Yeast Pretreatment / Propagation

Chris Richards

SVP Sales & Business Development

Lallemand Biofuels & Distilled Spirits

The Alcohol School Concise

WIRSPA
West Indies Rum & Spirits Producers' Association Inc.

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Place: • Caribbean Distilling Seminar, St Lucia

Date: • Wednesday 17th April 2024

What are We Talking About?

- Wide range of processes

- Ranging from simple addition to complex aseptic propagation

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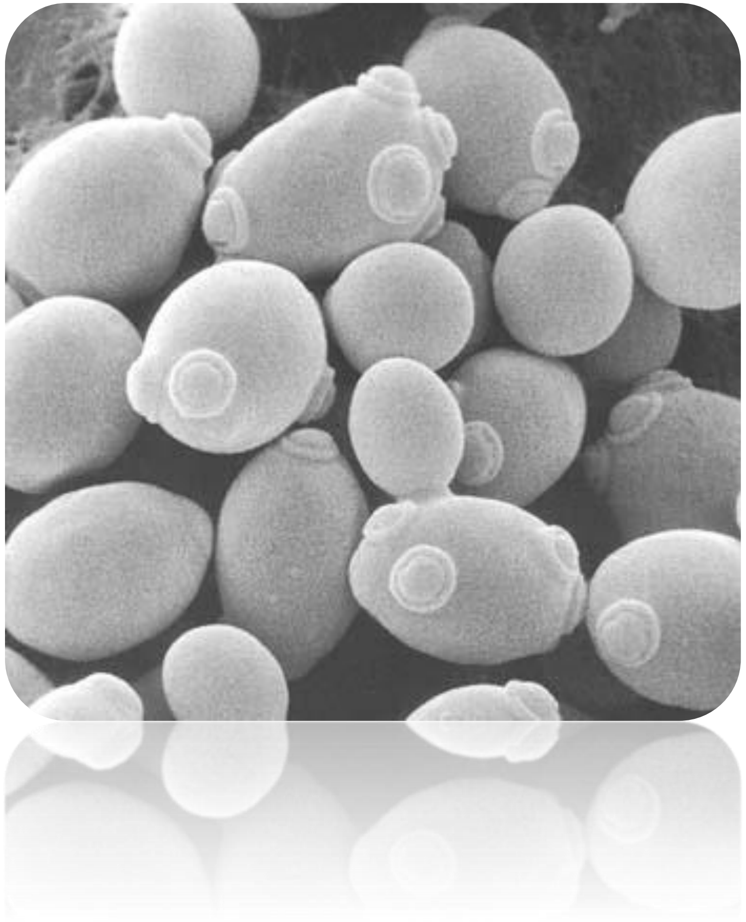
The Objective is?

- To provide to the fermenter
  - Yeast that is healthy, clean and active
  - The optimum quantity
  - At the optimum time



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Options

- Direct pitch
- Conditioning / simple propagation
- Continuous propagation / pre-fermentation
- Full in house propagation
- Yeast recycle

- What drives our choice?
    1. Fermentation design
    2. Feedstock
    3. Technical ability
    4. Economics
    5. Process performance / yield

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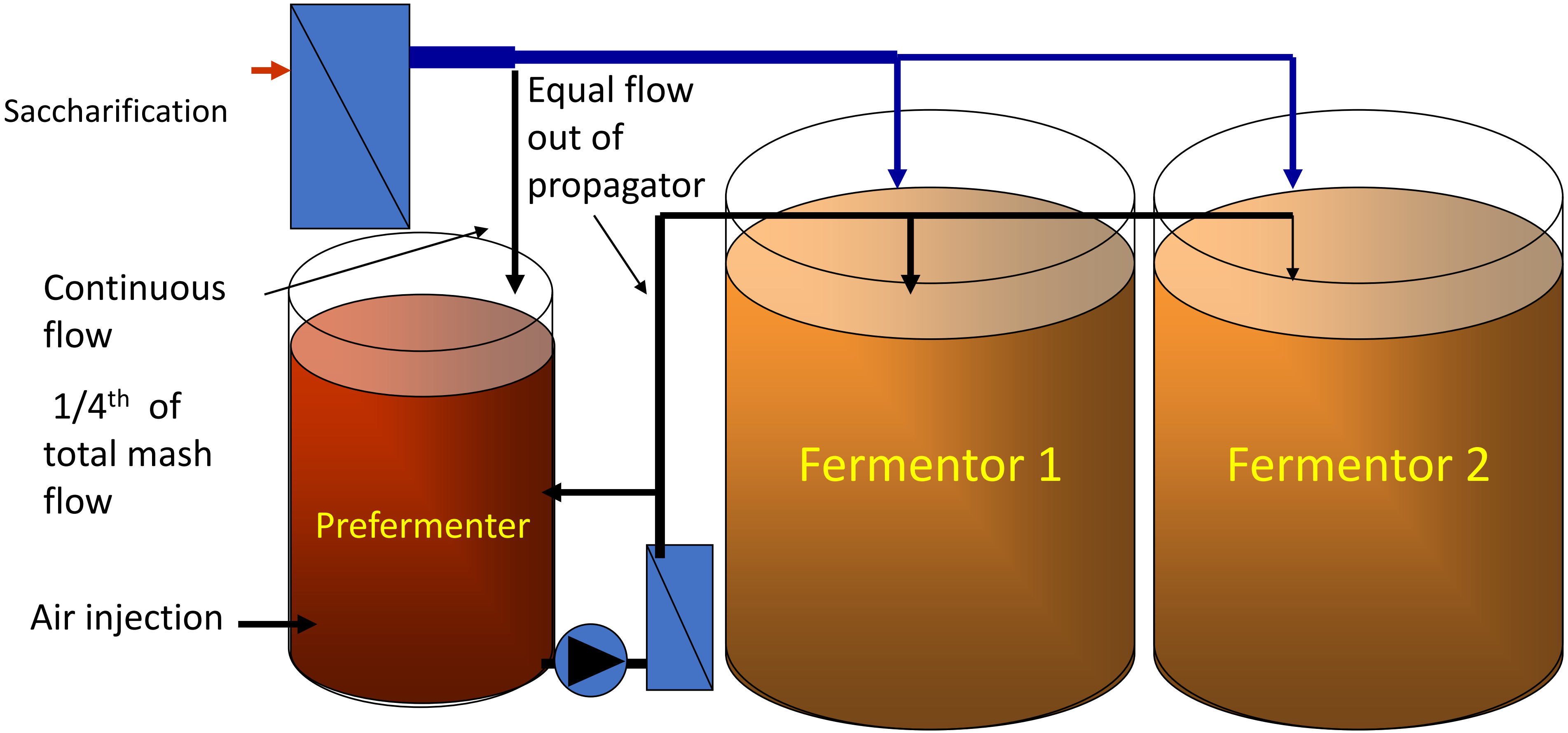
Fermentation Design

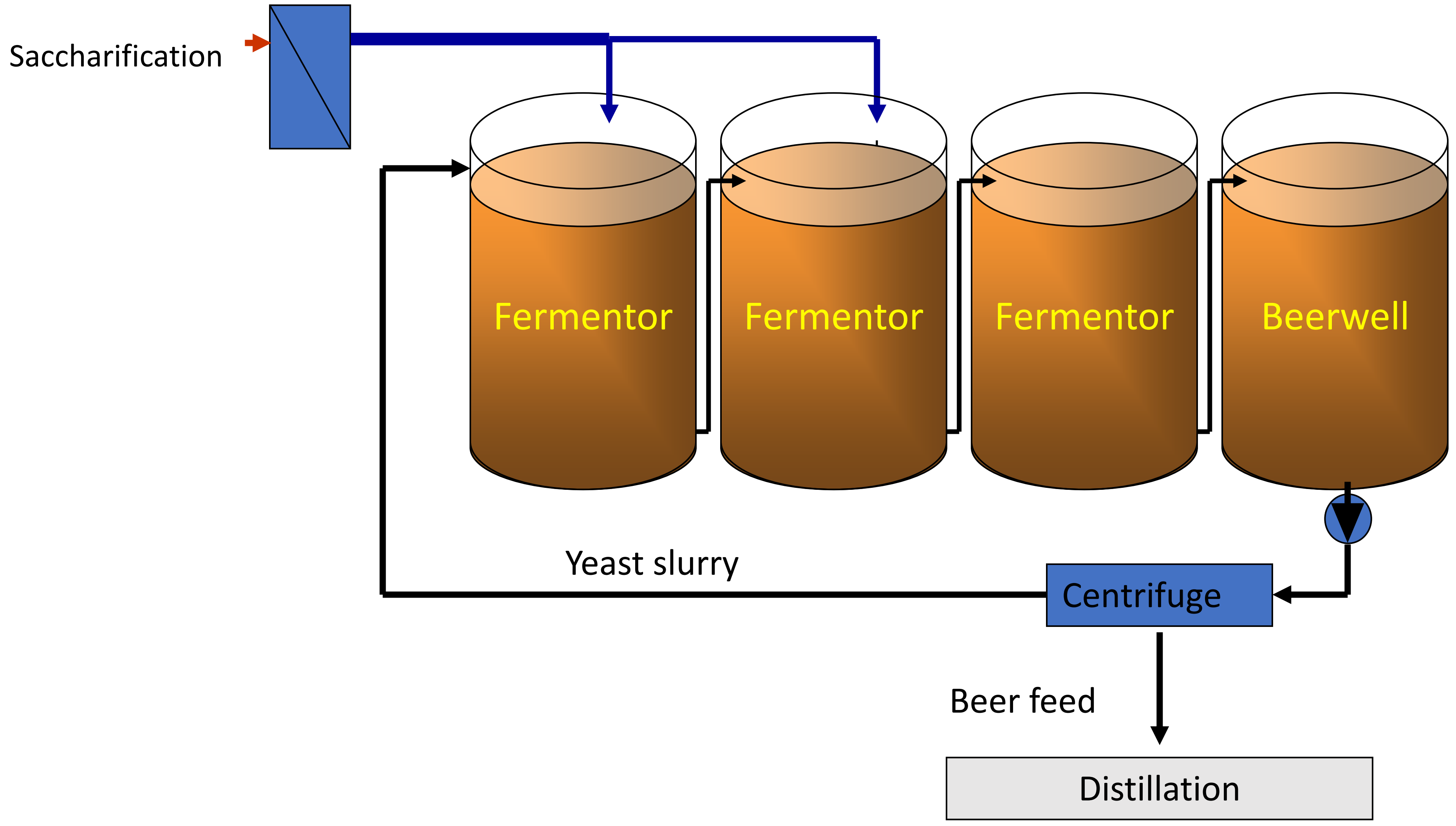
- Really only two choices here
- Continuous fermentation vs. batch fermentation
- Very obvious choice if you have continuous
- You need a consistent and permanent flow of yeast dose to the initial part of the train

Continuous Propagation



In theory, additions to Fermentor 2 would not be made.

