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Studies supporting the use of mechanical mixing in large scale beer fermentations

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Nomenclature

A	Cross-sectional area of the cylindroconical vessel (m^2)
D	Impeller diameter (m)
g	Gravitational constant (9.81 m^2/s)
H	Static head due to the height of liquid in the cylindroconical vessel (m)
H_A	Static head due to atmospheric pressure (m)
N	Impeller speed (rev/s)
n	Number of impellers (-)
P	Power (W)
Po	Power number of impeller (-)
Q_{CO2}	Volumetric CO_2 production rate (m^3 CO_2/m^3 liquid/s)
Re	Reynolds number (= ND^2/ν) (-)
t_m	Mixing time (s)
V	Volume of liquid (m^3)
v_S	Superficial gas velocity in the cylindroconical vessel (m/s)

Greek letters

ε_T	Local specific energy dissipation rate (W/kg)
$\bar{\varepsilon}_T$	Mean specific energy dissipation rate (W/kg)
λ_K	Kolmogoroff microscale of turbulence (m)
ν	Kinematic viscosity (m^2/s)
ρ	Liquid density (kg/m^3)

Subscript

| g | Under gassed conditions |

ABSTRACT

Brewing fermentations have traditionally been undertaken without the use of mechanical agitation, with mixing being provided only by the fluid motion induced by the CO_2 evolved during the batch process. This approach has largely been maintained because of the belief in industry that rotating agitators would damage the yeast. Recent studies have questioned this view. At the bench scale, it has been shown that yeast is very robust and is able to withstand very intense mechanical agitation under aerobic conditions without observable damage as measured by flow cytometry and other parameters. Much less intense mechanical agitation also reduces batch fermentation time for anaerobic beer production by about 25% compared to mixing by CO_2 evolution alone with a small change in the concentration of the different flavour compounds. These changes probably arise for two reasons. Firstly, the agitation increases the relative velocity and the area of contact between the cells and the wort, thereby enhancing the rate of mass transfer to and from the cells. Secondly, the agitation eliminates spatial variations in both yeast concentration and temperature, thus ensuring that the cells are maintained close to the optimum temperature profile during the whole of the fermentation time. These bench scale studies have recently been supported by results at the commercial scale from mixing by an impeller or by a rotary jet head (RJH), giving more consistent production without changes in final flavour. It is suggested that this reluctance of the brewing industry to use (adequate) mechanical agitation is another example where the myth of shear damage has had a detrimental effect on the optimal operation of commercial bioprocessing.

INTRODUCTION

In essence the brewing process has not changed since the early Middle Ages (Hewitt 2003). Barley is harvested, steeped in water, left to germinate in a warm environment (malting) before roasting or kilning to develop the characteristic colour of the malt and hence the resultant beer. The malt is then ground milled or hammer milled to produce either coarse or fine grist (flour), which is mixed with water and heated to between $40^oC - 70^oC$ in a stepwise manner. This process, known as mashing, converts the starch and various polymers of glucose into fermentable sugars. Once the solids or 'returns' have been filtered out, the resulting liquid wort is then boiled and sometimes hops added until the correct colour and flavour of the final product has developed. After cooling, the wort is supersaturated with air, then yeast is added and it is allowed to ferment, without further intervention, until the correct specific gravity (indicative of ethanol content) has been reached. After removing the suspension from the fermenter and filtering, the beer is allowed to mature before being packaged into casks or bottles prior to sale and consumption.

One of the traditional beliefs of the brewing industry has been that mechanical agitation during the fermentation step would damage the yeast. A measure of this attitude was the comment of one referee on an earlier paper of ours on the impact of agitation on brewing (Boswell et al. 2002); "Do the authors realize that Schlitz introduced stirrers into their fermenters and they closed down?" Both the statements are true and Schlitz, who were once one of the largest beer producers in Milwaukee ("The beer that made Milwaukee famous" was their slogan) introduced mechanical agitation in the early 1970s. Later, they made massive losses and were finally sold to the Stroh Brewing Co. in 1982. However, these events do not show a causal linkage. As a result of such attitudes, mixing, which is essential for any effective contact to be

made between the yeast and the nutrients/carbon source throughout the whole of the batch fermentation time, has been achieved, at best, only during the period of significant fluid motion induced by rising bubbles as CO_2 is generated during part of the anaerobic fermentation (Figure 1). Some lesser motion is also induced due to natural convection arising from temperature gradients either from heat release by the fermentation or from cooling of the walls of the fermenter, undertaken to maintain the desired operating time/temperature profile. Thus, for much of the fermentation time there is very limited fluid motion from any source, with Garcia *et al*. (1994) reporting spatial variations in yeast concentration and temperature in a commercial scale fermenter. Nevertheless, it is still usual to assume that the contents of large scale cylindroconical beer fermenters are well mixed both by practitioners of brewing and when the process is modelled, as pointed out by Boulton et al. (2007) and Hind (2000) respectively.

This paper considers recently published work at the bench scale that firstly queries the veracity of the belief set out above that yeast is 'shear sensitive'; and secondly, indicates what improvements might be achieved if mechanical agitation was implemented. Some very recent studies at the industrial scale supporting the bench scale findings are also outlined.

IMPACT OF FLUID MECHANICAL STRESS DUE TO AGITATION AND BURSTING BUBBLES ON *S. CEREVISIAE.*

Frequently, reference to ''shear damage'' is made to explain detrimental changes in bioprocessing (Hewitt & Nienow 2007) when mechanical agitation and aeration are introduced into a bioreactor. However, even animal cells, which were initially thought to be very sensitive to such forces because of the lack of a cell wall

(Cherry & Papoutsakis, 1986) have been shown to tolerate relatively high mechanical stresses due to turbulent flow in a stirred bioreactor with mean specific energy dissipation rates, $\bar{\varepsilon}_T$ up to 0.25 W/kg (Nienow, 2006). On the other hand, because the fluid mechanical stresses associated with bubbles bursting at the surface of the media have local specific energy dissipation rates, ε_T (W/kg) two to three orders of magnitude higher than those found under typical agitation conditions (Boulton-Stone & Blake 1993), the stresses arising can damage such cells. However, the damage can be essentially eliminated by the use of the surfactant, Pluronic F68, which prevents such cells attaching to bubbles so that when they burst, the cells are not in the vicinity of the very localised and intense stresses produced (Nienow, 2006).

