MICROBIAL CONTROL DURING THE WINEMAKING PROCESS

Dr. Nichola Hall
MN Grape Growers Association
2017 Cool Climate Conference
February 16th 2017
**MICROFLORA ASSOCIATED WITH WINE PRODUCTION STAGES**

<table>
<thead>
<tr>
<th>GRAPE</th>
<th>FERMENTATION</th>
<th>AGING</th>
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</thead>
<tbody>
<tr>
<td>• Non-fermentative yeast</td>
<td></td>
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<tr>
<td>• LAB</td>
<td></td>
<td></td>
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<tr>
<td>• AAB</td>
<td></td>
<td></td>
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<td>• Molds</td>
<td>• Fermentative yeast</td>
<td></td>
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RELATIONSHIP BETWEEN GRAPES AND WINE

JUICE CHEMISTRY

WINE CHEMISTRY

WINE MICROBIOLOGY

POPULATION WILL VARY DEPENDING UPON: VINTAGE, VINEYARD, CHEMISTRY (pH), SANITARY STATUS OF FRUIT
GRAPE MICROFLORA - WHY SHOULD WE CARE?

• Nutrient depletion
  – Vitamins and minerals can be consumed in the first few hours

• Production of microbial inhibitors

• Production of negative sensory compounds

• Control over fermentation process and wine style
CONTROL OF GRAPE MICROFLORA

• Once identified we can select the most appropriate control method
  – Biological
    • Organism introduction
      – Organism choice - Yeast and/or Bacteria
        » Inoculation time and rate, handling and acclimatization
        » Paying attention to the yeast lag phase specifics
  – Chemical
    • SO$_2$, Lysozyme, Tartaric Acid
  – Physical/Environmental
    • Settling, temperature management, hygiene
# Microbial Control of the Fermentation Process

## Alcoholic Fermentation
- Yeast strain selection
- Yeast preparation
  - Time, temperature and acclimatization
- Yeast addition
- Yeast nutrition, incl. Oxygen
  - Protection
  - Nourishment
- Temperature control
- Presence of inhibitors
  - VA, Ethanol, SMCFA

## MaloLactic Fermentation
- Bacteria strain selection
- Bacteria preparation
  - Timing, temperature
- Bacteria nutrition
- Bacteria addition
- Temperature management
- Chemical parameters
  - pH, FSO$_2$ and TSO$_2$, Ethanol
- Presence of inhibitors
  - High lactic acid, polyphenolics, pesticide residues, SMCFA
MICROBIAL CONTROL OF THE FERMENTATION PROCESS

Alcoholic Fermentation
- Yeast strain selection
- Yeast preparation
  - Time, temperature, acclimatization
- Yeast addition
- Yeast nutrition, incl. Oxygen
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MaloLactic Fermentation
- Bacteria strain selection
- Bacteria preparation
  - Timing, temperature
- Bacteria nutrition
- Bacteria addition
- Temperature management
- Chemical parameters
  - pH, FSO₂, TSO₂
  - Ethanol
- Presence of inhibitors
  - High lactic acid, polyphenolics, pesticide residues, SMCFA

GOOD FERMENTATION MANAGEMENT
AVOID MICROBIAL VOIDS

END OF ALF:
G:F, Ethanol, pH, Malic acid, VA

END OF MLF:
Malic acid, VA, pH, FSO2 (MSO2), TSO2
MICROBIAL FLORA DURING AGING

Should have none!
MICROBIAL CONTROL DURING AGING

- Hygiene
- Minimize oxygen
- Manage pH/\(SO_2\) levels
- Manage temperature
- Prophylactic or treatment dosage of Lysozyme and/or Chitosan/Chitin-Glucan
- Taste wine
- INTERVENE EARLY!

RECOMMENDED ANALYSIS:
Baseline microbiology, VA, FSO\(_2\), TSO\(_2\) and MSO\(_2\)
CONTROL IS THE BEST MEANS TO AVOID SPOILAGE!