Continuous Fermentation with Yeast Recycle*



* can only be done if yeasts maintain high viability (usually low alcohol levels)

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Pros & Cons – Prefermentor / Yeast Recycle

Pros

- Minimal cleaning
- High inoculation
- Fast fermentation
- Minimal yeast usage
- Easily automated

Cons

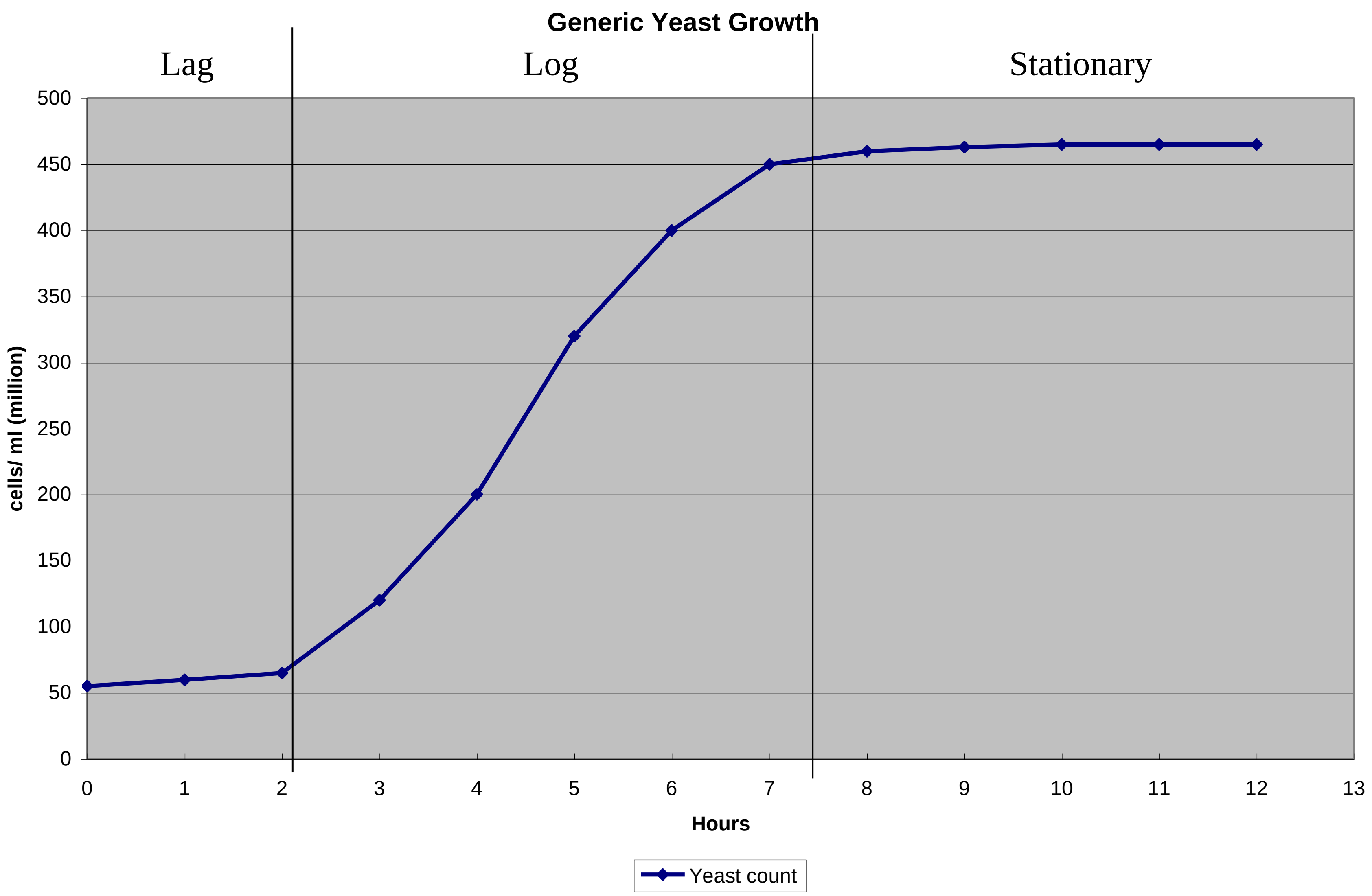
- Yeast is not always in good condition
- Yeast forced into stationary phase (low budding)
- Depletion of sterols & unsaturated fatty acids
- High potential for yeast mutation
- High risk of bacterial infection
- High risk of selection of faster-growing bacteria or wild yeasts
- Difficult to clean
- Very high stress on yeast in fermentor train due to steady state
- Lower alcohol limit on system

Batch Fermentation

- Here many more options are available to the producer

- Simplest – throw the yeast into the fermenter!
  - Simple, quick, easy
  - Poor rehydration – increases stress on yeast
  - Has significant lag phase

Growth Phases of Yeast



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Batch Fermentation

- Here many more options are available to the producer

- Simplest – throw the yeast into the fermenter!
  - Simple, quick, easy
  - Poor rehydration – increases stress on yeast
  - Has significant lag phase
    - Allows bacterial growth opportunity
  - Requires significant yeast dose

- Yeast should be added as soon as possible into the fermenter

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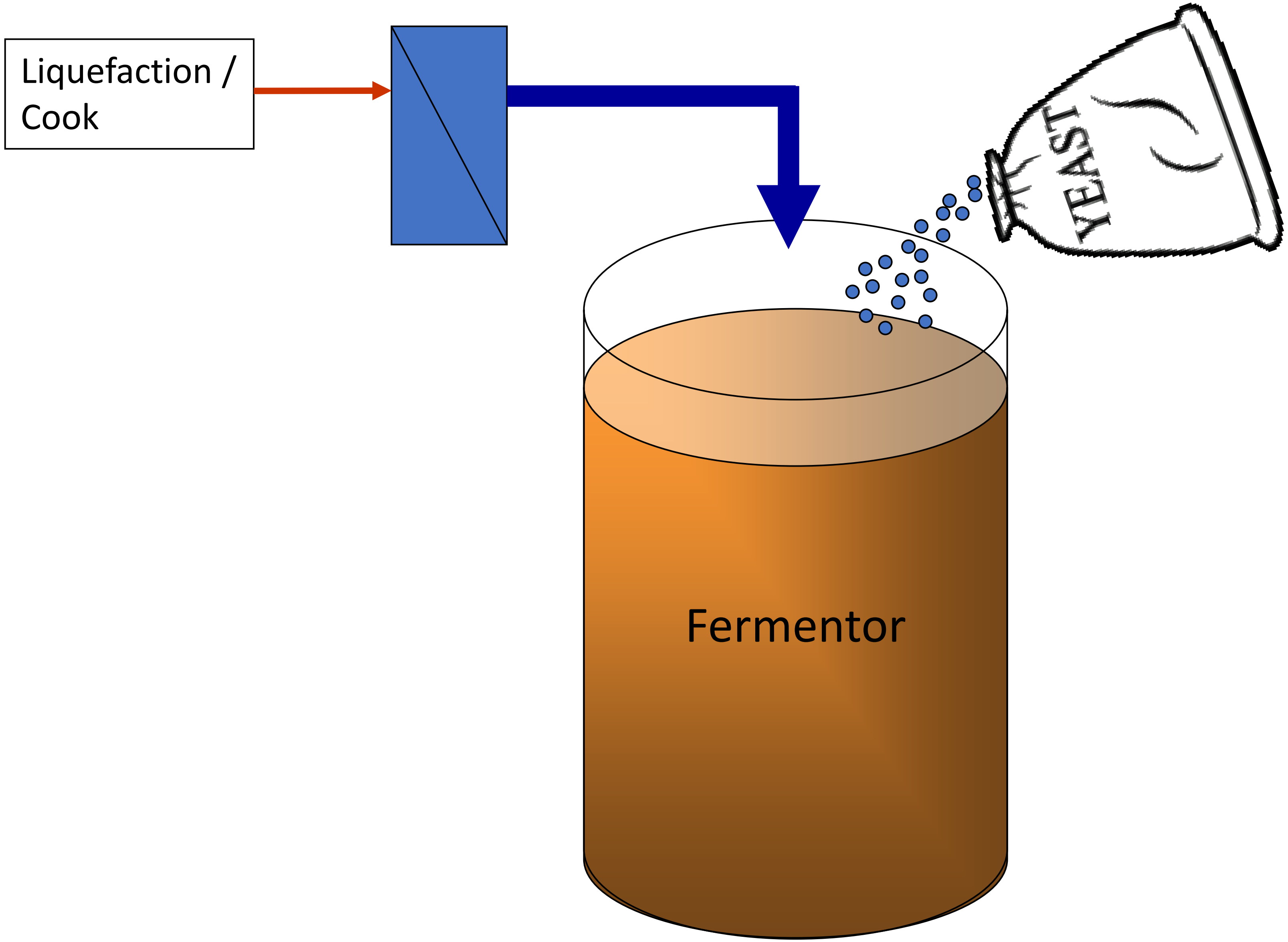
Direct Addition of Dry Yeast



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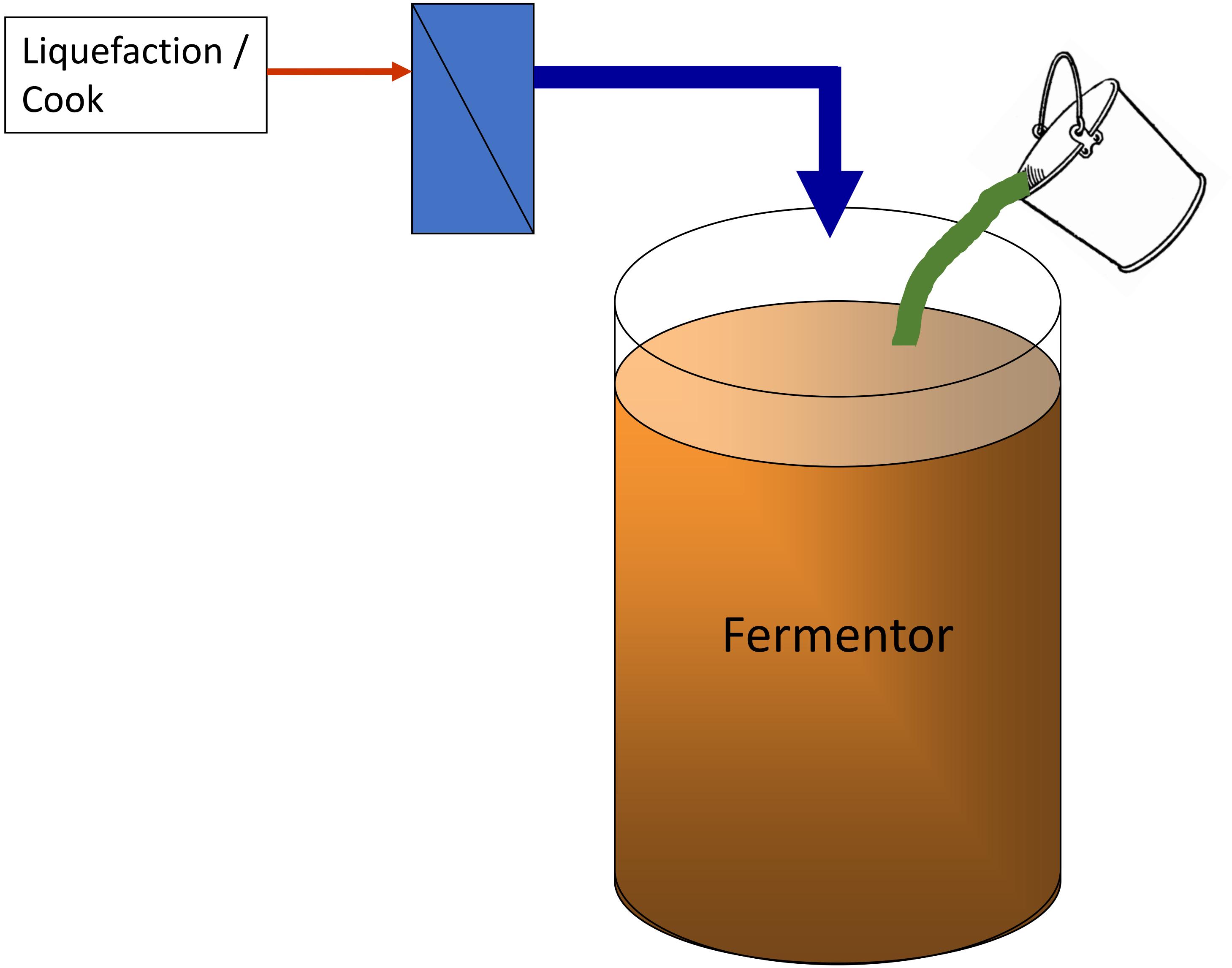
Next Option

