

# AN INVESTIGATION INTO THE DISTRIBUTION OF VIABLE YEAST MASS AND TEMPERATURE VARIATION IN CYLINDROCONICAL VESSELS DURING FERMENTATION

CHRIS BOULTON, UNIVERSITY OF NOTTINGHAM, UK.

The following scientific article examines the distribution and fermentation performance of yeast during primary fermentation in a production scale cylindroconical fermenter where, contrary to standard procedure, mechanical agitation is applied.

Vertical cylindroconical fermenting vessels fitted with external cooling jackets are commonly used for the large-scale production of lager beers. Such fermenters are not usually provided with a means of mechanical agitation. Adequate mixing of the vessel contents is reliant upon the natural agitation provided by CO<sub>2</sub> evolution and by convection currents. The latter may be encouraged by the application of differential cooling via a jacket located

towards the top of the vessel. It is assumed that during active primary fermentation, natural agitation is sufficient to ensure that the vessel contents are homogeneous such that yeast cells are evenly distributed throughout the fermenting wort. At the end of primary fermentation, it is further assumed that yeast begins to sediment into the cone due to a combination of the cessation of agitation via CO<sub>2</sub> evolution and the onset of flocculation in response to the exhaustion of fermentable sugars. During this period, the pool of the VDK precursor  $\alpha$ -acetolactate undergoes spontaneous oxidative decarboxylation to form diacetyl. The latter is then reduced by yeast to form acetoin and 2,3-butanediol. When the diacetyl has fallen to a pre-determined low concentration, further yeast sedimentation is encouraged by opening fully the valves supplying coolant to all jackets. Following a suitable time period during which the temperature of the green beer falls to ca 3-4 °C and yeast sedimentation is completed, the crop is removed and the vessel emptied.

A previous report has highlighted the difficulties of attemperating highly concentrated unstirred yeast slurries [1]. Others have confirmed these findings and suggested that inadequate temperature control of yeast sediments in



Figure 1. Photograph showing the stainless steel waterproof enclosure surrounding the biomass meter head amplifier and probe. Also shown is the stainless steel cable to which the enclosures were attached.

A PREVIOUS REPORT HAS HIGHLIGHTED THE DIFFICULTIES OF ATTEMPERATING HIGHLY CONCENTRATED UNSTIRRED YEAST SLURRIES. OTHERS HAVE CONFIRMED THESE FINDINGS AND SUGGESTED THAT INADEQUATE TEMPERATURE CONTROL OF YEAST SEDIMENTS IN FERMENTER CONES MAY HAVE DELETERIOUS EFFECTS ON YEAST CROP QUALITY.

fermenter cones may have deleterious effects on yeast crop quality [2]. The same authors suggest that there would be benefits to removing yeast crops early in the process and before the application of crash cooling. The tacit assumption of this suggestion is that significant amounts of yeast settle into the cone before the application of crash cooling.

Hitherto, it has not been possible to measure in real time the distribution of yeast in fermenter. A yeast biomass sensor has

been used for the control of yeast pitching and cropping [3, 4]. This device provides an instantaneous measure of viable yeast concentration and, in addition, it also incorporates an accurate thermometer. Here is described the results of an investigation in which yeast distribution and temperature variation have been measured using an array of similar biomass sensors submersed in a production scale cylindroconical fermenter.

The vessel used in these investigations was fitted with a small mechanically-driven bladed agitator. The effects of continuous →

