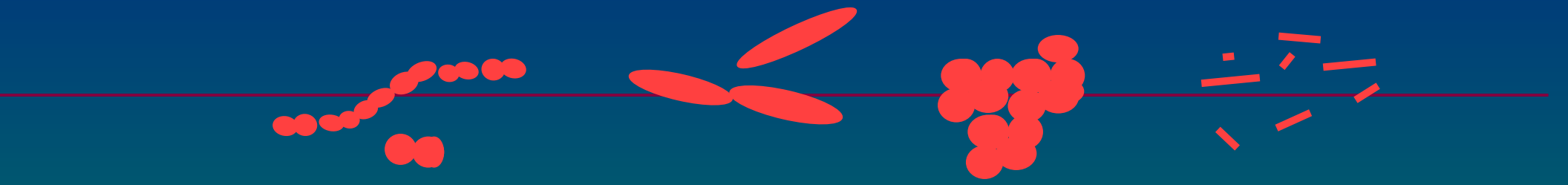
The Joy of Malolactic Fermentation



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Malolactic Fermentation

- "The Second Fermentation"
- 1913 Müller-Thurgau proved bacterial theory

$$C_4H_6O_5 \rightarrow C_3H_6O_3 + CO_2\uparrow$$

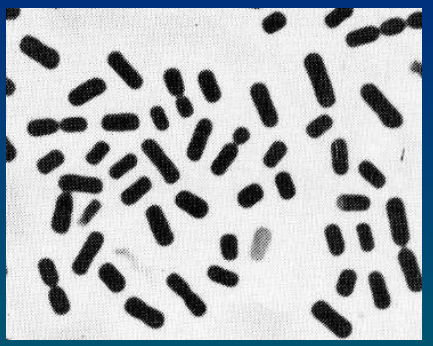
MALIC ACID — LACTIC ACID — CARBON DIOXIDE

- Lactic Acid Bacteria LAB- 4 genera:
  - Lactobacillus
  - Pediococcus
  - Leuconostoc
  - Oenococcus

