Best Practices for Cleaning and Sanitation in the Winery

Cory Marx, PhD Student, Oberholster Lab Group
University of California, Davis

UCDAVIS

Outline

- Introduction
- Winery Spoilage Microbes
- Cleaning and Sanitation Chemicals, Protocols
- Monitoring Strategies
- Supporting Experimental Data

UC Davis Teaching Winery

Winery Cleaning and Sanitation

- **Cleaning and sanitizing is a *preventative* process, not a *corrective* one**
- **Minor time investment compared to total winemaking process, often overlooked**
- **Established, written protocols ensure product quality and worker safety**

Benefits:

- Improved Product Quality
- Reduced Operating Cost
- Longer Equipment Shelf Life
- Safe Working Environment

Costs/Consequences:

- Spoiled/Unsaleable Product
- Damaged Reputation
- Damaged Equipment
- Hazardous Conditions

Winery Cleaning and Sanitation

- **Numerous materials, wide variation in equipment**
  - Stainless steel, plastic, concrete, rubber
  - Tanks, hoses, barrels, bottling lines, drains

Cleaning

- Process involving physical removal of organic and inorganic soils

vs.

Sanitizing

- Process involving inactivation and/or killing of microbes

Variety of fermenters in UC Davis teaching winery

Definitions

- **Disinfection –** Reduction in harmful/pathogenic cells (log 3, 99.9%)
- **Sanitation –** Effective elimination of potential spoilage microbes (log 6+)
- **Sterilization –** Elimination of all viable cells (log 12+)

An autoclave won't fit your fermentor…

Definitions cont'd

- **PPE –** Personal Protective Equipment
- **PEL –** Permissible Exposure Limit
  - OSHA defined, TWA and ceiling values
- **SDS (MSDS) –** Safety Data Sheet

OSHA pictograms found in SDS's. Part of GHS hazard communication

Common winery PPE

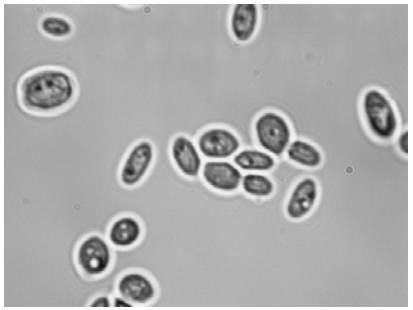
Safety

- **Proper chemical preparation, documentation**
  - Apply chemicals at recommended concentrations, temperatures.
  - First aid, fire, spill kits regularly inspected and stocked
- **PPE – appropriate for the given task**
- **Following established, written protocols**
- **Slips, trips, falls**

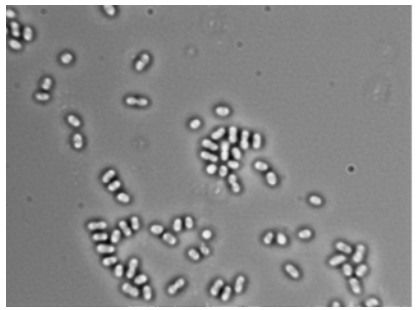
A situation you really, really do not want to be in….

Winery Microbes

- **Wineries have high microbial load, especially during harvest**
- **Both must and finished wine contain properties that place 'selective pressure' on microbial community**
  - Low pH, ethanol major population drivers
  - Spoilage microbes only group of concern. All *heat labile*
- **Spoilage microorganisms require a vector to travel through the winery (except for fruit flies)**
  - Vectors include workers, HVAC system, improperly sanitized equipment or tools.



S. cerevisiae

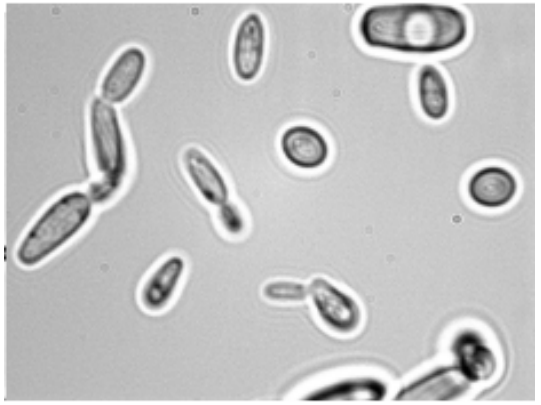


A. pasteurianus

A note on filtration: 0.45 µm is sufficient to remove commercial yeast (typically 5-10 µm), but not all bacteria, and/or some wild yeasts. Shouldn't be used to remedy bacterial infection!