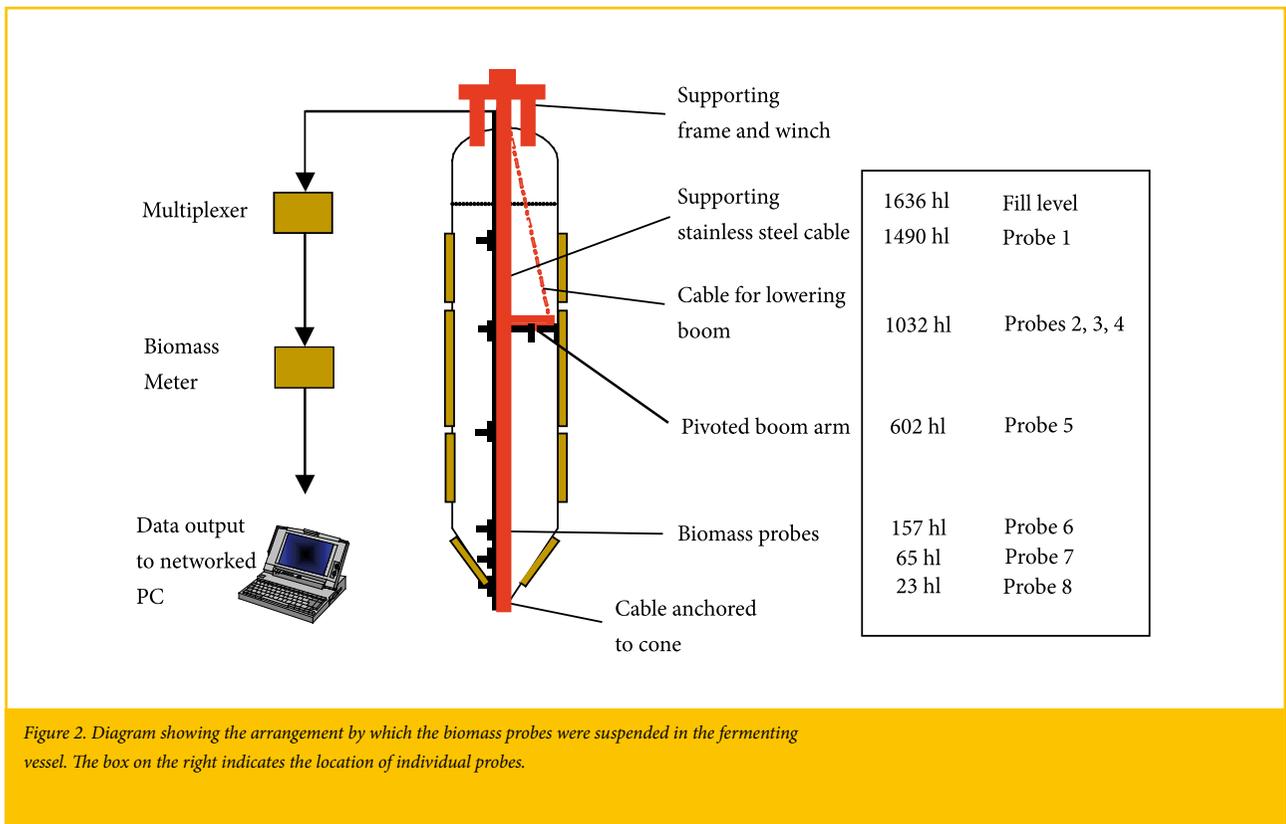


Figure 2. Diagram showing the arrangement by which the biomass probes were suspended in the fermenting vessel. The box on the right indicates the location of individual probes.

mechanical agitation applied throughout primary fermentation on yeast distribution and fermentation performance were investigated.

**MATERIALS AND METHODS**

Trials were performed using a cylindroconical fermenter with an operating capacity of 1,600 hl. The vessel was of all stainless steel construction with an aspect ratio of 3.9:1 and an internal cone angle of 72°. Cooling was provided by four

external jackets, three on the vertical walls and one located around the cone.

Biomass probes and associated head amplifiers were sealed in waterproofed stainless steel enclosures (Figure 1).

Eight individual probe and amplifier assemblies were inserted into the vessel, six located along the central vertical axis and three in the central transverse axis (Figure 2). Each

