Yeast Management

- Propagation
- Yeast crop
- Yeast Treatment
- Storage

What are the possible consequences of “bad” yeast management?

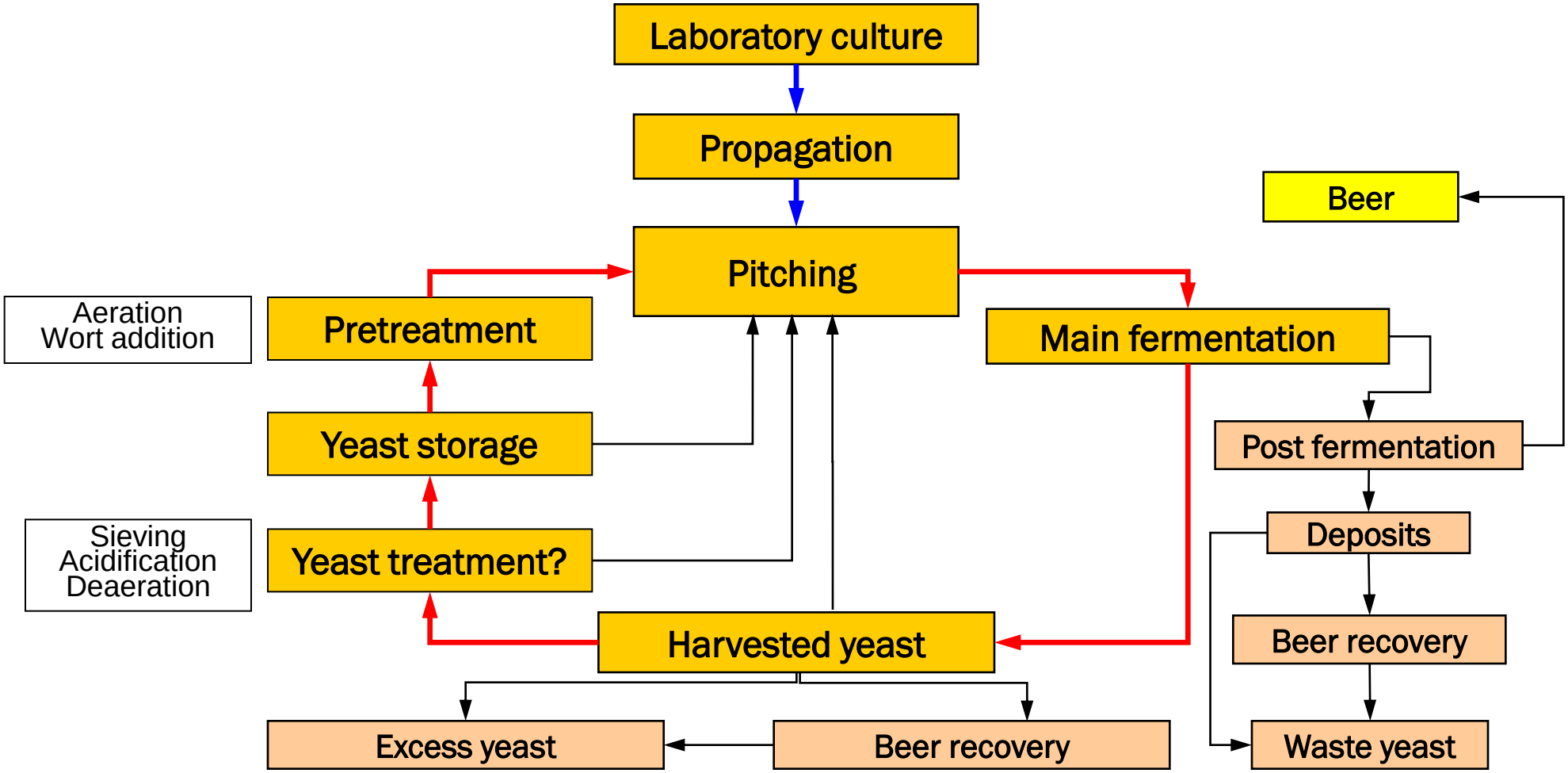
- Decrease in fermentation speed → capacity problems
- Growing differences between the final attenuation degree determined in the lab and the attenuation degree of the final product → economics, microbiological product quality
- Longer maturation times → diacetyl reduction rate
- Slow pH drop → contamination possible
- Beer aroma profile changes → concentration ratio of HA to esters changes in favour of HA
- Foam stability decreases → Proteinase A
- Turbidity problems → “invisible haze” caused by Glycogen excretion
- “Autolysis taste” → release of e.g. fatty acids into the product
- Less formation of reductones → flavour stability requirements?
- Unequal fermentation degrees

Types of Yeast Stress

- **Suboptimal Temperature / Temperature Shock** – (Shock potential at Δ 5K)
- **Osmotic Stress** – from worts with low extract to high extract contents
- **Oxidative Stress** – overaeration, formation of radicals
- **Lack of Nutrients** – Carbon source (sugars), Zinc, FAN (somtimes possible by overintensive sterilisation and resulting intensive flocculation of proteins), poor storage conditions (water)
- **Radical Change in pH** – yeast washing e.g. souring yeast with H_3PO_4 or H_2SO_4 to pH values <2.0
- **Effects of toxic substances** – high ethanol contents e.g. > 5% vol, residual disinfectants, preservatives
- **Dehydration/Hydration**

Source: Die Hefe in der Brauerei: Annemüller, Manger, Lietz

Paths of Yeast in a brewery



Yeast Propagation

- 1883 Emil Christian Hansen from Denmark first managed it to propagate yeast cultures.He isolated a single yeast cell and multiplied it step by step.
- This way of propagation has been continually improved and today it is possible to propagate special culture yeasts with special properties for the demands of each brewery. Today nearly every large brewery propagates yeast on their own in their laboratories and propagation plants.

