Fermentation

Amateur Winemaker Workshop

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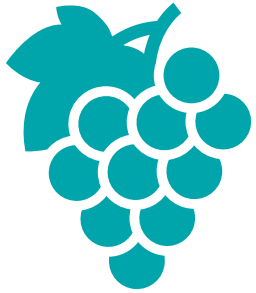
Quality wine ~



60 - 80% Vineyard

Start with healthy grapes picked at ripeness for the style of wine

40 - 20% Winemaker

The more you learn about microbes the more success you will see with quality of fermentation

What is winemaking?

Wine is simply crushed, fermented juice, a spontaneous product produced by spoilage

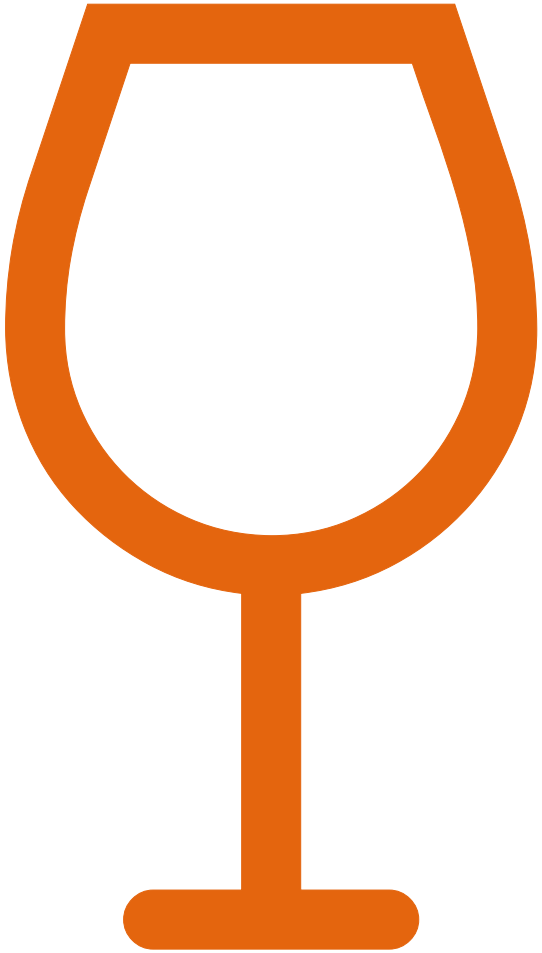
"Winemakers succeeded with combinations of folklore, observation, and pure luck" - Zoecklin, et al.

As technology increases along with trial and error, the more we learn about these microbes

Management not a dictatorship

Fermentation Equation

- Yeast + Sugar = Alcohol + CO2 + Heat



Fermentation ~

- An attempt to manage the constant chemical changes while allowing intrinsic fruit quality and the positive benefits of microbiological activity to emerge
    - It's not just about converting sugar to alcohol, although success is important
- Fermentation by-products are important
    - Sulfides/sulfites
    - Odor/flavor compounds
    - Esters
    - Terpenes
    - Mannoproteins

Yeasts ~

Kingdom: Fungi

Division: Ascomycota

Genus: Saccharomyces

Species: Cerevisiae / Buyanus

Good Wine Microbes ~

- "Beneficial" organisms
  - Responsible for desired conversions
    - Sugar to alcohol (primary fermentation)
    - Malic acid to lactic acid (secondary fermentation)
  - Responsible for desired mouthfeel and aromatics
    - Specialized commercial yeast and bacteria can produce certain pleasant aroma's
      - Toluraspora can contribute to mouthfeel

Bad Wine Microbes ~

- "Spoilage" organisms
    - Produce off-odors, hazes, off-flavors and other negative sensory effects
  - Yeast
    - Brettanomyces can contribute 4-EP, 4-EG (band-aid, barnyard, horse sweat, leather)
    - Kloeckera, cold fermentative yeast can contribute to ethyl acetate
  - Bacteria
    - Acetobacter, can contribute to acetic acid or VA
    - Mold and bacteria can produce cork taint in cork material or wood/paper material in winery
    - Lactobacillus, pediococcus can produce mouse urine, rancid nuts, etc.

