The Impact of CO_2 Induced Anaerobiosis and Cold Shock on the Flocculation Potential of Brewing Yeast

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INTRODUCTION

The basic role of yeast is to cause the fermentation of barley grains, which in turn produces beer. Fermentation is the anaerobic conversion of fermentable sugars, mainly maltose and glucose, by yeast, into ethanol, carbon dioxide and the characteristic aroma compounds found in beer.

Figure 1. A small modern brewing plant.
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The behaviour of yeast cells during fermentation, especially the clumping or flocculation of yeast cells, is extremely important for the brewing industry because they can affect the final flavour, texture, alcohol level and clarification of the beer (Jibiki et al., 2001). Top fermenting yeasts tend to produce a light frothy 'head' on the beer and are used to produce ales. Bottom fermenting yeasts are used to produce lagers which tend to retain more 'fizz'. There are many natural by-products of fermentation such as diacetyl and acetaldehyde. These natural by-products all contribute to the different aroma compounds and flavours found in certain types of beer.

The ideal flocculation percentage for brewers would be as close to 100% as possible, as that would signify complete cell flocculation. A flocculation percentage of 0 on the other hand would indicate that the yeast is fully dispersed throughout the beer, resulting in a cloudy product with a texture that is undesirable for consumers.

Production scale brewery fermentations are traditionally carried out at around 15°C under anaerobic conditions. This is considerably lower than the 25°C temperature used to quantify flocculation in a laboratory which is measured under aerobic conditions. Consequently, these laboratory conditions may not give a true

representation of the conditions that the yeast are subjected to during production scale brewery fermentations. Hence, it may be more appropriate to analyse flocculation performance at a temperature similar to that found during production scale brewery fermentations.

The aim of this study was to investigate the impact of anaerobic conditions and temperature on the flocculation potential of brewing yeast.

Fermentation was carried out at three different temperatures conditions: 4°C to represent cold storage, 15°C as the temperature at which fermentation is commercially performed and 25°C as the standard laboratory temperature.

Brewing Yeast

A brewing yeast cell goes through 4 stages: a lag phase, a growth phase(reproduction), a fermentation phase and a sedimentation phase. Cells are activated from dormancy when they are added (or pitched) to the wort. Once sufficient food reserves are built up, the yeast cells are then able to reproduce by budding, and fermentation commences. During fermentation the yeast is in suspension in the wort from 5-7 days. After this time, the yeast cells begin to flocculate and sediment.

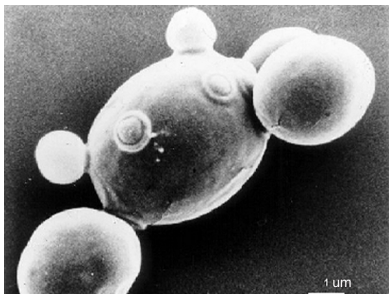


Figure 2. These are budding cells of the brewers yeast, Saccharomyces cerevisiae. Scars form where daughter cells have broken away from the parent cell. This unicellular fungus ferments sugars into alcohol. **Gordon Beakes © University of Newcastle upon Tyne.** Image courtesy Centre for Bioscience, the Higher Education Academy, ImageBank http://www.bioscience.heacademy.ac.uk/imagebank/.

Brewing yeast can be divided into two main types; ale and lager. These yeasts share many similarities, but they also have different electrical properties, hydrophobicities and surface chemical compositions (Dengis et al., 1995). The most marked difference between the two yeast strains however, is observed towards the end of fermentation and is due to the expression of cell surface proteins.

Saccharomyces cerevisiae (S. cerevisiae) is an ale yeast species. Four strains of this yeast were used in this study. Ale strains are generally more hydrophobic than lager strains, due to a higher concentration of surface proteins containing protonated amino and carboxylate groups. Ale fermentations are traditionally carried out over 5-6 days at between 12-18°C where the yeast cells rise to the top of the fermenter due to their affinity for CO_2 bubbles (Ritcey, 1997). Ale strains are given the phenotypic description New*FLO* (Stratford and Assinder, 1991). The New*FLO* phenotype strain is characterised by flocculation that is inhibited by mannose, maltose, glucose or sucrose.

