Maintaining Ideal Yeast Health: Nutrients Yeast Need

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Eric Molson

"What makes a great beer?

Fermentation. You put the yeast in, and then the fermentation takes about a week. A lot happens in that week. **The trick is to give the yeast exactly what it wants.** It's a mould and it wants to eat, and while it eats, it makes alcohol and gas."

Interview with Eric Molson; Globe and Mail, May 29, 2009

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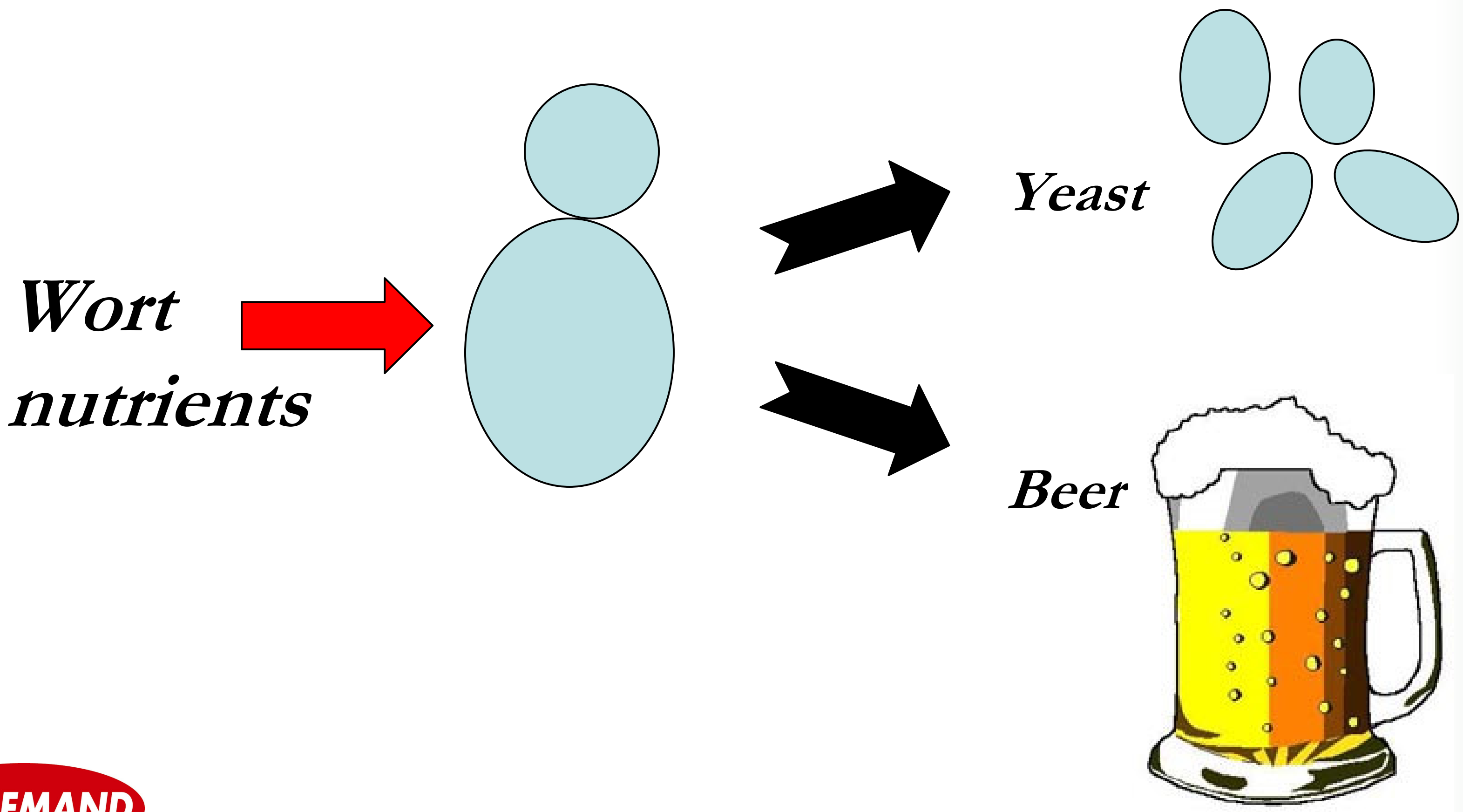
General concept



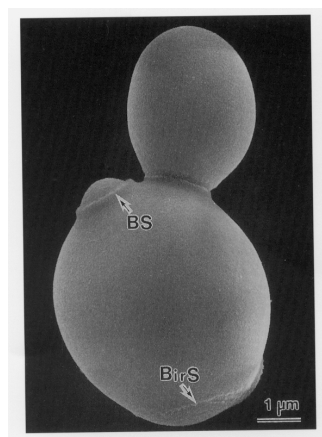
Importance of Yeast to Beer Characteristics

- Around 950 compounds are thought to give beer its character
- Of these, around 400 are produced by yeast!

WORT

(sugars, amino acids, etc.)

More yeast!



Yeast autolytic compounds

Phenols

Sulphur compounds

Aldehydes & ketones

Esters

Acids (organic & fatty)

Alcohols (ethanol & fusel oils)

CO_2

Glycerol

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Elemental requirements for growth of brewing yeast

Macroelements

H - cell water, organic material

O - respiration, cell water, organics

C - organic material

N - proteins, nucleic acids, coenzymes

S - proteins, coenzymes

P - nucleic acids, phosphoslipids

K - cell electrolyte, enzyme cofactor

Mg - enzyme cofactor, cell division

Microelements

Ca - flocculation

Mn - enzyme cofactor

Fe - cytochromes

Zn - alcohol dehydrogenase

Co - vitamin B12, coenzymes

Cu, Ni, Mo - specialised enzymes

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Yeast nutrients in wort

- **Carbohydrates** **(fermentable sugars)**
- **Nitrogen sources** **(mainly amino acids)**
- **Inorganic sources** **(P, S, Mg, Zn, other minerals)**
- **Oxygen** **(need around 25% saturation of wort)**
- **Yeast "foods"** **(vitamins, fatty acids, sterols)**

***NOTE**: The level and availability of such nutrients is very important in governing fermentation performance of brewing yeast*

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Wort composition influences:

- **Rate of attenuation**
- **Degree of attenuation**
- **Amount of yeast produced**
- **Flocculation**
- **Beer characteristics**

Carbon sources for brewing yeast

- Hexose sugars: **glucose, fructose**
- Pentose sugars: **no** (but possibly xylulose)
- Disaccharides: **maltose, sucrose, melibiose**
- Trisaccahrides: **maltotriose** (slowly)
- Tetrasaccharides: **maltotetraose** (possibly some strains)
- Oligosaccharides: **maltodextrins** (unfermented)
- Polysaccharides: **starch** (unfermented)
- Others: **ethanol, acetate, glycerol** (only respired)

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Nitrogen sources for brewing yeast

- Peptides
- Amino acids
- Ammonium salts (little present in wort)

Inorganic sources for brewing yeasts

Phosphorus, sulphur and potassium generally sufficient in wort

Metal cations may occasionally be deficient (and ratios of metal ions may be sub-optimal)

Essential bulk ions: K, Mg

Essential trace ions: Zn, Ca, Fe, Mn

Toxic trace ions: Pb, Cd, Cr, Hg, Cu etc.