➔ Insures continuous quality

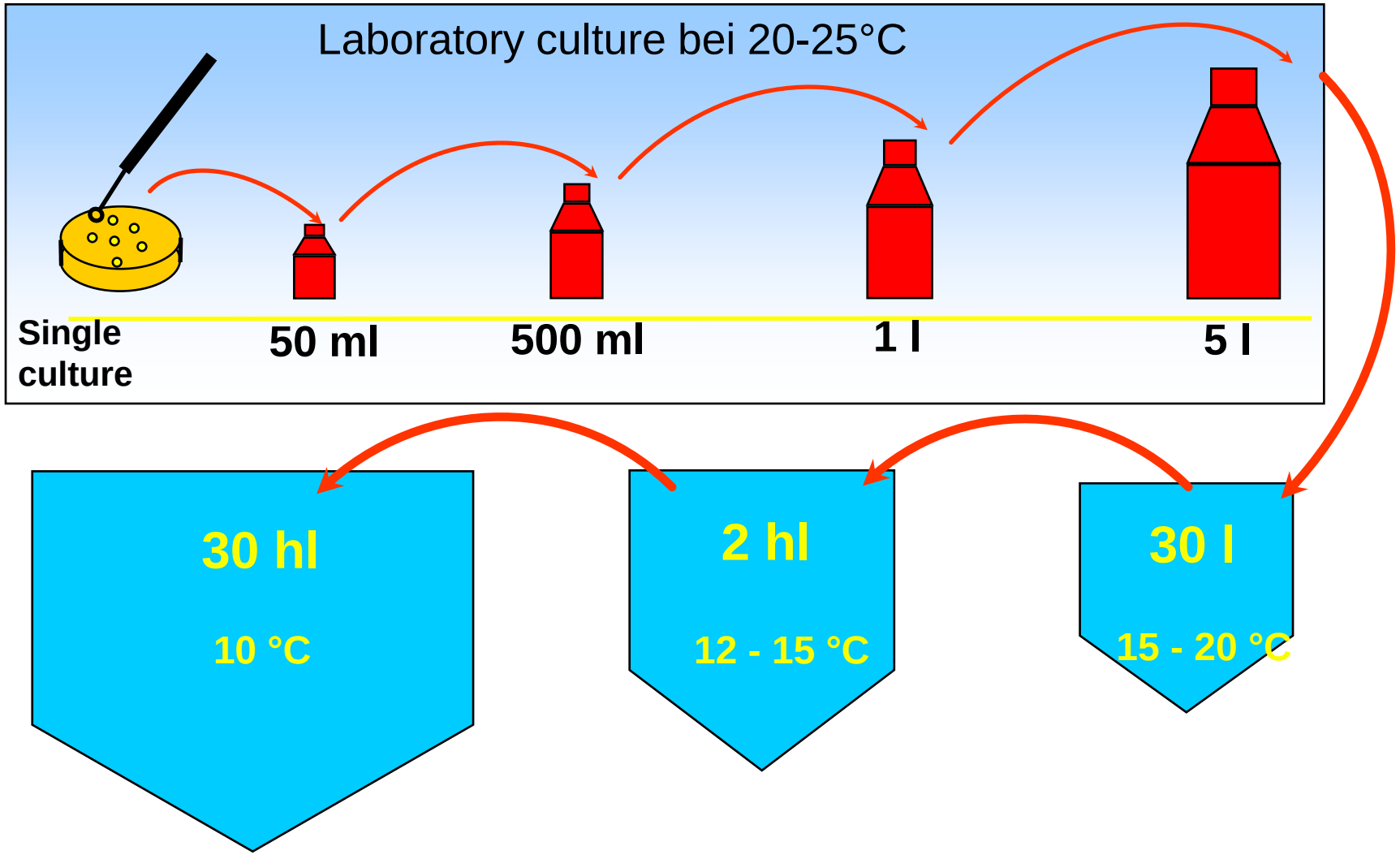
Goals of Yeast Propagation

- Sufficient biomass for pitching
- Optimum physiological conditions of yeast cells at pitching
  - high vitality
  - high viability
- Short propagation times → maximum specific growth rate of yeast cells
- Course of fermentation should be as fast as possible (with low pitching rate)

Three Steps in Propagation

- Isolation of suitable yeast cells
- Multiplication of the yeast in the laboratory until sufficiently vigorous fermenting yeast is obtained.
- Yeast multiplication in the brewery until a sufficient amount is obtained to pitch a complete brew

CONVENTIONAL YEAST PROPAGATION



Generation Time of Lager Yeast

Temperature [°C]	Generation time [h]
8	20 - 25
12	12 – 15
15	10 - 12
16	9 – 11
20	6 - 8
25	2 - 3