Given these findings with animal cells, it might be expected that yeast cells would be even less likely to be damaged since they are somewhat smaller than animal cells and, additionally, they have a mechanically strong cell wall. To see if this was indeed the case and given the perception of the brewing industry, studies were undertaken to determine the impact of fluid mechanical stress on *S. cerevisiae* cells in a chemostat (essentially a continuous stirred tank reactor) culture of 120 hrs under air sparged, aerobic conditions. During this time, both nutrient concentration and dissolved oxygen concentration, dO_2, were held constant (the latter by gas blending) whilst using various agitation speeds with either a Rushton turbine or paddle impeller (Boswell et al., 2003 a, b) under turbulent flow conditions ($Re > \sim 2 \times 10^4$). In addition, to check on the possibility of damage from bursting bubbles, a period of very high sparging rate was also employed.

The agitation conditions gave mean specific energy dissipation rates, $\bar{\varepsilon}_T$ of up to 6 W/kg where;

$$\bar{\varepsilon}_T = P / \rho V = nPo_g N^3 D^5 / V \qquad\qquad 1$$

and P (W) is the power input from n agitators of diameter D (m) and power number Po_g under aerated conditions running at a speed N (rev/s) in a medium of density ρ (kg/m^3). This value of $\bar{\varepsilon}_T$ is at the upper limit of that used at an industrial scale for aerobic fermentations (Amanullah et al., 2003) and very much higher than that of about 0.035 W/kg generated by the maximum rate of CO_2 release during a beer fermentation of 300 m^3 (Luyben, 1997) (Figure 2).

At the scale of cells, mixing is generally analysed by applying Kolmogoroff's theory of locally homogeneous isotropic turbulence. Thomas (1990) suggested that cells should remain unaffected by fluid mechanical stresses due to turbulence provided that they are smaller than the Kolmogoroff microscale of turbulence, λ_K, defined as,

$$\lambda_K = \left(v^3 / \varepsilon_T\right)^{1/4} \qquad\qquad 2$$

where ε_T is the local specific energy dissipation rate (W/kg) and v is the kinematic viscosity of the medium. Therefore, if $\bar{\varepsilon}_T$ is 6 W/kg, in a water based culture medium, then λ_K would be ~20 µm. Thus, yeast cells (~5 µm) are significantly below this Kolmogoroff microscale. Even at the maximum ε_T as found in the impeller region which is about 30 times the average (Nienow, 1998), λ_K (~ 9 µm) is greater than the cell size, so damage might not be expected.

Experimental results supported these considerations. During chemostat culture, specific cell mass, oxygen uptake rate and CO_2 evolution rate (and hence respiratory quotient) remained constant with time once steady state had been reached with respect to dO_2 and feed rate. During this time, the agitator speed was first held at a low level and then increased stepwise for 2-3 days, after which it was reduced, again

stepwise, back to the original one, to give $\bar{\varepsilon}_T$ ~ 0.045 and ~ 6 W/kg respectively. In addition, multi-parameter flow cytometry, which uses laser light and fluorescent dyes (Hewitt & Nebe-von-Caron 2001, 2004) to determine the physiological state of many thousands of cells, indicated that all cells were viable and therefore, damage was not detected.

On the other hand, flow cytometry showed that during the two to three days of agitation at $\bar{\varepsilon}_T = 6$ W/kg, the two sub-populations corresponding to single and dividing cells temporarily showed a complete reduction in the number of dividing cells over a period of 4 hrs. Towards the end of this time at 6 W/kg, the latter sub-population began to reappear and did so completely and rapidly once $\bar{\varepsilon}_T$ was reduced to 0.045 W/kg again.

For completeness, because of the very high stresses associated with bursting bubbles and their impact on animal cells, during chemostat culture, aeration rates of 1 and 3 vvm were used, both being much higher than the maximum of about 7×10^{-3} vvm due to CO_2 release estimated for a typical 300 m^3 beer fermentation (Garcia et al., 1995). The value of 3 vvm is even high for an aerobic fermentation (Nienow, 1998). Again, no change in normal fermentation parameters was detected nor did flow cytometry indicate any dead cells or a permanent change in the population.

IMPACT OF MECHANICAL AGITATION ON BREWING PERFORMANCE WITH MUNTON'S PALE ALE

The above study provided strong evidence that mechanical stresses due to agitation and sparging during aerobic fermentations did not permanently damage yeast cells at very much more intense levels than would be found in beer brewing even at full commercial scale. Therefore, it was decided to use mechanical agitation at

much lower intensities throughout a batch anaerobic fermentation to study (Boswell et al., 2002; Boswell et al., 2003b) the improvement, if any, that would result. These studies were undertaken in fermenters of 500 mL operating volume using the yeast strain, *S. cerevisiae* NCYC 1324 either without agitation or with different agitation speeds up to 600 rpm to give $\bar{\varepsilon}_T$ values up to 0.26 W/kg with a Rushton turbine. The wort (the carbon source) was prepared from a concentrate ('Hopped Light' wort, Munton's, Stowmarket, Suffolk, UK as used to make Munton's Pale Ale), the vessels were maintained at $12^\circ C$ and the pitching rate (the amount of cells introduced at the start of fermentation) was 1.5×10^7 yeast cells/mL wort. Estimates of $\bar{\varepsilon}_T$ due to CO_2 release vary during the whole fermentation process from zero initially (before the yeast becomes active and there is no CO_2 flow) to zero again (after all of the sugars in the wort have been effectively utilized) with a maximum at some point during the fermentation (Figure 2), which depends on the fermenter configuration. Large scale fermenters are typically cylindroconical vessels and $\bar{\varepsilon}_T$ due to CO_2 release can be approximated by the method of Vrieling (1978) for beer fermentations:

$$\bar{\varepsilon}_T = Q_{CO2} g H_A \ln\left[(H_A + 0.5H)/H_A\right] \qquad 3$$