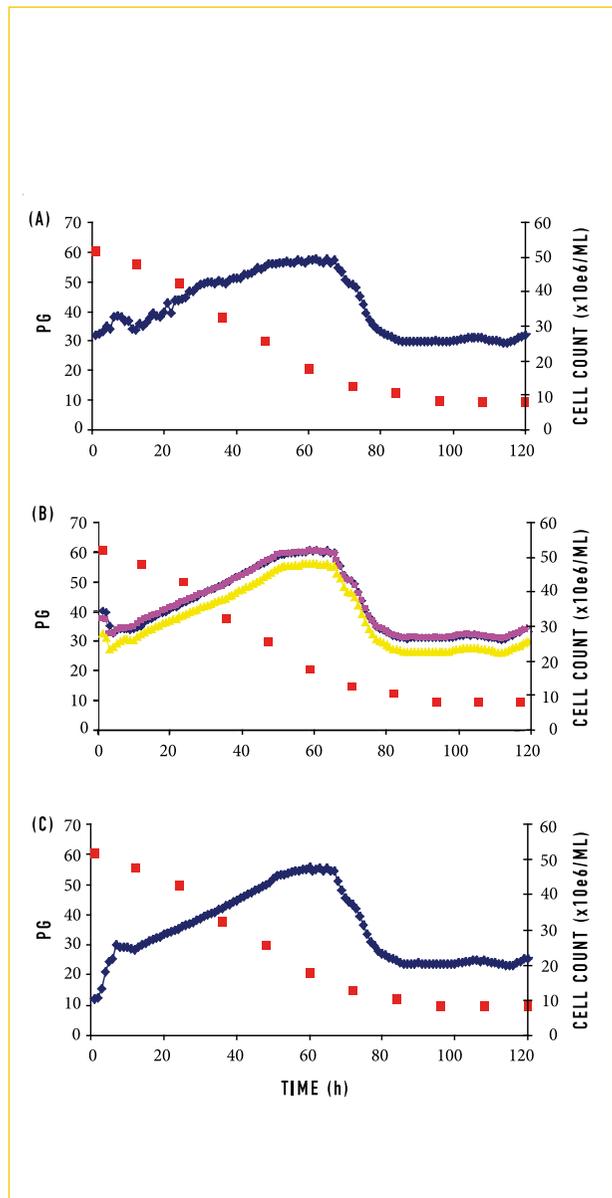


Figure 3. Graph showing the variation in viable yeast concentration with time from the probes located in the cylindrical portion of the fermenter. The PG profile is shown in red. Probe 1, upper cylinder [A]; probes 2,3,4, middle cylinder [B]; probe 5, bottom cylinder [C].

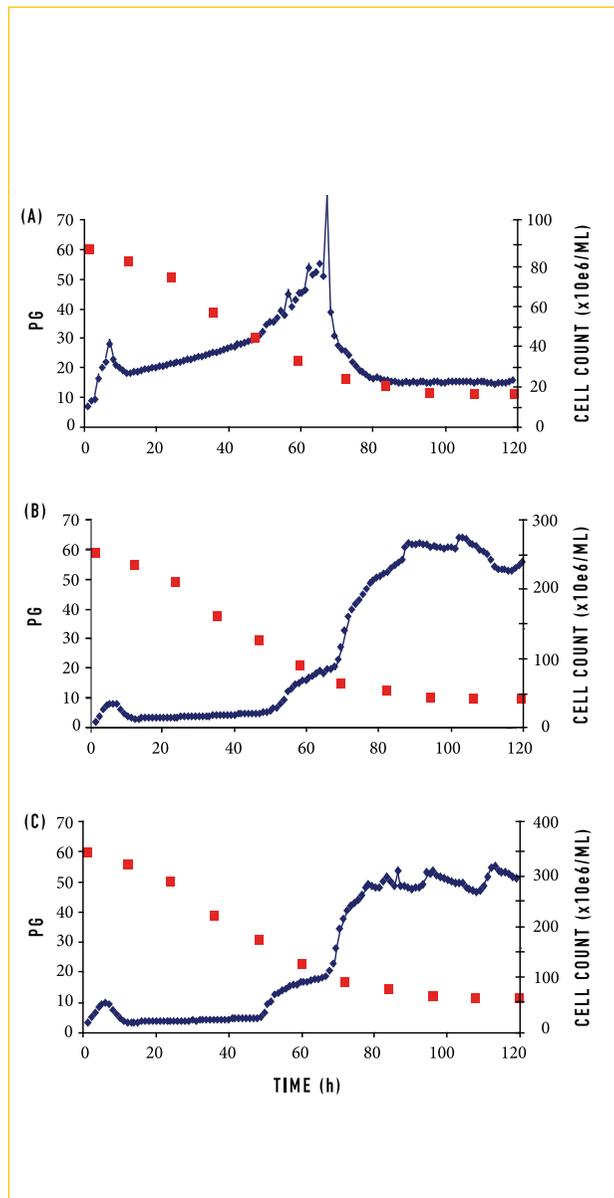


Figure 4. Graph showing the variation in viable yeast concentration with time from the probes located in the cone of the fermenter. The PG profile is shown in red. Probe 6, upper cone [A]; probe 7, mid-cone [B]; probe 8, lower cone [C].

unit was clamped onto a stainless steel cable attached to the base and top plate of the fermenter. The latter was provided with a winch to allow the biomass probes to be inserted into the vessel via the detachable part of the cone and, once installed, to apply tension to ensure that the probes remained stationary.