Zinc

- Faster fermentation
- Better flocculation
- Stimulates uptake of maltose and maltotriose
- Stimulation of protein synthesis and yeast growth
- Protection of enzymes
- Stabilisation of protein and membrane system

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Zinc Concentration in Wort

- During wort preparation approx. 95 % of zinc from malt are lost with spent grain
- In modern stainless steel breweries the zinc concentration is not sufficient (<0.2 mg/L)

⬇

Slow or stuck fermentation

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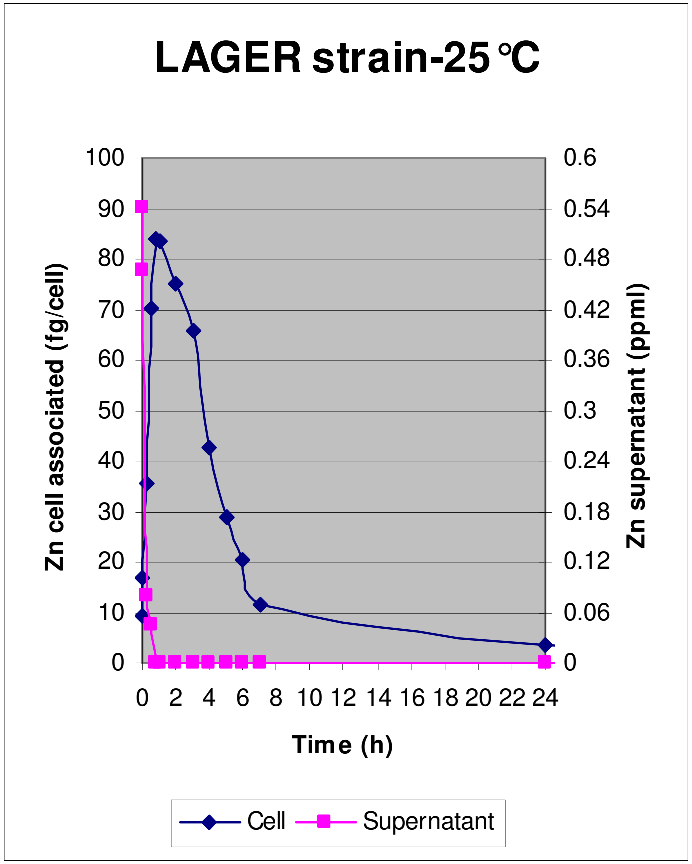
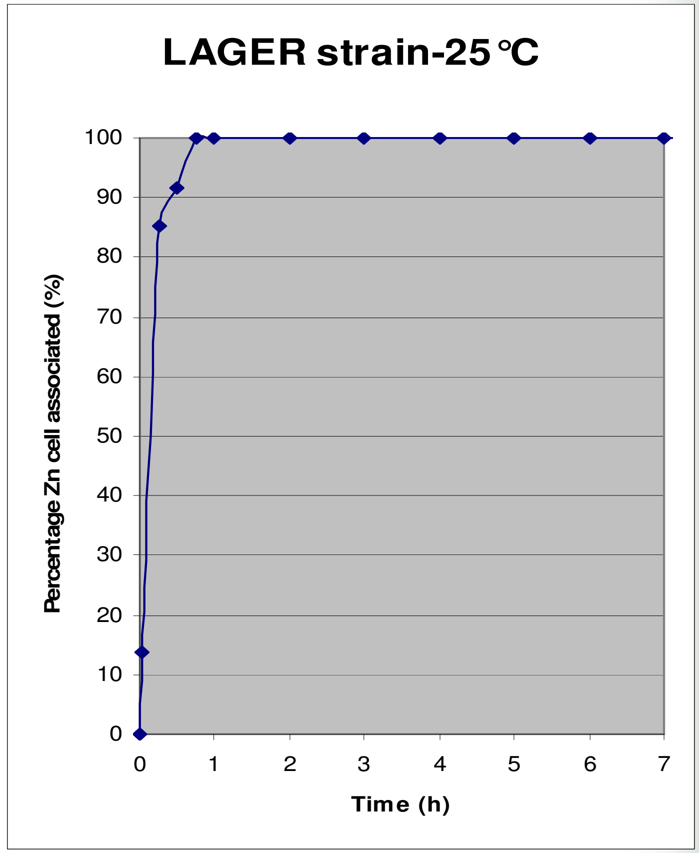


Lager brewing yeast strain – lab conditions



- Zinc wort content is almost zero after 1 hour
- Zn is entirely accumulated into cells which redistribute the metal among growing yeast

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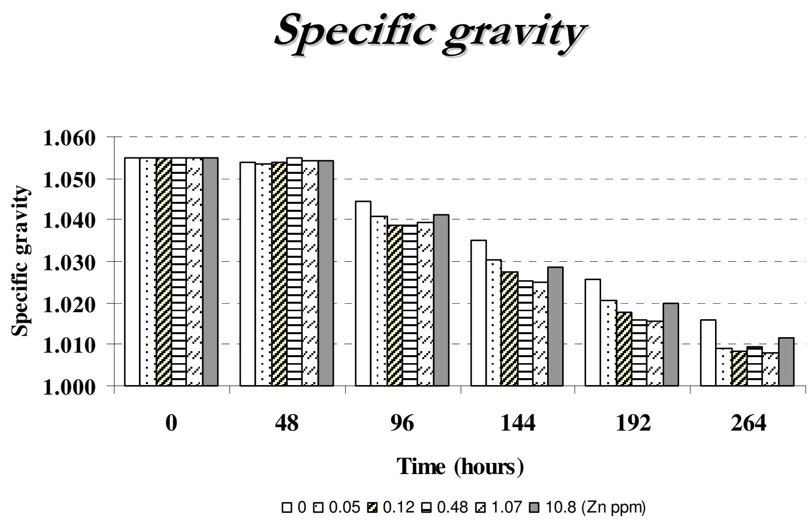
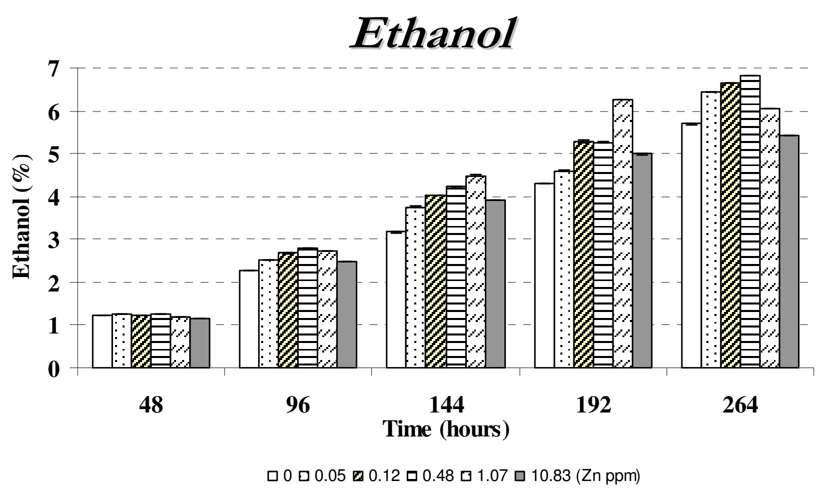
G. Walker, ASBC 2007, Victoria, Canada

Zn & fermentation performance





- Low zinc (0.05 ppm) delayed sugar utilization
- High zinc (10 ppm) slightly delayed sugar utilization – but same after 14 days fermentation
- 0.4 ppm-1.0 ppm Zn resulted in fastest fermentation rates

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G. Walker, ASBC 2007, Victoria, Canada

Magnesium

- Absolutely essential (growth, fermentation)
- Cell Mg correlates with viability/vitality
- Required for glycolysis (stimulates fermentation)
- Maintains structural integrity of yeast (stress-protectant)
- Ca/Mg ratio is important – (a major consideration for commercial yeast food formulations)

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Vitamin sources for brewing yeasts

Wort generally sufficient in folic acid, thiamine, riboflavin, pantothenic acid, niacin, inositol and biotin

Biotin, however, may occasionally be deficient

Can supplement wort with "yeast foods"

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Typical levels of vitamins in wort

VITAMIN	LEVEL IN WORT /100ml	METABOLIC ROLES
Biotin	0.5µg	Carboxylations
Thiamin (B1)	60µg	Decarboxylations
Pantothenate (B5)	~50µg	Acetylations, CoA
Nicotinic acid (B3)	1000µg	Coenzymes
Riboflavin (B2)	50µg	Coenzymes
Pyridoxine (B6)	85µg	Amino acid metabolism
Inositol	10mg	Membranes

