Cleaning and Sanitation in the Winery

PRWCA VA Prevention Seminar

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Outline

- Introduction
- Winery Spoilage Microbes
- Cleaning and Sanitation Chemicals
- Protocols
- Monitoring Strategies
- Special Topics/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+)

Common winery PPE

- **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

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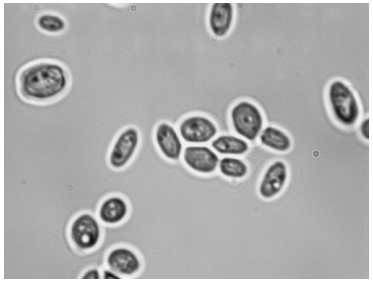
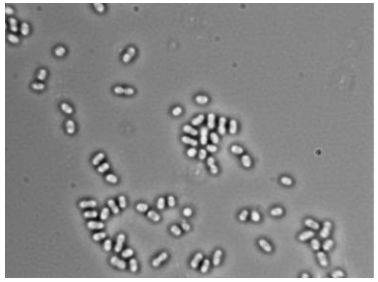
Winery Microbes

- **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 *alone* remedy bacterial infection!

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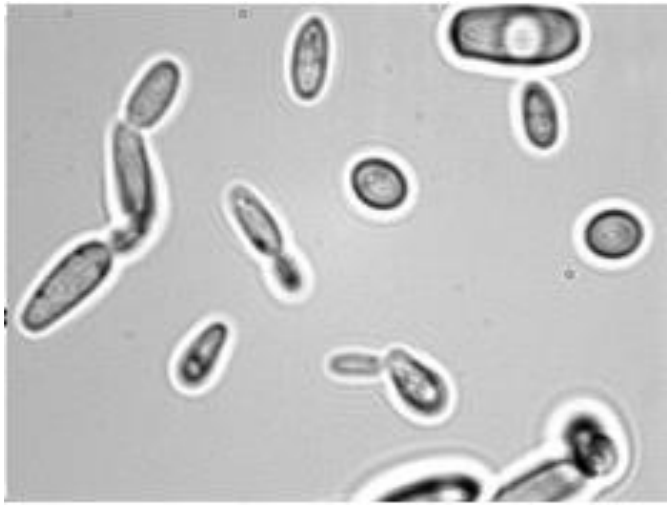
Winery Microbes cont'd.

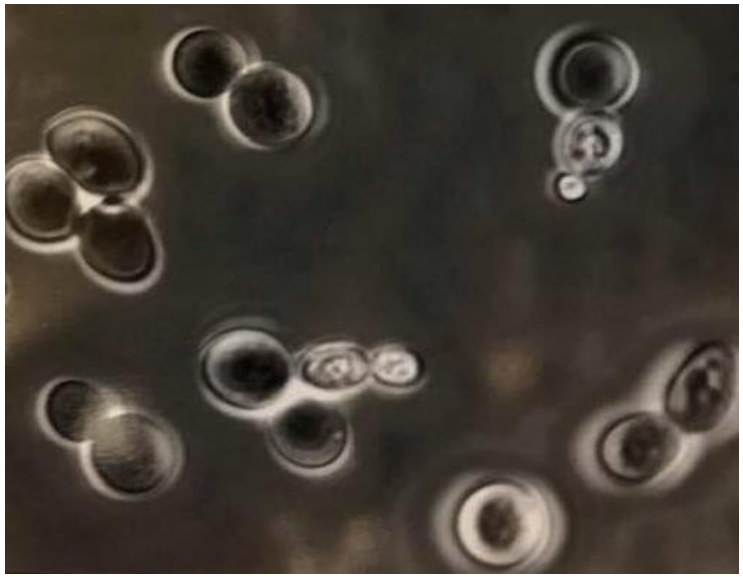
- **Yeasts, molds, lactic and acetic acid bacteria primary culprits in wine spoilage.**
  - Common spoilage yeast genera: *Saccharomyces, Brettanomyces, Zygosaccharomyces*
  - Common bacteria genera: *Lactobacillus, Acetobacter, Oenococcus, Pediococcus*
- **Many organisms can exist in the planktonic (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.

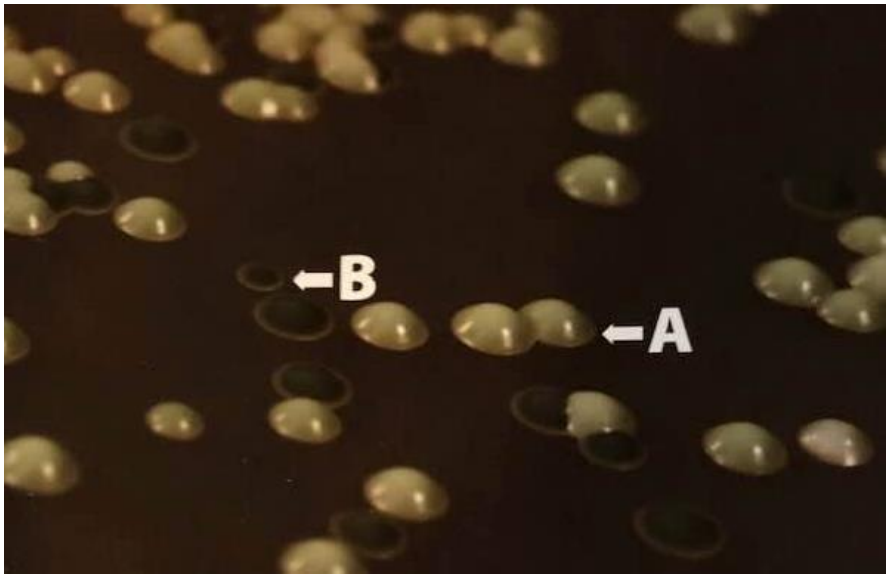


Dekkera bruxellensis (teleomorph of *Brettanomyces*)

Assessing Microbial Spoilage: The basics

- Often assessed first by sensory
- No substitution for plating, microscopy
- Molecular methods exist for external analysis (ex. Scorpion)
- Have ID guides on hand!

