone, like other sanitizers, works based on contact time and concentration. Treatment levels of at least 2 - 2.5 mg/L ozone in barrel are recommended.

Ozone is a strong irritant and uncontrolled exposure may result in inflammation of eyes, nose throat and lungs. Limits for ozone exposure have been set by the

Occupational Safety and Health Administration (OSHA). The legal maximum concentration for an 8–hour continuous exposure is 0.1 mg/L whereas the limit for short–term exposure is 0.2 mg/L for 10 minutes (Khadre et al., 2001). Staff should be well trained and use proper ozone safety monitors.

Hot Water and Steam

Delivered at temperatures >82°C/180°F, both hot water and steam are near ideal sterilants.

- Hot water/steam have excellent penetrative properties and works against all wine/juice microorganisms,
- Hot water and steam are noncorrosive and leave no residue.
- Both hot water/steam may more rapidly degrade gaskets compared to other techniques. The most frequent application for hot water/steam is for sterilization of bottling lines.
- Temperatures greater than 82°C (180°F) are recommended for no less than 20 minutes as monitored at the farthest point from the steam source (i.e., the end of the line, fill spouts, etc.). The sterilization cycle begins when the temperature at that point reaches recommended.
- When steam is used to sterilize tanks, the recommendation is to continue until condensate from valves reaches temperatures greater than 82°C x 20 minutes.
- Dismantling valves, racking arms, etc., and soaking in hot water, while desirable for cleaning, may not yield the time and temperature relationships necessary for sanitation.

Other applications of hot water/steam include barrel cleaning. Typical temperatures range from 60-80°C (140-176°F) used in conjunction with high pressure delivery systems. Recalcitrant precipitates often require temperatures >85°C/185°F and/or steaming. Malfeito-Ferreira et al. (2004) used steam for effective decontamination of barrel staves to an interior depth of 2 mm.

Ultraviolet Light (UVL) and Photon Sterilization Technology (PST)

Although UVL is directly effective against microbes, it has very low penetrative capabilities, and even a thin film of water will serve as an effective barrier between radiation and microbes. Photon sterilization technology (PST) systems work by generating photons from UV rays created by a series of fluorescent tubes. PST is effective against both airborne microbes and those present on contact surfaces. Although relatively new to the wine industry, such systems have been used in the food industry for sometime.

Dry Ice Blasting and Ultrasound/HPU

High pressure application of dry ice pellets is becoming more popular for barrel cleaning and sanitation. The *Rajeunir* (Fr. "rejuvenate") system uses dry ice to effect removal of surface contaminants (tartrates, etc) without significant (1.25 mm) abrasion.

Conversion of Electrical Energy to Lethal Ultrasonic Sound Waves

Yap, et al., 2007 reported the use of high-power ultrasound (HPU) for barrel cleaning including precipitate removal and control of resident microbial populations including *Brettanomyces/ Dekkera*. HPU generates a stream of "micro-bubbles" that, upon cavitation, generate high energy shock waves that impact/disrupt particulates on surfaces.

- HPU is used as an energy source for both cleaning and sanitizing, especially for barrels.
- Advantages of HPU systems include reduced energy costs as well as reduced chemical input.
- HPU may have processing applications for red must extraction.

Table 2. Winery Cleaners and Sanitizers: Advantages and Disadvantages

1. Caustic Detergents (NaOH/KOH):

Advantages:

- Effective/efficient at cleaning heavily deposits including tartrates and stains
- Antimicrobial activity
- Can potentially be used multiple times
- KOH is environmentally friendly

Disadvantages:

- Potentially corrosive on stainless steel above recommended concentrations
- Health and safety risks.

2. Trisodium Phosphate (TSP)

Advantages:

- Effective in the case of lightly soiled equipment
- Phosphates help soften water

Disadvantages:

- Limited solubility in ambient-temperature water
- Not a good destaining agent
- Not environmentally friendly (phosphate content)

3. Phosphoric Acid

Advantages:

- As necessary to reduce/remove mineral deposits and rust
- Soften water

Disadvantages:

- Potentially corrosive towards stainless steel
- Limited detergency

Sanitizers/Sterilants

1. Halogens: Chlorine

Advantages:

- Effective against a broad spectrum of microbes and organics.

Disadvantages:

- Not recommended for use in the winery due to risk of haloanisole formation/contamination (see environmental TCA).