To rehydrate the yeast prior to addition

1. Add dry yeast to warm water (35-38^{o}C/95-100^{o}F)
2. Yeast to water ratio 1:10
3. Agitate gently to homogenous mixture
4. Allow to stand 10-15 minutes
5. Add to mash

- This allows the yeast to rehydrate under the best conditions
    1. Low osmotic stress
    2. Correct temperature
    3. Optimal cell wall condition

Direct Addition of Rehydrated Yeast Slurry



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Rehydration

- Resistance to dehydration and rehydration stress is strain dependant
- Rehydration in general recommended
- Rehydrate dry yeast at 35-38^{o}C in ten times its weight clean, sterilized (boiled) tap water for 15-20 minutes

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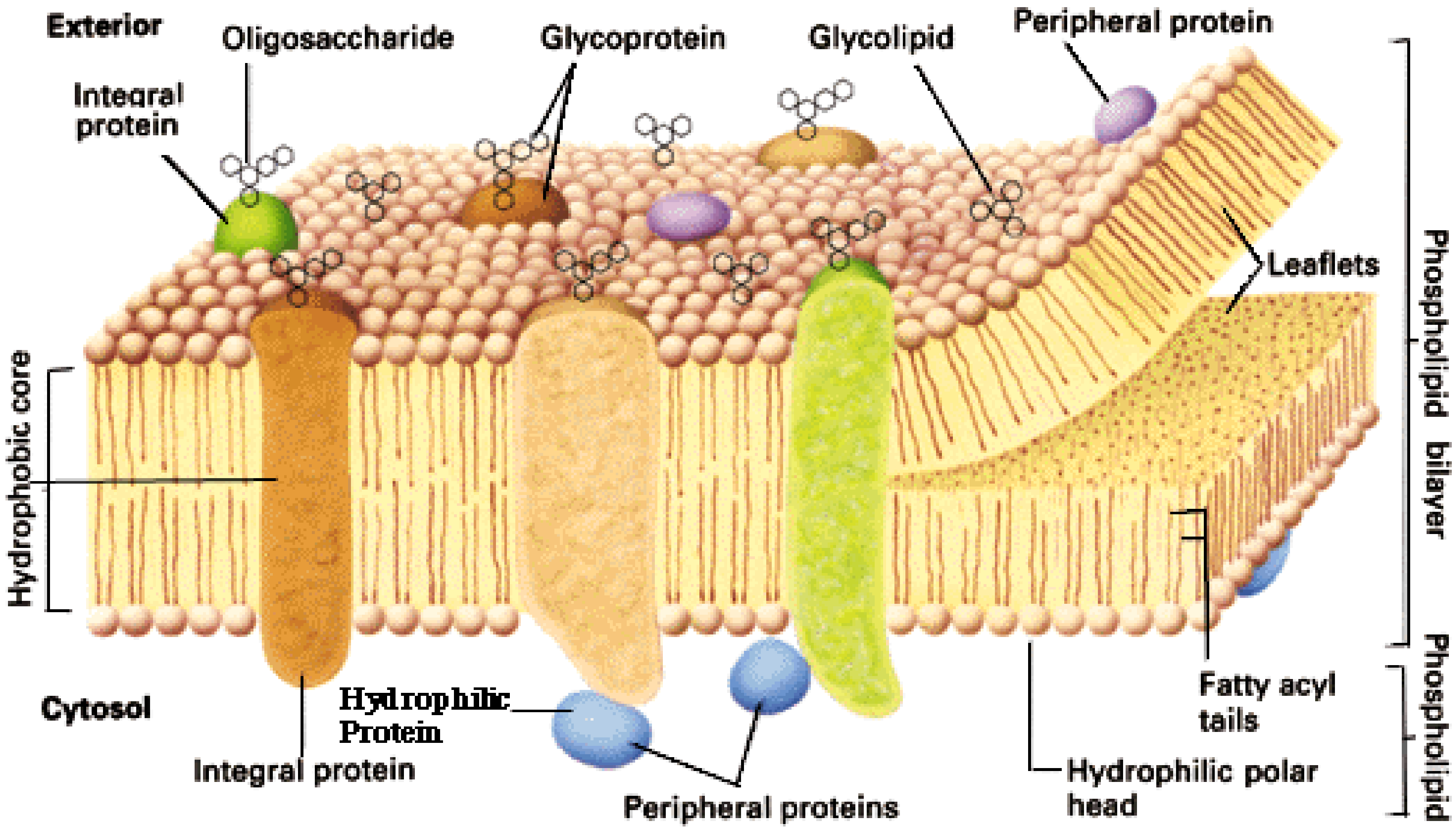
Rehydration

- Upon Rehydration dry cell membranes undergo a transition from gel to liquid crystal phase
  => membrane packing defects, cell leakage
- Viability increases at rehydration temperatures higher than phase transition temperature (Tm)
- The dryer the membrane the higher is the phase transition temperature (depending on membrane composition)

Cell Membrane



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Membrane Phase Transition

- Membrane packaging defects
- Causes damage of the cell membrane
- Leakage of cytoplasmic contents
- Death of cell

Implications

- Still reasonably simple but some care needed during rehydration
- Good rehydration will improve yeast health & performance
- Important not to go over on temperature
- Don’t let the yeast slurry stand too long beyond 15 minutes
- Yeast slurry should be added as soon as possible into fermenter

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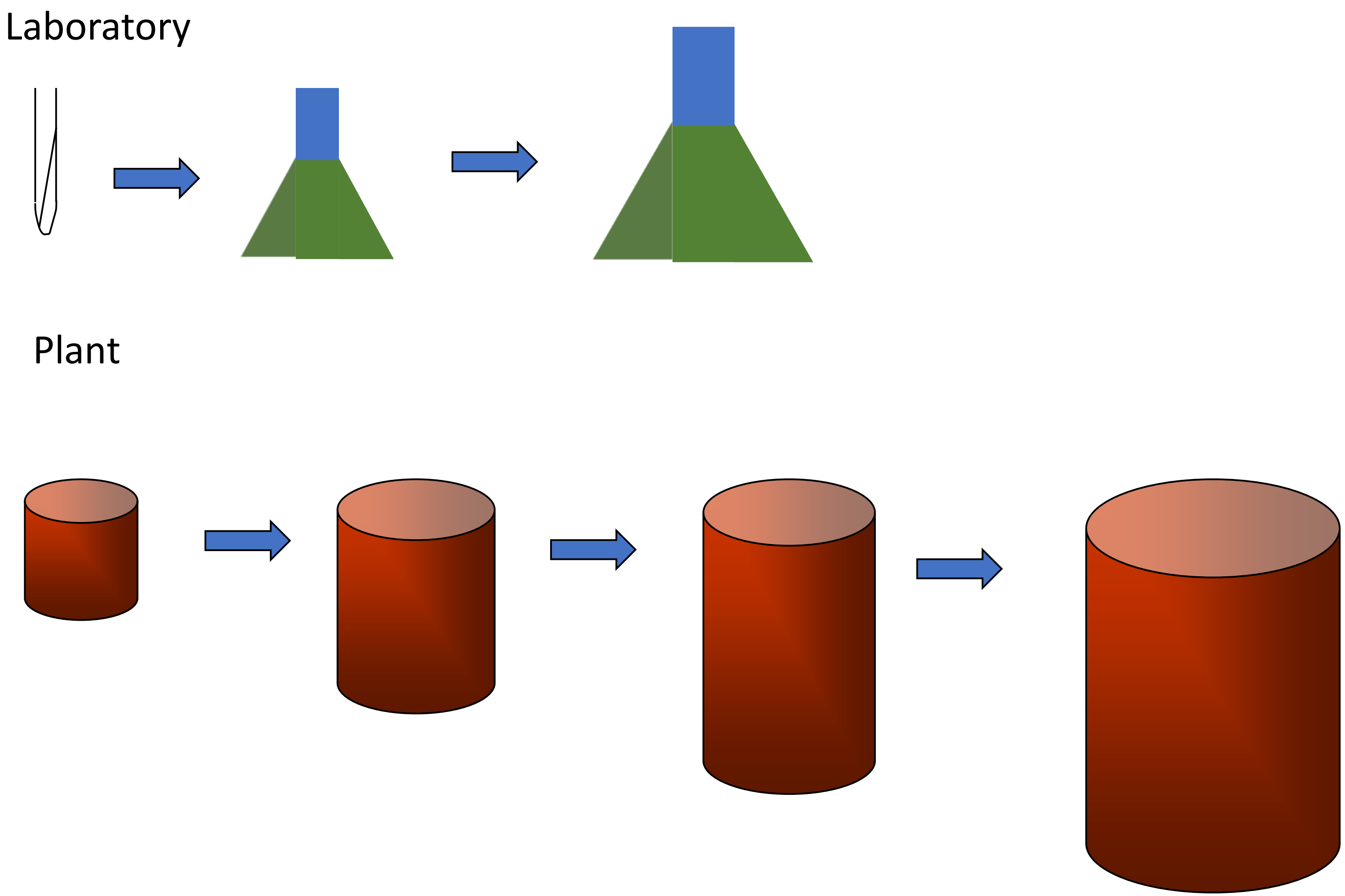
Full In-House Propagation



Pros & Cons

Pros

- Perceived low cost
- Can maintain “own” Strain

Cons

- High risk of contamination
- Multiple stages / multiple cleaning
- High variability
- No back-up
  - What do you do if “bad” batch
- High level of technical ability required
- Higher capital & operating costs

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Yeast Conditioning

- In this case yeast is allowed a single step Conditioning / growth phase prior to addition to the Batch fermenter

- Generally considered a method of increasing yeast mass to economically improve dosage quantity to fermenter

- Multiple variations of this process
  - Continuous
  - Semi Continuous
  - Multiple dose / Semi Continuous
  - Single Batch

