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Comparison of ease of use, process, and reproducibility of two different fermentation systems: bioreactor and shaking flask

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Objective

This study aimed to compare a multi-strain Kveik yeast (MSK) and a single-strain commercial version (SSK) growth during aerobic propagation at 45°C using two different fermentation systems: a bioreactor and a incubator shaker. Additionally, the best system will be identified for propagating a wet yeast starter from a multi-strain Kveik culture for a brewery in Norway.

Keywords
Minifors 2 bioreactor, Multitron Standard Incubator Shaker, INFORS HT, fermentation, high-temperature yeast propagation

Introduction

Kveik are traditional Norwegian farmhouse ale yeasts, typically maintained as mixed-strain cultures (consortia) of *Saccharomyces cerevisiae* [1]. These multi-strain Kveik cultures ferment wort sugars extremely rapidly and are noted for their high tolerance to fermentation stresses (especially elevated temperature and ethanol) while exhibiting strong flocculation and little to no phenolic off-flavor production. Kveik strains efficiently utilize maltose and maltotriose to completion, often fermenting to high attenuation within 1–2 days under warm pitching conditions (30–40°C). Genomic analyses reveal that kveik constitute a distinct domesticated beer-yeast lineage with an admixed ancestry (one parent in the Beer 1 ale clade) and classic domestication signatures, including expanded maltose-transporter (MAL) gene copy numbers and loss-of-function mutations in PAD1/FDC1 that eliminate 4-vinylguaiacol (phenolic) production. By blending multiple Kveik strains, brewers harness the synergistic advantages of these phenotypes to achieve robust, efficient fermentations and unique aroma/flavor profiles in innovative brewing applications [2,3].

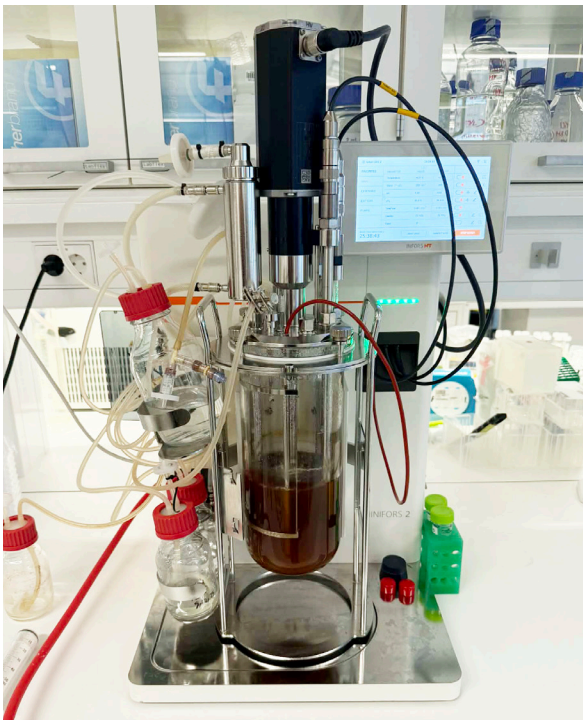

Figure 1. Minifors 2 bench-top bioreactor (INFORS HT) set up

Fermentation systems range from simple shake-flask incubators to fully controlled stirred tank bioreactors. Shake flasks (orbital shakers with flasks) are inexpensive and versatile for strain screening and media optimization [4], but they provide no active pH/DO control or fixed aeration, so oxygen transfer becomes limiting and CO_2 can accumulate at high cell density [5]. In contrast, bioreactors (fermenters) are closed, instrumented vessels that maintain precise control of temperature, dissolved oxygen, pH, and nutrient feed, and they support fed-batch or continuous modes. These features enable much higher cell densities and yields [6]. Consequently, incubator shakers are ideal for rapid small-scale experiments, whereas bioreactors are used for scale-up to production (e.g., large-scale brewing or biomanufacturing) with tight process control.

