REDUCING DIACETYL: ONE BREWERY’S EXPERIENCE WITH APPLICATION OF ALDC

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AGENDA

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2
How is VDK formed?

3
Diacetyl Reduction: Traditional vs. Enzyme Addition

4
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5
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6
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1.
WHAT IS DIACETYL &
WHY IS IT SO IMPORTANT?

WHAT IS DIACETYL

Vicinal Diketone (VDK)

Diacetyl is a Vicinal Diketone - commonly referred to as “VDK”

Primary feature is the ketone, characterized as carbon atom joined to an oxygen atom by a double bond and to other carbon or hydrogen atoms by single bonds: >C=O

Note: aldehydes also have the same C=O functional group.

VDKs have 2 of these functional groups in close proximity. They are adjacent or in the vicinity (vicinal) to one another.

Most problematic is diacetyl (2,3 butanedione), but there is also 2,3 pentanedione.

VDKs are the result of yeast metabolism and possible bacterial contamination

Most important is their organoleptic effect on beer.

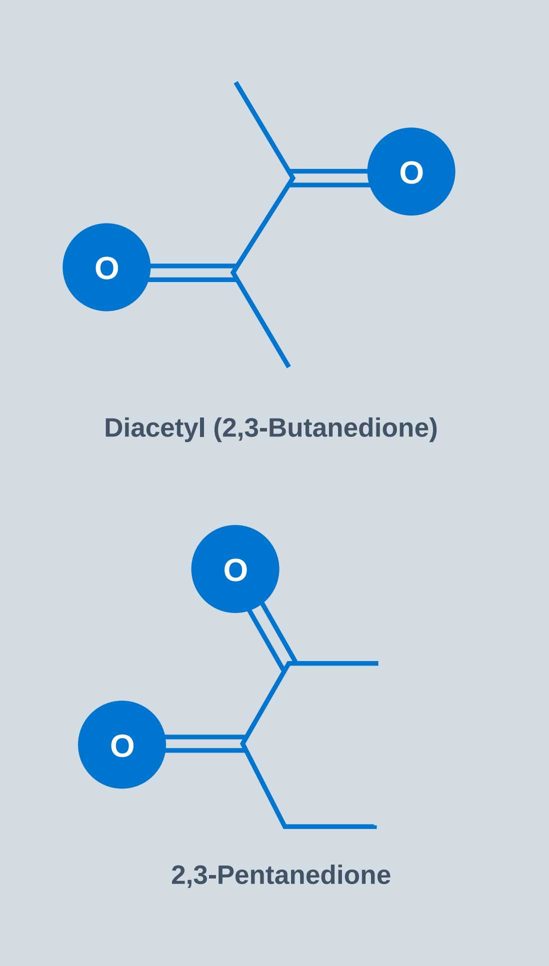


Figure 1: VDKs

WHY THE FOCUS?

Vicinal Diketone (VDK)

Why is it important?

Diacetyl (2,3-butanedione) and 2,3-pentanedione are among the most important immature beer aromas.

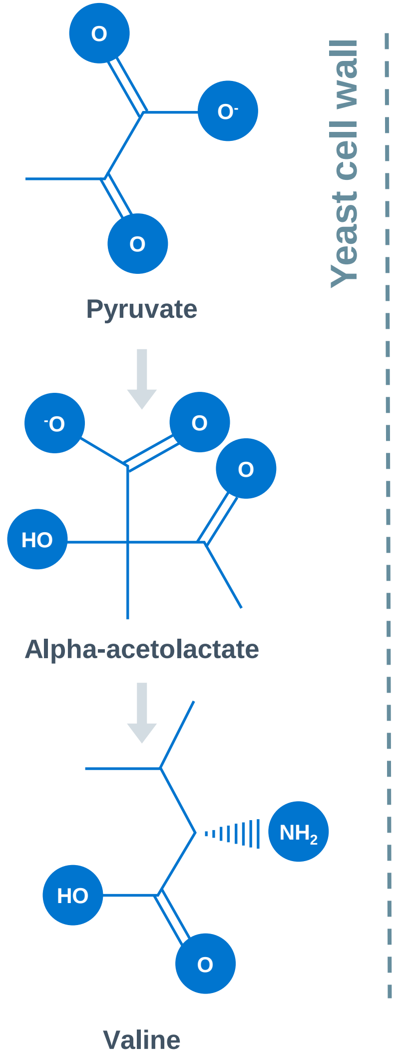
The reduction of VDKs during beer conditioning is an important indicator of the progress of beer maturation.

