Sulfur dioxide and Sanitation

Thomas Collins, PhD
Washington Winegrowers Convention, 06 February 2018

Chemistry of Sulfur Dioxide

- $H_2O + SO_2 \leftrightarrow HSO_3^-$ $pK_{a1} = 1.77$
- $HSO_3^- \leftrightarrow SO_3^{2-}$ $pK_{a2} = 7.21$
- SO_2 "molecular form"
- HSO_3^- "bisulfite form"
- SO_3^{2-} "sulfite form" (only a trace at wine pH)

- Each form reacts differently based on its own specific chemistry.

Molecular Form Functions

- Kills wild yeast and bacteria (wounds them mostly)
- Effective hydrogen peroxide trapping agent
- Is volatile and detectable by sensory evaluation
  - Pungent metallic odor
- Due to the volatility of the molecular form, concentration decreases over time, particularly at lower pH values

Bisulfite Functions

- Decolorizes anthocyanin by binding to them
  - Disrupts conjugated system

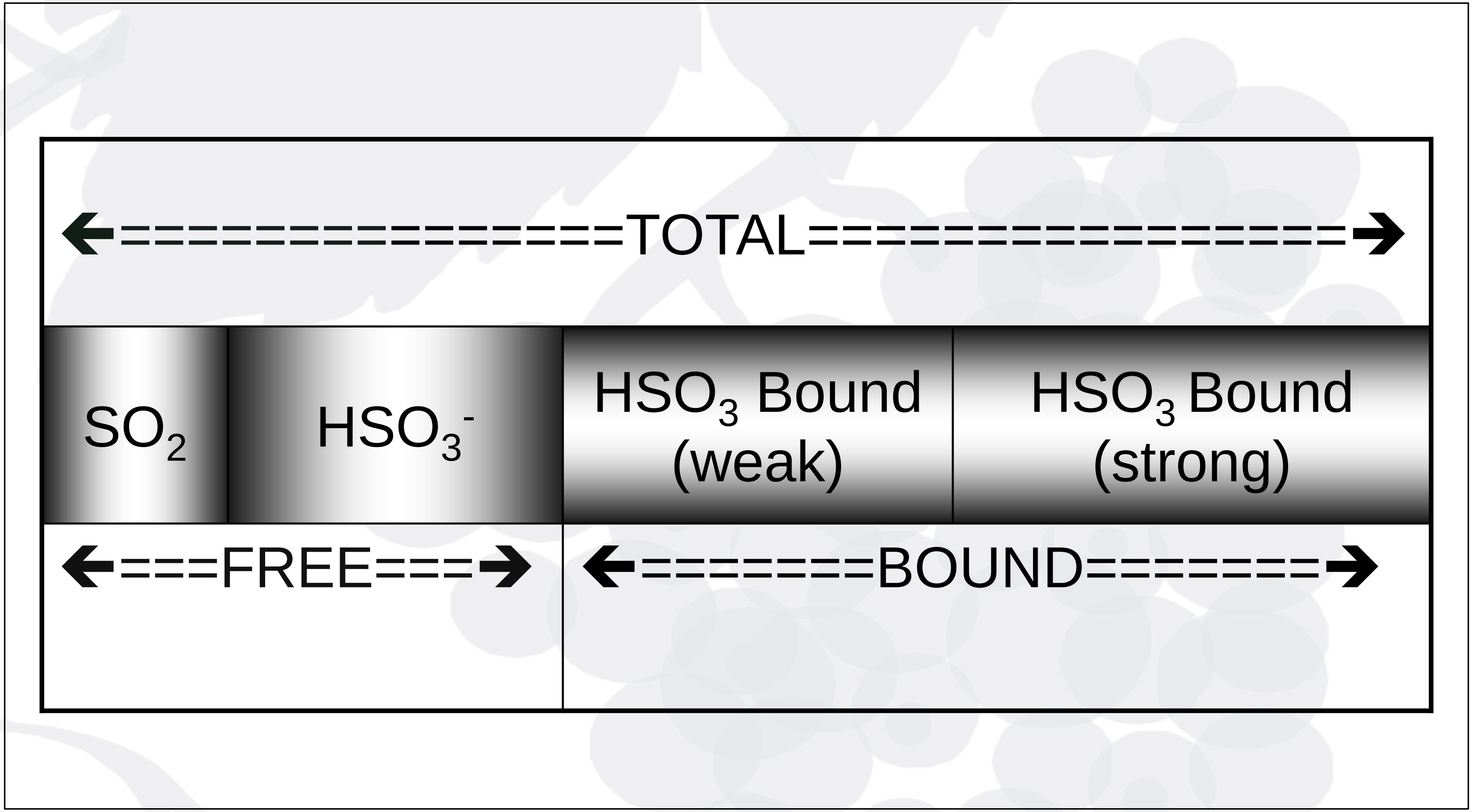
- Inhibits oxidative enzymes
  - Behaves as competitive inhibitor

- Is easily covalently bound by acetaldehyde, keto-acids anthocyanin and sugar.

Bisulfite Functions

- Bound form disrupts normal equilibrium.
- Establishes new equilibrium of bound and free forms.
- Forces analytical methodologies to measure bound and free
  - Current methodologies have difficulty estimating bound versus free forms because of differential disassociation constants of bound forms

