



**WORLD BREWING CONGRESS**

August 13–17, 2016 • Denver, Colorado, U.S.A.

#ElevateBeer



# Serial Re-Pitching: Two Case Studies

Maria Josey

International Centre for Brewing and Distilling

Heriot-Watt University, U.K.

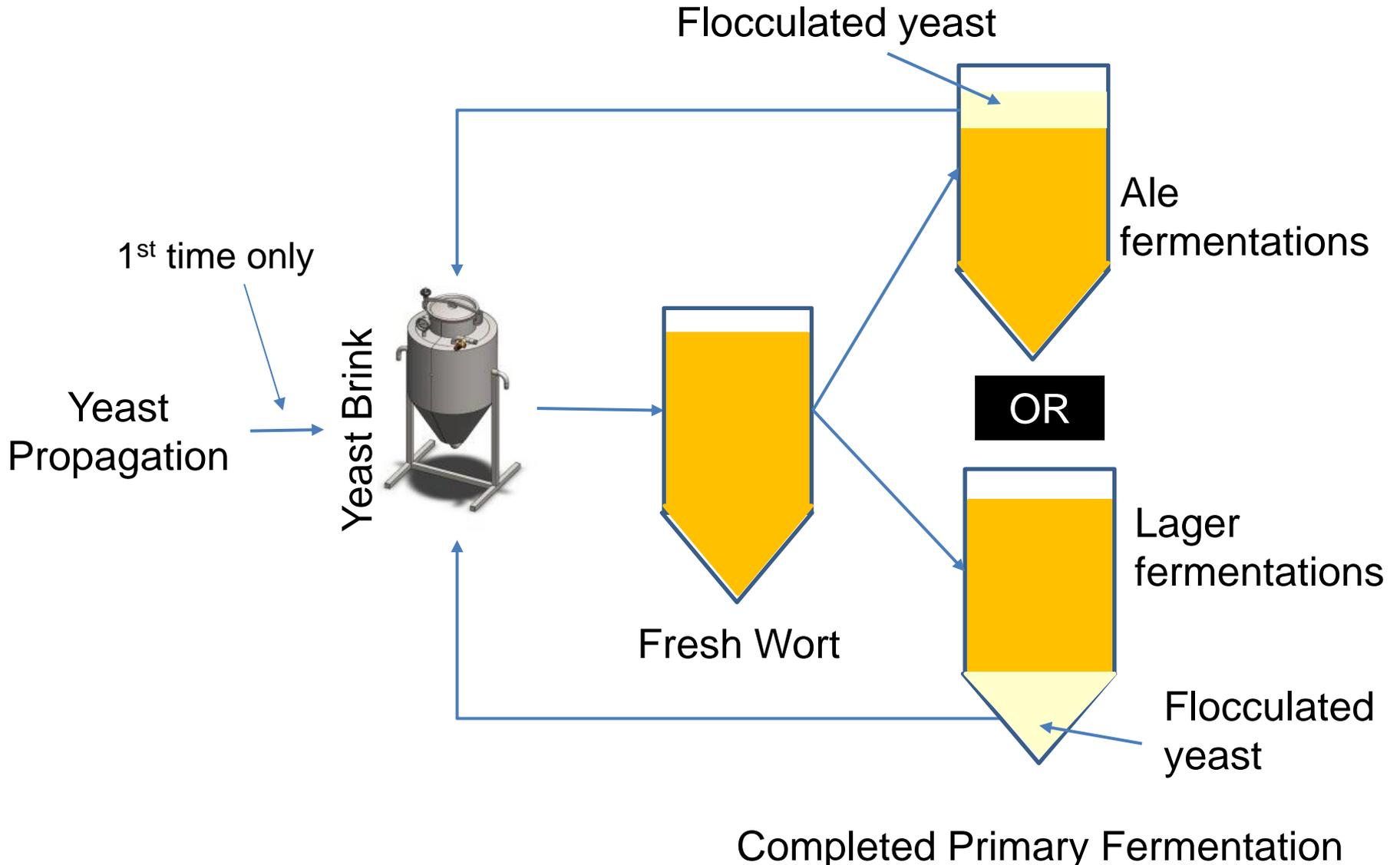


Institute of Brewing & Distilling





# What is serial re-pitching?





# Benefits of serial re-pitching

- Saves time propagating yeast
    - Takes multiple days
  - Cost saving
  - Reduces first crop yeast use
    - New yeast crops exhibit abnormal fermentation profiles
- 
- **Are breweries maximizing their crop number?**



# CASE STUDY: RE-PITCHED LAGER FERMENTATIONS





# Lager Case Study: Experimental Design

**Industrial Lager  
Crop Collected  
(3<sup>rd</sup> crop)**

1<sup>st</sup> time only

**Cells Quantified**

**Pitched into  
12 P Wort\***

\*Malt bill: Lager malt (73%),  
rice (25%), Crystal (or  
Munich) (2%)

