Yeast Prop Optimization

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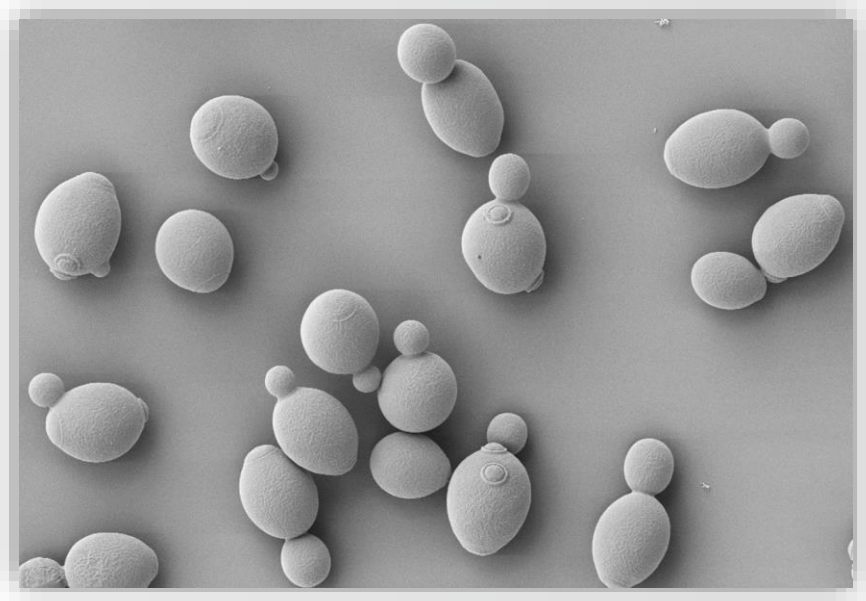
LALLEMAND BIOFUELS & DISTILLED SPIRITS

Presentation Outline

- Definition and Purpose of Propagation
  - Requirements
- True vs Biofuel Propagation
  - Steps and Considerations
  - Propagation Pitfalls
- Propagation Optimization
  - From Prop Profiling to Engineering



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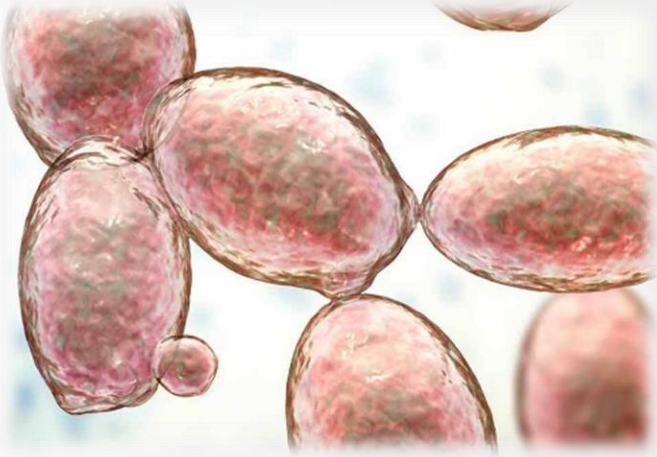
Definition and Purpose



- Definition

  The process of increasing population of organisms by natural reproduction

- Purpose of a yeast propagation
  - Rehydration, conditioning and yeast biomass increase
    - Yeast cells double every **90 minutes** (in ideal conditions)
    - A prop time of 6-10 hours will allow you to use 25% of your yeast input (vs dry pitch)
- An effective propagation will:
    - Reduce lag phase
    - Increase fermentation kinetics and reduce fermentation time
    - Offer competitive inhibition