Output from the individual probes was collected via a multiplexer and two Model 720 biomass meters (Aber Instruments, Science Park, Aberystwyth, UK). The data was

routed via a serial output feeding a network card allowing data collection and manipulation using a remote computer. Standard production fermentations were monitored. These used a malt wort of 15° Plato. The wort was collected over seven hours in two 800 hl brew lengths, pumped at a rate of ca 350 hl/h. The wort was oxygenated at the paraflow to a concentration of 25ppm. Yeast, ca 2,500 kg of slurry, was pitched in-line after transfer of the first 50 hl of wort of the first brew length. The target pitching rate was  $15 \times 10^6$  viable →

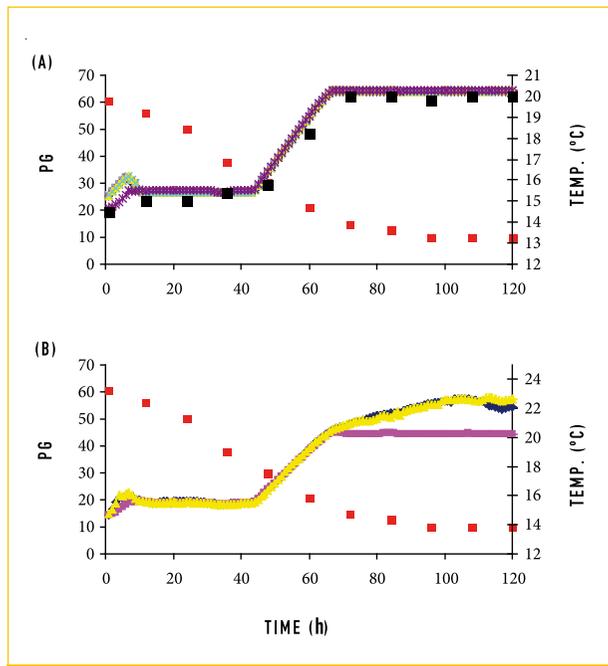


Figure 5. Graph showing variation in temperature with time. The gravity profile is shown in red. Output from the probes located in the cylindrical body of the vessel is shown in [A] together with point measurements taken from the body thermometer used by the attemperation control system. Output from the probes located in the cone is shown in [B].

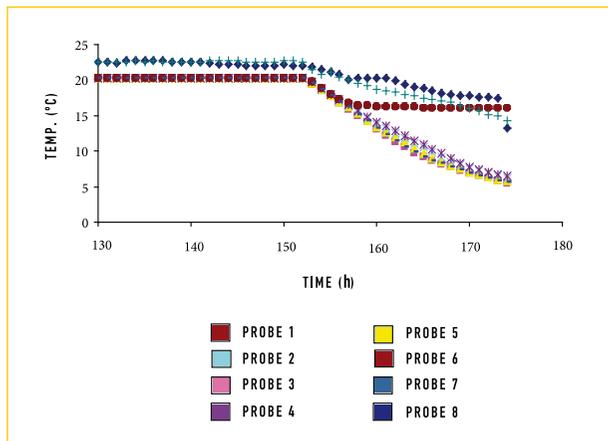


Figure 6. Temperature outputs during crash cool.