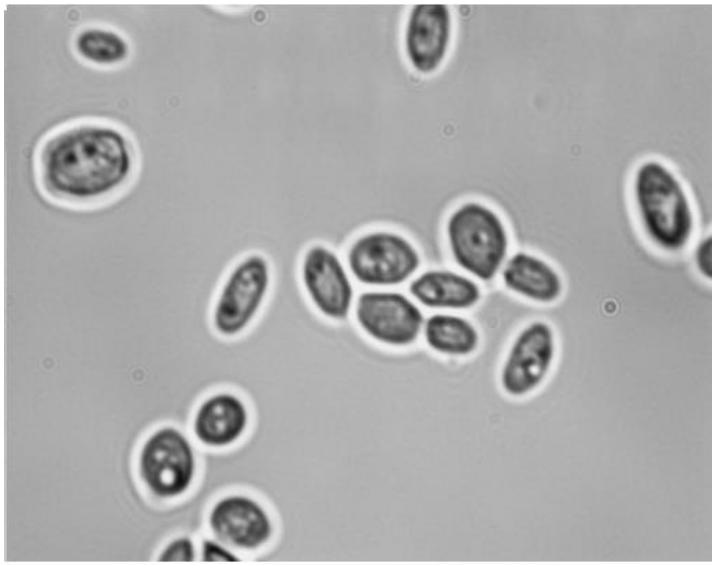




S. cerevisiae (A) and *K. thermotolerans* (B). Very difficult to determine difference under light microscope (left) but easy on agar plates

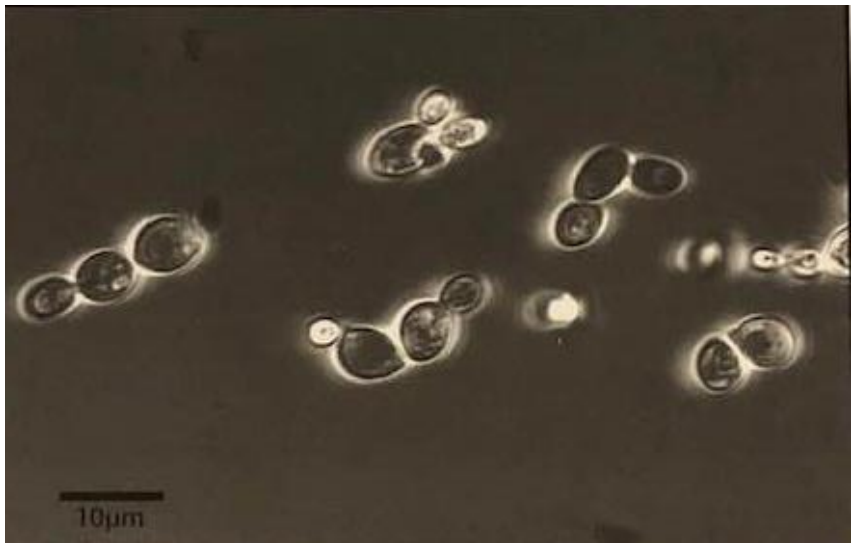
Images on this and next slide from *Illustrated Guide to Mirobes and Sediments in Wine, Beer, and Juice,* by Charles G. Edwards (Winebugs LLC and Gusmer Enterprises inc. 2005)

Assessing Microbial Spoilage: The basics

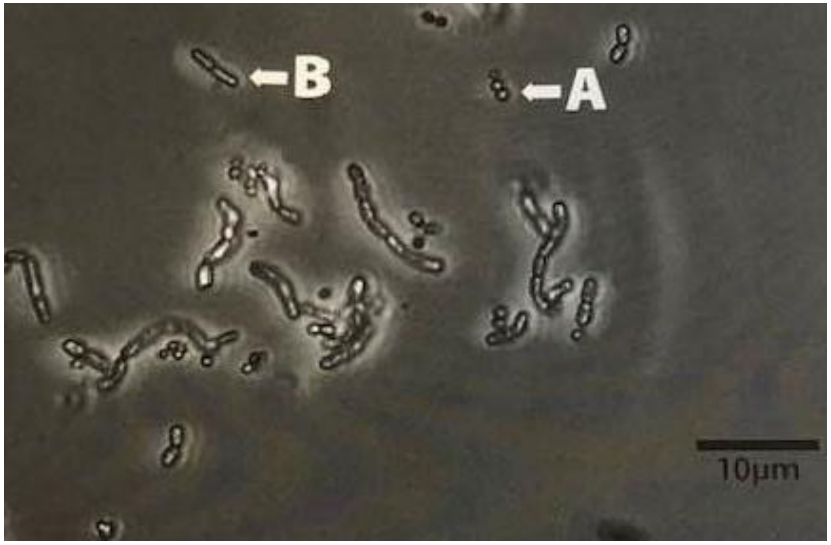


S. cerevisiae

VS.



B. bruxellensis



P. Parvulus A) and *L. brevis (b)*

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

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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!)
  - Dedicated equipment required
- **Heat/Steam**
  - Temperatures > 185^{oF} are sufficient to inactivate winery spoilage microorganisms
  - Heat is a full-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
- **Others (UV, ultrasonic, etc.)**

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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

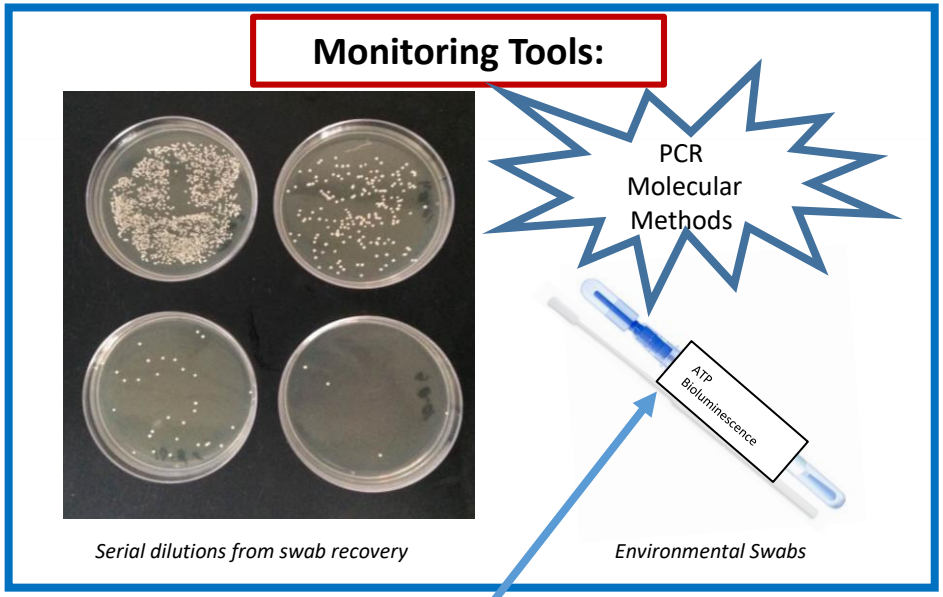
Cleaning Choices:

- 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

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



Note: Not all ATP readers are created equal. Always determine thresholds <u>that work for your winery.</u>

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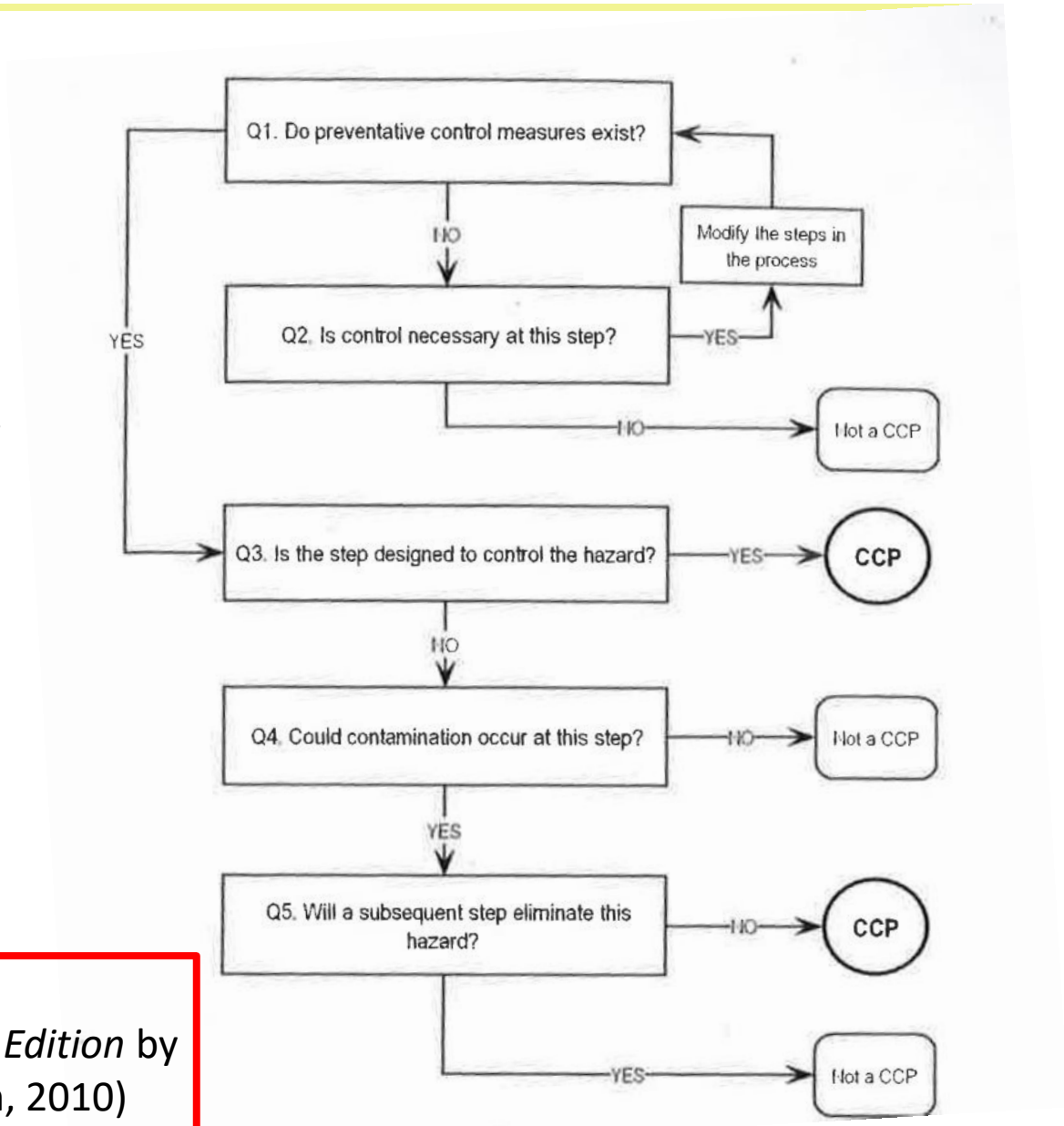
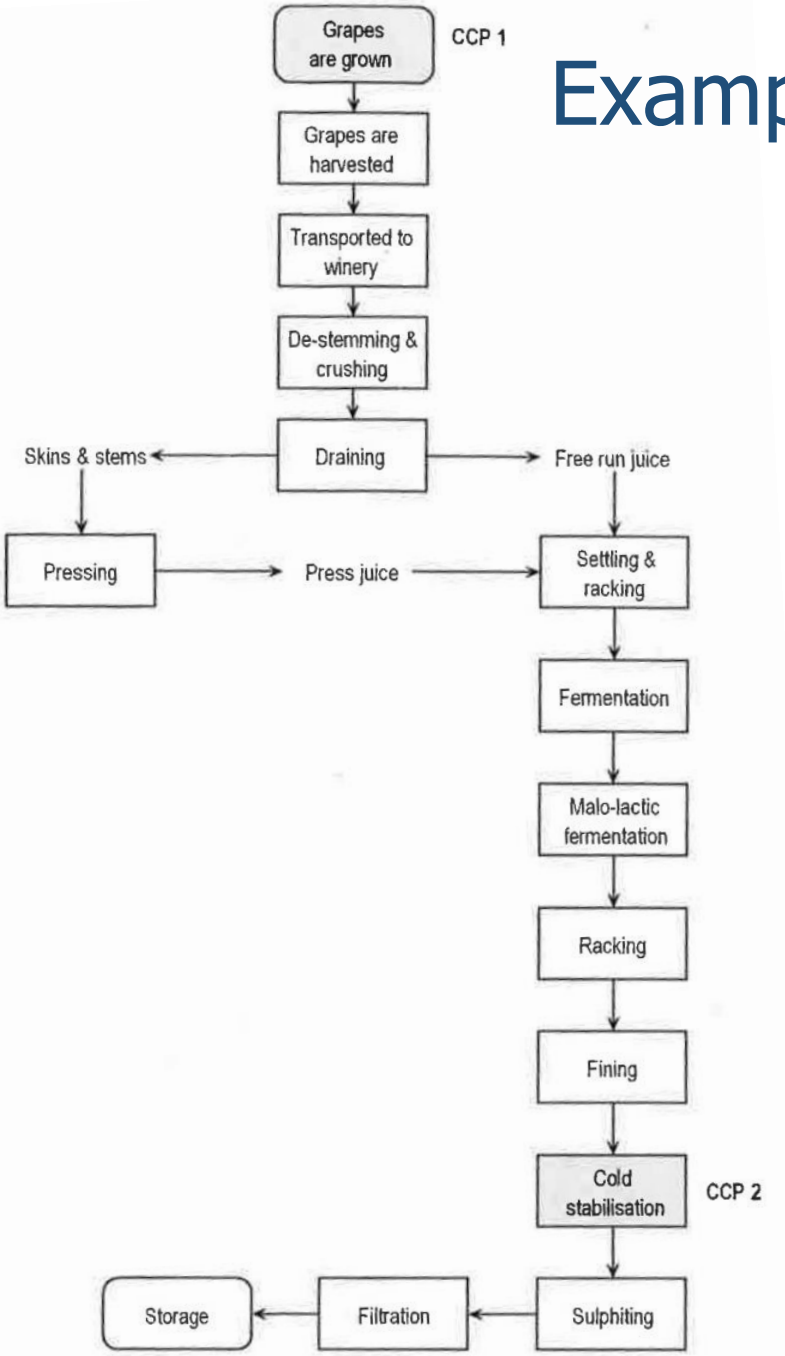
HACCP, ISO, and formalized SOP's