Flocculation can be defined as the "phenomenon wherein yeast cells adhere in clumps and either sediment rapidly from the medium in which they are suspended or rise to the surface" (Stewart and Russell, 1981). The most widely accepted hypothesis to explain the mechanism of flocculation is the lectin-like theory of flocculation (Miki, 1982). This hypothesis suggests that specific lectin-like cell surface proteins known as zymolectins, selectively bind to proteins containing mannose polysaccharides (mannoproteins) present on the walls of neighbouring yeast cells (See Fig. 3).

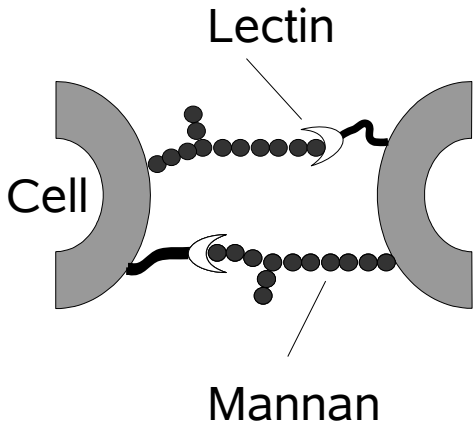


Figure 3. In the lectin-like theory of flocculation, lectins appear at the cell surface in the stationary phase and specifically bind to mannan carbohydrates of adjacent yeast cells (after Touhami et al, 2003).

Flocculation is influenced by the properties of the yeast cell wall and the nature of the culture medium or wort. It is important that strain variability is taken into account when considering culture temperature and composition as individual yeast strains respond differently to environmental conditions. In addition to temperature, the concentrations of ethanol, trace elements and especially dissolved oxygen can all affect flocculation. Anaerobic conditions lead to the differential expression of cell wall mannoproteins (Smart, 2003). It is presumed that this change in expression alters cell wall composition and structure, favouring lectin-receptor interactions (Smart, 2003).

METHODS

Ale yeast strains were cultured at a range of temperatures both aerobically and anaerobically and their ability to flocculate determined.

The results from two ale yeast strains are reported here (**SCB7**, **SCB8**, Scottish Courage Brewing Ltd, Technical Centre, Edinburgh, UK.) Each strain was grown aerobically on a Yeast Peptone Dextrose (YPD) plate at 25°C, then a starter culture produced by inoculating a single colony from each growth plate into 100ml of YPD in a 250ml sterile flask. The flasks were then put in an orbital shaker at 120 rpm at 25°C for 48 hours and the cell concentration determined by counting the cells under a microscope on a special slide called a haemocytometer (haemocytometers have a calibrated grid commonly used for counting blood cells, hence the name). A volume of this cell suspension was then inoculated into 100ml of YPD in a 250ml sterile flask to obtain a final cell concentration of $1x10^6$ cells/ml.

The yeast cells were then incubated for 48, 72 and 96 hours in YPD at 25°C, 15°C or 4°C, shaking or static, in either aerobic or CO_2 induced anaerobic conditions. The choice of 4°C was intended to represent cold storage, 15°C as the temperature at which fermentation is traditionally carried out and 25°C as the standard laboratory temperature. Anaerobic incubation was achieved using an Oxoid Anaerogen® pack and a 3.5L gas jar.

Cell populations were harvested by centrifugation and the flocculation potential measured.

The sedimentation rate, which is dependent upon the viscosity of the medium, floc size and mass is the standard method of analysis (Stratford, 1992). A major commercially used test of this type is called the Helm's test. This method was used in this study. The optical density (OD) of a yeast solution is measured over a set time period. The quantification of cell sedimentation therefore gives an

indication of the physical behaviour of the strain in liquid culture, and the rate and degree to which it flocculates.
Data was analysed by comparing the % flocculation against the fermentation time at different temperatures under aerobic or anaerobic conditions. Fisher's protected least significant difference test (Fisher's PLSD) was used to determine significant differences between the groups.