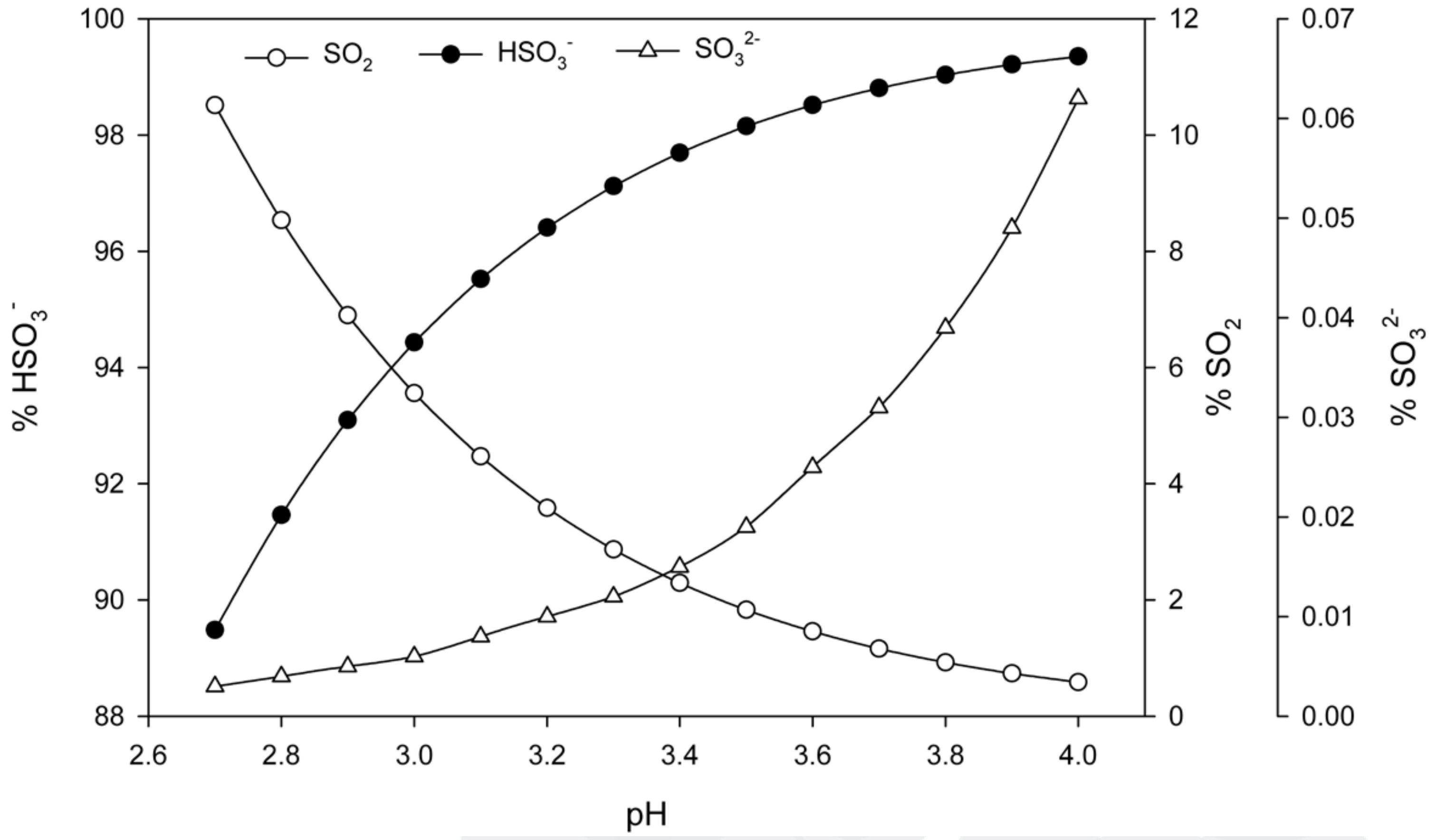
SO_2 Equilibrium



Sulfite Ion Form

- The antioxidant form
- Low concentration
  - (0.006 % - 0.06%) from pH 3.0 to pH 4.0
- It reacts directly with dissolved oxygen
- However it reacts more slowly than more powerful antioxidants found in wine such as phenolic compounds