In this equation, Q_{CO2} is the volumetric flow of CO_2 in $(m^3/s)/m^3$ wort produced during the fermentation, H_A is the static head due to atmospheric pressure and H is the head due to the height of liquid in the fermenter. This approach assumed that the CO_2 was generated throughout the vessel and therefore on average, the evolved gas rose against a pressure due to half the static head. However, for present purposes given the other approximations involved in the model, $\bar{\varepsilon}_T$ from CO_2 evolution can be estimated sufficiently accurately (Boswell, 2003b) by the relationship:

$$\bar{\varepsilon}_T = v_s g \qquad\qquad\qquad 4$$

where $v_s = Q_{CO2}(V/A)$ and V and A are the volume and cross-sectional area of the fermenter. In addition, $V/A = \sim \alpha T$ where α is the fermenter aspect ratio (= H/T) and H and T are its height and diameter respectively. Equation 4 also applies to a gas being introduced at the base of a vessel and since here it is generated *in situ* but at a location which is not actually known, it has been suggested (Hind, 2000) that a value of H/2 should be assumed for the aspect ratio . Thus, it can be seen that $\bar{\varepsilon}_T$ increases with scale due to both increasing size and in general, aspect ratio. For typical beer fermenters of the order of 400 to 500 m^3 of aspect ratio $\sim$ 4 to 5 and a maximum CO_2 evolution rate of 1.2 x 10^{-4} (m^3/s)/m^3 (Garcia et al., 1994), $(\bar{\varepsilon}_T)_{max}$ values during a typical commercial scale batch fermentation are of the order of 0.045 W/kg, a value similar to that proposed earlier (Luyben, 1997).

During the fermentation studies (Boswell et al., 2002; Boswell et al., 2003b), standard parameters (specific gravity, dry cell weight, fermentable sugars, and flavour compounds) were monitored (Figure 3 and 4, not all data shown). There was a threshold for $\bar{\varepsilon}_T$ of $\sim$ 0.03 W/kg (of the order of the estimated $(\bar{\varepsilon}_T)_{max}$ at the large commercial scale) below which there was no significant difference between stirred and unstirred fermentations. Above that, for all values of $\bar{\varepsilon}_T$ up to 0.25 W/kg, the overall batch fermentation time was significantly reduced from ~160 to ~100 h, with increased dry cell weight, an increase in higher alcohols, a reduction in esters and with the % of ethanol produced unchanged. These changes can be ascribed to improved transport of substrates in and desorption of products out across the cells cytoplasmic membrane. There are two reasons for this. Firstly, the agitation increases the relative velocity between the dispersed solids (here the cells) and secondly

because, as could be seen, the cells were better suspended under agitated conditions, the effective area available for mass transfer was increased (Nienow, 1997)...

The use of multi-parameter flow cytometry indicated that up to 0.03 W/kg, there were ~ 6% dead cells after 160h, at which time the attenuation limit (all sugars had been utilised) had been reached. After 100h at higher agitation intensities when the attenuation limit had again been reached, there were 9% dead cells. These results implied that significant savings in batch times and therefore increases in productivity could be obtained by the use of gentle mechanical agitation throughout a batch fermentation at only a little above the level of $\bar{\varepsilon}_T$ found from CO_2 evolution without a significant loss of cells (Boswell et al., 2002; Boswell et al., 2003b). However, at the bench scale, there was a 3-5 fold increase in the accumulation of isobutanol and up to a 6 fold increase in isobutyl acetate whilst ethyl acetate concentrations remained unchanged. Similar results with respect to batch times and flavour compounds had been found previously (Vrieling, 1978); Masschelein *et al.*, 1981; Okabe *et al.*, 1992).

IMPACT OF MECHANICAL AGITATION ON BREWING PERFORMANCE WITH GROLSCH LAGER

The above work clearly shows the potential for reducing batch time and hence increasing the productivity of large scale beer fermentations without damaging the yeasts by introducing mechanical agitation. This enhancement at the bench scale was explained by the increased rate of transfer of nutrients, partly due to enhanced area of contact due to cell suspension and partly due to an increased slip velocity between the cells and the wort (Nienow, 1997). However, at the large scale, in addition to the above potential for improvement, the use of agitation should also reduce temperature and possibly other concentration gradients giving conditions closer to the relatively

homogeneous conditions always found at smaller scales at comparable mean specific energy dissipation rates (Nienow, 1998).

Earlier work by Garcia *et al*. (1995) indicated that during lager fermentations, which are performed at lower temperatures than pale ale, significantly lower temperatures exist at the end of a fermentation in the cone of the cylindroconical vessel when cooling via a jacket was only applied to the cone region. Thus, the cooling jacket tends to over-cool the wort and this tendency for a lower temperature to form will be enhanced by the increasing density, leading to density stratification; and the low flow rate of liquid generated by either bubble evolution or natural convection will do little to alleviate it.

Thus, work was done firstly to investigate the effect of mechanical agitation on the production of a lager beer (Grolsch lager wort, Coors Brewery Ltd, Burton-upon-Trent, UK) at a constant lower temperature compared to the earlier work with Munton's Pale Ale. Secondly, a rig was constructed to allow experiments at the bench scale with this lager beer that simulated the temperature variations at the large scale indicated by Garcia et al. (1995). These latter experiments were conducted with and without mechanical agitation.