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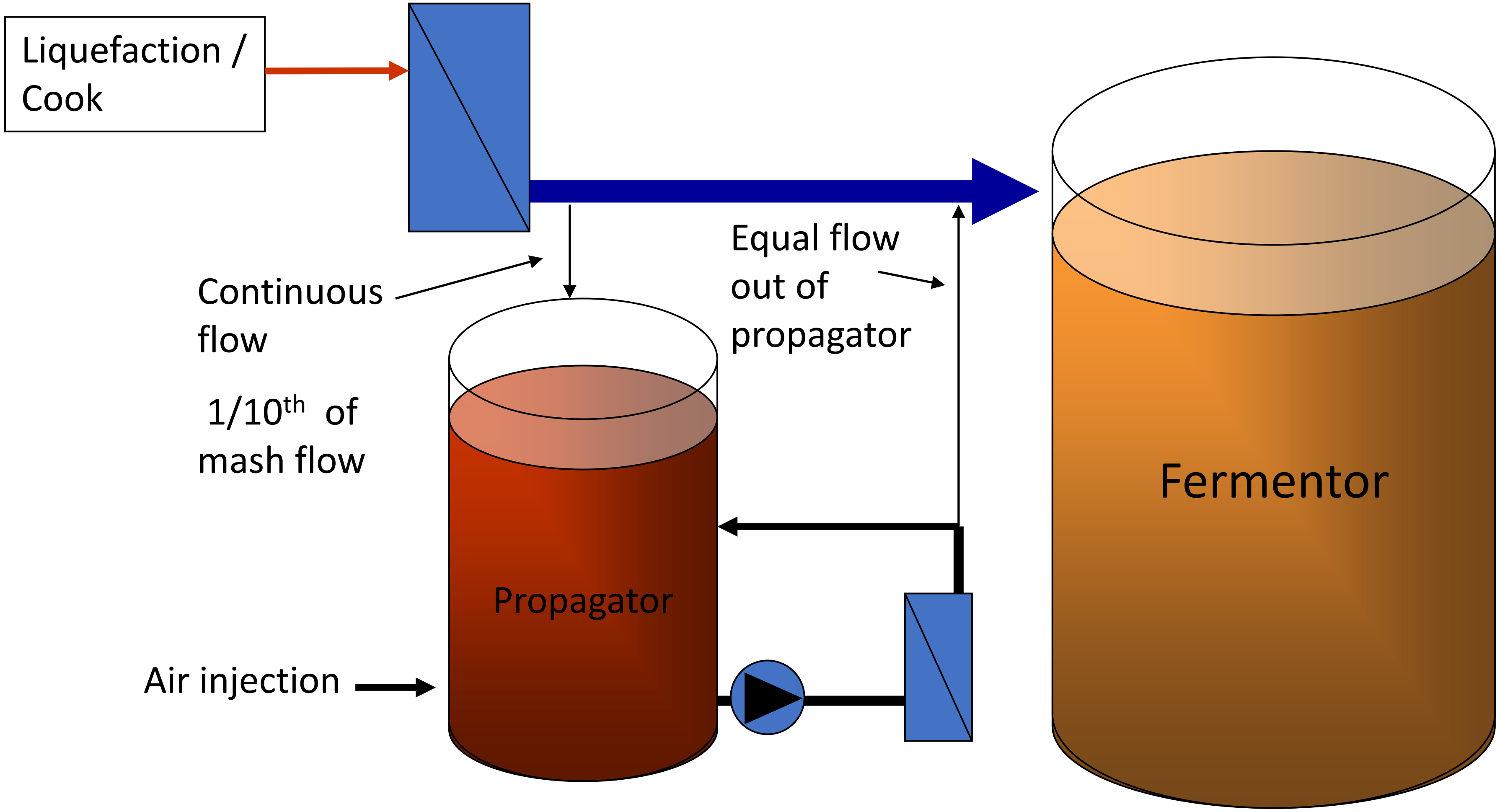
Continuous Process Flows



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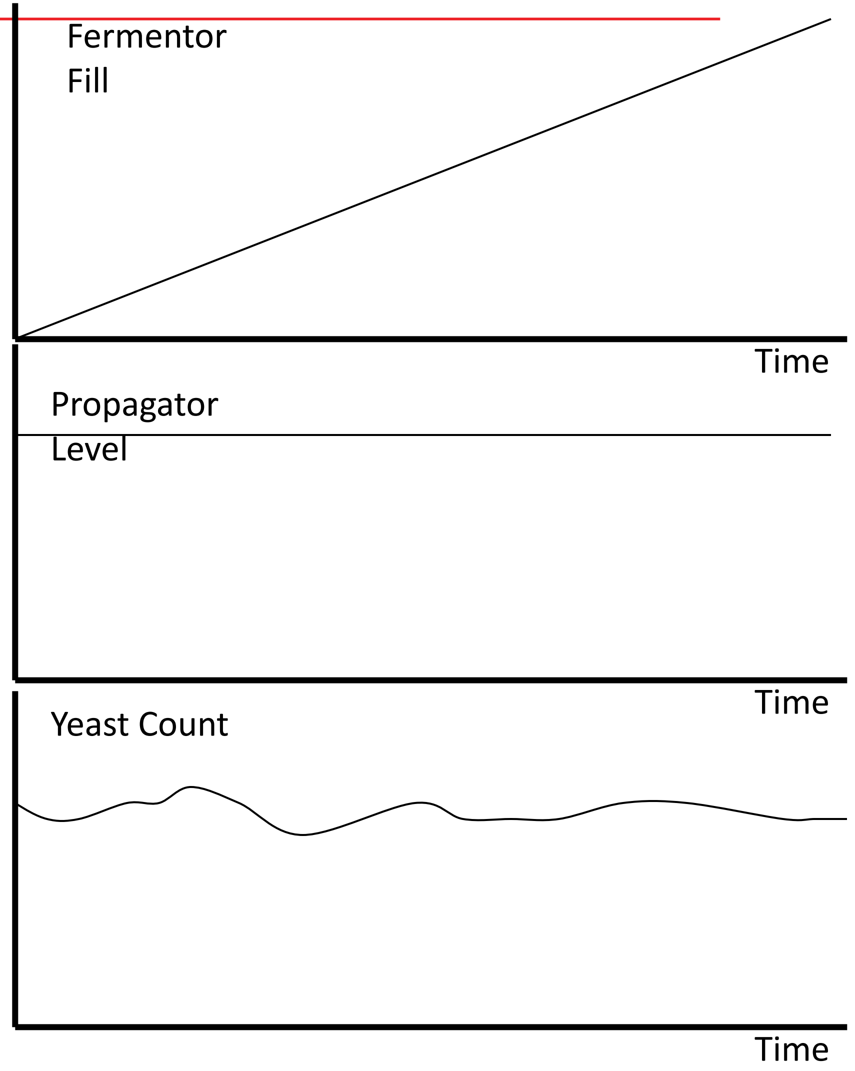
Continuous Propagation

- Propagator maintains level & yeast count
- Flow in matches flow out
- Feed to fermentor throughout its fill time
- 100% yeast in fermentor at 100% fill



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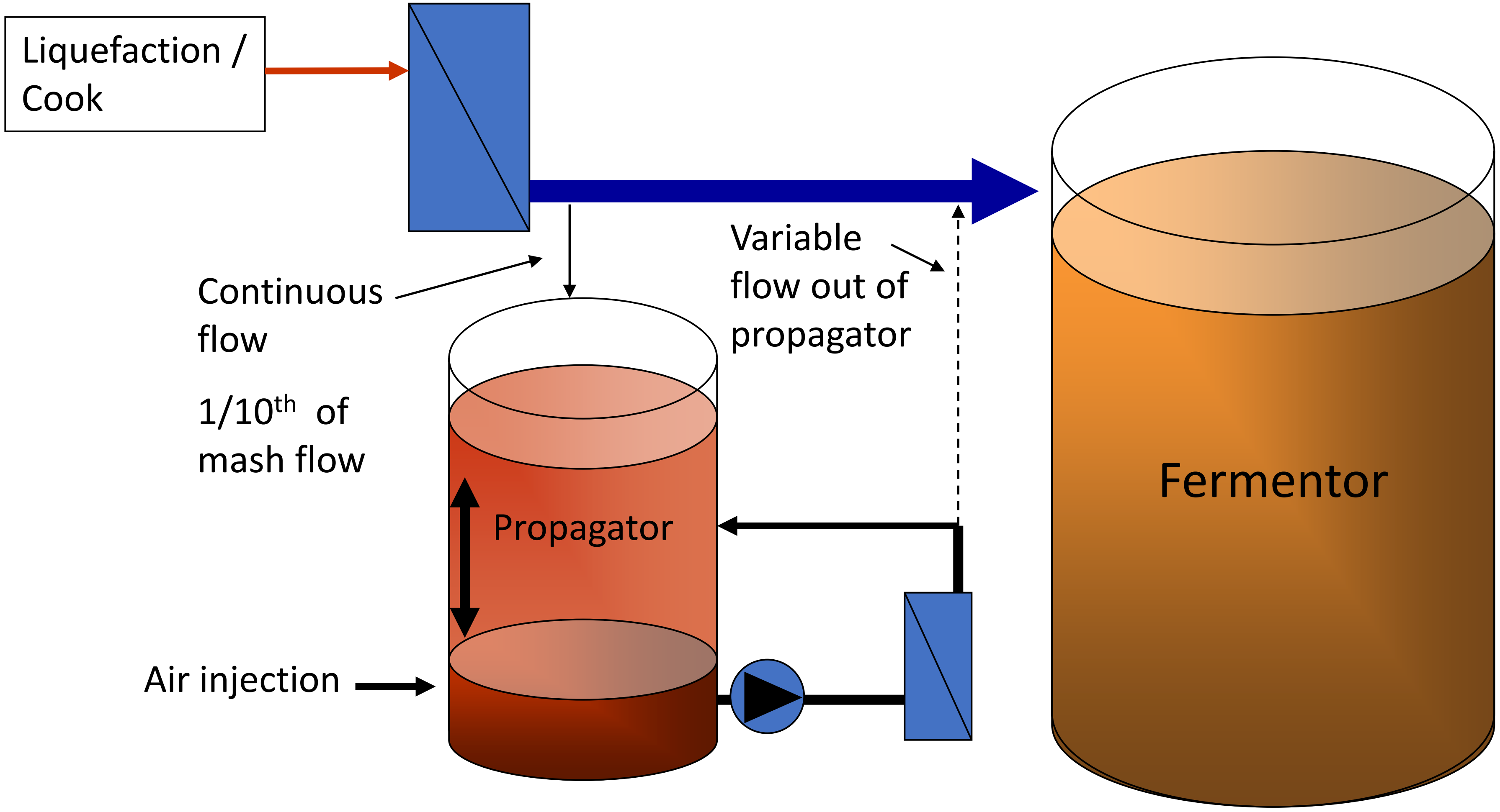
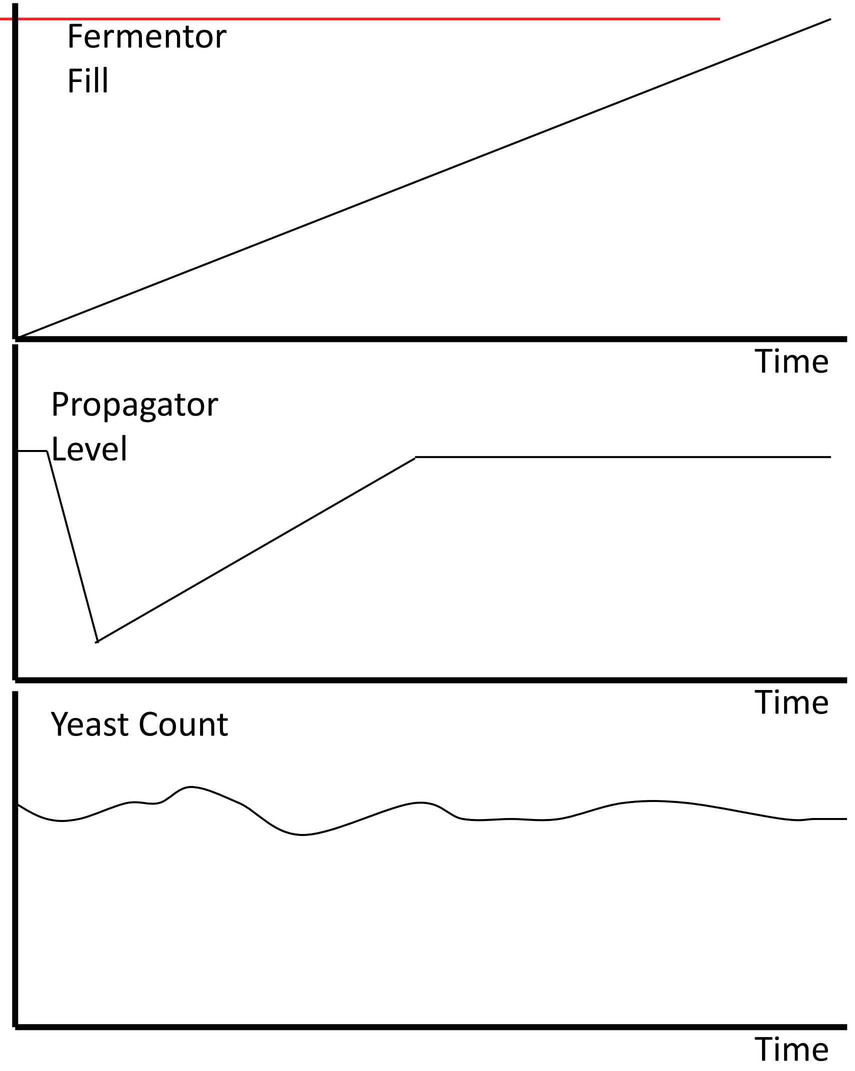
Semi-Continuous Process Flows