Enzyferm, a biotechnology startup focused on fermentation technologies and owner of the "Kveik Yeastery" brand that produces multi-strain Kveik yeasts for laboratory experiments, needs a reliable, consistent, and easy-to-use fermentation system. This system will be employed for research and development experiments. As a startup with a limited budget, the cost and efficiency of the system are crucial. The system will be used for product validation, strain development, and culture propagation for small-scale breweries. In this experiment, we aimed to compare the two mentioned systems in terms of reproducibility, ease of use, and process convenience. The experiment is based on the company's core hypothesis that multi-strain yeast can withstand thermal stress better than single-strain yeast. The highest extreme temperature level for Kveik cells, 45°C, was used in this experiment. The viability and growth results will serve as standards for comparing the two systems, in addition to the performance of the two biological samples. Ultimately, the most reliable and efficient system will be chosen to produce an inoculum (minimum 5 billion cells/mL) for 500 L of ale brewing at a brewery in Norway.

Methods and materials

Two fermentation systems were tested:

- System A: Bioreactor
- System B: Incubator Shaker

Each system was tested with Single-Strain Kveik (SSK) and Multi-Strain Kveik (MSK) yeasts, each with two experimental replicates (I and II). Samples were taken at 3h, 5h, 24h, and 28h, with two sampling replicates (S1 and S2) and two technical replicates per sample (T1 and T2) for the growth test.

System A (Bioreactor) parameters

The MSK and SSK propagations were done in a 2 L Minifors 2 bench-top bioreactor (INFORS HT), (Figure 1) with pH and pO_2 sensors (Hamilton). Temperature was set to 45°C prior to inoculation. pH was set to 5.50 and kept constant with 2.0 M NaOH and 1.0 M HCl (Sigma-Aldrich) during fermentation. The oxygen level was maintained at 70% by 1.0 L/min aeration to the system and was further adjusted by the EVE program (v. 2025 H1 R1) automatically to change stirrer speed from 100 to 1000 rpm. One liter of media was utilized for each experiment. Inoculation was done by the feed pump. Samples for the growth test (10 mL) were taken from the sampling port equipped with the Super Safe Sampler for sterility maintenance and sample consistency.

System B (Incubator Shaker) parameters

Four bottom flat 250 mL Erlenmeyer flasks were used for each experiment (MSK-I, MSK-II, SSK-I, and SSK-II) in a Multitron Standard Incuabtor Shaker (INFORS HT), (Figure 2). The incubation temperature was 45°C, before and after inoculation, with 40% humidity, and 220 rpm shaking speed, 25 cm orbit. Each flask was filled with 200 mL of media. Samples were taken manually from the top (2 mL) for the growth test.

Inoculum

Inocula were prepared from 0.25 g of active dried yeast of each biological sample into the YPD (yeast extract peptone dextrose) broth and incubated for 24 h at 31°C. An equivalent volume of the fresh culture to 1 million cells/mL then underwent centrifugation for 5 min (3000 rpm) to remove the metabolite traces and possible dried yeast antioxidant additives, and the pellet was directly dispersed into the incubator shaker flasks or 100 mL of media in a bottle for the bioreactor experiments.

Media

YPM (yeast extract peptone maltose) was used after autoclaving (121°C, 20 min) as media for all of the experiments. 900 and 200 mL of media were used for the bioreactor and the shaking flask experiments, respectively.

Growth test

The growth test was done by taking samples in specific time intervals (3, 5, 24, and 28 h) after inoculation. The viable cells were then counted under a microscope utilizing a Bürker-Türk hemocytometer slide (200x magnification) and a 1:1 trypan blue (0.4% in PBS) ratio. And reported as million cells/mL of media.