And, spoilage occurs when we don’t or can’t exert control!
MICROBIAL SPOILAGE- A DEFINITION

Spoilage is considered to have occurred if the growth or metabolism of a microorganism imparts an off-aroma or mouthfeel character to juice or wine or, there is a change in the physical appearance.
MICROBIAL CONTROL

Environmental

- Hygiene
- Temperature
- Humidity
- Controlled Fermentations
- Nutrients deserts

Chemical

- pH
- MSO$_2$
- Chitosan/Chitin-Glucan
- Lysozyme
- DMDC (Velcorin)
- Sorbic Acid
- CO$_2$
- Alcohol

Physical

- Filtration
- Thermal
- UV
MICROBIAL CONTROL

Environmental
- Hygiene
- Temperature
- Humidity
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Chemical
- pH
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Physical
- Filtration
- Thermal
- UV
SULFUR DIOXIDE

Total SO$_2$

Free

- (M)SO$_2$
- Bisulfite HSO$_3^-$
- Sulfite SO$_3^-$

Bound

- Bound to sugars, phenolics, aldehydes, etc.
SO$_2$ AS AN ANTI-MICROBIAL

![Graph showing the percent in given form (%) of SO$_2$, HSO$_3^-$, and SO$_3^{2-}$ versus pH.](image)
SO₂ enters the cell (as it doesn’t have a charge) and undergoes a rapid pH-driven dissociation at cytoplasmic pH, yielding sulfite and bisulfite. These molecules bind with essential proteins leading to cellular death.

SO₂ is either Microstatic or microcidal depending on concentration.
### RELATIONSHIP BETWEEN pH and SO$_2$

<table>
<thead>
<tr>
<th>pH of wine</th>
<th>% as Molecular SO$_2$</th>
<th>Free SO$_2$ concentration (ppm) for 0.8 ppm Molecular SO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.00</td>
<td>6.06</td>
<td>14</td>
</tr>
<tr>
<td>3.10</td>
<td>4.88</td>
<td>18</td>
</tr>
<tr>
<td>3.20</td>
<td>3.91</td>
<td>22</td>
</tr>
<tr>
<td>3.30</td>
<td>3.13</td>
<td>28</td>
</tr>
<tr>
<td>3.40</td>
<td>2.51</td>
<td>35</td>
</tr>
<tr>
<td>3.50</td>
<td>2.00</td>
<td>44</td>
</tr>
<tr>
<td>3.60</td>
<td>1.60</td>
<td>55</td>
</tr>
<tr>
<td>3.70</td>
<td>1.27</td>
<td>69</td>
</tr>
<tr>
<td>3.80</td>
<td>1.01</td>
<td>87</td>
</tr>
<tr>
<td>3.90</td>
<td>0.81</td>
<td>109</td>
</tr>
<tr>
<td>4.00</td>
<td>0.64</td>
<td>125</td>
</tr>
</tbody>
</table>
ADJUVANTS TO SO$_2$

• LYSOZYME
  – Gram positive (Lactic acid) bacteria
    • Initial fining effect then lysis of LAB cell walls

• Chitosan/Chitin-Glucan
  – Brettanomyces, Lactic Acid and Acetic acid bacteria
    • Fining then lysis of cell walls/ membranes of cells

• DMDC
  – Most effective against Yeast

• Sorbic Acid (Potassium Sorbate)
  – Yeast
    • pH and ethanol influences it fungicidal nature
HYGIENE IS A MEANS OF MICROBIAL CONTROL, PRODUCT INTEGRITY, EQUIPMENT MAINTENANCE, ENVIRONMENT(AL) MANAGEMENT AND CONSERVATION!
5S PRINCIPLE

• 5S methodologies
  – Sort (Seiri)
    • Return, retain, trash
  – Straighten (Seiton)
    • Organize and arrange
  – Shine (Seiso)
    • Systematic cleaning
  – Standardize (Seiketsu)
    • Uniform procedures and operations
  – Sustain (Shitsuke)
    • Adhere to
CLEANING... IS A PROCESS!
HYGIENE STEPS

- CLEANING CAN BE:
  - RINSE
  - WASH
  - RINSE
  - NEUTRALIZATION STEP
  - SANITATION
    - POTENTIAL RINSE STEP

- CLEANING CAN BE:
  - RINSE
  - WASH
  - SINGLE PASS RINSE
    - SANITATION
EFFICIENCY IS ACHIEVED BY:

WATCH RULE

Balanced interactions for optimized efficiencies!
WATER

- Water quality
- Water quantity
  - 10% of volume

Can your process water be re-used?
(MECHANICAL) Action

• Key Factor for success, especially in difficult to reach areas

• Forces the contaminants off the surface
  – Used in conjunction with the dissolving properties of the cleaning solution
  – Reactions are often equilibrium controlled, constant circulation is required