Sulfur Dioxide Forms



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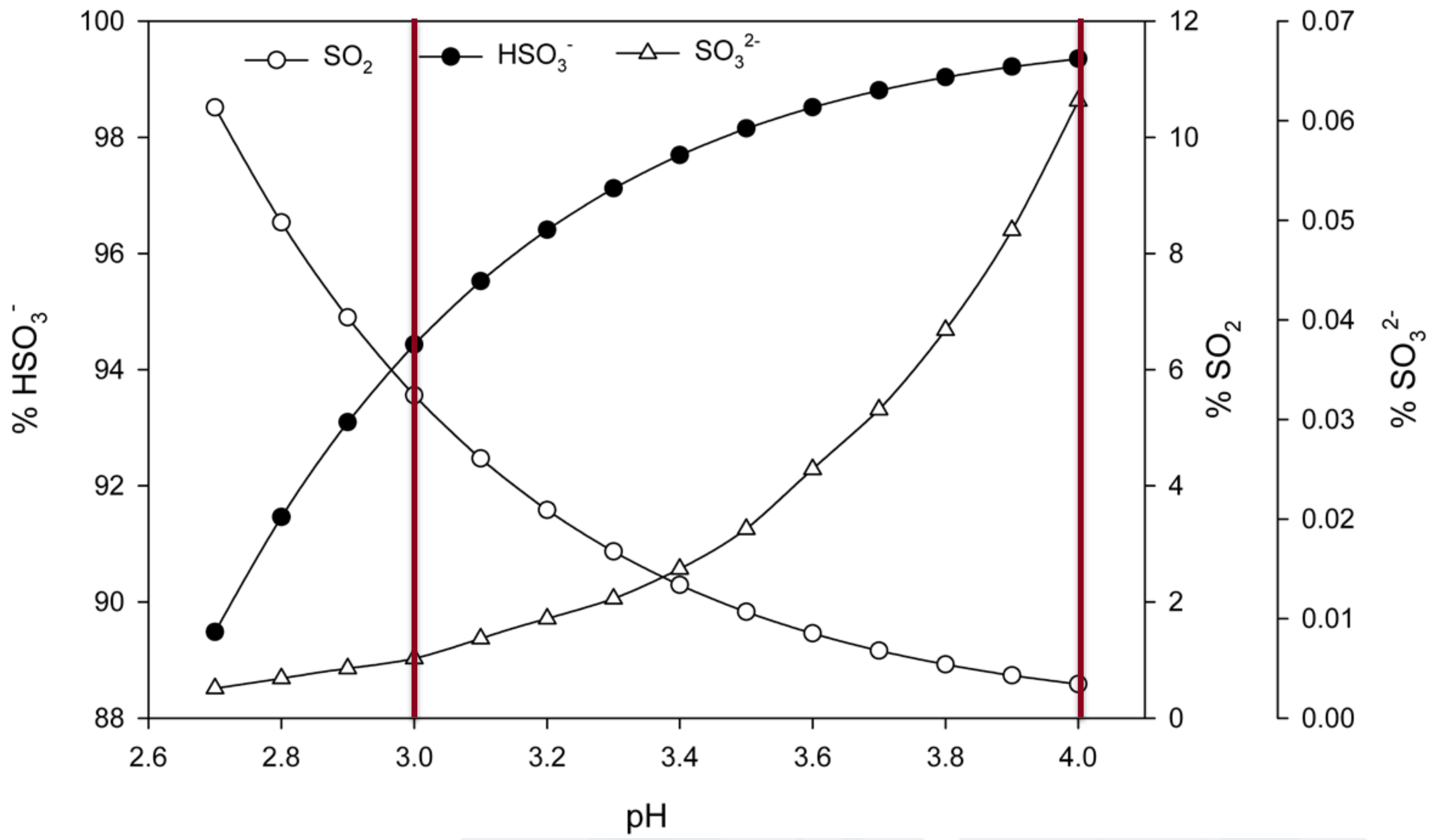
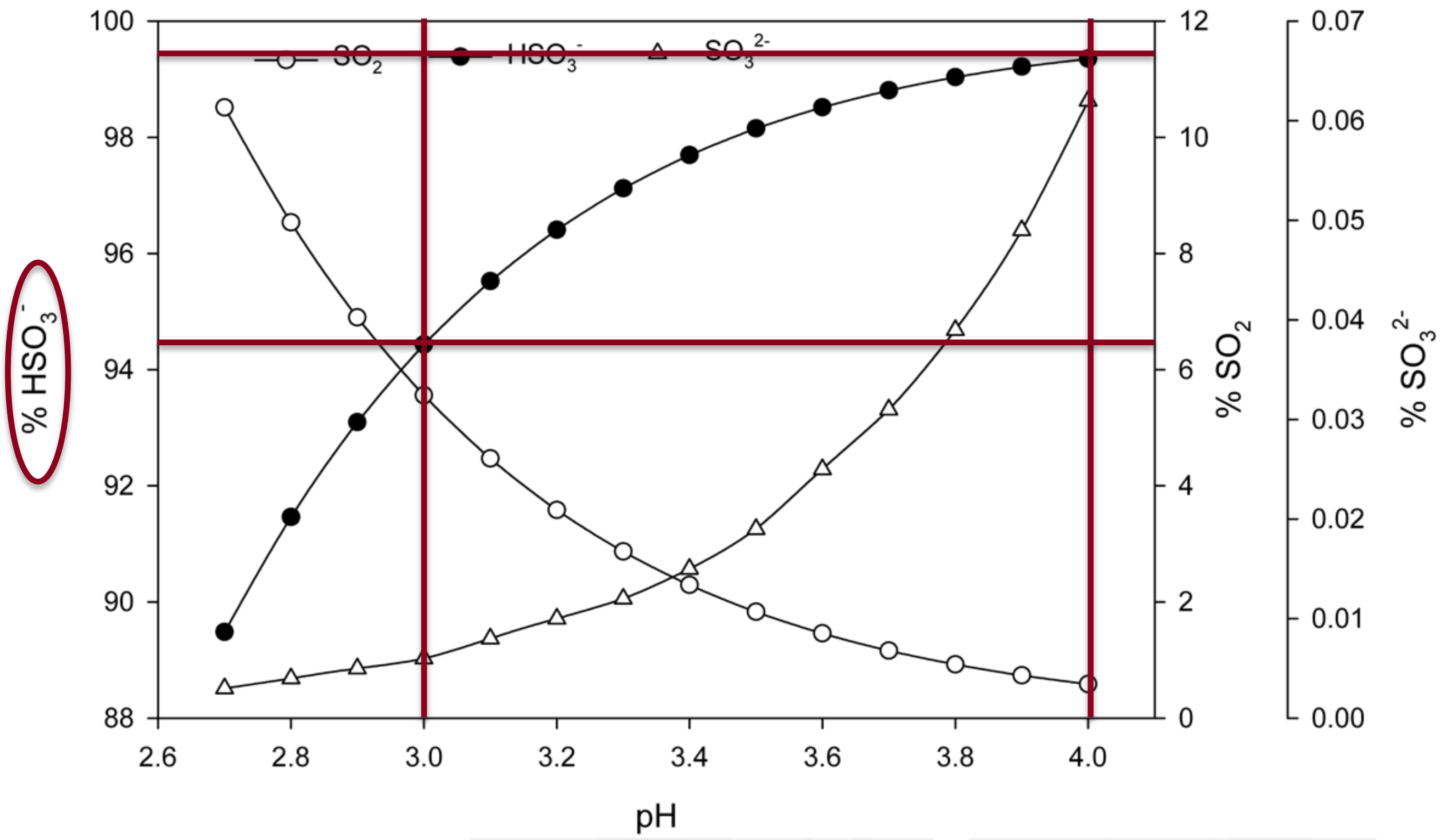


