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Decrease in Hydrogen Sulfide Content during the Final Stage of Beer Fermentation Due to Involvement of Yeast and Not Carbon Dioxide Gas Purging

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We observed a rapid decrease in hydrogen sulfide content in the final stage of beer fermentation that was attributed to yeast and not to the purging of carbon dioxide (CO_2) gas. The well known immature off-flavor in beer due to hydrogen sulfide (H_2S) behavior during beer fermentation was closely investigated. The H_2S decrease occurred during the final stage of fermentation when the CO_2-evolution rate was extremely small and there was a decrease in the availability of fermentable sugars, suggesting that the exhaustion of fermentable sugars triggered the decrease in H_2S. An H_2S-balance analysis suggested that the H_2S decrease might have been caused due to sulfide uptake by yeast. Further investigation showed that the time necessary for H_2S to decrease below the sensory threshold was related to the number of suspended yeast cells. This supported the hypothesis that yeast cells contributed to the rapid decrease in H_2S during the final stage of beer fermentation.

[**Key words:** hydrogen sulfide, beer, fermentation, yeast, fermentable sugar]

Hydrogen sulfide (H_2S) is a well known, volatile sulfur compound that strongly masks desirable characters in beer. When the concentration of H_2S is greater than the sensory threshold of 0.005 mg/*l*, it imparts a rotten-egg smell and contributes to off-flavor (1). Many studies have examined the effects of yeast strains, fermentation conditions, and gene modification on the production of H_2S during fermentation (2–6). During the cell maturation cycle, H_2S seems to be released from yeast cells and assimilated during the budding cycle (7), but the decrease in H_2S content during the late stage of beer fermentation, after yeast growth, is said to be mainly attributed to CO_2 purging. Keeping the H_2S concentration below the sensory threshold is one of the most important goals for high quality beer production. A further goal is to shorten the maturation and fermentation periods to control the H_2S concentration.

When the Suntory Kyushu-Kumamoto brewery was started, we experienced a sulfury note in our beer in lager tanks. We found that excessive amounts of H_2S remained in the green beer at the end of fermentation. To reduce the high levels of H_2S below the sensory threshold remaining in green beer, we had to extend the maturation time before filtration. In order to produce a high quality beer within the designed brewing schedule, it was important to know how to reduce H_2S formation. The results of this study indicate that a new mechanism, other than CO_2 purging, was associated with the H_2S decrease in the later stages of fermentation.

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MATERIALS AND METHODS

Yeast We used *Saccharomyces carlsbergensis* strain BH-449 for the beer production.

Wort The wort was brewed with two-row barley malt and an original gravity of 12 w/w%. The target of limit attenuation (LA) was 85.5%, and it was controlled by saccharification temperature and the duration of the mashing process. If a higher LA of wort was necessary, then a longer saccharification rest was adopted.

Fermentation condition The dissolved oxygen content before yeast pitching was adjusted to 10 mg/*l* by aeration. The yeast was pitched at the rate of 12×10^6 cells/ml. The fermentation temperature was 12°C and there was no counter pressure in the head space of the fermentation tank. All trials were conducted in commercial-scale fermentation tanks and the batch size was 180 m^3, unless indicated otherwise.

Hydrogen sulfide The H_2S in the fermenting wort and beer was analyzed by the head-space GC/FPD method reported by Nagami *et al.* (8). The H_2S in the exhausted carbon dioxide (CO_2) was analyzed with a gas-detector tube system 4 LB (Gastec Corporation; Ayase, Kanagawa).

Yeast cell count The number of yeast cells was counted with a hemocytometer.