Splitting hairs ~ focus on desired outcome

- Many beneficial microbes can produce components that can be considered a part of spoilage
  - Stressed yeast produce sulfides as a by-product (rotten egg, can corn, garlic/onion…)
- Many spoilage microbes can produce components that can be associated with quality
  - Brettanomyces = expensive French wine
  - Non-saccharomyces fermented yeast contribute to mouthfeel
  - Acetic acid produced by acetobacter can contribute to aromas and mouthfeel

What do micro-organisms need ~

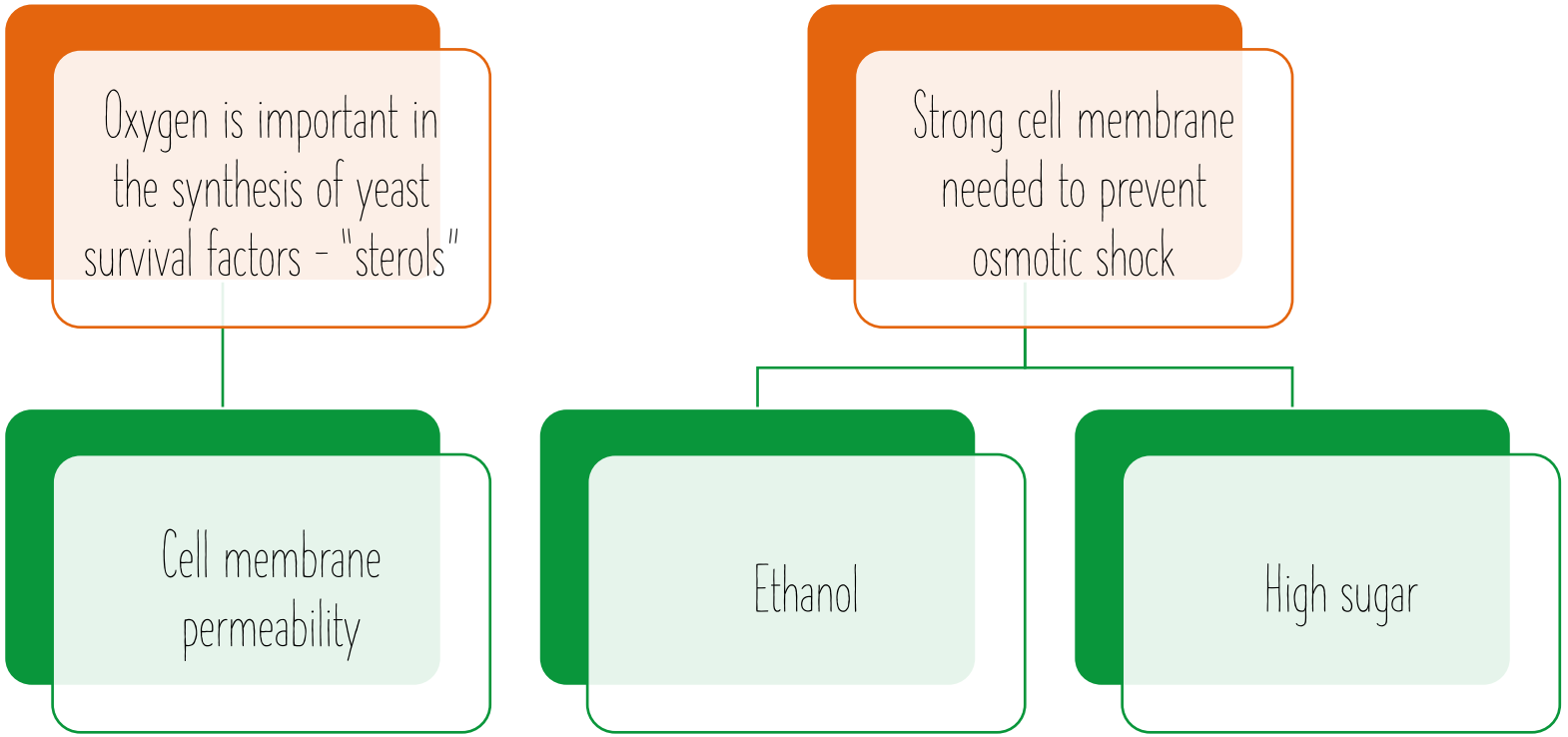
Atmospheric requirements

- Optimal temperature
- Oxygen (sometimes)

Food / nutrients

- Carbohydrate source (sugar or other carbohydrate)
- Nitrogen
- Vitamins
- Minerals
- Unknown!