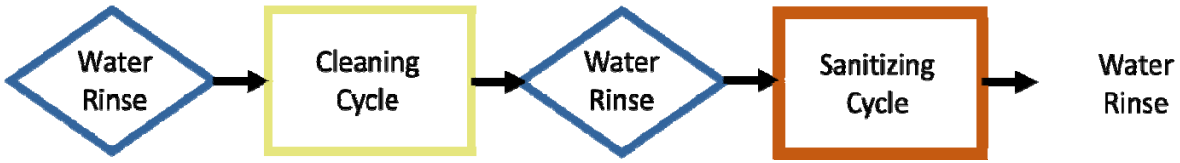
Winery Microbes cont'd.

- **Yeasts, molds, lactic and acetic acid bacteria primary culprits in wine spoilage**
  - Common spoilage yeast genera: *Saccharomyces, Brettanomyces, Zygosaccharomyces*
  - Common bacteria genera: *Lactobacillus, Acetobacter, Oenococcus, Pediococcus*
- **Many organisms can exist in the planktonic (suspended in medium) or sessile (surface-associated) state. The latter is referred to as a *biofilm***
  - Biofilms more difficult to inactivate/remove. It is **not** sufficient to only inactivate the cells within the biofilm



Dekkera bruxellensis
(teleomorph of *Brettanomyces*)

The Cleaning and Sanitizing Process



Basic 5-Step Cleaning/Sanitizing Protocol

Water Rinse 1 – Room temperature water pre-rinse to remove gross soil. Can be followed by warm water rinse. Hot water may 'cook' on debris, and should be avoided at this stage

Water Rinse 2 – Remove cleaner residue, loosened debris, neutralize. Using room temperature water facilitates application of some sanitizers

Water Rinse 3 – Remove sanitizer residue (if necessary)

Cleaning and Sanitizing Strategies

Cleaning Choices:

- Manual (Hose, buckets, brushes)
- Semi-Automatic (spray ball, mobile sprayer)
- CIP (Clean-in-place)
- COP (Clean-out-of-place)
- Immersion

Rodem CIP vs COP systems

Common Cleaning Chemistries

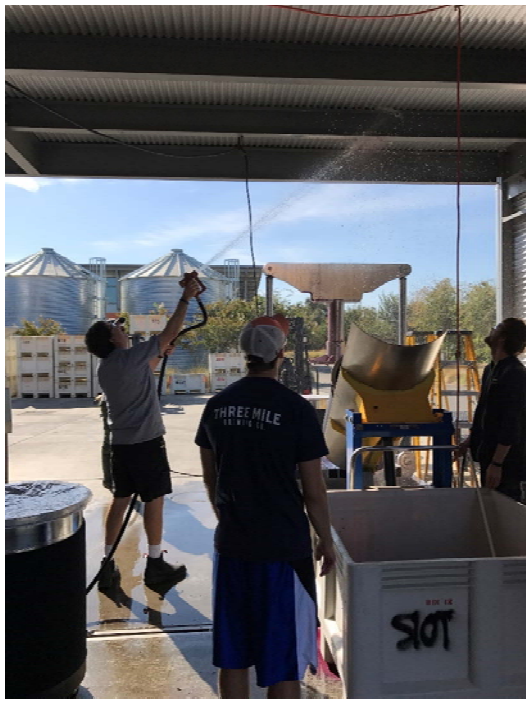
- **Built Cleaners versus Base Chemicals**
- **Caustics**
  - NaOH, KOH
  - Capable of dissolving soils
  - Have biocidal activities (at typical 1-2% concentration)
- **Non-caustic Alkaline Products**
  - Often Sodium carbonate/Potassium percarbonate-based
  - TSP, hydrogen peroxide, sodium metasilicate common in formulations
- **Acid Cleaners**
  - Phosphoric/Nitric acid-based cleaners

NaOH pellets pull moisture from the air. Containers must be kept closed tight!