Considered to be an undesirable contributor to the flavor of finished beers.

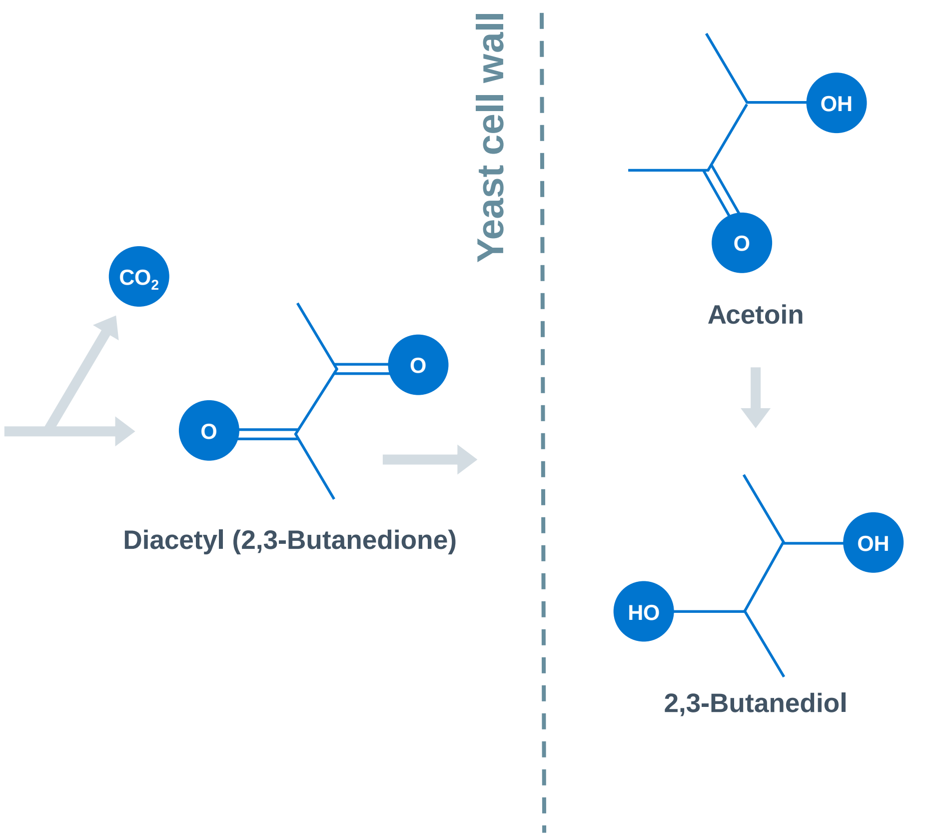
- In lager beer, diacetyl is generally considered a defect, but a low level of detectable diacetyl may be acceptable in some beer styles.
- Diacetyl is also produced by pediococcus and some lactobacillus. Therefore, an indication of contamination.
- Sensory threshold for the total VDK content for a fully matured beer is 0.1mg/l = 100 ppb.
  - Diacetyl (2,3-butanedione) = butterscotch, buttery, popcorn.
  - 2,3-pentanedione = honey-like flavor.

2.
HOW IS DIACETYL FORMED ?

HOW IS DIACETYL FORMED?



The reaction from pre-cursor to diacetyl is a chemical reaction. It takes place outside the yeast cell and it is the rate limiting step in the entire process. Yeast will quickly absorb diacetyl once it is formed—to get NAD+ back.