The impact of mechanical agitation to aid suspension of yeast and produce a more homogeneous environment throughout the whole batch time.

The first experiments with the Grolsch Lager were essentially the same as those undertaken as described above with the Munton's Pale Ale. However, the temperature was maintained at 12°C throughout the batch runs and the experiments were conducted in 5L fermenters. The results obtained essentially showed the same trends as with the Munton's Pale Ale. Agitation reduced the overall batch

fermentation time from about 104 to 80 hrs (by about 1 day), increased the dry cell weight of yeast, changed slightly the flavour compounds by enhancing the production of higher alcohols and suppressing esters and left the percentage of ethyl alcohol unchanged whilst a similar quantity of maltose was consumed (Table 1). An estimate of the amount of CO_2 produced showed a reduction when the fermentation was agitated. A retrospective review (McLeod, 2007) of the earlier work of Boswell et al. (2002) suggested an equivalent reduction in CO_2 with Munton's Pale Ale when agitated.

Using mechanical agitation to eliminate spatial temperature variations

The temperatures reported by Garcia et al. (1994) varied from ~ 5°C in the conical base of the commercial cylindroconical fermenter at the start and end of the fermentation when the CO_2 evolution rates were low, to ~ 12°C, the desired operating temperature, which was the same everywhere in the vessel during the period of peak evolution. These conditions were simulated at the bench scale using a stirred fermenter with plug flow loop (STR-PFR). This combination has been successfully used for other scale down studies (Amanullah et al. 2003) and has been particularly successful at mimicking large scale *E. coli* fermentations (Onyeaka et al. 2003; Hewitt et al. 2007). The work reported here is the first in which temperature gradients in a bioreactor have been simulated. Here, the volumes of the STR (5L Electrolab fermenter) and PFR were 4L and 0.4L respectively. The PFR consisted of five equally sized glass cylinders each containing a removable stainless steel static mixer element (Kenics, Chemineer, UK), giving a total liquid volume in the PFR of 544ml. The static mixers were used to eliminate radial concentration gradients and encourage plug flow. The STR/PFR volumetric ratio of 10:1 was selected to give a similar ratio to

that between the volumes of the cylinder and cone respectively of a large scale fermenter (Garcia et al., 1995). Wort was circulated by peristaltic pump between the stirred fermenter with a temperature fixed at 12°C (with agitation set to give an $\bar{\varepsilon}_T$ of either 0.044 or 0.28 W/kg) and the chilled PFR in which the temperature was varied as set out below.

Temperatures were controlled in the STR, PFR and transfer line with a cooling jacket through which antifreeze was circulated. This jacket was constructed by coiling silicon tubing (0.6mm external diameter) around the outside of the vessels and line. Antifreeze was passed through the tubing in a co-current direction to the flow of the fermentation broth and the temperature of the anti-freeze was maintained at -10°C using a chiller unit. The whole rig is shown schematically in Figure 5.

The circulation time in a large fermenter was calculated from Equ 5 for the mixing time, t_m, in a bubble column (Van't Riet and Tramper, 1991);

$$t_m = 11(H/T)(gv_sT^{-2})^{-0.33}$$ 5

using the assumption that the circulation time was one quarter of the mixing time (Van't Riet and Tramper, 1991). For typical values of CO_2 evolution rate throughout the course of a fermentation applied to the 300 m^3 scale, this leads to the circulation times shown in Figure 6. The flow rate in the scale down rig was then varied to match those estimated circulation times. The change of flow rates gave a temperature profile with time in the PFR that matched quite well that reported by Garcia et al. (1994) (Figure 7), with variations between the inlet and the outlet. The temperature in the STR remained constant throughout.

The results (Table 2) can be summarised as follows. Comparing the results under agitated conditions in the STR plus circulation through the low temperature PFR (12°C at the base of the PFR rising to 12°C before re-entering the STR) with

those without circulation, the fermentation time was longer (110-120-h versus ~ 95 h), the amount of fermentable sugars converted to ethanol was less, the dry cell weight was considerably less and the flavour compounds were less. In addition, the amount of CO_2 was greater (~50% compared to ~ 40% (data not shown)) whilst consuming a similar amount of maltose. Overall, this simulation showed that the presence of the low temperature in the base of the cone at the industrial scale led to a poorer performance compared to that obtained when it was eliminated by improved mixing throughout the batch time.

DISCUSSION AND CONCLUSIONS

The above studies show that at the bench scale, intense agitation did not damage the yeast as measured by fermentation parameters and flow cytometry under aerobic conditions. Similar insensitivities to mechanical agitation under aerobic conditions using these experimental and analytical tools have recently been shown for *E. coli* (Hewitt et al. 1998) and *Corynebacterium glutamicum* (Chamsartra et al. 2005). Even animal cells, which do not have a cell wall, are now recognized as being more robust than it was first thought and many such cell lines have been shown to be able to be agitated at $\bar{\varepsilon}_T$ values up to 0.25 W/kg without a reduction in cell viability or productivity (Nienow 2006).

It has also been shown by flow cytometry that when producing beer under anaerobic conditions at $\bar{\varepsilon}_T$ levels from mechanical agitation up to ~ 0.25 W/kg, the amount of dead yeast cells produced compared to unagitated conditions was essentially the same. In addition, agitation intensities from ~ 0.03 to ~ 0.25 W/kg improved beer productivity as indicated by reducing the fermentation time by 1-2 days in both a Munton's Pale Ale and a Grolsch lager fermentation, compared to

fermentation without mixing except by CO_2 evolution. Mechanical mixing also reduced the proportion of fermentable sugars going to CO_2 and slightly modified the proportions of the flavour compounds produced, particularly an increase in higher alcohols and a reduction in esters.

