

# Alternative Method for **DETERMINING THE PHYSIOLOGICAL CONDITION OF YEAST**

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-KMA-



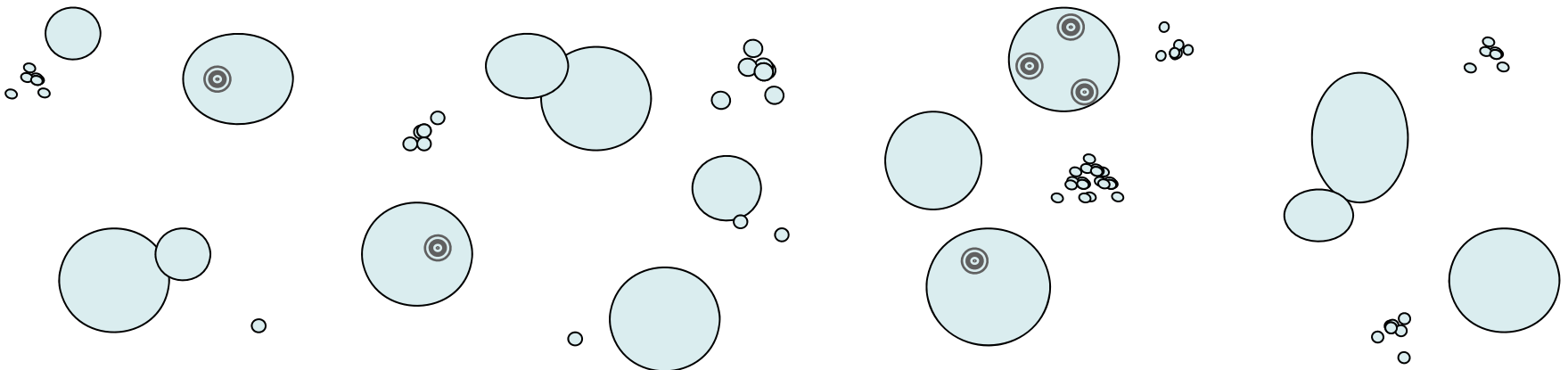
Yeast influences the product quality and efficiency significantly!

## Routine laboratory analysis

- Testing viability with staining methods (methylene blue)
- Determination of cell count (with Thoma cell counter)

## Problem

- These analyses alone are insufficient!



### Analysis of Yeast Vitality According to HEGGART

Method based on:	Example	Direct	Practicability
<b>Metabolic activity</b>	Vitality staining	----	----
	Microcalorimetry	----	----
	Reduction of vicinal diketones (VDK)	----	----
	Protease activity of yeast	----	----
	Magnesium ion release test (MRT)	----	----
	Specific oxygen uptake	----	----
	Acidification power test	----	----
	Intracellular pH value (ICP)	----	X
<b>Measurement of cellular components</b>	Adenosine triphosphate (ATP)	----	----
	Adenylate energy charge (AEC)	----	----
	NADH (fluorometric)	----	----
	Glycogen and trehalose	----	----
	Sterols and unsaturated fatty acids	----	----
<b>Fermentation capacity or glycolytic flow rate</b>	Glycolytic flow rate	X	----
	CO <sub>2</sub> measurement	X	??????
	Rapid fermentation	X	----

## Yeast Metabolism

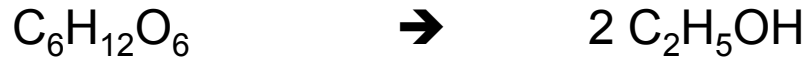
### Oxidative sugar decomposition



### Oxidative ethanol decomposition



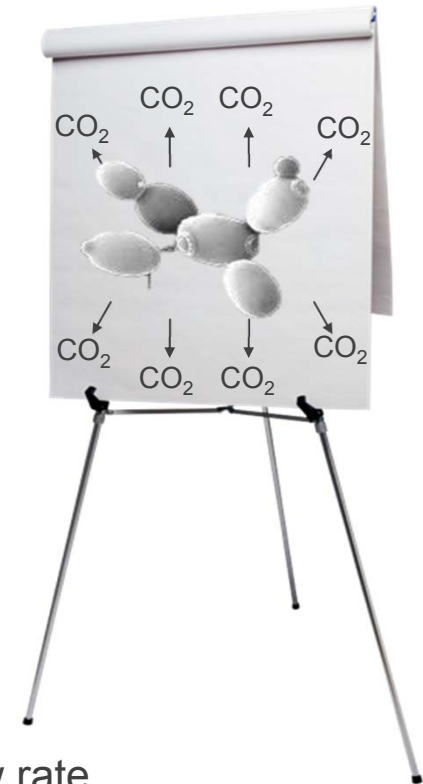
### Fermentation



+ 6 CO<sub>2</sub>

+ 2 CO<sub>2</sub>

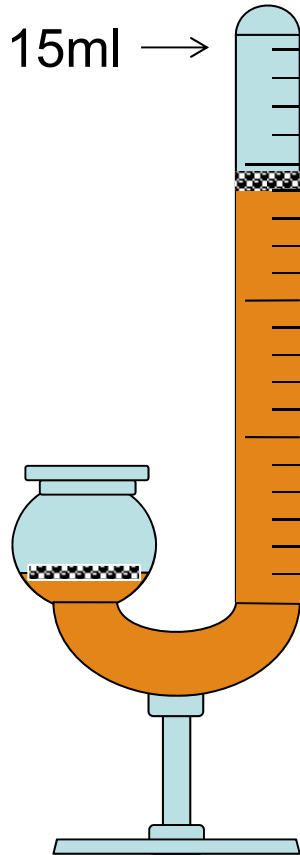
+ 2 CO<sub>2</sub>



## Methods based on CO<sub>2</sub> measurements

1961	HLAVÁČEK	Fermentometer method
1980	NARZIß / BACK	30 min pressure accumulation test
2000	HEGGART	CO <sub>2</sub> measurement using glycolytic flow rate
2011	KMA	Fermentation saccharometer according to Einhorn

## Einhorn Fermentation Saccharometer



Fermentation substrate	Maltose solution
Sample preparation	Centrifugation: 750 g, 5 min; Adjustment with water to 200 million YC/ml
Mixing ratio	6 ml yeast suspension (200 million YC/ml) +14 ml maltose solution (10 % [m/v])
Equilibration time	60 min at 28 ° C (incubator)
Incubation temperature	28 ° C
Fermentation time	max. 120 min