Rotating cleaning chemicals (Alkaline/Acid treatment, or active ingredients) can be a smart choice for fighting microbial buildup

Cleaning

- A proper cleaning regimen is the most important aspect of a cleaning and sanitation program and should do the bulk of the ‘work’ in the process
- There is no substitute for mechanical energy in removing soils loosened during the cleaning process. Hoses, brushes, spray balls, and jets are all effective means of generating the mechanical force required to remove soils and dislodge biofilms
- Common contact times range from 10 to 20 minutes, but vary depending on the specific chemical and concentration applied. Manufacturers may recommend specific contact times for a given formulation
- Use appropriate concentrations and temperatures!



Cleaning every aspect of the facility sometimes includes a ceiling-spraydown!

Sanitizing Chemistries

- **Peracetic Acid (PAA) [w/ or w/o added H_2O_2]**
  - Breaks down to acetic acid, oxygen, water. Can use as no-rinse sanitizer
  - Effective at low temperatures
  - Less effective against some yeast and molds, must store cold, can be expensive
- **Ozone**
  - Broad spectrum, strong oxidizer
  - Breaks down to molecular oxygen (O2)
  - Half-life important! (24hrs as a gas, but seconds dissolved in water!)
- **Heat/Steam**
  - Temperatures > 185^{oF} are sufficient to inactivate winery spoilage microorganisms
  - Heat is the most broad-spectrum technique, but requires dedicated equipment and can have high energy costs

Sanitizing Chemistries cont'd.

- **Cl^- - based compounds**
  - Broad-spectrum activity
  - TCA issues with hypochlorites. No evidence of taint issues with chlorine dioxide
  - Important to maintain pH < 7 (must thoroughly rinse alkaline cleaners!)
  - Hazardous to health! Requires PPE
- **I^- -based compounds**
  - No-rinse formulations available.
  - Can stain equipment, temperature sensitive (can't be used with >120^{oF} water)
- **Quaternary Ammonium Compounds (QUATs)**
  - Has residual activity. Can be left on surfaces that won't be used immediately
  - Potential sensory impact, affected by water quality, relatively narrow-spectrum
- **SO_2 (acidified to pH ~3)**
  - Inexpensive. Frequently already stocked in winery **BUT** corrosive to metals, hazardous to health. Not generally recommended as a primary sanitizing agent

Sanitation

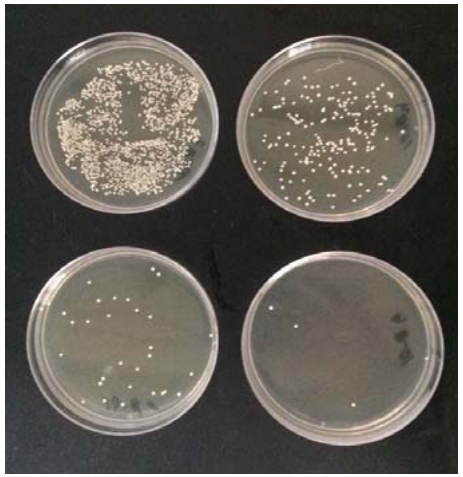
- Sanitizing cycles are secondary to cleaning, essentially provide extra level of assurance
- Sanitizing chemicals are extremely susceptible to residual organic matter, and effectiveness is greatly diminished in the presence of soil remaining from the cleaning process
- Many sanitizing chemicals contain formulations that *must* be rinsed; the quality of the process water used to rinse a sanitizer is *critically* important
- As with cleaners, contact time, temperature, and concentration are critical for the successful application of *all* sanitizers. Consult manufacturer's recommendations and *validate*