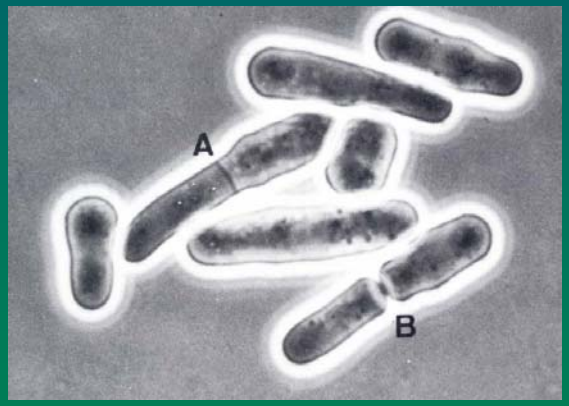
Malolactic Fermentation

Oenococcus/Leuconostoc

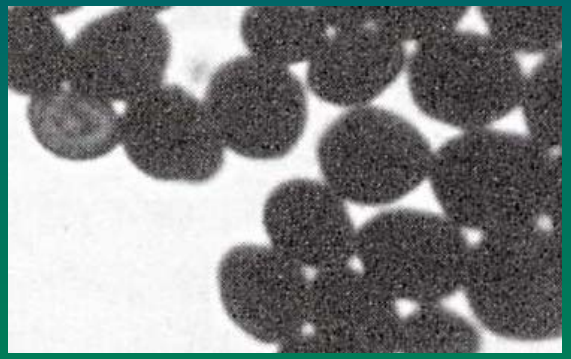
Pediococcus



Lactobacillus



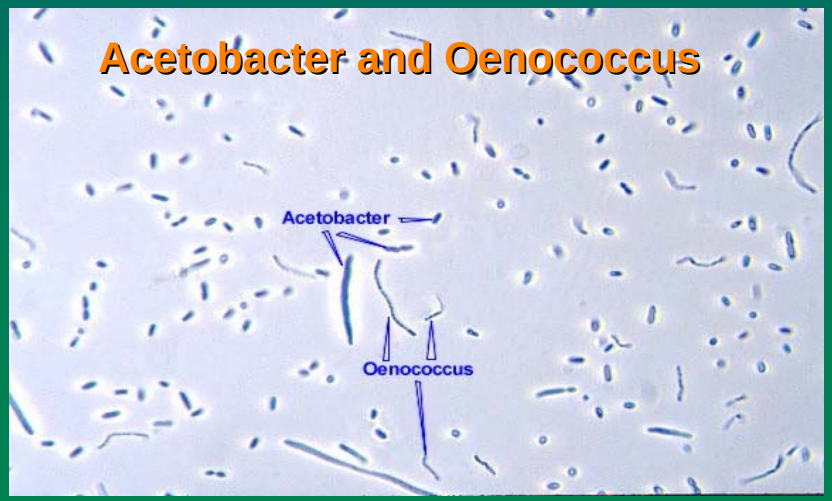
Schizosaccharomyces pombe



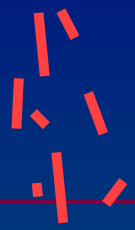
Saccharomyces cerevisiae

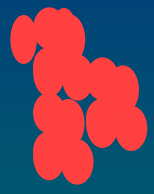
Genetically modified with bacterial DNA

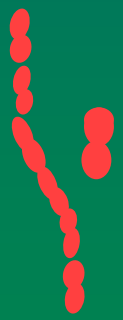
Microflora Found in Must & Wine



Taxonomy of LAB





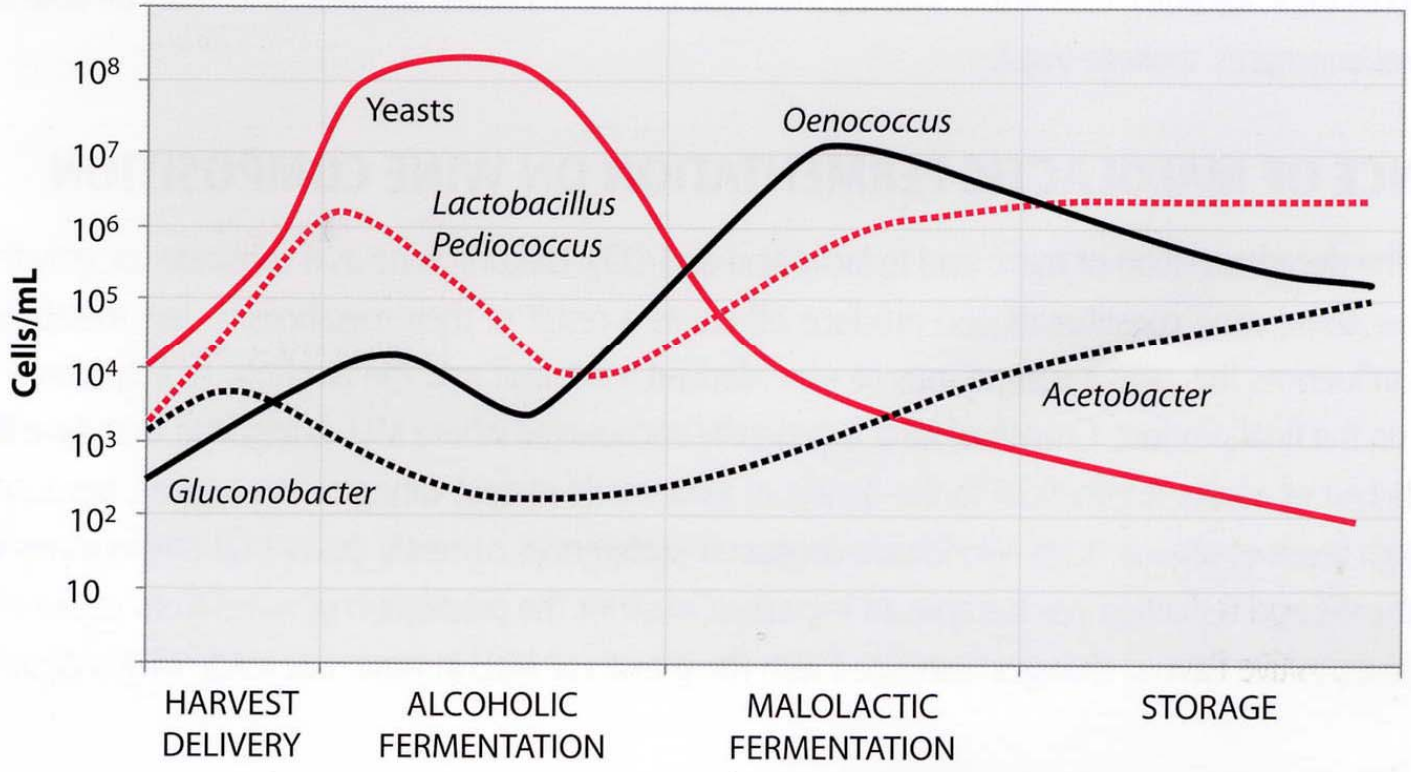


- All LAB
  - Gram Positive
  - Non-mobile & non-sporulating
  - Facultative Anaerobes
  - Convert Malic Acid to Lactic Acid
  - Require rich medium & fermentable sugars
    - Different genera utilize glucose differently

Growth of LAB

- Few LAB found on fruit, leaves, stems
- Usually found on winery equipment

Figure 1. Growth cycle of lactic acid bacteria in wine during vinification and storage