Evaluation	Very good	Sufficient	Insufficient
Lag/log phase transition	1 ml CO <sub>2</sub> in 20 min	1 ml CO <sub>2</sub> in max 50 min	Less than 1 ml CO <sub>2</sub> in 50 min
Fermentative capacity	10 ml CO <sub>2</sub> in 80 min	10 ml CO <sub>2</sub> in max 120 min	Less than 10 ml CO <sub>2</sub> in 120 min

## Gas Production Monitoring System



- Wireless data transfer
- Online monitoring of accumulated pressure
- Parallel measurements
- Adjustment of system pressure
- Provides sum of accumulated pressure
- Regulation of pressure release intervals
- Routine laboratory analysis
  - cleanability,
  - safety,
  - resistant to changes in temperature
  - size
- Low price

## Measurement Parameters

### Attributes

- Uncomplicated method
- Time required to conduct experiment: 2 – 4 hours
- Low cell count for inoculation
- Test conditions  $\cong$  brewery conditions

Yeast strain

Fermentation  
media

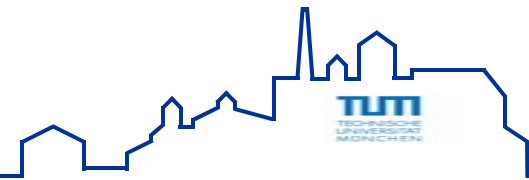
Headspace  
volume

Required  
temperature

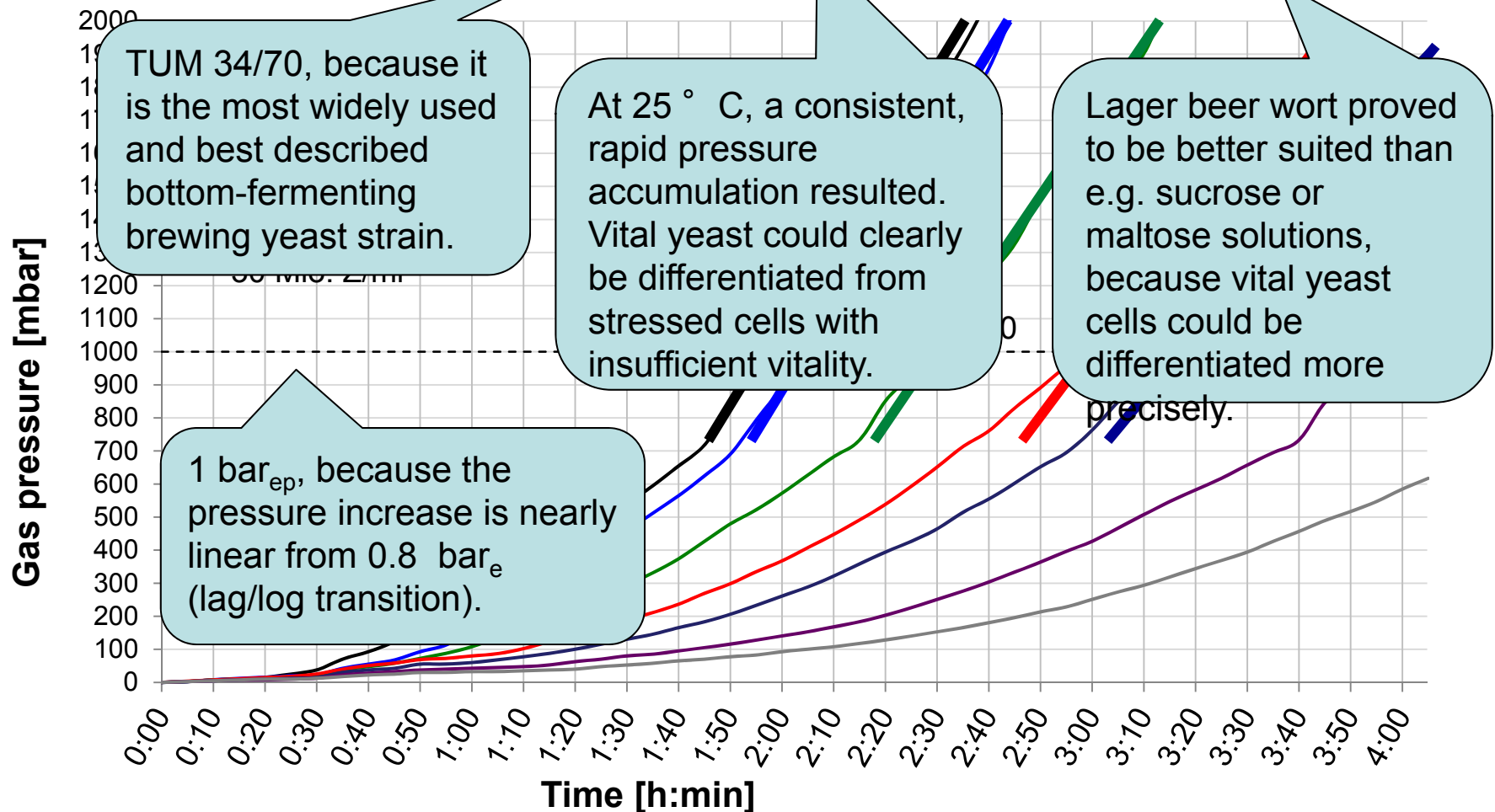
Lag/Log  
phase

Cell  
count





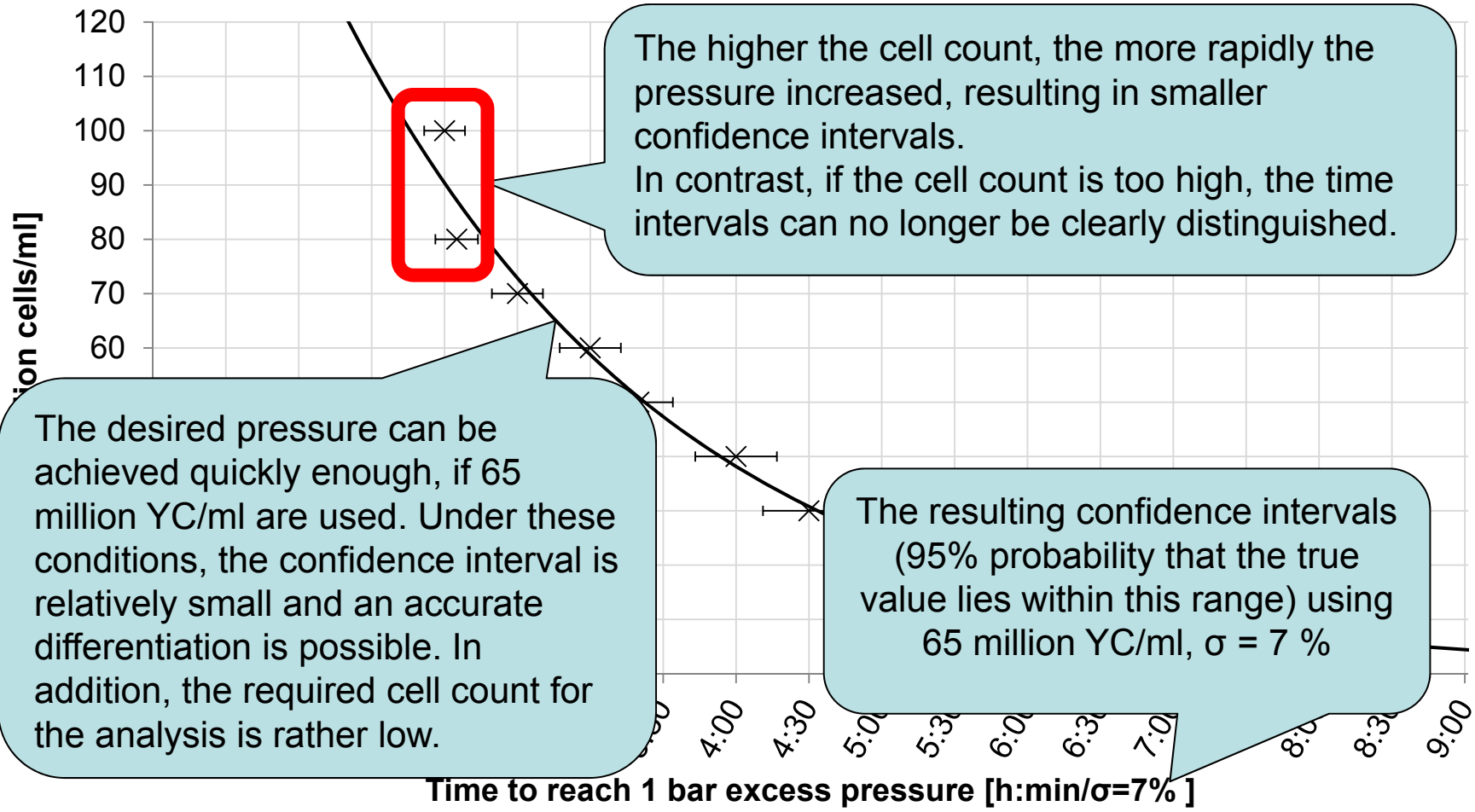
Determination of Cell Count (TUM 34/70, 25 ° C, Lager Beer Wort, 12 ° P)







## Pressure Increase to 1 bar<sub>ep</sub> in Correlation with Cell Count



The higher the cell count, the more rapidly the pressure increased, resulting in smaller confidence intervals. In contrast, if the cell count is too high, the time intervals can no longer be clearly distinguished.

The desired pressure can be achieved quickly enough, if 65 million YC/ml are used. Under these conditions, the confidence interval is relatively small and an accurate differentiation is possible. In addition, the required cell count for the analysis is rather low.