Apparent extract (A-Ex), alcohol concentration, original extract (O-Ex), and apparent attenuation (AA) of beer These were

analyzed by a SCABA Beer Analyzer (FOSS Analytical; Hilleroed, Denmark). The apparent extract concentration (A-Ex, w/w%) was calculated from the specific gravity of the sample and the alcohol concentration (w/w%) was measured with a ceramic sensor using the oxidation of evaporated vapor from the sample. The original extract (O-Ex) is an index estimating the original, unfermented wort concentration, and is calculated from the alcohol and A-Ex in the beer. The apparent attenuation (AA, %) is an index of the degree of apparent fermentation in the sample and is defined as:

$$AA = 100\ (O\text{-}Ex - A\text{-}Ex)/O\text{-}Ex \quad (9).$$

Limit attenuation (LA) The LA (%) is an index of the degree of apparent fermentation of the forced-fermented sample with an excessive yeast dosage to exhaust fermentable sugars. The limit extract (L-Ex, w/w%) is equal to the A-Ex of the forced-fermented sample. LA is defined as:

$$LA = 100\ (O\text{-}Ex - L\text{-}Ex)/O\text{-}Ex \quad (9)$$

Fermentable carbohydrates in beer The fermentable carbohydrates in the beer were analyzed with HPLC, a refractive-index detector, and the EBC method (9).

Dissolved CO_2 concentration The dissolved CO_2 concentration was measured by a CO_2 Gehaltmeter, type DGM (Norit Haffmans; Venlo, Netherlands).

Decrease in hydrogen sulfide concentration with different numbers of suspended yeast cells following primary fermentation To test whether centrifugation could control the number of suspended yeast cells, green beer was drawn from the fermentation tanks into each of three pilot tanks (50 *l*) at the end of primary fermentation. As the beer was being transferred, the first tank was not centrifuged, whereas 85% of the beer and 95% of the beer in the second and third tanks, respectively, were centrifuged. The yeast cell counts in the three pilot tanks were 55, 7.0, and 3.5×10^6 cells/ml respectively. The pilot tanks were maintained at 12°C and the H_2S was periodically analyzed.

RESULTS AND DISCUSSION

Changes in hydrogen sulfide during beer fermentation When the Kyushu-Kumamoto brewery was started up, the fermentation period was 116 h, and the green beer was transferred to maturation tanks when the AA was 71%, but greater than 0.06 mg/*l* of H_2S still remained; therefore, the maturation time before filtration was extended to 4–7 d to decrease the H_2S concentration below the sensory threshold. To reduce H_2S production and to produce a high quality beer within the designed brewing schedule, we conducted multiple fermentation trials considering the dissolved oxygen in the wort, yeast pitching rate, fermentation temperature, pressure in the head space, and the duration of the fermentation period. We observed a rapid decrease in H_2S when the fermentation period was extended to 140 h (Fig. 1). In this trial, the H_2S concentration in the green beer at the end of fermentation was 0.014 mg/*l*, and the AA was 85%. Additional trials indicated that this method was reproducible.

Depletion of fermentable sugar as a key factor of the rapid decrease in hydrogen sulfide In this series of trials, we found an interesting feature related to the rapid H_2S decrease (Fig. 2A). Although the fermentation conditions were not intentionally changed, the yeast-growth rate was greater in one batch than the other. The maximum yeast-cell count of the faster batch and the normal batch were 52×10^6 and 47×10^6 cells/ml, respectively. The rapid decrease in H_2S in the normal batch started at approximately 140 h, whereas it started at approximately 120 h in the faster batch. In contrast, the rapid decrease in H_2S began at an AA of approximately 85% in both batches (Fig. 2B) suggesting that AA was associated with the decrease in H_2S and that the decrease should always begin at the same AA independent of

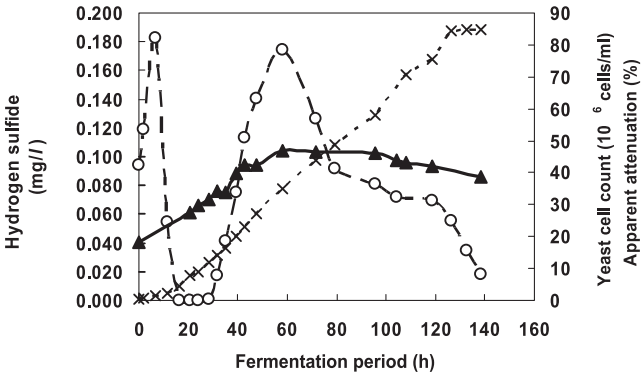

FIG. 1. H_2S behavior during beer fermentation with an extended fermentation period. Axes: horizontal, fermentation period in h; left vertical, H_2S content in mg/*l*; right vertical, yeast cell counts in 10^6 cells/ml and AA in %. Symbols: open circle, H_2S content; closed triangle, yeast cell counts; cross, apparent attenuation.