Importance of Oxygen ~



Importance of proper rehydration ~

- Specific to yeast brands:
  - Based on commercial production of yeast
  - Recommended rate
  - Temperature of water for rehydration
    - No chlorine
  - Time of rehydration
  - Acclimation

Rehydration Nutrient ~

- Sterol rich, containing inactivated yeast and autolysates
- Builds cell wall to increase cell viability
- No inorganic N
- Provides a micronutrient rich environment for yeast to reestablish function during rehydration
- Use of rehydration nutrient and proper rehydration are the two most important factors for success

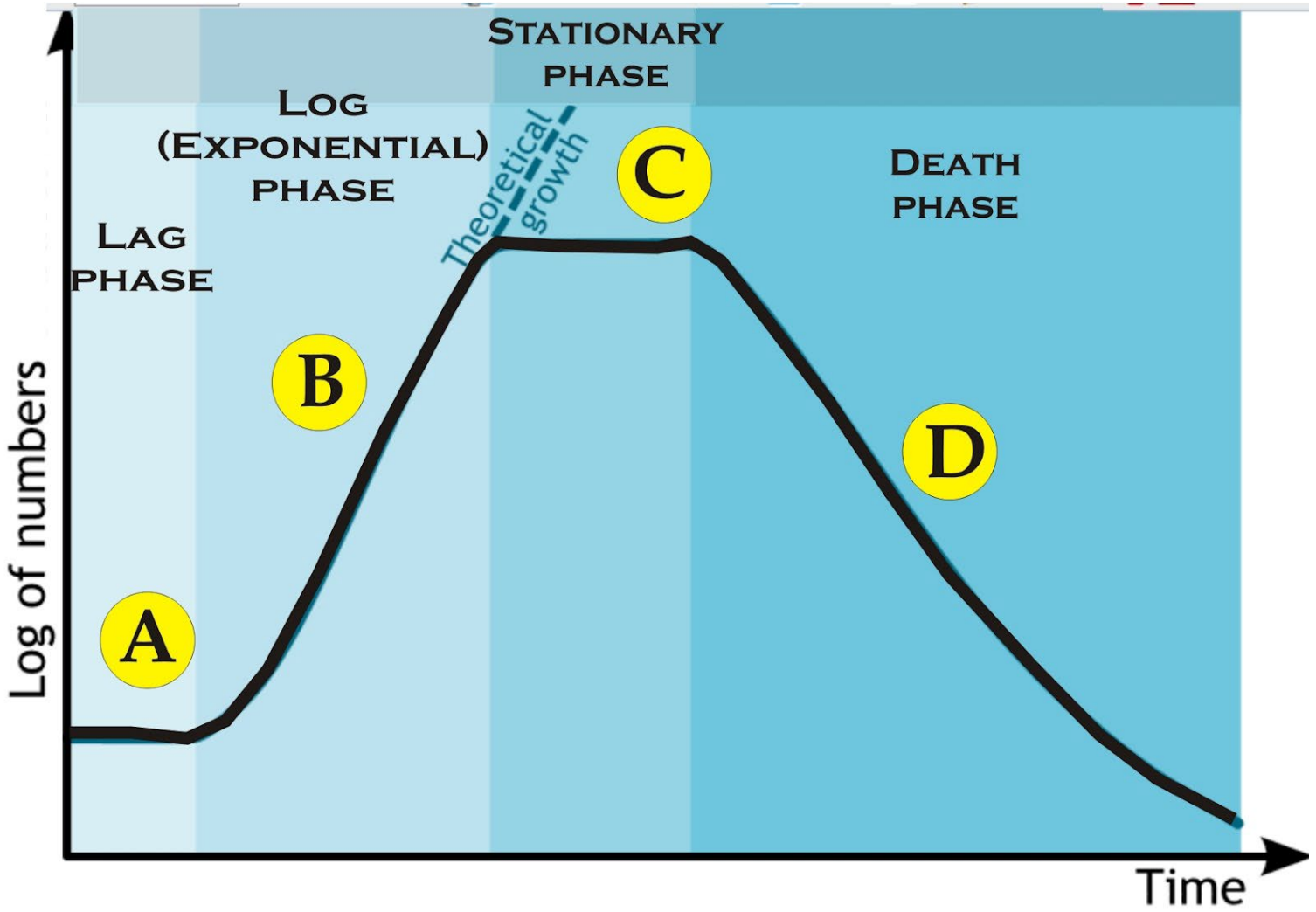
Sterols ~

Necessary for membrane permeability, effects cell's ability to release ethanol from the cell

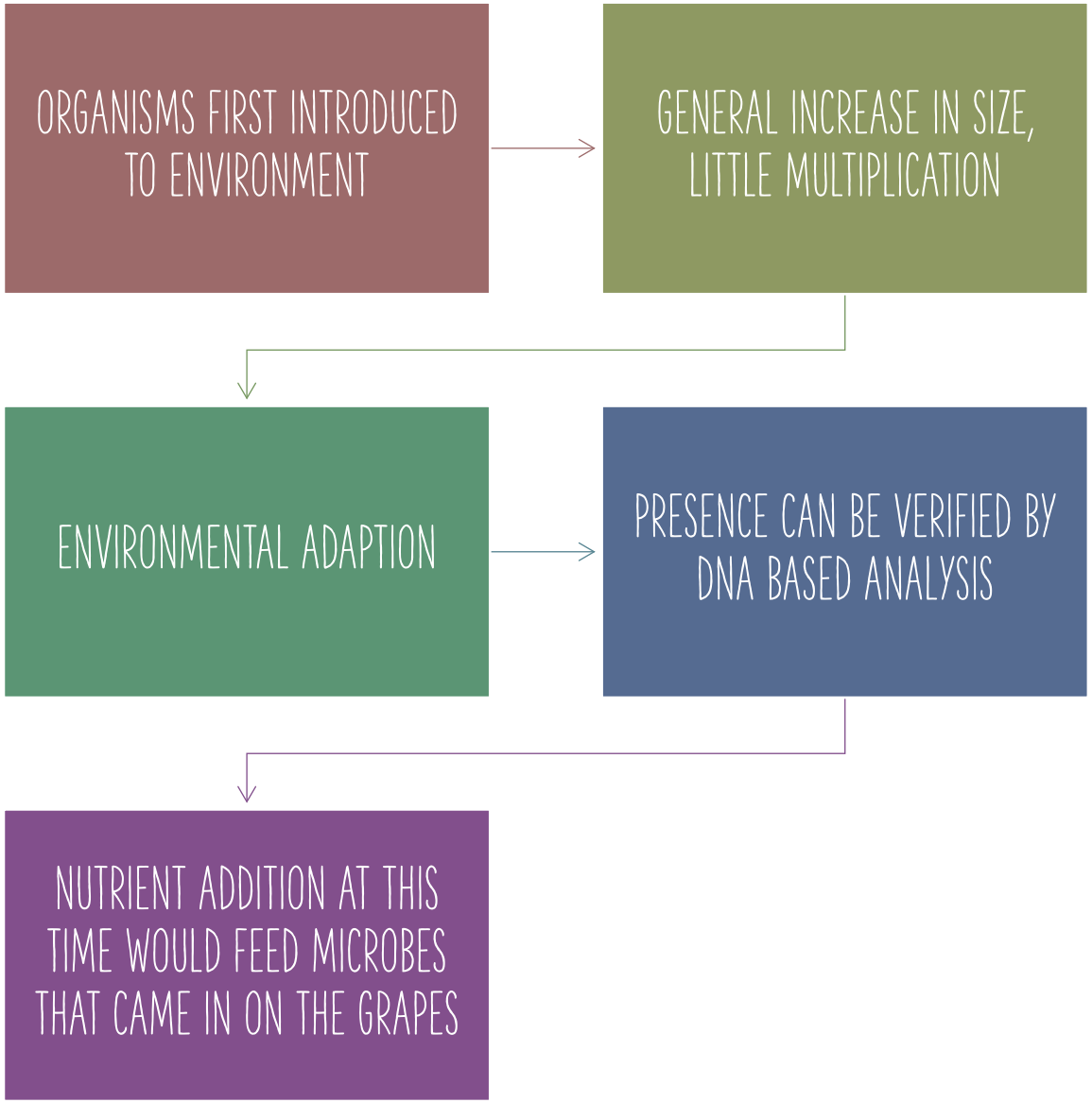
Will increase the viability of yeasts and prolong their fermentative activity