Yours truly putting his money where his mouth is using PAA

Protocol Development, Monitoring Strategies

- The long-term success of cleaning and sanitation programs depends on written, reproducible protocols
- Employee checklists, instructions are helpful to ensure all steps of the protocol are followed
- A monitoring strategy should be developed to validate the protocol
  - ATP and environmental swabbing cheap, easy to use options
  - pH meters and strips, temperature tape and thermometers are handy for cleaning/sanitizing operations

Monitoring Tools:



Serial dilutions from swab recovery

PCR
Molecular Methods

Environmental Swabs

Chemical Screening: Planktonic cells

		Yeast				Bacteria			
Treatment	*Organism*	*S. cerevisae*	*B. bruxellensis*	*Z. bailii*		*A. pasteurianus*	*L. casei*	*P. parvulus*	*O. oeni*
	2% NaOH								
Cleaners	**1% KOH**								
	2% KOH								
	NaOH-Based								
	KOH-Based								
	Sodium Percarbonate-Based								
	Potassium-Carbonate Based								
	Na, K Carb, EDTA, chlorides								
	Biocleaner								
Sanitizers	**1% H2O2**								
	100 ppm PAA								
	200 ppm PAA								
	100 ppm PAA + 1% H2O2								
	20 mM KHSO4								
	20 mM KHSO4+ 1% H2O2								
	40 mM KHSO4+ 1% H2O2								
	20 mM KHSO4 + 2% citric acid								
	Quaternary Amonium -Based								
	Iodophor								
	100 ppm ClO2								

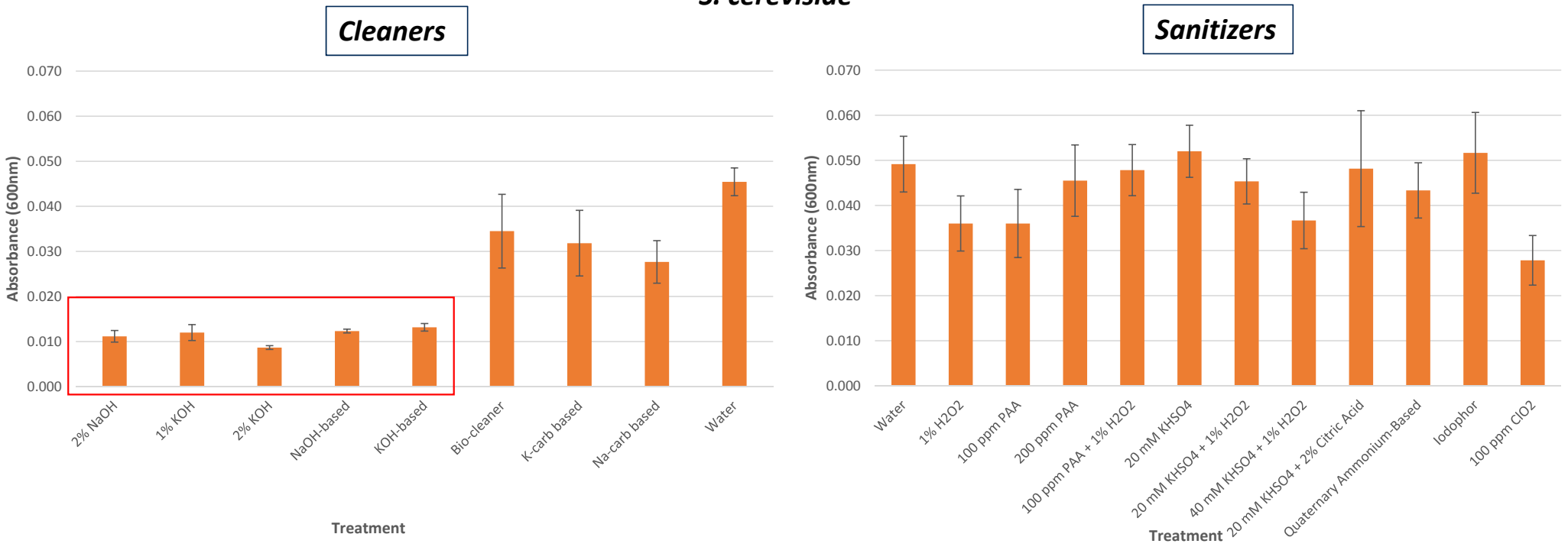
Results of chemical screening assay for planktonic cells. Shaded area represents a treatment that resulted in no culturable cells in at least one time points (6 points, 5-30 minute sampling). Products marketed as cleaners are shaded pink, sanitizers are shaded orange