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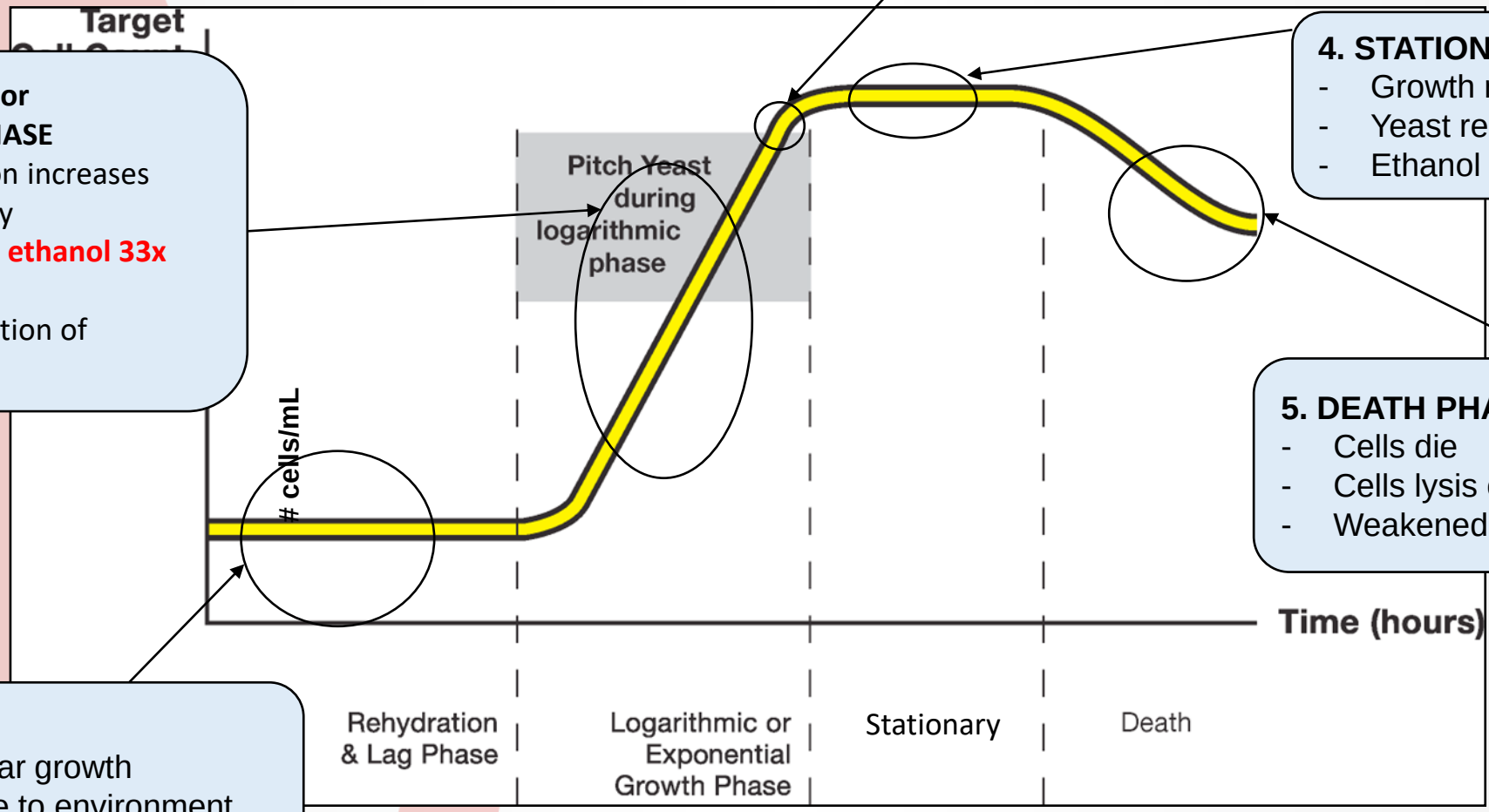
Growth Curve Phases

3. DECELERATION PHASE
- Cells counts begin to level off
- Budding decreased substantially
- Increase of inhibitory metabolites

4. STATIONARY PHASE
- Growth rate stops
- Yeast remain active
- Ethanol production remains

2. LOGARITHMIC or EXPONENTIAL PHASE
- Cell population increases logarithmically
- **Cells produce ethanol 33x faster**
- Fast consumption of nutrients



