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Fed-Batch System for Propagation of Brewer's Yeast

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ABSTRACT

A novel high-density yeast propagation system has been developed, which produced yeast that performed as well as cropped yeast in commercial brewery trials. This process is capable of producing yeast concentrations 13 times greater than traditional yeast propagation approaches used in breweries to date. The system is based on a controlled fed-batch yeast fermentation, which can produce pitching yeast in as little as 24 h. A demonstrator plant was installed in a regional brewery and yeast produced from the test-platform was used to pitch commercial brews. Plant-scale trials have shown that the yeast propagated using this new system had the same fermentation profile compared to control fermentations that used cropped yeast. Volatile analysis showed no significant difference between the control and experimental beers. The experimental beers tasted true-to-type and were released to trade. The new process allows for smaller pitching volumes while maintaining overall beer quality.

KEYWORDS

Bioprocessing; brewing; distilling; fed-batch; propagation; yeast

Introduction

Traditionally brewers re-use yeast cropped from fermentation to pitch subsequent brews.[1] In many breweries, this process cannot continue indefinitely due to the occurrence of spontaneous mutants and the increased risk of contamination. Some brewing yeast strains are susceptible to such genetic drift; however, others are more resilient and can remain stable over extended periods of time. While the negative effects of serial re-pitchings have been reported by researchers (e.g., petite generation and flocculation mutations), others have indicated little change in lager yeast serially re-pitched up to 135 times.[2–5] Also, it was noted that extents of deterioration can vary between yeast strains.[6] Where such introductions are necessary, they generally happen after approximately ten fermentation cycles.[7] Large brewers generally propagate their own proprietary yeast strains, whereas smaller breweries rely on yeast purchased from yeast suppliers.

In brewery fermentations, the yeast undergoes a lag phase during which little yeast growth takes place. This is followed by a vigorous growth phase where yeast reproduce and finally a fermentation phase, where growth slows down and the sugars in the wort are fermented.[8] For a successful beer fermentation, the yeast must attain sufficient cell numbers in order to convert the sugar in the wort to alcohol. Brewer's yeast is capable of growth under strictly anaerobic conditions only when there is a supply of sterols and unsaturated fatty acids.[9,10] In wort, this is not usually the case. Unsaturated fatty acids are produced from saturated fatty acids in the presence of oxygen and sterols are produced from squalene in the presence of oxygen and sufficient oxygen is generally supplied to result in approximately a 3 to 4 doubling of the pitching yeast numbers.[11] Oxygen is therefore added for yeast cell reproduction and so that the cells can produce stable cell walls. A high level of expression of genes involved in fatty acid and ergosterol biosynthesis has been shown to occur within this period and unexpected near complete repression of many genes involved in early glycolysis and alcohol metabolism.[12] Pitching rates for beer fermentations range from 5–20 million cells/mL wort.[12] For example, should 10×10^6 cells ml^{-1} be pitched into air-saturated wort, then the maximum yeast count attained would be of the order of $80–100 \times 10^6$ cells ml^{-1}. This maximal yeast count is normally achieved 18 to 24 h after pitching for ale yeasts.[13] The yeast from this propagation vessel is transferred to the primary beer fermentation when it reaches exponential phase, however the beer produced from this first-generation yeast would usually be blended with beer having the correct flavor profile. The yeast crop from the second generation would however produce beer with a typical flavor.[13]

Particular yeast strains produced by batch fermentation with continuous aeration of wort can be grown to cell densities upwards of 300×10^6 cells ml^{-1}. Yeast propagated in

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breweries is normally aerated intermittently in wort and generally reaches a cell density of approximately 100×10^6 cells ml^{-1} in an attempt to limit the production of off-flavours in the propagated yeast. As during yeast propagation, the aim is to obtain maximum yield of yeast but also to keep the flavor of the beer similar to a fermentation with the correct flavor profile, so that it can be blended into the production stream.[13]

In some cases, the need for "blending-off" of the beer produced from first generation yeast is no longer required. Wackerbauer et al.[14] have used acetaldehyde as a marker compound for successful propagation.[15] A low level of acetaldehyde at the end of propagation is desirable as this compound is an off-flavor in beer and it is not desirable that it be carried over to the beer fermentation. The formation of acetaldehyde is most likely due to the Crabtree effect in brewing yeasts. It is thought to be due to the limited respiratory capacity of yeast cells, this leads to an overflow of reaction from pyruvate, through acetaldehyde and on to ethanol and carbon dioxide.[16,17] The term overflow metabolism has been used to describe this seemingly wasteful strategy in which cells incompletely oxidize their growth substrate.[17] The specific growth rate should ideally be maintained at maximum oxidative growth rate in order to maximize biomass yield and productivity in fed-batch yeast fermentations.[18] Propagation in wort with a high sugar concentration does not allow full respirative yeast metabolism.[19] In contrast, fed-batch systems used for baker's yeast production maintain the sugar concentration in the growth medium at a low concentration to ensure that the yeast are maintained in a respiratory metabolism.[20]

The rise in craft brewing has led to the need for use of multiple yeast strains within small and regional breweries. Many of these breweries do not have the facility to propagate their own yeasts. The lack of propagation facilities can be circumvented by the use of active dry yeast (ADY) or the provision of liquid yeast by some suppliers. The former has the disadvantages of high cost, low viability (approximately 70%), contamination with lactic acid bacteria and some loss in viability on reconstitution.[21] Its ease-of-use, the lack of the necessity to oxygenate the first beer fermentation and the lack of a requirement for "blending-off" of the beer produced from first generation yeast, are major advantages of ADY. Purchased liquid yeast is generally propagated under conditions identical to those of the fermentation and eliminates the need for "blending-off" the first beer.[22]

Active dry yeast is produced using molasses-based fed-batch fermentations. Such yeast cultures are 100% viable and free from contamination. It is the non-sterile downstream processing, i.e., concentration of and drying, that introduces contamination and reduced yeast viability in ADY. Furthermore, during the processing of active dry yeast, the liquid medium is removed entirely from the yeast culture. This process, combined with the heat treatment during the drying process, removes all volatile products, including beer off-flavours, from such preparations indicating why fermentations performed using ADY do not have to be blended-off.