1.6 hL

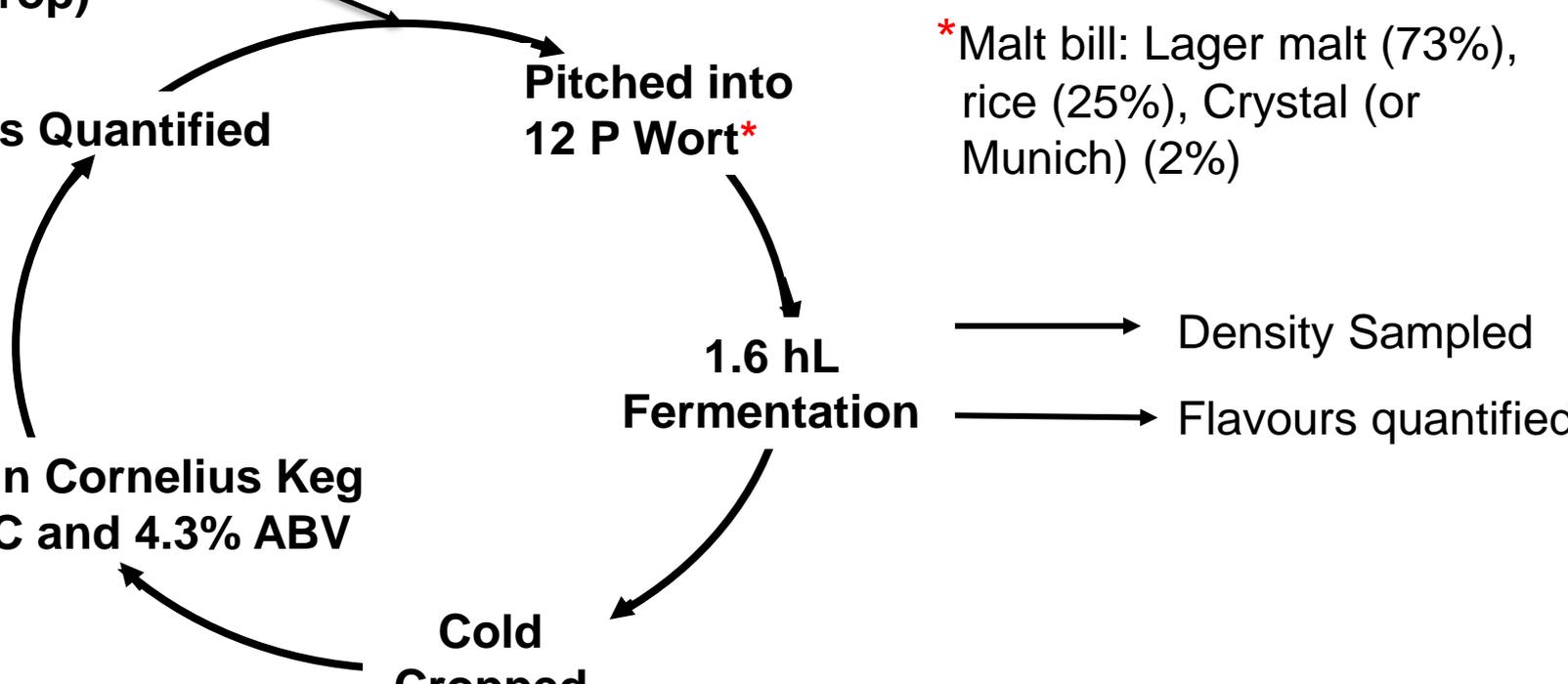
**Fermentation**

→ Density Sampled

→ Flavours quantified

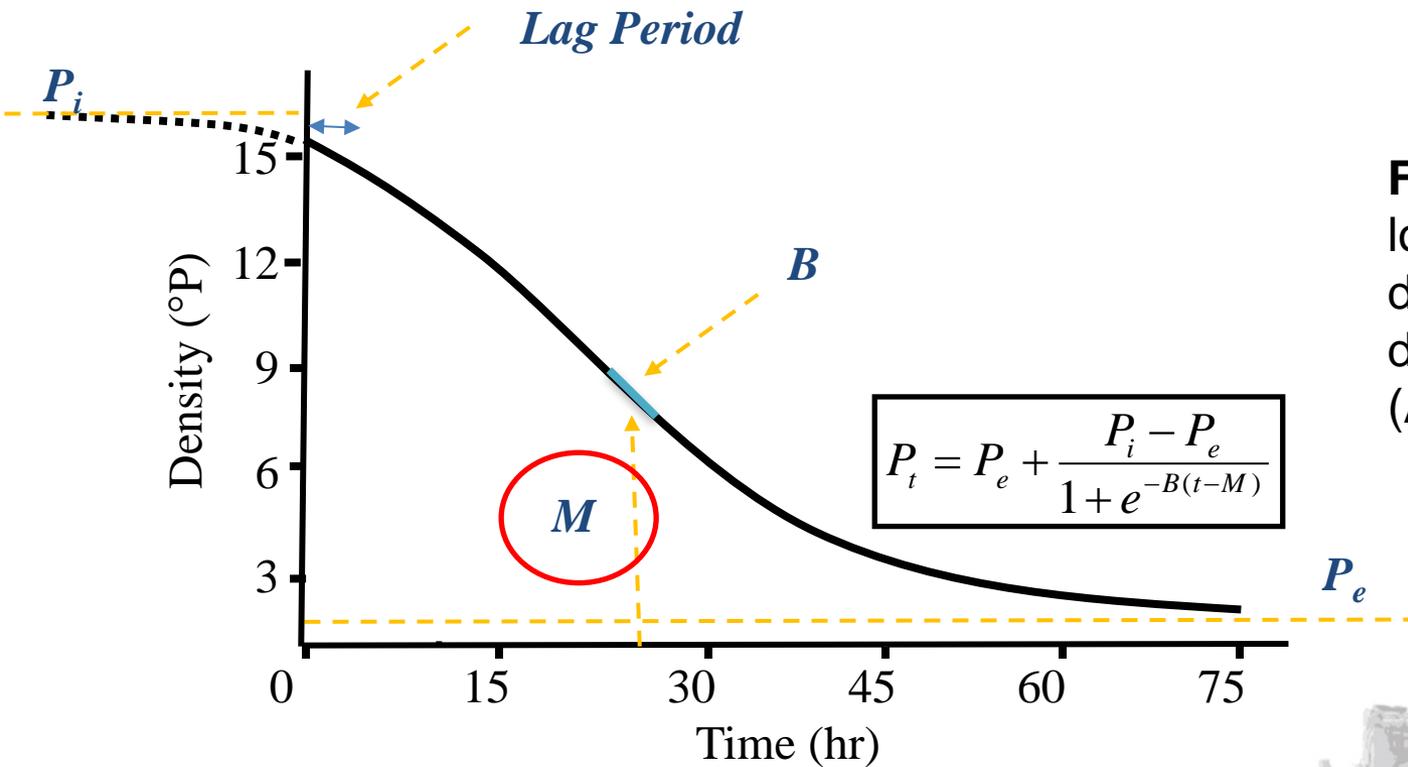
**Stored in Cornelius Keg  
at 11 °C and 4.3% ABV**

**Cold  
Cropped**





# Nonlinear Regression Analysis



**Figure 1.** Nonlinear logistic describing density attenuation during fermentation (ASBC Yeast-14)



# Significance of density attenuation curves?

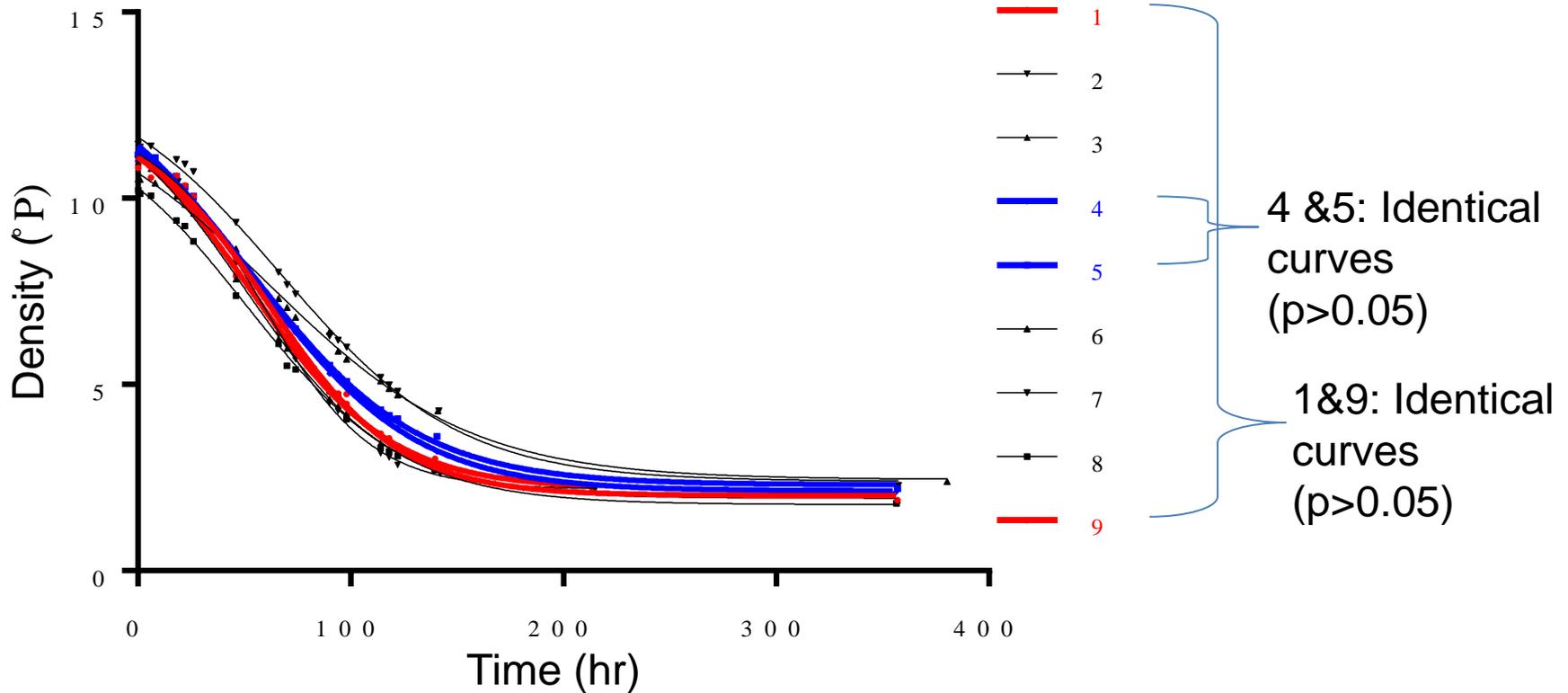
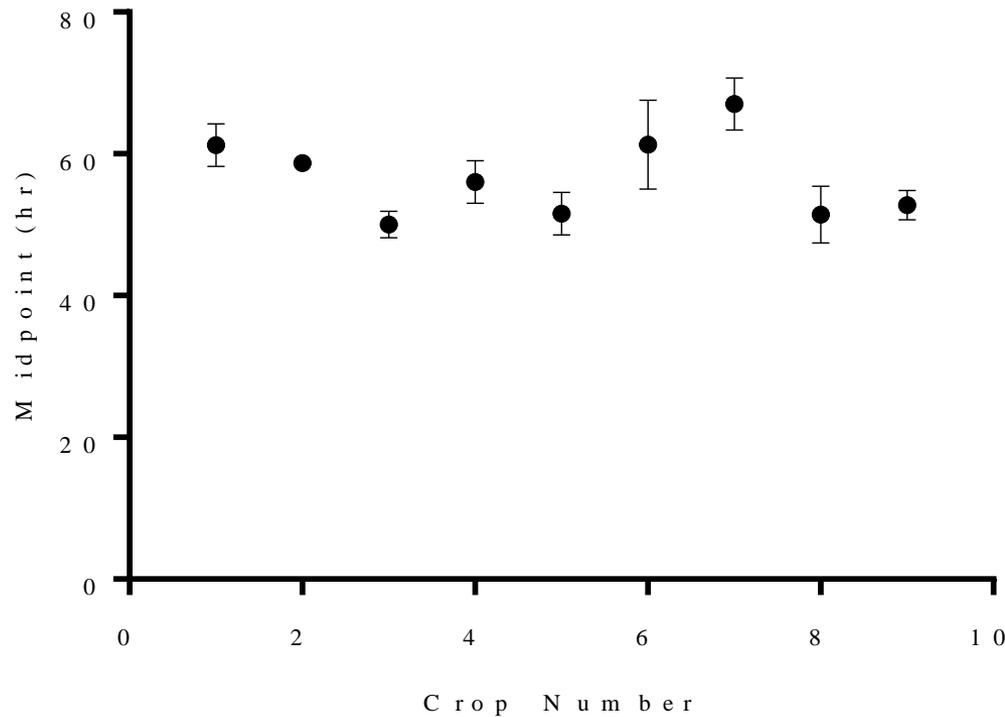


Figure 2. Modelled density attenuation curve for nine re-pitched fermentations, the re-pitched number is noted by individual colours.