5. DEATH PHASE
- Cells die
- Cells lysis can occur
- Weakened cellular membranes

1. LAG PHASE
- Very low cellular growth
- Cells acclimate to environment
- Length of the phase variable

- **Propagation**: phases 1 & 2 (sometimes 3)
- **Fermentation**: phases 1-5

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Oxygen Requirements

- Yeast propagation is an aerobic process – providing proper aeration is essential
  - Dissolved oxygen is introduced by air inductors or compressed air and spargers
  - In props, yeast expend energy to create biomass rather than ethanol

	Aerobic respiration	Anaerobic respiration
Oxygen required?	Yes	No
ATP yield	32 ATP	2 ATP
End products	CO_2 and water	CO_2 and ethanol

  - **16x more energy** is generated during respiration per glucose molecule than fermentation

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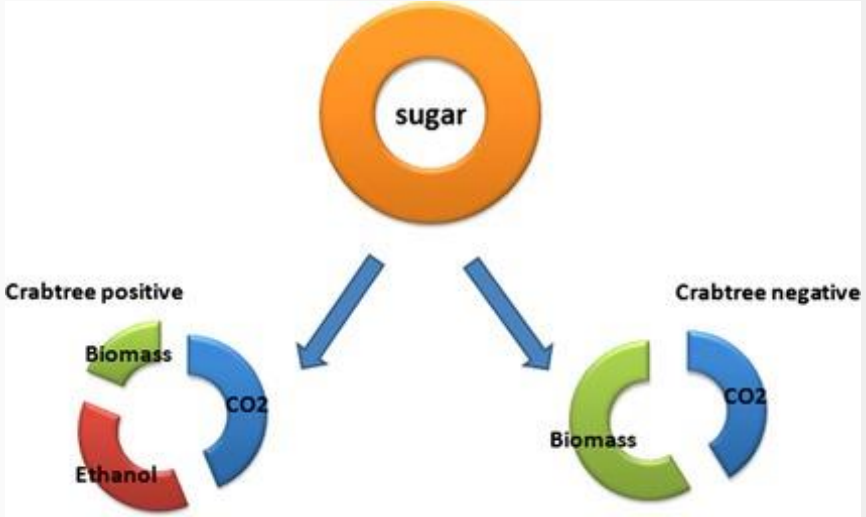
Nutritional Requirements: Carbon and Nitrogen

- Carbon source – glucose
  - Glucoamylase required for saccharification
  - Glucose levels of ~2% at the beginning of propagation are required
    - Higher levels can induce yeast to produce ethanol through inhibition of oxygen consumption
- Nitrogen source – urea
  - Nitrogen is a building block for protein – target rate of 500 ppm nitrogen addition
    - Ammonia represents a good yeast assimilable nitrogen source during fermentation
    - Proteases and nutrient options are also often recommended



Dashko et al., 2014

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Propagation vs. Fermentation

Respiration: Yeast energy production (biomass)