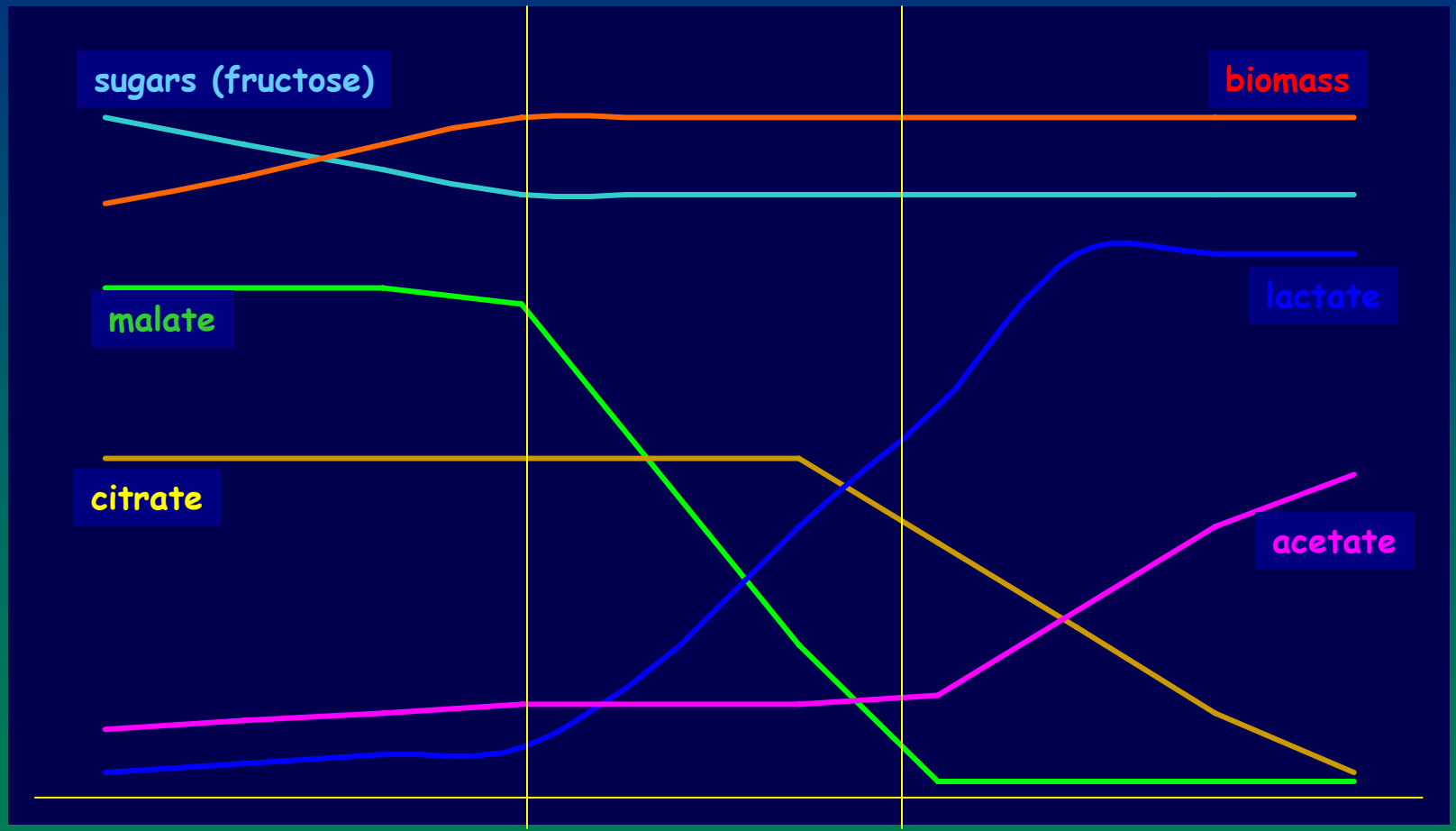
MLF Decisions – Why to …

- Desired character of final wine?
  - Acid level reduction
    - Depends on concentration of Malic Acid
    - Rough estimate with paper chromatography
  - Masking of vegetal character by diacetyl
  - Increased body by increased production of dextrans and glucans
  - Increased flavor complexity
    - Buttery, nutty, honey vanilla, leather, spices toasty, more body, smoother tannins

Diacetyl – Concentration
The Butter Factor

- To encourage high levels:
  - MLF occurs late in fermentation
  - Lower MLF culture inoculation rate
  - Lower end of temperature range
  - Lower pH wines
  - High Citric Acid concentration
  - Reduce contact time with yeast and bacterial lees

Diacetyl – Concentration
The Butter Factor



CELL GROWTH
sugar catabolism
no malate catabolism
no citrate catabolism
slight acetate production
slight lactate production

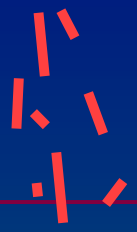
STATIONARY PHASE I
no sugar catabolism
malate catabolism
no citrate catabolism
no acetate production
lactate production

STATIONARY PHASE II
no sugar catabolism
no malate catabolism
citrate catabolism
acetate production
no lactate production

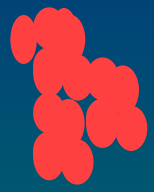
MLF Decisions – Why not …

- Potential problems:
  - Volatile acidity always increases, especially in high pH wines
  - pH is always increased
  - Cost to produce wine increased
  - Time to produce wine increased
  - Sulfur dioxide addition delayed
  - NOT recommended for sweet wines

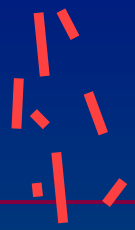
Wine Spoilage by LAB



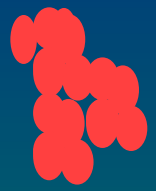
- Spoilage issues involve
  - Wine conditions above 3.5 pH &
  - Indigenous Lactobacillus and Pediococcus (not Oenococcus)
  - Off odors:
    - Cheesy, milky, metallic, earthy, mousy, sweaty, wet leather, rotten fruit
  - Physical changes:
    - Loss of color
    - Ropiness
    - Bitterness
    - Tartaric acid decomposition

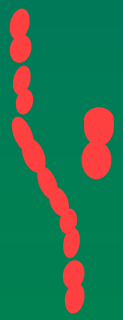


Wine Spoilage by LAB

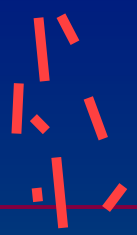
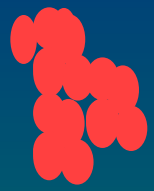


- Spoilage issues involve
  - Production of Histamine
    - May cause rx at levels >0.1 mg/L in sensitive people
      - Rashes, respiratory problems, runny nose
    - Hi pH >3.5 = increased Histamine levels
    - Inoculation with commercial cultures reduces population of “wild” LAB
    - Post MLF cells should be removed ASAP to reduce histamine production