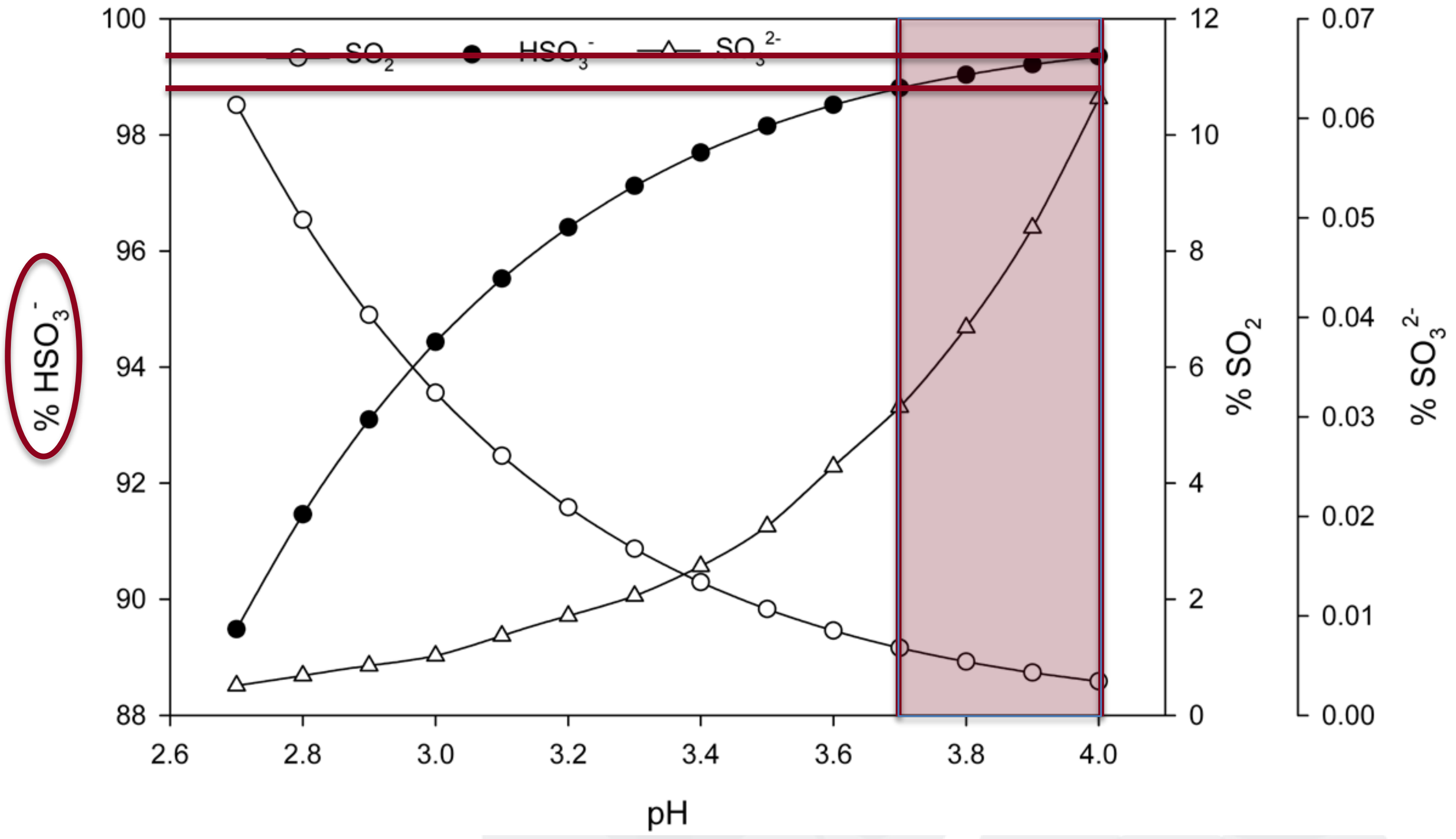
Sulfur Dioxide Forms

Between pH 3.0 and pH 4.0, bisulfite makes up 94-99% of the free SO2 present



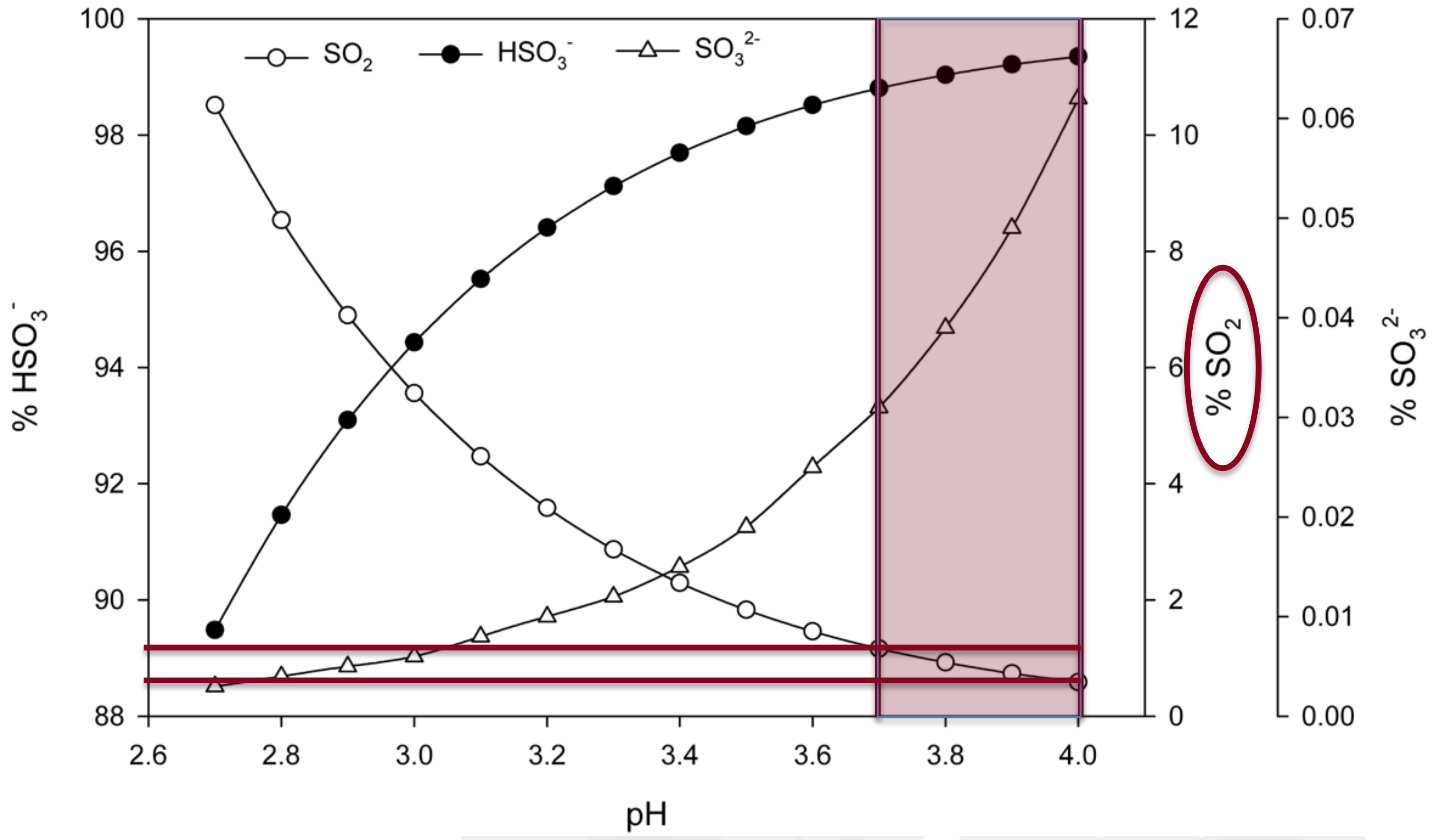
Sulfur Dioxide Forms

Between pH 3.7-4.0, bisulfite is 99⁺% of the free SO_2