UCDAVIS

Chemical Screening: Biofilms

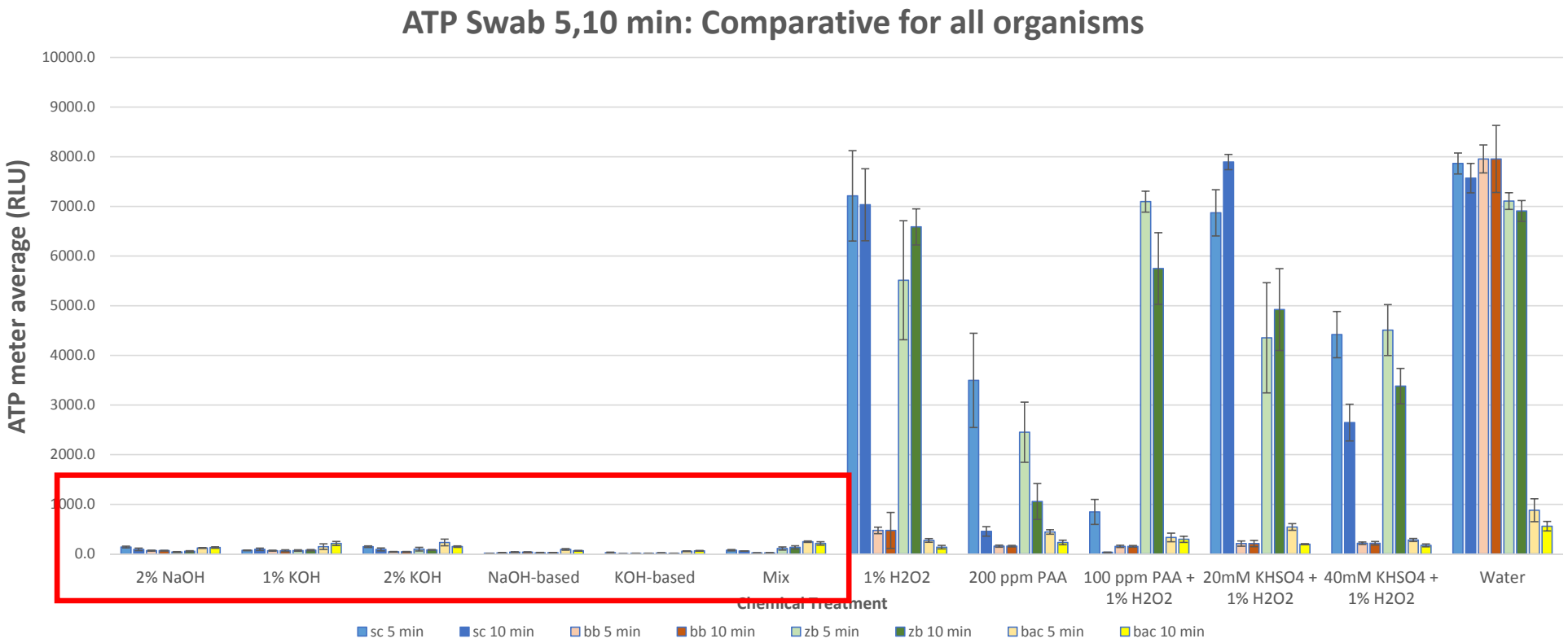
Difference from biofilm-free absorbance at t = 10 minutes