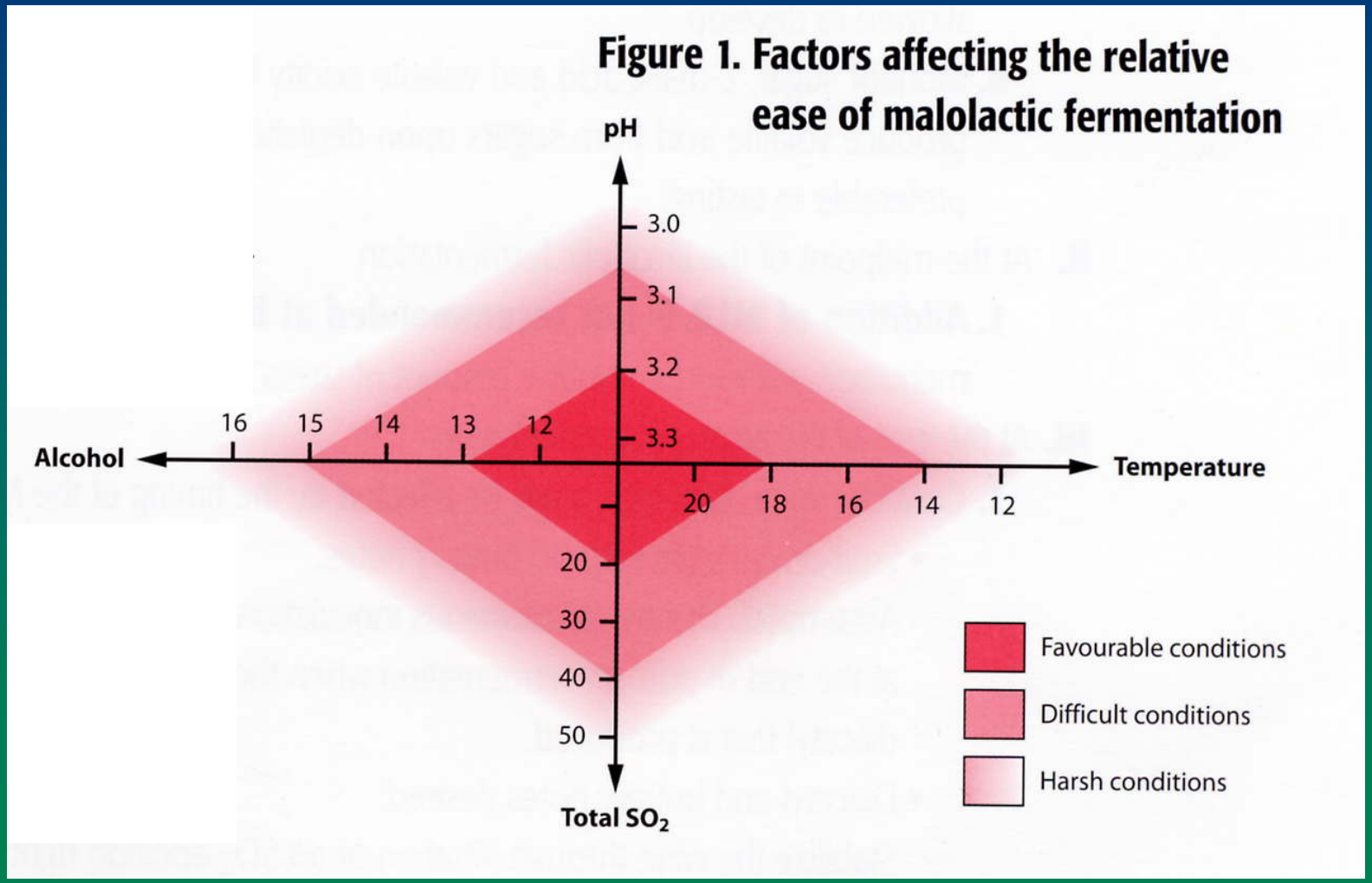


Wine Conditions vs MLF

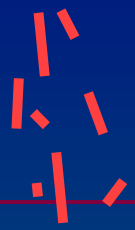



	Limiting Conditions	Ideal Conditions
Temperature	< 60F	65-68F
pH	<3.1	>3.2
Alcohol	>13.5%	<12.5%
Total SO2	>30 mg/L	<20 mg/L
Free SO2	>20 mg/L	<10 mg/L
Inoculation	Aged, clarified wine	Late yeast fermentation

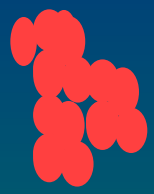
Wine Conditions vs MLF



MLF Decisions – Timing



- Inoculation near end of sugar fermentation usually best
  - Warmer temperatures (60-65 F)
  - Lower CO2 concentration
  - Deteriorating yeast cells
    - contribute micronutrients
    - Absorb inhibitory compounds
      - pesticides, decanoic acid
  - Higher concentration of flavor compounds
    - Diacetyl, acetoin & 2,3 butanediol



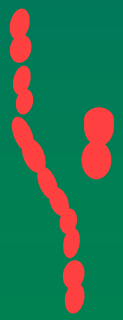
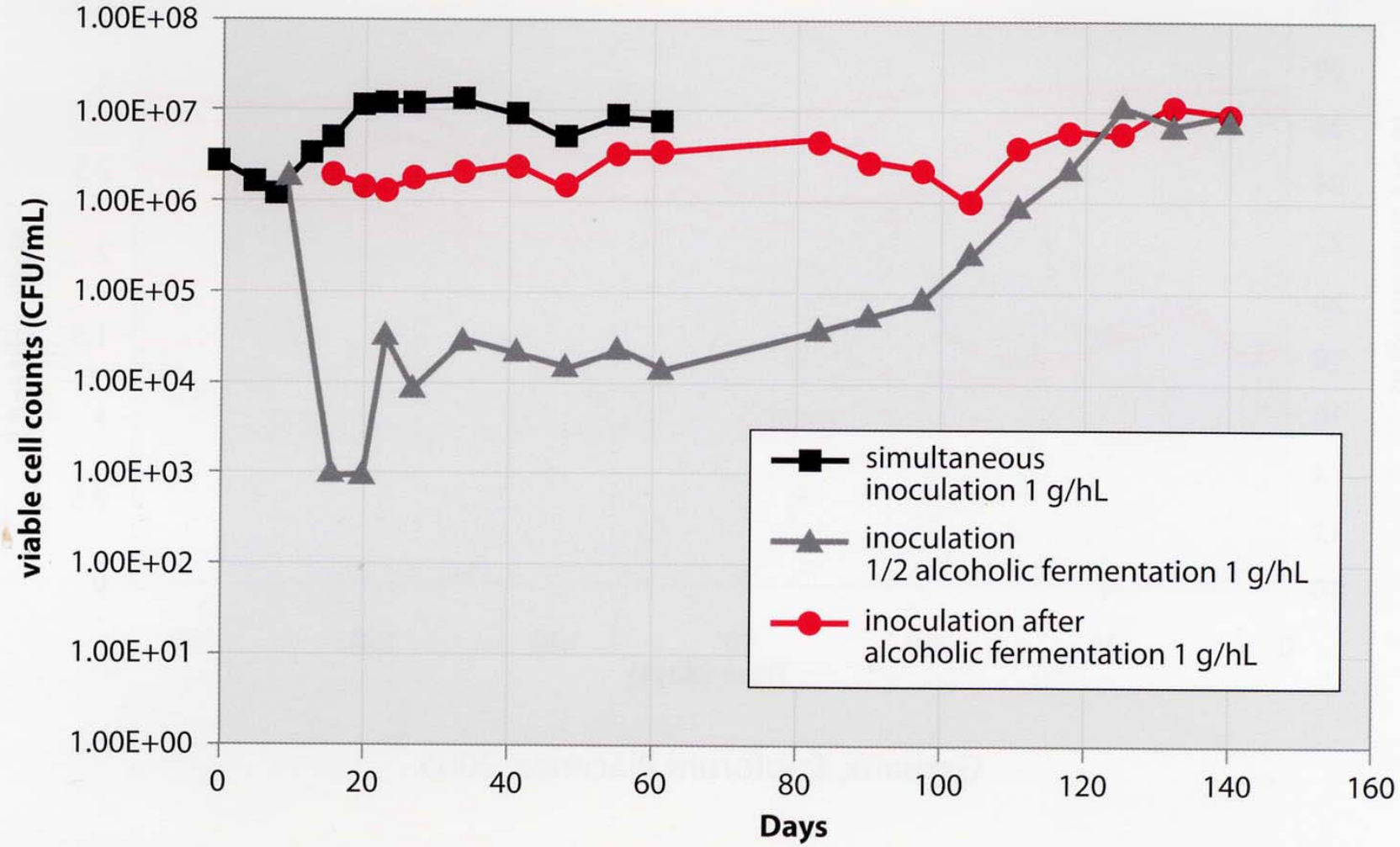