• Hoses and piping
  – Turbulent flow
  – Flow rate
    • 5-7’ / sec
      – 1.5” hoses = 24-34 gpm
      – 2.5” hoses = 69-96 gpm
    • Movement is upstream, not downstream
(MECHANICAL) **Action**

- **Tank surface flow rates**
  - 27L (7.1 gallons)/min/m circulated - light soil
  - 30L - medium soil
  - 32L - heavy soil

Rates sufficient for tank surface **flow and volume** to enable cleaning to occur!
(CONTACT) **TIME**

- Cleaners do not work instantly
  - Takes time to penetrate the soil

- Consider
  - How cleaner is being applied
  - Spraying, soaking, foam, gels
CONCENTRATION

• Amount used dependent upon:
  – Water quality and quantity
  – Soil quantity
  – Soil quality
  • Damp, dried or baked
  – Temperature of cleaning water

If a little works- A lot is *not* better!
### IN PRACTICE - HOW MUCH CLEANER DO YOU NEED?

<table>
<thead>
<tr>
<th>CONDITIONS</th>
<th>EVALUATIONS</th>
<th>MY SCORE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 POINT</td>
<td>2 POINTS</td>
</tr>
<tr>
<td>Level of soil</td>
<td>Light</td>
<td>Moderate</td>
</tr>
<tr>
<td>Water hardness</td>
<td>Soft-Moderate</td>
<td>Hard</td>
</tr>
<tr>
<td>Water temperature</td>
<td>Recommended Range</td>
<td>10°F cooler than recommended</td>
</tr>
<tr>
<td>Interpretation of score</td>
<td>3 points Use low end of recommended range</td>
<td>4-6 points Use medium level of recommended range</td>
</tr>
</tbody>
</table>

Score card was designed for AiRD Products.
HEAT (TEMPERATURE)

• Each detergent has an optimum temperature at which it works best
  – Too cool
    • Ineffective
  – Too hot
    • Denature soil and without surfactants bake it on...

• Significant effect on aqueous cleaning success
  – Load carrying capacity is higher in warmer water
  – Increasing above ambient increases efficiency
  – Reduces contact (reaction) time
CONSIDERATIONS FOR CLEANER SELECTION & CONCENTRATION

• Type of soil present
  – Visible, invisible
  – Loose, baked, stain, biofilm

• Amount of soil present
  – Low, moderate, heavy

• Material composition
  – SS, wood, PP, ?
IDENTIFICATION OF SOILS
PROPERTIES

Cleaning requirements

For your operation how clean is clean enough?

Physically clean
Chemically clean
Microbiologically clean
A WORD OF CAUTION

- CAUSTICS

- Highly corrosive and reactive
- Exothermic reactions
- Increases surface tension
- Hard water + heat = scale
- Denatures and chars soils
- Potentially redeposits it
BUILT CLEANERS

• Should be suitable for the job
• Should be safe
• Respectful

• Active cleaning agent
• Adjuvants
  – Surfactants
  – Chelation aids
  – Rinse aids
CLEANING IS A PHYSICO-CHEMICAL PROCESS!
EFFICIENCY IS ACHIEVED BY:

WATCH RULE

Balanced interactions for optimized efficiencies!
PRIOR TO CLEANING

• The first stage of the cycle is always a warm water rinse as soon as equipment has been emptied
  – Deals with majority of water soluble materials
  – ~145°F
  • By doing a good job with this rinse you can cut back on the level of detergent you are using

There is a stage before this initial rinse and that is the dry cleaning phase…sweep, shovel, etc to remove as much of the visible solids as possible.
A CLEAN TANK
EQUIPMENT
THE 5S PRINCIPLE...
CLEANING EQUIPMENT

• Cleaning equipment

How often do you clean your cleaning equipment?

– PIG’s
5S PRINCIPLE IN ACTION
Gordon Taylor, DavenLore
WE ARE NOT DONE YET...

• Clean before use and after

• No ideal cleaner
  – This is why we rotate different cleaners in for different jobs
    • Cleaner choices should still be appropriate

• RINSE
  – Removes residual cleaner and prepares for sanitation
SANITATION

• Generally with chemicals or heat
  – Other industries: UV or radiation

• Reduction of non-pathogenic, vegetative cells on clean surfaces to 99.999%

• Complies with FDA and EPA
  – FDA
    • GMP
  – EPA
    • ...