Certain sterols, either incorporated into or formed by wine yeast during the growth phase, provide important protection against ethanol toxicity

Life Cycle of a microbe ~



A. Lag Phase ~


ORGANISMS FIRST INTRODUCED TO ENVIRONMENT
GENERAL INCREASE IN SIZE, LITTLE MULTIPLICATION
ENVIRONMENTAL ADAPTION
PRESENCE CAN BE VERIFIED BY DNA BASED ANALYSIS
NUTRIENT ADDITION AT THIS TIME WOULD FEED MICROBES THAT CAME IN ON THE GRAPES

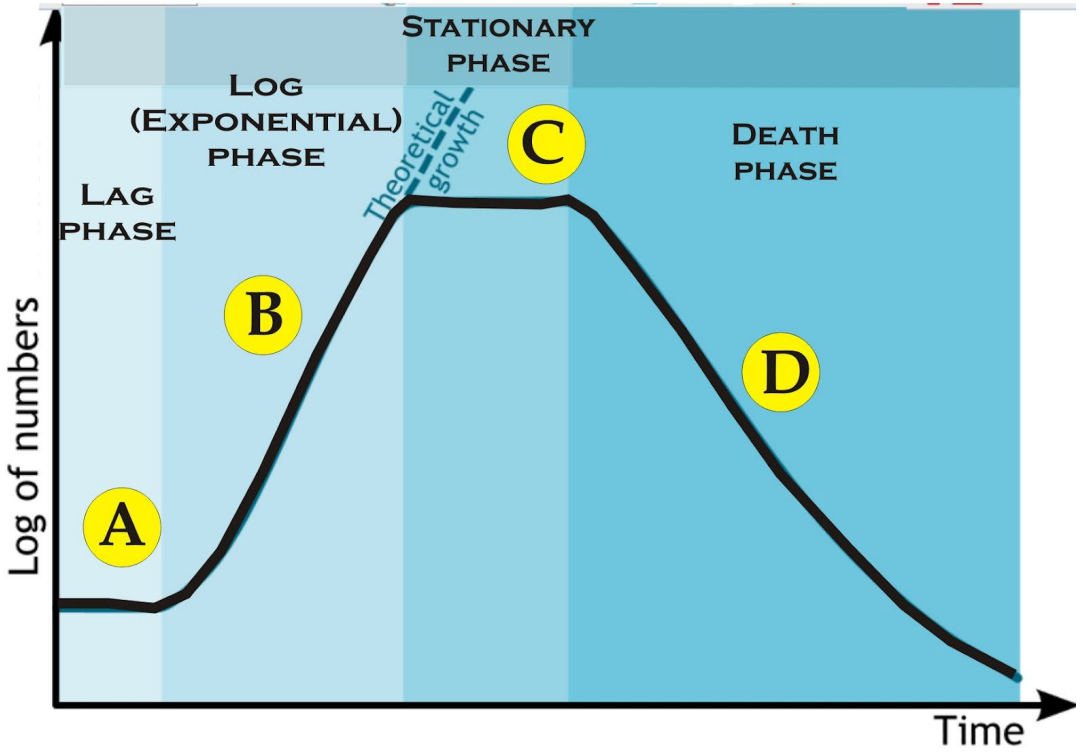
B. Log Phase ~

Rapid multiplication

Nutrients widely available

Favorable environmental conditions present

Winemaking indicators become present (Brix, temperature, sensory, carbon dioxide)