In-house fed-batch fermentations have not found favor with brewers, especially those who use a single yeast strain and use their propagators sporadically.[23] As a consequence only few publications exist[19,20] that implement fed-batch fermentations to pitch yeast in breweries. Currently the majority of the fed-batch fermentation research in brewing is towards fermentation of the wort for production of high-gravity beers.[24–29] The use of an in-brewery fed-batch propagation system may redress the disadvantages associated with active dry yeast, while maintaining the advantages associated with this high cell density process. Here we describe the construction of simple skid-mounted, fed-batch propagation system and its installation into a commercial brewery handling multiple yeast strains. Beers produced by fed-batch propagation with those produced using cropped yeast are compared.

Experimental

Yeast strain and culture conditions

Carlow Brewing Company's proprietary ale yeast *Saccharomyces cerevisiae* "Liffey-1," was used during this study. It was maintained on malt extract agar (Oxoid) slopes and grown in malt extract liquid medium (Muntons, Stowmarket, United Kingdom). A 100 ml aliquot of liquid medium and yeast grown to late log-phase was inoculated into a 500 ml shake flask. When the yeast reached late log-phase, it was used as the inoculum for the batch phase of aerobic propagation. Yeast strains were stored on malt extract slopes at 4 °C and sub-cultured weekly. The flasks were incubated for 48 h at 30 °C on an orbital shaker. Cell counts and dry cell weight (DCW) measurements[30] were taken at 5.75, 25 and 48 h. Yeast viability was determined using the methylene blue staining method.[31] Media constituents were optimized in shake flask aerobic cultures grown on an orbital shaker.

Laboratory-scale fermentation in a stirred tank reactor (STR)

In order to perform fed-batch cultures, it is common to start the process with a batch phase. This offers the advantage of acclimatizing the yeast to the new process conditions and starts the fed-batch without a lag phase. Both the batch and fed-batch phases were aerated. The lab-scale cultures were grown in an automated 1.2 L RALF™ bioreactor from BioEngineering AG, (Wald, CH). The system implements on-line pO2, pH and temperature control, including automated anti-foam addition. The pH was controlled with 3 mol/L NaOH and HCl solutions (Fisher Scientific, Dublin), and foam was contained with a formulation of silicone-based antifoam 30% in water (Sigma-Aldrich, Arklow, Co. Wicklow, Ireland). To meet high-oxygen transfer requirements, a steel sintered sparger was implemented, yielding in a k_La value of $0.045\,s^{-1}$.

On-line gas analysis was performed with a Tandem™ gas analyzer from Magellan Instruments Ltd (Norfolk, UK). The data was logged with an automated data acquisition system (DAq) developed using National Instruments

Table 1. The composition of media optimized for batch and fed-batch fermentations.

	Batch		Feed	
	Medium 1 (g/L)	Medium 2 (g/L)	Medium 3 (g/L)	Medium 4 (g/L)
Wort (solids content)		132		
Malt extract	132			350
Yeast extract			5	10
Glucose			300	
Ammonium sodium phosphate	2.5	2.5		
Dipotassium phosphate	2.5	2.5		
Ammonium phosphate	1	1		
Magnesium sulphate	0.5	0.5		
Manganese sulphate	4×10^{-4}	4×10^{-4}		
Zinc ion (Zn^{2+})	1.5×10^{-4}	1.5×10^{-4}		
Thiamine hydrochloride	4×10^{-4}	4×10^{-4}		
Biotin	2×10^{-6}	2×10^{-6}		
Pantothenic acid	4×10^{-4}	4×10^{-4}		

LabVIEW software (Austin Texas). The feed medium, composed of glucose and yeast extract (Table 1), was added with a remotely controlled peristaltic pump (IPS from ISMATEC, Wartheim, DE). All trials were controlled at a pH value of 5.0 and a temperature of 28 °C. The vessel was autoclaved before each trial at 121 °C for 20 min. Sampling was performed automatically using a preparative chromatography fraction collector (Gilson Inc, Middleton WI, USA) connected to a recirculating peristaltic pump (Perista, ATTO Corp, Tokyo, JP). The flowrate was controlled automatically with a proportional–integral controller developed within NI LabVIEW and using a feedback signal from a weighting scale (PM2500 DeltaRange™, Mettler-Toledo, Greifensee, CH). The developed software controller maintained the pump flowrate to match a defined exponential flow profile (presented below).

Plant-scale propagation in a 20-L stirred tank reactor

For plant-scale trials, the process developed in the laboratory-scale STR was scaled up by a factor of 18 to obtain sufficient quantities of yeast to pitch commercial beer fermentations. The medium in which the batch phase of the propagation process was performed (see Table 1) was sterilized *in-situ* for 15 min at 121 °C. The cultures were grown at 28 °C in a STR equipped with a triple 6-blade Rushton-type agitator, the system has on-line pO_2, pH and temperature control, including automated anti-foam addition. The agitator was set at 1000 rpm. A solution of 25% aqueous ammonia (Fisher Scientific) was used to maintain automatic control of the pH to pH = 5.0; no acid control was required.

Software development and controller design

The growth controller design is based on a modification of the feed-forward controller described by Dabros et al.[32] and Brignoli et al.[33] where the design takes account of volume changes in the system. The feed rate was derived from the cell and substrate balance equations below

$$\frac{d(V_x)}{dt} = \mu\left(V_x\right) \tag{1}$$

where x is the cell concentration at a particular time t, the specific growth rate μ and the reaction volume is V.

$$\frac{d(VS)}{dt} = FS_F - \frac{1}{Y}\mu Vx \tag{2}$$

where S is the substrate concentration in the reactor, S_F is the sugar concentration in the feed supply and Y is the biomass yield. Equation 3 is described as follows:

$$V\frac{dS}{dt} + S\frac{dV}{dt} = FS_F - \frac{1}{Y}\mu V_0 x_0 e^{\mu t} \tag{3}$$

where x_0 is the initial cell concentration and V_0 is the volume at the end of batch phase. Assuming that the substrate concentration is kept close to zero during the fed-batch phase, the feed rate F can be derived as

$$F = \frac{\mu V_0 x_0 e^{\mu t}}{YS_F} \tag{4}$$

The feed-forward model is derived with the assumption that the flowrate of the substrate during the fed-batch phase is proportional to the biomass growth. The initial flowrate can be deduced from the conditions at the end of the batch phase. Equation (4) was used to derive the flowrate at any time during the feeding phase. Typically, the final volume of the fermentation was comprised of 66.6% batch and 33% fed-batch volumes. All programs were generated in NI LabVIEW™ and were coded in-house.