SCOTT LABORATORIES
WHY DOES THE EPA GET INVOLVED?

• 4 acts that influence winery operations
  – Water pollution control act, Clean air act, Resource conservation and recovery act and Federal Insecticide, Fungicide and Rodenticide act (FIFRA)
    • FIFRA deals with pesticides
  – Sanitation aids are classes as pesticides due to their anti-microbial nature
    • Cleaners do have anti-microbial activity, but that is not their main function
HEAT

• Food grade!
  – Penetrates well, kills most microorganisms, penetrates irregular surfaces, suitable for CIP and relatively inexpensive (once set-up), non-toxic
  – Bake on residues (leading to biofilm formation), may form scale, inappropriate for general use, scalding hazard, contact time sensitive, must be generated (energy intensive process)

<table>
<thead>
<tr>
<th>TEMPERATURE (°F/C)</th>
<th>TIME (MINUTES)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200/93</td>
<td>20</td>
</tr>
<tr>
<td>180/82</td>
<td>30</td>
</tr>
<tr>
<td>160/71</td>
<td>40</td>
</tr>
<tr>
<td>140/60</td>
<td>60</td>
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CHEMICAL LABELS

Product must be used in accordance with approval
## CHOOSING SANITIZERS

<table>
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<tr>
<th>TYPE OF SANITIZER</th>
<th>ADVANTAGES</th>
<th>DISADVANTAGES</th>
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</table>
| Iodine            | Broad spectrum  
Effective at low temp  
Inexpensive  
Does not leave film  
Test strips available | May corrode metal and weaken rubber  
Unstable, dissipates quickly  
Narrow pH range (acidify)  
Sensitive to organic load  
Stains  
Irritant |
| QUAT’S            | Non-corrosive  
Residual activity (if not rinsed)  
Can be applied as a foam  
Test strips available | Inactivated by most detergents  
Inactivated by hard water  
Not broad spectrum  
Effectiveness varies with formulation |
| Ozone             | Strong oxidizer  
Broad spectrum  
Breakdown products friendly | Expensive and must be generated  
Unstable and cannot be stored  
May corrode some materials  
Inactivated by organic materials |
## CHOOSING SANITIZERS

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</table>
| CHLORINE DIOXIDE | Broad spectrum  
Strong oxidizer  
Less corrosive than Cl  
Less effected by organic residue  
Generate on site from packets  
No free Cl ion (TCA) | Unstable and cannot be stored  
Worker safety issues via generation  
High generator costs |
| PEROXY COMPOUNDS | Broad spectrum  
Good for (bacterial) biofilms  
Stable  
Effective at low temperature  
Breakdown | Expensive  
Inactivated by some metals  
May corrode some metals  
Product?? |
| SULFUR DIOXIDE  | Inexpensive  
Fairly effective | Irritant  
Registered for use |
DEVELOPING PROCEDURES

• Set up a schedule
• Assign tasks
• Procedure
  – When C&S should be conducted
  – What C&S chemicals are to be used
  – How the solutions should be prepared
  – How are they applied
  – What is the correct sequence of use
  – By whom
• MONITOR & DOCUMENT!
EXAMPLE- SSOP

- Wearing proper PPE
- Measure out X gallons of X°F potable water into clean non-reactive container
- Measure out X ounces of X brand cleaning solution
- Carefully mix to ensure homogenous solution
- Check concentration is within range (state range)
HEAVY TARTRATED TANKS

1. Circulate warm water (104-140°F) for 10 mins.
2. Drain solution from tank.
3. Mix 2% solution of Cleanskin®-K in chemical mixing drum in warm water (104-140°F).
4. Remove door seal and clean door seal in Cleanskin®-K solution with stiff brush.
5. Circulate solution for 15 mins through spray ball.
6. Lower spray ball in the tank to clean around door - circulate for 15 mins.
7. Inspect tank - repeat steps five and six with new solution if required.
8. Drain solution when tank is clean.
9. Refit door seal.
10. Single pass water rinse.
OVERVIEW

• Keep abreast of your wines
  – Know their chemistry and have baselines
  – Know the risks associated with the different organisms
    • Exploit their weaknesses!

• Have procedures and protocols

• Follow WATCH rules

• Use winery specific chemicals for wine based soils and winery equipment
THANK YOU

Questions

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Katiec@Scottlab.com