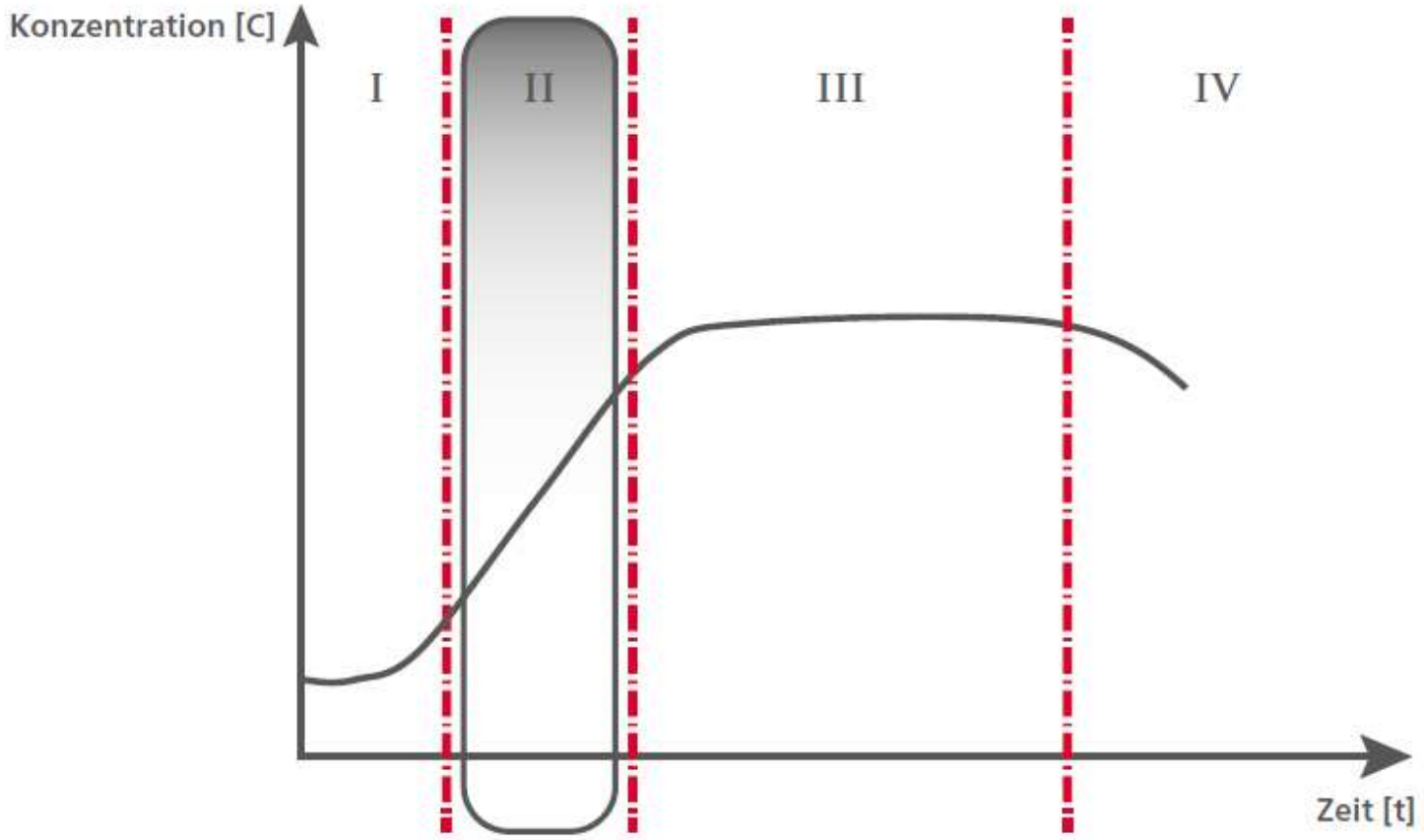
Propagation

- The operation must be performed under sterile conditions right through to pitching the yeast in the brewhouse wort
- Intensive sterile aeration or oxygenation of the yeast is necessary for rapid yeast growth
- Brewing wort should be used for yeast propagation as the hop bittering compounds exert an inhibiting effect on the growth of bacteria

Propagation Steps

Step	Target Initial Pitching cells/ml	Target Final Yeast Conc. cells /ml	Temp °C	Aeration Style	Transfer Time	Dilution for next step
0.1 hl Carlsberg Flask	~50 million	~130 million	20	Continuous First 24 hours	At ~7 % Plato	10 x
1 hl	~13 million	~130 million	17	Periodic	At ~7 % Plato	10 x
10 hl	~13 million	~130 million	14	Periodic	At ~7 % Plato	10 x
100 hl	~13 million	~130 million	e.g. 11	Periodic	At ~7 % Plato	10 x
1000 hl	~13 million	50-60 million	e.g. 11	Wort Aeration	At ~7 % Plato or full fermentation	5 x
5000 hl	~13 million	50-60 million	e.g. 11	Wort Aeration	Full fermentation	

Ideal Transfer – Log Phase



I = Lag Phase, II = Log Phase, III = Stationary Phase, IV = Decline Phase

Source: GEA

Carlsberg Flask

Application

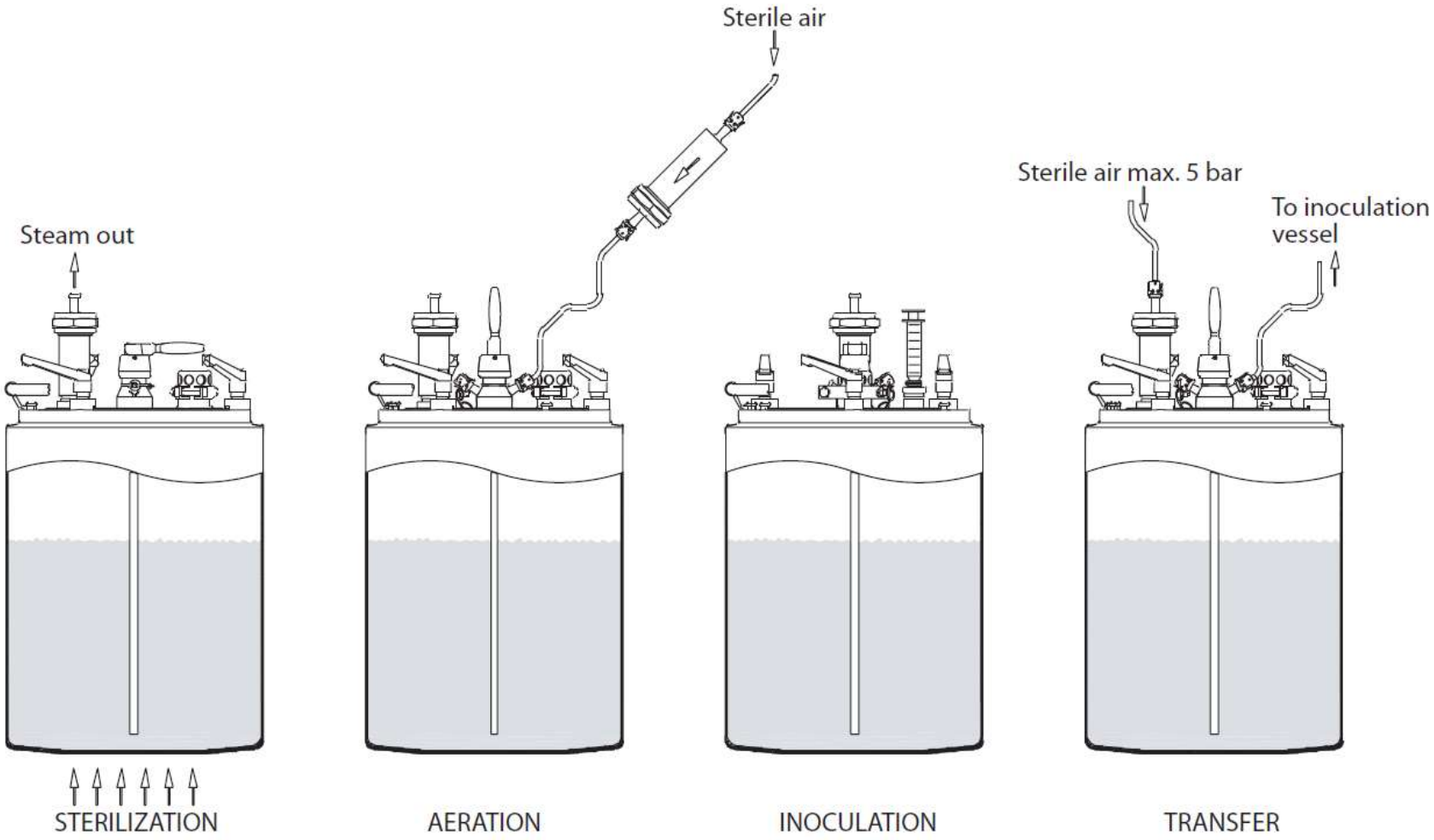
The Carlsberg Flask is used to sterilise wort and propagate pure yeast culture for yeast propagation plants from a laboratory scale.