Semi Continuous Propagation

- Propagator maintains yeast count
- Slug dose at start of fill (4%)
- Feed to fermentor throughout fill time once propagator regains level
- 100% yeast in fermentor at 100% fill



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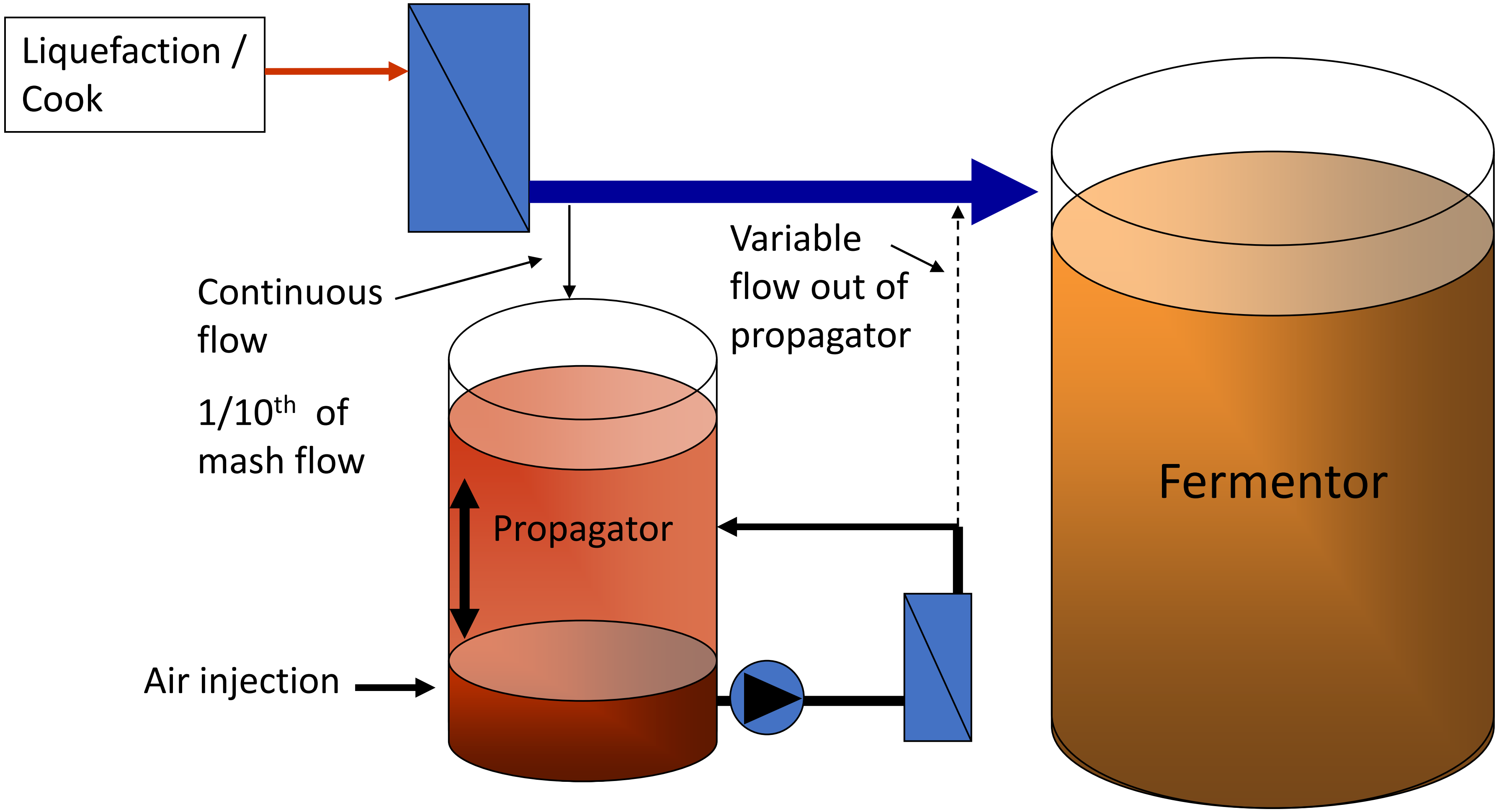
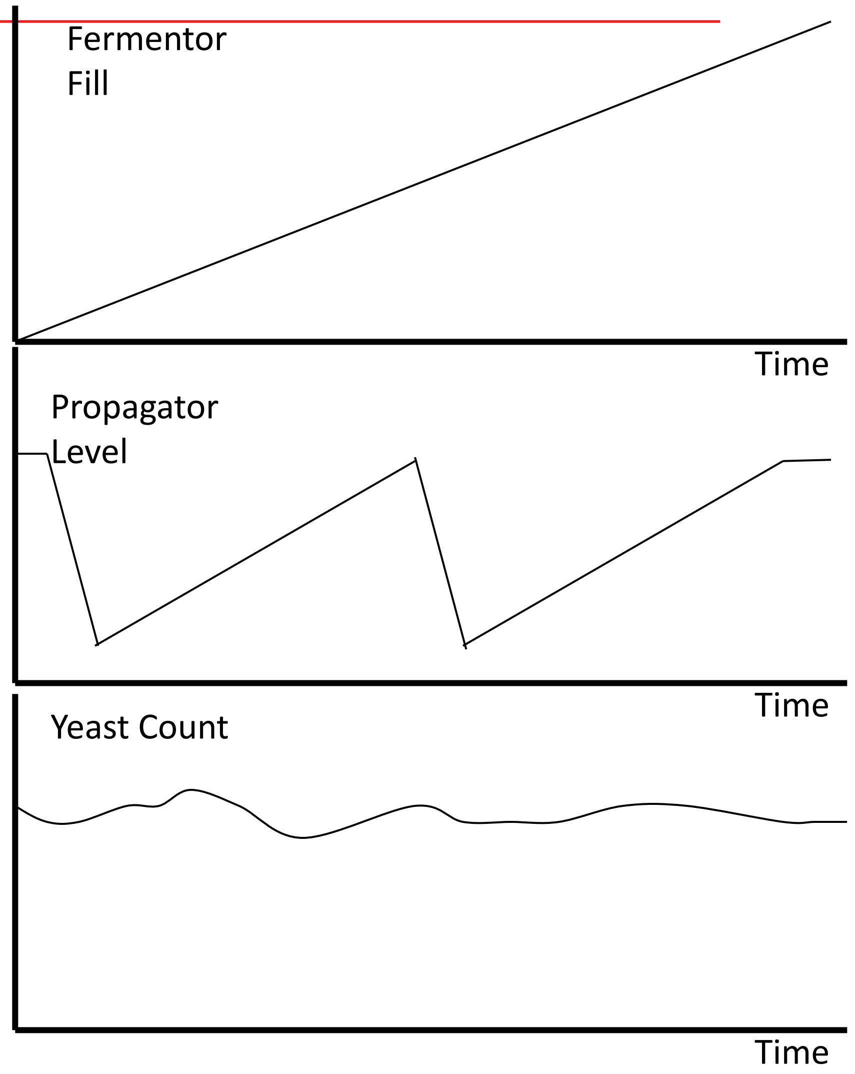
Semi-Continuous Multiple Batch



Semi Continuous Multiple Batch

- Propagator maintains yeast count
- Slug dose at start of fill (4%)
- Second Slug dose once propagator regains fill level
- 100% yeast in fermentor at 100% fill



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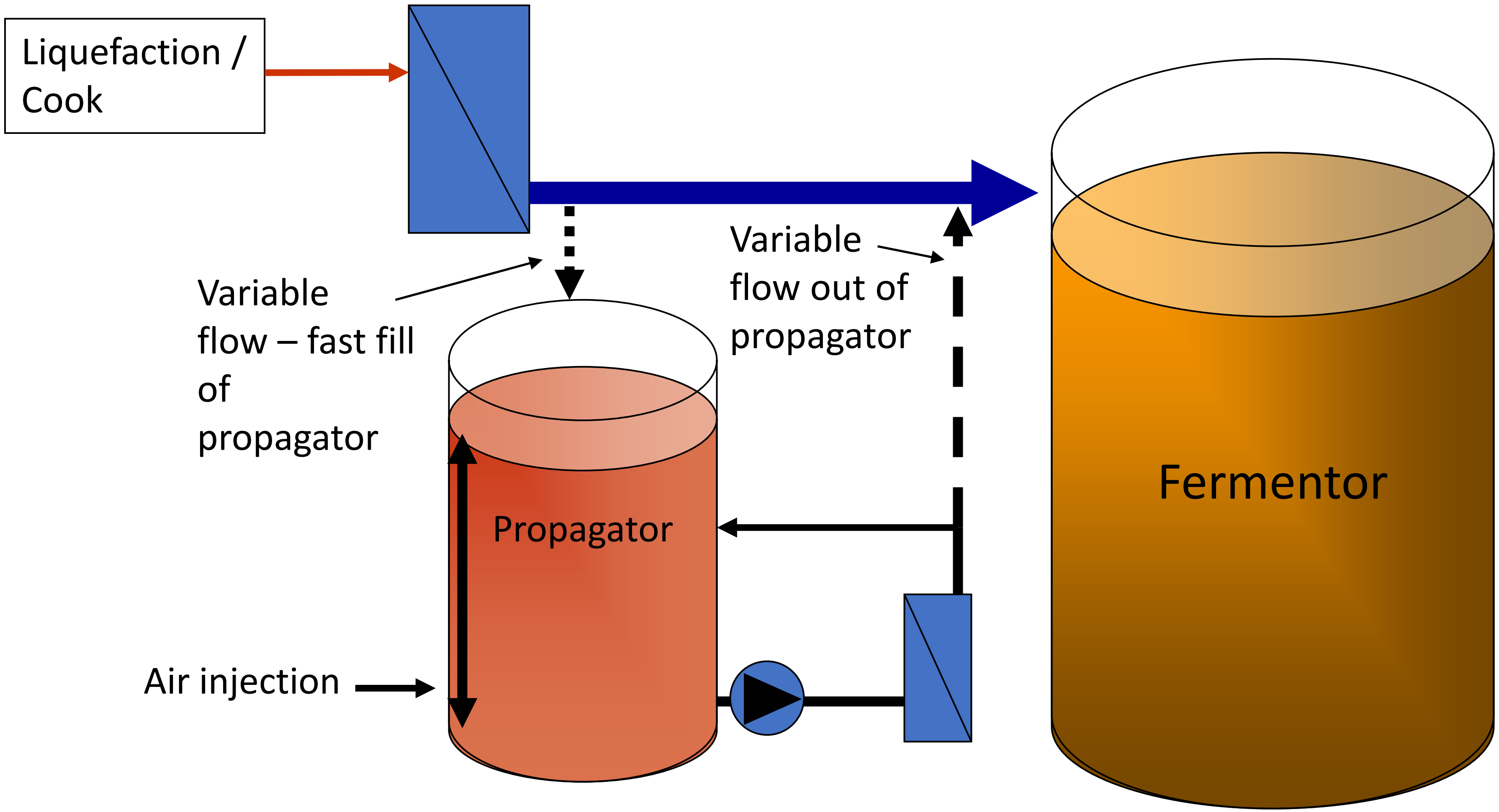
Single Batch Process Flows