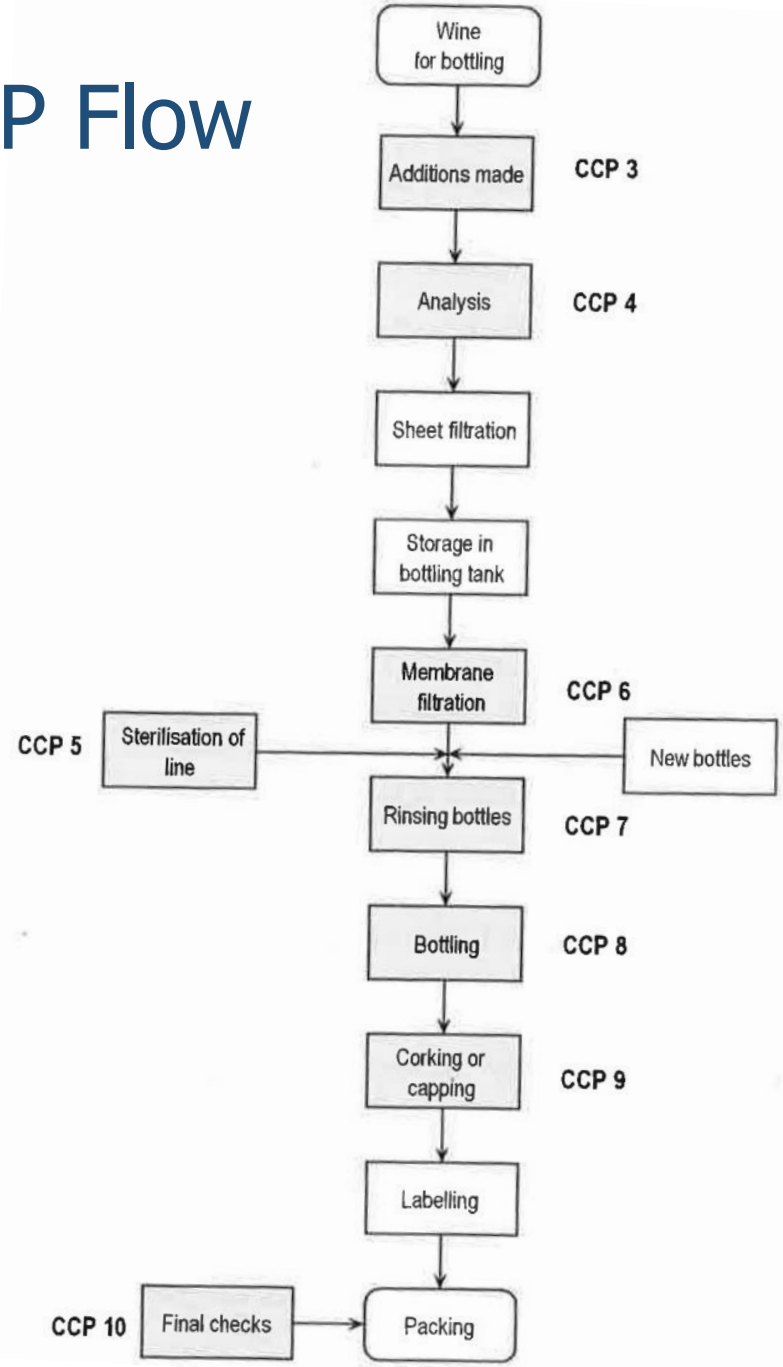
- HACCP defined in *Codex Alimentarius*
- Established methods for critically assessing and developing product stream and protocols
- **Write down SOP's**
- Use checklists