Volatile analysis

Samples were analyzed externally using Headspace-Gas Chromatography (HS-GC) analysis for selected esters (ethyl acetate, isoamyl acetate, 2-phenylethyl acetate), higher alcohols (1-propanol, isobutanol, 2-methyl-1-butanol, 3-methyl-1-butanol, 2-phenyl ethanol), vicinal diketones (diacetyl, diacetyl total, pentanedione-2,3, pentanedione-2,3 total) and acetaldehyde by Versuchs-und Lehranstalt für Brauerei (VLB, Berlin, D) according to the method of Rettberg et al.[34]

The sum of the concentrations of these higher alcohols was expressed as combined higher alcohols in µg/ml.

Beer production and tasting

Ale and stout brews were produced in a regional brewery having a brewlength of 80 hl. Control brews were pitched using cropped *Saccharomyces cerevisiae* "Liffey 1" strain and experimental beer fermentations were pitched using the same strain grown in the plant-scale STR. Both control and experimental fermentations were pitched into cylindroconical fermenters having the same dimensions. The final beer was tasted by the brewery's taste panel.

Results

Shake flask growth medium optimization

The objectives of the shake-flask trials were to determine the optimal concentration of malt extract for yeast growth and to generate data for fitting a correlation between dry cell weight (DCW) and yeast cell count. For shake flask trials, malt extract was used as the base medium. Laboratory trials with media made using malt extract alone, at various densities, were used to determine the optimum level of malt extract for growth.

Figure 1(a) and 1(b) shows that the biomass yield increases with increasing malt extract concentration. This data agrees with other reports of increased biomass yields with increasing wort strength.[35] Altered fermentation characteristics have been reported for yeast subjected to stress prior to pitching.[36] As malt extract densities of 1040 kg m^{-3} allow maximal biomass yields while limiting any stress caused by increasing malt extract concentration, 1040 kg m^{-3} was chosen as the optimum malt density for yeast propagation. Malt extract density of 1040 kg m^{-3} was seen to provide optimum biomass growth, this was selected for progressing the study towards bioreactor operation. Higher malt extract densities of 1060 kg m^{-3} and 1080 kg m^{-3} produced lower comparative biomass yields.

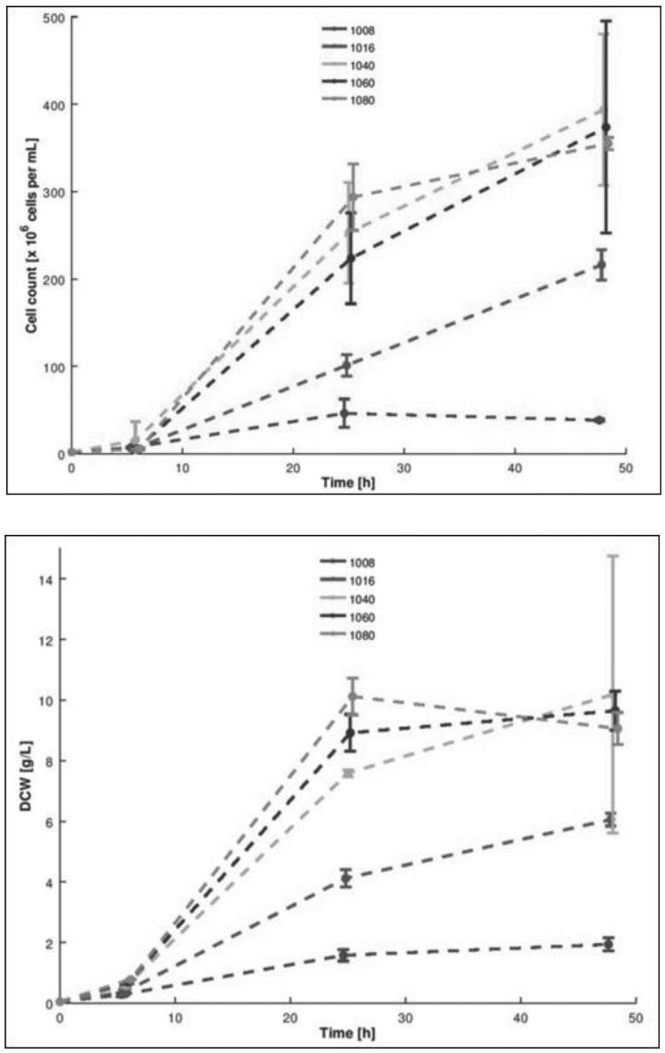

Figure 1. (a) Yeast growth, monitored using yeast cell count, at various concentrations of malt extract over time. (b) Yeast growth, monitored using dry cell weight, at various concentrations of malt extract over time.

Correlation of cell counts versus dry cell weights

Data from shake flask trials along with data generated during the initial fed-batch trials were used to fit a regression model between cell counts and DCW. This correlation was used to estimate cell counts from DCW measurements collected in subsequent experiments. DCW measurements were preferred because they showed less variance and reduced the error of biomass estimation. To develop the model, a total of 57 samples within a range of 0 to 40 g/L DCW were assessed, and a statistical analysis of the results is presented in Figure 2. It was noticed that the cell counts were more spread at high values, therefore an additional and more accurate model with 44 samples was fitted in a range of 0 to 15 g/L (more common in brewing than high cell densities). The confidence and prediction intervals were calculated for a two-tailed 95% probability based on a T-distribution. The prediction intervals indicate, with 95% confidence, the range within a new value could fall from the regression line. Whereas the confidence intervals indicate with 95% confidence the range from the regression plot that the experimental data would fall within.