2. Chlorine Dioxide

Advantages:

- Broad spectrum of antimicrobial activity or a wide pH range
- Active against existing biofilms and inhibits formation of new
- Chlorine is not a product/byproduct using current methods

Disadvantages:

- Initially costly
- Vapors from activation step can be a safety hazard

3. Iodophores

Advantages:

- Broad spectrum antimicrobial (including spore-formers) activity
- Effective at low concentration levels (25 ppm)
- Non-corrosive and easy to use

Disadvantages:

- Possible odor and/or flavor concerns if it gets into product
- Most effective in a narrow pH range
- Stains surfaces

4. Quartenary Ammonium Compounds (QUATS)

Advantages

- Stable over a wide range of pH and temperatures.
- Non-corrosive
- Good protection against mold growth

- May be applied directly to surfaces as a foam or gel application and without rinsing provides long-term residual action.

Disadvantages:

- Lack of broad-spectrum activity against microbes (especially Gram-negative bacteria)
- Inactivated by salts and low pH
- Leaves residual film; best for external and non-product contact surfaces.

5. Acidulated Sulfur Dioxide

Advantages

- Inexpensive with long shelf life

Disadvantages:

- Must be acidulated (pH (3-4) for antimicrobial activity.
- Health and safety concerns with the formulated solution
- Very corrosive

6. Peroxides (Sodium Percarbonate)

Advantages

- Effectively cleans and bleaches lightly soiled equipment
- Dissolves rapidly in water
- Rinses off easily
- Considered a strong fungicide

Disadvantages:

- Not as effective as caustic cleaners on heavily soiled equipment

Peroxides (Peroxyacetic Acid)

Advantages

- Strong antimicrobial activity over wide pH range
- Highly effective against broad range of microbes
- Degrades biofilms

- No post-rinse required
- Environmentally friendly

Disadvantages:

- Cost/application.
- As a concentrate, health and safety issues.
- Corrosive to metals such as copper and aluminum

7. Ozone

Advantages:

- Strong antimicrobial activity over a broad pH and temperature range
- Requires very little contact time
- Breaks down biofilms

Disadvantages:

- Poor solubility in water, very short half-life
- Corrosive
- Reacts with organic material
- Health and safety concerns that require safety monitoring
- Cannot be stored for later use
- Initial cost for equipment

8. Hot Water/Steam

Advantages:

- Strong antimicrobial activity
- Sanitizes cracks and non-contact surfaces via heat conduction
- Improves effectiveness of winery soil removal especially when combined with high pressure applicators

Disadvantages:

- Expensive energy costs, maintenance and installation
- Requires more time to sanitize a surface than chemicals
- Can encourage biofilm formation by baking material on surfaces

- May disperse microbes
- May penetrate control boxes leading to equipment malfunction

Sanitation Monitoring

Validating the effectiveness of a sanitation program should be an ongoing concern at every stage in the winemaking process. Each winery should have a HACCP program (hazard analysis and critical control points) for sanitation. Efforts to detect ineffective cleaning and sanitization include the following:

- evaluation of slippery surfaces
- presence of odor
- evaluation of rinse water
- swab test for sterility
- biochemical monitoring

Swab testing involves application of a sterile cotton swab over a defined surface area for a defined period of time.

- The swab is then transferred to a sterile diluent (e.g., peptone) and shaken for a defined period of time prior to membrane filtration.
- The membrane is then transferred to appropriate agar media for growth. Using swab sampling does not allow for complete recovery of microbes.

Other Direct Contact Tests: Where surfaces are flat and smooth, agar plates filled with the appropriate media can be pressed directly against the sanitized surface. In theory, viable cells are transferred directly to the agar plate. Variables affecting success include contact time and pressure. Commercially available direct contact "kits" are available. Various "tapes" have been used in a manner similar to agar plates. In this case, "tape" is applied to surface and subsequently reapplied to agar surface.

Brett Sniff tests: Several companies supply nutrient vials that contain growth media to both support *Brettanomyces* growth and the precursors to form aroma intensive metabolites. A defined volume of wine from one or more barrels is transferred to these kit vials using sterile methods and stored in a warm place for 24-48 hours. An evaluation of the odor produced in these vials can be used as presumptive evidence of viable Brett.