- The precursor of diacetyl is 2-acetolactate - an intermediate in the biosynthesis of valine, and leucine, from pyruvate.
- 2 -acetolactate is excreted from the yeast and is converted to diacetyl by a spontaneous chemical oxidative decarboxylation reaction, taking place in the beer.
- The yeast cell is capable of reducing diacetyl into acetoin and further into 2,3-butanediol during maturation.
- It is the decomposition of the α-acetolactate to diacetyl in the beer which is the rate limiting step in the maturation process.
- The reduction of diacetyl into acetoin, inside the yeast cell, is 10x faster. This reaction provides energy and NAD+, vital for glycolysis, to the yeast cell.

3.
DIACETYL REDUCTION: TRADITIONAL VS. ENZYMATIC METHODS

TRADITIONAL VS. ENZYMATIC

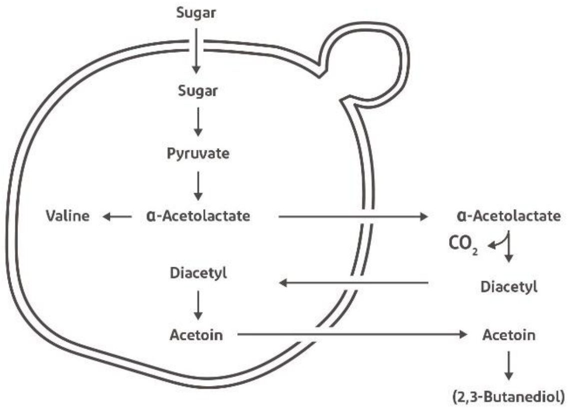
How ALDC application works to reduce diacetyl and maturation time

Enzyme application by-passes diacetyl and 2,3-pentanedione generation.

Converts **α-aceto-lactate** and α-aceto-hydroxy-butyrate **directly into the flavorless compounds acetoin** and 2,3-pentanediol.

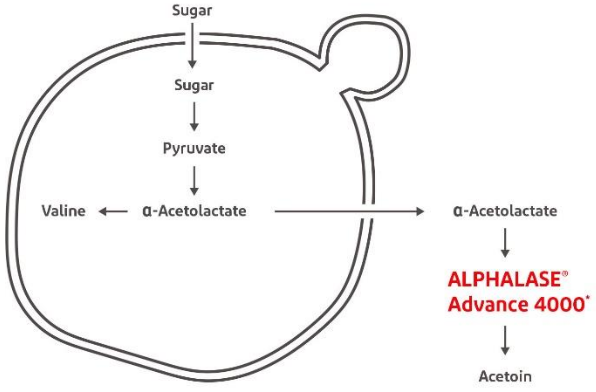
Please note that both processes are taking place simultaneously.

Traditional



Slow removal of diacetyl by yeast (extracellular oxidation)

Enzymatic



Action pattern of ALPHALASE® Advance 4000 during fermentation

* Generation and reduction of diacetyl and 2,3-pentanedione with and without the presence of ALPHALASE® Advance 4000 during fermentation. The two reaction patterns compete with one another.

Traditional Method:
- Slow
- Influenced by temperature and pH
- Wort composition
- Wort gravity
- Needs sufficient amount of yeast in suspension
- Needs viable and vital yeast in suspension
- Tank geometry

ALDC Addition:
- Add enzyme in cold wort
- The enzyme converts pre-cursor directly to end product.
- Shortens maturation period.
- Lower diacetyl (VDK) levels
- More consistent diacetyl (VDK) levels.
- Beer is ready to transfer earlier, reducing process times.