In Figure 2(a) and 2(b) the intervals express that the "real" regression line is embedded in this interval with a probability of 95%. A statistical "sample" was measured and the regression line between cell counts and DCW approximated. This approximation is based on the assumptions that the true event (counts) is measured as a Gaussian distribution. There is therefore a 95% chance to find the "real" regression line between 2 boundaries and those are within the confidence intervals.

Laboratory-scale fermentation stirred tank reactor (STR) trials

Batch fermentation

An example of batch growth using "Medium 1" (described in Table 1) in a laboratory-scale fermentation is shown in

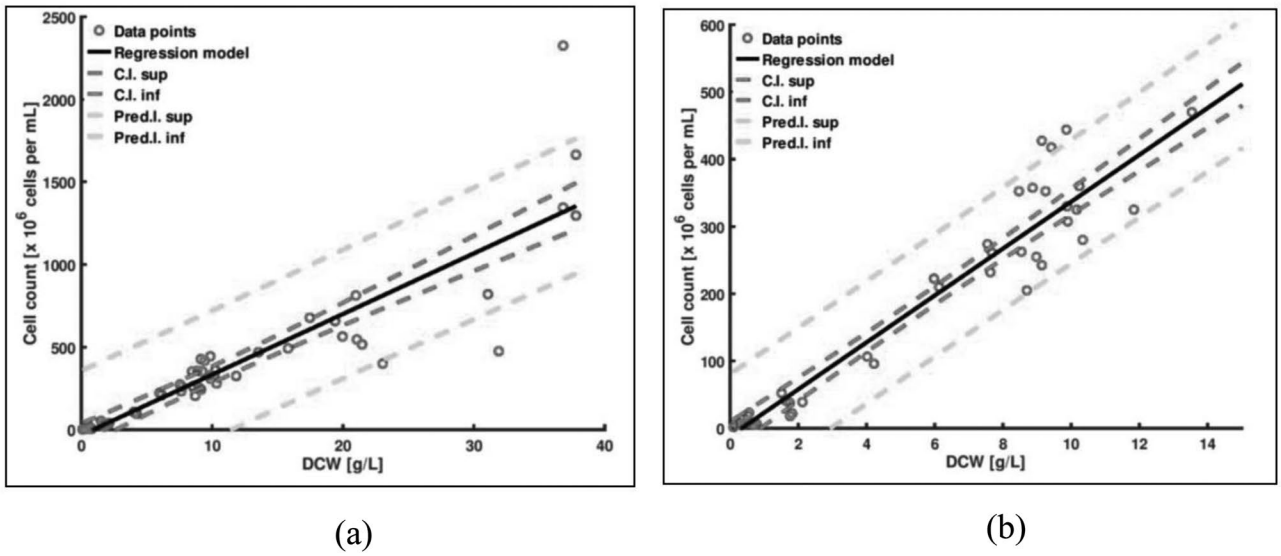

Figure 2. Correlation of cell counts vs. dry cell weight for (a) high concentrations (Adj, R^2 = 0.81) and (b) low concentrations (Adj, R^2 = 0.93) of cell growth during the bioprocess (displaying 95% prediction and confidence intervals). Confidence interval superior boundary (C.I. sup.), confidence interval inferior boundary (C.I. inf.), Prediction interval superior boundary (Pred.I. sup) and Prediction interval inferior boundary (Pred.I. inf) are noted on the graphs.

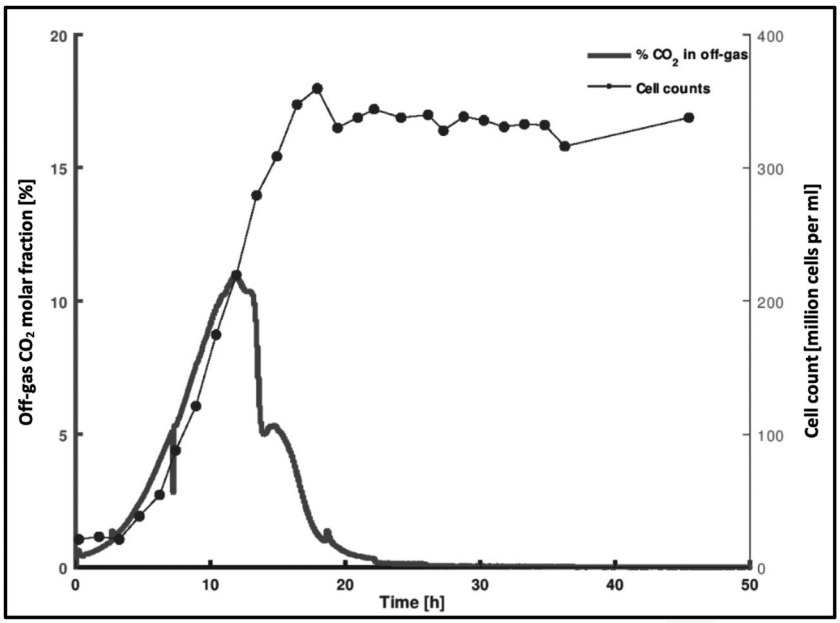

Figure 3. Figure shows yeast cell density and off-gas analysis for 1.2 L laboratory batch bioprocess, indicating maximum cell density of 3.2 × 10^8 cells ml^{-1}. The initial batch medium volume was 0.7 L and the final volume after feeding was 1.2 L.

Figure 3. The batch process was capable of attaining cell densities in excess of 3×10^8 ml^{-1}, 20 h post-inoculation. The batch phase was considered to be completed when the CO_2 evolution ceased, and the culture entered stationary phase.

Fed-batch fermentation

Figure 4 shows both the batch and fed-batch phases in a laboratory fermenter. Fed-batch mode was initiated after the carbon dioxide evolution rate (CER) slows significantly at time = 17 h. The end of fed-batch phase was indicated by a drop in CER at 40 h. The final cell density in the experiment shown in Figure 4 was approximately 1.3×10^9 cells ml^{-1} and no acetaldehyde was detectable at the end of fermentation (40 h).