RESULTS
For populations of the SCB7 ale yeast strain, when incubated aerobically at 25°C there was a significant increase in flocculation potential, however when incubated anaerobically at 25°C, there was a significant decrease in flocculation potential.

For SCB8, under both aerobic and anaerobic conditions at 25°C, the flocculation potential was maintained throughout the time period assessed at approximately the same value that was achieved after 48 hours, thus there was no significant change.

At 15°C, the flocculation potential of SCB7 progressively decreased under both aerobic and anaerobic conditions. With SCB8, the flocculation potential peaked at the 72 hour time point under both gaseous conditions, but there was no further increase in the flocculation potential with any further incubation.

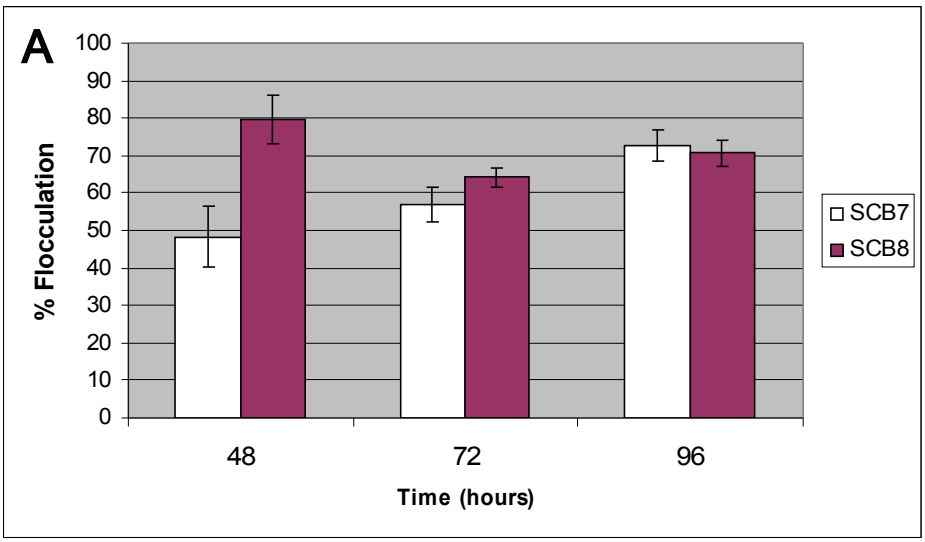
For SCB7 under aerobic incubation at 4°C there was a progressive increase in flocculation potential from 72 to 96 hours. Under anaerobic incubation at 4°C, flocculation progressively increased. Aerobic and anaerobic incubation of SCB8 at 4°C lead to a progressive increase in the flocculation potentials achieved under both gaseous conditions.

DISCUSSION
The yeast cell wall is a dynamic structure that can adapt in response to many physiological and morphological changes. The composition of the cell wall changes when it is exposed to different growth conditions: including temperature, pH, aeration, the mode of cultivation and the available carbon source.