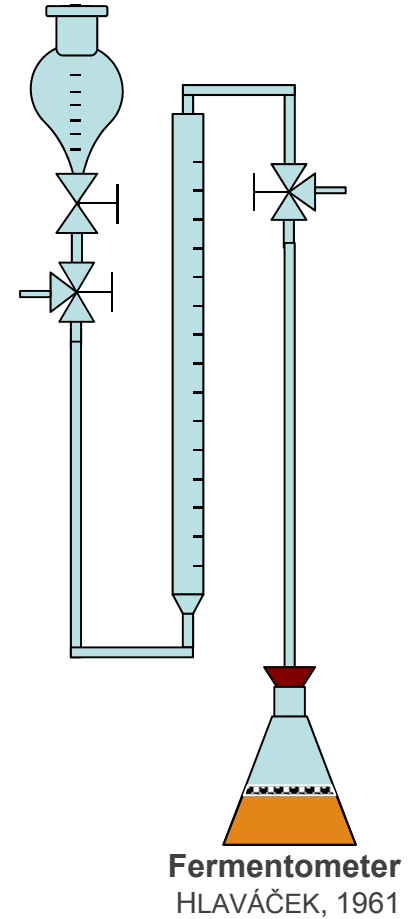
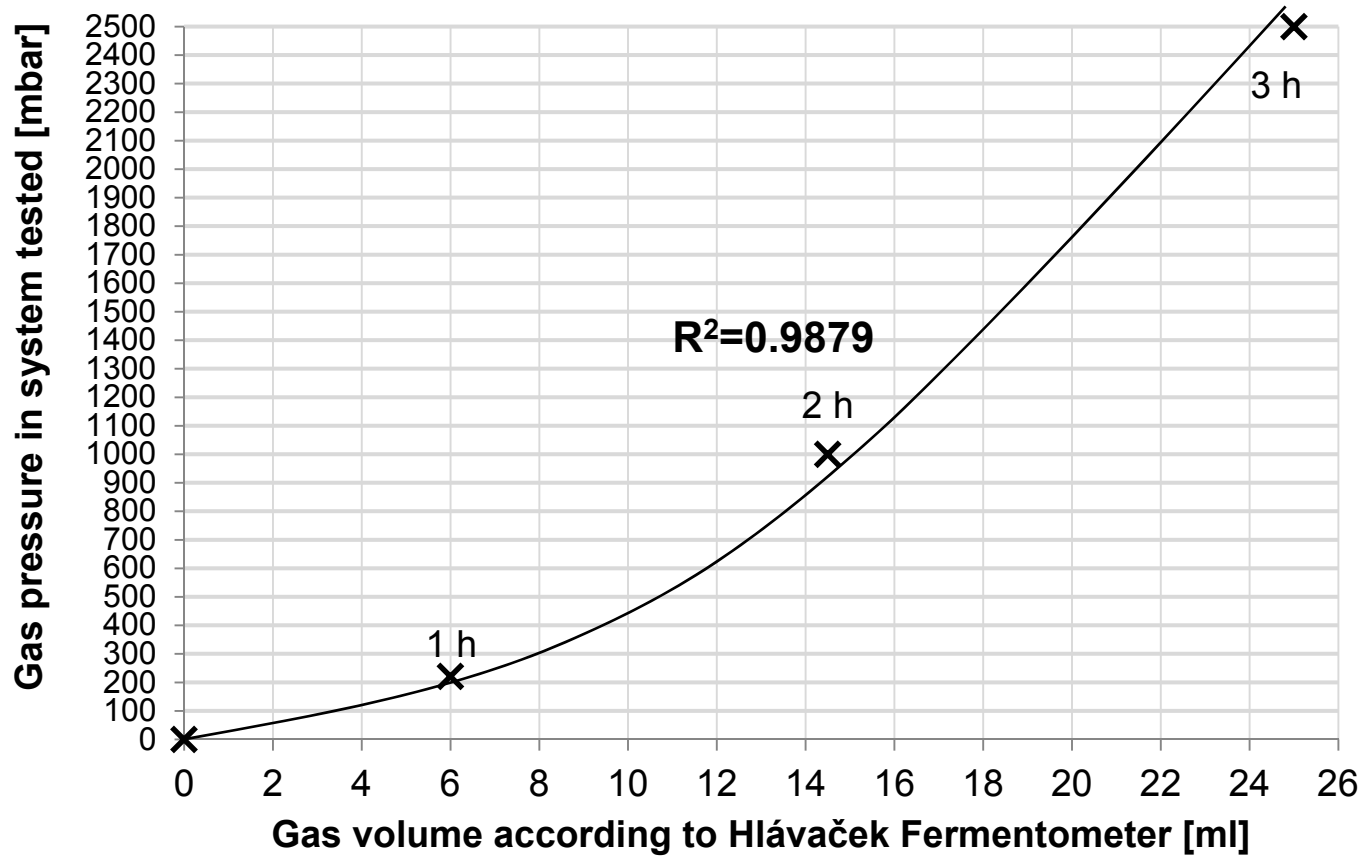
The resulting confidence intervals (95% probability that the true value lies within this range) using 65 million YC/ml,  $\sigma = 7\%$

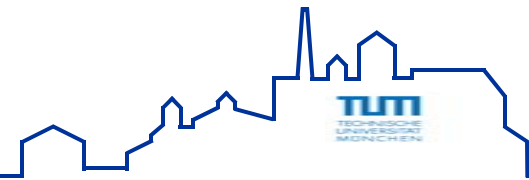
## Parameters Used in Conducting the Experimental Trials

Parameter	Value	Comment
Volume (ml)	200	Wort and yeast
Temperature (°C)	25	In a water bath or refrigerator
YCA (million per ml)	65	Here: harvested yeast TUM 34/70
Fermentation media	12 °Plato wort	Here: Weihenstephan „Original“ wort
Goal (h:min)	Time to reach 1 bar <sub>ep</sub>	Exponential increase in CO <sub>2</sub> pressure
YCA to headspace ratio	118,18 million YC/ml-headspace	For appropriate adjustment if different reactor sizes are used
Analytical error	(+/-) 7%	Confidence interval for the stated conditions