Figure 5. Survival of malolactic bacteria after inoculation at different stages of alcoholic fermentation



1999 Late Harvest White Rieslaner, pH 3.3, Alcohol 12.5% (v/v), 13°C

Uninoculated/Spontaneous MLF

- Why?
  - you forgot to buy MLF culture
  - you like to take big chances
  - it just happened
  - flavor complexity potential
  - increased body

Uninoculated/Spontaneous MLF

- How?
  - Hi pH wines, used barrels, warm temperatures, red wines
  - Minimal SO2 addition
    - <25 gm/L at crush
    - No addition until MLF complete
  - Keep on lees until complete
  - Monitor with chromatography weekly after end of alcoholic fermentation

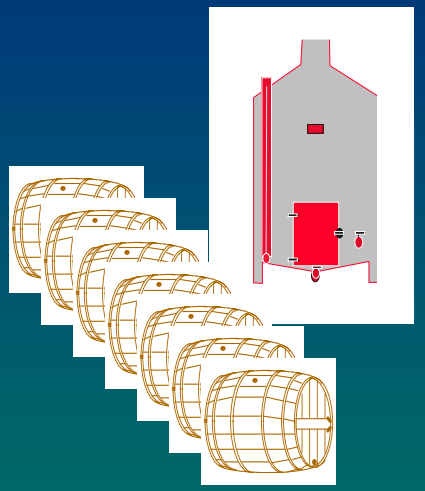
Using Starter Cultures

- FOLLOW DIRECTIONS OF PRODUCER
  - Purchase fresh each year, store frozen
  - Use proper amount of culture
  - Use nutrient addition, esp. if marginal wine
  - Rehydrate if suggested
  - Maintain optimum temperature
  - Occasional stirring is good
  - Monitor weekly with paper chromatography
  - Rack and sulfite wines immediately
    - Sur Lees styles are exception
    - Monitor at each stirring for problems

Using Starter Cultures

1. DIRECT

DIRECT INOCULATION CULTURES



2. Build-Up

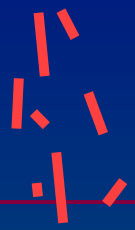
BUILD-UP CULTURES

1 rehydrated sachet (68-86°F clean water)
slight stirring, avoid air, let suspension rest 15 minutes

Equal parts H_2O & juice (pH>3.3, no SO_2), 1% of final wine volume, add active dried yeast & ML nutrient. Wait for mix to complete alcohol fermentation. (3-5 days)

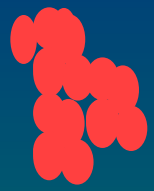
4-8% of final wine volume (pH>3.2, 65-80°F). Wait for ~1/2 conversion L-malic before expanding (1-3 weeks)