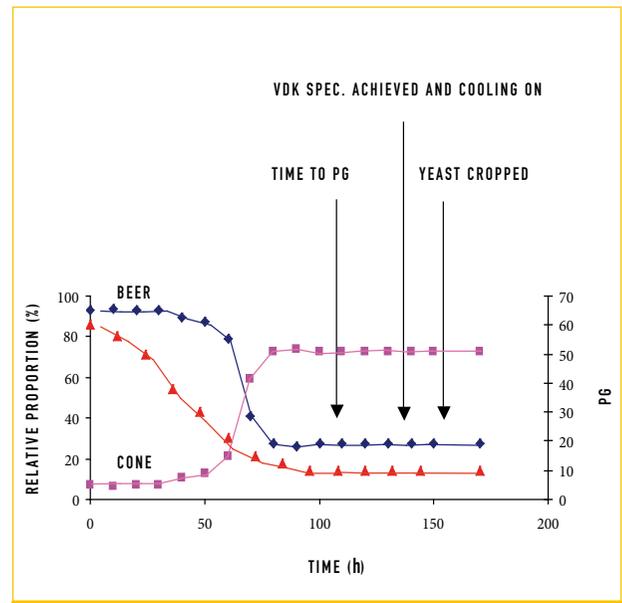


Figure 7. Graph showing the relative proportion of the total yeast population located in the body of the fermenter and in the cone during the course of fermentation.

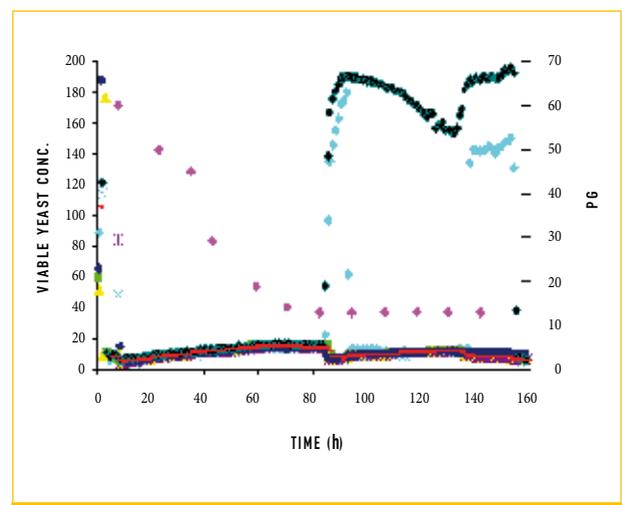


Figure 8. Plot of viable yeast concentration from all probes and PG during an agitated fermentation using a moderately flocculent yeast strain. The Agitator was switched on at time zero, the wort collection was completed at eight hours and the agitator was switched off at 82 hours.

cells/ml. During fermentation, a stepped attemperation profile was used.

The yeast was a moderately flocculent lager strain.

**RESULTS AND DISCUSSION**

The variation in yeast concentration recorded from the five probes located in the cylindrical part of the fermenter together with the wort gravity attenuation profile, obtained from analysis of off-line samples, is shown in Figure 3a, b and c. During the first 10-12 hours after collection was completed, the output from individual probes changed rapidly and with no apparent coherent pattern. This suggested that during this initial phase, the contents of the vessel were not homogeneous and that there were localised regions of relatively higher and lower yeast concentration flowing past individual probes. From approximately 12 hours onwards, the output from all of these probes was similar throughout the remainder of fermentation indicating that at any instant, the yeast concentration in the cylindrical portion of the vessel was relatively constant. During the first 60-70 hours of fermentation there was a gradual increase in viable yeast concentration, presumably reflecting growth.

The maximum observed yeast count of approximately 50 million cells/ml was achieved at around 65 hours. After this time, the yeast count decreased sharply. Output from the three probes located in the cone is shown in Figure 4a, b and c. As with the previous set of data, there was evidence of heterogeneity during the very early phase of fermentation. After approximately 50 hours, when the wort gravity had decreased to half of the initial value, a transient peak in yeast concentration was observed from the probe located at the top of the cone (Figure 4a). At the same time, the yeast concentration measured by the two

lower cone probes was observed to increase sharply (Figure 4b and c). The time of this increase coincided with the decline in yeast concentration observed from the probes located on the main body of the vessel. This suggests that the transient peak in yeast concentration measured at the upper cone probe reflected yeast biomass falling from the upper body of the fermenting wort, passing this probe and forming a sediment in the cone.

The latter was evidenced by the coincident increase in yeast concentration measured at the probes located in the lower portion of the cone.

The temperatures measured at each individual probe, together with that obtained from the controlling body thermometer, are shown in Figures 5a and b.