ALPHALASE® ADVANCE 4000

Usage Guidelines

ALPHALASE® Advance 4000 is an α-acetolactate decarboxylase (ALDC) recommended to be dosed at the beginning of the main fermentation

Recommended dosage
(in g per hl cold wort)

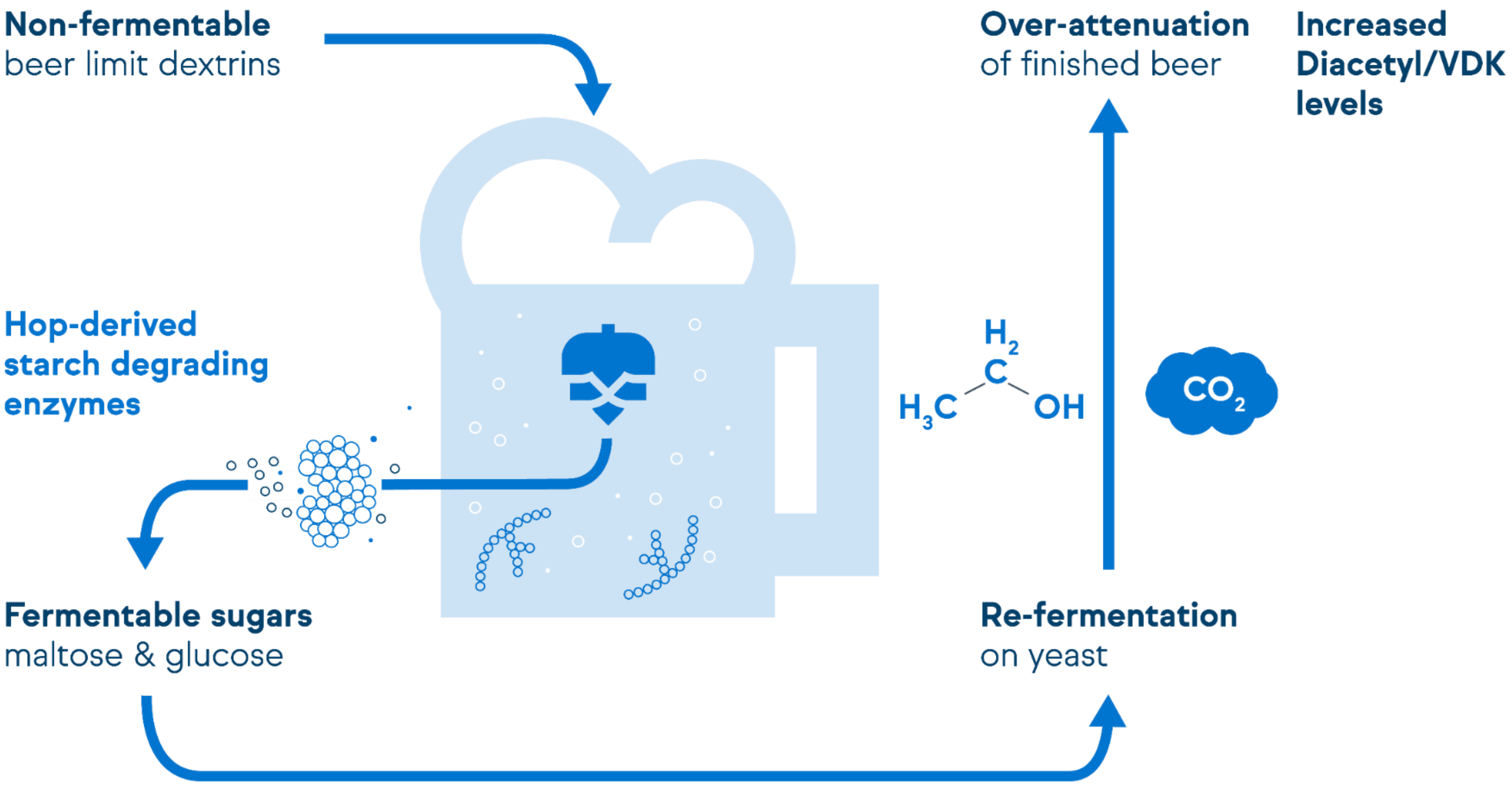
ALPHALASE® Advance 4000

RAW MATERIAL	DOSAGE GUIDELINE
All-malt wort – 12°P	0.5-0.7 g/hl
All-malt wort – 16°P	0.7-1.0 g/hl
Wort including 0-100% adjunct*	0.5-1.0 g/hl

*Depending on the adjunct type and local conditions
Optimal dose will depend on local conditions and experiences gained over time