Problem ML Fermentations

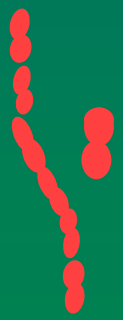


Must monitor with chromatography or better

- evidence of gas or reduced total acidity is not good enough



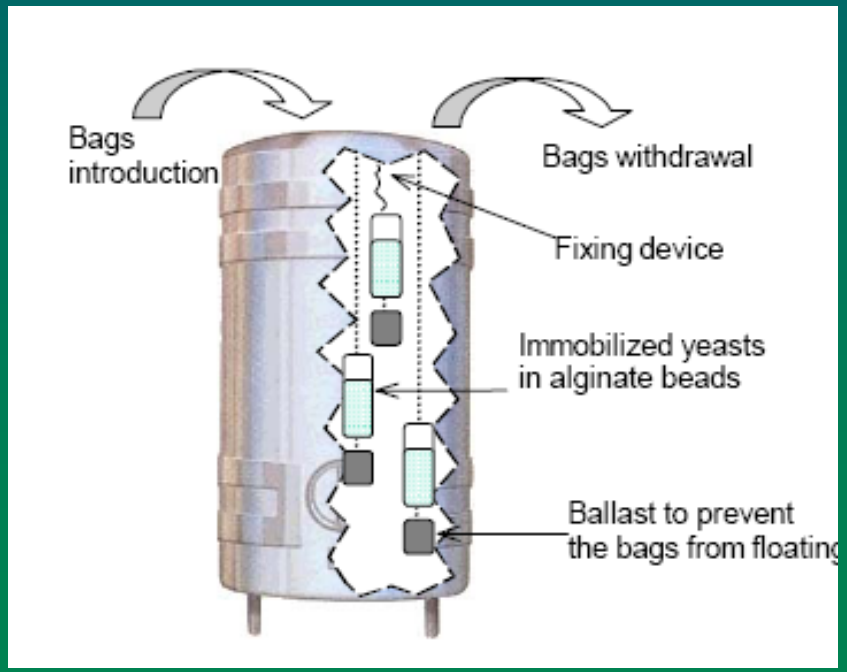
- Consider
  - Warming slightly if lower than optimum wine temperature
  - Racking or stirring to resuspend packed lees
  - Reinoculation (after 3-4 weeks)



Alternative Microbes

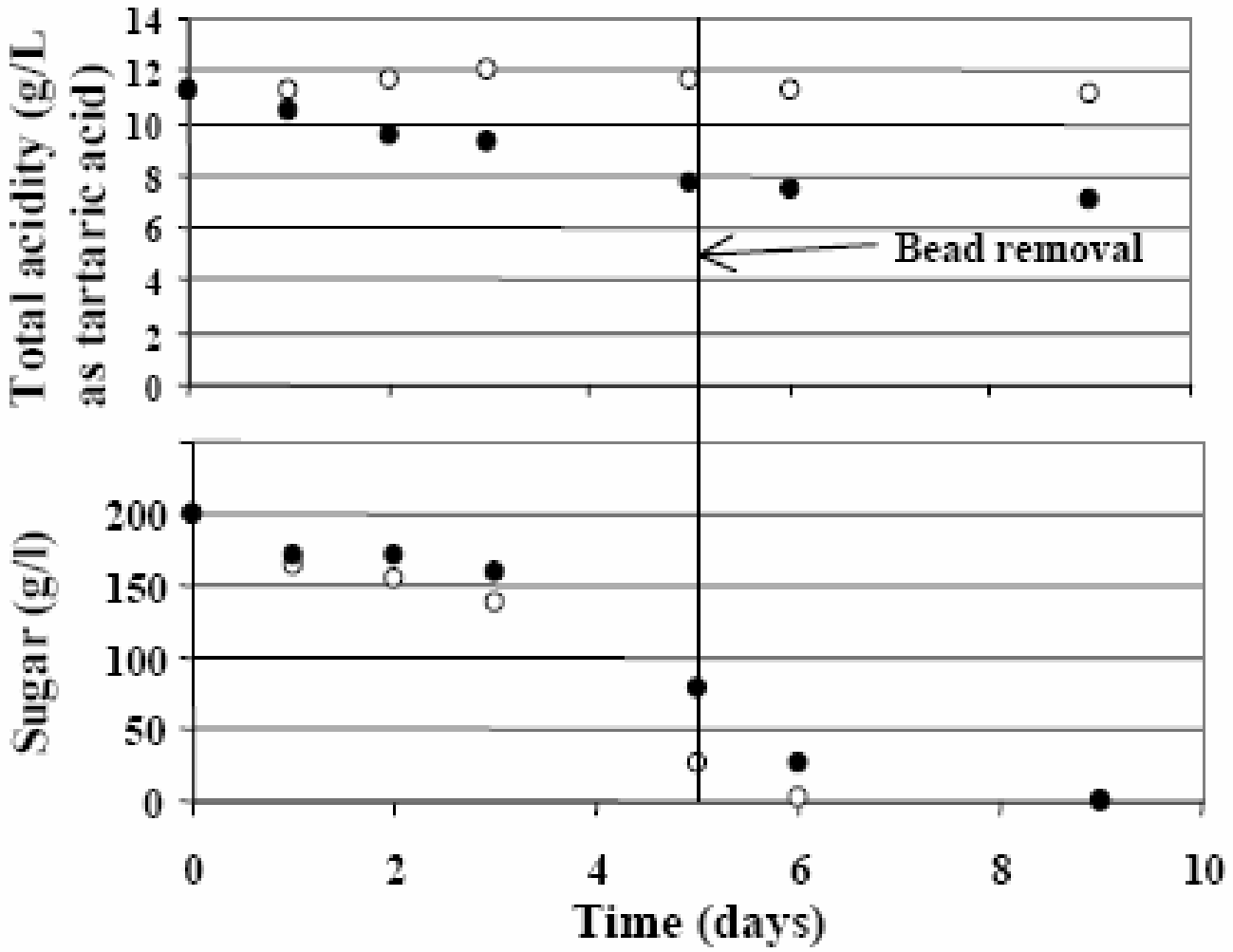
Schizosaccharomyces pombe – ProMalic

- Considered spoilage yeast – can product off odors during late alcoholic fermentation
- Metabolizes malic acid to ethanol – no lactic acid produced
- Encapsulated product remove when malic acid is depleted

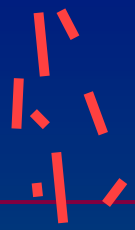


ProMalic Trial:

T.A. & R.S. during vinification of Azal white wine using ProMalic and *S. cerevisiae* (black circles) and a control with only *S. cerevisiae* (open circles). Initial RS 200g/L, 3.12 pH, fermentation at 16°C and 50ppm SO_2 added to the must.