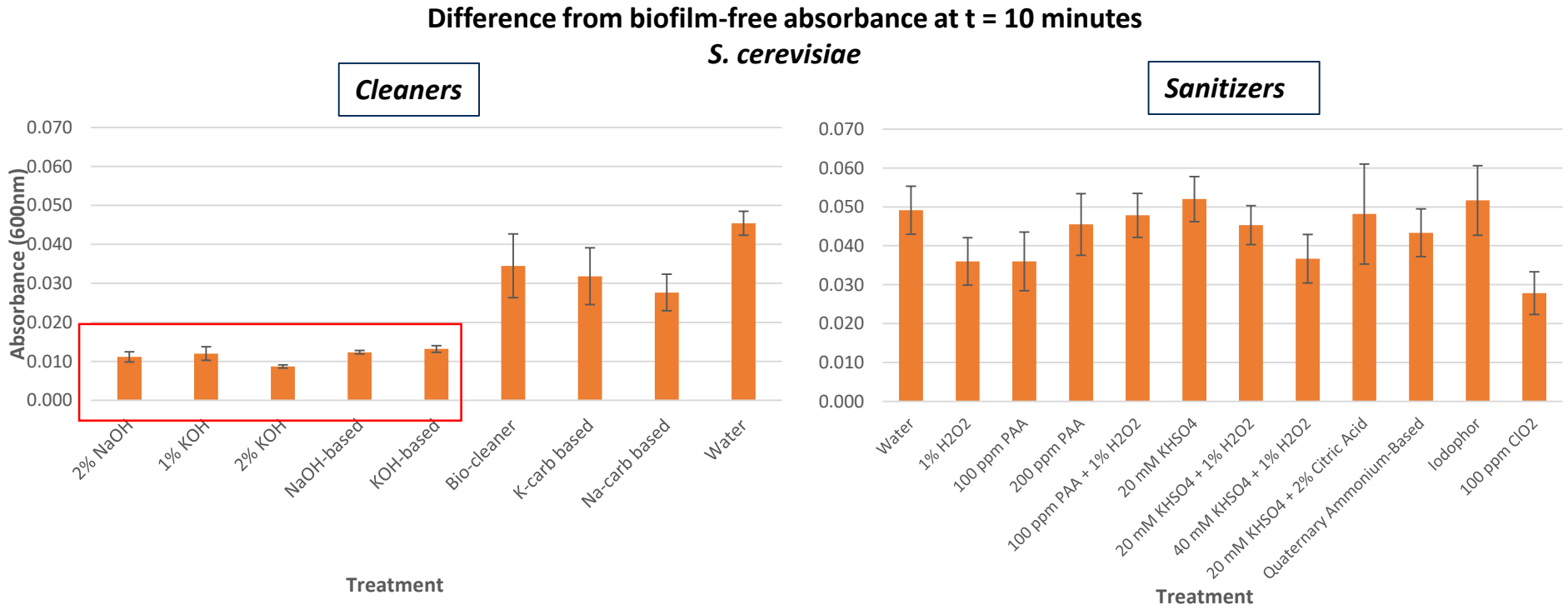
HACCP graphics reproduced from *Understanding Wine Technology 3rd Edition* by David Bird (DBQA publishing, Britain, 2010)

Example CCP Flow





Biofilm Management



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

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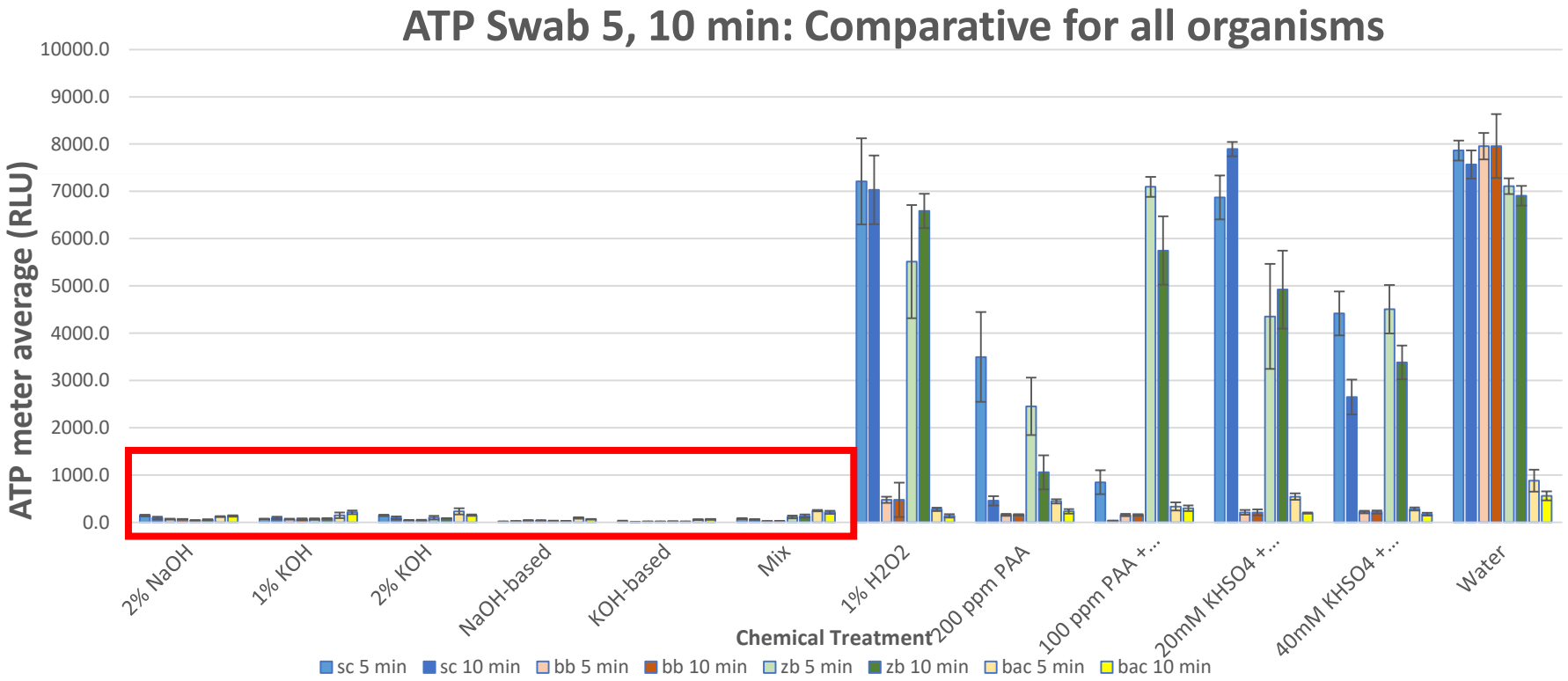
ATP Swabbing of Biofilms on SS