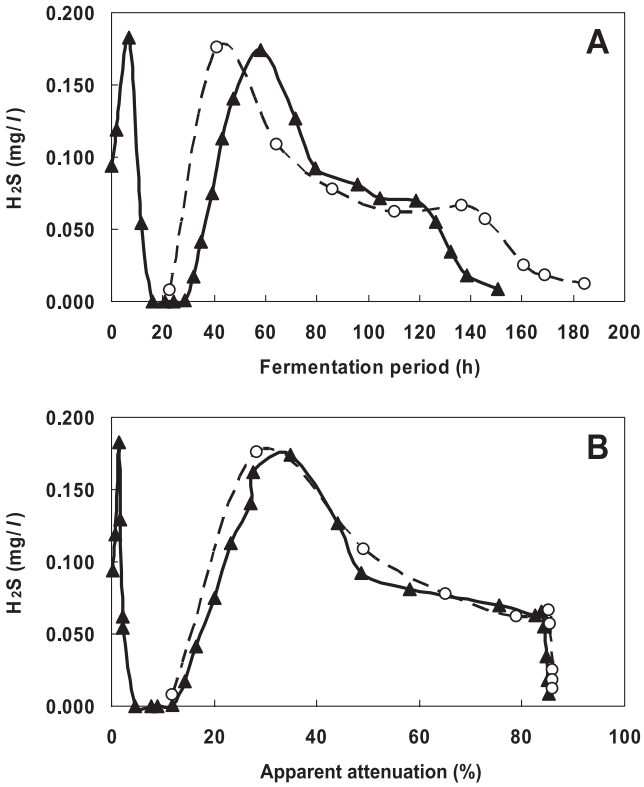

FIG. 2. (A) H_2S behavior at different fermentation speeds. Axes: horizontal, fermentation period in h; vertical, H_2S content in mg/*l*. Symbols: open circle, H_2S changes in slower-fermentation batch; closed triangle, H_2S changes in faster-fermentation batch. (B) H_2S behavior at different fermentation speeds. Axes: horizontal, AA in %; vertical axis, H_2S content in mg/*l*. Symbols: open circles, H_2S changes in slower-fermentation batch; closed triangles, H_2S changes in faster-fermentation batch.

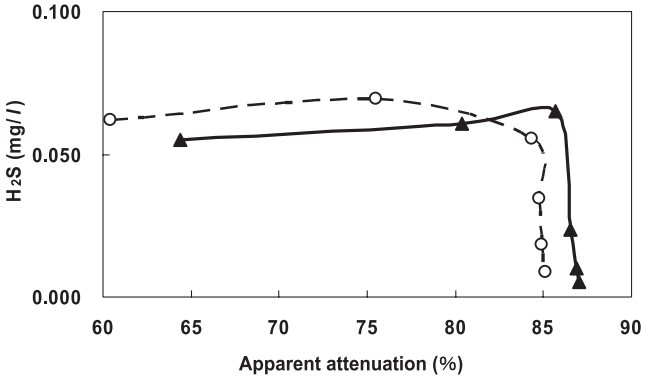

FIG. 3. H_2S behaviors during late-stage fermentation in two batches with different wort limit attenuation. Axes: horizontal, AA in %; vertical, H_2S content in mg/*l*. Symbols: open circles, H_2S changes in batch with 85.4% LA of wort; closed triangles, H_2S changes in batch with 87.5% LA of wort.