4.
DRY HOPPING

HOP CREEP, EXPLAINED



If you do not pasteurize your dry hopped beers, you may encounter[8]:

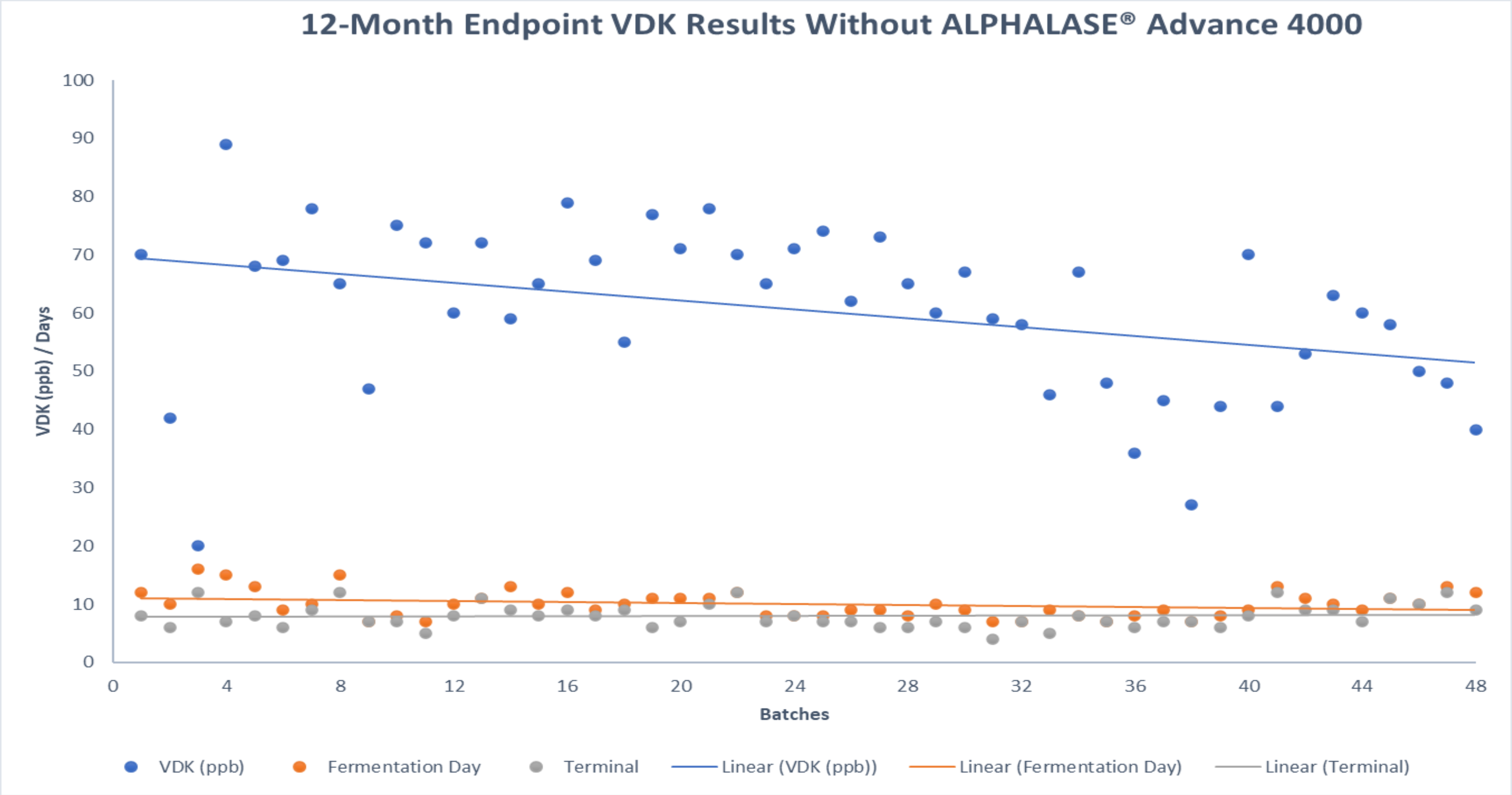
- % RDF increase
- AE decrease
- ABV increase
- CO_2 increase