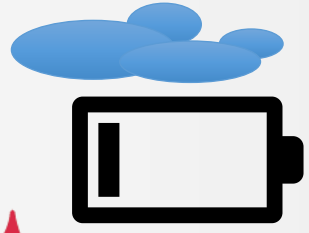
Fermentation: Ethanol production

Respiration

+ Sugar $\xrightarrow{O_2}$ H_2O + CO_2 +

Fermentation

+ Sugar $\xrightarrow{\cancel{O_2}}$ Ethanol + CO_2 +



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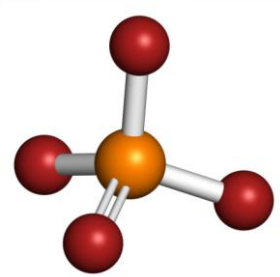
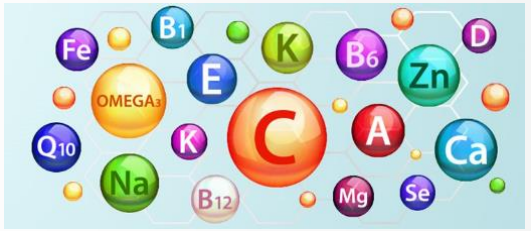
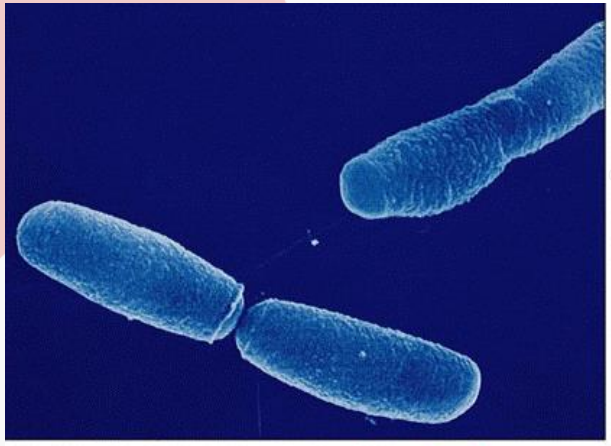
Other Requirements

- Nutritional: Phosphate  Vitamins & nutrients 

- Antimicrobials:
  - Target Gram Positives, Gram Negatives or broad spectrum


(a) Rod-shaped bacterium before penicillin. SEM 1 μm


(b) The bacterial cell is lysing as penicillin weakens the cell wall. SEM 1 μm

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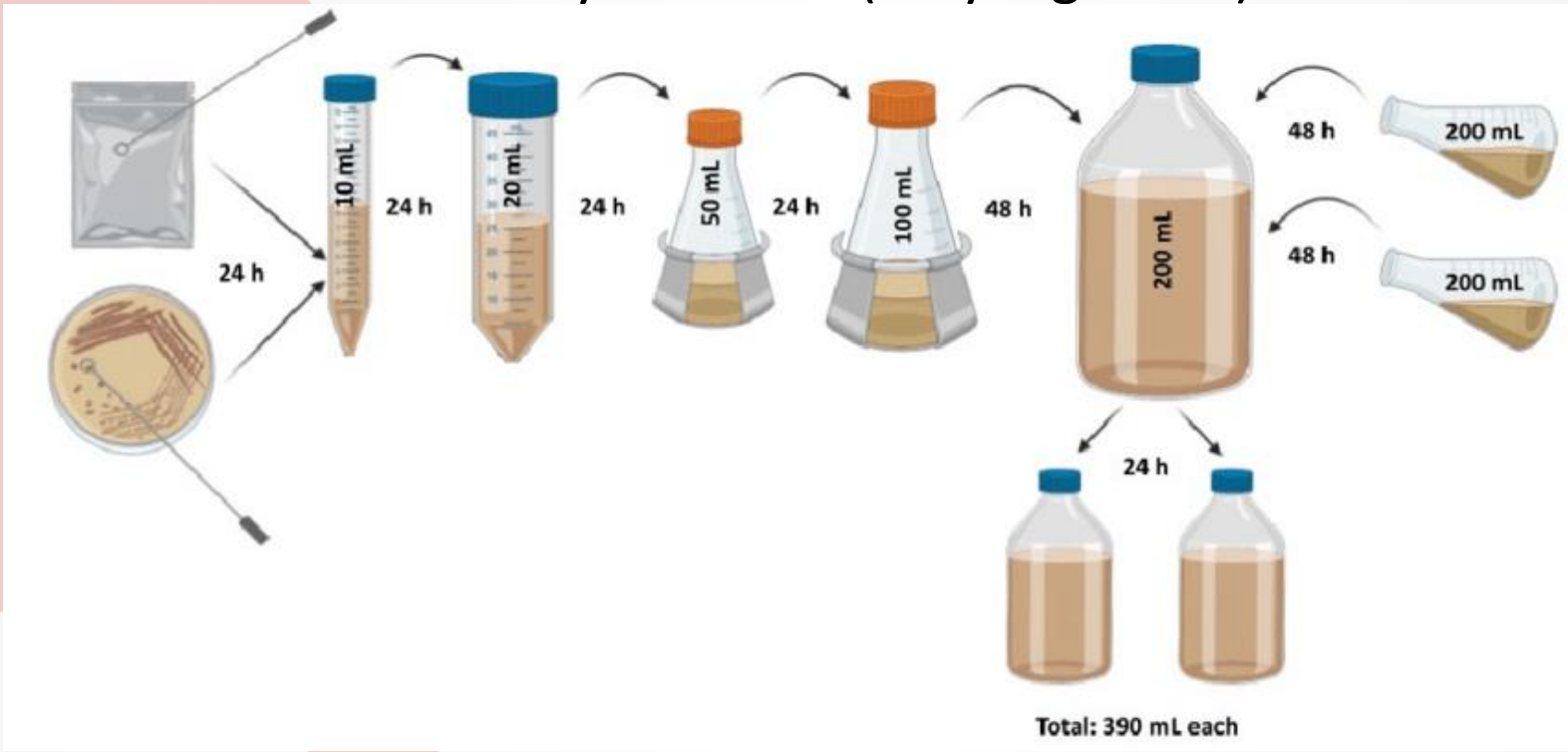
True Propagation