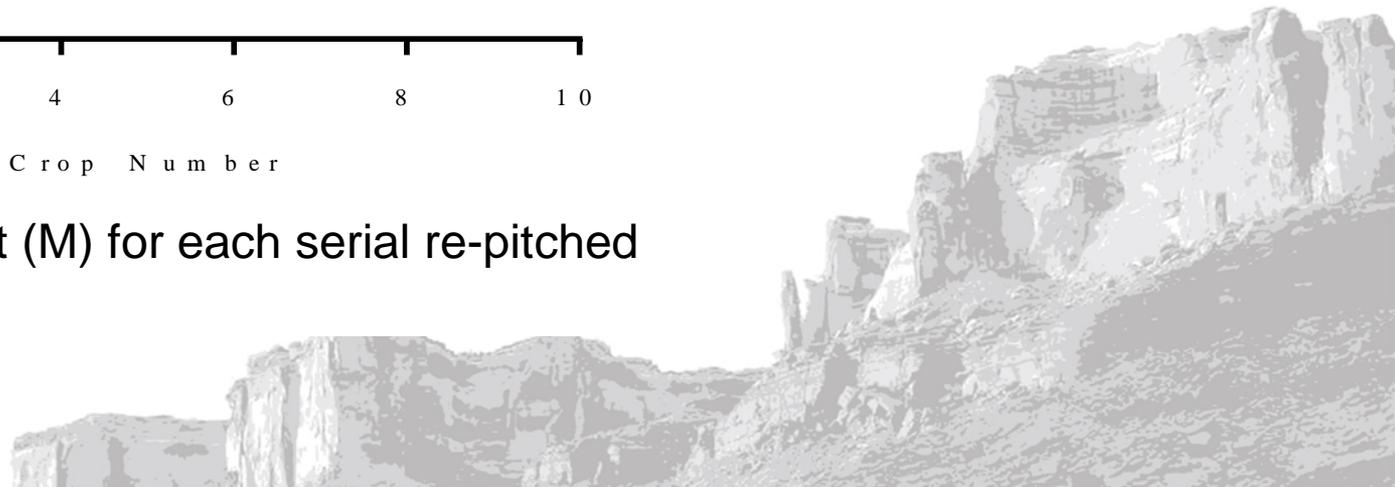


# Did the length of the fermentation change between crop number?



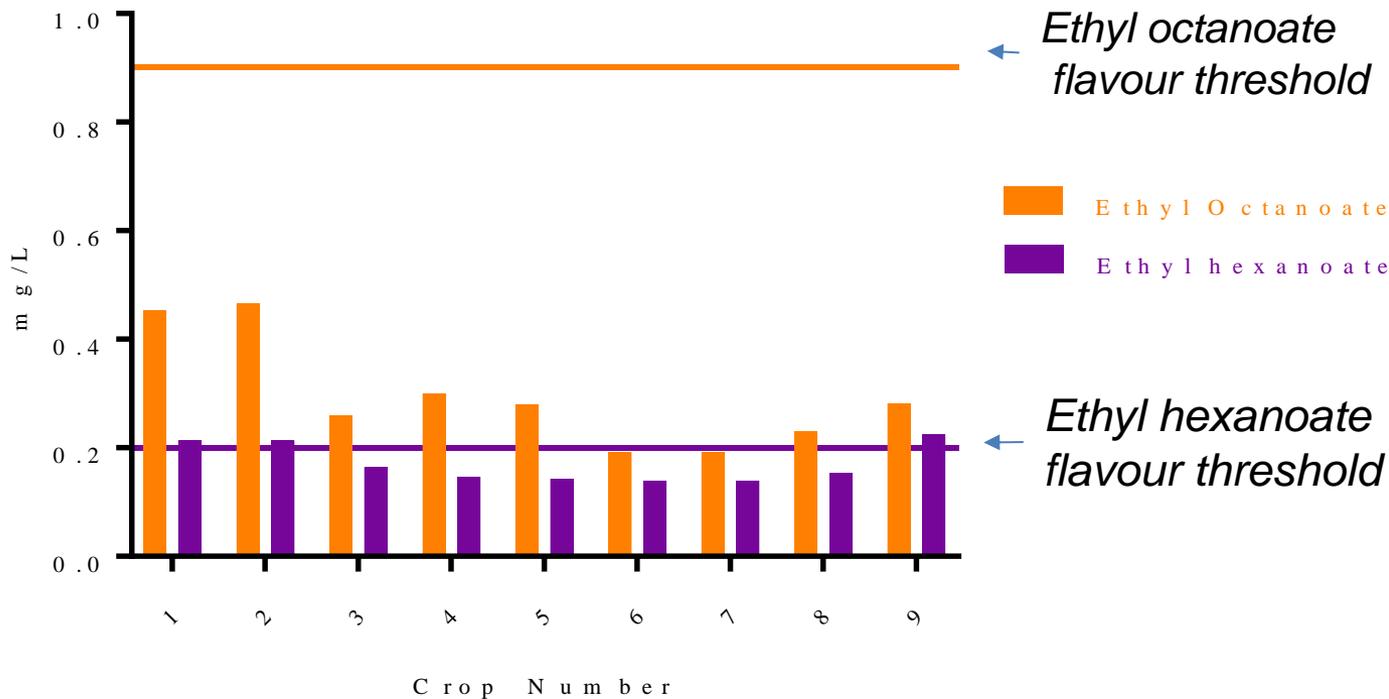
An F-Test on the slope showed the crop number had no significant ( $p > 0.05$ ) correlations to on the midpoint

**Figure 3.** Midpoint (M) for each serial re-pitched lager fermentation





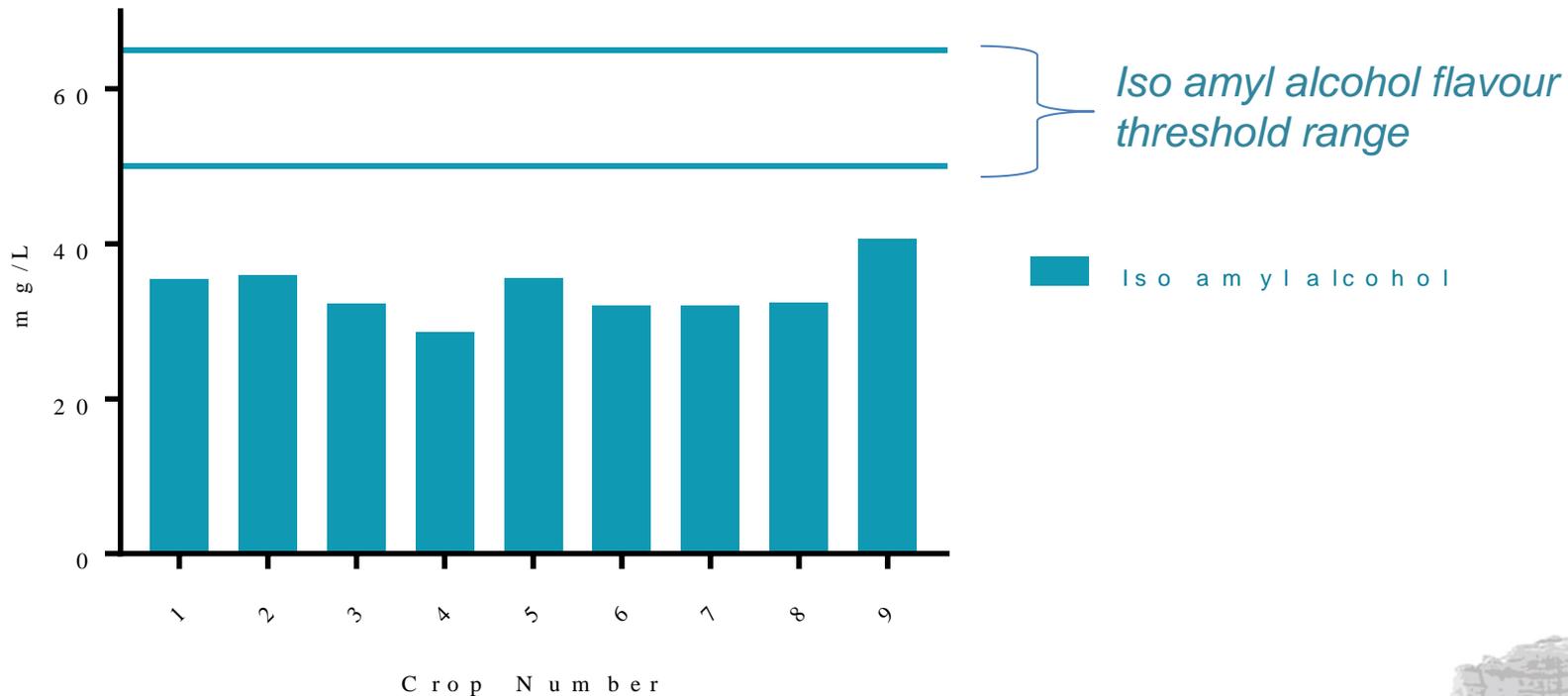
# Ester levels post fermentation with respect to crop number



**Figure 4.** Ethyl ester levels in the beer at hour 122 in fermentation for each fermentation with an increasing crop number



# Iso amyl alcohol levels compared to crop number



**Figure 5.** Iso amyl alcohol levels in the beer at hour 122 in fermentation for each fermentation with an increasing crop number



# Was the crop number maximized?

- Density attenuation had no effect on crop number
  - **up to crop number 9**
- Flavour compound levels not correlated to crop number with the exception of ethyl octanoate, which was below flavour threshold