A scale down study using an STR-PFR configuration to simulate spatial temperature variations previously reported in the literature for commercial scale lager beer fermenters have also shown to give a poorer performance compared to when the temperature is constant throughout the fermenter. In particular, without temperature variations and with the continuous application of mechanical agitation throughout the batch fermentation of $\bar{\varepsilon}_T$ values equivalent to those produced by CO_2 evolution at its peak, the fermentation time was shorter, the amount of ethanol produced was greater and the amount of CO_2 was reduced. Such improvements should be achievable at the large scale by mechanical agitation.

Two studies at the commercial scale of beer production have recently appeared. Boulton et al. (2007) have reported the impact of using an axial flow impeller at ~ 0.03 W/kg discharging downwards at 5° from horizontal just above the cone of a 150 m^3 beer fermenter at Coors Brewery at Burton, UK. Experiments with a variety of yeasts of different flocculation characteristics were used and the local concentration of cells at various points was measured as well as the local temperature by 9 Aber biomass probes (Aber Instruments, Science Park, Aberystwyth, UK) positioned throughout the fermenter. It was found that without mechanical agitation, with both strong and weak flocculating yeasts, very high yeast concentrations existed in the cone, even when CO_2 evolution was still quite vigorous after about 50 hrs; and remained so for the remaining time of the 120 hrs fermentation. In addition, temperature variations were found, though contrary to the work of Garcia et al.

(1994), temperatures were some 2.5 °C higher at the base. This higher temperature was ascribed to the high concentration of yeast in the cone leading to a locally high metabolic heat evolution that could not be dispersed because of the lack of adequate fluid motion (Boulton et al., 2007).   Agitation largely eliminated these variations and as in the work reported here, the fermentation time was reduced by about 1.5 days with no indication of yeast damage. In addition, the beer was found to be 'true to type' (taste similar to that produced without stirring). The difference in the findings related to flavour between the industrial scale study and the current work at the bench scale may arise because at the large scale, there are extensive pre-and post-processing steps which are relatively slow and which also impact on the final flavour too. Boulton et al. (2007) also showed that the use of mechanical agitation made the batch time and other process parameters more consistent.

The use of a rotary jet head (RJH) system to provide mechanical mixing (Nordkvist et al., 2007 & 2008) in 6 different beer fermentations up to 150 m^3 gave similar results to those of Boulton et al. (2007) in that the fermentations were more consistent, the beer was again 'true to type' and fermentation times were reduced by between 10 and 20%.

Overall, it appears the 'shear myth' is again impacting negatively on a bioprocess, in this case, the brewing of beer; and that mechanical agitation of beer fermenters could significantly improve batch consistency, enhance beer production whilst remaining true to type, and without loss of yeast viability.

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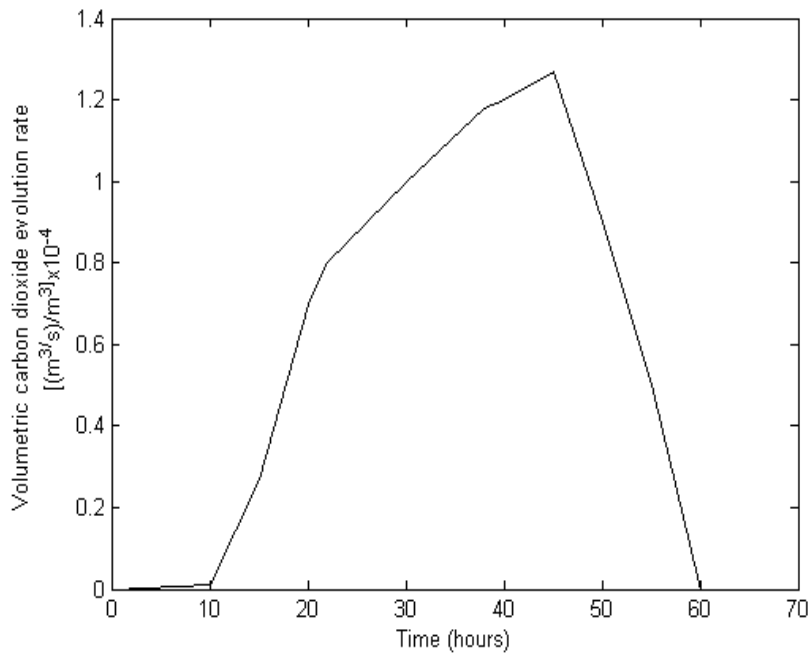
a)
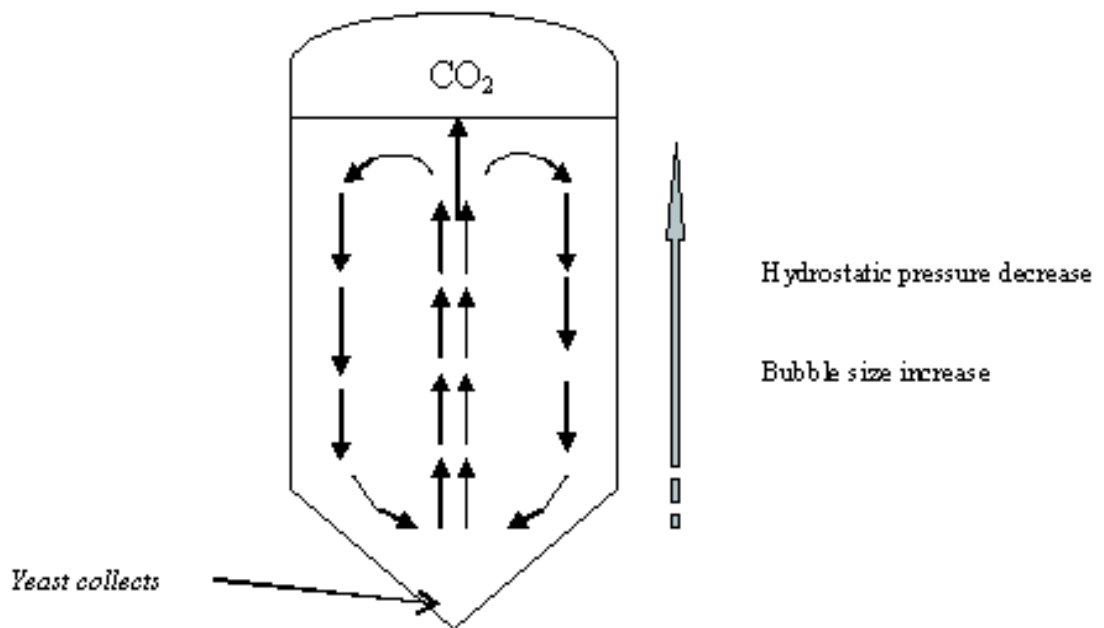
b)