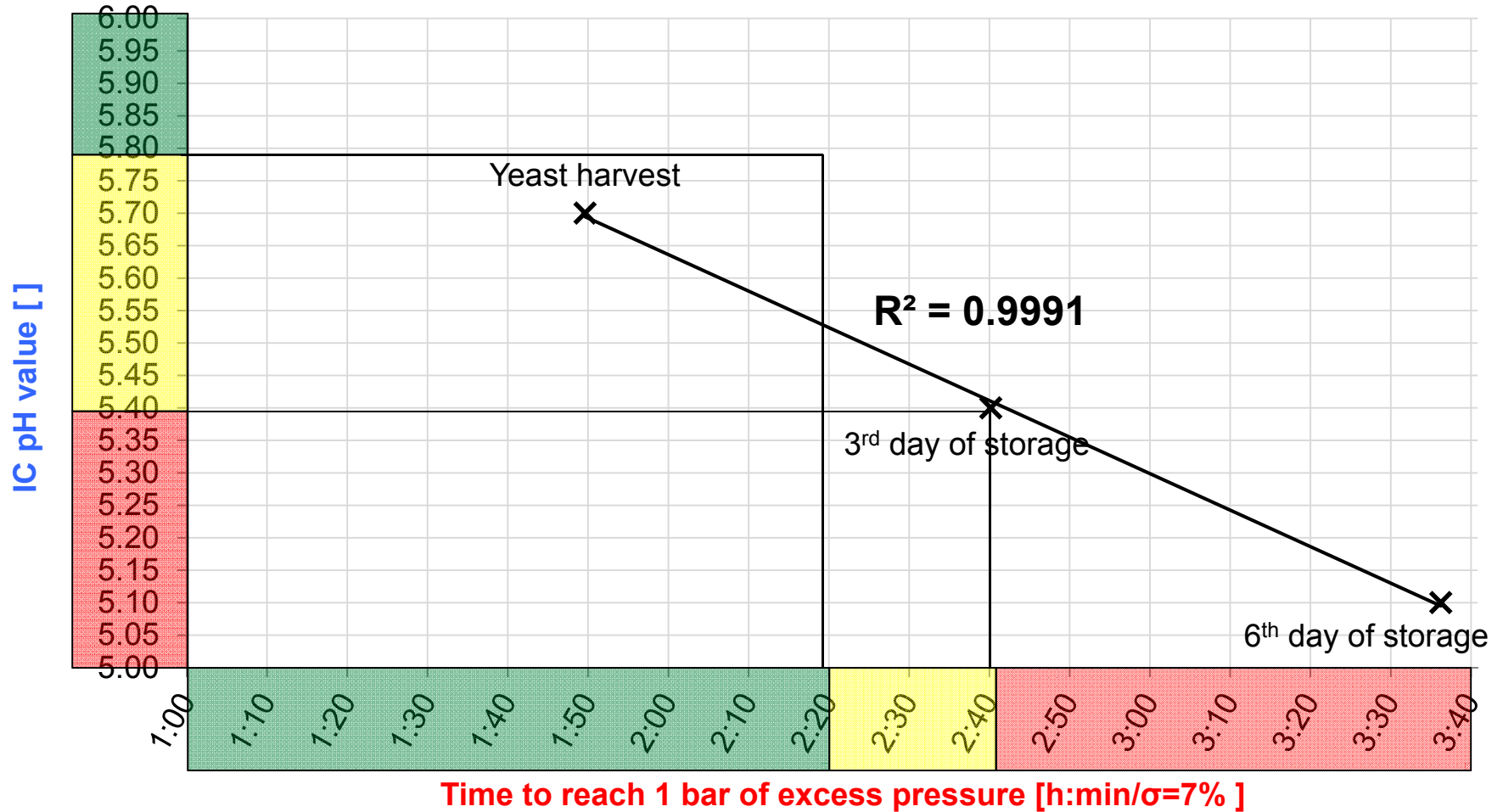


Correlation of Pressure Increase & Measurement of CO<sub>2</sub> Volume

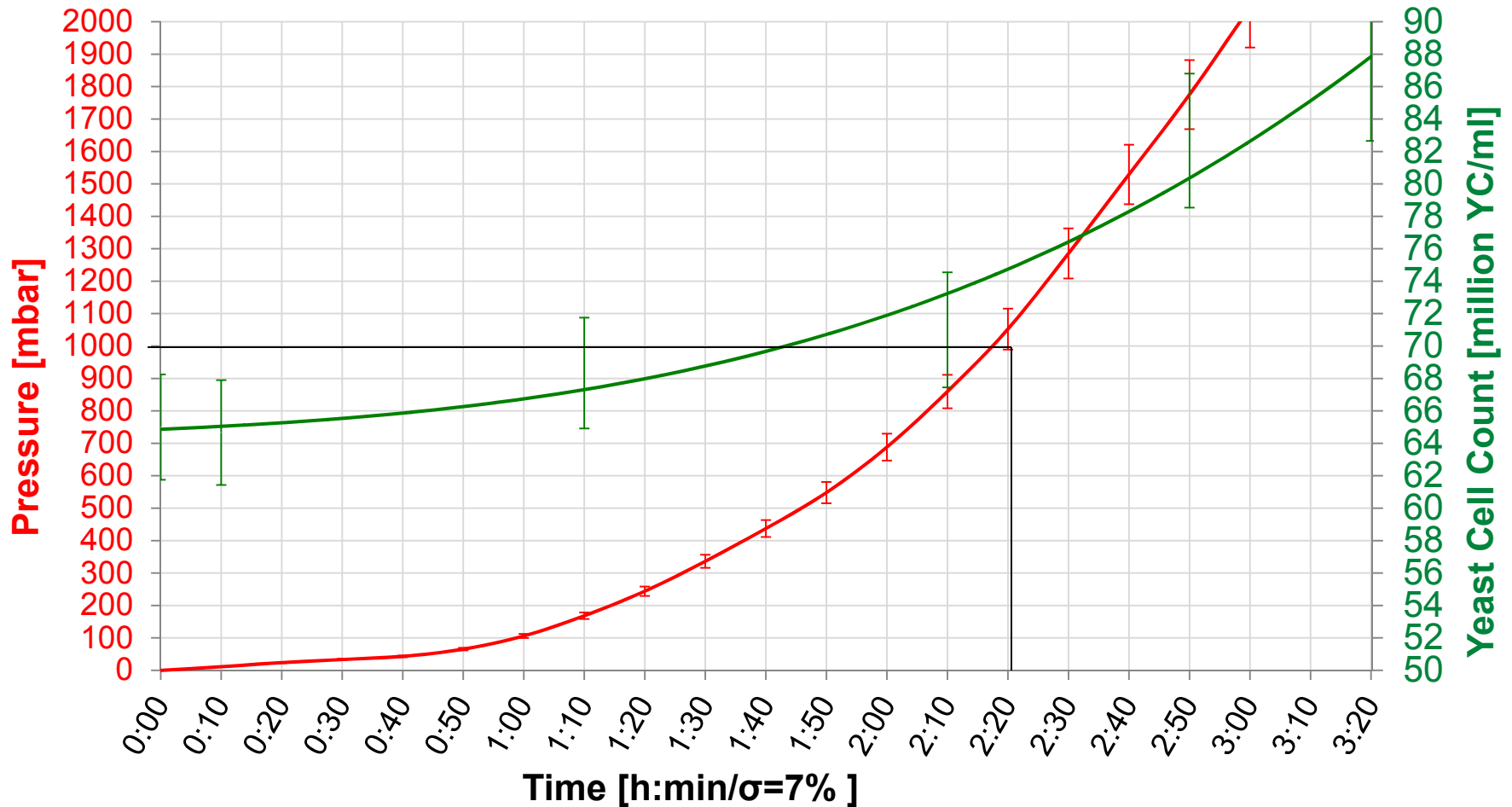




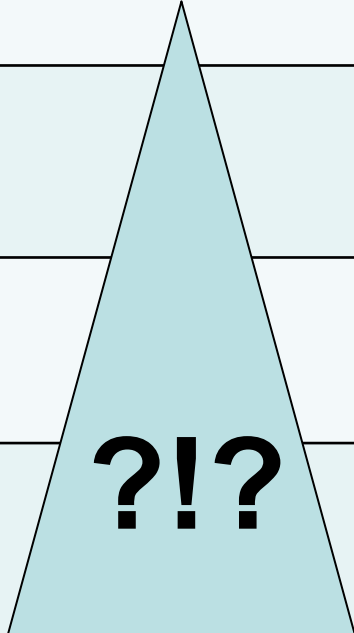
Correlation to the ICP (Intracellular pH Value) Determination