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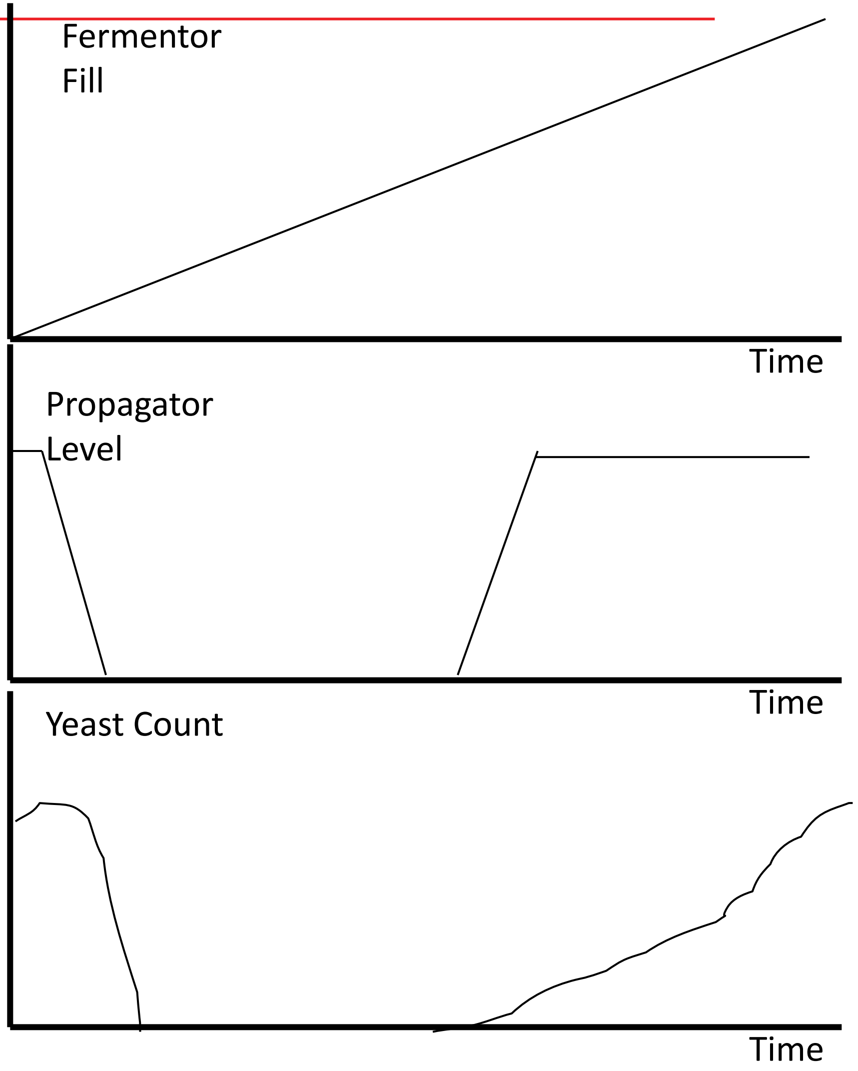
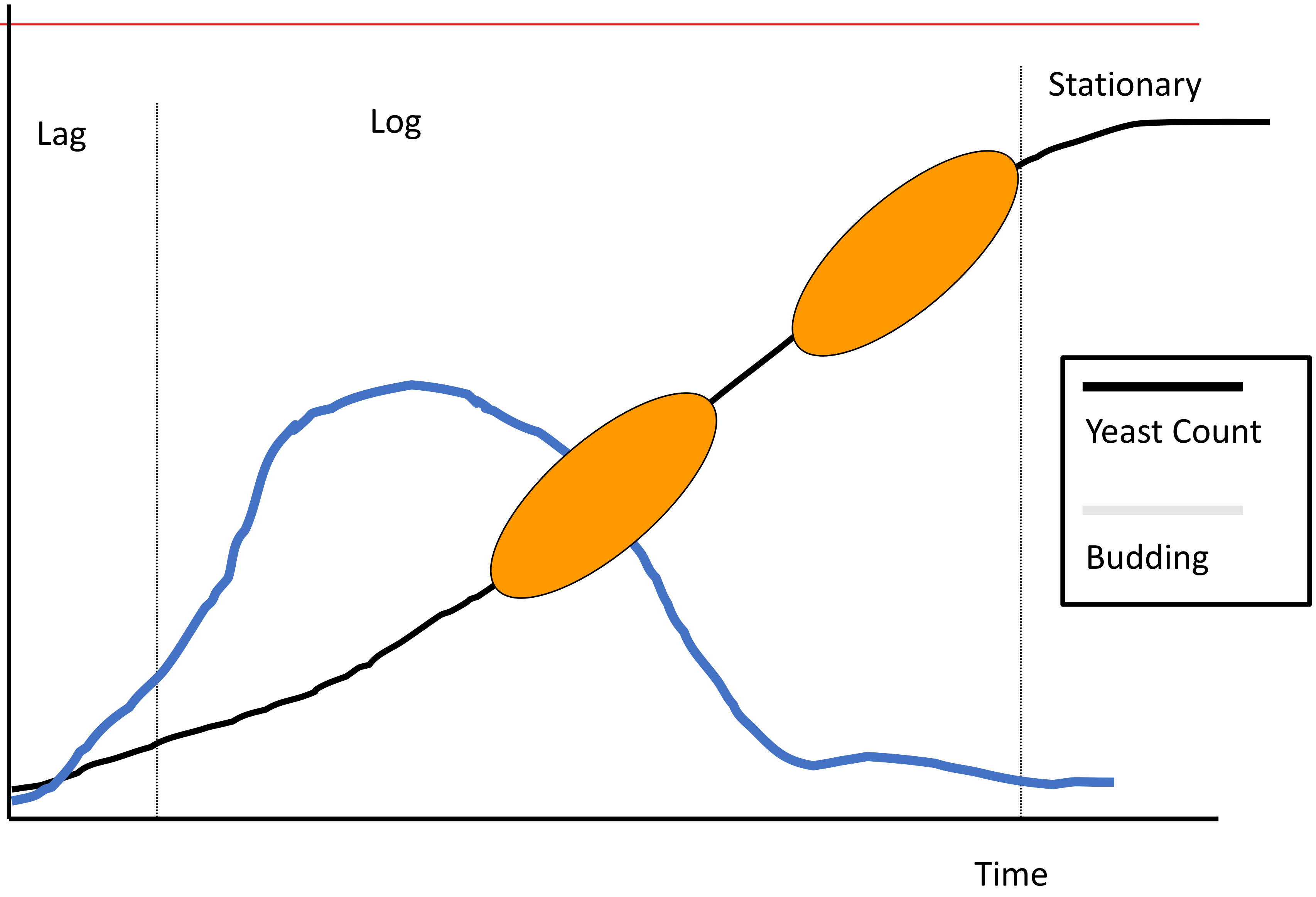
Single Batch Propagation

- Yeast count increases following dilution
- Propagator emptied at start of fill (4%)
- Feed to propagator is fast fill & stop
- 100% yeast is in fermentor at 5% fill
- Ability to optimize propagator time



Propagation Optimization



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Pros & Cons (Batch Fermentation Systems)

	Continuous
Cell numbers at start	☹
Sterol reserve	☹
Time yeast in	☹
Log phase	☹
Infection risk	☹
Mutation wild yeast	☹
Viability	☺
Vitality	☹
Hygiene	☹
Optimization	☹
Automation	☺
Reduced lag phase	☺
Yeast value	☹
Handling	☺

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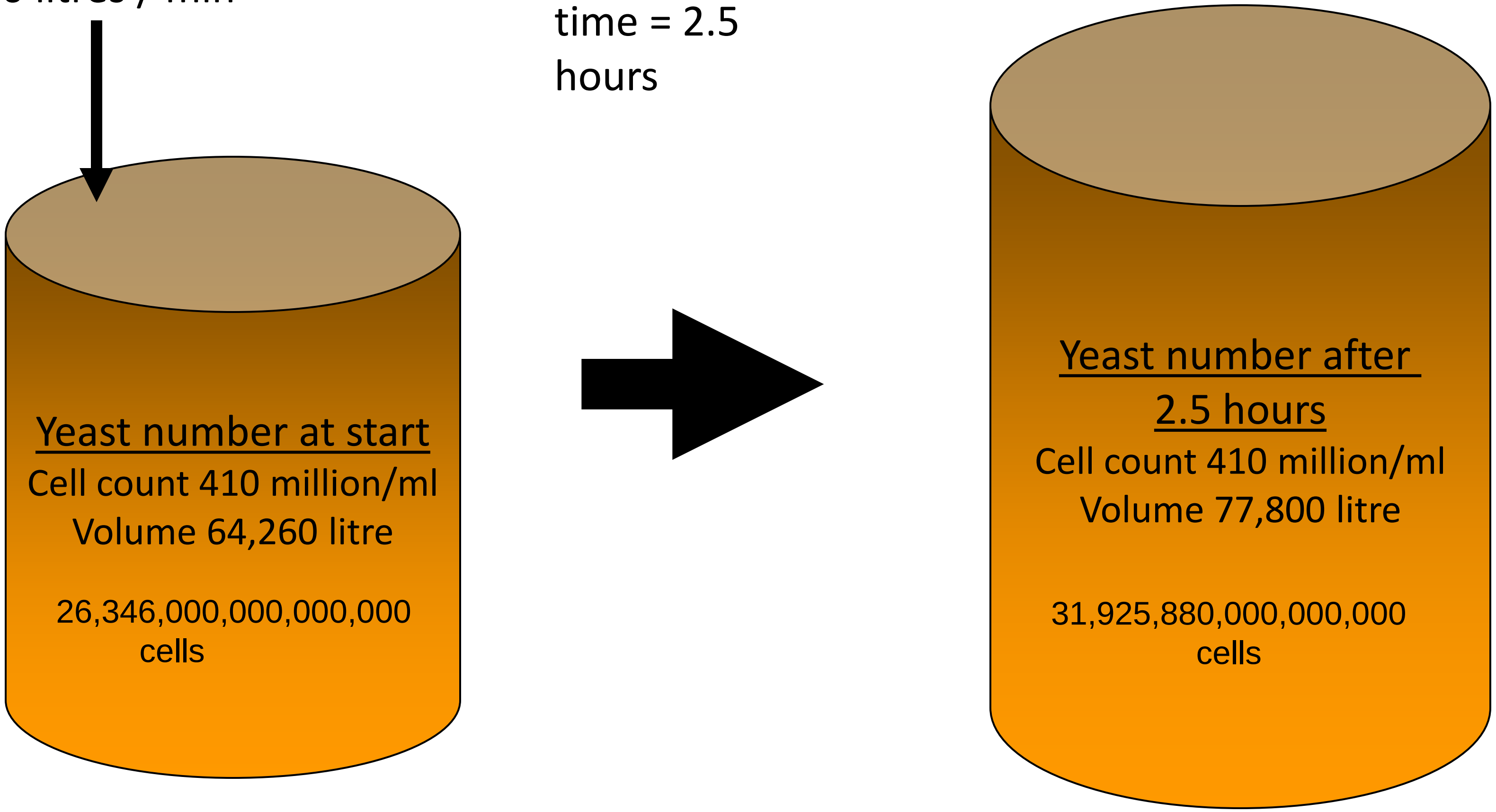
Is Stationary Phase Forced?