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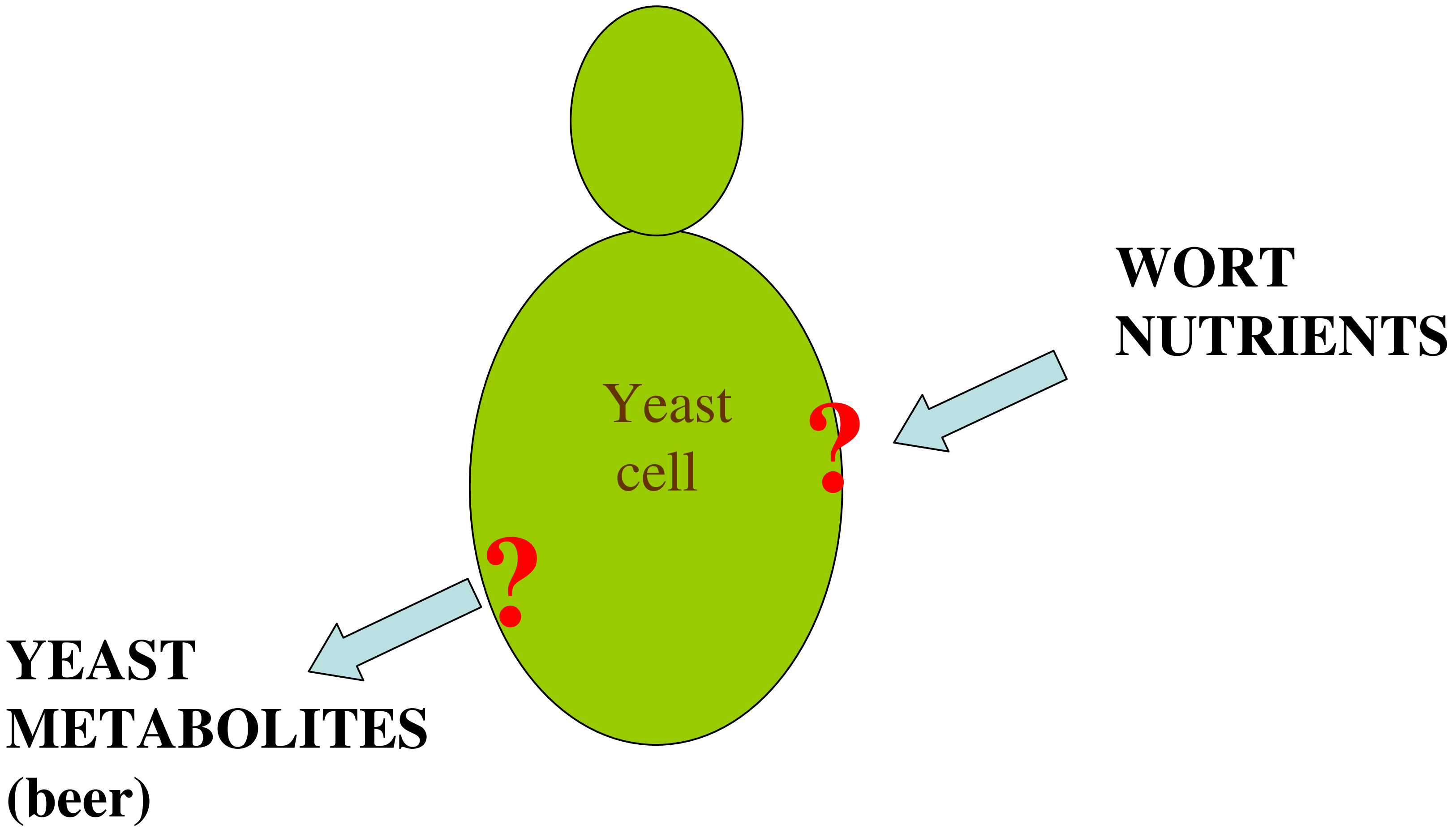
Yeast transport strategies for wort nutrients



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Translocation of wort nutrients into brewing yeast

CELLULAR BARRIERS

capsule, cell wall, periplasm, plasma membrane, organelles

PHYSICO-CHEMICAL BARRIERS

chelation, adsorption, molecular size, binding (e.g. nutrient unavailability in wort particles)

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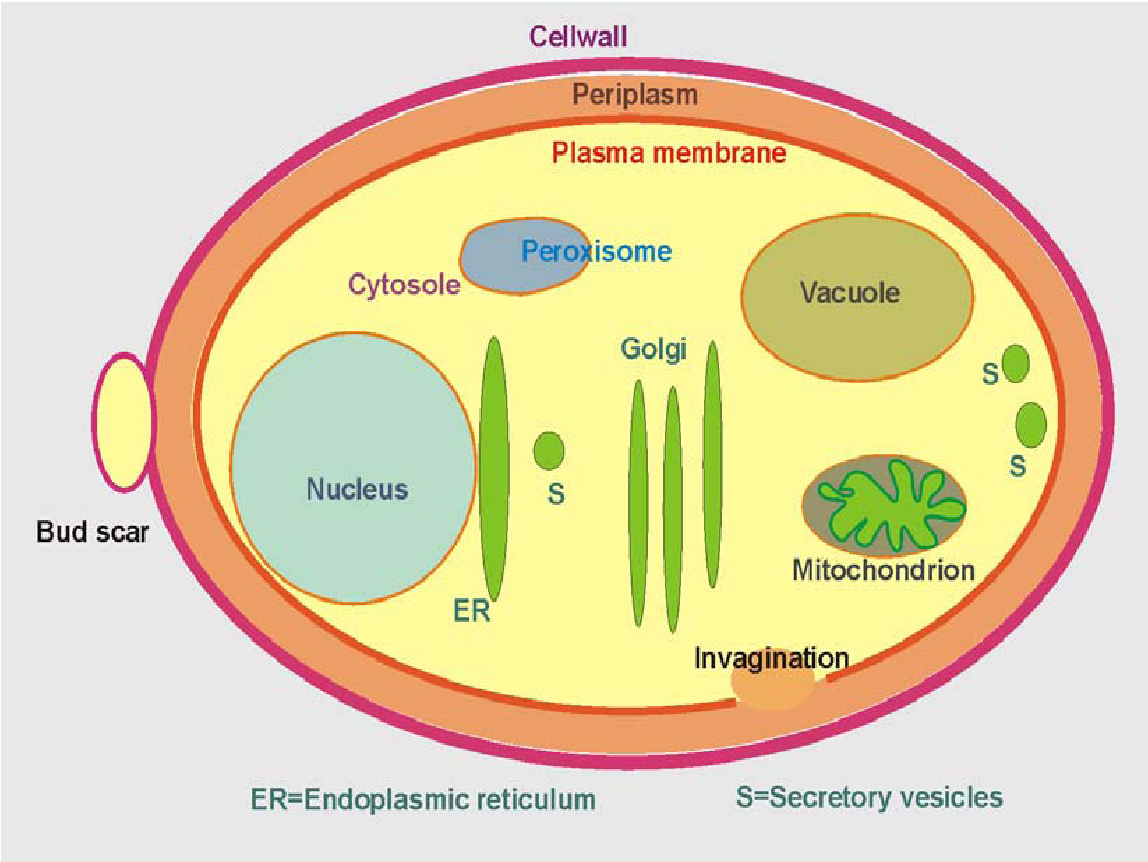
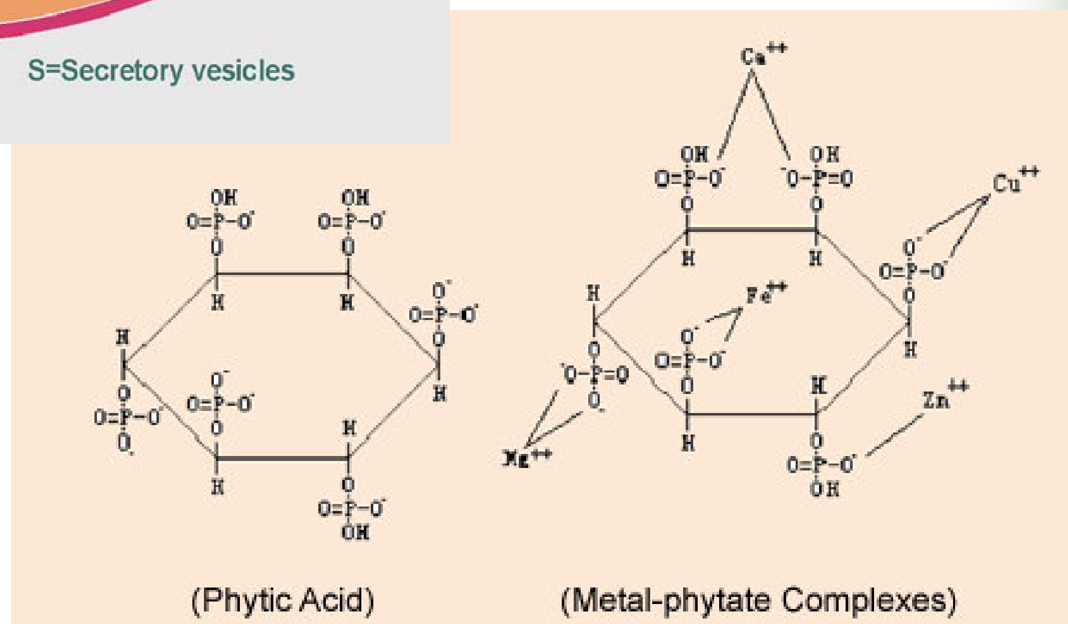

Cellwall
Periplasm
Plasma membrane
Peroxisome
Cytosole
Vacuole
Golgi
S
S
S
Nucleus
Bud scar
Mitochondrion
ER
Invagination
ER=Endoplasmic reticulum
S=Secretory vesicles