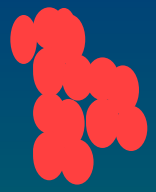


ProMalic
Pros & Cons



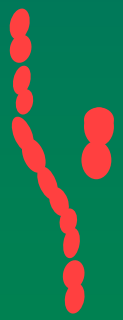
Pros:

- Reliable, fast, easy, no lactic acid, more acid reduction, tolerates SO2 & very low pH



Cons:

- Expensive, cannot expand culture, must remove from fermentation or potential for flavor problems, must handle bags several times a day.



Genitically Modified Yeast

Saccharomyces cerevisiae with bacterial malolactic gene implanted

- Developed by Hennie JJ Vuuren at U. of British Columbia, 2001
- Converts malic acid to lactic acid + CO_2
- Very new product, not much information available
- Must use low inoculum – the gene is inactivated by ethanol

Genetically Modified ML01 Yeast Pros & Cons

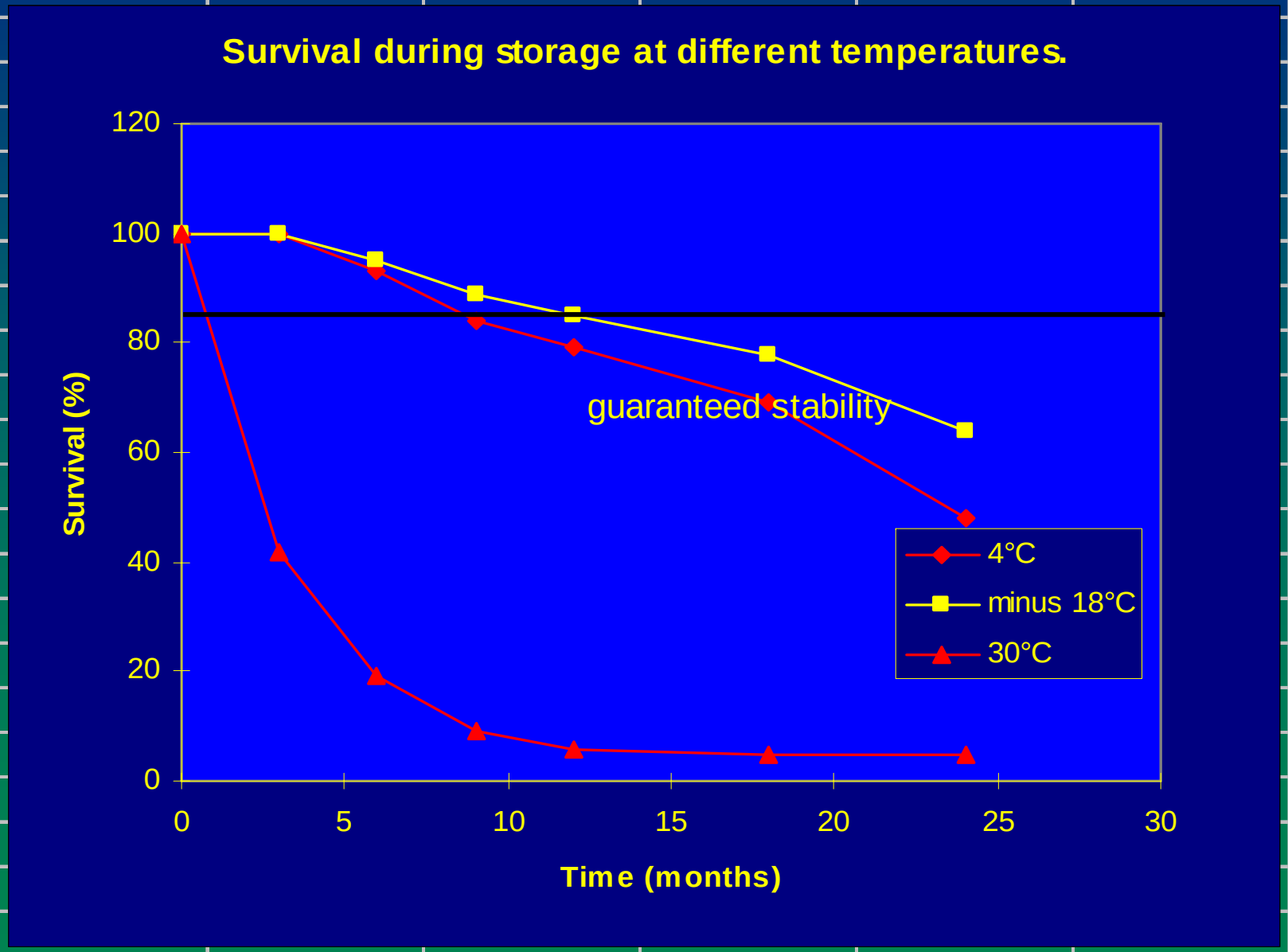
Pros:

- Easy, lactic acid complexity, tolerates SO2 & very low pH,

Cons:

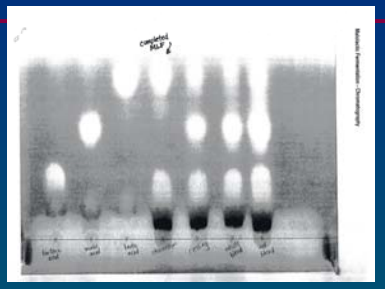
- Timing of malic metabolism is critical, may still have to use bacterial inoculation if malic is not depleted.