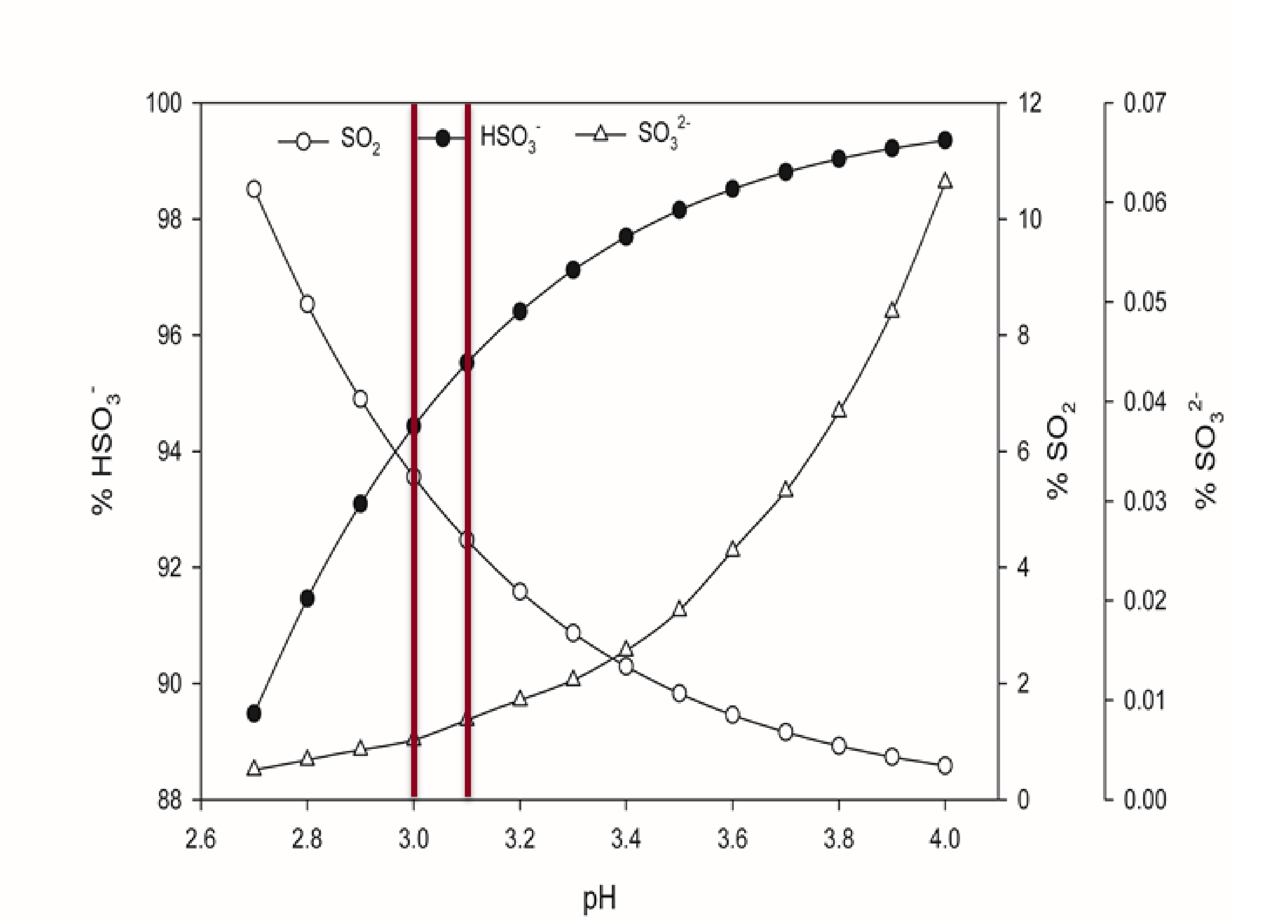
Sulfur Dioxide Forms

Between pH 3.7-4.0, molecular SO_2 is <1.5% of the free SO_2



REMINDER: Analyze Free and Total SO_2 after Addition

pH	Free SO_2 needed for 0.5 ppm molecular SO_2	Free SO_2 needed for 0.8 ppm molecular SO_2
3.0	9	14
3.1	11	17