- True propagation is the process of manufacturing yeast for commercial use
  - Requires high concentration of dissolved oxygen and low glucose
  - Special equipment and experience required
  - True pure culture is not widely available (very high cost)



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Biofuel Propagation

Batch

- Start every prop with fresh yeast
- CIP yeast prop between each fermenter addition
- Slight tendency for infection

Continuous and Semi-continuous

- Retain same inoculum over longer periods of time
- Spoon feed nutrients continuously
- Continuous feed or “Slug” feed to fermenter
- Highest tendency for infection

Direct Pitch

- Yeast pitched directly to fermenter after hydration
- No prop necessary
- Low tendency for infection
- Longest lag phase

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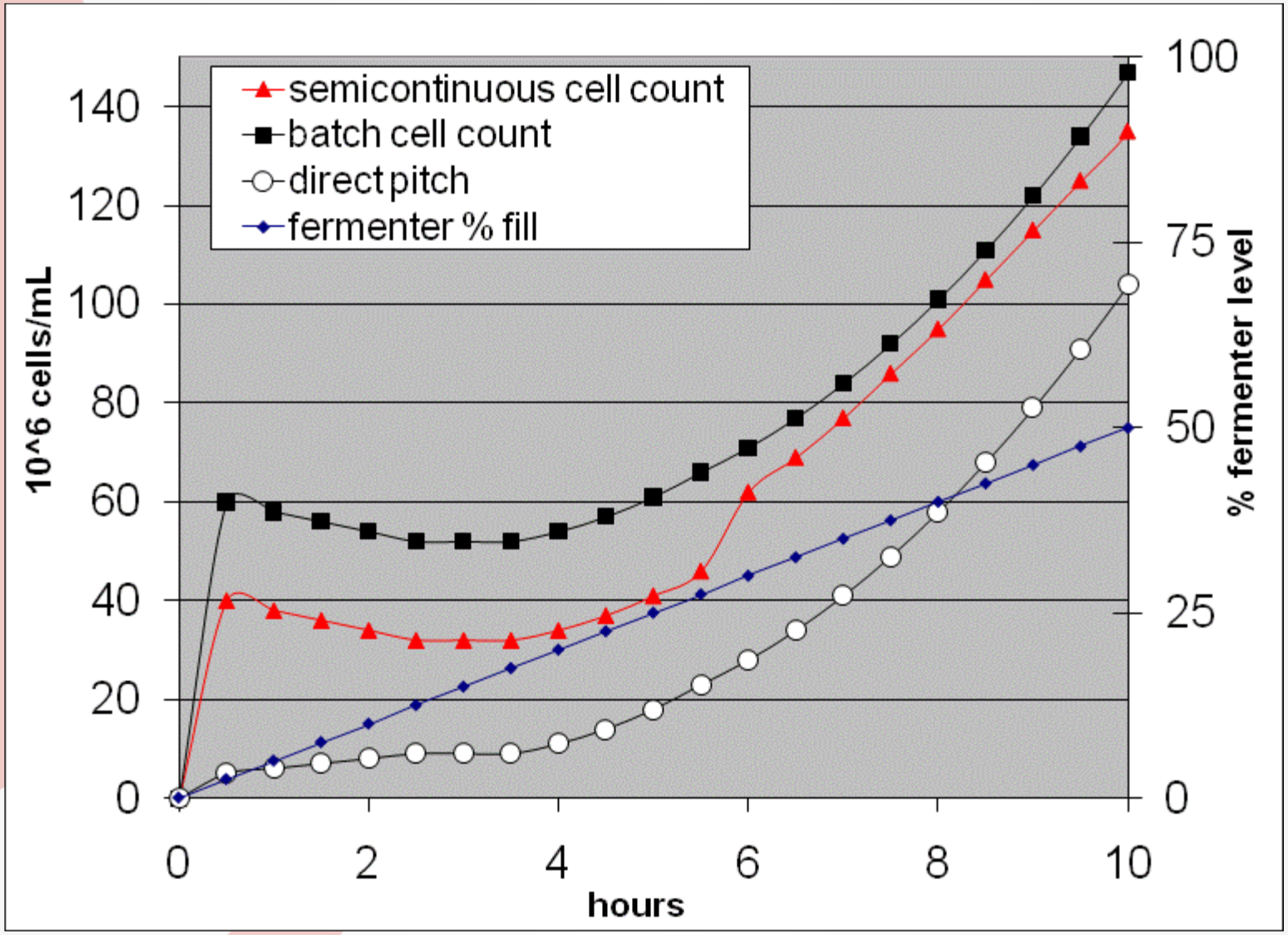
Yeast Count per Propagation Type



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Biofuel Propagation

Theory:

- Start with smaller yeast inoculum
- Fill prop tank with dilute fermentation mash
- Increase cell mass/decrease lag time

Reality:

- Temperature and time not optimized
- Mash dilution & nutrition not always appreciated
- Not truly an aerobic process
- Increased infection potential
- Additional time and labor

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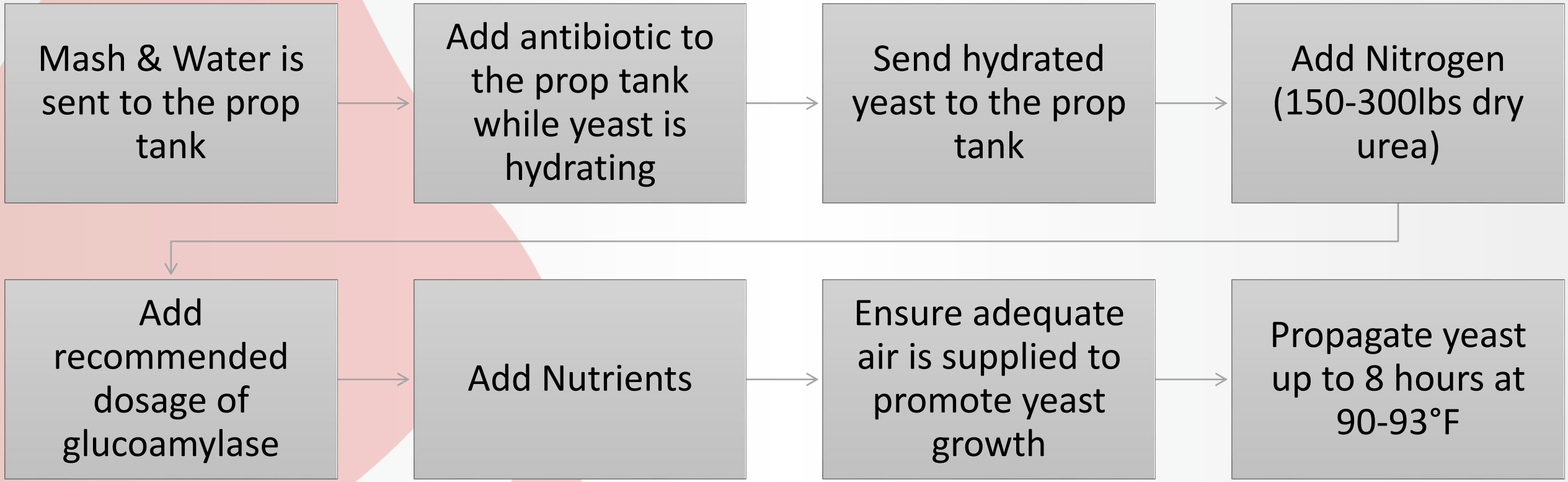
Propagation Steps