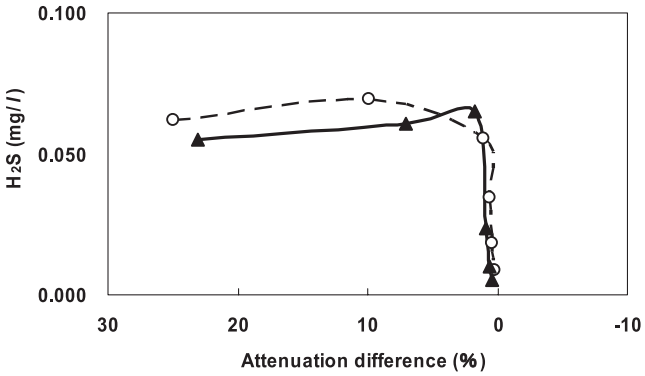

FIG. 4. H_2S behaviors during late-stage fermentation in two batches with different wort limit attenuation. Axes: horizontal, attenuation difference in %; vertical, H_2S content in mg/*l*. Symbols: open circles, H_2S changes in batch with 85.4% LA of wort; closed triangles, H_2S changes in batch with 87.5% LA of wort.

the LA of the wort. We investigated the changes in H_2S in batches in which the LA of the worts were 85.5% (control) and 87.4% (test). In the test batch, a longer saccharification rest was adopted to get a higher LA of the wort. The rapid decrease in H_2S started at 85.7% AA in the test batch, whereas it started at 84.4% AA in the control batch. This confirmed that the H_2S decrease started at a higher AA in a higher LA wort (Fig. 3). Next, we calculated the attenuation difference (AD). The AD was defined as the difference between the LA of the wort and the AA. So the AD is similar to the residual, fermentable sugar at the end of the fermentation. The results showed that the rapid decrease in H_2S started at a similar AD in the test batch (1.7%) and the control (1.1%) in spite of the different LA (Fig. 4). This suggested that the residual fermentable sugar was related to the H_2S decrease during the final stage of fermentation. We investigated the relationship between the assimilation rates of the fermentable sugars (glucose, maltose, and maltotriose) and the rapid decrease in H_2S. Once the fermentable-sugar assimilation rate dropped below 0.05 w/w%/h, we observed a rapid H_2S decrease (Fig. 5) suggesting that the decrease in H_2S was triggered by the depletion of fermentable sugars.

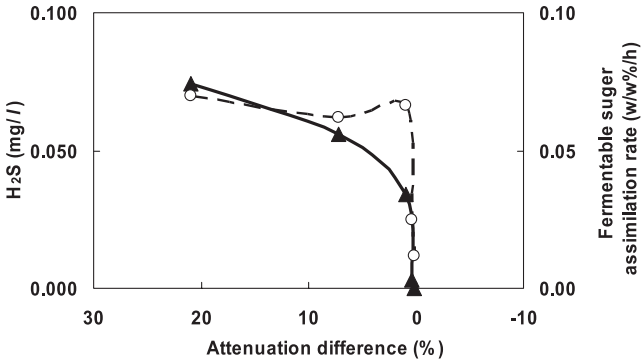

FIG. 5. H_2S behavior and fermentable sugar assimilation rate during late-stage fermentation. Axes: horizontal, attenuation difference in %; left vertical, H_2S content in mg/*l*; right vertical, fermentable sugar assimilation rate in w/w%/h. Symbols: open circles, H_2S content; closed triangles, fermentable sugar assimilation rate.