Total feed =

90 litres / min

Dilution Effect

Yeast doubling time = 2.5 hours



However, if the original cells were able to double in that time, and the final volume increased by 13,400 L, the theoretical cell count should be 677 million/ml.

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Pros & Cons (Batch Fermentation Systems)

	Continuous	Semi- contin.	Single batch
Cell numbers at start	☹	😐	☺
Sterol reserve	☹	☹	☺
Time yeast in	☹	☹	☺
Log phase	☹	☹	☺
Infection risk	☹	☹	☺
Mutation wild yeast	☹	☹	☺
Viability	☺	☺	☺
Vitality	☹	☹	😐
Hygiene	☹	☹	☺
Optimization	☹	☹	☺
Automation	☺	😐	😐
Reduced lag phase	☺	☺	😐
Yeast value	☹	☹	☺
Handling	☺	☺	☹

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Single Batch - Implications

1. More yeast required
2. More cleaning costs & times
3. Difficult to automate
4. More manual handling
5. Lag phase at start of each propagator

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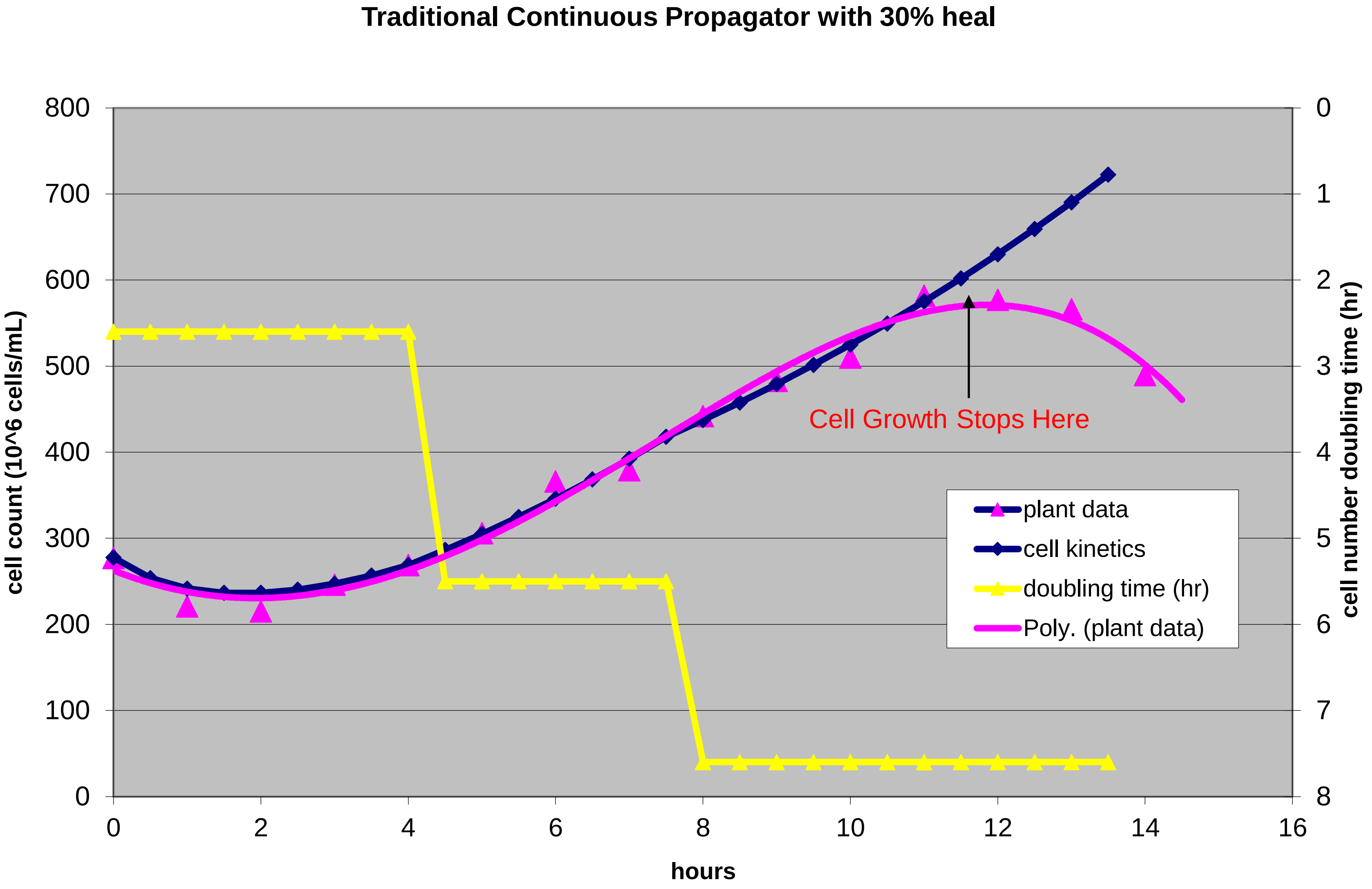
Actual Plant Data



Kurt Kohler, 2004

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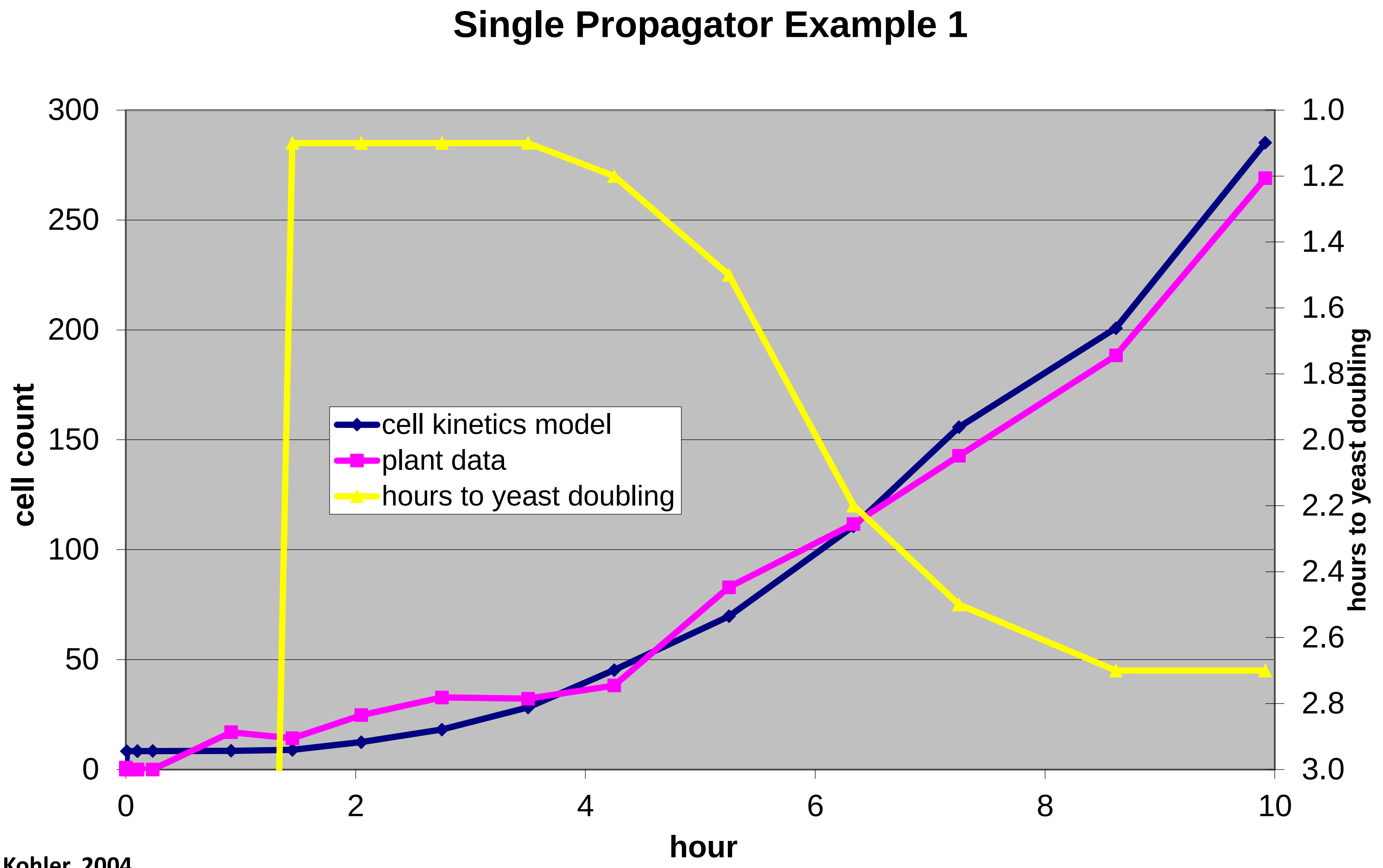
Actual Plant Data



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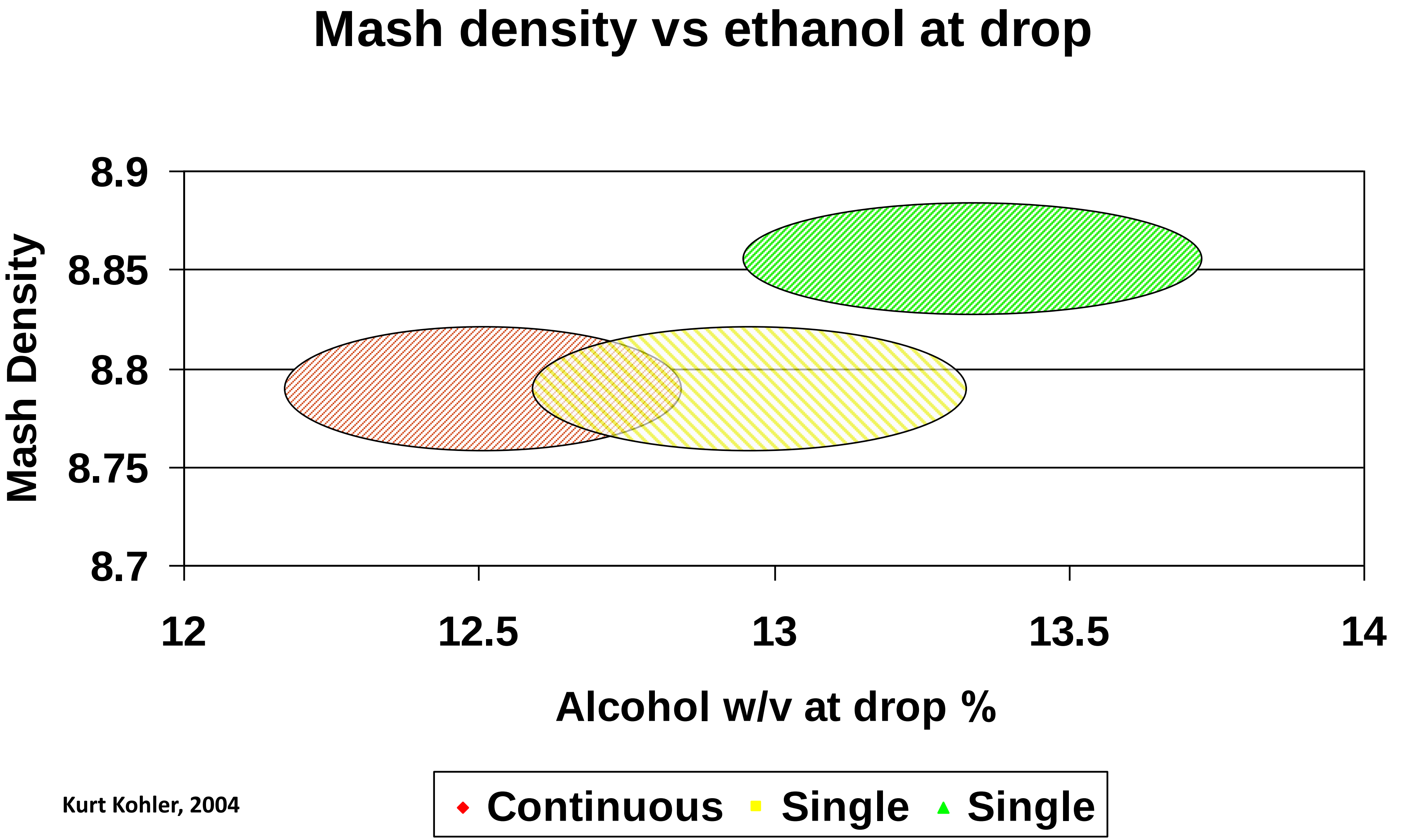
Actual Plant Data