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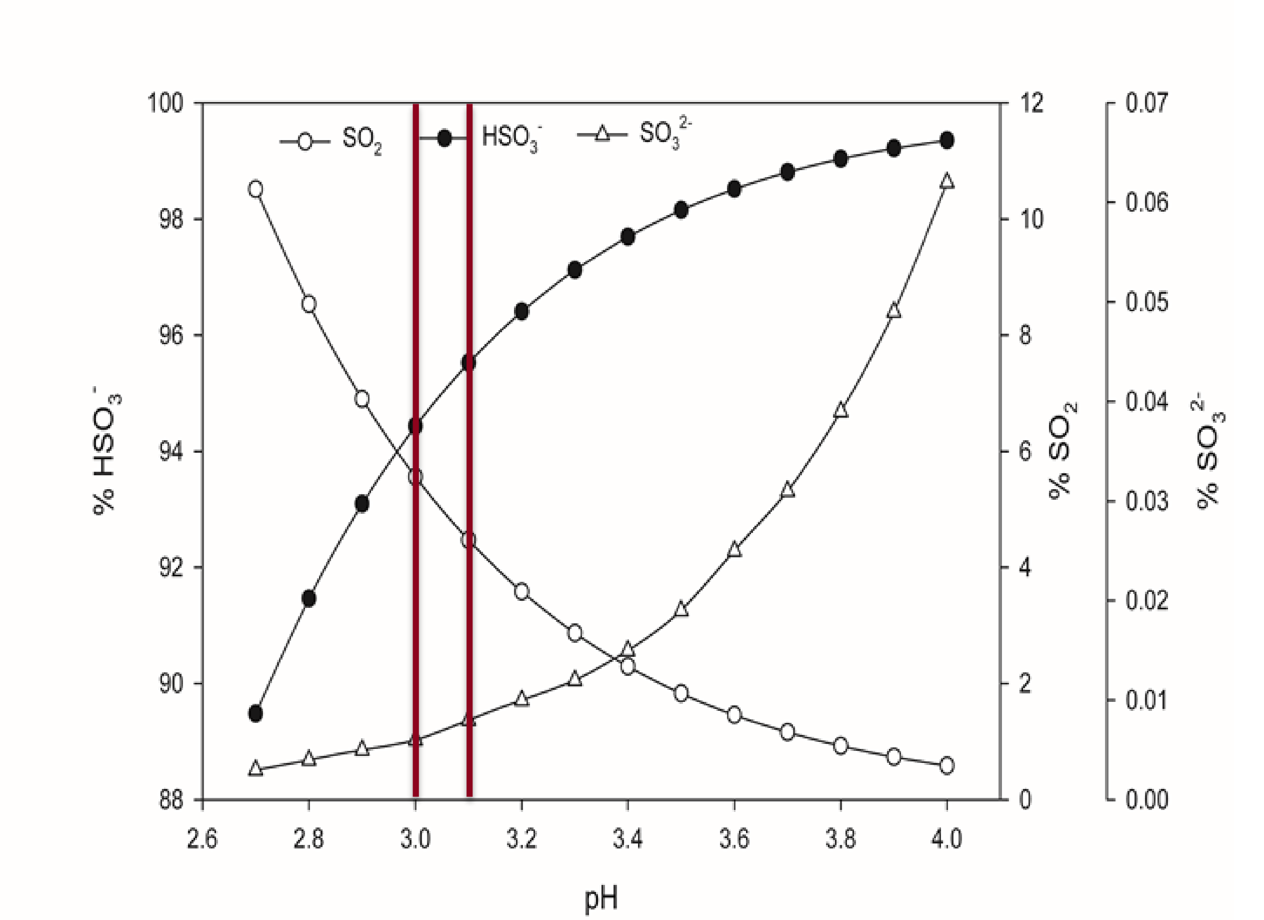
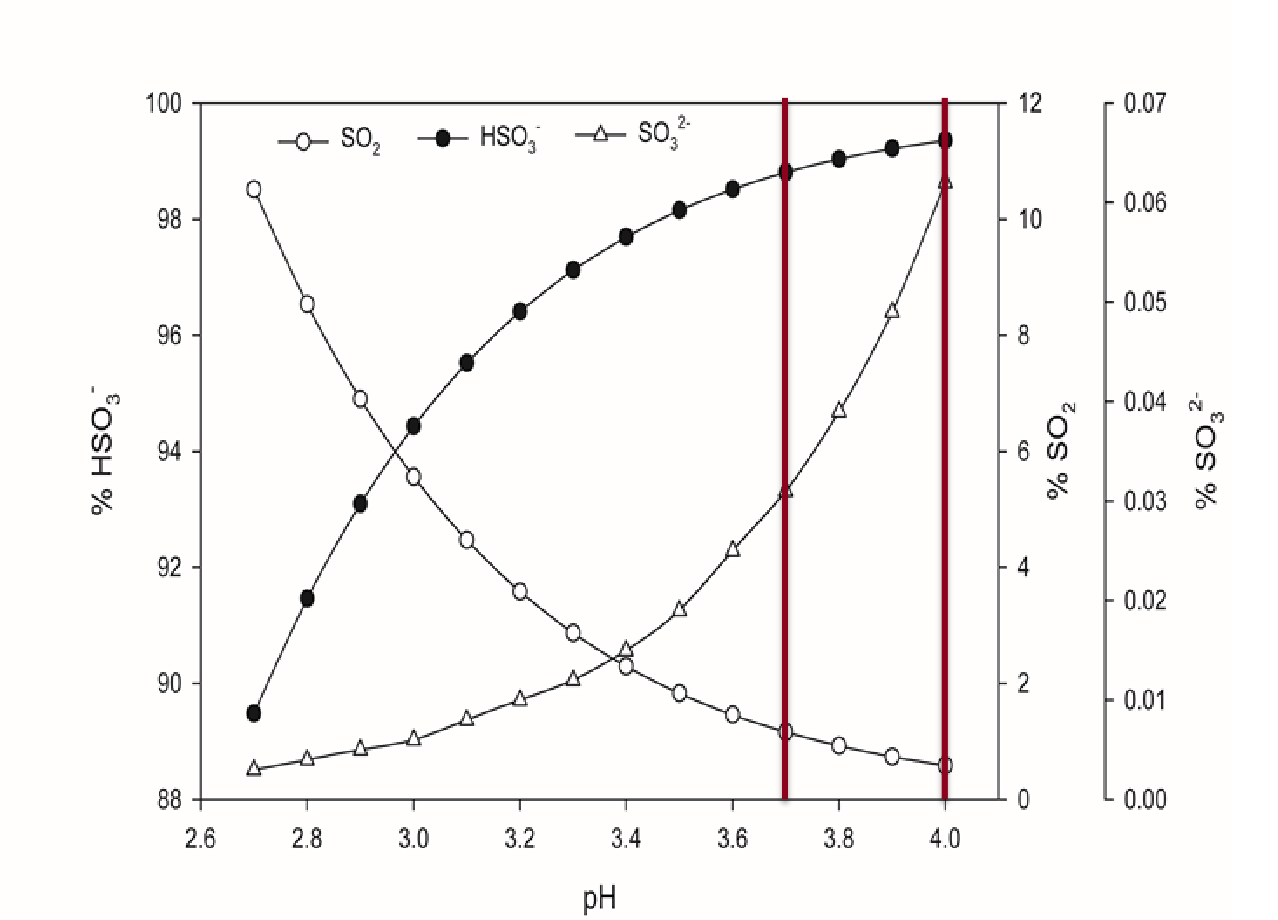


The volatility of molecular SO_2 is such that maintaining adequate free SO_2 is difficult in wines with low pH