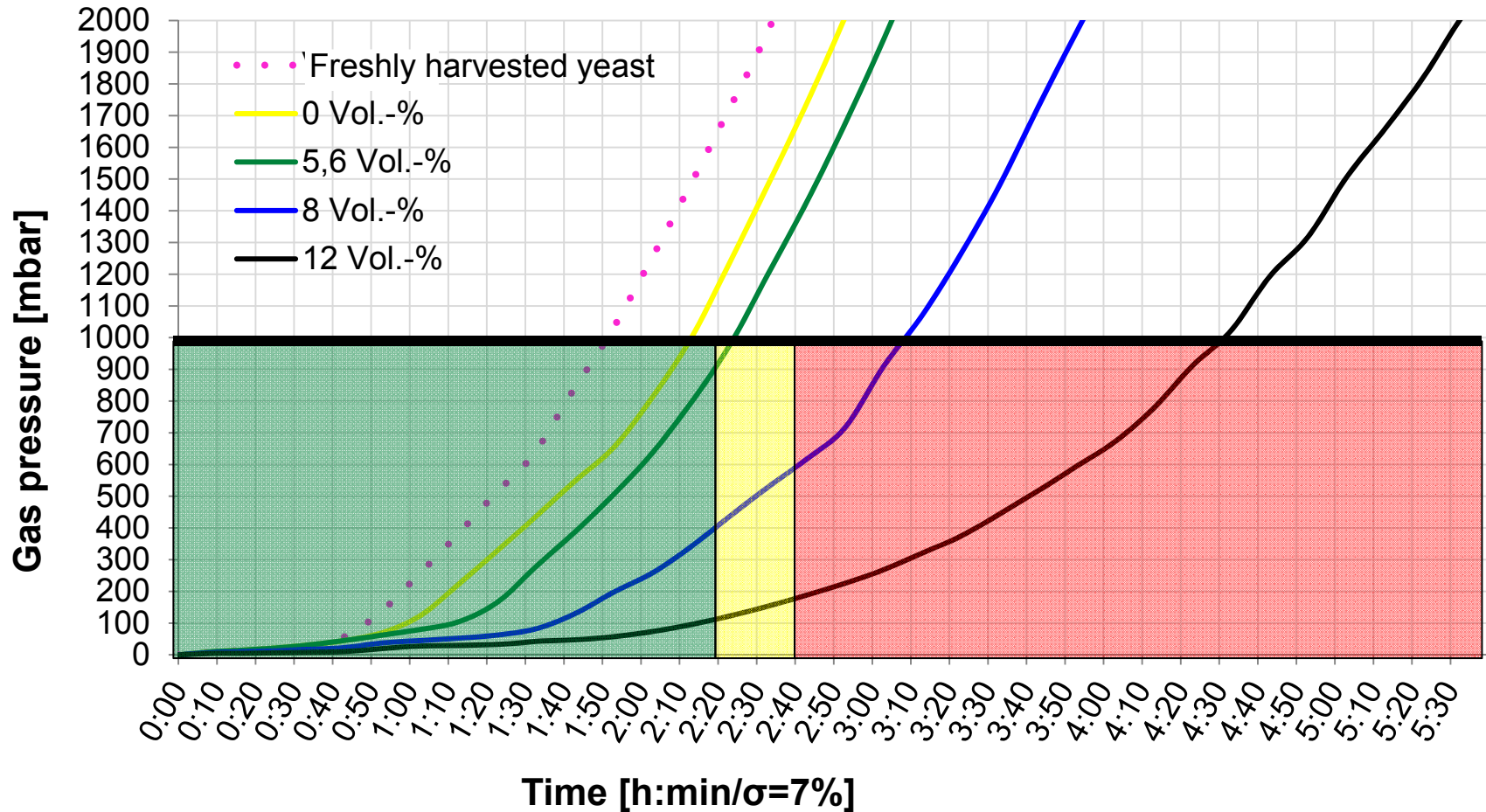
CO<sub>2</sub> Pressure in Relation to Total Yeast Cell Count (YC)