Contribution of yeast to the decrease in hydrogen sulfide during the final stage of fermentation There are few studies available in the literature concerning the H_2S decrease at the end of beer fermentation, but it has been attributed to CO_2 purging (8). After 145 h, the rate of CO_2 evolved, calculated from the decrease in the A-Ex rate was at its lowest. We hypothesized that the purging effect should also become smaller because of the depletion of fermentable sugar (Fig. 6A); however, there was a dramatic decrease in H_2S at this stage (Fig. 6B). This result suggested that something more than CO_2 purging was contributing to the H_2S decrease. Based on this result, we suspected that yeast cells could be assimilating the H_2S in green beer. To clarify the contribution of yeast cells to the decrease in H_2S, we investigated the balance of H_2S in the fermentation tank (Fig. 7). The H_2S balance in the fermentation tank was expressed as:

$$\Delta GB = Y - P$$

where ΔGB was the change in H_2S in green beer during a defined period; Y was the H_2S released from yeast cells during a defined period, and P was the H_2S purged from the fermenting wort during a defined period. To obtain P, we measured the amount of H_2S in exhausted CO_2, during a defined period, by a gas detector tube as described before. During alcohol fermentation, when one mole of alcohol is produced there is one mole of CO_2 produced at the same time. So, the exhausted CO_2 volume could be calculated from the A-Ex consumption rate, which is easily converted to the alcohol production rate. The fermentation volume was 180 m^3. From these calculations, we obtained P in mg/h/m^3 and the ΔGB during the final stage of fermentation was measured. From these two calculations, we determined the contribution of the yeast to the H_2S decrease. The material-balance analysis showed that the change in H_2S in the green beer was −215 mg/h/m^3, and the H_2S purged was 4 mg/h/m^3. From these data, we calculated the amount of H_2S released from the yeast cells, Y, as −211 mg/h/m^3.

$$Y = \Delta GB + P = -215 + 4 = -211 \quad (mg/h/m^3)$$

The results showed that the yeast cells did not release H_2S, but rather assimilated the H_2S during this stage. This demonstrated that the H_2S decrease was mainly through up-

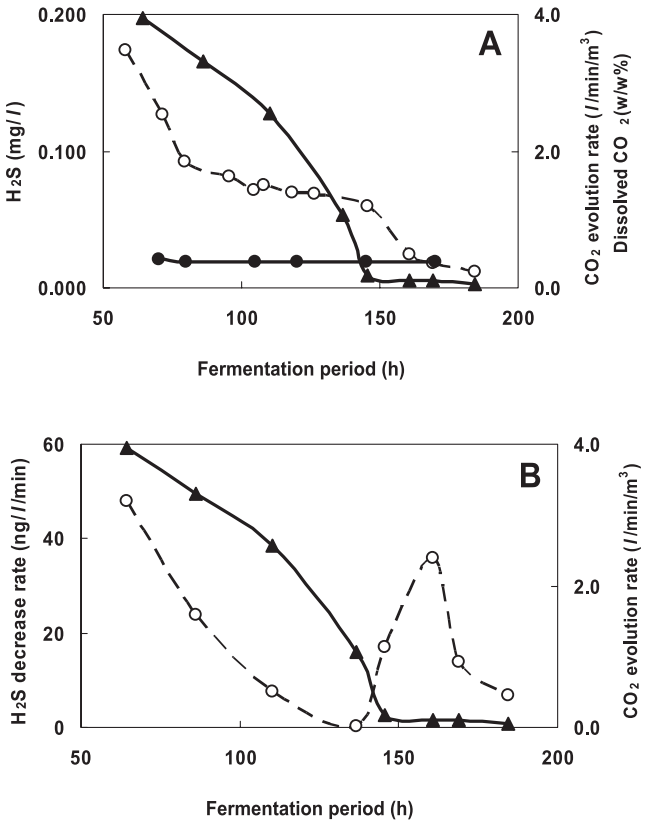

FIG. 6. (A) H_2S, CO_2 evolution rate, and dissolved CO_2 changes during fermentation. Axes: horizontal, fermentation period in h; left vertical, H_2S content in mg/*l*; right vertical, CO_2 evolution rate in *l*/min/m^3-fermenting wort and dissolved CO_2 content in w/w%. Symbols: open circle, H_2S content; closed triangle, CO_2 evolution rate; closed circle, dissolved CO_2 content. (B) Decrease in the rate of H_2S and the CO_2 evolution rate during fermentation. Axes: horizontal, fermentation period in hours; left vertical, H_2S decrease rate in ng/*l*/min; right vertical, CO_2 evolution rate in *l*/min/m^3-fermenting wort. Symbols: open circles, H_2S decrease rate; closed triangles, CO_2 evolution rate.