Figure 1 a) Variation in carbon dioxide evolution rate over time (adapted from Garcia

et al., 1994.); b) Assumed flow pattern created by natural carbon dioxide evolution in

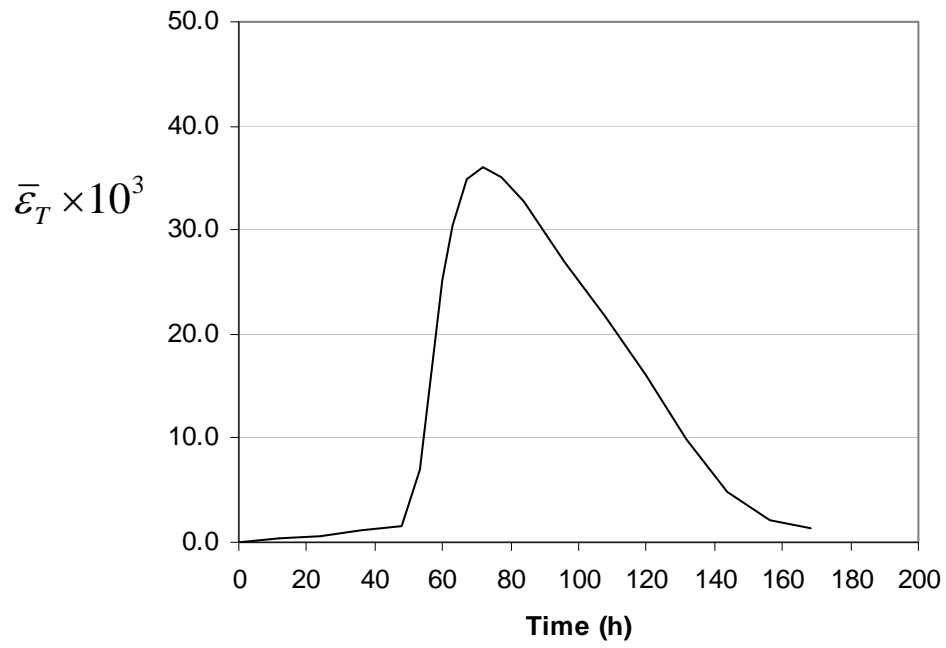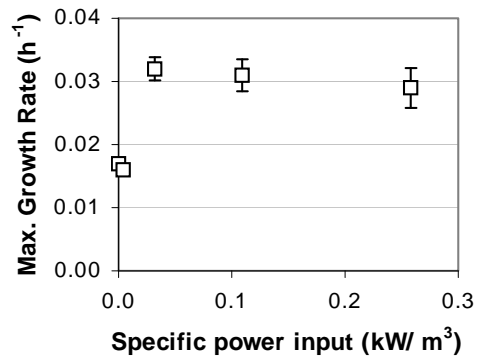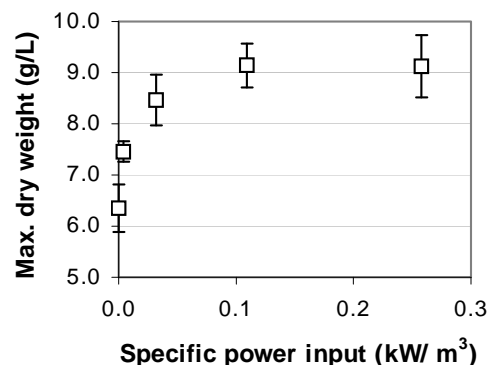a cylindroconical vessel (redrawn from Lewis and Young, 2001)

Figure 2. Estimated changes in mean specific energy dissipation rate, $\bar{\varepsilon}_T$, due to natural CO_2 evolution in a 300m^3 brewing fermentation (after Luyben (1997) using $\rho = 1020$ kg/m^3).
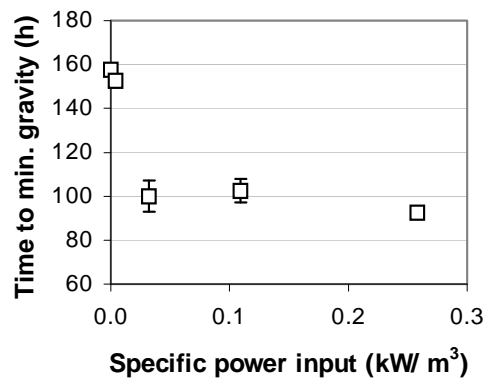
a)

b)

c)

Figure 3. Relationship between specific power input (kW/m^3) and growth rate (a);

maximum dry cell weight (b); and fermentation rate (c). Error bars, S.E. of mean of at

least two experiments.