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

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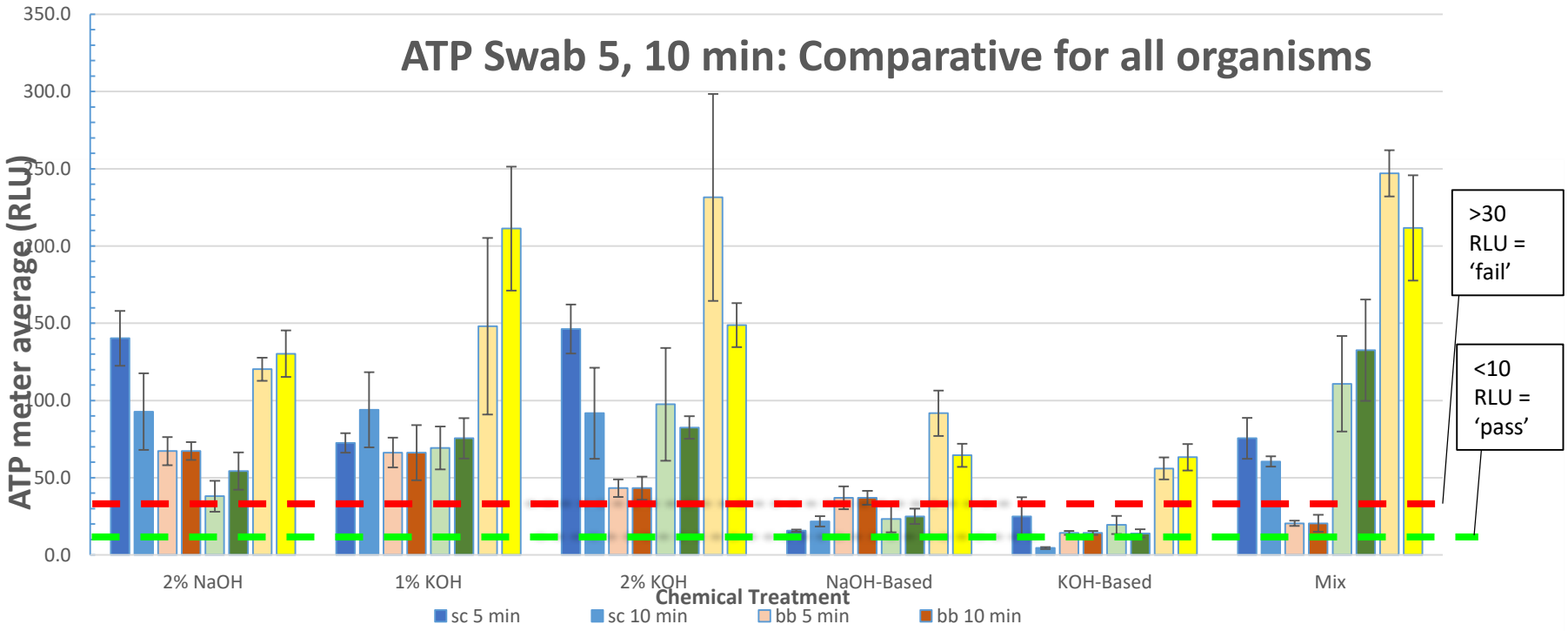
ATP Swabbing of Biofilms on SS



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Optimizing Concentrations/Contact Time

- Manufacturer's recommend concentrations for applying their chemicals to inactivate cells. Where do these come from?
- Wineries are FDA regulated facilities, so most recommendations come from *E. coli, Salmonella*, etc.
- Used Minimum Inhibitory/Biocidal (MIC/MBC) concentration assay to determine concentration required to inactivate microbes in planktonic and biofilm state
- At manufacturer's recommended cleaner concentrations, treatments were sufficient to inactivate yeast and bacteria tested

96-well microtiter plate

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'Heat Map' for *S. cerevisiae*

S. cerevisiae MIC/MBC in 50% grape juice medium											
NaOH	KOH	KOH-based	NaOH-based	Peracetic Acid	Mix	H2O2	KHSO4	KHSO4 + H2O2	ClO2	GC	SC
8.00%	8.00%	8.00%	8.00%	0.0800%	50.00%	8.00%	8.00%	8.00%	0.0250%		
4.00%	4.00%	4.00%	4.00%	0.0400%	25.00%	4.00%	4.00%	4.00%	0.0125%		
2.00%	2.00%	2.00%	2.00%	0.0200%	12.50%	2.00%	2.00%	2.00%	0.0063%	3	3
1.00%	1.00%	1.00%	1.00%	0.0100%	6.25%	1.00%	1.00%	1.00%	0.0031%	4	4
0.50%	0.50%	0.50%	0.50%	0.0050%	3.13%	0.50%	0.50%	0.50%	0.0016%	5	5
0.25%	0.25%	0.25%	0.25%	0.0025%	1.56%	0.25%	0.25%	0.25%	0.0008%	6	6
0.13%	0.13%	0.13%	0.13%	0.0013%	0.78%	0.13%	0.13%	0.13%	0.0004%	7	7
0.06%	0.06%	0.06%	0.06%	0.0006%	0.39%	0.06%	0.06%	0.06%	0.0002%	8	8

- Concentration Decreases from top to bottom, show in percent (0.08% = 800 ppm)
- Green cells = Treatment effective at inhibiting *and* inactivating cells
- Yellow cells = Treatment only inhibited cells, but did not inactivate (growth on agar)
- Red cells = Treatment did not inhibit growth in wells or agar.

