Yeast Propagation – A Practical Approach

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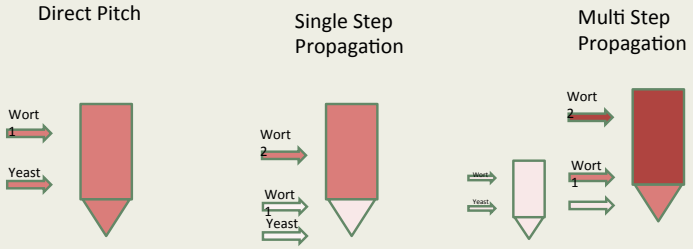
Overview

- General
- Planning
- Phase 1 Process
- Phase 2 Process
- QC

Main Goal of talk is to understand why:
Do not put 2L yeast into 3 gal for 5 days then add to 20 BBl

Inoculation Options



Propagation

Purpose – To efficiently expand a small culture population to achieve the desired pitch rate in the final FV

Pros

Lower initial yeast cost
- 1 off
- More strains
- Higher pitch rates

Cons

Labor cost
Media cost
Increase tank residence time
QC Concerns
% prop media in final product
Logistics – Media/Tank availability

Propagation Systems

Fed Batch

Incrementally feed culture throughout propagation
+ Higher yields - Aerobic
- High Process control needs
- High QC concerns

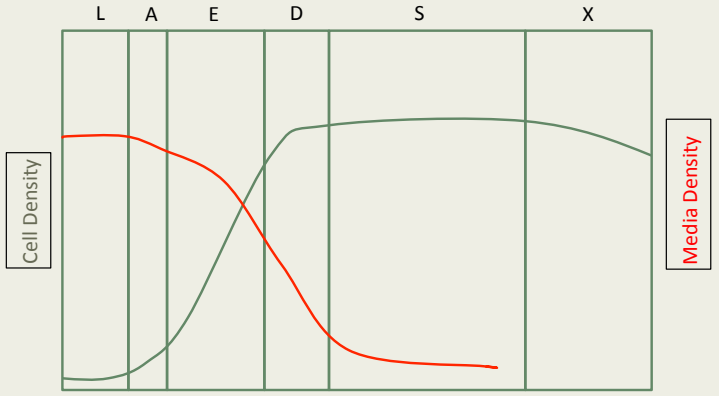
Continuous

Remove culture and add back media through out propagation
- Not applicable
- High Process control needs
- High QC concerns

Batch

Single additions of media at each step (1:5-1:10)
+ Less process control
+ Lower QC concerns
+ Lower flavor contribution
- Lower growth - Anaerobic

Batch Fermentation



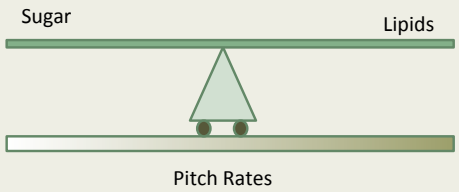
L - Lag Phase
A - Acceleration Phase
E - Exponential Phase
D - Deceleration Phase
S - Stationary Phase
X – Death Phase

Cell Growth

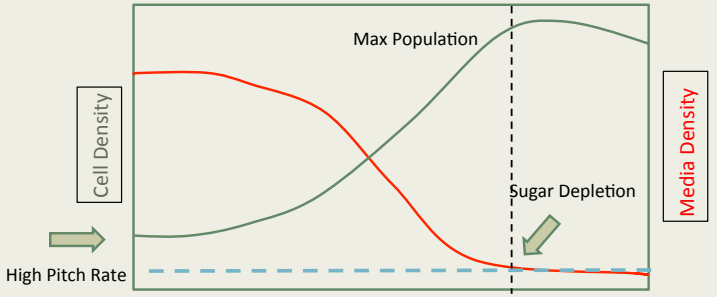
Cell growth is typically limited by depletion of a nutrient.