(For example: Added at day four during fermentation)

Internal

5.
CASE STUDY

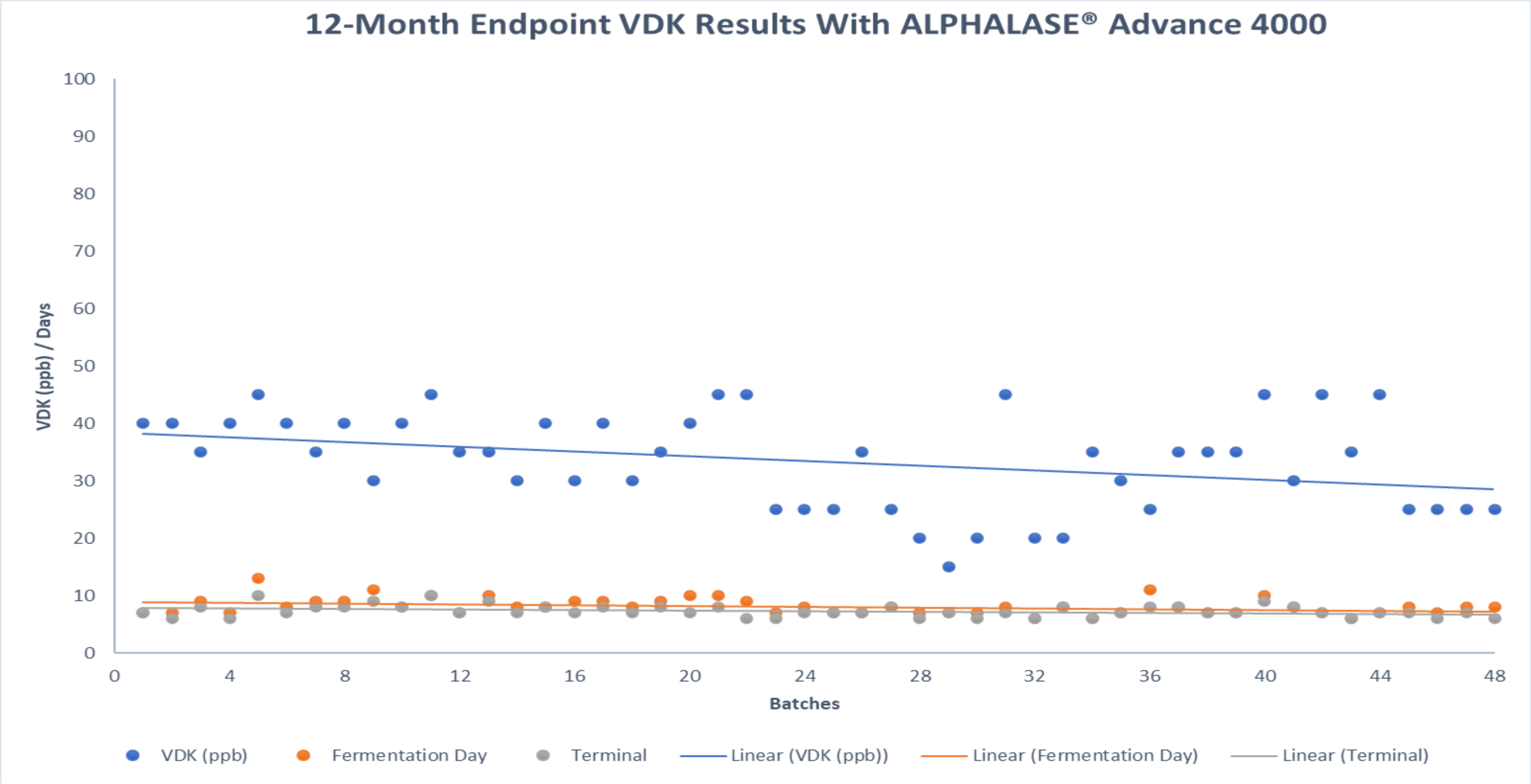
BEFORE: VDK REDUCTION WITHOUT AA-4000



VDK Specification:
70 ppb
ASBC Method
Beer-25 B.