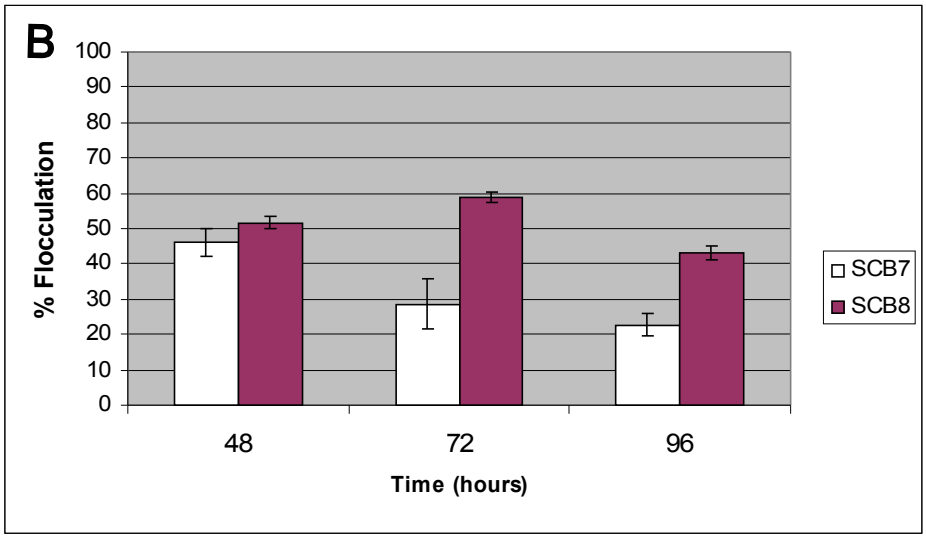
The Helm's test is currently performed with incubation under aerobic conditions at 25°C, whereas commercial brewing occurs under anaerobic conditions at 15 °C. Lawrence and Smart (in press) investigated the impact of CO_2 induced anaerobiosis on flocculation performance and found that in all yeast strains assessed, the rate of onset and extent of expression of flocculation was modified in response to this changing gaseous condition. They proposed that the quantification of flocculation during CO_2 induced anaerobiosis gave a more representative and predictive assessment of production scale brewery fermentation than the current method performed under aerobic conditions.

Aerobic Incubation of Ale Yeast Strains at 25°C, 15°C and 4°C
Under aerobic incubation at 25°C, 15°C and 4°C, both strains demonstrated a flocculation profile different to the other. This may have been because the reduction in temperature had opposing effects on each strain, either inducing or repressing the formation of a cell wall component involved in flocculation. The flocculation response to incubation under different temperature conditions is therefore presumed to be strain specific.

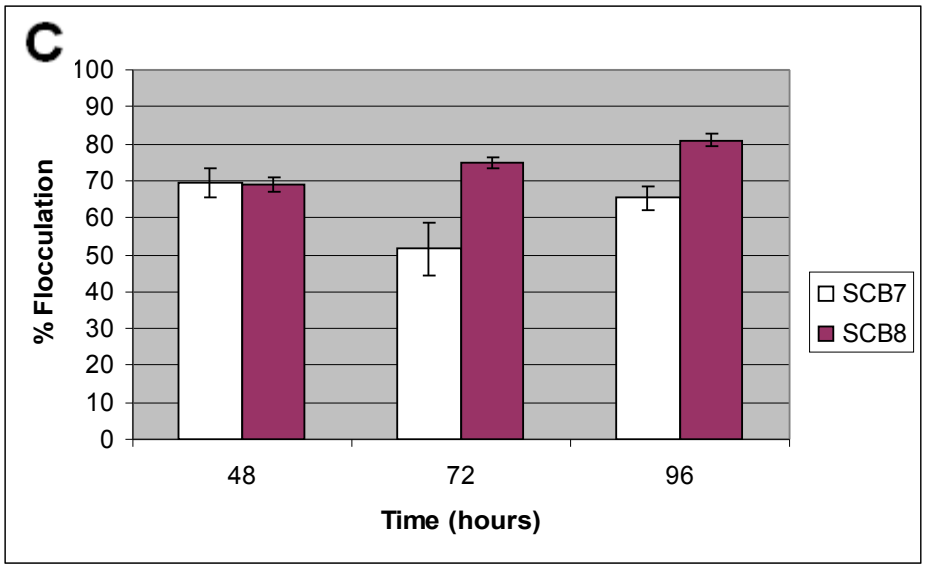
Anaerobic Incubation of Ale Yeast Strains at 25°C, 15°C and 4°C
At 25°C, the yeast cells were shaken during incubation. At 15°C and 4°C however, the yeast cells remained static. Agitation can have two opposing effects depending on how vigorous the yeast cells are shaken. A higher flocculation rate can occur as a result of enhanced particle collision rates, but depending on the