Features

+ Hygienic design
+ Wort sterilization in autoclave, or external heat source
+ Suitable for wort aeration
+ Provides safe conditions during transfer of yeast culture
+ Easy to clean
+ Handy construction
+ Easy to transport

Source: Alfa Laval

Propagation



Source: Alfa Laval

Influences on Propagation

- Temperature
- Oxygen concentration
- Dissolved CO_2 concentration
- Lipids (unsaturated fatty acids)
- Sterols
- Carbon source
- Nitrogen source (e.g. free amino acids)
- Mineral substances providing good yeast viability: Potassium, Sodium, Calcium, Magnesium, Copper, Iron, Manganese, Zinc, Sulphate ,Phosphate, Nitrate
- pH

Influence of Temperature

- Optimum temperature for yeast growth (not in propagation!):
  - Bottom fermenting yeast: 26.8° c – 30.4° c
  - Top fermenting yeast: 30° C - 35° C (*Walsh and Martin*)

- > 30° C ➔ faster yeast cell growth, no higher cell count

- Be careful with high temperatures ➔ temperature shock at pitching
- Lower flocculation rate in propagation at higher temperatures
- Lower temperatures in propagation ➔ yeast starts to agglutinate.
- Optimal temperatures for propagation: 20-28° C
- Higher temperatures in propagation (e.g. 30° C) accelerate the propagation but nearly have no influence on final cell count

Pasteur Effect

" Oxygen inhibits fermentation and reduces the rate of glycolysis"
discovered in 1857 by Louis Pasteur

Yeast is a facultative anaerobe microorganism
→ two different metabolic pathways for energy generation

At low oxygen concentration:

+ product of glycolysis, (pyruvate), is turned into ethanol and carbon dioxide, and the energy production efficiency is low (2 moles of ATP per mole of glucose).

At high oxygen concentration:

+ pyruvate is converted to acetyl CoA that can be used in the citric acid cycle, which increases the efficiency to 38 moles of ATP per mole of glucose.

→ 15 times as much glucose must be consumed anaerobically as aerobically to yield the same amount of ATP!!

Source: Teknisk mikrobiologi 2003

Crabtree Effect

Named after the English biochemist Herbert Grace Crabtree

The **Crabtree effect** phenomenon:

Yeast, Sacharomyces cerevisiae, produces ethanol aerobically in the presence of high external glucose concentrations rather than producing biomass via the tricarboxylic acid cycle

Same effect for fructose reported, for maltose less intense*

Effect can be observed at concentrations

> 0.1 g Glucose /liter

*Source: Malting and brewing science, Hough, Briggs, Stevens,Young

Influence of Oxygen

Disadvantages of too much supplied oxygen are:

- Cost-intensive (high energy demands)
- Foam formation in the propagation plant
- Damage of the foam positive substances
- Decreased redox-potential in the final beer
- Oxidative stress

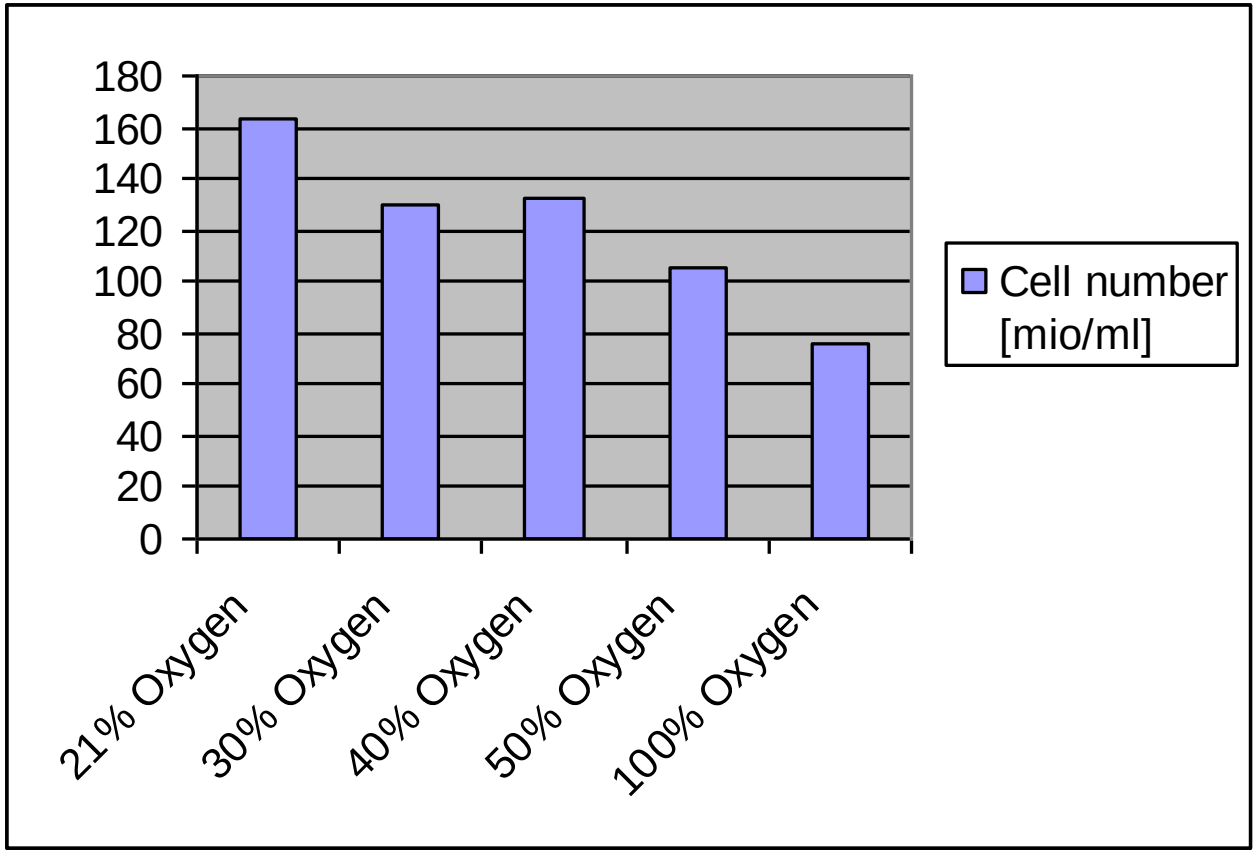
Deficiency in oxygen:

- Limited aerobic growth → no removal of CO_2
- Low yield factors
- Long doubling times
- Foam problems

+ Sterile air with 21% oxygen has a much better yeast growth than 100% pure oxygen

Martin V Quain, D.E. and K.A. Smart. Brewing Yeast Oxidation Stress Responses: Impact of Brewery Handling, ref. In Brewing Yeast – Fermentation Performance – 2nd Edition

Influence of Oxygen



Maximum Cell Number with Different Amounts of Supplied Oxygen

Methner, EBC 1999

Propagation: Aeration control

Air supply depends on:

+ Number of cells/biomass present in the propagator
+ Phase of propagation (log-phase or lag phase)
+ Specific oxygen transmission rate of the propagator (has to be determined in place by step response)

In practice often found:

+ Aeration control by foam
+ Aeration control by oxygen content of exhaust gas
+ Aeration control by a experience based program

Relation between Wort Gravity and Cell Count and Final Mean Cell Volume at the End of Propagation

Yeast	Wort original gravity (° P)	Cell count (Mio/ml)	Final mean cell volume (µm³)
Ale yeast 662	7.5	131	212
	10	134	261
	12.5	129	267
	15	137	270

Cahill, ASBC 2000

Relation between free Amino Acids and Yeast Growth

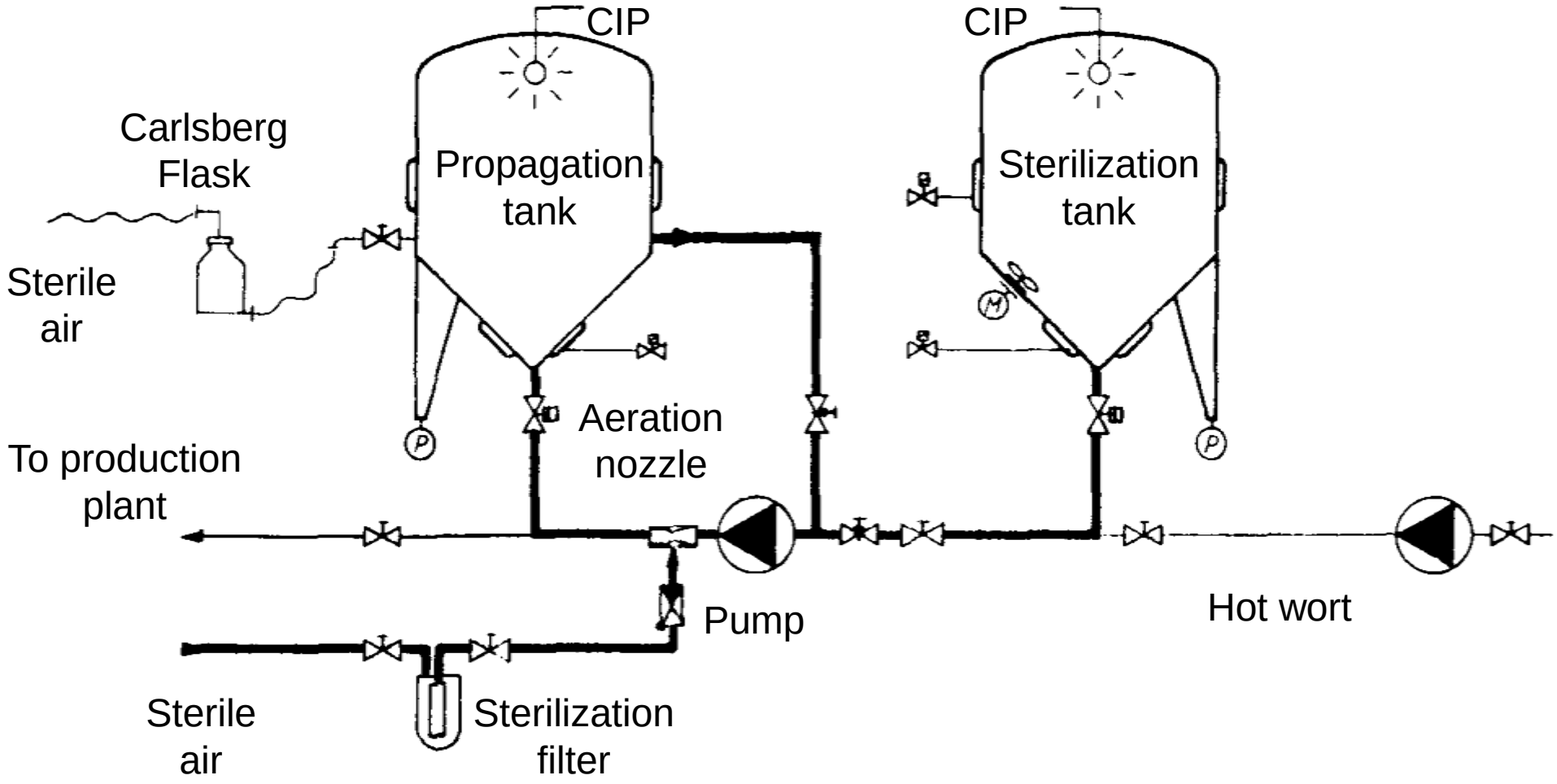
Free amino acids in pitching wort in mg/l	Yeast growth in million cells/ml
110	~ 30
130	~40
150	~55