## Future work

- Wort analysis along with fermentation monitoring

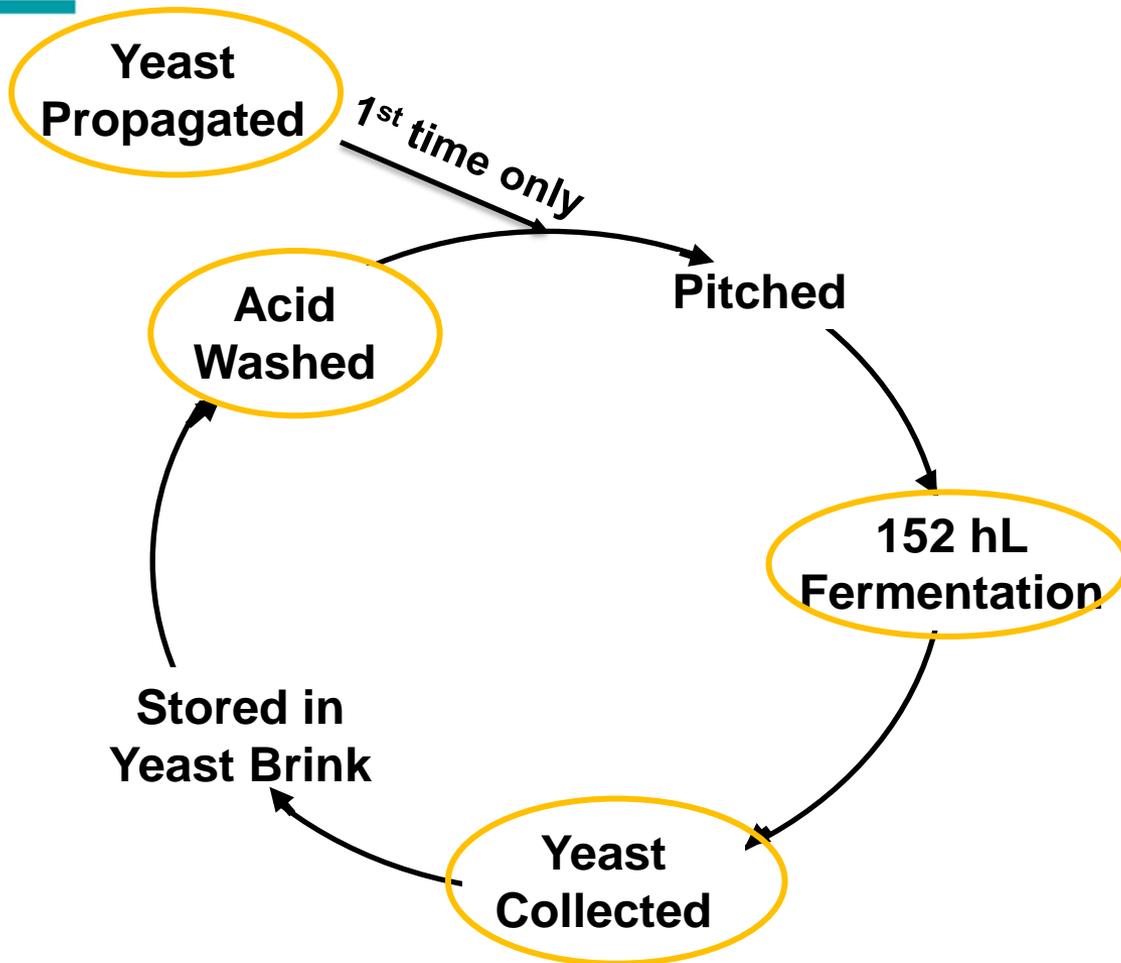


# CASE STUDY: RE-PITCHED ALE FERMENTATIONS





# Ale Case Study: Experimental Design

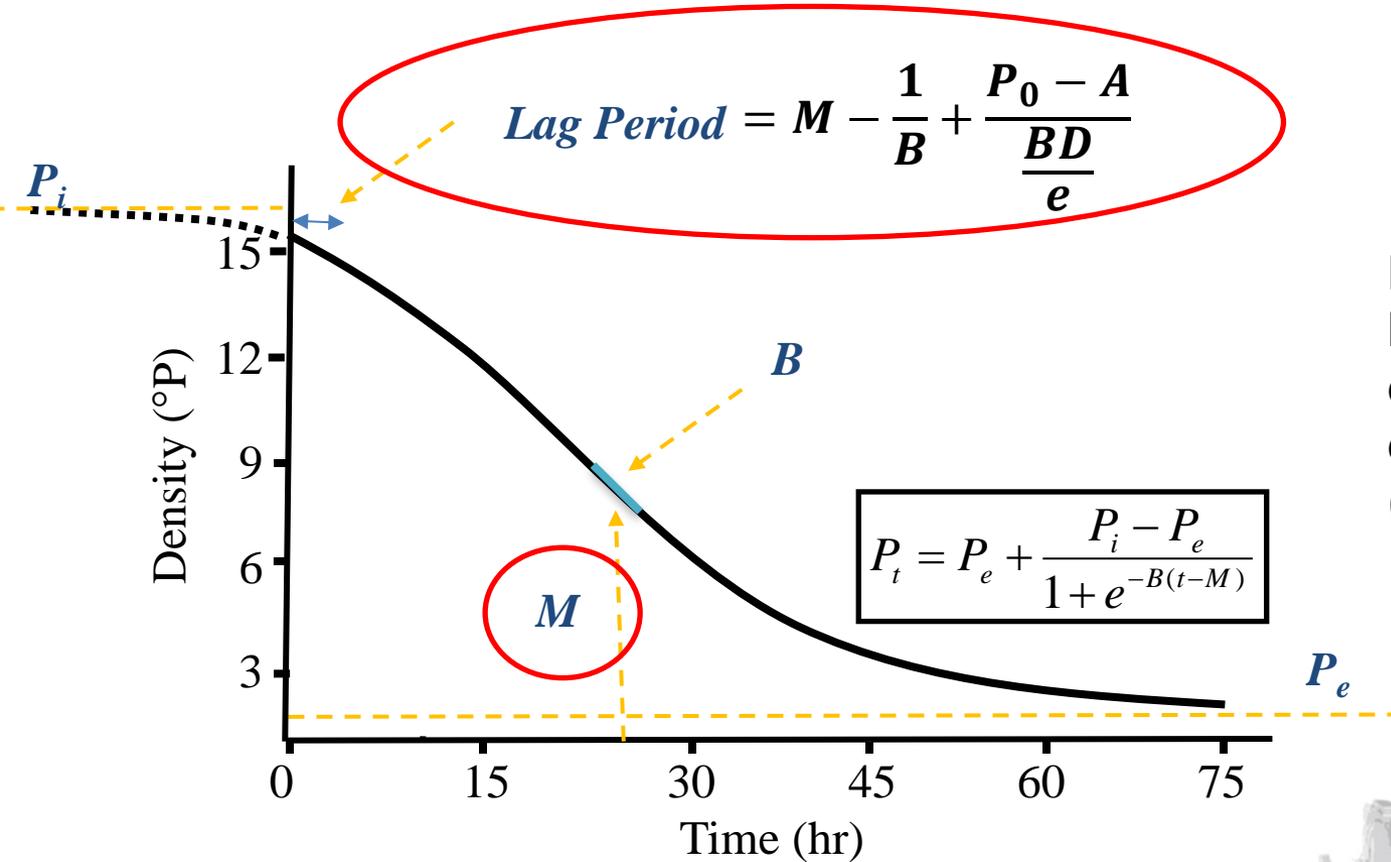


## Fermentation Monitoring

- Density monitored
- Sugar analysis t=0 and 95% complete
- Flavour analysis t=0 and 95% complete
- Initial absorbance (600nm)