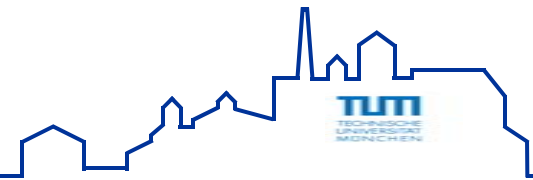


## Evaluation of Alternative Methods for the Determination of Yeast Vitality

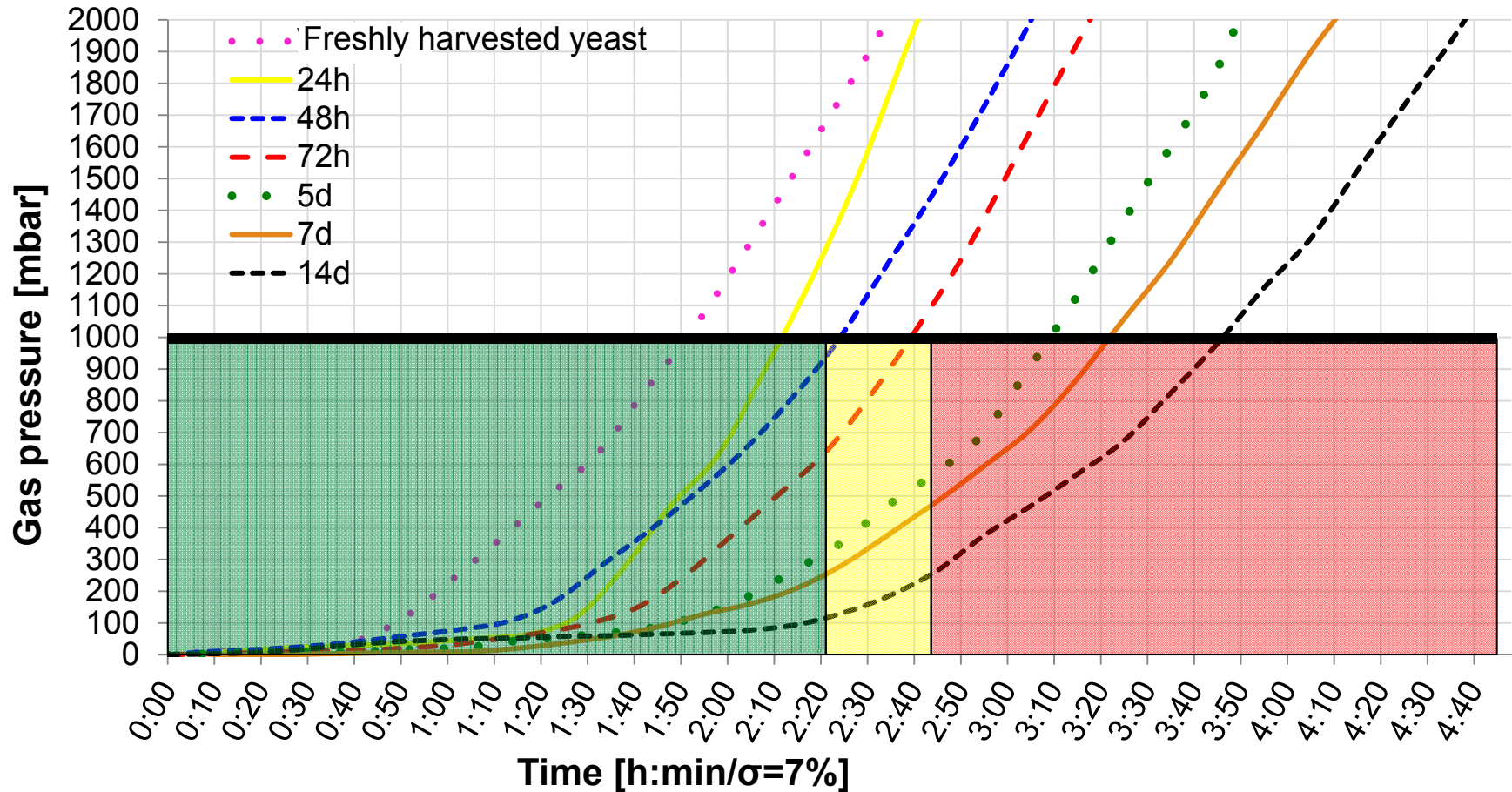
Vitality (Fermentative Capacity)				
Method	Good	Sufficient	Insufficient	Significance & routine lab analysis
ICP Value	$\geq 5,8$	$\geq 5,4 \dots < 5,8$	$< 5,4$	
<i>KMA / Hutzler</i> Einhorn Fermenter	10 ml CO <sub>2</sub> in 80 min	10 ml CO <sub>2</sub> in 80 min	Less than 10 ml CO <sub>2</sub> in 120 min	
HLAVÁČEK (ml CO <sub>2</sub> )	$\geq 25$ after 3 hours	----	$< 25$ after 3 hours	
<i>KMA / Silva</i> Time to reach 1 bar of excess pressure	$< 2:20$	$\geq 2:20 \dots \leq$ 2:40	$> 2:40$	

Yeast Stored for 48h in Various Alcohol Solutions

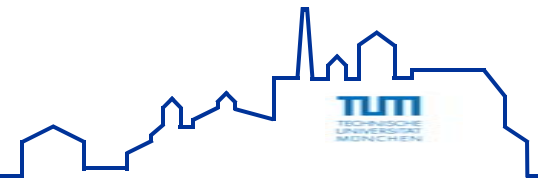




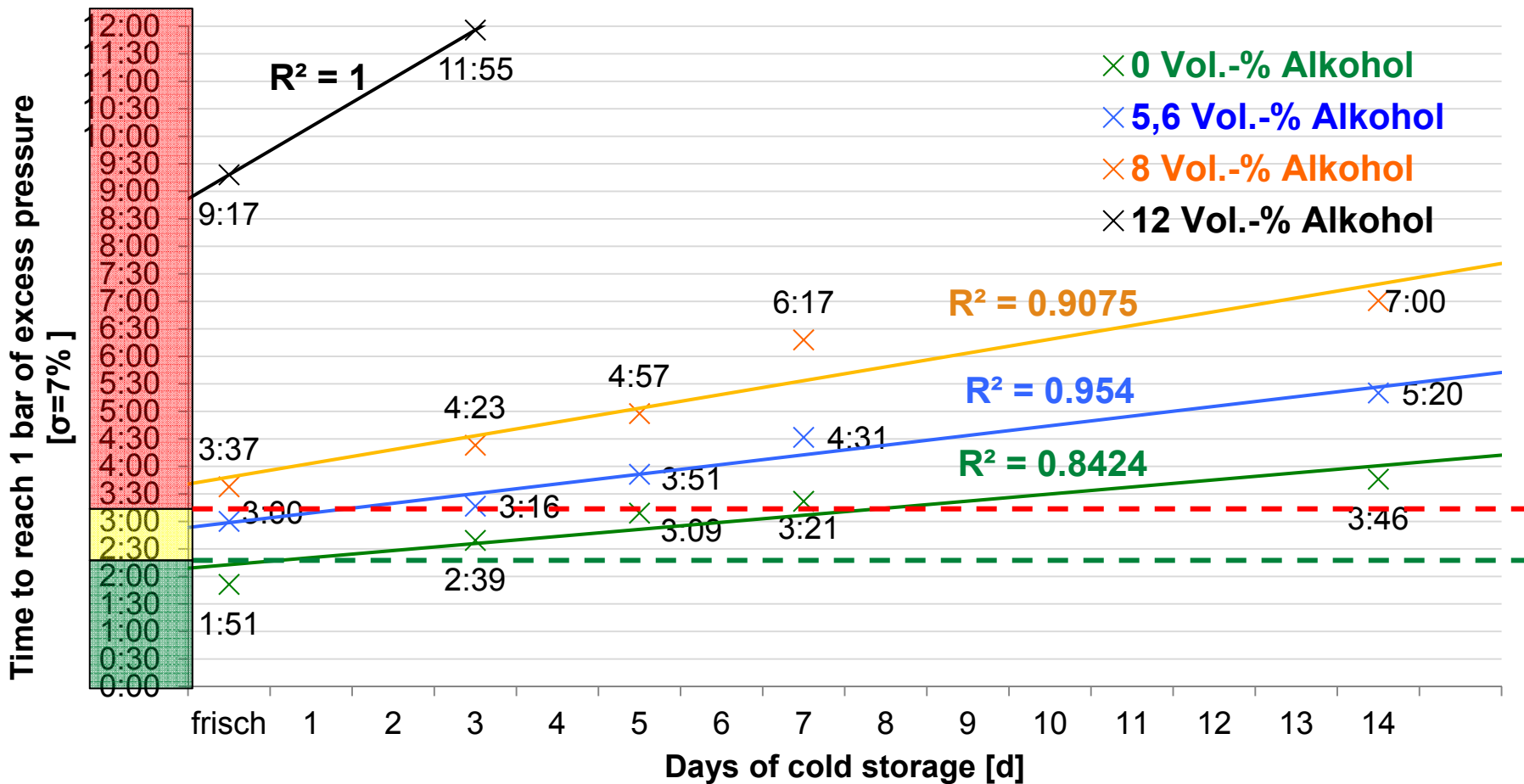
Harvested Yeast Stored at 4 ° C (5.6 % alc. by vol.) for Several Days