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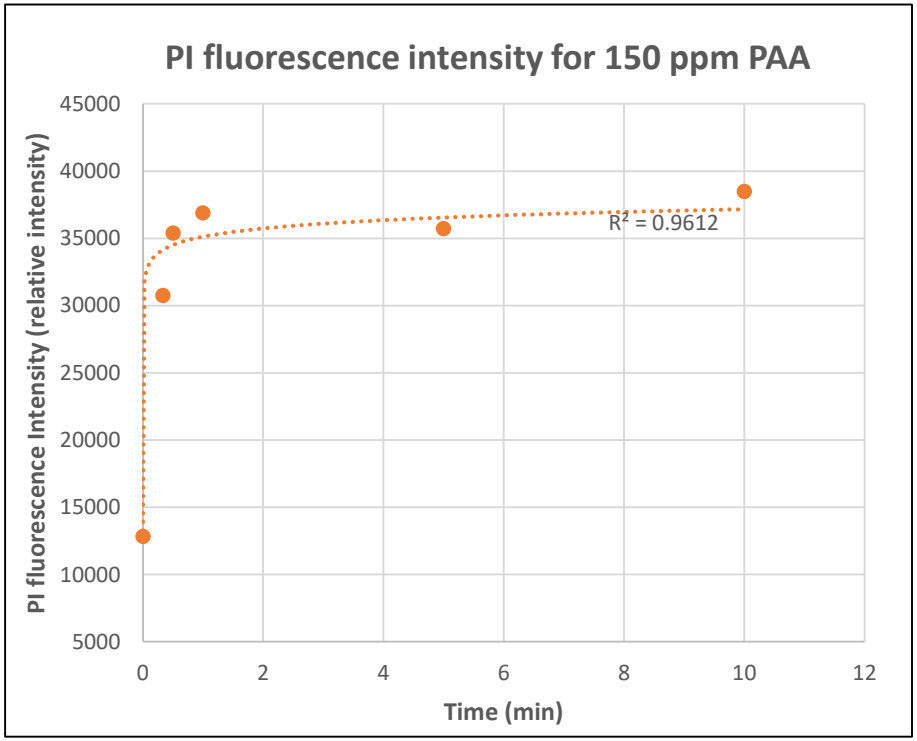
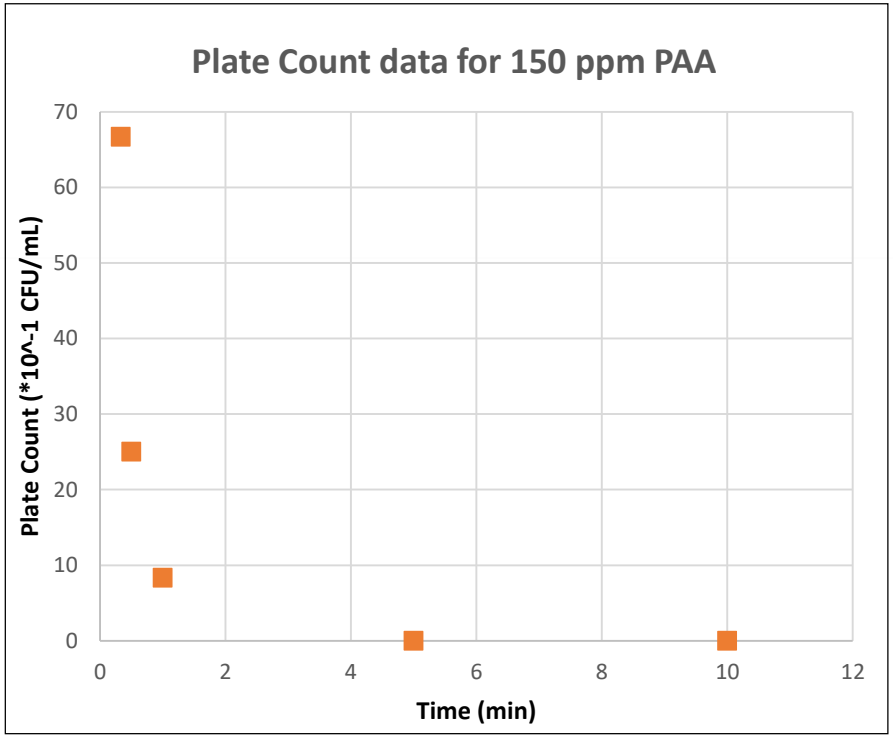
Time-kill experiments suggest only 5 minutes required to inactivate S. cerevisiae populations





Propidium Iodide (PI) fluorescence intensity and plate count data for time-kill experiments.

But…does this hold up in the winery?

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Optimizing Concentrations/Contact Time

Sample Legend:

ATP — Plate Count

Sample Locations:

TV1 – Interior of valve post. ATP and Plate swabs collected on opposite sides of valve

TV21 – Interior lip of port. Swabs collected on adjacent portions of lip.

TV22—Underside of upper tank surface, between port and walls.

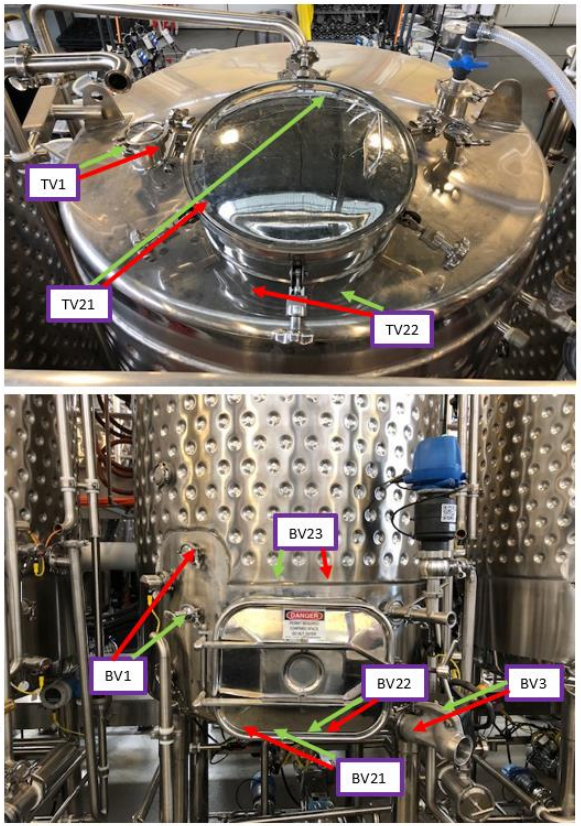
BV1—Interior of valves. Since swabs require ~10 cm^2 surface area samples were taken from different valves.