Ca++
Fe++
Cu++
Zn++
Mg++
(Phytic Acid)
(Metal-phytate Complexes)

PHYSICO-CHEMICAL BARRIERS

- **Zinc chelates with phytic acid, proteins & polyphenols**
- **These complex compounds are very stable**
- **Only ionic zinc is available to yeast**
- **Regular AAS is a destructive method, which measures total zinc not available zinc**
- **Ion-exchange separation separates complex and ionic zinc before AAS**

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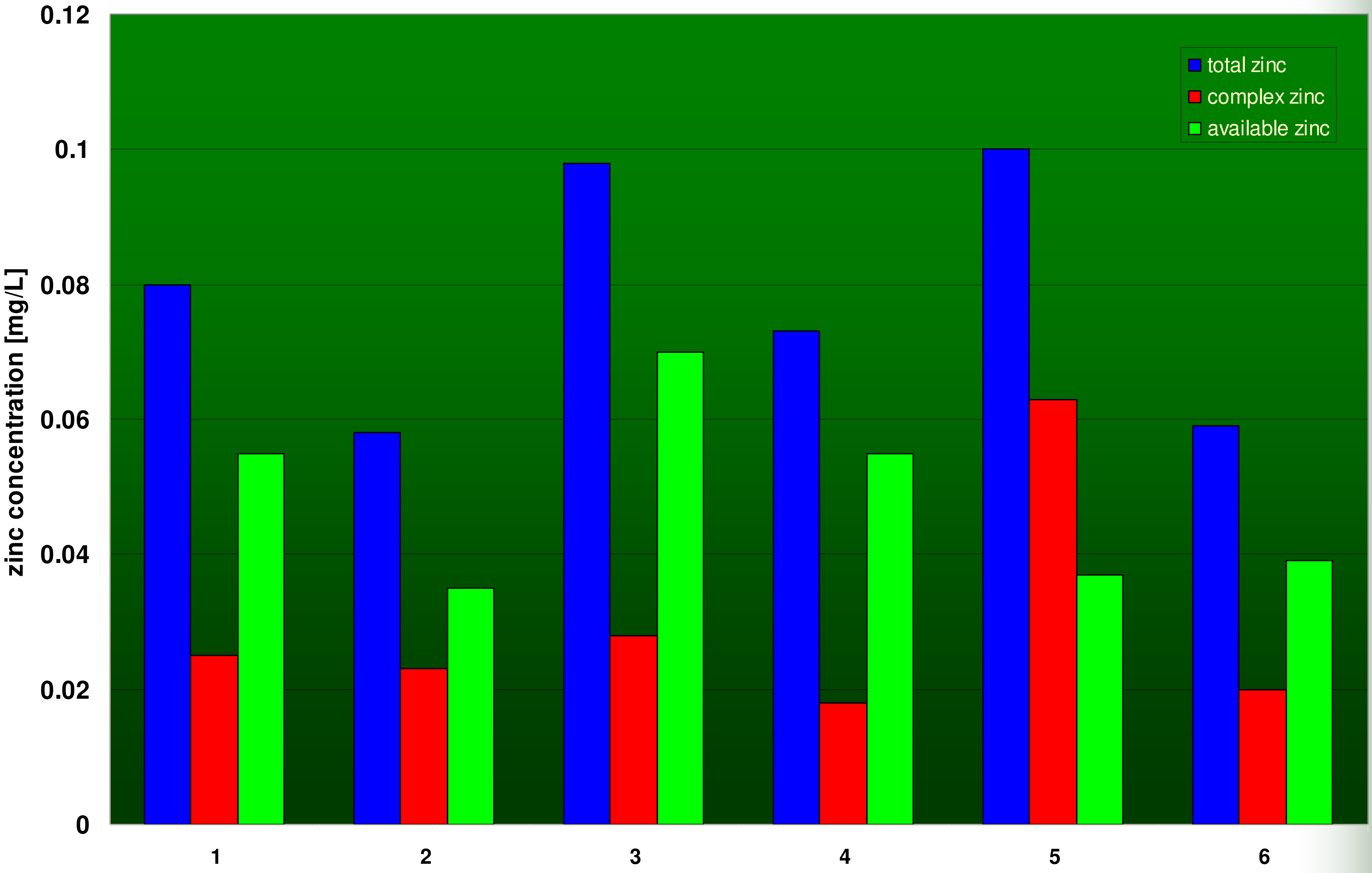
Total and complex zinc concentration of industrial wort samples



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Derived from: Vecseri-Hegyes, B., P. Fodor, et al. (2005). "The Role of Zinc in Beer Production, I (Wort Production)." Acta Alimentaria **34**(4): 373-380.

Consequences of low assimilable nitrogen wort

Low available nitrogen, especially in very high gravity wort, below a threshold of around 150mg/L, may lead to "stuck" fermentations

Amino acid uptake by brewing yeast

- **GROUP A (fast)**
  Glu, Asp, Asn, Gln, Ser, Thre, Lys, Arg
- **GROUP B (intermediate)**
  Val, Met, Leu, Isoleu, His
- **GROUP C (slow)**
  Gly, Phe, Tyr, Try, Ala, NH_3
- **GROUP D (little or no)**
  Pro

NOTE: A variety of transport permeases exist for (active) amino acid uptake by brewing yeast

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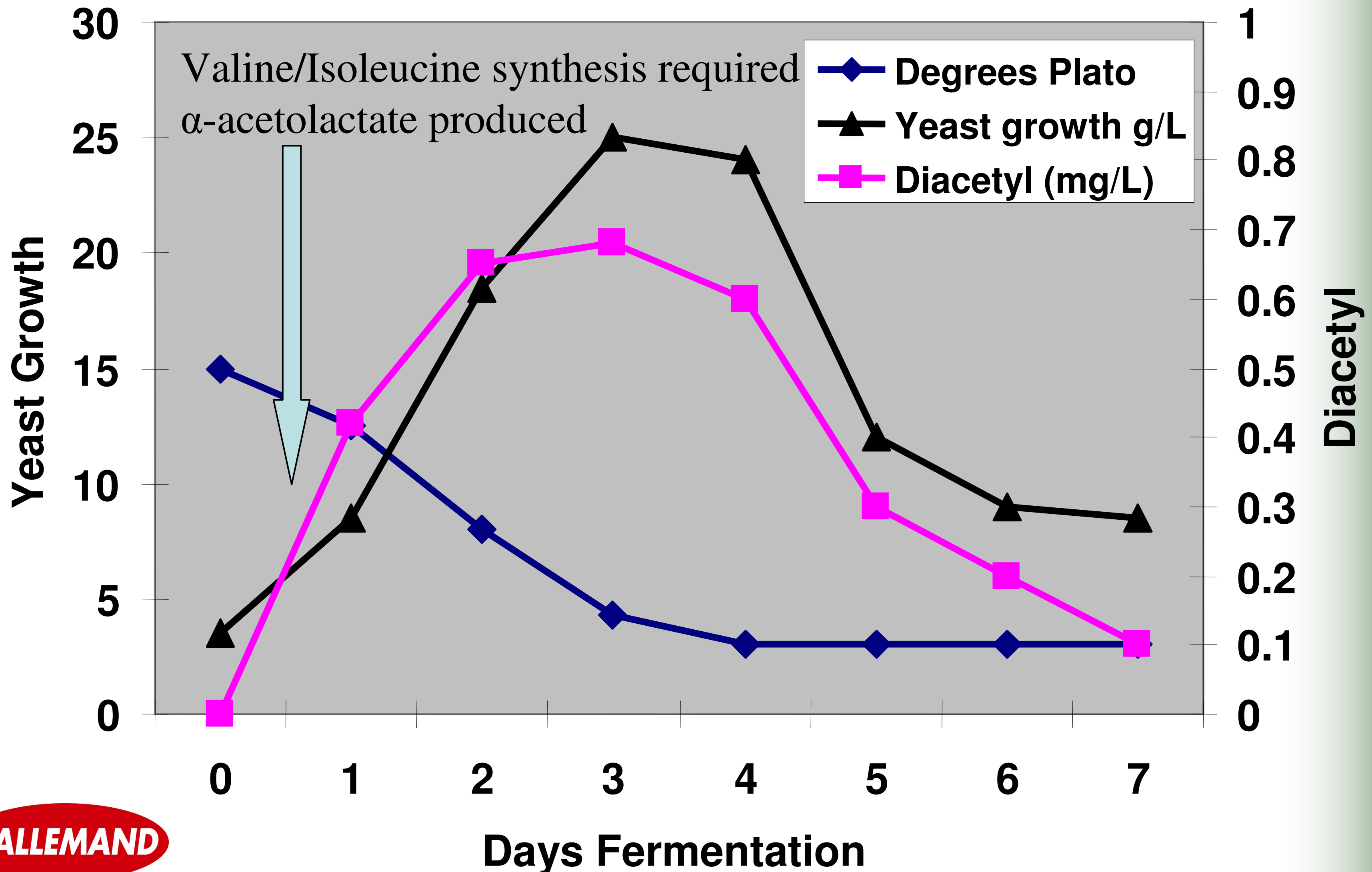
Yeast growth vs. Diacetyl Levels



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Mechanisms for yeast food enhanced reduction of diacetyl levels?