- wort should contain around 200 mg/l FAN → 80 – 120x10^6 cells per ml
- free amino acid consumption from pitching wort to the final beer should be between 100-140 mg/l

Equipment of Propagation Plants

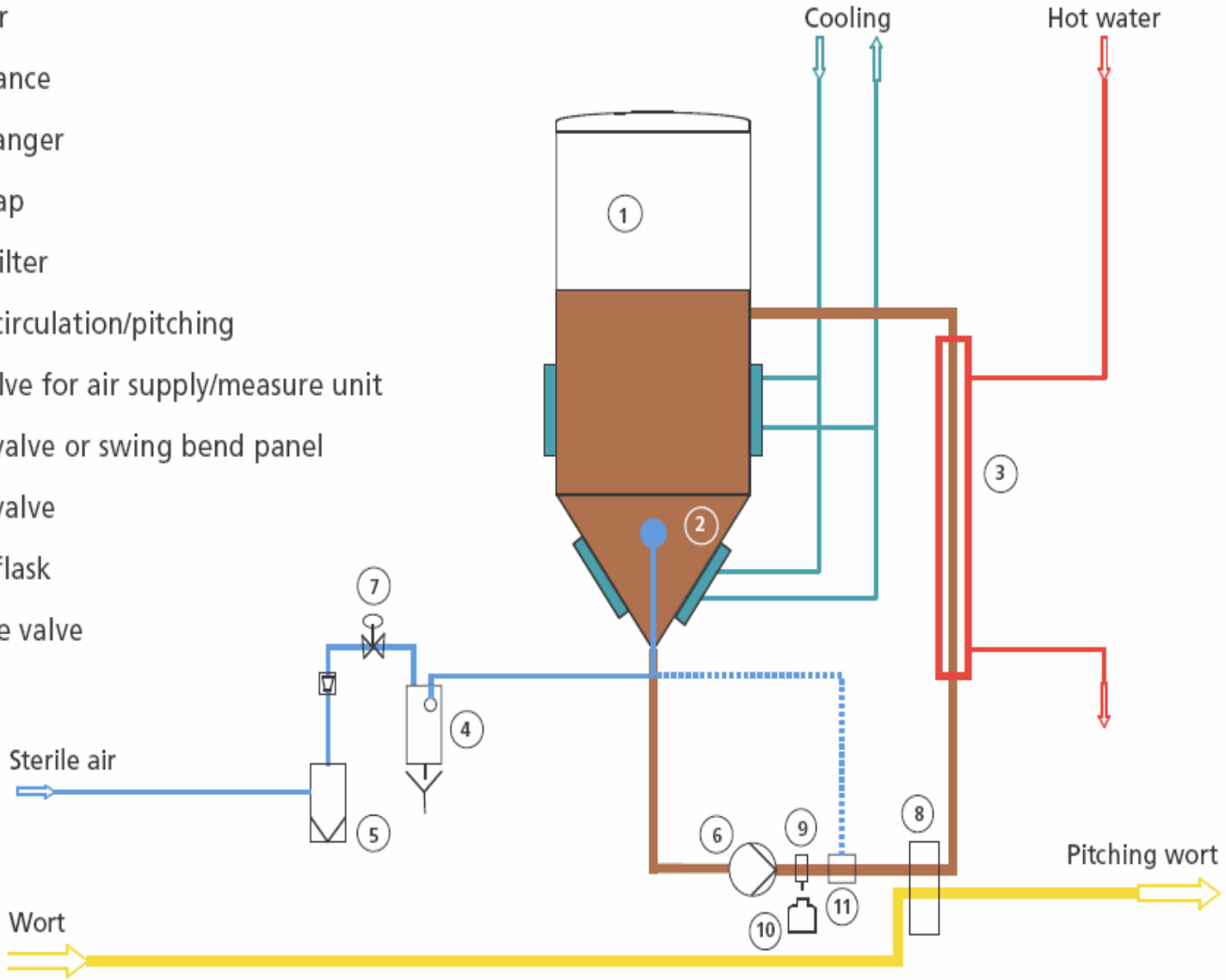
+ Fill height inspection
+ Temperature control devices
+ Pressure control device
+ Gauge for the yeast cell concentration
+ Oxygen measurement device