Freeze Dried Starter Culture Survival During Storage



Lab Evaluation of MLF

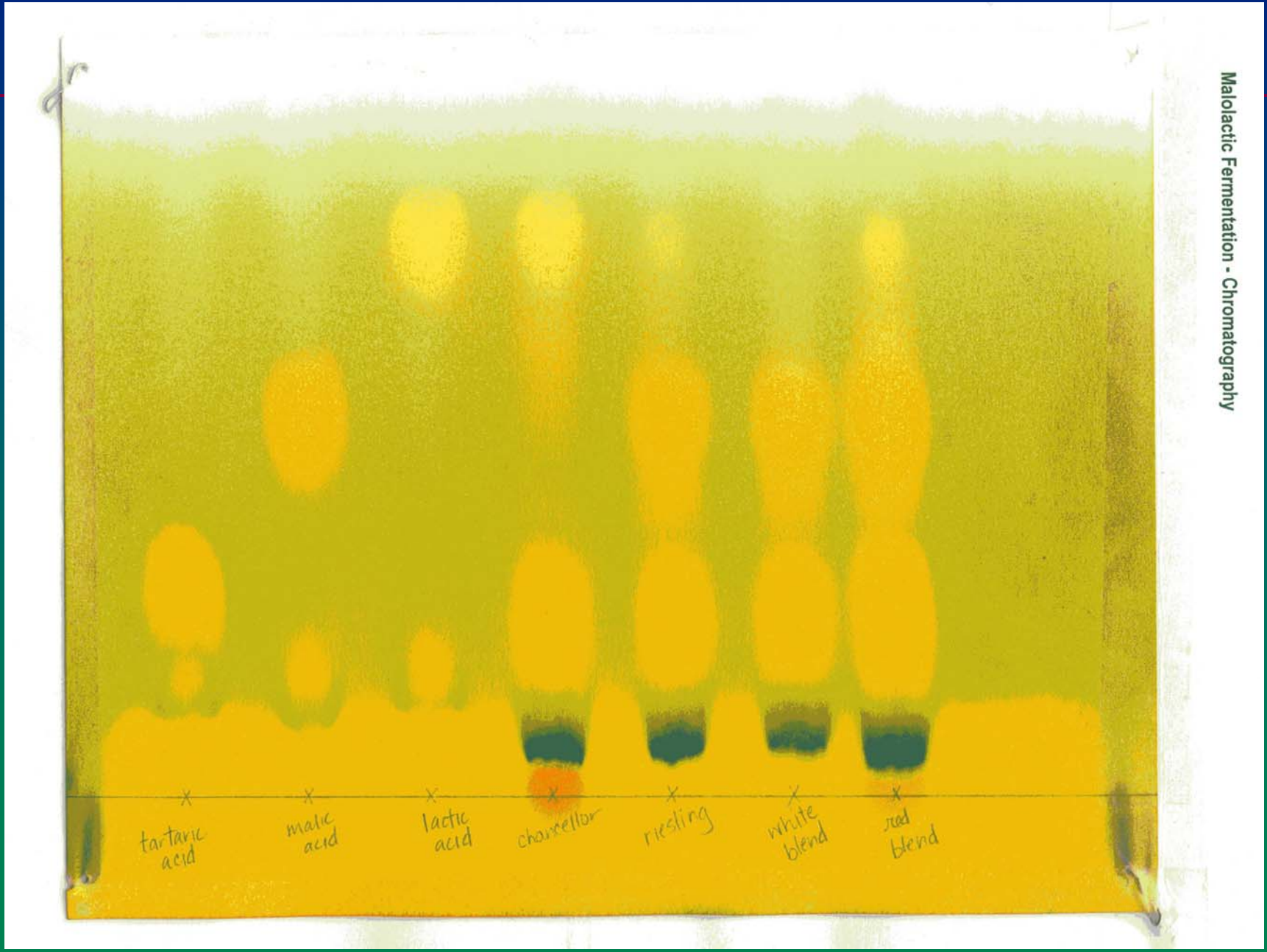
- Paper Chromatography:
  - Easy
  - Inexpensive ($40-45$/kit)
  - Moderately accurate



- Enzymatic Analyses:
  - Difficult
  - Expensive ($25/test)
  - Very Accurate

- Reflectoquant type systems
  - Somewhat difficult
  - Expensive ($500 +$1/test)
  - Varying Accuracy

Paper Chromatography



Commercial Malolactic Cultures

Producer	Culture	Liquid	Freeze Dried
Lallemand, Inc. Dist by: Scott Labs (707) 765-6666 www.scottlaboratories.com/home.asp	10 different cultures		X
Chrs. Hansen, Inc Dist by: Gusmer Enterprises Phone: (715) 258-5525 www.filtermaterials.com/Gusmer/index.asp	Viniflora CH-35 Viniflora CH-16 Viniflora Oenos		X X X
Vinquiry www.vinquiry.com Tel: 707-838-6312	5 different strains	X	X

Commercial Malolactic Cultures

Producer	Culture	Liquid	Freeze Dried
Pickering Winery Supply (415) 474-1588 http://www.winerystuff.com/	44-40 54-40		X X
BioSource Flavors, Inc. Muskego, WI 53150-0777 Phone: 414 422 9085 www.biosourceflavors.com/contact.htm	Leuconostoc oenos		X

Commercial Malolactic Cultures

Producer	Culture	Liquid	Freeze Dried
Vinquiry (Lallemand product) Tel: 707-838-6312 www.vinquiry.com	ProMalic Yeast Strain		X
American Tartaric Products Larchmont, NY 10538 Phone: 914-834-1881 http://www.americantartaric.com/	ML-01 Yeast Strain (Genetically modified)		X

Comparative Cost

Commercial Culture	Cost /gallon of wine
Malolactic Bacteria Cultures	$0.10 - $0.20 / gallon
ProMalic – S. pombe yeast	$0.30-$0.50 / gallon
ML-01 Genetically enhanced yeast	$0.036 / gallon

2005 Frontenac Wine Samples

Sample# 1 Frontenac + Oak & MLF	Sample#2 Frontenac Standard Red Method
HARVEST: Brix 24: pH 3.4; TA 1.05	**HARVEST:** Brix 24: pH 3.4; TA 1.05
WINE: RS: 0.3% pH: 3.9 TA: 0.735% VA: 0.07% ETOH: 12.8% MLF (chromatography) Complete	**WINE:** RS: 0.2% pH: 3.74 TA: 0.953 % VA: 0.04% ETOH: 13.7% MLF (chromatography) Negative

Famous Purdue Boilers