Enzymatic methods: Bioluminescence is the process by which a molecule in the excited state emits light which is then measured photometrically (Hartman et al., 1992). This process can be used to measure the amount of ATP produced by microorganisms during the course of growth. In theory, measurement of this compound should provide an estimate of viable cell numbers since higher populations of microorganisms produce more ATP. As a single yeast cell will have generally more ATP than a bacterial cell, the detection limit for yeast could be as low as 10 cells (Hartman et al., 1992). The luciferin–luciferase assay commonly used to measure ATP is as follows:

Luciferin + enzyme + ATP + Mg^{2+} → Luciferin–enzyme–AMP + pyrophosphate

Luciferin–enzyme–AMP + O_2 → Oxyluciferin + enzyme + AMP + CO_2 + light

Because this assay can be completed in just a few minutes, the technique has been used increasingly *in lieu* of traditional swab and plate methods. Several luminometers and test kits are commercially available. Once the monitor is purchased, the cost per test including sample collection container, swab, and reagents can be inexpensive. While this method has been used in the alcoholic

and non–alcoholic beverage industry (Thompson, 2000), a major drawback lies in the translation of ATP measurements to viable cell counts (Hartman et al., 1992). ATP production will vary between microorganisms, their physiological state (injured, starved, etc.), and this assay can not distinguish between ATP produced by yeast or other microorganisms. As such, interpretation of the results can be difficult, at times.

Molecular methods: Numerous approaches have evolved as attempts to characterize microbes, based upon fundamental similarities or differences (polymorphism) in their genomes. These involve direct comparison at the gene level, or secondarily characterizing proteins encoded by those gene(s). In either case, nucleic acids (DNA and RNA), as well as the proteins that they encode, are extracted and amplified and subsequently separated. Results are then compared to those from reference species, or to available data bases.

Three strategies have evolved to directly compare similarities/differences between isolates by examination of their respective genomes: (1) DNA harvested from isolates is digested by use of restriction enzymes to yield variously-sized DNA fragments; (2) Amplification of specific or randomly-selected regions by polymerase chain reaction (PCR); and (3) nucleotide sequencing of selected amplified areas. In the first two, fragments are then separated electrophoretically and patterns compared against those of other isolates or data bases.

Table 3 Comparison of sanitation monitoring methods

Method	Advantages	Disadvantages	Recommended frequency*
Visual monitoring	• **Quick, all employees are involved** • **No cost**	• **Subjective, cannot be used to reliably monitor sanitation**	**Each application**
Bioluminescence (ATP assay)	• **Results are "real time" and quantitative**	• **Does not indicate viability of microbes present** • **Difficult to correlate ATP and microbial swabbing results**	**Daily/Weekly**
Direct swabbing and culturing	• **Quantifies microbes and can be used to establish trends on contact surfaces over time.**	· **Slow, results taker 4-7 days**	**Weekly/Monthly/ Quarterly**

Safety issues

Sanitation typically uses strong agents such as oxidants (peroxides, ozone), caustics (NaOH or KOH), and/or acidic chemicals (phosphoric acid) as well as pressurized hot water and/or steam. Further, slippery floors resulting from discharge of detergents represent ongoing safety concerns. Employees regularly in contact with sanitizing chemicals should thoroughly trained (and retrained as necessary) in their safe use. Further, they should issued personal protective equipment (PPE) including water–repellant aprons and boots (non-skid soles) in addition to goggles and appropriate gloves.

The employee's "Right to Know" is a cornerstone of Federal and State health and safety regulations. Thus, part of any employee training program should include

identification of the health and safety information concerns associated with any chemical or operation. This information is contained in the Material Safety Data Sheets (MSDS) or Safety Data Sheets (SDS) that are obtained from suppliers or on-line. The compendium of MSDS/SDS relevant to the worksite must be made available to any employee at any time (24/7). To comply with regulations, it is recommended that they be kept alphabetized in clearly identifiable and displayed binders in the work area.

Fermenters and/or storage tanks are considered as "confined spaces." When it is necessary for workers to enter, special health and safety regulations apply. These include preliminary forced-air ventilation of the tank to reduce carbon dioxide levels followed by verification that have safe levels have been reached. Current National Institute for Occupational Safety and Health (NIOSH) definitions of "safe" for oxygen is >19.5% (v/v) whereas for carbon dioxide the permissible exposure level (PEL) is <5,000 ppm (on-line: 2008). Carbon dioxide and oxygen are easily measured by use of readily available and moderately-priced meters and probes. Once cleared for entry, employee(s) must be equipped with harnesses and tethered to the outside through side/bottom manhole where at least one worker remains on-station during the entire operation. Because of safety concerns, tank placards must include a confined space warning.

Practical Summary of Winemaking Issues

- The winemaker must understand the difference between cleaning and sanitation.
- The only means of determining the effectiveness of a sanitation program is to monitor the effectiveness.
- It is essential that those attempting to consistently craft fine wines understand the importance of sanitation and establish a HACPP plan.

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