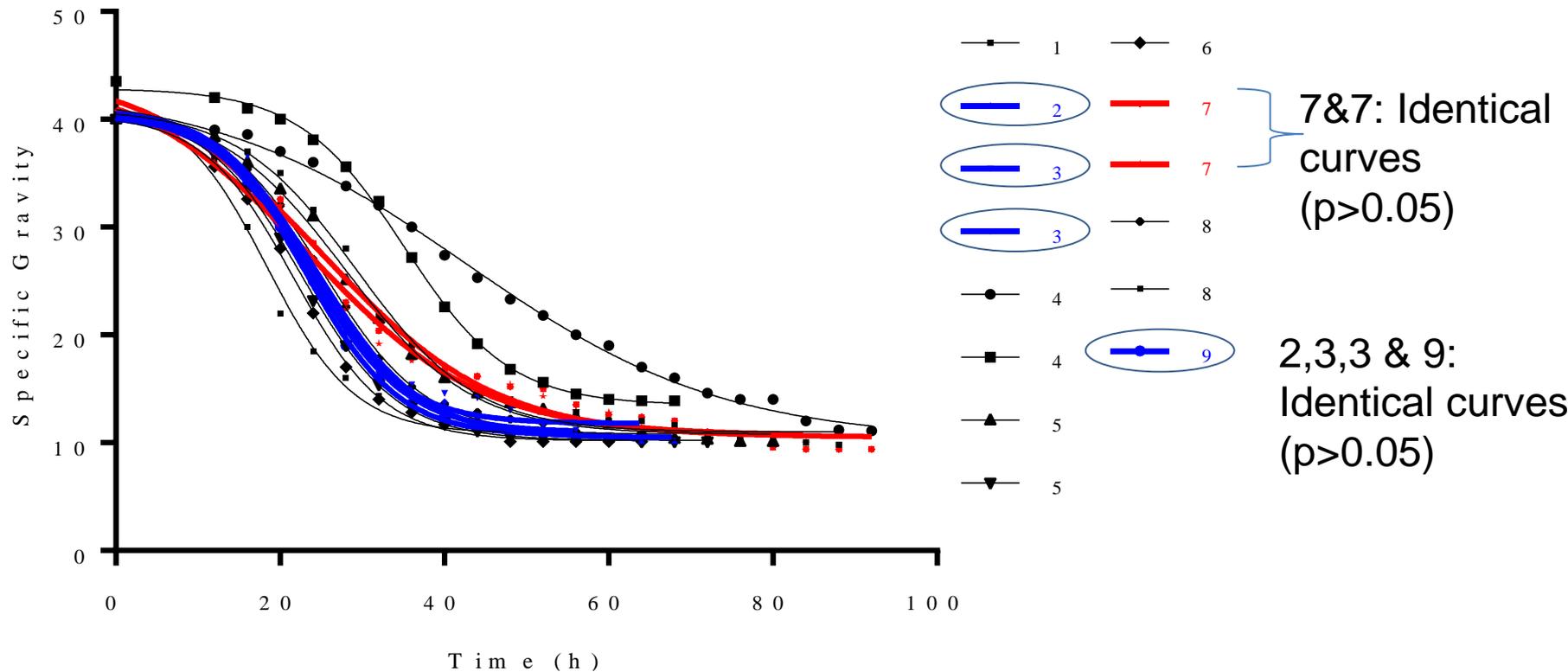
# Nonlinear Regression Analysis



**Figure 1.** Nonlinear logistic describing density attenuation during fermentation (ASBC Yeast-14)



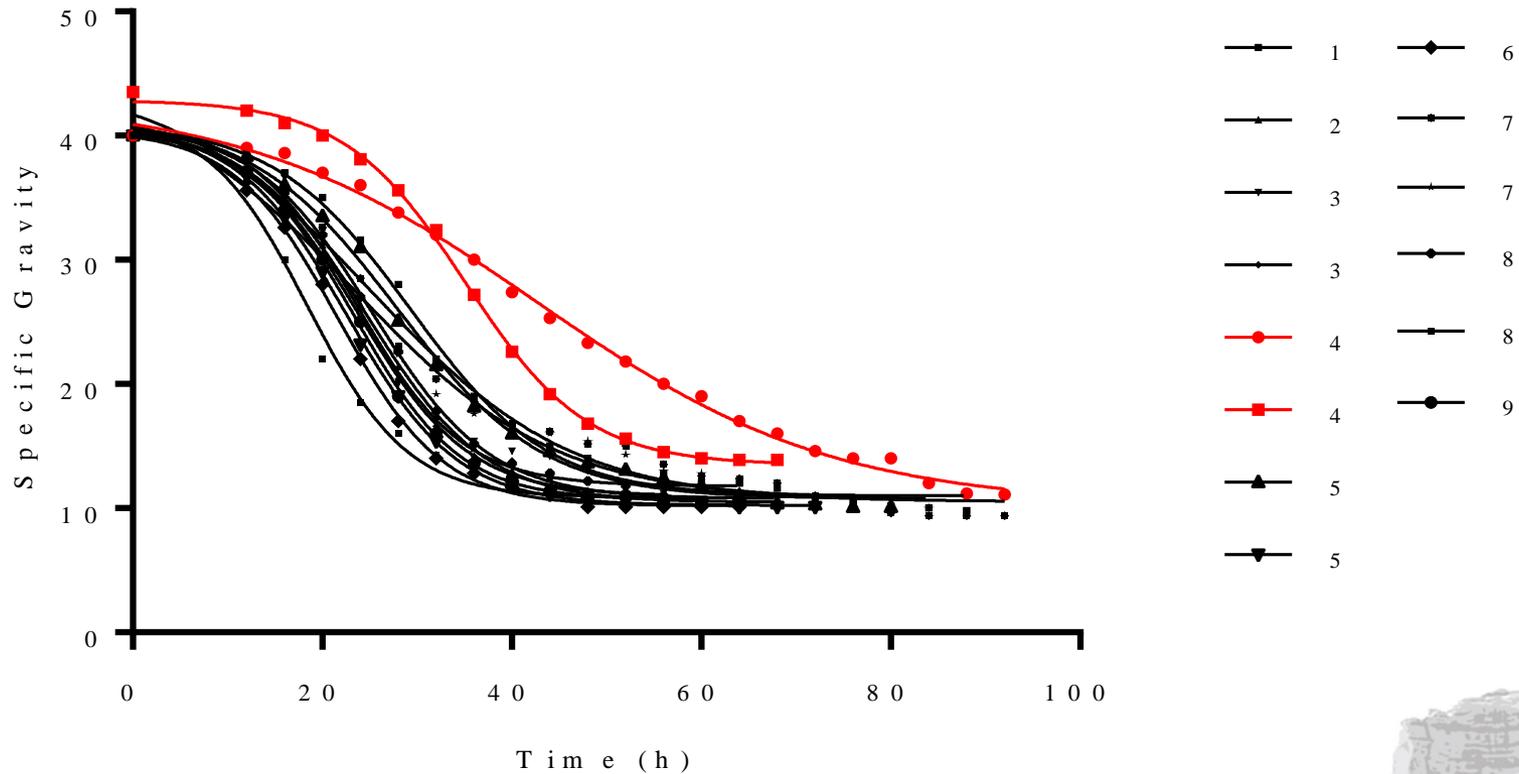
# Significance of density attenuation curves?



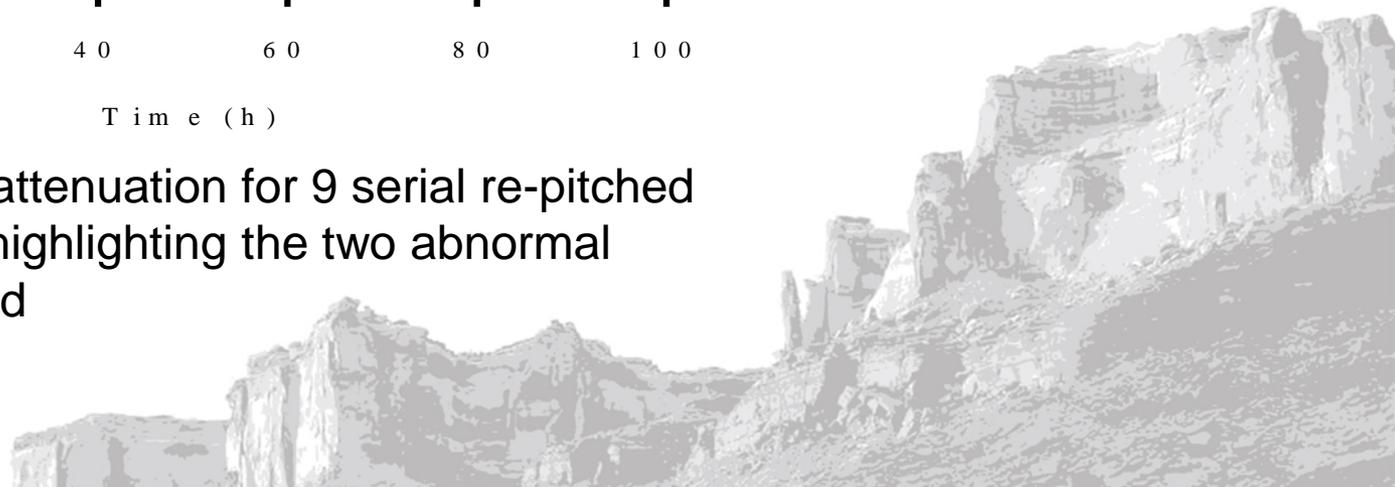
**Figure 6.** Specific gravity attenuation data with a yeast crop that was re-pitched up to 9 times that was modelled using a four parameter nonlinear regression.



# Two abnormal fermentations

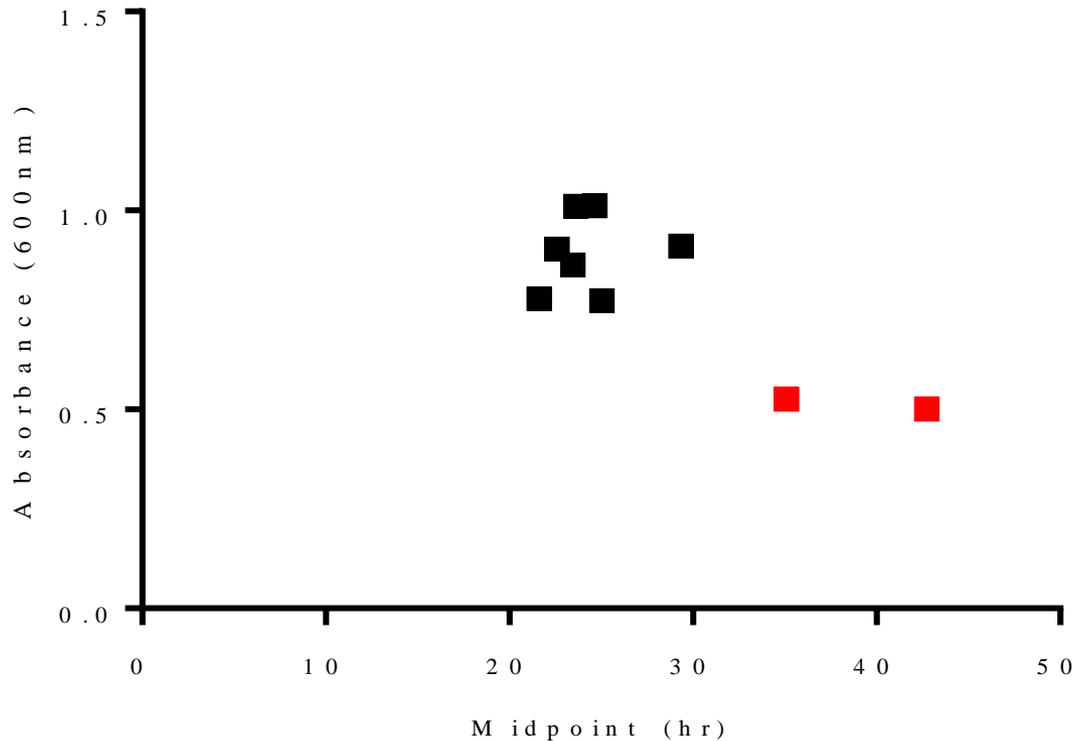


**Figure 7.** Density attenuation for 9 serial re-pitched ale fermentations highlighting the two abnormal fermentations in red