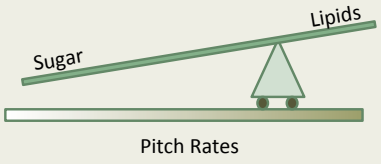
- Nitrogen
- Minerals
- Vitamins
- Carbon (Sugar)
- Oxygen (Lipids - Sterols)

Pitch Rate is a large factor



Sugar Limiting Growth





Sterol Limiting Growth

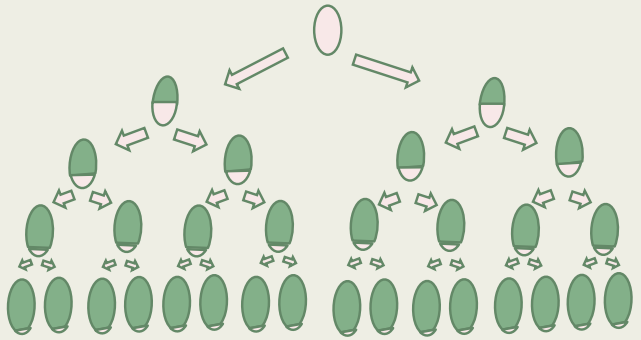
Sterol - Ergoterol

Structural lipids with the following roles:
- Regulate membrane fluidity
- Regulate membrane permeability
- Influence activity of membrane bound enzymes

Biosynthesis requires:
- Energy - Glycogen
- Oxygen

Max Cellular level 1.0%
Depleted 50% with each budding
Minimum level to maintain cellular function 0.1%

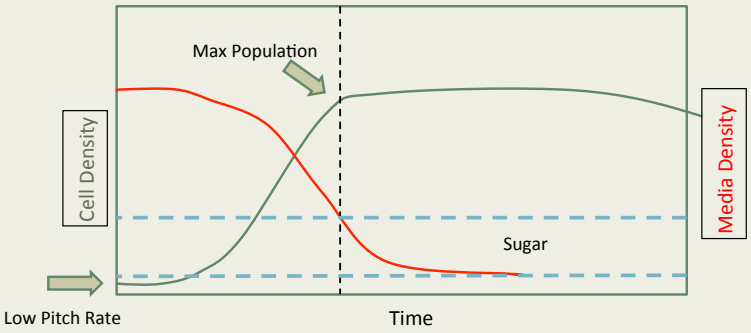
Sterol Limiting Growth

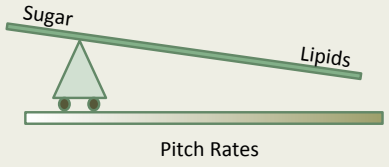


Doubling	Sterol Level
0	1.0%
1	0.5%
2	0.25%
3	0.125%
4	0.0625%

Sterol content will limit growth to ~ 3.25 doublings

Sterol Limiting Growth





Cell Growth Equation

Equation : $O_{CD} \times 2^{D} = F_{CD}$

O_{CD}	Original Cell Density
D	# of Doublings
F_{CD}	Final Cell Density

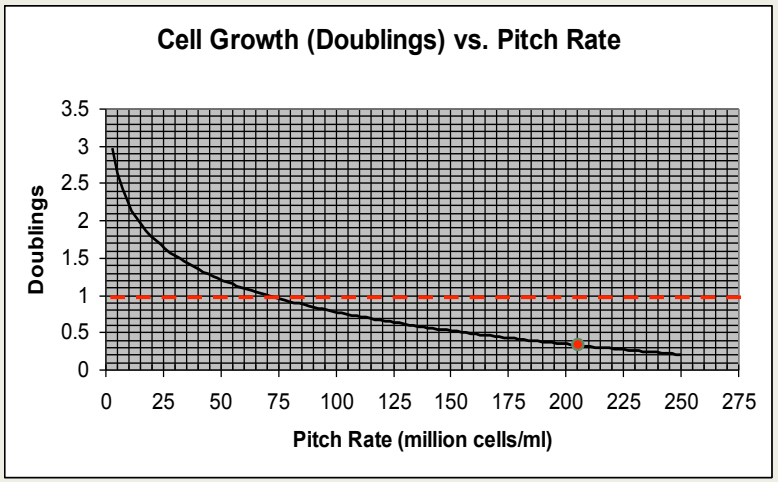
Doublings	Fold Increase (x)
3.25	9.51
3	8.00
2.5	5.66
2	4.00