α- acetolactate → (Oxidative Decarboxylation; (Slow)) → diacetyl → (Yeast reductase - fast) → acetoin

By-product of valine and isoleucine synthesis by yeast

↑

Amino acids supplied by yeast foods May reduce requirement for synthesis of valine/isoleucine (and thus α- acetolactate and diacetyl production)?

↑

Requires functioning cell membrane to allow for uptake of diacetyl prior to reduction

Provision of usable nitrogen source plus other nutrients results in a more vital cell that is enhancing diacetyl uptake and reduction?

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Yeast nutrition
potential problems for fermentation

- **Spectrum/availability of wort sugars**
  - **e.g. High glucose worts may slow fermentation**
- **Insufficient FAN**
  - **Stuck fermentation, change in flavour profile**
- **Metal ion availabitiy**
  - **e.g. Low Zn, excess Ca, insufficient Mg, toxic Cu**
- **Vitamin deficiency**
  (e.g. pantothenate - sulphur off-notes)
- **Stressed yeast - impaired nutrient uptake**
  (ethanol, pH, CO_2 , temp., starvation, osmotic pressure….)

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Yeast Foods

Yeast "foods"

Multifunctional roles

- **Alleviate CO_2 inhibitory effects**
- **Extra sources of assimilable nitrogen (hydrolysed protein)**
- **Extra sources of vitamins**
- **Extra sources of metal ions (to increase bioavailability)**

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Commercial Yeast Foods
Available Components

- Amino acids (FAN)- as protein hydrolysate or added as individual amino acids
- Low molecular weight peptides
- $NH4^+$
- Growth factors (vitamins)
- Protein (non-utilisable) – active in CO_2 nucleation?
- Sulphur/Phosphorous
- Zn/Cu/Mn/Ca (enzymatic and structural roles)
- Yeast 'hulls' (broken cell walls) as sterol/FA source, nucleation site
- Ergesterol – expensive

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Typical Yeast Food Effects*

- **Improved alcohol yield (increased extract utilization)**
- **Reduction in fermentation time (12-24h)**
- **Enhanced yeast vitality – Protect against stress**
- **Enhanced diacetyl removal/consistent diacetyl specification**
- **Control of undesirable flavour compounds**

** Depending on the yeast food composition*

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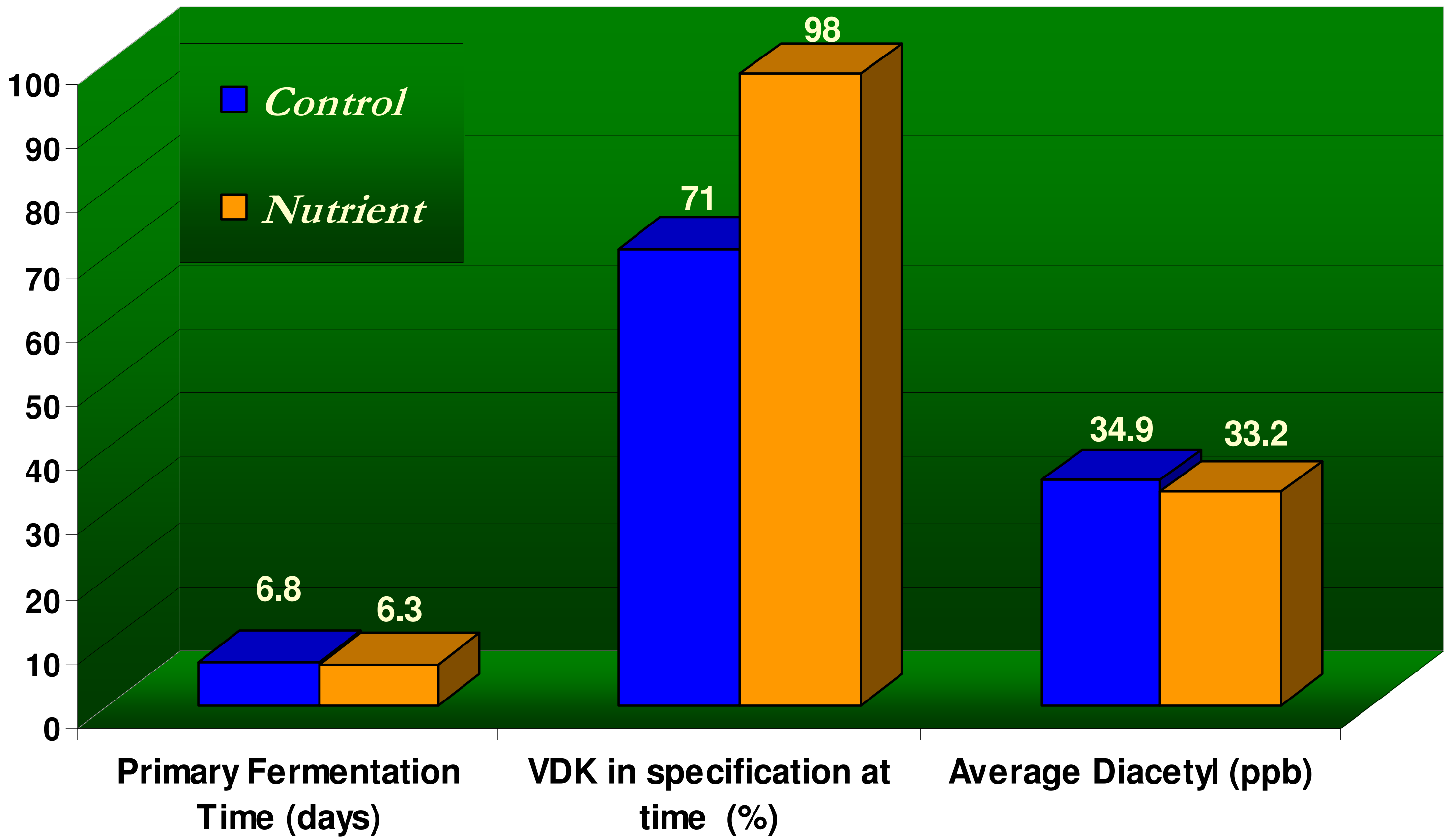
Nutrient Trial Results
Eastern European Brewery A