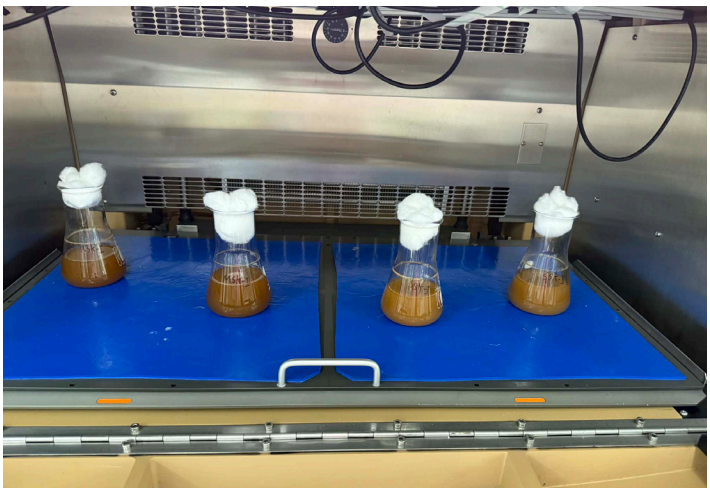

Figure 2. Multitron Standard Incubator Shaker (INFORS HT) set up

Results

Growth trends

In the bioreactor (A), both SSK and MSK cultures showed steady and consistent growth throughout the 28h propagation. MSK-I demonstrated the highest reproducibility, maintaining stable readings (1.6-1.7 million cells/mL across time points). SSK-I and SSK-II showed moderate growth, with values ranging from 1.0–1.5. Replicates between S1 and S2, and technical replicates (T1/T2), were highly consistent, indicating excellent reproducibility (Figure 3).

In the incubator shaker (B), the overall growth was lower, particularly after 24 hours, where values dropped below 1.0 for several SSK samples. MSK strains again performed more robustly than SSK, maintaining readings around 1.0–1.3. SSK-II displayed the most variable behavior, with noticeable decreases at later time points (Figure 3).