Initial Pitch Rate

$O_{CD} \times 2^{D} = F_{CD}$

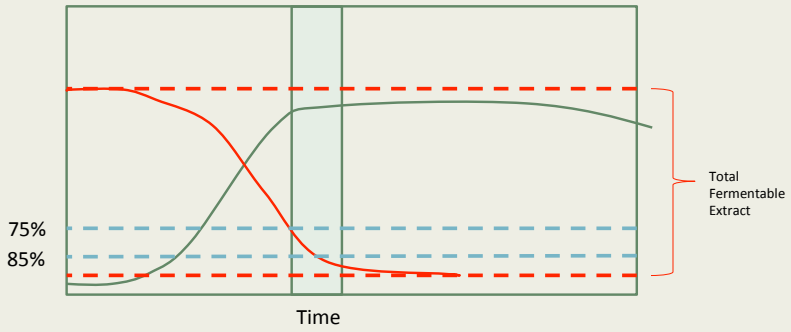
Initial Pitch Rate (1E6 ml-1)	Final Cell Density (1E6 ml^{-1}) per Doublings				
	1.25	**1.75**	**2.25**	**2.75**	**3.25**
3	7	10	14	20	29
6	14	20	29	40	57
9	21	30	43	61	86
12	29	40	57	81	114
15	36	50	71	101	143
18	43	61	86	121	171
21	50	71	100	141	200
24	57	81	114	161	228

Conservative Growth Estimator



Keep Prop Pitch Rates Between
Max 50E6 cells ml^{-1}
Min 12E6 cells ml^{-1}

Timing of Propagation Step



Pitch Rate and Temperature Dependent

Make propagation steps when:
- Population is high – Not Too Early
- Metabolism is active – Not Too Late

Typically recommend ~75-85% (6-9 P^{0})reduction of fermentable extract

Timing of Propagation Step

~Time to deplete 75%-85% Fermentable Extract @ 68F

PITCH RATE (1.0E6 ml^{-1})	Time (Hr)
6	**>48**
12	**48**
18	**48**
24	**36**
30	**36**
36	**24**
42	**24**

Propagation Goals/Planning

Identify parameters and then work backwards

Fermentation
- Volume
  - Density
- Inoculation Rate
  - Density
  - Temperature

Equipment
- Brewhouse limitations
- FV Setup
- FV Availability
- Media Availability

Goals/Planning – Volume/Equipment

3 Questions

1 - Final Volume of Brew?
Batch Volume

2 - Density?
*10% Propagation media @ 3-5 P°

3 - Fermentation Temperature?

*Blended Density = $((P_{vol} \times P_P)+(W_{vol} \times W_P))/(W_{vol} \times P_{vol})$
P - Prop, W – Wort

Goals/Planning – Volume/Equipment

Examples

Example	Final Wort Volume (Bbl)	Wort Density (P)	Adjusted Densitiy (P)	Fermentation Temp. (F)
1	7	15	14	66
2	7	14	13	52
3	10	18	17	68

Goals/Planning – Final Inoculation Rates

General Rule of Thumb 1.0E6 ml^{-1} P^{-1}

Lower Pitch Rates are OK due to active metabolism

DENSITY*	FERMENTATION TEMPERATURE (°F)	PITCH RATE (1.0E6 ml^{-1})
<15P°	>65	6.00-12.00
15-19P°	>65	12.00-15.00
>19P°	>65	>18.00
<15P°	<60	12.00
15-19P°	<60	18.00
>19P°	<60	>24.00

Goals/Planning

Examples

Example	Final Wort Volume (Bbl)	Wort Density (P)	Adjusted Density (P)	Fermentation Temp. (F)	Inoculation Rate (1E6 cellsml^{-1})
1	7	15	14	66	6.00E+06
2	7	14	13	52	1.20E+07
3	10	18	17	68	1.50E+07

Goals/Planning – Equipment Limitations

Brewhouse Limits
- Smallest Batch
- Media Availability
- Media Type
  - 10% of total beer

Fermenter
- Batch Size
- Headspace Total Volume
- Sample ports!!!
- Temp control
- # of fermenters
- FV Availability
  - Additional residence time

Goals/Planning – Calculations

Example	Final Wort Volume (Bbl)	Wort Density (P)	Adjusted Density (P)	Fermentation Temp. (F)	Inoculation Rate (1E6 cellsml^{-1})
1	7	15	14	66	6.00E+06
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#1 Do it by hand (laborious)
- Growth vs. Pitch Rate
- Growth Equation

#2 Pitch Rate & Growth Calculator
http://www.wyeastlab.com/pitch_rate.cfm

Goals/Planning – Calculations

Step 1
- Enter Slurry Volume (L)
- Enter Step 1 Wort Addition Volume (BBl)
- Check Pitch Rate

Step 2
- Enter Final Wort Volume
- Check Pitch Rate

Step 3
- Confirm & Make Adjustments

Many different ways to achieve the same goal.