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Propagation Targets

Timing	Count	Viability	Budding	Ferm Level
• Typically, 6.5 hrs to 8 hrs • Prop profile	• 225 to 325 (million cells/mL)	• 90% or greater	• 15% to 30% • High margin of error	• Enough to start circulating pump & dilute residual caustic

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Propagation Pitfalls

- Condition 1. Not enough yeast
  - **Symptoms:** Low counts at prop transfer; Increased lag phase in fermentation; Bacteria proliferation, Less viability at the end of fermentation
- Condition 2. Improper CIP
  - **Symptoms of Caustic Residue:** High pH and sodium concentration; Elevated glycerol and reduced overall performance
  - **Symptoms of Infection:** Increased organic acids; Decrease in nutrients; Low cell mass and potential for ferm contamination
- Condition 3. Training and SOP
  - Instructions should be CLEAR and all personnel should be TRAINED to identify possible problems

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Propagation Pitfalls: Condition 4. Improper Additions

Mixing yeast with antibiotics	• Testing up to 25 ppm • Concentration: Too much of anything rule
Mixing yeast with glucoamylase	• Starting fermentation without proper nutrients
Mixing yeast with protease	• Yeast are made of 40 - 45% protein
Mixing yeast with urea	• Too much of anything rule
Getting additions to the prop	• Antibiotics clumpy • Variant amounts of GA can make a big difference