Ethyl acetate
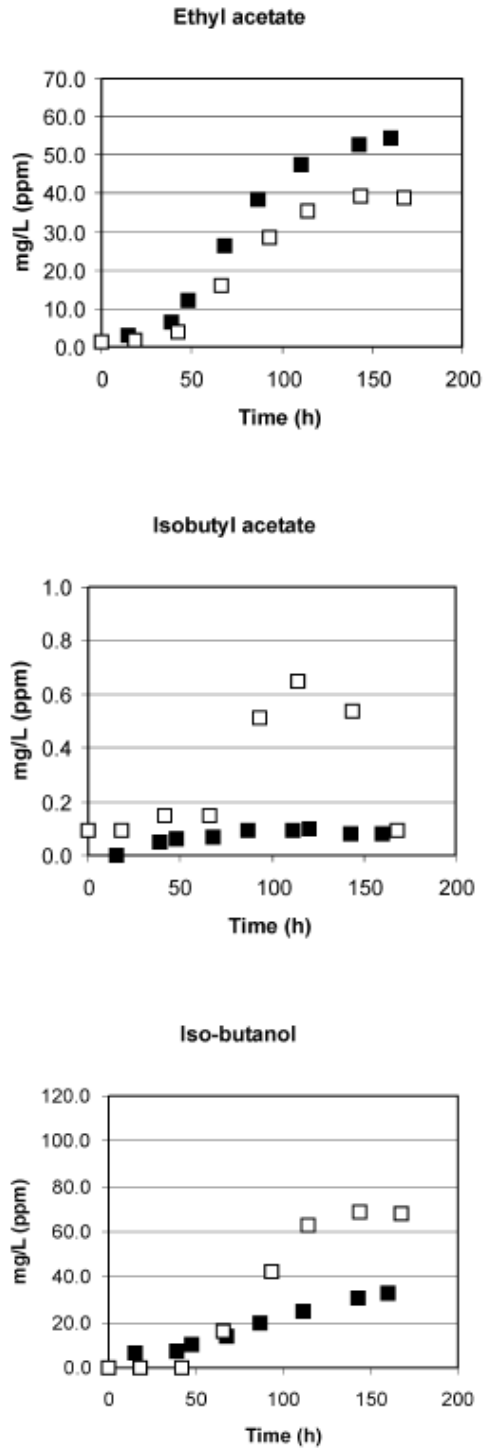

Isobutyl acetate

Iso-butanol

Figure 4. The effect of mechanical agitation rate on the formation of selected volatile flavour compounds over time. Filled symbols indicate non-agitated conditions and unfilled symbols indicate fermentations agitated with a Rushton turbine with $\bar{\varepsilon}_T$ of up to ~ 0.25 W/kg.
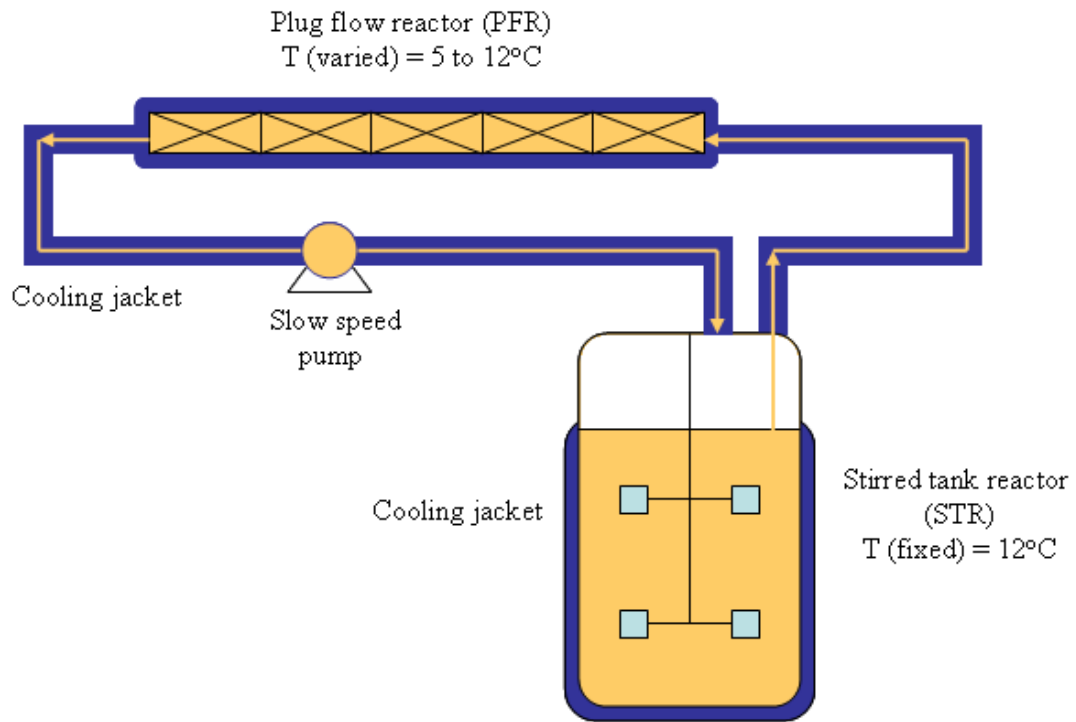
Figure 5. Schematic of scale-down model to simulate temperature variations in an industrial scale brewing fermenter.
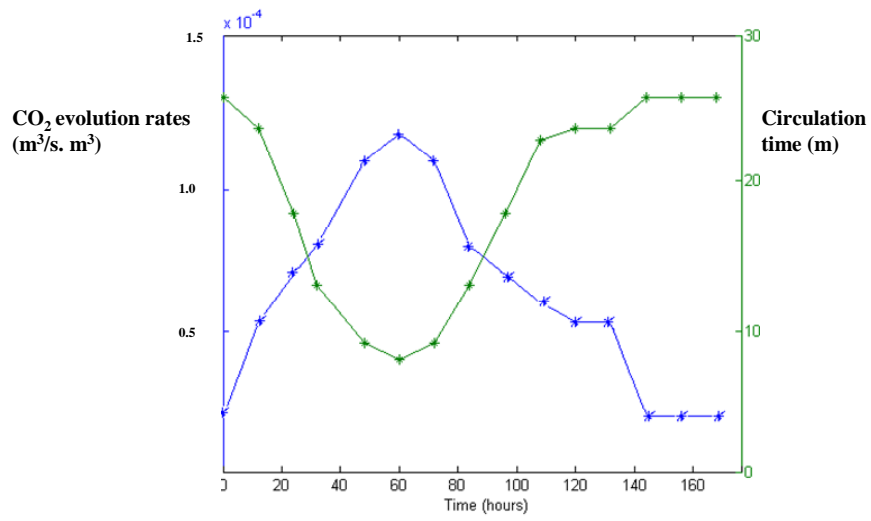
Figure 6. Profile for liquid circulation times through the PFR calculated from typical values for carbon dioxide evolution rate.
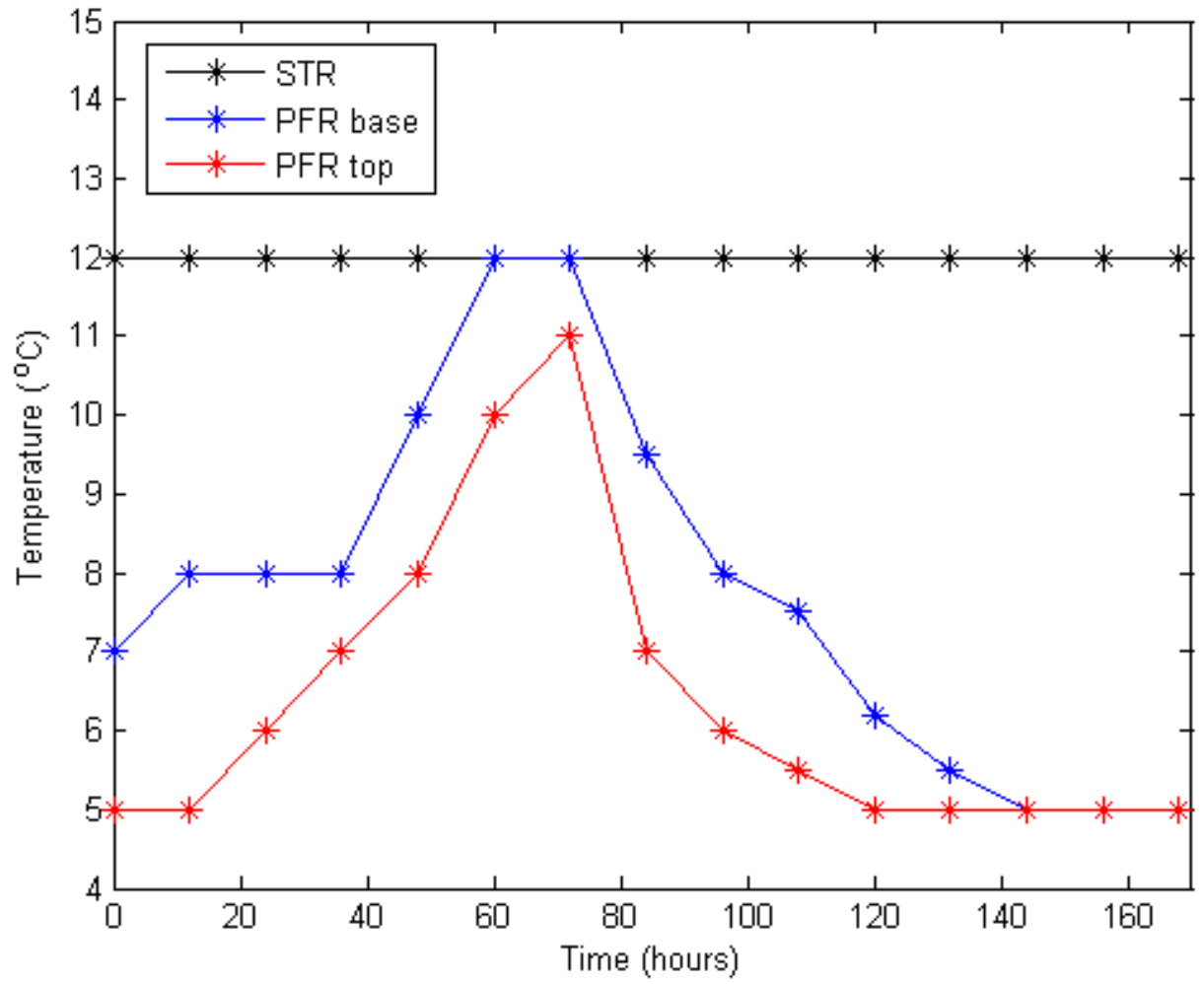
Figure 7. Measured temperatures in different parts of the scale-down model.