a) 25ºC

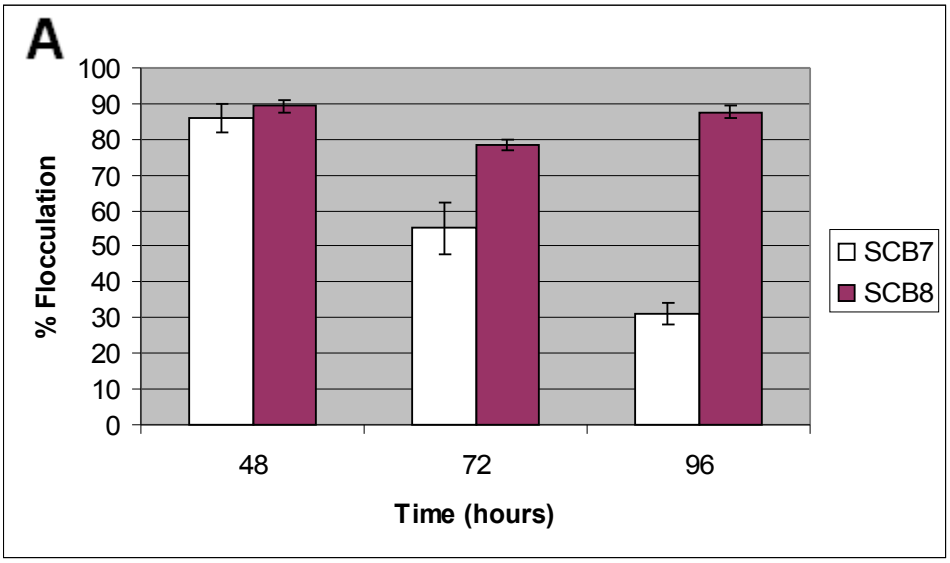


b) 15ºC

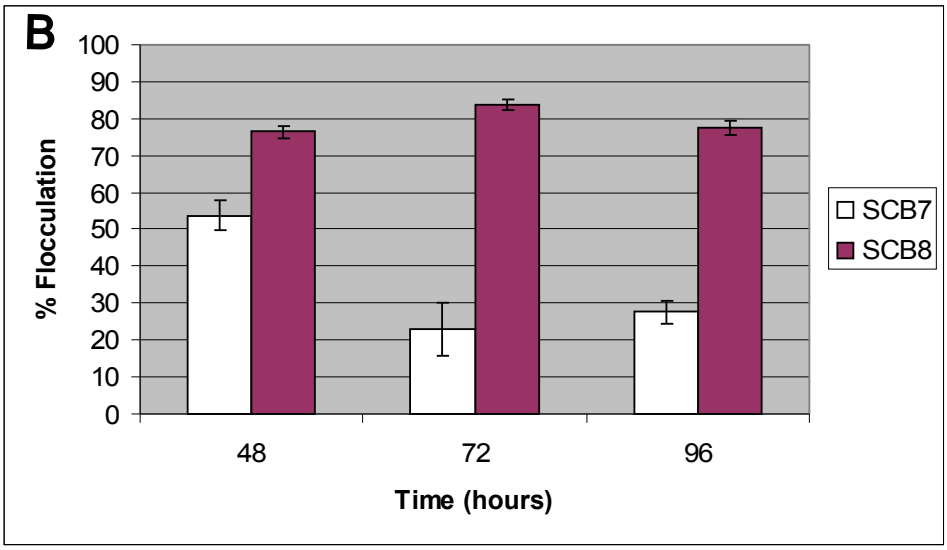


c) 4ºC

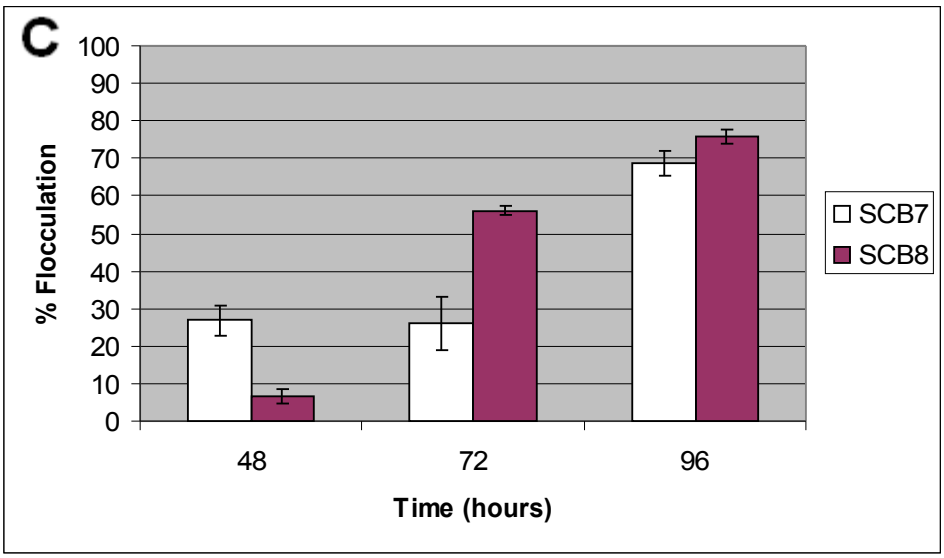
Figure 4. Flocculation potential of ale brewing yeast strains in aerobic conditions at 25ºC, 15ºC and 4ºC. Yeast populations were grown in aerobic conditions in YPD, for 48, 72 and 96 hours. Cells were harvested by centrifugation and flocculation was measured using a modified version of the Helm's test. The data represents the mean of triplicate analysis. Error bars represent the standard error.



a) 25°C



b) 15°C



c) 4°C

Figure 5. Flocculation potential of ale brewing yeast strains in anaerobic conditions. Yeast populations were grown in YPD at 25°C, for 48, 72 and 96 hours. Anaerobic conditions were achieved by incubation in an anaerobic gas jar containing an Oxoid Anaerogen® pack inside. The data represents the mean of triplicate analysis. Error bars represent the standard error.

strength of the agitation, it can also cause particle breakage, leading to a decrease in floc size and a slower rate of sedimentation (Domingues *et al.*, 2000). Thus, agitation may have had an affect on the flocculation potentials achieved under each temperature condition.

At all temperature conditions, SCB8 achieved a higher flocculation potential under both aerobiosis and CO_2 induced anaerobiosis than SCB7, indicating that SCB8 may be more stress tolerant than SCB7. Both strains achieved their highest flocculation potentials under aerobic incubation at 25°C. There was no pattern between the flocculation profiles of SCB7 and SCB8 observed under incubation at each different temperature. This response is likely to be the consequence of a strain specific effect.