C. Stationary Phase ~

Growth and death rate are equal

Caused by nutrient deficiency, toxicity and/or environment stressor

Yeast switch from growth to metabolizing sugar to alcohol

Budding

Occurs during the LOG PHASE of growth cycle

Oxygen rich environment

- Under optimal conditions, a single mother cell may bud many times during the cultivation period
- Under fermentation, a single mother cell will bud only 3-4 times, each time the cell buds and separates the mother cell's membrane is depleted in half

During STATIONARY PHASE of growth cycle

Oxygen becomes limiting and budding ceases

Most fermentations are completed during this phase

Focus moves from population increases to nutrient consumption (sugar --> alcohol)

D. Death Phase ~

Cell walls break down

Number of living, active cells decreases

Parameters for picking yeast ~

- Fermentation to dryness
- Reasonable rate of fermentation
- Predictable fermentation characteristics
- SO2 tolerance
- Good ethanol tolerance
- Aggressiveness
- Flocculation
- Sugar tolerance
- Temperature
- Varietal selection
- By-products (thiols, esters, etc.)
- MLF compatibility
- Nutrient requirements
- Metabolize malic acid

Causes of hydrogen sulfide ~

- Pantothenic acid
- Low YAN / high YAN
- Residual sulphur / pesticides / copper ion residues
- Stress to yeast (temperature)
- Clarification level
- Concentration of sulfur dioxide / sulfites
- Storage on lees (reduction)
- Yeast strain genetics
- Yeast biomass
- Oxygen availability
- Yeast viability

Nutritional Goal ~

- Based on fermentation plan:
  - Goal is to produce enough biomass without excess and maintain biomass in good shape throughout fermentation.
  - Fermentation is a challenging environment for yeast, accumulation of alcohol and metabolic wastes, yeasts metabolically adapt and react to stress of fermentation.

Nitrogen Composition of Grape Must:

- Ammonia: 3-10 %
- Amino Acid: 25-30 %
- Peptides and proteins: 30-50 %

Yeast Assimiable Nitrogen (YAN) ~

- YAN dependent on the following:
  - Yeast strain
  - Fermentation conditions
  - Sugar
  - Temperature
  - Aeration
- The higher the YAN is to start will increase cell density, fermentation rate and heat production.
- Direct correlation between YAN and AF duration, the more YAN = quicker AF and larger biomass of cells.

Calculations

~

- Fermentable sugar:
  - Sugar (g/L) = Brix x 10 x Specific Gravity

- Rate of yeast increases with Brix, so does the amount of Nitrogen to feed biomass
- As sugar increases, so does the level of nitrogen required due to osmotic pressure on yeast causing stress. (Brix level only, not individual yeast requirements)
  - 21 Brix = 250 ppm N
  - 23 Brix = 300 ppm N
  - 25 Brix = 350 ppm N
  - 27 Brix = 400 ppm N

Nitrogen Requirements for Yeast ~

High --> 1.25

A high nitrogen demanding strain has a strong tendency to produce more biomass, correlating to a bigger need during the stationary phase.

Medium --> .9

Low --> .75

Lower biomass production

YAN Calculation ~

- Yeast Assimiable Nitrogen (YAN)
  - YAN = Alpha amino N + (ammonia)(.8225)

  - At pH 3.0, only 70 % ammonia utilized
  - At pH 4.0, 90 % ammonia utilized

Nutritional Requirement Calculation ~

- Nitrogen required for Brix level and individual needs of the yeast
- Minus the YAN level in must/juice
- Nitrogen overage or deficit?
  - Take a look at desired wine style to make final decisions on additions

Two Forms of Nitrogen ~

ORGANIC SOURCE - HEALTH OF CELL - "HEALTHY FOOD"