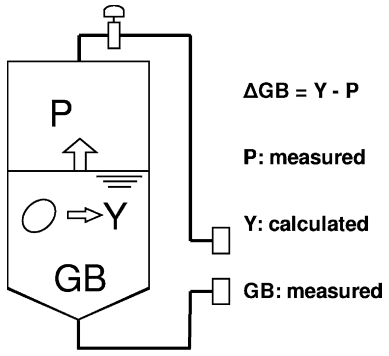

FIG. 7. H_2S balance analysis in the fermentation tank during the final stage of fermentation. The following equations were used: $\Delta GB = Y - P$. Abbreviations: ΔGB, changes in H_2S in green beer during a defined period; Y, H_2S released by yeast during a defined period; P, H_2S purged from fermenting wort during a defined period. ΔGB and P were measured, and Y was calculated from both figures.

take by yeast, when the CO_2-purging effect was very small. These results contradict the generally accepted idea that the decrease in H_2S during late-stage fermentation is mainly attributable to CO_2 purging. We propose that yeast cells take up H_2S in green beer during the final stage of fermentation.

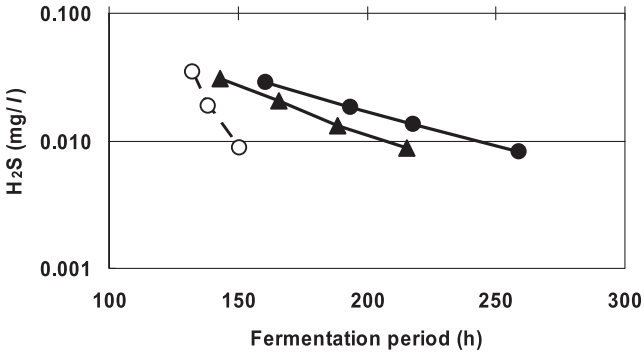

FIG. 8. H_2S decrease at 12°C with various numbers of suspended-yeast cells. Axes: horizontal, fermentation period in h; vertical, H_2S content in mg/*l*. Symbols: open circles, yeast count 55×10^6 cells/ml; closed triangles, 7.0×10^6 cells/ml; closed circles, 3.5×10^6 cells/ml.

To support this hypothesis, we studied the relationship between the number of suspended yeast cells and the rate of H_2S decrease in a 50 *l* pilot-scale tank under conditions similar to the end of fermentation. The results showed that as the number of yeast cells increased, there was a higher rate of decrease in H_2S (Fig. 8).

We confirmed these findings using another lager yeast strain (data not shown). As described, it was possible to obtain acceptable levels of H_2S at the end of fermentation by extending the fermentation time to 48 h. When the assimilation rate of fermentable sugars became less than 0.05 w/w%/h, there was rapid decrease in H_2S, which might have been caused by yeast uptake. If the fermentation time was not extended, then 4–7 additional days were necessary to obtain acceptable H_2S levels in the maturation tank. Although the fermentation and maturation temperatures were the same in this experiment, the number of yeast cells in the maturation tank was much smaller than in the fermentation tank even at the end of fermentation. This might be a possible reason for the difference in the decrease in H_2S efficiency.

It is generally accepted that during their growth phase, yeast take up sulfate and then reduce it to sulfite and then sulfide, which is used for sulfur-containing amino acid synthesis. When metabolism slows, sulfite and sulfide are released from the yeast cells. When budding occurs, the H_2S in the fermenting wort decreased very rapidly, probably through yeast uptake. Although the yeast in our experiments was not in the growth phase, there may have been enough amino acid biosynthesis such that the sulfide demand was larger than the intracellular sulfide supply through the reduction of sulfate and sulfite. Further studies will be needed to confirm this hypothesis.

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