Goals/Planning – Calculations

		#1	#2	#3
Step 1	Cell Count Slurry (Billion cells/ml.)	1.20E+09	1.20E+09	1.20E+09
	Volume of Slurry (L)	1.00	2.00	2.00
	Volume of Wort #1 (Bbl)	1	2	1
	Pitch Rate #1(Million Cells/ml)	10.23	10.23	20.45
Step 2	Vol of Wort Added #2 (Bbl)	7	6	2
	Total Volume In Fermenter (Bbl)	8	8	3
	Pitch Rate #2 (Million Cells/ml)	5.89	11.78	23.26
Step 3	Vol. Wort Added #3 (Bbl)			10
	Total Volume In Fermenter (Bbl)			13
	Pitch Rate #3 (Million Cells/ml.)			14.54

Propagation

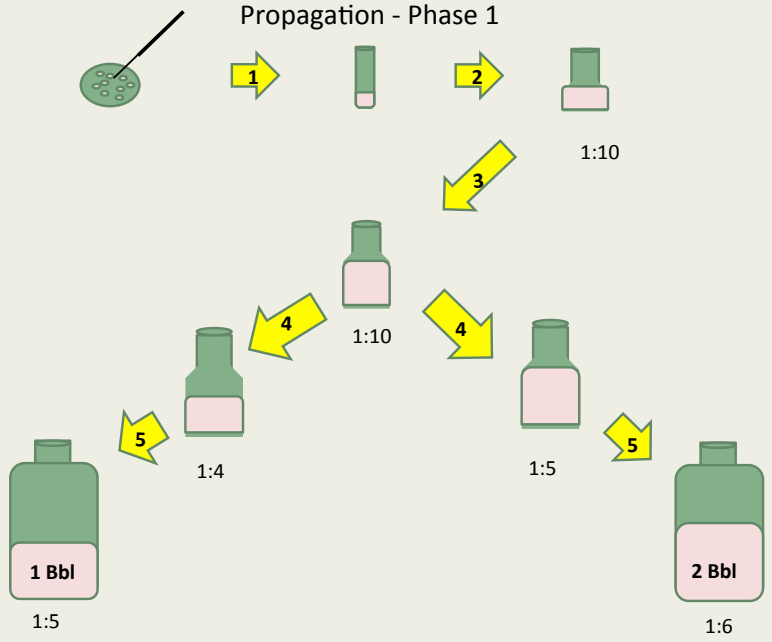
Phase 1 –
- Single morphology working culture to inoculum for FV
- Mainly Lab work utilizing aseptic technique
- Lab equipment needed
- Series of steps to reach volume for tank inoculation
- Each step 24-48 hours
- May not be practical for most breweries
- Can be substituted with commercial culture

Phase 2 – One or more steps in FV
- Use yeast from Phase 1 or commercial culture
- Brewery work
- Practical for most breweries

Propagation - Phase 1

Equipment
- Sterilizer
  - Autoclave/Pressure Cooker
- Working culture
  - Slant or Plate with isolate
- Inoculation needle
- Positive flow hood work station
- Incubator
- Sterile Test Tubes with media
- Sterile Flasks with media
- Shaker table
- Sanitized large vessel with sterile media

Propagation - Phase 1



Propagation - Phase 1

1 Bbl Prop

Process	Inoculum	Media (ml.)	Step Increase	Start CC	Finish CC	Total Volume ml.	Time (Hr.)
Step 1	1 CFU	10	-	-	5.00E+07	10	48
Step 2	10	100	11	5.00E+06	5.00E+07	110	48
Step 3	110	1000	11	5.00E+06	5.00E+07	1110	48
Step 4	1100	3000	4	1.25E+07	1.00E+08	4110	48
Step 5	4110	10000	5	2.31E+07	1.00E+08	14110	24-36

Total Time – 228 hour (9 Days)
Inoculates 1 BBl at 12E6 cells ml^{-1}
Equals 1 L of commercial yeast

2 Bbl Prop

Process	Inoculum	Media (ml.)	Step Increase	Start CC	Finish CC	Total Volume ml.	Time (Hr.)
Step 1	1 CFU	10	-	-	5.00E+07	10	48
Step 2	10	100	11	5.00E+06	5.00E+07	110	48
Step 3	110	1000	11	5.00E+06	5.00E+07	1110	48
Step 4	1100	5000	6	1.67E+07	1.00E+08	6110	48
Step 5	6110	20000	5	2.00E+07	1.00E+08	26110	24-36