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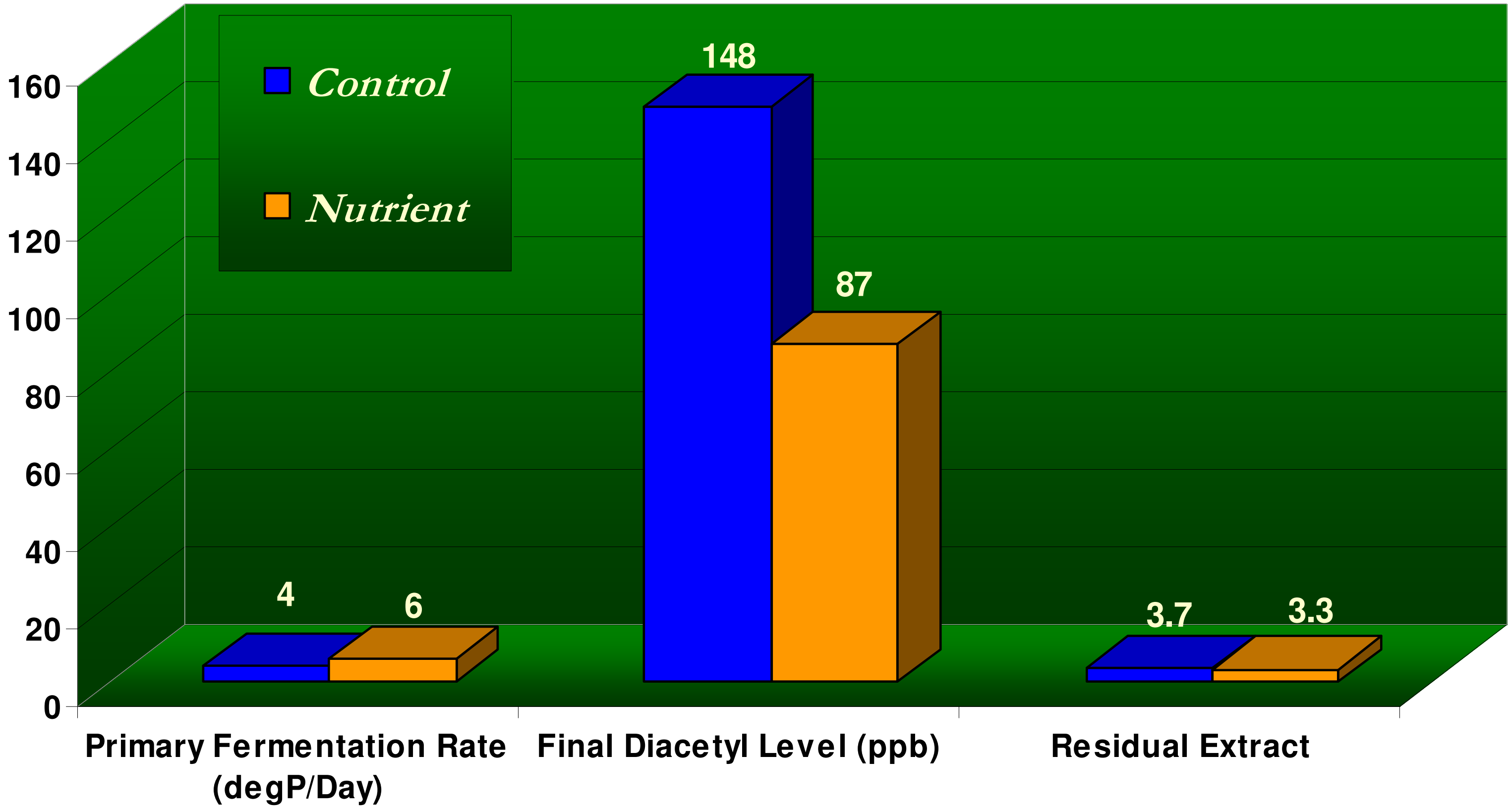
Nutrient Trial Results
Eastern European Brewery B



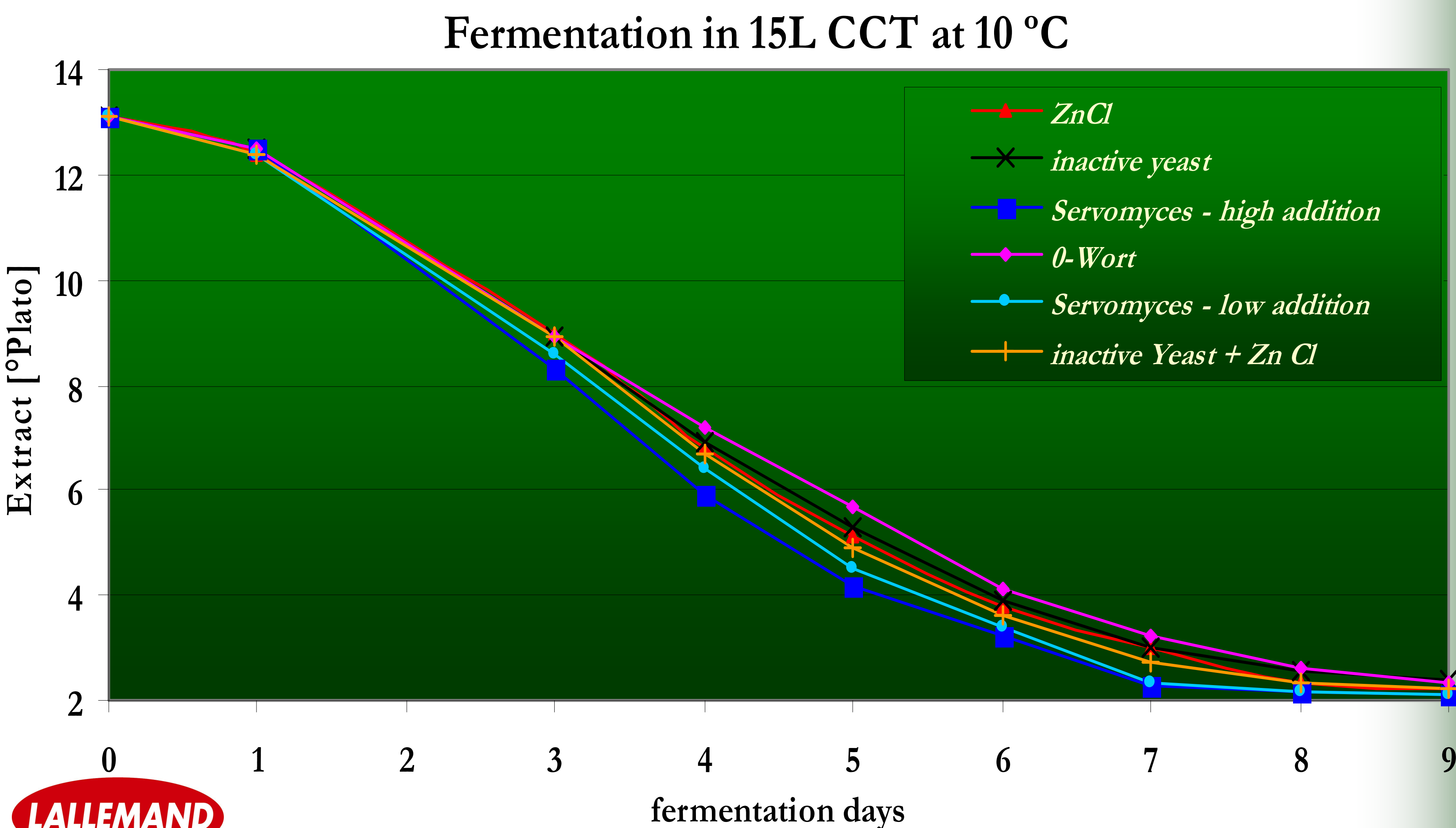
Servomyces Weihenstephan Trials

15L lab trials

- Sample 1: no zinc addition (O-wort)
- Sample 2: addition of 0.3 mg/L ZnCl2
- Sample 3: addition of 8 mg/L inactive yeast
- Sample 4: addition of 8 mg/L SERVOMYCES
- Sample 5: addition of 2.3 mg/L SERVOMYCES
- Sample 6: addition of 8 mg/L inactive yeast + 0.3 mg/L ZnCl2
- Initial yeast zinc pool: 4.1 mg/100 g

	0-wort	$ZnCl_2$	inactive yeast	SERVOMYCES High Addition	SERVOMYCES Low Addition	inactive yeast + $ZnCl_2$
Initial wort [mg/L]	0.10	0.35	0.11	1.22	0.36	0.34
Crop yeast [mg/100 g]	2.67	4.15	2.63	11.61	5.13	3.11