Volatile analysis of laboratory STR fermentations

In laboratory experiments, at the end of the aerobic batch phase, acetaldehyde levels were typically in the region of 200 mg/L, as can be observed in Figure 5. In contrast at the end of the fed-batch trials, still in laboratory trials, acetaldehyde was no longer detectable. It was not obvious whether the disappearance of

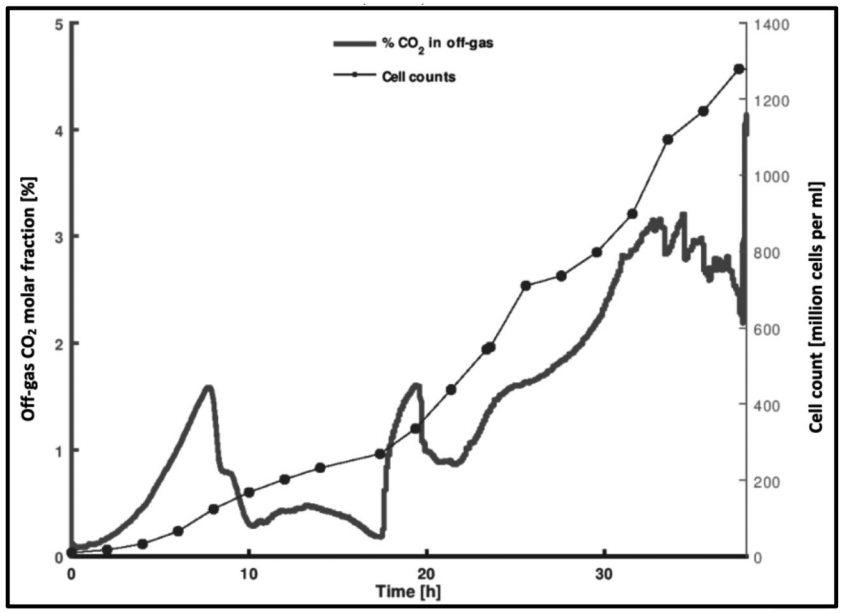

Figure 4. Figure illustrates yeast cell density and off-gas analysis for 1.2 L laboratory fed-batch bioprocess, showing maximum yeast cell density reaching 1.3×10^9 cells ml^{-1}.

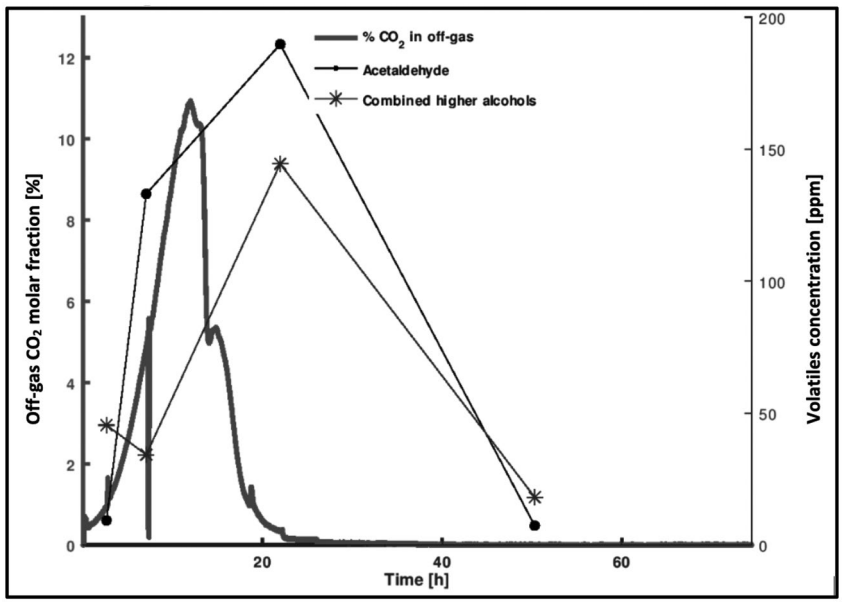

Figure 5. Figure shows the 50-h profile of off-gas CO_2, acetaldehyde and higher alcohols in the 1.2 L fed-batch bioreactor.

acetaldehyde during the fed-batch phase was due to stripping by air supply to the fermenter or of its re-assimilation by the fed-batch grown cells.

To determine the root cause of this phenomenon, a batch growth trial was performed where the STR was aerated long after growth had ceased (i.e., after CO_2 evolution ended). Figure 5 shows that even in the absence of the fed-batch phase acetaldehyde and other volatiles compounds were produced during the aerobic batch phase and the higher alcohols were lost from the fermentation medium. This suggests that air stripping is the mechanism by which volatiles are removed during the fed-batch phase.

Plant-scale fermentation propagation trials

With the propagation media for both batch and fed-batch phases of the high-density propagation process optimized, alongside a knowledge of the specific growth rates and feeding regimes, the laboratory fermentations could be scaled-up. Some of the laboratory process parameters had to be modified when working within the constraints of a brewery, both in terms of scheduling and regulatory restrictions. For example, ammonium hydroxide was used to control the pH and wort (diluted to 1040) was used instead of malt extract. The target time was set at 24 h for the