Total Time – 228 hour (9 Days)
Inoculates 1 BBl at 22E6 cells ml^{-1}
Inoculates 1 BBl at 12E6 cells ml^{-1}
Equals 2 L of commercial yeast

Propagation - Phase 2

Media
Temperature
Oxygen
Management
Multiple Batches

Propagation - Phase 2

Media

All malt wort should have all essential nutrients

Density
- 10-14 P^o
- Higher levels may not increase growth & increase alcohol stress
- Lower levels may reduce growth

Color
- Consideration for blending (10% total wort)

Hop Aroma and IBU
- Consideration for blending (10% total wort)

Availability

Pils or Blonde Ale Best

Propagation - Phase 2

Temperature and Oxygen

Temperature

Yeast (Ales and Lagers) grows optimally at 85F^o
- May not be optimal for flavor contribution
- Difficult to control rate of fermentation and timing of steps

Ales 68F^o

Lagers 62-65F^o
- Within 10F^o of fermentation temp avoid shock
- Slightly less flavor contribution
- Slower growth

Oxygen

Important nutrient for sterol synthesis
12-15 ppm
Diacetyl

Propagation - Phase 2

Management

Sample Ports!!!
- Run blind without samples

Sample frequently (4 hr.)
- Density
  - 75-85% Reduction of fermentable extract
- pH
  - Good early indicator of performance
  - pH will drop prior to density
- Cell Count
  - Low confidence in representative sample
  - Time consuming
- Viability
  - Useful
- Assess kinetics

Propagation - Phase 2

Examples

Example	Final Wort Volume (Bbl)	Wort Density (P)	Adjusted Density (P)	Fermentation Temp. (F)	Inoculation Rate (1E6 cellsml^{-1})
1	7	15	14	66	6.00E+06
2	7	14	13	52	1.20E+07
3	7	18	17	68	1.50E+07

Propagation - Phase 2

		#1	#2	#3
	Final Pitch Rate (Cells ml^{-1})	5.89	11.78	14.54
	Final FV Volume (Bbl)	8.00	8.00	13.00
Step 1	Volume of Slurry (L)	1.00	2.00	2.00
	Volume of Wort (Bbl)	1	2	1
	Density of Wort (P)	12	12	12
	Confirm Density Drop 75% of Fermentable sugar (~Time & ~P)	48+ Hrs.	48+ Hrs.	36 Hrs.
		4.80	4.80	4.80
Step 2	Volume of Wort (Bbl)	7	6	2
	Density of Wort (P)	14	13	12
	Confirm Density Drop 75% of Fermentable sugar (~Time & ~P)			24-36 Hrs.
				5.76
Step 3	Volume of Wort #1 (Bbl)			10

Multiple Batches

Brewing multiple batches into one tank over multiple days

Example:
- 10 BBl Brewhouse
- 20 BBl FV
- Brew 10 BBl Day 1
- Brew 10 Bbl Day 2

Multiple Batches

#1 Pitch quantity of yeast for entire 20 BBl FV volume on day 1
Aerate batch 1
Do not aerate batch 2

#2 Ale Pitch quantity of yeast for day 1 vol
Aerate batch 1
Assume 1 doubling in 24 hours
Aerate batch 2

#2 Lager Pitch quantity of yeast for day 1 vol
Aerate batch 1
Temp @ 62
Assume 1 doubling in 24 hours
Aerate batch 2
Temp 52

Quality Control

What to test?
When to test?

Microbial
Cell Density
Culture Health

Quality Control

Identify critical points vs. practical points

Each step
- 1 generation
- Media
- Not practical

Phase 1 Many steps
Phase 2 One – two
Sensitivity of test
- Testing too early is not going to represent final population

Timing of results
- Results 3-4 days
- Some test results after fermentation complete.

Stop propagation after Phase 1 for testing

Quality Control

Microbial
- Plating O2, Ana wild yeast media
- HLP difficult with high cell density

Cell count
Viability
Results will determine use in Phase 2

Establish specifications

Wyeast Specifications at Packaging

Viable Cell Count	1.2E9 Cells ml^{-1}
Viability	>90%
Culture Purity	Single morphology
Anaerobic Bacteria	<1 CFU 7.5E7 Cells
Aerobic Bacteria	<1 CFU 7.5E7 Cells
Wild Yeast/ Mold	<1 CFU 7.5E7 Cells

Monitor yeast performance/ standard testing protocols

Thanks

Contact Info:

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