Data: Single Lager Beer Brand, Urban Chestnut Brewing Co., (St. Louis, USA) - 2022

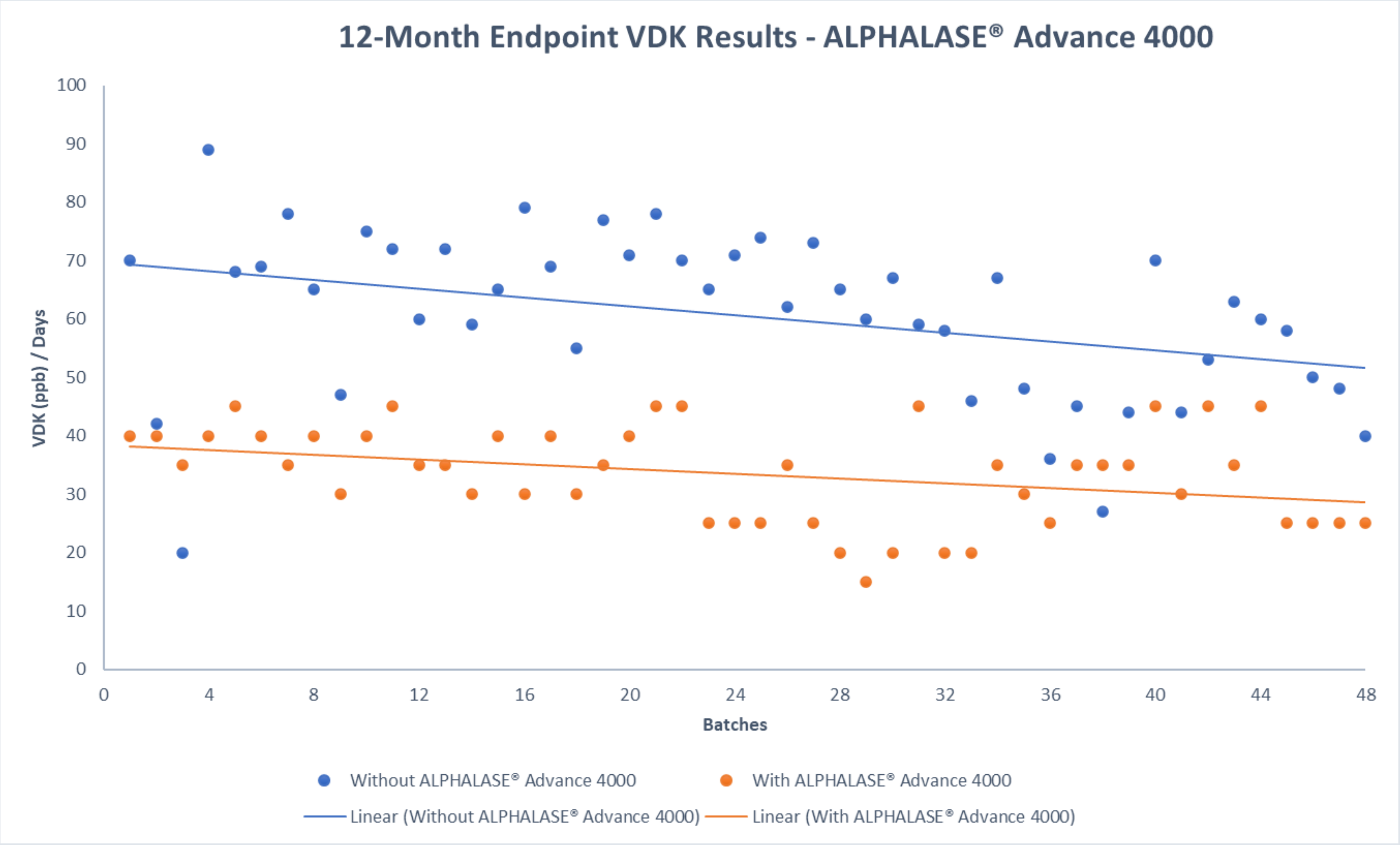
AFTER: VDK RESULTS WITH ADDITION OF AA-4000



VDK Specification:
50 ppb
ASBC Method
Beer-25 B.