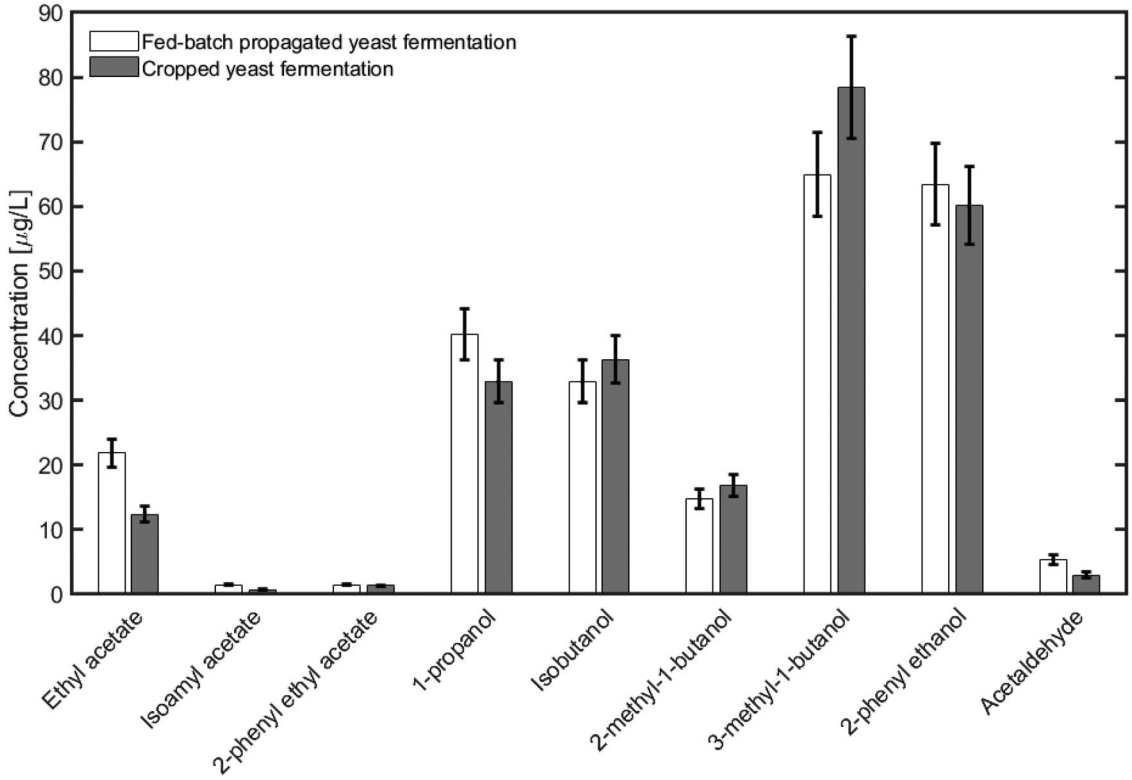

Figure 6. Volatile analysis of production scale fermentation of experimental yeast (high density fed-batch propagation) vs. control sample from standard commercial production with a cropped yeast.

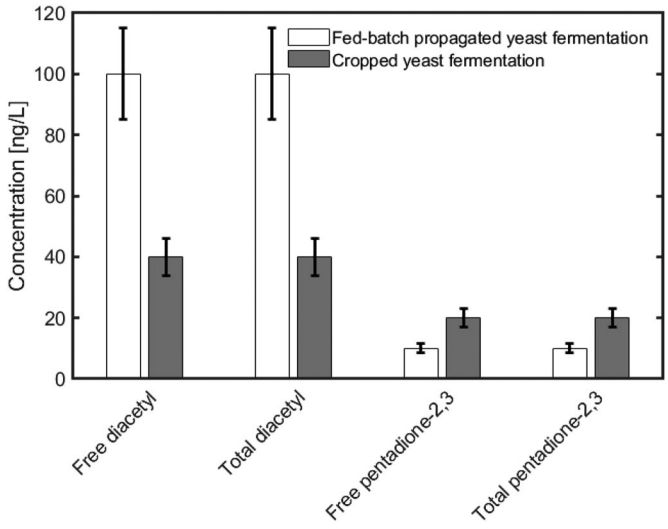

Figure 7. Vicinal diketone analysis of production scale fermentation of experimental yeast (high density fed-batch propagation) vs. control sample from standard commercial production with a cropped yeast.

entire propagation process. The batch phase was shortened to 15 h, a time that allowed the culture to attain high cell density in the batch phase, while the cells were still in exponential phase. When the specific growth rate was increased to 0.17 h^{-1}, this resulted in an increased rate of feeding and the entire process could be attenuated to 24 h. The batch phase feed medium was changed to Medium 3 (Table 1), which contained glucose as the carbon substrate and was amended with yeast extract. A high glucose concentration (300 g/L) was employed to prevent an excessive volume increase during the feeding phase.

India pale ale (IPA) trial

Typical yeast cell counts at the end of the fed-batch phase were lower than those for the optimized process, 1×10^9 cells ml^{-1} versus up to 1.3×10^9 yeast cells ml^{-1} for laboratory fermentations. With a shorter fed-batch time, the level of acetaldehyde at the end of fed-batch was typically in the region of 37 μg/ml versus zero for the laboratory trials.

A control India Pale Ale (IPA) brew (12.6°P) was pitched with cropped yeast from a previous fermentation. A second experimental brew was pitched with the fed-batch propagated yeast in a separate fermentation vessel (FV) having the same dimensions as that of the control brew. Both fermentations reached their attenuation limit after 4 days and samples were taken for volatile analysis at the end of fermentation (Figure 6). Ester levels were similar in the experimental brew versus the control reference. Combined higher alcohols were similar in the control and the experimental brews, 224.6 μg/ml versus 216.2 μg/ml, respectively. The acetaldehyde level was lower in the experimental beer compared to the control sample (3.0 μg/ml versus 5.4 μg/ml) (Figure 7). At the end of fermentation diacetyl levels were lower in the control brew compared to the experimental. Free and total diacetyl levels in both the control and experimental beer fermentations were the same indicating that all of the precursor of diacetyl (i.e., alpha acetolactate) had been converted to free diacetyl. The level of pentadione-2,3 was lower in the experimental compared to the level of pentadione-2,3 in the control. Again, both