During the early part of fermentation, there was again evidence of heterogeneity seeming to confirm that the vessel contents were not properly mixed until approximately 12 hours into the fermentation. After this initial period, the temperature was apparently uniform throughout the entire vessel. The mid-point stepped increase in temperature coincided with the time at which the yeast began to settle in the cone. Perhaps unexpectedly, the achievement of the second temperature plateau was accompanied by a second more pronounced phase of increase in yeast concentration in the cone. The presence of the high concentration of yeast in the cone was confirmed by the fact that the temperature in the cone was significantly higher than the set-point. The lack of efficient attemperation in the cone persisted throughout crash cooling (Figure 6). In addition, a band of beer at the top of the vessel, presumably located above the top cooling jacket, failed to cool appreciably even during and after the crash cool.

From the plots of yeast concentration measured at each probe over time, it was possible to construct a plot showing

	TIME TO VDK (DAYS)	RANGE
AGITATED TRIALS (MEAN OF 6 FERMENTATIONS)	3,7	+/- 0.28
CONTEMPORARY CONTROLS (MEAN OF 6 FERMENTATIONS)	5,2	+/- 0.44

Table 1. The effect on cycle times, shown as time to attain the desired VDK specification, for mechanically agitated fermentations and compared with standard contemporary non-agitated control fermentations which used similar wort and yeast. Each data set represents the mean of six fermentations.

the approximate distribution of yeast biomass throughout the vessel during fermentation (Figure 7). Throughout the first 50 hours of fermentation, approximately 85-90% of the total yeast in the vessel was evenly distributed throughout the beer in the body of the vessel. After 50 hours, when the wort was roughly 60% attenuated, the yeast began to move downwards into the cone. At ca 65 hours, equivalent to 84% attenuation, 50% of the total yeast in the vessel was in the cone. At ca 75 hours, equivalent to 90% attenuation, some 70% of the total viable yeast population was in the cone. After this time, there was no further apparent change. Complete attenuation of the wort was not observed until ca 110 hours had elapsed and the VDK specification was not reached until ca 140 hours into the fermentation. At this time, crash cooling was applied and this had no apparent effect on the remaining suspended yeast. The yeast crop was removed at ca 160 hours.

The results shown were for a typical fermentation. In subsequent trials, the effects of serial re-pitching throughout nine generations were investigated. Essentially, similar results (not shown) were obtained to those described already.

These results suggested that it would be beneficial to provide mechanical agitation during primary fermentation in order to prevent premature yeast sedimentation. The effects of mechanical agitation on yeast distribution were investigated using a mechanically-driven three-bladed impeller (700 mm shaft, side-mounted at junction of side wall and cone and inclined downwards 6° to the horizontal. The rotation speed was 230 rpm). The impeller was switched on immediately after the yeast was pitched during wort collection and turned off when attenuation gravity was achieved. Results using the moderately flocculent yeast strain are shown in Figure 8.

The results indicated that throughout primary fermentation the contents of the vessel were mixed sufficiently well such that output from all eight probes was essentially identical. The usual lag in decline seen at the start of fermentation was abolished. Yeast sedimentation occurred very soon after the agitator was switched off.

The effects of mechanical agitation on vessel cycle time is shown in Table 1.

The results indicated that compared with contemporary unstirred control fermentations the cycle time was reduced by approximately 1.5 days. Based upon the range of

results, there was some indication that the agitated trial fermentations were more consistent.

## CONCLUSIONS

The results suggest that for the combination of yeast strain, wort quality and fermenter described here there

**YEAST SEDIMENTATION BEGAN AT A TIME WHEN THE WORT WAS ONLY 60% ATTENUATED. MORE THAN 70% OF THE TOTAL VIABLE YEAST HAD MOVED INTO THE CONE BEFORE WORT ATTENUATION WAS ACHIEVED. PREDICTABLY, ATTEMPERATION OF THE PACKED YEAST MASS IN THE CONE WAS INADEQUATE. THIS MUST BE A CAUSE FOR CONCERN REGARDING PITCHING YEAST AND BEER QUALITY SINCE THE CROP WAS NOT REMOVED FOR A FURTHER 85 HOURS.**

was significant heterogeneity of vessel contents throughout most of the process. The lack of homogeneity during the early phase of fermentation probably reflects the fact that injecting a burst of concentrated yeast slurry into the wort stream flowing into the base of the vessel produced a relatively non-turbulent fill. The lack of appreciable CO<sub>2</sub> and heat evolution during this early period would produce little or no natural mixing action. From ca 12 to 40 hours after pitching, the relative concurrence of output from each probe suggested that during this period mixing was adequate and that vessel contents were homogeneous.