Table 1. Material balance for Grolsch lager mixing studies without and with mechanical mixing at varying $\bar{\varepsilon}_T$ values showing the conversion of the total sugars to various beer components

	INPUT	OUTPUT				
$\bar{\varepsilon}_T$	Maltose (g/L)	Ethanol (g/L)	Dry cell weight (g/L)	Ethyl acetate (mg/L)	Isobutanol (mg/L)	Total output (g/L)
0 W/kg	52.1	45.5	4.1	53	60	49.7
0.044 W/kg	48.8	45.2	5.6	42	122	51.0
0.275 W/kg	47.0	45.6	5.6	30	122	51.4

Table 2. Material balance for Grolsch lager fermentations in the STR at 12 °C and $\bar{\varepsilon}_T$ = 0.275 kW/m^3 and with circulation from the STR through the PFR at lower temperatures showing the conversion of the total sugars to various beer components

$\bar{\varepsilon}_T$	INPUT	OUTPUT				
	Maltose (g/L)	Ethanol (g/L)	Dry cell weight (g/L)	Ethyl acetate (mg/L)	Isobutanol (mg/L)	Total output (g/L)
0.275 kW/m^3 STR with no PFR	49.3	45	5.2	34	105	50.3
0.275 kW/m^3 STR with PFR circulation	47.3	33	2.1	23	60	35.2