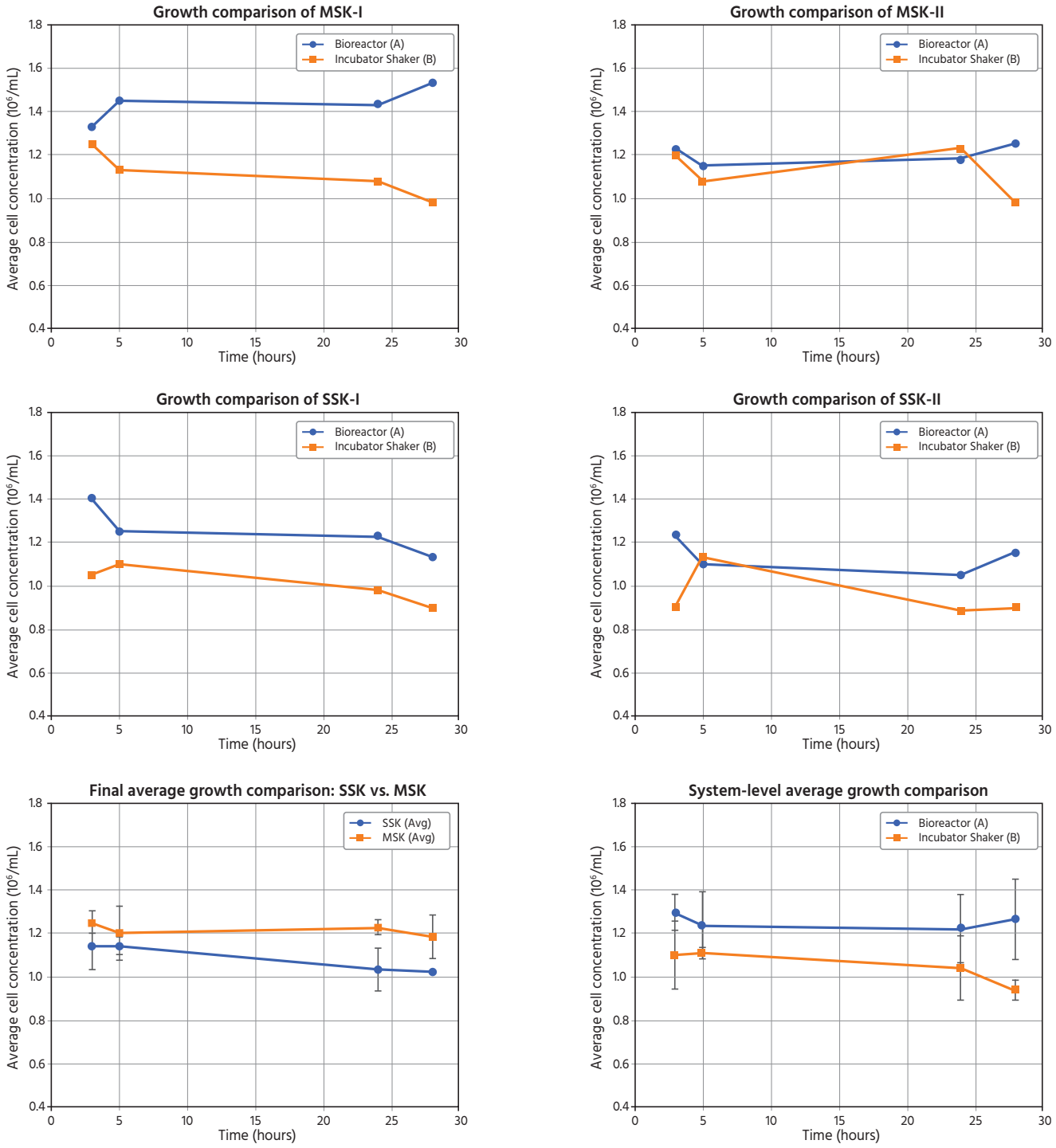

Figure 2. Growth comparison of each experimental replicate (I and II), biological samples (MSK and SSK), and the two different systems (bioreactor and shaker). NB: The x axis shows Time (hours), and the Y axis demonstrates average cell concentration (millions/mL)

Reproducibility

Reproducibility was assessed between each sample in a system separately using CV% (coefficient of variation) (Table 1), and then all were combined to achieve overall consistency among all experiments (Table 2), thereby determining the most consistent system. The smaller the CV%, the more consistent the system is. For sampling, reproducibility was assessed based on the Pearson Correlation Coefficient (PCC), and, since the data is from one set, Intraclass Correlation (ICC, one-way random, single measurement) was performed (Table 3). In both measurements, a value closer to 1 is considered ideal and will follow the linear correlation; thus, it is more consistent and reproducible.

System	Sample	Mean	SD	CV%
Bioreactor	SSK	1.175	0.122	10.37%
Bioreactor	MSK	1.316	0.174	13.25%
Shaker	SSK	0.978	0.143	14.63%
Shaker	MSK	1.113	0.124	11.13%

Table 1. CV% of each biological sample within the two systems.

System	Mean	SD	CV%
Bioreactor	1.245	0.165	13.26%
Shaker	1.045	0.149	14.25%

Table 2. Total CV% of the two systems.

System	PCC	ICC
Bioreactor	0.67	0.93
Shaker	0.45	0.91

Table 3. Sampling consistency (S1 vs. S2) in each system.

Process and ease of access

While the incubator shaker offers simpler and faster setup in addition to lower operational cost, the bioreactor system provides greater control over key parameters, e.g., aeration, agitation, and temperature. Using a shaker in parallel experiments can save valuable time but, on the other hand, increases the chance of contamination compared to the bioreactor.

Discussion

This study is the comparison of the two fermentation systems, bioreactor (A) and shaker flasks in a incubator shaker (B). The main focus, though, is on the reproducibility of fermentation cell growth measurements between two biological samples, a multi-strain Kveik yeast (MSK) and a single-strain isolate from the same culture (SSK) at 45°C, the upper growth limit temperature. Across all analyses, the bioreactor system demonstrated superior reproducibility, showing higher consistency between sampling replicates (S1 vs. S2) and more stable values across 28 h. The improved performance of System A likely reflects the benefits of controlled aeration, mixing, and temperature stability, which reduce microenvironmental variation and sampling noise.

In contrast, the incubator shaker demonstrated more variability between sampling replicates, particularly for the SSK sample. Although technical replicates remained consistent, the higher value difference and less linear correlation between S1 and S2 (PCC = 0.45) indicate that possibly utilization of shaker cultivation at high temperature introduces greater variability, especially when biomass and oxygen demand increase. Different yeast cells also influenced reproducibility. The multi-strain Kveik culture (MSK) consistently showed higher agreement between sampling replicates in both systems, suggesting that the strain diversity within MSK provides functional redundancy and greater physiological stability under thermal stress. On the other hand, the single-strain (SSK) displayed higher variability, most noticeably in the incubator shaker, indicating that single-strain may be more sensitive to environmental fluctuations and less robust at 45°C.

In summary, these findings highlight that, as expected, both system design and biological composition influence reproducibility. Bioreactors provide more reliable environments for quantitative fermentation measurements, while multi-strain Kveik cultures maintain greater robustness under high-temperature fermentation. Even though the bioreactor has higher costs, and cleaning, preparation, and sterilization are time-demanding in comparison to the shaking flask, the higher reproducibility and sensitivity of the system, specifically in extraordinary situations, such as higher temperature, benefits our final goal.