REMINDER: Analyze Free and Total SO_2 after Addition

pH	Free SO_2 needed for 0.5 ppm molecular SO_2	Free SO_2 needed for 0.8 ppm molecular SO_2
3.0	9	14
3.1	11	17
3.7	40	64
3.8	49	81
3.9	63	101
4.0	79	128



At high pH, volatility of molecular SO_2 is not the issue, the issue is having enough free SO_2 to reach adequate levels of molecular SO_2

Cleaning and sanitation

- Sanitation refers to the reduction of population of micro-organisms present on a surface
  - Usually defined as a 3-log reduction, i.e., 99.9% of the original population is removed (killed)
  - 1 cell left for each 1000 in the initial population

Cleaning and sanitation

- Sanitation is often achieved through the application of chemical agents
  - Acidic agents reduce pH to levels that are lethal to most cells (<2.0)
  - Basic agents increase pH to greater than 10.0
  - Oxidizing agents can disrupt cell membranes

- Heat can also be used to achieve sanitation
  - Water above 165°F will kill most organisms
  - 180°F required to kill some spores
  - Low pressure steam can be used as well
  - Time and temperature are critical to success

High pH winemaking impacts on sanitation

- Used at appropriate concentrations, sanitizing agents are not directly impacted by high pH (3.7-4.0) wine residues
  - Sufficient chemical agents to establish conditions for sanitation

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- Indirect effects of higher pH winemaking
  - Greater diversity of microbial population
  - Higher populations of microbial organisms
  - Ability of many wine related organisms to create protective biofilms

Sanitation treatments

- Guzzon, et al, 2013, evaluated the effectiveness of aqueous ozone against a wide range of wine related microorganisms
  - At low cell counts (10^3 CFU/mL), aqueous ozone was capable of eliminating all of the organisms tested
  - At higher cell counts (10^5-10^6 CFU/mL), greater concentrations of ozone were required (7mg/L) and some organisms could not be eliminated even at that concentration of ozone

Guzzon, et al, Australian Journal of Grape and Wine Research, 2013 (19), pp. 180-188

Effectiveness of aqueous ozone (from Guzzon, et al, 2013)

	Initial cell concentration (CFU/mL)				
	10^6	10^5	10^4	10^3	10^2
Brettanomyces bruxellensis	5	1	1	1	1
Acetobacter aceti	VC	5	5	2.5	1
Gluconobacter oxidans	7	2.5	2.5	1	1
Hanseniaspora uvarum	7	2.5	1	1	1
Metschnikowia pulcherrima	VC	VC	VC	1	1
Oenococcus oeni	2.5	1	1	1	1
Pediococcus pentosaceus	5	2.5	1	1	1
Saccharomyces cerevisiae	7	2.5	1	1	1
Zygosaccharomyces bailii	7	7	2.5	2.5	1

Concentration of ozone (mg/L) required to eliminate several wine relate microorganisms

The authors noted a correlation between ozone required and ability to form biofilms

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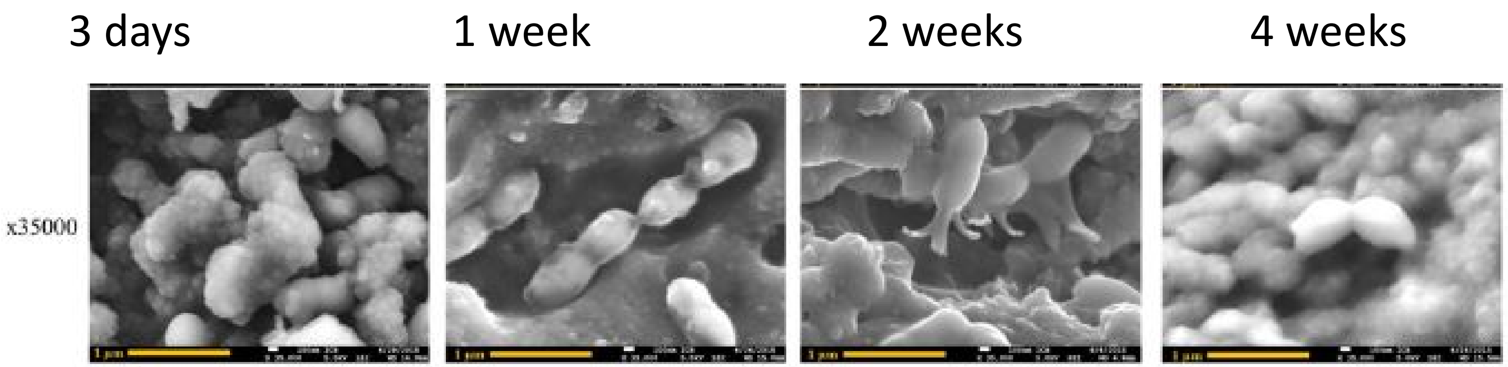
Biofilms

- Many wine related organisms can form or participate in biofilms
- Biofilms often involve multiple species of micro-organisms, protected by a polysaccharide coating
- Biofilms are resistant to many chemical cleaning agents
  - Mechanical scrubbing action required to break up the protective coating
  - Organisms beneath the coating are protected from chemicals and even heat
- Biofilms often found in the scum or foam line in tanks

Oenococcus oeni biofilm formation



Oenococcus oeni can form biofilms on steel and on wood surfaces when population are high enough

Surface adhesion and the protective material around the cells provide additional resistance to cleaning/sanitizing attempts

Mechanical scrubbing often required to disrupt the biofilm

Bastard, et al, *Frontiers in Microbiology*, 7, (2016), Article 613

Heat

- Heat is an effective sanitizing agent
  - Most wine-related micro-organisms can be killed with hot water (>165°F) or steam, with sufficient contact time
  - Contact time is temperature dependent, with shorter times at higher temperatures
- Contact time should be based on when the surface temperature reaches the desired temperature
  - It takes some time for equipment to reach the desired temperature

Time/temperature guidelines for sanitation	
Temperature	Time
200°F	20 minutes
180°F	30 minutes
160°F	40 minutes
140°F	60 minutes
Practical Winery & Vineyard, 1989	

Steam

- Low pressure steam has been used for sanitation of bottling lines and for barrel sanitation

- Contact time for use of steam for bottling lines begins when live steam emerges from the far end of the equipment

- Barrels can be surface treated with steam
  - Wood is a good insulator
  - Wood temperature decreases rapidly below the surface layer

Proper Sanitation

- Sanitizing agents must be used in sufficient concentration to be effective
  - Reduction in concentration used may save money, but result in ineffective control of microbial populations

- Similarly, time and temperature recommendations must be followed; with lower temperatures, longer contact time is usually required.