Two-tank Propagation-Plant – With Sterilization



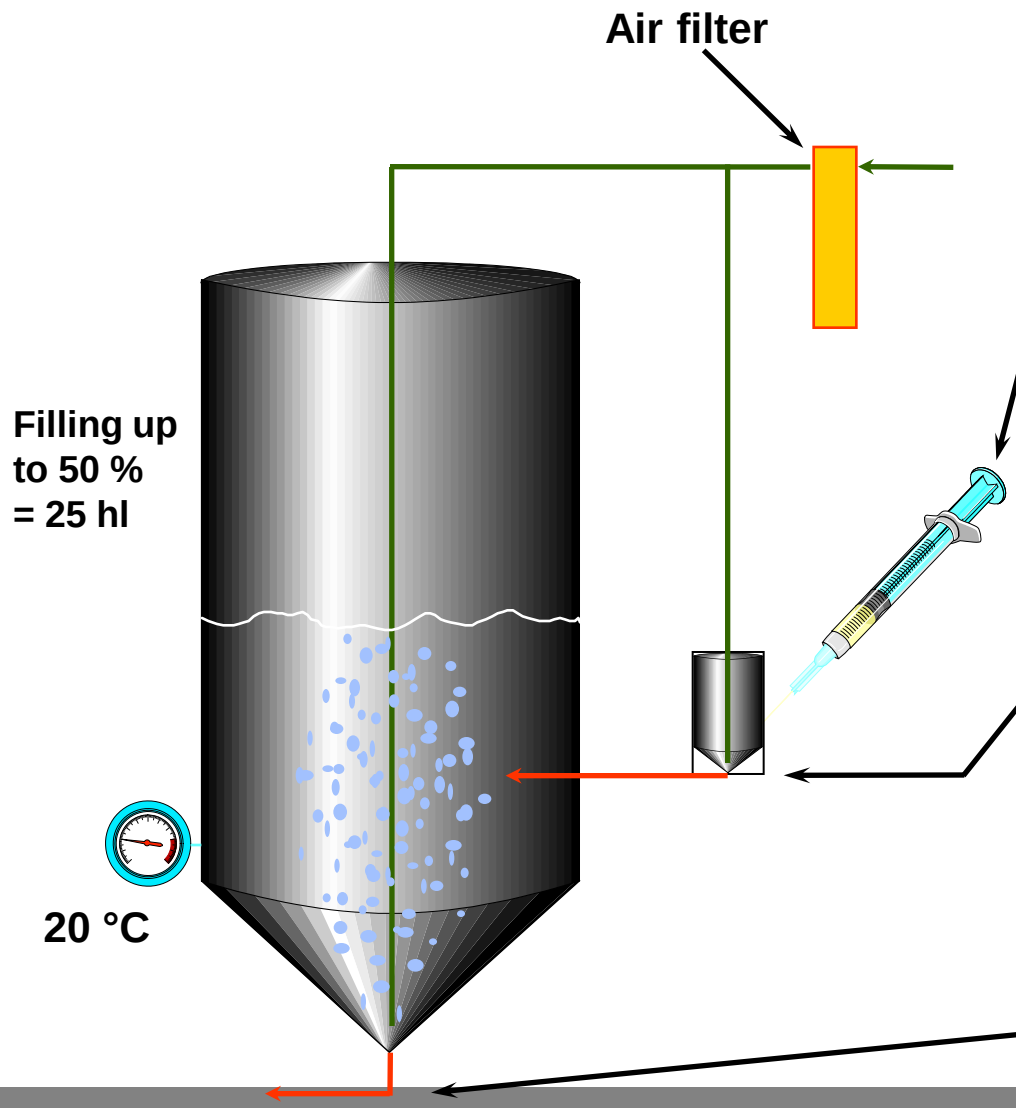
Kunze

1 Propagator
2 Aeration lance
3 Heat Exchanger
4 Product trap
5 Sterile airfilter
6 Pump for circulation/pitching
7 Control valve for air supply/measure unit
8 Mixproof valve or swing bend panel
9 Sampling valve
10 Carlsberg flask
11 CIP Impulse valve



GEA

Single-tank Propagation-Plant



Laboratory:

Adding 50 ml culture
in a ten liter Carlsberg flask
Ratio 1 : 200

Interval aeration for 24 h at 20°C

Plant:

From the Carlsberg flask at high Kräusen in a 25 hl propagator
Ratio 1 : 250 to 1 : 300

Interval aeration for 36 - 48 h at 20°C

Pitching:

Pitching at high Kräusen
with 500 hl wort
Ratio 1 : 20

Repeated Fed Batch Method

- Periodical removal of a specific amount of the yeast and a subsequent refilling of the vessel with wort

  ➔ Shortens the lag-phase of the yeast growth
- Temperature approx. 20 ° C
- Constant oxygen concentration of 0,2 mg/l at high head stage
- Removal of 20% of the total propagation wort over a period of 3 hours ➔ stable operating state
- Also possible removal of 40% of the total propagation wort over a period of 4,5 hours
- Removal of 40% of the total propagation wort over a period of 3 hours is too much ➔ total yeast cell count decreases

Two Tank Procedure (Assimilation Procedure)

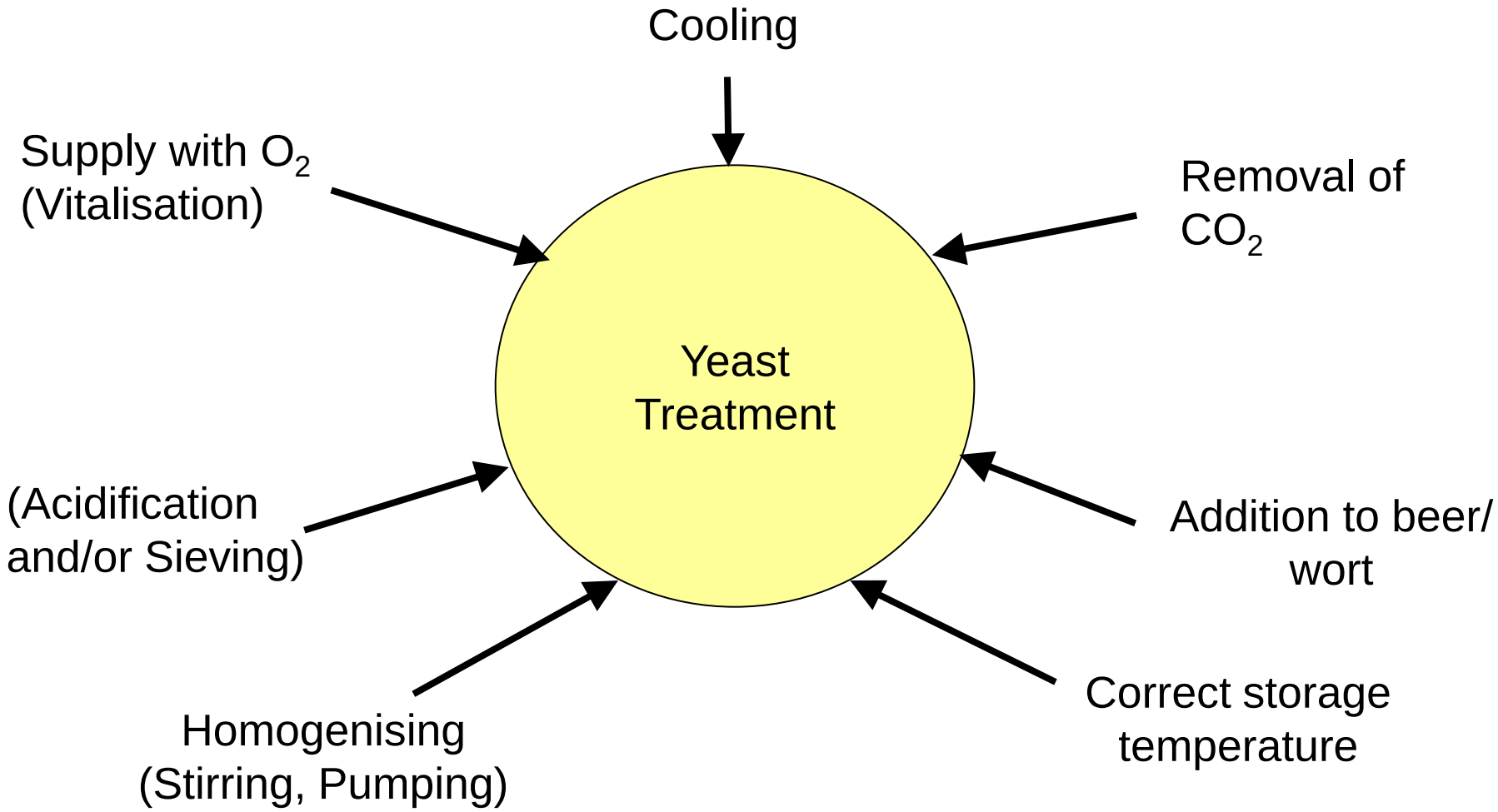
- Two propagators → connected by venturi nozzle
- Plant equipment:
    - Agitator
    - Heating/ cooling jacket
    - Measurement equipment: Oxigen, temperature, pressure, pH
- Aeration while pumping from one tank to second tank
- Temperature: 8 - 14°C
- 80 – 85 % used for pitching at ($E_{app.}$ → 6 - 7 %)
- 15 – 20 % remain in prop.tank→ topped wtih wort
- Disadvantages higher space requirements, costs