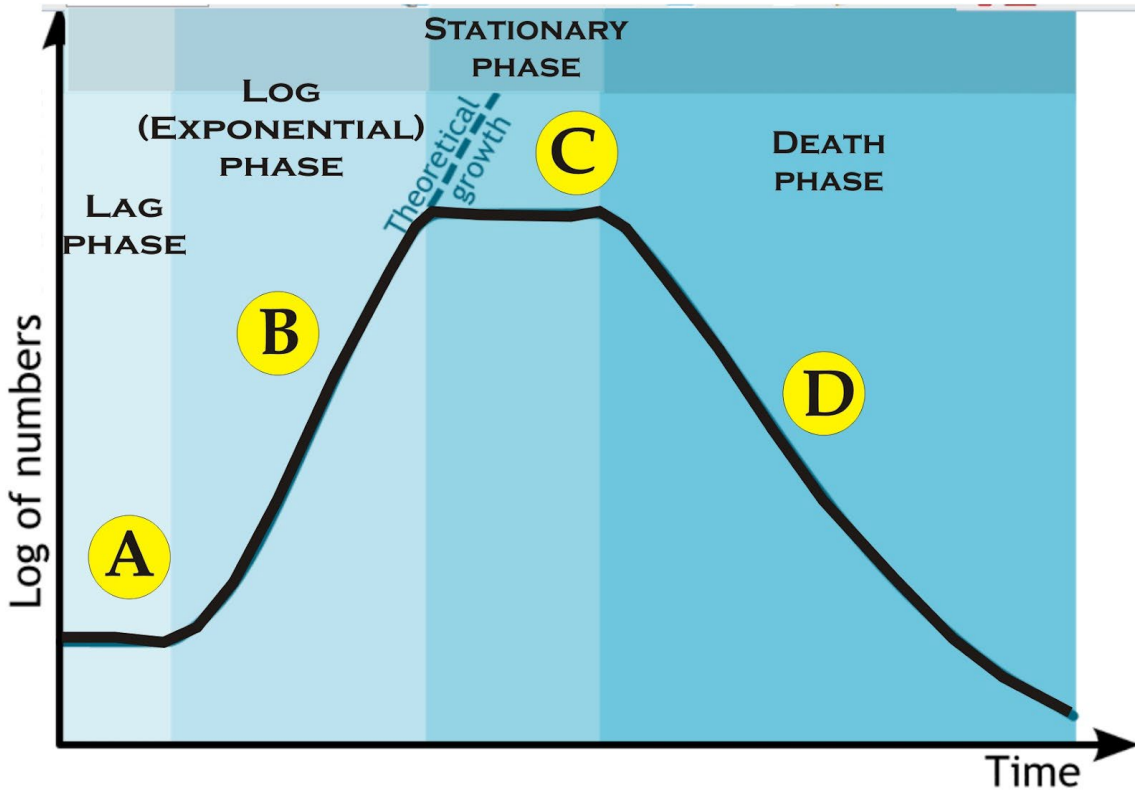
AMINO ACIDS

INORGANIC SOURCE - ENERGY CONSUMED BY THE CELL - "JUNK FOOD"

DIAMMONIUM PHOSPHATE

Nitrogen and life cycle phases

- A. Lag phase
- B. Log phase- add nitrogen at the start, 2-3 Brix drop
- C. Stationary phase- add nitrogen at 1/3 of fermentation or beginning of this phase
- D. Death phase- no nutrients below 8 brix



Common Pitfalls with Yeast:

- Follow rehydration instructions exactly, this will make a difference on your biomass.
- Acclimate your yeast to temperature of juice or must.
- Don't use expired packages or opened more than a season.
- Refer to must YAN and yeast requirements.
- Sulfide issue early, make a note to not keep yeast lees present post-fermentation.
- Avoid temperature fluctuations
- Oxygen at beginning can be helpful
- Avoid stalling at +1 degree Brix as dead yeast cells falling to bottom
- Use proper rate of yeast, ensure biomass needed
- Check for dryness