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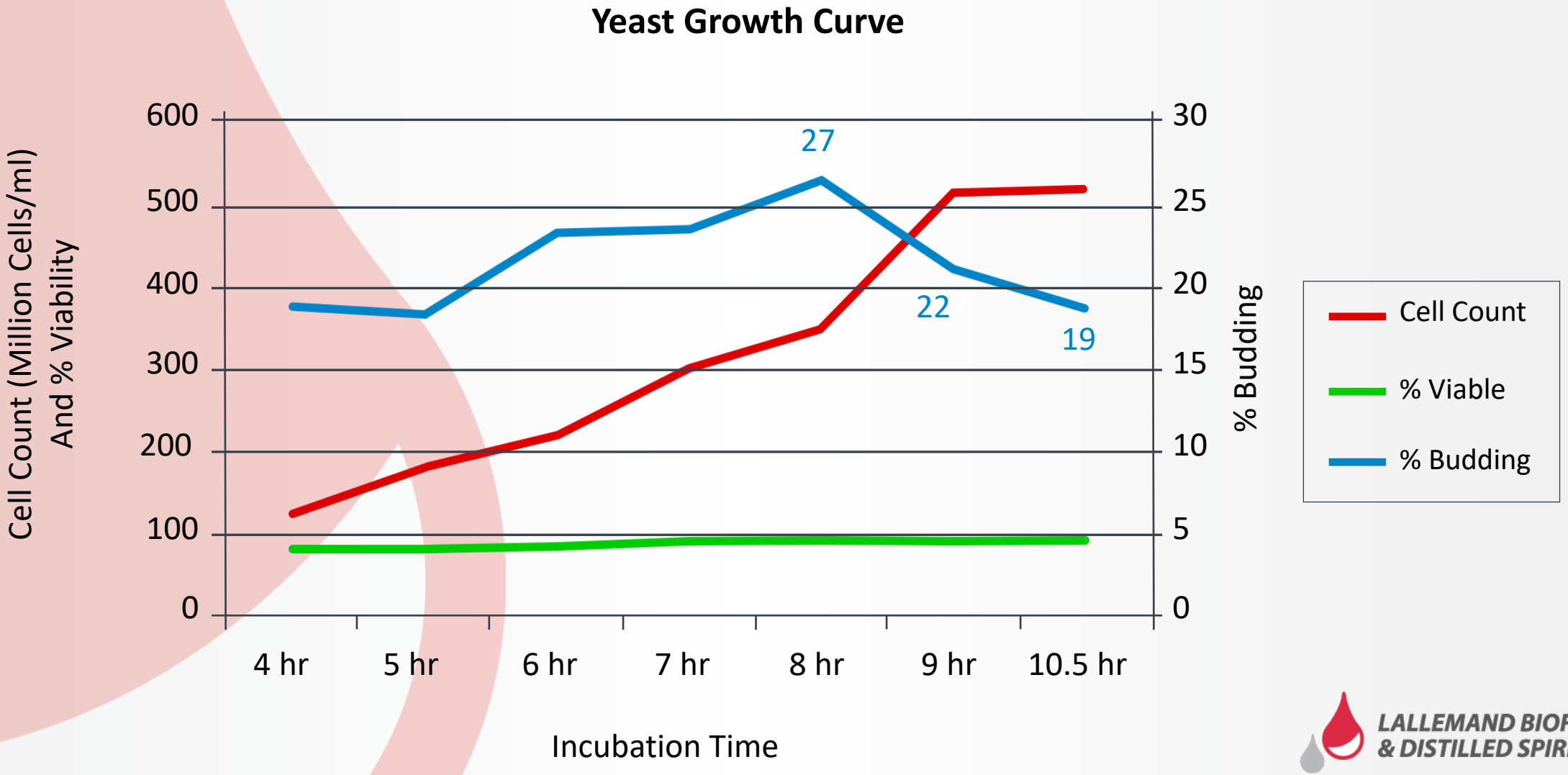
Propagation Optimization: Prop Profiling



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Propagation Optimization Considerations

- Most plants have been operating for 10+ years with designs even older
  - Technology has come a long way since start-up
- Industry has done a great job optimizing their process
  - Denatured Ethanol Yields 3.0+ gal/bu
  - Energy Efficiencies
  - Carbon Intensity Score Reduction
  - Coproduct Diversification
- Technology providers offered upgrades and “bolt-ons”
- Yeast and enzyme innovation

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Yeast Propagation Optimization: An Engineering Case Study

- Start-from-scratch design of yeast prop between Homeland Energy Solutions and LBDS
- **Fish bowl concept – 30,000 gal tank**
- **Plentiful oxygen supply**
  - Dedicated system
  - Filtered and cooled
  - 4 sparger design
  - Dissolved oxygen probe on each prop
    - Starts out saturated > 4000 ppb
    - End of propagation ~ 7 hours (50 – 70 ppb)

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Yeast Propagation Optimization: An Engineering Case Study

- **1 year payback on $3M investment**
  - Reduction in yeast cost/gallon
  - Reduction in other fermentation ingredients

- **Ethanol Producer 2019 Collaboration of the Year Award**

2019 ETHANOL PRODUCER AWARDS

COLLABORATION OF THE YEAR

COLLABORATION OF THE YEAR
Technology Advancement through Partnership
Homeland Energy Solutions and Lallemand Biofuels & Distilled Spirits

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Thank you!

Any Questions?

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