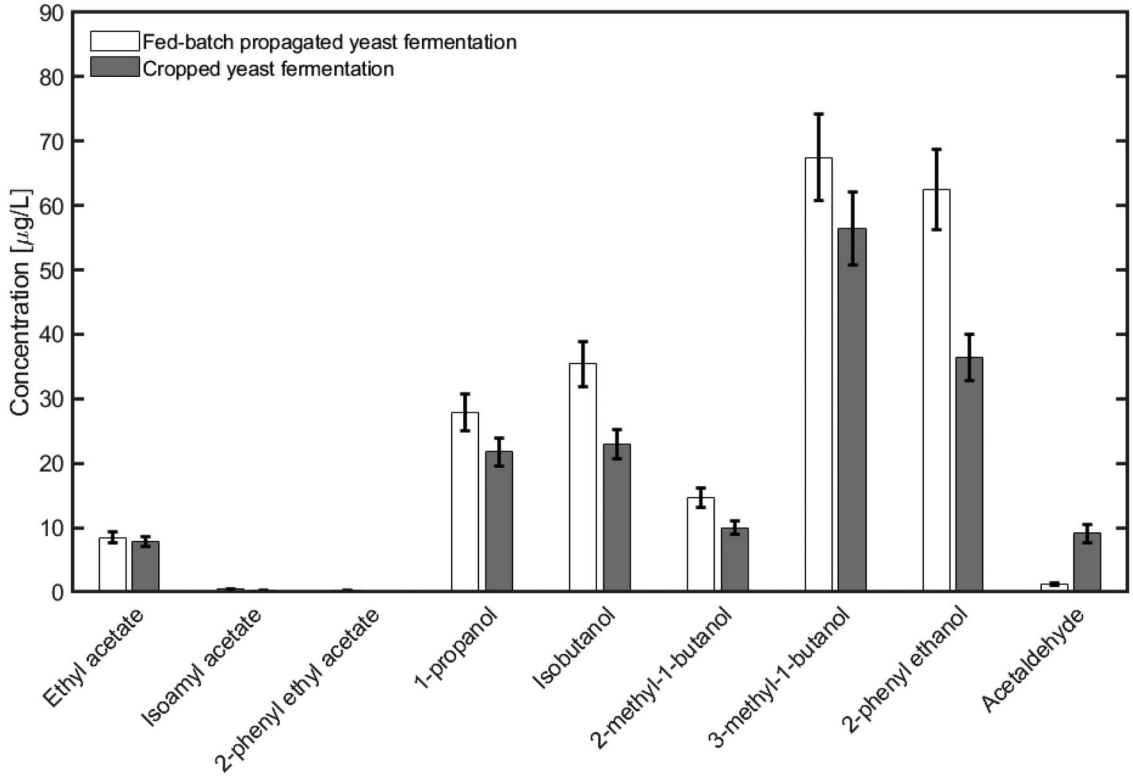

Figure 8. Volatile compounds at the end of primary fermentation of a stout for experimental (high density fed-batch propagated yeast) versus control beer (pitched with cropped yeast).

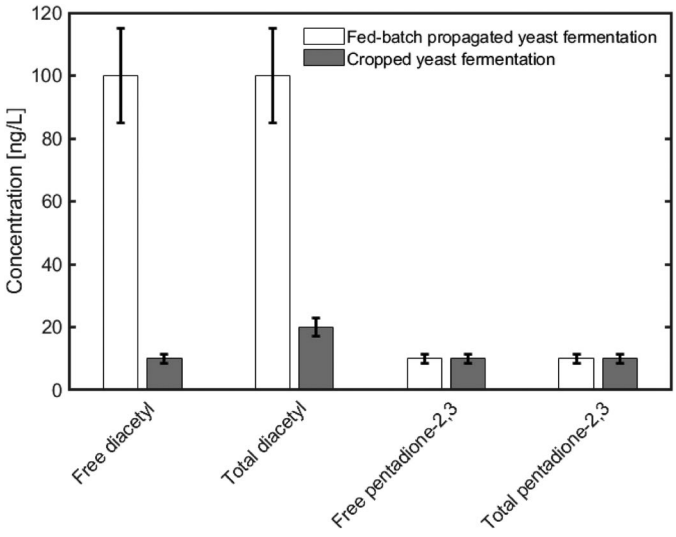

Figure 9. Vicinal diketone analysis at the end of primary fermentation of a stout for experimental (high density fed-batch propagated yeast) versus control beer (pitched with cropped yeast).

total and free pentadione-2,3 were the same at the end of beer fermentation. Following maturation, all the vicinal diketones fell below the flavor threshold.

Stout trial

A control Stout brew (10.4°P) was pitched with cropped yeast from a previous fermentation. A second experimental brew was pitched with the fed-batch propagated yeast in a separate fermentation vessel (FV) having the same dimensions as that of the control brew. Both fermentations reached their attenuation limit after 4 days and samples were taken for volatile analysis at the end of fermentation (Figure 8). Ester levels were similar in the experimental versus the control brews. Combined higher alcohols were higher in the control sample compared to the experimental one, 208 µg/ml versus 148 µg/ml, respectively. The acetaldehyde level was lower in the experimental beer compared to the control sample (<1.25 µg/ml versus 9.0 µg/ml). The level of free diacetyl and total diacetyl was the same at the end of primary fermentation in the experimental beer. The levels of diacetyl were higher than those of the control at the end of primary fermentation (Figure 9). Free and total pentadione-2,3 levels were the same in both the control and experimental beers at the end of primary fermentation.

Following a diacetyl rest, both beers were tasted by a trained panel. No difference in the taste of the beers was detected by the panel. Both beers were subsequently released to trade.

Discussion and conclusions

Yeast is grown in a yeast propagation vessel in breweries, and it is transferred to the primary beer fermentation vessel when it reaches exponential phase. However, the beer produced from this first-generation yeast would usually be blended with beer that had the correct flavor profile for the beer produced, meeting production process control requirements. Customers expect a specific beer to have a consistent flavor. Generally the higher-generation yeast produce the consistent flavor for beer produced.[13] This process

Table 2. Comparison of the various methods of introducing propagated yeast to a brewery.

Feature	Conventional brewery propagation	Fed-batch propagation	Active dry yeast
Capital cost	High	Low	None
Operating cost	Medium	Less CIP, energy, water etc.	High
Quality	Low (cell numbers)	High (cell numbers)	Low (60% viability) and invariably contaminated with bacteria
Physical size	Very large	Small footprint	Small footprint for make-up tank
Flexibility	Inflexible 48 h process	Variable processing times as specific growth rate is a process parameter	No propagation required
Supply chain	Can use proprietary yeast	Can use proprietary yeast	Restricted to yeast strains supplied by suppliers
Blending-off of first pitching	Blending usually required	Blending should not be required	Blending not required

Table 3. Comparison of various methods of pitching spirit fermentations.