Servomyces
Weihenstephan Trials



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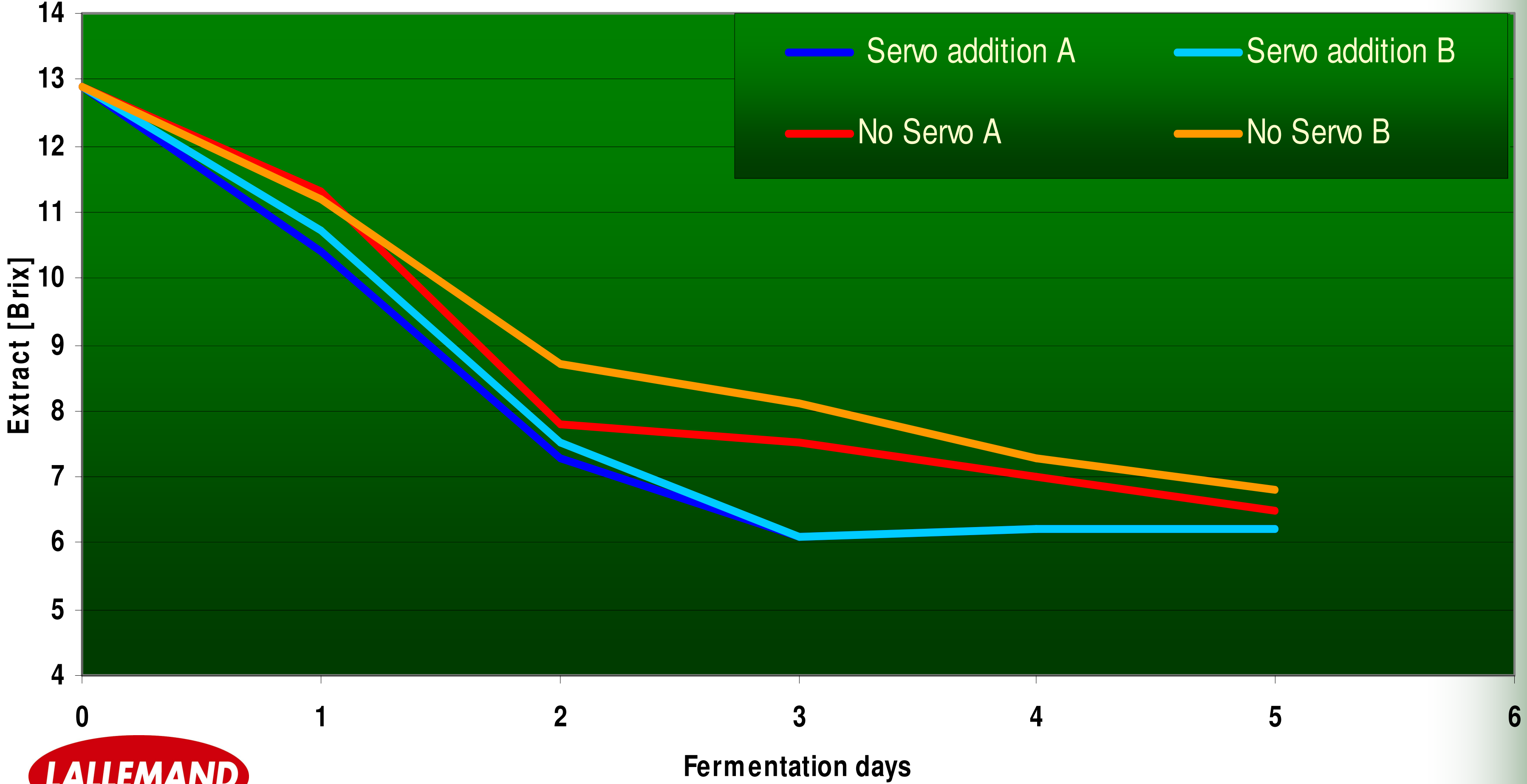
Servomyces Trial Results

US Brewery



Propagation

- **With each cell division the internal zinc pool is divided between mother and daughter cell**
- **Fermentation 4-5 times biomass increase**
- **Propagation 15-20 times biomass increase**
  => very low internal zinc pool

➢ **Slower, less efficient propagation**

➢ **Fermentation problems in 1. generation beer**
  - ➢ **Slower, stuck fermentation**
  - ➢ **Longer diacetyl rest**
  - ➢ **Less flocculent yeast**

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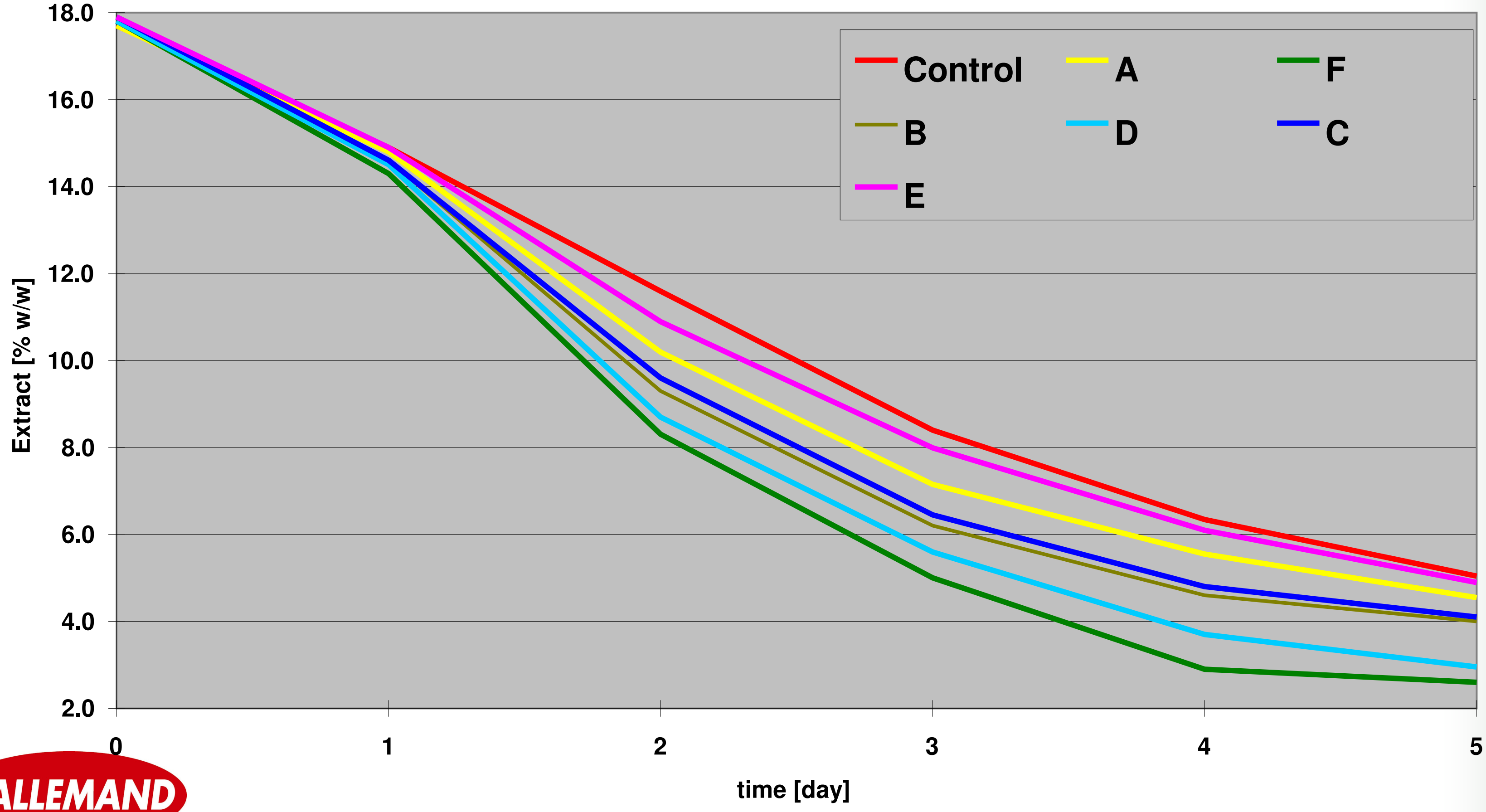
The Impact of Nutrients on High Gravity/Adjunct Brew

- **60% malt / 40% adjunct / 18°P / Gen 1**



Serial Repitching

- **Repeated stress for the yeast**
- **Decline in yeast activity**
- **Resistance to stress is strain dependent**
- **Contamination**
- **Genetic drift/petite mutation**