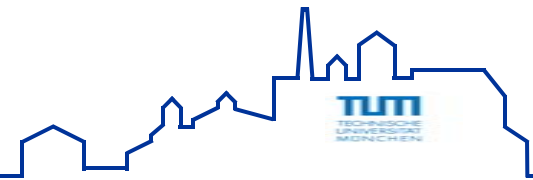




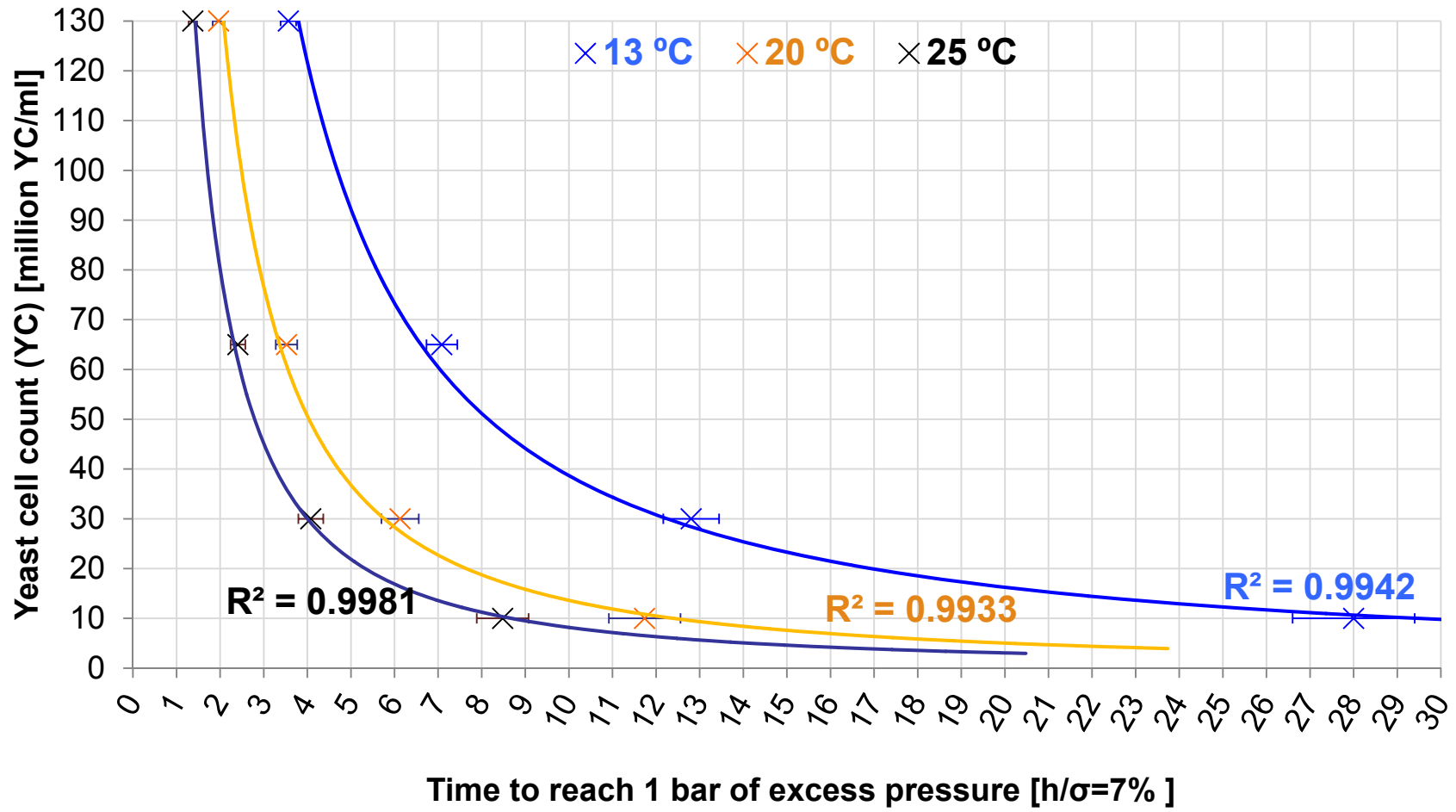


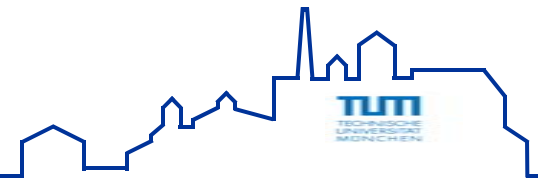
## Reaction of Cold Stored Yeast (4 ° C) at Different Alc. Concentrations





Lag Phase at Various Yeast Cell Counts & Fermentation Temperatures





## Conclusion

- With this alternative method of analysis, yeast vitality can be determined simply and rapidly with a high level of accuracy.
- The method proposed here can be directly correlated with established vitality measurement methods (e.g. with the ICP method).
- By analyzing the brewing yeast on a regular basis, important knowledge can be obtained which can then be applied to optimize fermentation processes.

## Future Research and Discussion

- At the moment, the method is being improved by applying induced stress factors, in order to detect potential weaknesses more precisely.
- The technology will subsequently be tested in a commercial brewery on an industrial scale, in order to improve yeast propagation and pitching practices.
- Furthermore, this method will be used to evaluate standard data and mathematical formulas.

# Thank you for your attention!

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## Acknowledgement: Mr. Willian Silva