Feature	Cream yeast bulk	In house fed-batch propagation	Active dry yeast
Capital cost	Storage vessels required	Low	None
Operating cost	Lower than ADY	Less CIP, energy, water etc.	High
Quality	High viability but invariably contaminated with bacteria	High yeast cell numbers pure culture	Low (60% viability) and invariably contaminated with bacteria
Physical size	Large storage tanks	Small footprint	Small footprint for make-up tank
Flexibility	No propagation required	Variable processing times as specific growth rate is a process parameter	No propagation required
Supply chain	Restricted to yeast strains supplied by suppliers	Can use proprietary yeast	Restricted to yeast strains supplied by suppliers
Ethanol yield	Reduction in yield due to bacteria contamination	No loss in yield due to yeast contamination by bacteria	Reduction in yield due to bacteria contamination

not only generates extra work to ensure the required blending, but it is also wasteful in capital expenditure as the yeast propagation plant has be very large to accommodate such low-density propagations. In contrast, the process described here produces high density yeast cultures that have little or no volatile off flavours carried through to the beer fermentation. The process plant can be much smaller than conventional process plants. Advantageously, the entire process can be scaled down to a skid, allowing for easy on-site transport.

Laboratory fermentations were used to determine the process parameters and feeding methodology to enable efficient growth via the tricarboxylic acid cycle (TCA) aerobic metabolism and bypassing the Crabtree effect. An exponential feeding profile based on the culture material balance was used as a feed-forward model to achieve open-loop control of the specific growth rate.

As can be seen in Figure 4, after 14 h growth in an aerobic batch phase the rate of CO_2 production slowed, indicating an approach to the end of the batch phase. The CO_2 evolved here is from both fermentation of sugar to CO_2 and ethanol and CO_2 produced from respiration. When feeding was initiated, it resulted in an immediate increase in the rate of CO_2 production as the respiratory metabolism only commenced. During this respiratory phase, the CO_2 is generated from an active tricarboxylic acid cycle and not from overflow metabolism, which is the fermentative branch that produces acetaldehyde, ethanol, and CO_2. From Figures 4 and 5, it can be seen that the aerobic batch phase was complete after approximately 20 h. In the experiment described in Figure 4 feeding took place, where in the experiment described in Figure 5 there was no feeding. After 40 to 50 h both trials had very low levels of acetaldehyde. From this it was concluded that the low levels of acetaldehyde at the end of fed-batch fermentations was due to stripping of these volatiles from the fermenter caused by the aeration of the STR.

The removal of acetaldehyde by stripping the volatile compounds from a batch fermentation does not provide a strategy for the removal of unwanted volatiles from the propagation culture, as in the absence of a carbon source, yeast cells were observed to autolyze rapidly causing large losses in viability (data not shown). The provision of a carbon source, as is the case with a fed-batch fermentation, overcomes this loss in viability. Yeast populations at the end of the fed-batch phase were seen to be typically almost 100% viable.

The optimized process achieved a cell count of 1.3×10^9 cells per ml in a 1.2 L lab-scale bioreactor (Figure 4), a 13-fold increase compared with conventional intermittently aerated batch propagations attaining cell densities of 1×10^8 cells per ml. Along with cell concentration improvements, beer quality was improved due to the fed-batch bioprocess, as acetaldehyde levels were seen to be reduced in the fed-batch fermentation. The aim of yeast propagation is to obtain maximum yield of yeast, while ensuring the flavor of the beer is similar to a normal fermentation so that it can be blended into the production stream. Often the first-pitching can produce a different flavor profile compared to a fermentation pitched using cropped yeast.

This can often be related to the high level of acetaldehyde contained in the propagated yeast and other fermentation by-products such as higher alcohols. Acetaldehyde in beer is considered an off-flavor with a reported flavor threshold of 10–20 mg/L,[37] however many tasters can detect this compound at much lower levels.[38] Its presence in beer results in 'grassy' off-flavours, therefore it is of great importance to ensure only small concentrations of acetaldehyde are carried over to the final beer from the pitching yeast.[14] Furthermore, the SO_2 level of the matrix used will play an important role, as the major binding partner for SO_2 present in beer is acetaldehyde.[39] In the process developed in this study, a very high level of acetaldehyde is produced during the batch phase, but this is significantly reduced following the fed-batch phase.

Finally, it should be noted that the volume of fed-batch grown propagation to be pitched into the first beer fermentation will be 13 times lower than that of a conventional yeast propagation, thus the overall quantity of unwanted volatiles moving forward to the first beer fermentation is greatly reduced. A summary of the process improvements due to applying in-house fed-batch propagation compared with conventional systems is provided in Table 2, along with implications to the distilling industry in Table 3.

For distilleries, yeast is produced in specialized yeast production units, separate from the distillery, using a fed-batch system usually using molasses as the carbon source for growth. Distilleries do not reuse this yeast and the spent yeast is disposed of in the pot ale fraction as a waste product. There is a commonly held belief that the flavor compounds produced by yeast contribute little to the final overall flavor of the spirit produced. Recently however, this view has been challenged, as discussed in the literature.[40] With craft distilling now mirroring the growth trajectory of craft brewing, there is a growing demand for bespoke yeasts. For many years, brewers have maintained that they use their own yeast to produce beers in the establishment of provenance of their beer brands. This is not the case for distilleries. The adoption of the process outlined in the paper will also allow distillers to use bespoke yeast that can be propagated in the distillery and facilitate the use of such yeast for marketing purposes.

A wort-based medium has been applied to grow yeast cells to a high cell density. Such high-density yeast propagation has been shown to be suitable as pitching yeast in a brewery. In this work we successfully implemented a fed-batch propagated yeast in a commercial brewery with no negative impact on final product quality. In addition, the yeast production methods would be equally applicable to distilling yeast.

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Disclosure statement

D. Donnelly is the director of Dandonnellytek Limited, Seamus O'Hara is CEO of The Carlow Brewing Company, L. Blanchard, M. Dabros, S. O'Hara, D. Brabazon, G. Foley, and B. Freeland declare no conflict of interest.

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