S. cerevisiae



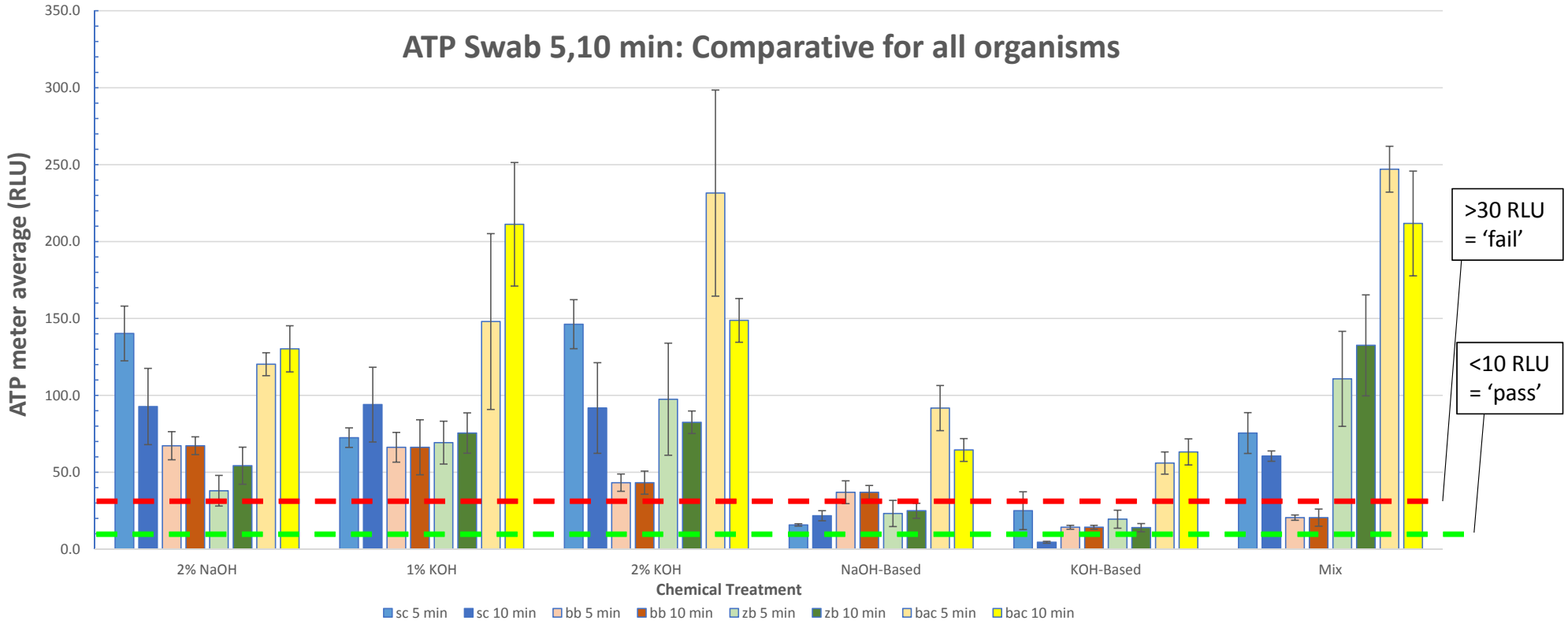
Results of assay investigating impact of various cleaners and sanitizers on biofilms. Given the analysis conditions (biofilms grown in 50% grape juice for 10 days), only the caustic-based cleaning chemicals provided a significant reduction in biofilm over water rinses

ATP Swabbing of Biofilms on SS



Results from biofilm swabbing trials on visually clean surface demonstrates need for cleaning step. Sanitizing chemicals alone result in high ATP results

ATP Swabbing of Biofilms on SS



Summary

- A proper cleaning regimen is the most important aspect of a cleaning and sanitation program and should do the bulk of the ‘work’ in the process
- The basic five-step cleaning and sanitizing protocol provides a foundation for developing a successful program for a winery of any size
- There is no right or wrong choice for cleaning and sanitizing chemicals. The choice should depend on budget, the application (surface, soil load), regulations or personal preference, but *should be validated by a monitoring program*
- Even in the case of visually clean surfaces, a cleaning step cannot be omitted or the risk of contamination from biofilms will occur
- Written protocols for cleaning and sanitation are key in ensuring the long-term success of a regimen