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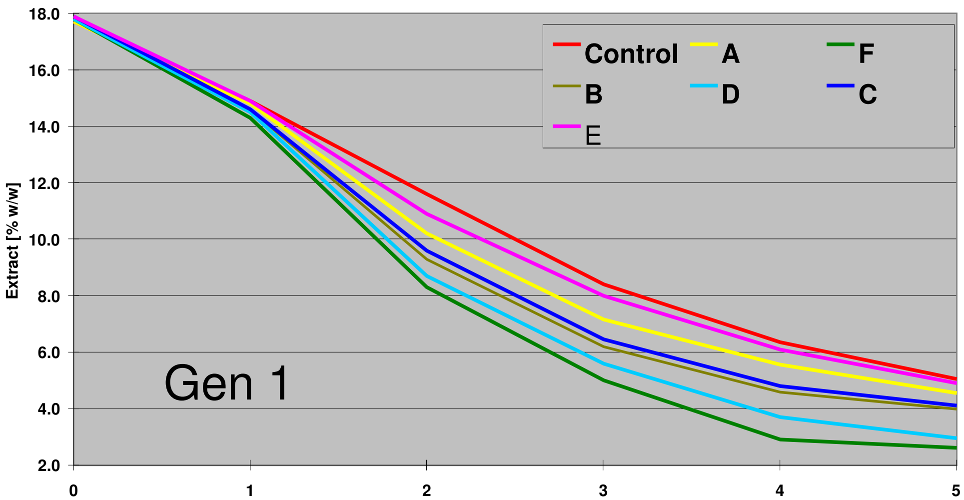
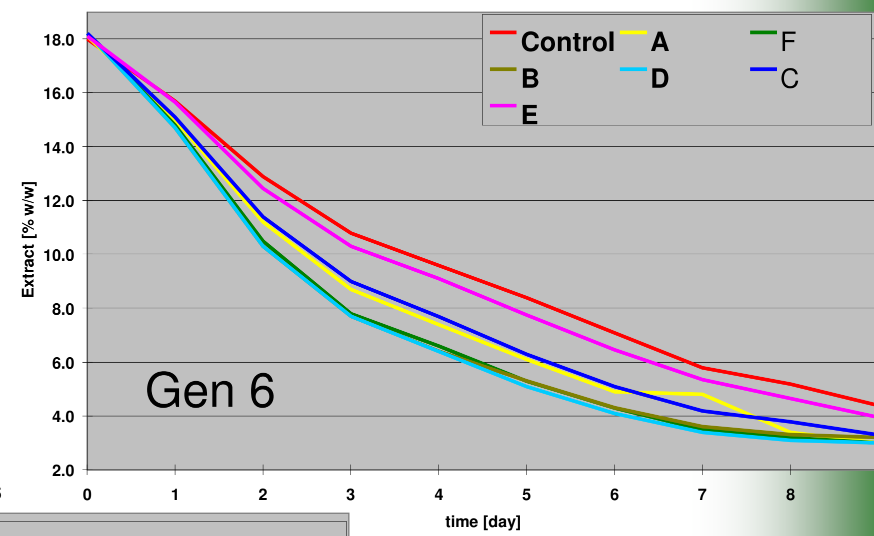
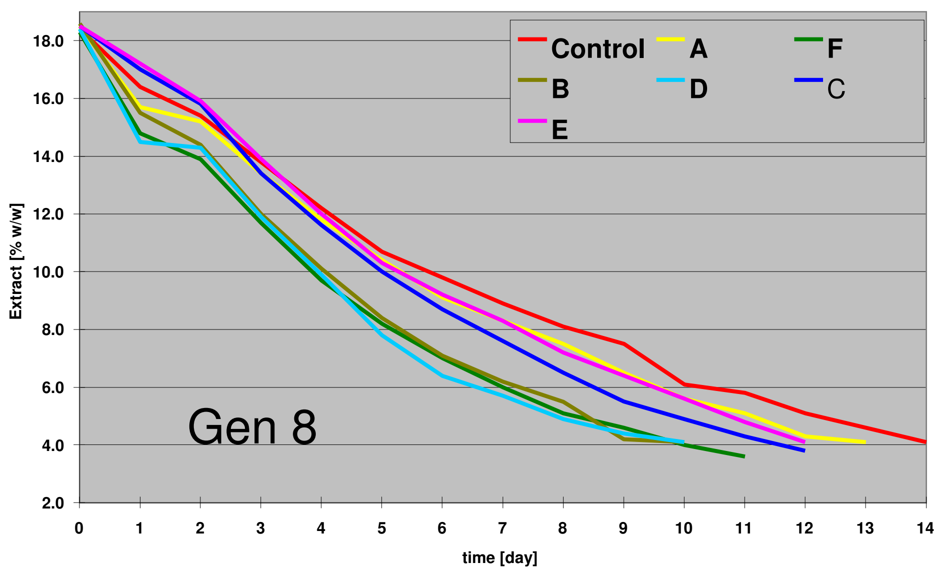
The Impact of Yeast Generation on the Use of Nutrients

High Gravity/Adjunct Brew







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Nucleation Sites

Effect of dissolved CO_2

- **Inhibits growth and metabolism**
- **Decreases multiplication rate**
- **Reduces biomass yield**
- **Increases lag phase**
- **Inhibition of nutrient uptake**
- **Loss of cell division and bud formation**

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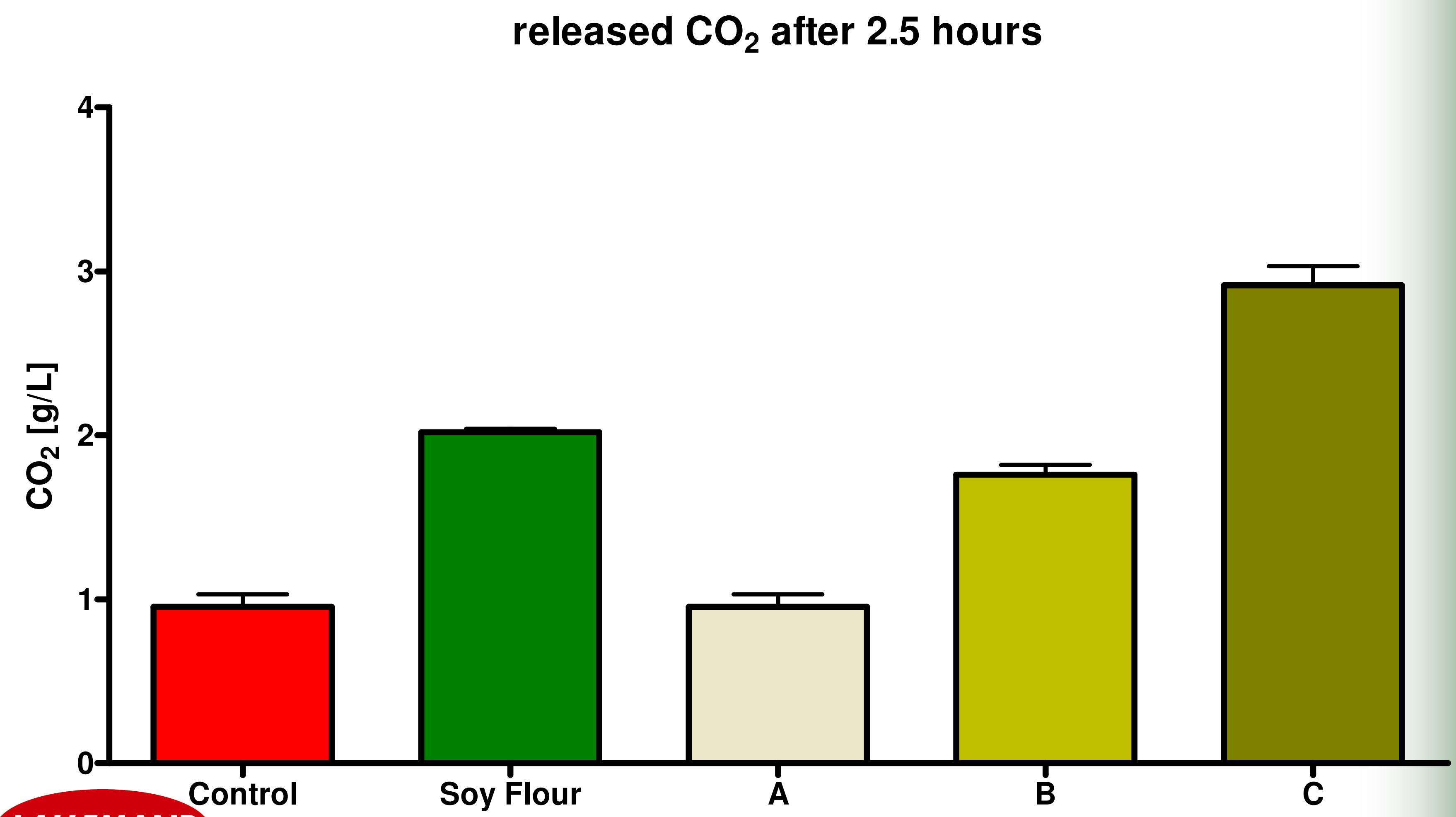
Nucleation Sites

- **Particles working as nucleation sites reduce the solubility of CO_2**
- **Most nucleation in a fermenter occurred from particles that sedimented to its bottom**
- **Particle size is a likely determinant of the particle's ability to be an effective CO_2 nucleator**
- **The more porous a particle is, the most efficient it is as a nucleation site**
- **Soy flour may be ruled out due to allergen issues**

Nucleation Sites – Degassing Activity



Why Nutrients?

- Sufficient supply of nutrients to the yeast
- Predictable, fast and consistent fermentations without the formation of undesired flavours
- Higher alcohol yields
- High gravity brewing and increased amounts of adjuncts can result in slow or stuck fermentation
- Insufficient nutrient supply will also result in formation of undesired flavours

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