Yeast Cropping

- Yeast crop as soon as possible because of unfavourable conditions in cone:
  - high CO_2 concentration
  - no nutrients
  - high static pressure

  → Risk of excretion low molecular peptides and fatty acids → autolysis
- Recommended: 2 – 3 crops per CCT during main fermentation
- Avoiding shear forces → gentle pumping (rotary piston pump)
- External heat exchanger for homogeneous cooling

Yeast treatment



Storage carbohydrates of yeast: Glycogen and Trehalose

- These substances function as important carbon and energy reserves in the following states of metabolism:
  - starving yeast cells
  - sporulating cells
  - germinating spores
  - in vegetative cells in the stationary phase
  - during the mitotic cell cycle under conditions of carbon and energy limitation
  - Under stress glycogen can be released by yeast → turbidity problems

Yeast Storage

- Yeast uses slight amounts of sugars for keeping up its vitality during storage:

  0.2 % extract/d (20 °C; 10^6 yeast cells/ml beer)
- As soon as no more fermentable sugars are available yeast use their storage carbohydrates (glycogen) for survival.
- The longer yeast is stored, the more important the cool storage temperatures.
- The re-addition to wort can lead to a loss of certain substances (shock-excretion) resulting in:
  - Prolongation of the lag-phase
  - Decrease of the fermentation rate

Short Time Storage

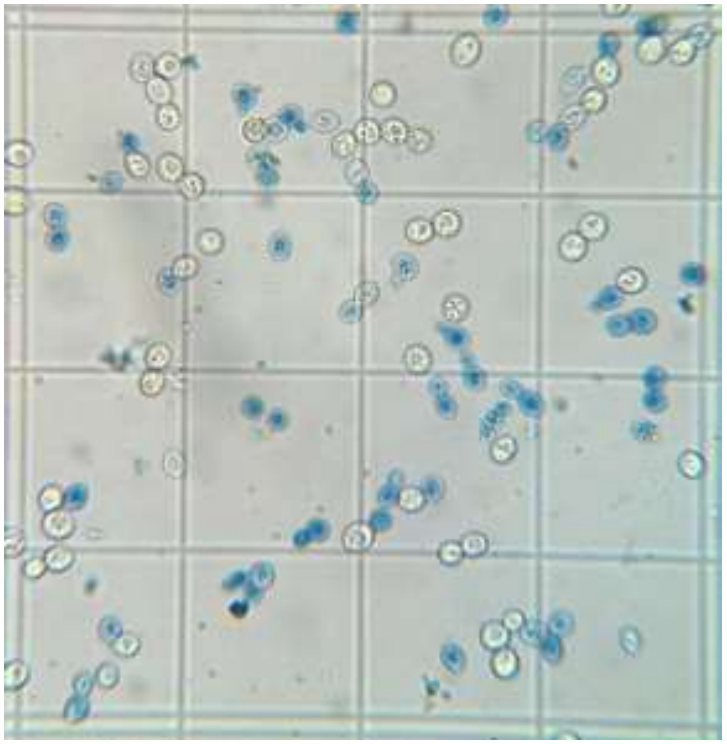
+ Between brew breaks storage under wort or beer with rest extract
+ Low temperature 1 – 4 °C
+ Slow pressure release and removal of CO_2
+ Gentle aeration for preparing next brew
+ Storage longer 12 h **no** aeration but slow pressure release < 4 °C

Viability and vitality control

- Viability (Percentage of living/dead cells)
- Vitality (activity of living yeast cells)

Viability

- Viability of a yeast population
- Viability ➔ number of living cells of the total population
- Determination of active cells
- Methods based on replication or on staining
- Some Example Methods:
  - Counting Chamber with Methylene Blue
  - Fluorescence Staining
  - Flow Cytometry
  - Coulter Counter



Vitality

Vitality of a yeast population:

+ Based on biochemical activity of a yeast population
+ Based on biochemical activity of single yeast cells and on their statistical distribution in a population
+ Some Example Methods
  - CO_2 Production Measurement
  - Flow Cytometry
  - ICP Intracellular pH Measurement

Thank You for your Attention!

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FIBGP

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