- After chemical sanitation, tanks should be rinsed with clean potable water
  - Some agents are consider “no rinse” sanitizers and do not require a rinse post-use

Testing sanitation

- As with cleaning, it is important to test the efficacy of sanitation efforts
  - For chemical sanitizers, test the concentration of the sanitizer remaining in solution after the sanitation procedure
  - Residual levels should be above the minimum concentration required for control
  - If little or none remains, the sanitation may not be effective
- ATP tests or swabbing/plating can be used to test the populations remaining after sanitation

Thank you!

Chlorine based

- Hypochlorite (bleach) and other sanitizing agents that create the hypochlorite
  - These were the most widely used agents until the link to TCA production was worked out
  - Hypochlorite is a strong oxidizing agent and chlorinator
    - Reacts with amino acids, proteins and lipids
    - Affects protein function
    - Reacts with lipids in cell membranes making the membranes more permeable
  - Efficacy is affect by pH and concentration
    - HOCl is more effective than OCl^-

Chlorine based

- Hypochlorite is a direct chlorinator
  - Reaction of hypochlorite with phenolic compounds yields chlorophenols
- Chlorophenols are toxic to most fungi and bacteria
  - Pentachlorophenol was widely used as a wood preservative on this basis
- Many fungi and some bacteria detoxify chlorophenols by methylation, creating chloroanisoles
- Long-term use of chlorinated cleaning and sanitizing agents have left older winery cellars with chlorophenols in cellar drains and in wood structural components

Chlorine based

- Chlorine dioxide (ClO_2) has supplanted hypochlorite as the primary chlorine based sanitizing agent
- Chlorine dioxide is a strong oxidizer, but not a direct chlorinator
  - Sanitizing action based primarily on oxidation reactions
  - Does not directly create chlorophenols when it reacts with phenolic compounds
- Chlorine dioxide is generated at the winery
  - A number of ways it can be generated, this is typical:

$$2NaClO_2 + HOCl + HCl \rightarrow 2ClO_2 + 2NaCl + H_2O$$

Chlorine based

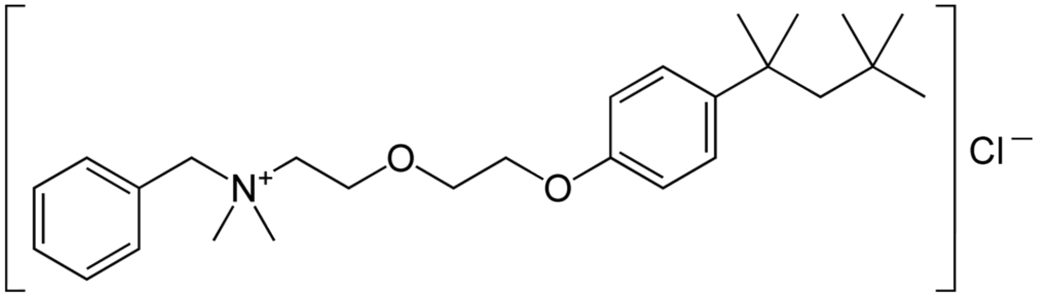
- Chlorine dioxide is colorless to brown gas, readily soluble in water
  - Explosive when present at concentrations above 5% (hence on-site generation
  - Generators limit the level produced to well below 5%

- Control of wine related organisms achieved at concentrations greater than 1 mg/L, with contact times of 1-10 minutes

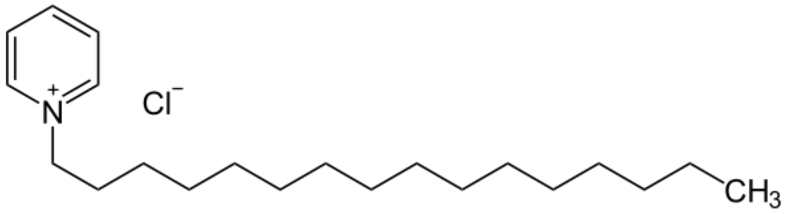
- Often used as a no-rinse sanitizer; residual ClO_2 gas maintains sanitation in closed tanks for up to several days

Quaternary ammonium compounds

- Quaternary ammonium compounds are stable cationic surfactants
  - Can act as cleaners and sanitizers
  - Quats have a lipophilic section and an ionic section
  - Interfere with cell membranes
  - 200-400 ppm concentrations for effectiveness



Benzethonium chloride



Cetylpyridinium chloride

Peroxyacetic acid (PAA)

- Peroxyacetic acid is a colorless liquid with slight aroma of acetic acid
  - Strong oxidizing agent, corrosive in high concentrations
  - Effective antimicrobial at low concentrations (<85-300 ppm), with contact times of less than 10 minutes
  - Effective over broad pH and temperature ranges
  - Registered as a no-rinse sanitizer
    - Residual PAA can maintain sanitation over several days in a closed tank
    - Breaks down to water, acetic acid and oxygen
    - Residual acetic acid does not contribute significantly to volatile acidity in wines
- Concentrated solution requires closed engineered system for dilution to sanitation concentrations

Ozone

- Ozone (O_3) is a colorless to pale blue gas with a strong odor similar to bleach
  - Used in dilute water solutions for sanitation, as air concentrations between 0.1-1.0 ppm can be smelled and can cause irritation to eyes and lungs
  - As ozone is not readily stored, it is produced on-site as needed and typically placed into cold water solutions
    - More soluble in low temperature water—ozone readily gases off in hot water
  - Ozone is a strong oxidizing agent, disrupts cell membranes
  - Residual ozone water and gas can maintain sanitation in a closed tank for a short period of time

Ozone

- Because of ozone's respiratory effects at low levels, there are strict regulatory exposure limits for workers using ozone

- Ozone can react with phenolic components in wood, degrading the lignan structural polymer
  - Oxidized compounds in the wood may also react with wines subsequent put into treated barrels