Kurt Kohler, 2004

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Value to an Ethanol Plant

- On a daily basis a 190 million litre plant will realize increased alcohol production of approximately 17,000 litres
- Which at a price of € 0.49/litre would net an extra revenue of € 8,330 or € 2.85 million per annum
- Some US plants are now achieving 20% v/v ethanol using very high gravity (VHG) fermentations (only in batch fermentation)

Converted from Kurt Kohler, 2004

Yeast Handling as a Cost Center

- As part of the operations, it is critical to understand the cost implications of each step

- The following slides follow a costing exercise at a facility in USA

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Propagator Operations

- The Propagator is cleaned
  1. CIP fluid made up to strength
  2. CIP fluid is heated up
  3. CIP fluid is circulated
  4. Propagator is circulated
  5. CIP fluid is "recovered"

- The Propagator filled
  1. Additions made (yeast, nutrients, antibiotics, enzymes etc)
  2. Agitation of propagator started (if agitator present)
  3. Aeration started
  4. Dilution water added
  5. Sample taken for initial tests

- Propagator is circulated
  1. Circulation pump is run
  2. Aeration is carried out
  3. Agitation (if present) is run

- Propagator is sent to fermentor
  1. Sample taken for drop analysis
  2. Pump run to sent to fermenter
  3. Tank and lines flushed

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What is Estimated as a “Cost” Normally?

- The Propagator is cleaned
  1. CIP fluid made up to strength
  2. CIP fluid is heated up
  3. CIP fluid is circulated
  4. Propagator is circulated
  5. CIP fluid is “recovered”
- The Propagator filled
  1. Additions made (yeast, nutrients, antibiotics, enzymes etc)
  2. Agitation of propagator started (if agitator present)
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  5. Sample taken for initial tests
- Propagator is circulated
  1. Circulation pump is run
  2. Aeration is carried out
  3. Agitation (if present) is run
- Propagator is sent to fermentor
  1. Sample taken for drop analysis
  2. Pump run to sent to fermenter
  3. Tank and lines flushed

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Cost of CIP Operation? – Caustic Cost

- Assume that after one clean the caustic strength drops by 1% (from 4% to 3%)

- Dependant on the caustic tank size the value of replacement caustic can be measured

- From plant data (200 million litre capacity)
  - Tank size = 175,100 litre (filled to 50%) = 87,539 litre
  - Volume of caustic (100% strength) required = 875 litres
  - Caustic required (purchased strength 50%) = 1750 litres
  - Cost per ferm = $414
  - Cost per annum = $ 232,100

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Cost of CIP Operation? – Heating Cost

- Assume that after previous clean there is a need to reheat caustic back to 88°C (190°F) from 77°C (170°F)
- Assuming same heat coefficient as water, the BTUs can be calculated (1 BTU = energy to raise 1lb water 1°F at atmospheric pressure)
- From plant data (200 million litre capacity)
  - Tank size = 175,100 litre (filled to 50%) = 87,539 litre
  - BTUs required= 3,859,826 BTU
  - BTUs per annum = 2,161,502,430 BTU
  - Cost per annum ($5/million BTU) = $10,807

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Cost of CIP Operation? – Pumping Cost

- Assume that
  - product is circulated for 2 hours per clean
  - Use both CIP pump & Prop recirculation pump
  - Both pumps 100 HP
  - Cost of electrical energy $0.07 / Kwh

- From plant data (200 million litre capacity)
  - 83,522 Kwh for each pump per annum
  - Pump average loading = 75%
  - Cost per annum (per pump) = $4,142
  - Total cost = $8,284

Total CIP Costs

- Caustic replacement = $ 232,100
- CIP heating costs = $10,807
- Electrical energy = $8,284
- TOTAL COST FOR CLEANING = **$251,191**

This cost is “hidden” in caustic & energy budgets!

This is a direct cost of propagation

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Other Hidden Direct Costs

- Energy (per annum) = $ 56,895
  - Other pumping costs - $ 16,568
  - Cooling - $5,949
  - Agitation - $1,242
  - Aeration - $33,136

- Lab consumables (per annum) = $ 2,744

- Man hours = approx 2 hours per fermentor = 1120 hrs per annum (but not costed as no manpower reduction is possible through elimination)

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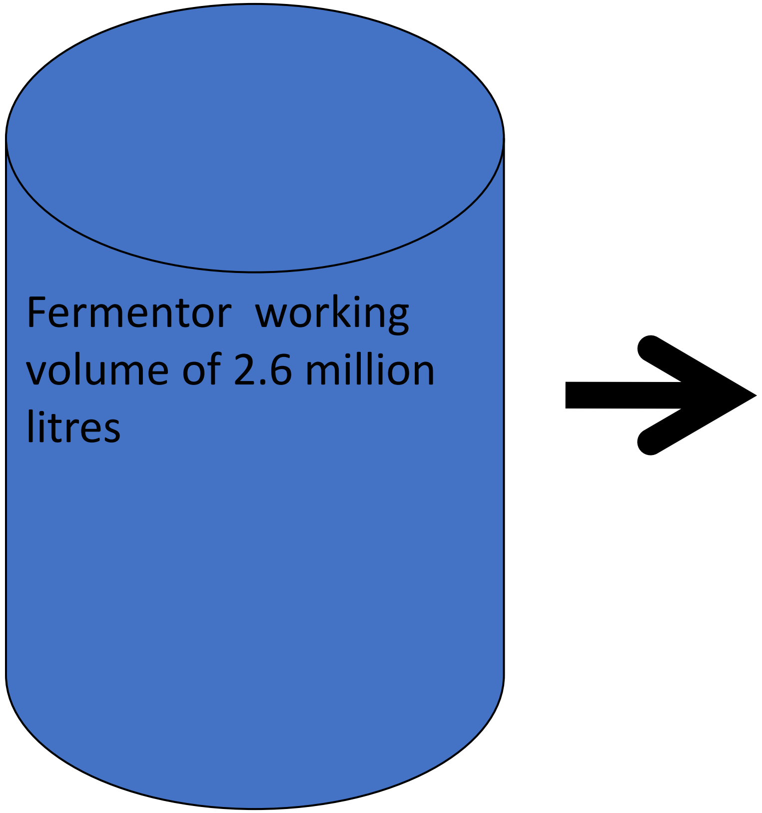
Potentially Total Hidden Direct Costs!

$310,830

There are other indirect costs

Dilution Water from Propagator = Lost Throughput!



2,486,450 litres mash from fill line

94,625 litres mash from propagator

18,925 litres water from propagator

If no propagation is done, then no dilution water is used. The water could be replaced with mash thus creating additional throughput/productivity

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Value of Additional Throughput

- Based on a bushel (25.4 kg) occupying 65.4 litres (at 31% solids)
- 18,925 litres replaced allows 290 additional bushels per fermentor (7366 kg)
- Expected yield 2.8 gal / bushel (417.2 litres/tonne)
- Additional throughput = 812 gal (3,073 litres) per ferm
- Additional throughput per annum = 454,720 gal (1.72 million litres)
- Dependant on cost of make ranges from $200,000 – $400,000

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Other Indirect Costs of Propagation - Contamination

- Potential contaminations
- Assume
  - One fermentor every two months loses 1% wt/vol more ethanol due to propagated yeast performance (contamination, operation error etc).
  - At $2.00 per gal
- Loss of 8,500 gal (32,172 litres) per fermentor
- Equates to $17,000 per fermentor
- Loss per annum = $102,000

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Other Indirect Costs of Propagation - Yield

- Potentially increased yield benefits
- Assume
  - Minor yield benefit of 0.002 gallon / bushel (0.3 litres per tonne)
  - $2.00 per gallon
- Gain of 48,211 gallon (182,479 litres) per annum
- Equates to $96,422 per annum

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All the Calculations Put Together

Category	Product / item	Amount / ferm	Unit cost	Cost per fermentor	Cost per annum
Prop Additions	Cake Yeast	136.08 kg.	$ 4.41/kg.	$600.00	$336,000.00
	Antibiotics - Lact V	0.50 kg.	$ 183.00/kg.	$91.50	$51,240.00
	Gluco Amylase	24.75 kg.	$ 3.00/kg.	$74.26	$41,586.55
	Urea	22.68 kg.	$ 0.46/kg.	$10.50	$5,880.00
	Neotrol	0.50 kg.	$ 55.07/kg.	$27.54	$15,419.60
	AYF 1000	25.00 kg.	$ 3.85/kg.	$96.25	$53,900.00
Sub total				$803.80	$504,026.15
Water	Water replacement			$713.31	$399,451.87
	Water	5000.00 Gal	$ 3.00/ 1000 gal.	$15.00	$8,400.00
Sub total				$728.31	$407,851.87
Prop utilities	Caustic	462.56 Gal	$ 0.90/gal.	$414.45	$232,094.11
	Heat			$19.30	$10,807.51
	Pump Energy			$44.38	$24,852.54
	Cooling			$10.62	$5,949.02
	Agitation			$2.22	$1,242.63
	Air Blowers			$59.17	$33,136.72
	Operator hours	0.83 Hours	$ 0.00/ hour.	$0.00	$0.00
	Maintenance hours	0.16 Hours	$ 0.00/ hour.	$0.00	$0.00
	Lab Man hours	1.00 Hours	$ 0.00/ hour.	$0.00	$0.00
	Lab consumables	1 quantity	$ 4.90 per prop	$4.90	$2,744.00
Sub total				$555.05	$310,826.53
Yield	See yield calculation			$170.46	$95,458.18
Sub total				$170.46	$95,458.18
Contamination	See calculation below			$179.93	$100,761.41
Additional treatment	Antibiotics - Lact V			$3.92	$2,196.00
Sub total				$183.85	$102,957.41
Total				$2,441.47	$1,421,120.14

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Conclusions

- Choose the system that is best suited to your needs & abilities as well as the availability of product & format
- Look at the process benefits and if considering change ensure the cost benefit is measurable
- Look at all the costs not just the inputs
- Your yeast is the "heart" of your distillery and its health and effectiveness produce your yield

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Happy yeast are good for business!

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