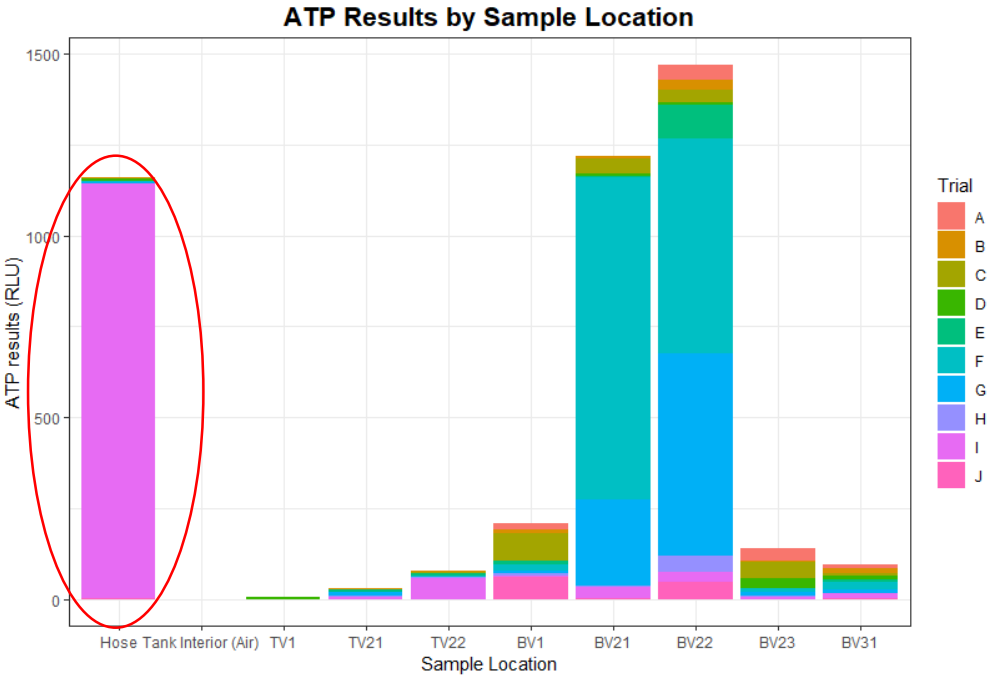
BV21—Bottom interior lip of tank port.

BV22 – Gasket of the port door.

BV23 – Interior surface of tank wall above port

BV3 – Upper (plate count) and lower (ATP) surfaces of the valve interior





Not exactly….

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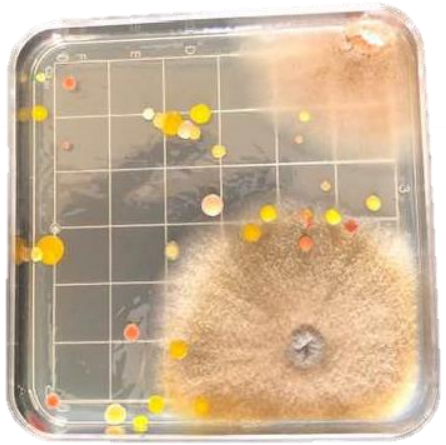
Major Areas of Microbial Contamination

- Studies have shown that winery tank surfaces and bottling lines are generally the cleanest parts of the winery.
- Drains and the area around the bung of barrels are notorious for high levels of microbial contamination.
- Pumps, hoses, thieves, *and the winery process water* are all capable of quickly spreading contamination.



Environmental swab of a winery tank after cleaning/sanitation (BV22 on next slide)

Strategies for Fermentation-Soil Protocols

- Pay attention to critical areas: valves, gaskets, ports—places in the shadow of spray balls or mechanical agitation. Take care in cleaning floors, especially with hose water.
- Develop a strategy for monitoring the success of a protocol—as we have seen, visual clean doesn't cut it!
- *Written* protocols and checklists make sure nothing has been missed.

Perfex color-coded brushes have bristles molded into the handles. The bristles don't fall out, and microbes don't have a place to hide

Strategies for Barrel Management

- Brettanomyces identified to penetrate barrel staves up to 9 mm
  - For thermal treatments: Inactivated at 55C with D-value of 12-80 sec.
  - At 60C ~2 min , at 70C <1 min for effective thermal inactivation
  - Appear to penetrate deeper into French barrels, especially with higher toast levels

- **Hot water**
  - Normal protocol: Fill barrels with 50-70C Water, soak for variable time
- **Steam**
  - 12 min required to produce no culturable cells at 9 mm depth.
  - Takes approximately 4 min for barrel to reach 55C at 9 mm depth.
- **Ozone**
  - 1 mg/L for 30 min
  - Hindered by flow diffusion into staves, reacts easily with organics in barrel
- **Chemical Control, Ultrasonic, SO2**

Supporting details from:

- Cartwright, Z.M., Glawe, D., and Edwards, C.G. (2018) *Reduction of Brettanomyces bruxellensis Populations from Oak Barrel Staves Using Steam*, Am. J. Enol. Vitic. 69:4
- De Lourdes Alejandra Aguilar Solis, M., Gerling, C., and Worobo, R. (2013) *Sanitation of Wine Cooperage using Five Different Treatment Methods: and In Vivo study,* Appellation Corness Research Focus 2013-3

Strategies for Barrel Management

- Cross contamination
  - Barrel sampling and topping often the culprit for spreading contamination
  - During sampling, frequently sanitize wine thief
  - It is critical that wine for topping be microbially stable. Consider using filtered wine, or plating samples before spreading among barrels
  - Consider carrying 70% ethanol to spray outer of bung prior removing, adding

A dirty wine barrel. Spills like the wine staining the bung hole are a big reason for finding high spoilage microbe populations there that can make their way into the wine

Some Takeaways

- 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
- Pay attention to critical surfaces (gaskets, hoses, drains, etc.). You won't fully sanitize a tank if the gaskets are left on, plus you may damage them over time if left on/closed
- Written protocols for cleaning and sanitation are key in ensuring the long-term success of a regimen