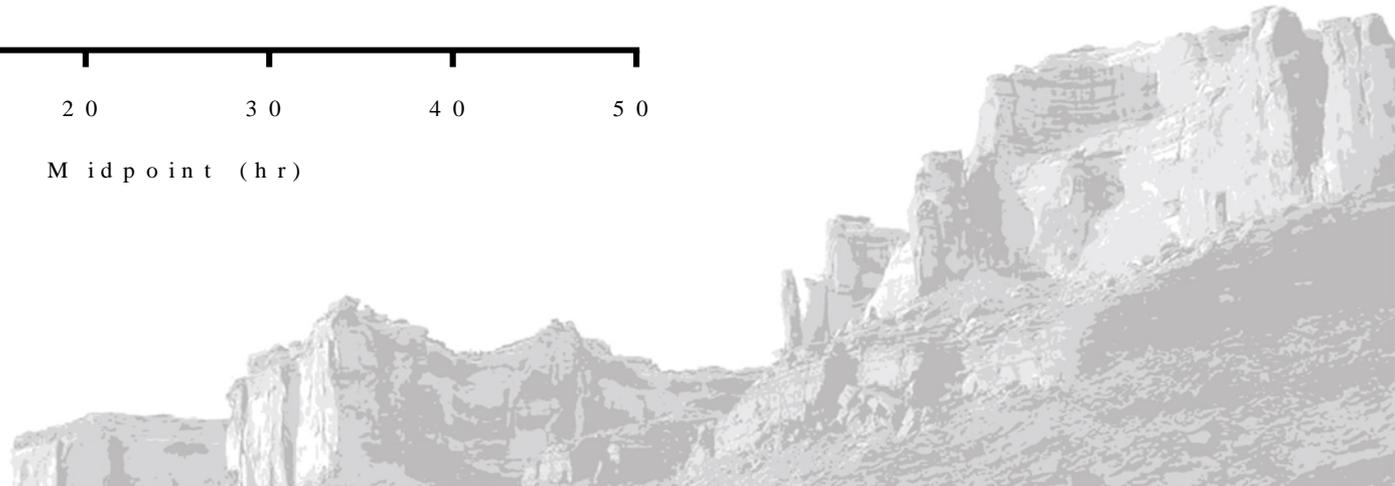




# Abnormal fermentations a result of under pitching

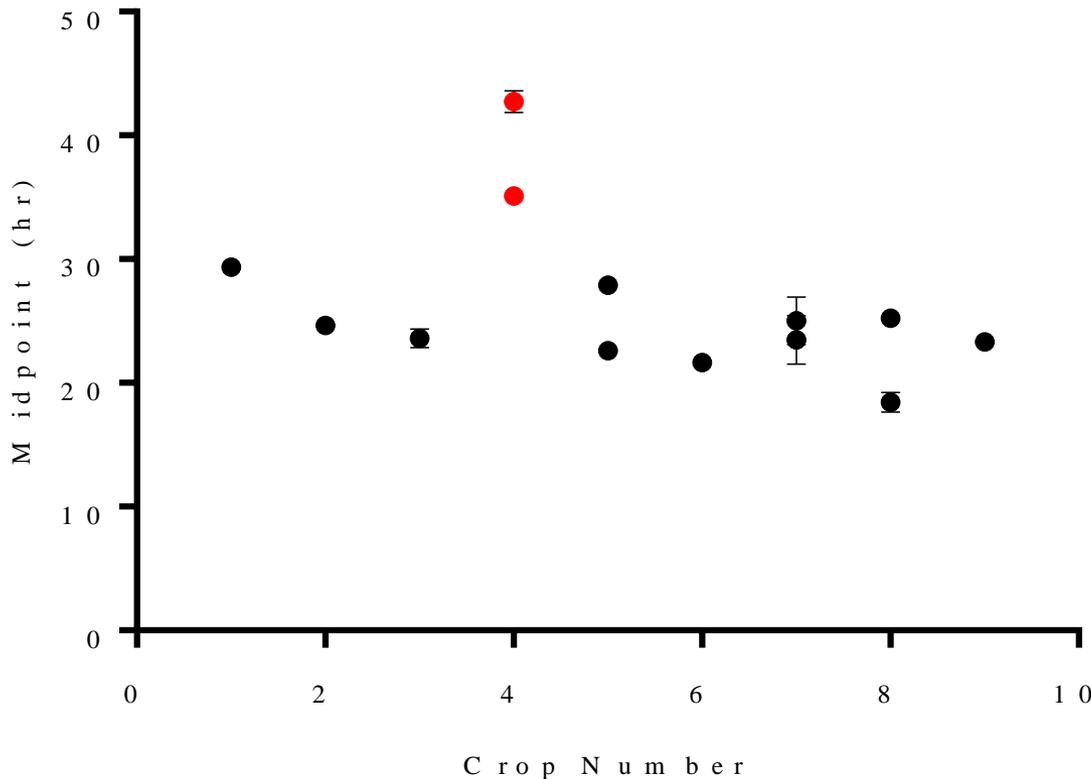


**Figure 8.** The midpoint of the fermentation compared to the initial absorbance relating to the quantity of yeast at the start of fermentation. The two data points in red are the noted abnormal fermentations



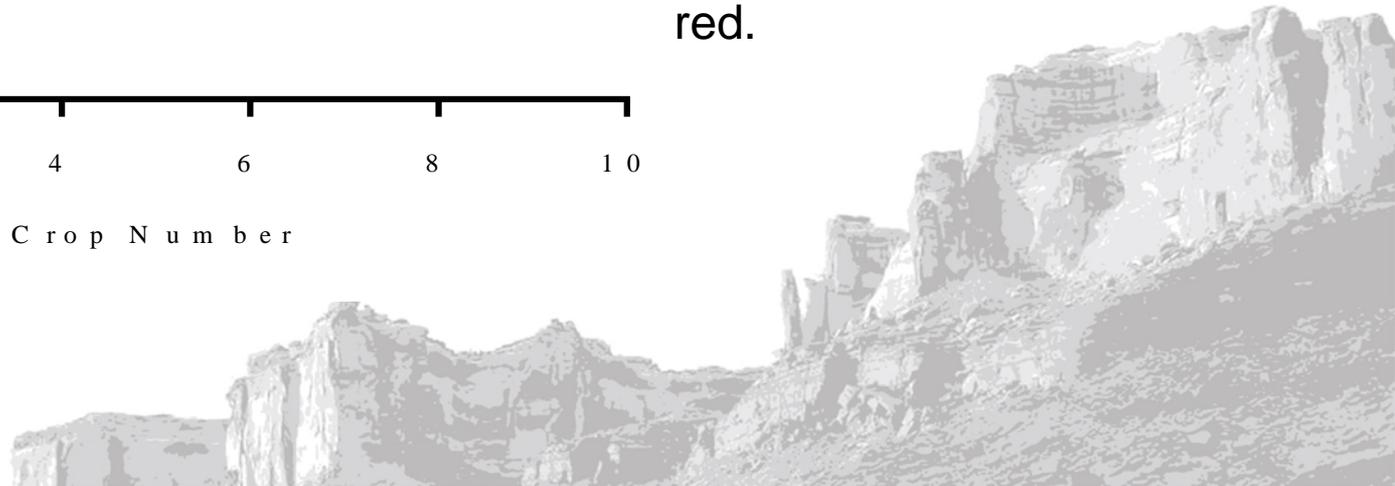


# Midpoint (hr) for each serially re-pitched fermentation



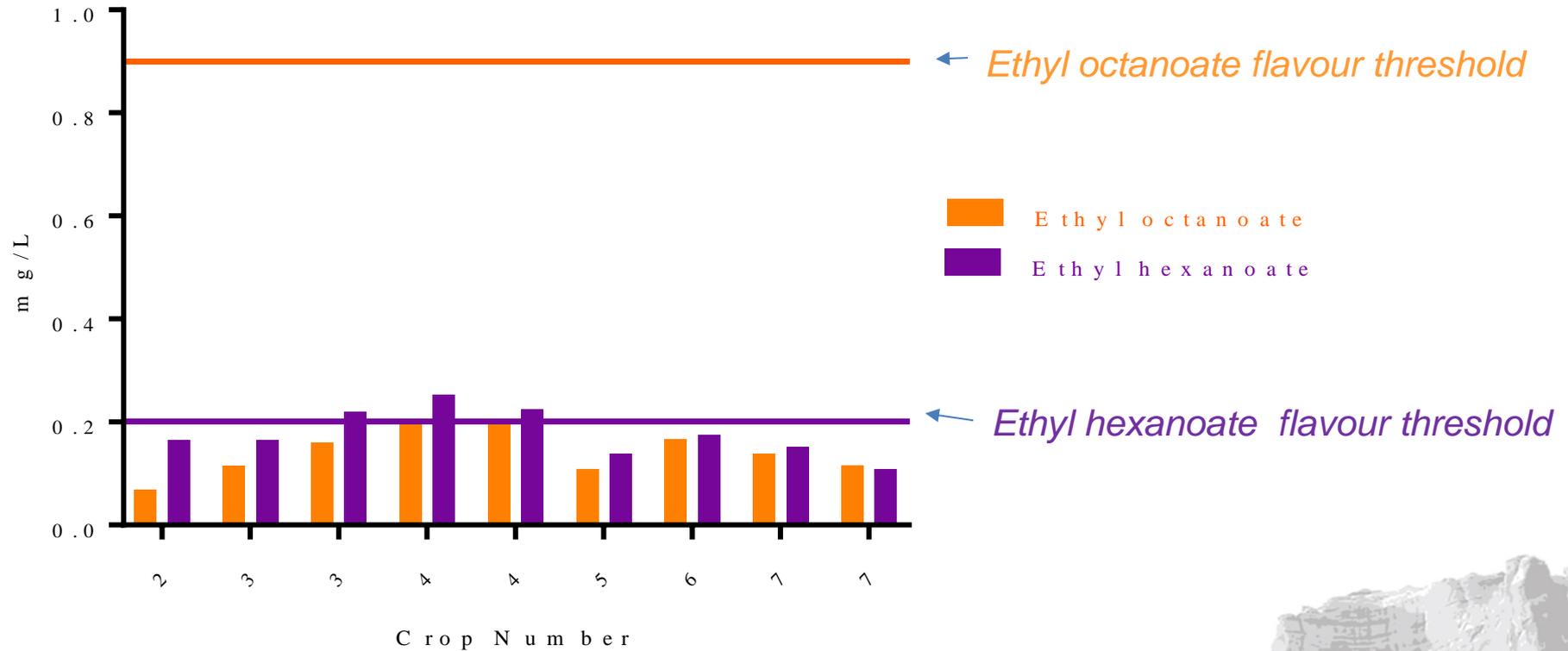
An F-Test on the slope showed the crop number had no significant ( $p > 0.05$ ) correlations to on the midpoint