Yeast sedimentation began at a time when the wort was only 60% attenuated. More than 70% of the total viable yeast had moved into the cone before wort attenuation was achieved. Predictably, attemperation of the packed yeast mass in the cone was inadequate. This must be a cause for concern regarding pitching yeast and beer quality →



**THE RESULTS INDICATED THAT COMPARED WITH CONTEMPORARY UNSTIRRED CONTROL FERMENTATIONS THE CYCLE TIME WAS REDUCED BY APPROXIMATELY 1.5 DAYS. BASED UPON THE RANGE OF RESULTS, THERE WAS SOME INDICATION THAT THE AGITATED TRIAL FERMENTATIONS WERE MORE CONSISTENT.**



since the crop was not removed for a further 85 hours. VDK specification was not achieved until 70 hours after the majority of the yeast had settled into the cone. This suggests that the bulk of the yeast would be relatively inaccessible to the main body of beer and therefore probably not involved in diacetyl reduction. By inference, the relatively small proportion of suspended yeast that could not be induced to settle out either by exhaustion of fermentable sugars or by crash cooling was presumably responsible for elimination of diacetyl during the latter part of the fermentation.

These results confirm those of Quain et al [2] that in the interests of good yeast management, it would be sensible to remove the yeast crop soon after the time at which the wort becomes fully attenuated. Of equal importance,

preliminary results suggest that the application of mechanical agitation during primary fermentation had a beneficial effect. This was manifest as a significant reduction in overall cycle times, but, perhaps more importantly, there was a suggestion that this approach represents a practical method for achieving more consistent fermentation performance and by inference, although this requires to be demonstrated, more consistent beer.

The majority of large capacity fermentation vessels are not provided with mechanical agitators. The costs of retro-fitting these are prohibitive especially where cooling jackets have to be bridged. In addition, such devices are difficult to clean and may be sources of infection. Gas rousing is inefficient and may purge volatiles from beer and its use cannot be recommended. In order to reap the benefits of agitation, an alternative means of providing efficient mixing of the contents of large vessels is desirable. One such approach is the use of a loop system in which fermenting wort is removed from the base of the cone and returned to a point higher up the vertical side of the vessel. Continuous recirculation of the vessel contents through such a loop system in conjunction with a rotary head of the type used for CIP provides a highly efficient method of mixing. Since the loop can be made part of the CIP route, there is no risk of infection. A suitable system employing this arrangement has been developed by the Danish company Iso-Mix (Copenhagen, Denmark), now part of the Alfa-Laval company. Trials using this approach have proven very promising. <sup>1</sup>

#### ACKNOWLEDGEMENTS:

The author wishes to thank Bob Todd, John Carvell and Nick Martin of Aber Instruments for the design and construction of the biomass meters and probes used in this investigation. Trials were performed at the Alton Brewery of Coors Brewers UK Limited. The author thanks the directors of Coors Brewers for permission to undertake the trials and Ann Peters, Chris Wilson and John Wright of Coors for invaluable technical assistance.

#### REFERENCES:

- <sup>1</sup> Cahill, G., Walsh, P.K. & Donnelly, D. Proceedings of the European Brewery Convention Congress, Cannes, 1999, 695-702.
- <sup>2</sup> Quain, D.E., Powell, C., Hamilton, A., Ruddlesden, J.D. & Box, W. Proceedings of the European Brewery Convention Congress, Budapest, 2001, 328-396.
- <sup>3</sup> Boulton, C.A., Maryan, P.S., Loveridge, D. & Kell, D. Proceedings of the European Brewery Convention Congress, Zurich, 1989, 653-661.
- <sup>4</sup> Boulton, C.A. & Clutterbuck, V.J. Proceedings of the European Brewery Convention Congress, Oslo, 1993, 509-516.