Conclusion

Among the strains, multi-strain Kveik (MSK) demonstrated superior adaptability and growth stability. The bioreactor system proved more effective and reproducible for propagating both single- and multi-strain Kveik yeasts under aerobic conditions. Therefore, it was utilized for multi-strain yeast propagation to use as a starter (inoculum) for a brewery (our customer) in Norway.

Acknowledgments

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References

1. Dippel K, Matti K, Muno-Bender J, et al. Co-Fermentations of Kveik with Non-Conventional Yeasts for Targeted Aroma Modulation. Microorganisms. 2022;10(10):1922. doi:10.3390/microorganisms10101922
2. Foster B, Tyrawa C, Ozsahin E, et al. Kveik Brewing Yeasts Demonstrate Wide Flexibility in Beer Fermentation Temperature Tolerance and Exhibit Enhanced Trehalose Accumulation. Front Microbiol. 2022;13:747546. doi:10.3389/fmicb.2022.747546
3. Preiss R, Tyrawa C, Krogerus K, Garshol LM, Van Der Merwe G. Traditional Norwegian Kveik Are a Genetically Distinct Group of Domesticated Saccharomyces cerevisiae Brewing Yeasts. Front Microbiol. 2018;9:2137. doi:10.3389/fmicb.2018.02137
4. Brauneck G, Engel D, Grebe LA, et al. Pitfalls in Early Bioprocess Development Using Shake Flask Cultivations. Eng Life Sci. 2025;25(1):e70001. doi:10.1002/elsc.70001
5. Kumar V, Tolosa M, Ge X, Rao G. Reinventing shake flask fermentation: The breathable flask. Biotechnol Bioeng. 2024;121(9):2706-2715. doi:10.1002/bit.28734
6. Ganjave SD, Dodia H, Sunder AV, Madhu S, Wangikar PP. High cell density cultivation of E. coli in shake flasks for the production of recombinant proteins. Biotechnol Rep. 2022;33:e00694. doi:10.1016/j.btre.2021.e00694

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Supplementary Data

System A (bioreactor):

			Sample replicates			
			S1		S2	
Biological sample	Experiment rep.	Test timing	T1	T2	T1	T2
SSK	I	3h	1.4	1.4	1.2	1.2
		5h	1.3	1.2	1.2	1.2
		24h	1.5	1.1	1.1	1.2
		28h	1.1	1.0	1.2	1.2
SSK	II	3h	1.2	1.2	1.3	1.2
		5h	1.3	1.1	1.0	1.0
		24h	1.0	1.1	1.1	1.0
		28h	1.2	1.2	1.1	1.1
MSK	I	3h	1.6	1.2	1.2	1.3
		5h	1.4	1.6	1.4	1.4
		24h	1.6	1.4	1.3	1.4
		28h	1.7	1.6	1.4	1.4
MSK	II	3h	1.2	1.4	1.2	1.1
		5h	1.1	1	1.3	1.2
		24h	1.1	1.2	1.2	1.2
		28h	1.4	1.1	1.3	1.2

Supplementary Data

System B (Incubator Shaker):

			Sample replicates			
			S1		S2	
Biological sample	Experiment rep.	Test timing	T1	T2	T1	T2
SSK	I	3h	1.2	1.1	1.0	0.9
		5h	1.0	1.2	1.1	1.1
		24h	1.1	1.1	0.9	0.8
		28h	0.9	0.9	0.9	0.9
SSK	II	3h	1.1	1.0	0.7	0.8
		5h	1.3	1.2	1.0	1.0
		24h	0.9	0.8	0.9	0.9
		28h	0.8	0.8	1.0	1.0
MSK	I	3h	1.3	1.2	1.2	1.3
		5h	1.1	1.1	1.2	1.1
		24h	1.0	1.0	1.2	1.1
		28h	1.0	1.0	0.9	1.0
MSK	II	3h	1.4	1.2	1.1	1.1
		5h	1.1	1.1	1.0	1.1
		24h	1.1	1.3	1.3	1.2
		28h	1.0	0.9	1.0	1.0