**Figure 9.** The midpoint for each re-pitched fermentation with the two abnormal fermentations in red.





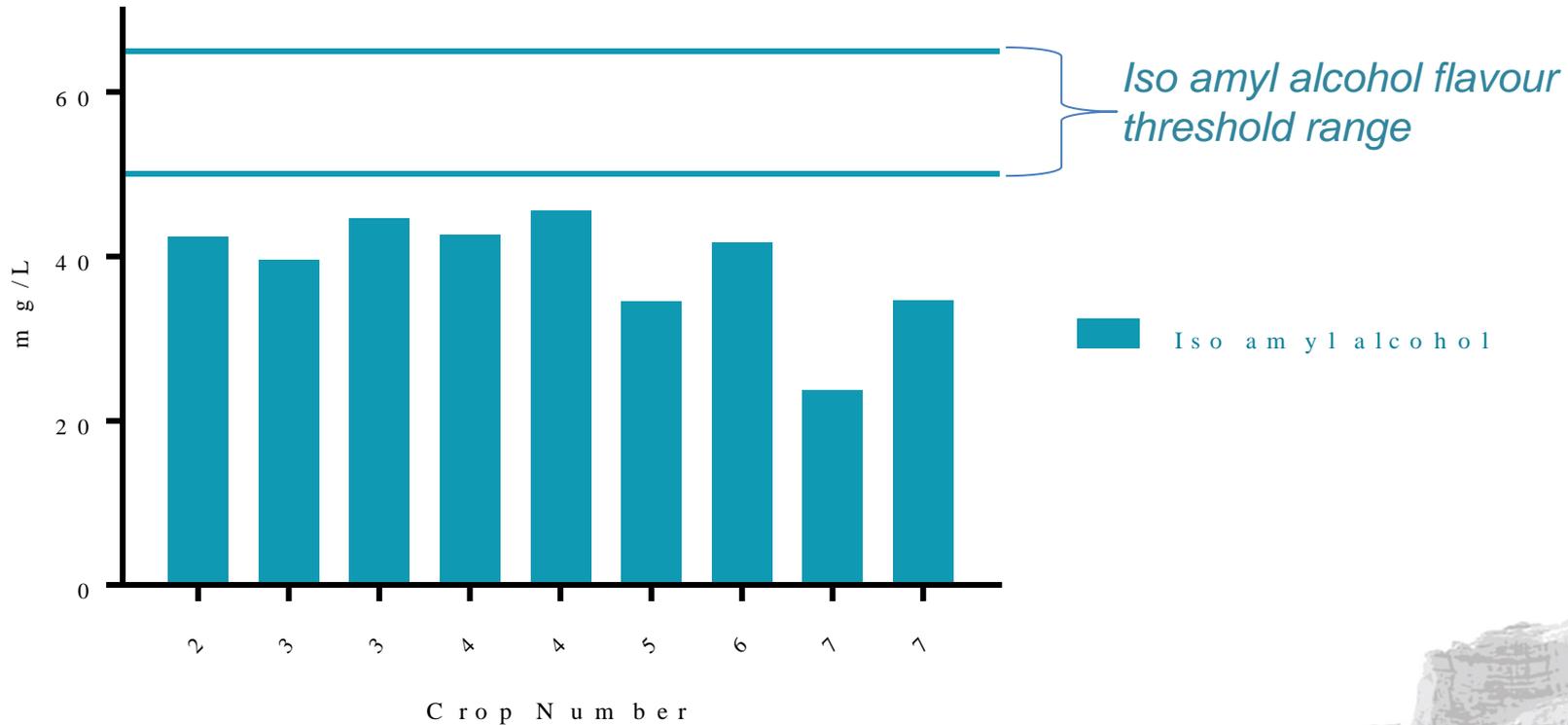
# Ester levels post fermentation with respect to crop number



**Figure 10.** Ethyl ester levels in the beer at 95% complete fermentations as the crop number increased.



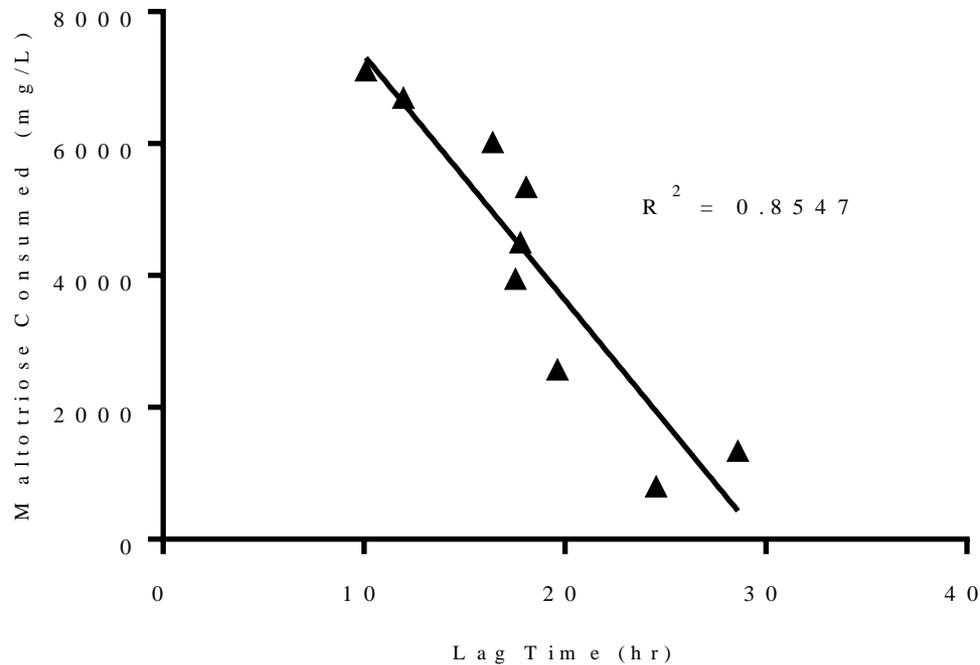
# Iso amyl alcohol levels with respect to crop number



**Figure 11.** Iso amyl alcohol levels in the beer at 95% complete fermentations as the crop number increased.



# Trends in maltotriose consumption with lag period



- As the lag time increased, the maltotriose consumed by 95% complete fermentations decreased

**Figure 12.** Linear correlations between maltotriose consumed and the lag time of the fermentation



# Was the crop number maximized?

- Same as the lager case study, the density attenuation not indicative of drifting in one direction based on crop number **up to crop number 9**
- Flavour compound levels were not correlated to the crop number

## Future work

- Maltotriose deserves further investigation





**WORLD BREWING CONGRESS**

August 13–17, 2016 • Denver, Colorado, U.S.A.

#ElevateBeer



# Summary

- With both case studies, there was no indicators that the changes in density attenuation or flavour profiles were due to the crop number
- Potential to investigate extending the crop number





# Literature

- Bühligen, F., P. Lindner, I. Fetzer, F. Stahl, T. Scheper, H. Harms and S. Müller (2014). "Analysis of aging in lager brewing yeast during serial repitching." Journal of Biotechnology **187**: 60-70.
- Bühligen, F., P. Rüdinger, I. Fetzer, F. Stahl, T. Scheper, H. Harms and S. Müller (2013). "Sustainability of industrial yeast serial repitching practice studied by gene expression and correlation analysis." Journal of Biotechnology **168**: 718-728.
- Cutaia, A. J., A.-J. Reid and R. A. Speers (2009). "Examination of the Relationships Between Original, Real and Apparent Extracts, and Alcohol in Pilot Plant and Commercially Produced Beers." Journal of the Institute of Brewing **115**(4): 318-327.
- Jenkins, C. L., A. I. Kennedy, J. A. Hodgson, P. Thurston and K. A. Smart (2003). "Impact of Serial Repitching on Lager Brewing Yeast Quality." Journal of the American Society of Brewing Chemists **61**(1): 1-9.
- Miller, K. J., W. G. Box, D. M. Jenkins, C. Boulton, R. S. T. Linforth and K. A. Smart (2013). "Does Generation Number Matter? The Impact of Repitching on Wort Utilization." Journal of the American Society of Brewing Chemists **71**(4): 233-241.
- Powell, C. and T. Fischborn (2010). "Serial Repitching of Dried Lager Yeast." Journal of the American Society of Brewing Chemists **68**(1): 48-56.
- Powell, C. D. and A. N. Diacetis (2007). "Long Term Serial Repitching and the Genetic and Phenotypic Stability of Brewer's Yeast." Journal of the Institute of Brewing **113**(1): 67-74.
- Smart, K. A. and S. Whisker (1996). "Effect of Serial Repitching on the Fermentation Properties and Condition of Brewing Yeast." Journal of the American Society of Brewing Chemists **54**(1): 41-44.
- Somani, A., F. Bealin-Kelly, B. Axcell and K. A. Smart (2012). "Impact of Storage Temperature on Lager Brewing Yeast Viability, Glycogen, Trehalose, and Fatty Acid Content." Journal of the American Society of Brewing Chemists **70**(2): 123-130.
- Speers, R. A., P. Rogers and B. Smith (2003). "Non-Linear Modelling of Industrial Brewing Fermentations." Journal of the Institute of Brewing **109**(3): 229-235.
- Speers, R. A. and S. Stokes (2009). "Effects of Vessel Geometry, Fermenting Volume and Yeast Repitching on Fermenting Beer." Journal of the Institute of Brewing **115**: 148-150.
- Speers, R. A., Y.-Q. Wan, Y.-L. Jin and R. J. Stewart (2006). "Effects of Fermentation Parameters and Cell Wall Properties on Yeast Flocculation." Journal of the Institute of Brewing **112**(3): 246-254.



# Thanks and acknowledgments

- International Centre for Brewing and Distilling (ICBD)
  - Alex Speers (ICBD and Dalhousie)
  - Dawn Maskell
  - James Bryce
  - Annie Hill
  - Matthew Pauley
  - Margaux Huismann
- Scottish breweries
  - Production crew and technical staff
- Institute of Brewing and Distilling



Institute of Brewing & Distilling





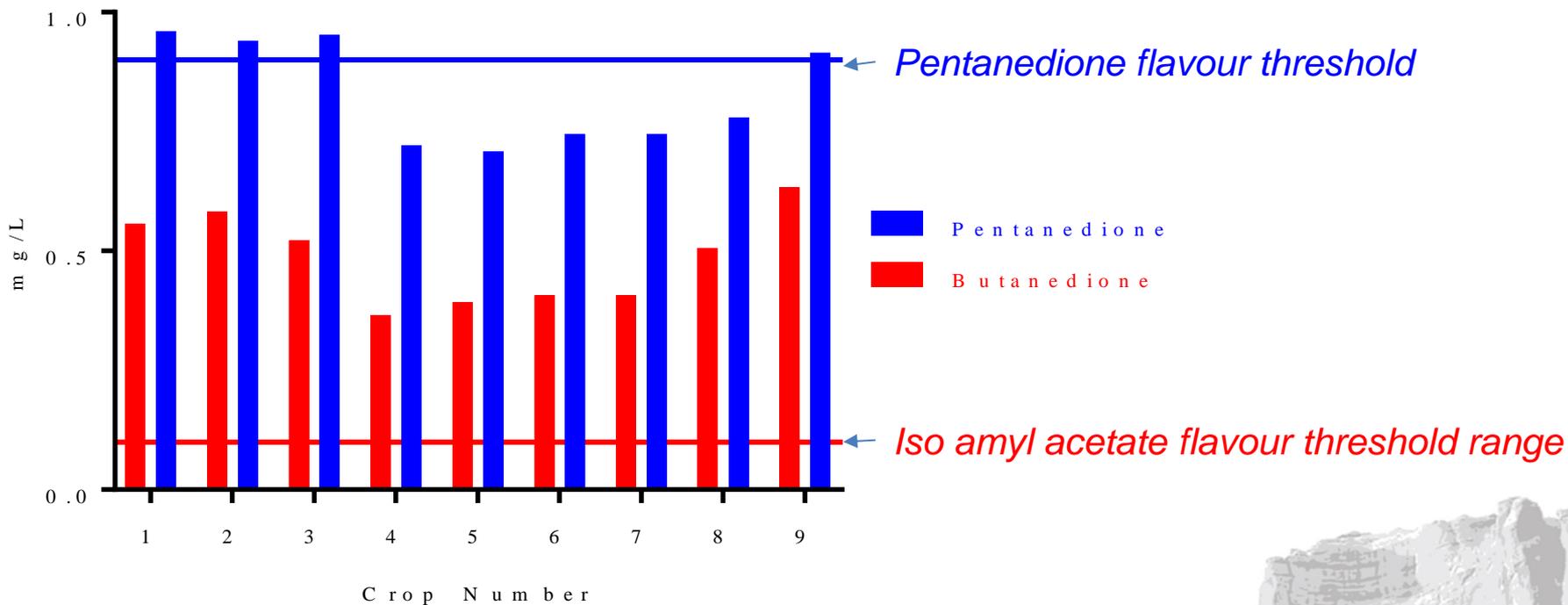
# Thank you for listening!