Previous research carried out by Lawrence and Smart (unpublished data), has shown that the rate of onset and extent of expression of flocculation of two different lager yeasts, SCB2 and SCB3 also appears to be strain dependant. The flocculation profile of these lager yeasts when incubated under aerobic conditions, completely differed from those seen under incubation in CO_2 induced anaerobic conditions. Therefore the results from this study are in agreement with the observations of other laboratories. These studies suggest that it may be necessary to incorporate temperature as well as CO_2 induced anaerobiosis when analysing the flocculation performance of yeast, to better represent the conditions found during production scale brewery fermentations.

CONCLUSION

The flocculation potential of ale yeast strains was indeed influenced by CO_2 induced anaerobiosis and temperature, although the rate of onset and extent of expression of flocculation appeared to be strain dependant.
These findings indicate that it may be necessary to consider temperature as well as CO_2 induced anaerobiosis when analysing the flocculation performance of yeast strains under laboratory conditions. This would give a better representation of the conditions found during production scale brewery fermentations, so that a more accurate and predictive assessment of the performance of these attributes on flocculation potential can be made.

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Francine Upshon: Fran is 20 years old and studied Applied Biology at the University of Nottingham, based at the Sutton Bonington campus. She graduated in 2007 with an upper second BSc with honours degree. Fran studied a wide range of topics on her course including many plant, animal and microbial modules. She was particularly interested in the microbiology of the brewing process and is currently applying for a job within the brewing industry.