Data: Single Lager Beer Brand, Urban Chestnut Brewing Co., (St. Louis, USA) - 2022

SUMMARY OF DIFFERENCE IN VDK LEVELS



Data: Single Lager Beer Brand, Urban Chestnut Brewing Co., (St. Louis, USA) - 2022

6.
VALUE FOR THE BREWERY

01 - Reduced Process Time

- Side-by-side controlled production trial predicted a reduced ‘brew to cool’ time by ~2 days
- Use of ALPHALASE® Advance 4000 for all lager fermentations over 12-months supported that prediction with additional benefits

02 - Low Diacetyl Levels in Beer

- Consistent, more predictable Diacetyl Levels in Beer – Increasing quality standards.
- Use of ALPHALASE® Advance 4000 allowed us to reduce our VDK finish spec – from 70 ppb to 50 ppb on all fermentations.
- Before using ALPHALASE ® Advance 4000, getting from 70 ppb to 50 ppb took yet another 2-3 days post terminal gravity.

03 - Consistent Process Times

More predictable process time for lager fermentations

Greatly benefits production management and packaging scheduling immensely by using ALPHALASE® Advance 4000 consistently

04 - Increased Production with Same Equipment

At our Grove brewery, we have about 4000 ‘tank days’ a year (11 Unitanks * 365 days a year)

With ALDC, we have observed a reduction in ‘dead tank days’ of diacetyl rest by 2-5 days

This means a potential increase in production capacity of 11% - 33% by getting to the cold conditioning phase more efficiently

RESOURCES

1. Hannemann, W. (2002). ‘Reducing beer maturation time and retaining quality’, *MBAA TQ,* vol. 39(3), pp 149-155.

2. Gibson et al. (2018). ‘Diacetyl control during brewery fermentation via adaptive laboratory engineering of the lager yeast Saccharomyces pastorianus’, *Journ Ind Micro & Biotechnology*, vol. 45, pp 1103-1112.

3. Krogerus, K. and Gibson, B. (2013). ‘125th anniversary review: diacetyl and its control during brewery fermentation’, *J Inst Brew,* vol. 119, pp 86-97.

4. Bamforth, C. and Kanauchi, M. (2004). ‘Enzymology of vicinal diketone reduction in brewer’s yeast’, *J Inst Brew,* vol. 11(2), pp 83-93.

5. Godtfredsen et al. (1984) ‘Application of the acetolactate decarboxylase from lactobacillus casei for accelerated maturation of beer’, *Carlsberg Res. Commun*., vol. 49, pp 69-74.

6. Bruner et al. (2021). ‘Dry-hop creep potential of various saccharomyces yeast species and strains’, *Fermentation*, 7(2), 66. available at: https://doi.org/10.3390/fermentation7020066.

7. Bruner et al. (2022) ‘Hop creep: new insights, research, and approaches for managing it’, *Craft Brewers Conference and BrewExpo America,* Minneapolis, MN, 3 May 2022.

8. Shellhammer, T. (2019) Hop-derived enzymes and hop creep [Online]. Available at: https://www.mbaa.com/meetings/districtpresentations/DistrictPresentations/SHELLHAMMER, Hop-derived enzymes and hop creep.pdf (Accessed 30 Oct 2022).

Thank you!

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QUESTION & ANSWER

To prevent late diacetyl formation formed by dry hoping, how do I use the enzyme?

To obtain maximum diacetyl reduction, dose enzyme at the start of fermentation and additional dose right before dry hopping. This is to prevent diacetyl formation because ALPHALASE® Advance 4000 loses activity at an increased rate due the to lower pH at the end of fermentation. For further insight, see relevant description of mechanism by Godfredsen et al.