Maria Josey  
ICBD, Heriot-Watt University  
Edinburgh, U.K.  
Email: [mej2@hw.ac.uk](mailto:mej2@hw.ac.uk)





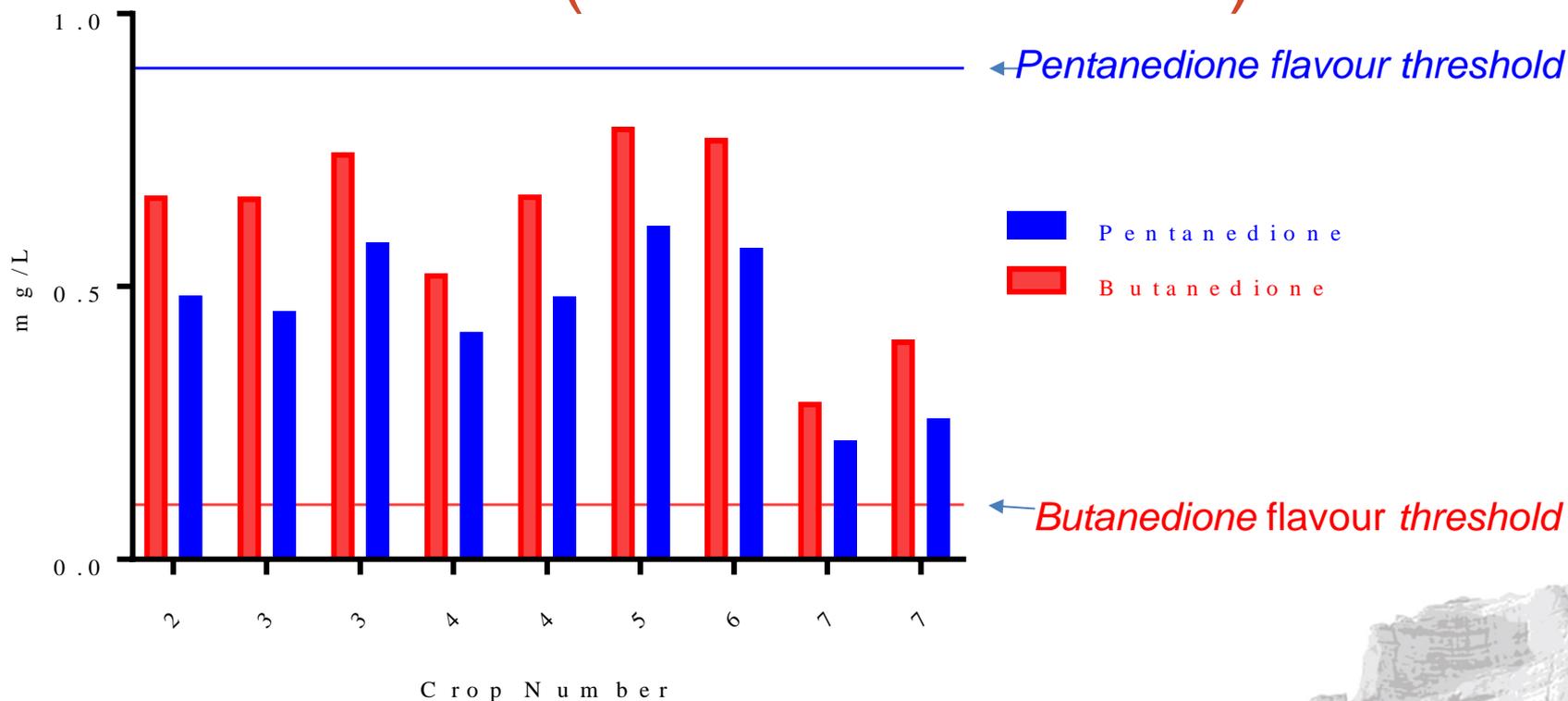
# Vicinal diketone levels post fermentation with respect to crop number (Lager Fermentations)



**Figure 5.** Vicinal diketone levels in the beer at hour 122 in fermentation for each fermentation with an increasing crop number



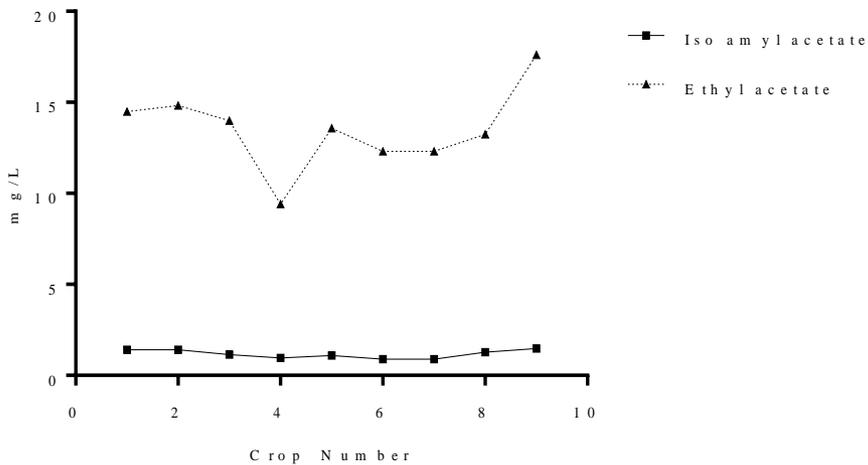
# Vicinal diketones present post fermentation with respect to crop number (Ale Fermentations)



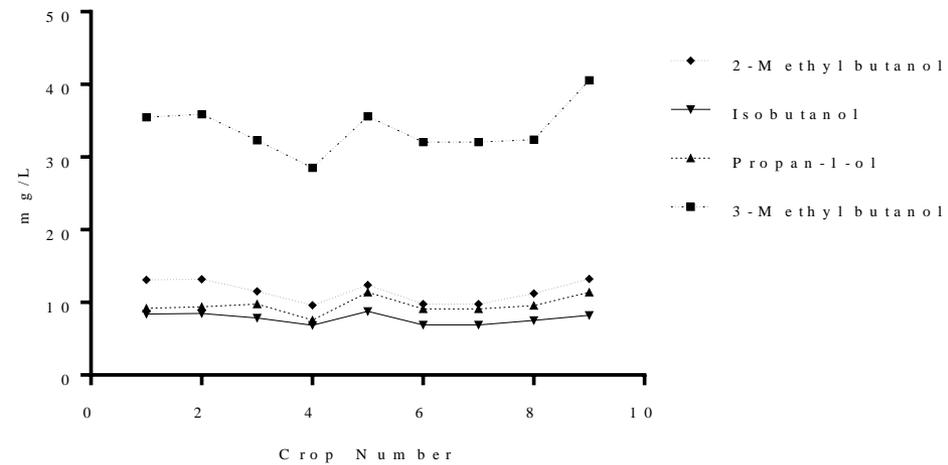
**Figure 11.** Vicinal diketone levels in the beer at 95% complete fermentations as the crop number increased.

# Esters and Higher Alcohols Analysed in the Serial Re-Pitching with Lager Yeast Case Study

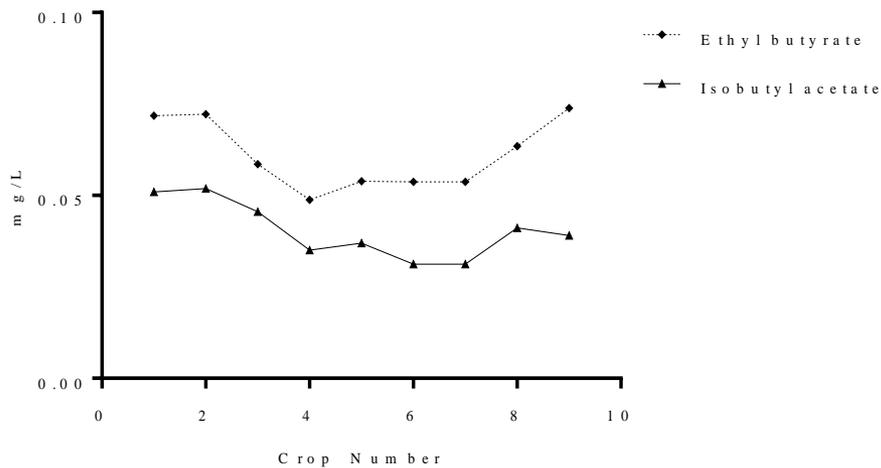
Ethyl Acetate and Iso Amyl Acetate



Higher Alcohols



Isobutyl Acetate and Ethyl butyrate



Ethyl Octanoate and Ethyl Hexanoate

