Accelerated Fermentation of Brewer's Wort by
Saccharomyces carlsbergensis

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A rapid procedure for wort fermentation with Saccharomyces carlsbergensis at 12°C is described. Fermentation time was reduced from 7 to 4 days with normal inoculum by shaking. Increasing the inoculation to 5 to 10 times normal and shaking resulted in complete fermentation in 3 days. Maximum yeast population was reached rapidly with the large inocula, but fermentation proceeded at approximately the same rate when inoculations in excess of four times the normal were used. Similar results were obtained with both small-scale (100 ml) and microbrew (2.4 liters) fermentations.

Many papers have been published concerning the reduction of the fermentation time and the attainment of maximum fermentation rate by an increase in inoculum and/or in temperature of incubation (1, 7, 9, 11, 16). Griffin (9) and Hudson (11) observed that the level of Saccharomyces cerevisiae in suspension was disproportionate to the amount of inoculum and that it was difficult to maintain higher yeast concentrations in the head. Others overcame this difficulty through the use of stirring (3, 10). The yeast in suspension undergoes a different metabolic pattern than the sedimenterd yeast, probably because the metabolism of the suspended yeast is not diffusion limited (13). Analyses of the beer produced by accelerated fermentation indicated increases in the amount of higher alcohols (8, 14) and the quantity of diacetyl (4).

Little information has been published on the effects of changes in the amount of inoculum and stirring upon yeast metabolism in brewery wort with Saccharomyces carlsbergensis at 12°C. In the present study, a rapid, small-scale fermentation for use in studying the effects of changes in malting and mashing procedures has been developed. The utilization of reducing sugar and nitrogenous substances and the production of alcohol and yeast biomass were used as parameters to measure degree of fermentation. A comparison of this type of rapid fermentation was made with a larger-scale microbrew prepared in a similar manner.

Materials and Methods
S. carlsbergensis strain MC-2 was obtained from the Miller Brewing Co. (Milwaukee, Wis.). Prior to inoculation the yeast was grown in a shaken culture in wort supplemented with 1% (wt/vol) maltose at 12°C until a log culture was obtained. Different inocula, from 2.5 g/liter of fresh yeast weight, corresponding to 500 mg/liter of dry yeast weight (normal inoculum), to 10 times this amount, were used. Stationary and shaker fermentations (160 rpm) with normal inoculum were used as controls. Rapid fermentation is defined here as the use of five times the normal inoculum with shaking for 3 days. Fermentations were carried out at 12°C, using 100 ml of wort per 125-ml Erlenmeyer flask. Experiments were done in duplicate or triplicate. A rapid microbrew fermentation stirred with a magnetic stirrer (90 rpm) was carried out with five times normal inoculum and 2.4 liters of wort in a 3-liter Erlenmeyer flask. For comparison a stationary microbrew was prepared with normal inoculum and 2.2 liters of wort in a 2.15-liter tube fermenter.

Hopped wort was prepared using 70% Larker malt and 30% corn grits by the method of Burkhart et al. (5). Wort was stored frozen until needed, when it was thawed and filtered sterilized. None of the wort used was aerated prior to inoculation. Maltose (1%) was added to the wort, and shaming was used to induce yeast growth.

At the end of fermentation, the beer samples were centrifuged at 7,000 x g at 4°C for 30 min, and the yeast cells were washed once with 0.1 M NaCl and once with distilled water. They were then dried (at 80°C) to constant weight, and the weight of dry yeast per ml of wort was calculated.

Beer and wort were analyzed for reducing sugar, formal nitrogen, dextrin, and alcohol content according to the methods of the American Society of Brewing Chemists (2). Total nitrogen in wort and beer was determined according to the procedure of
Johnson (12). Flavor evaluations were conducted with a taste panel of five members by comparing the test brew with a control, which was stationary microbrew. Two taste tests were run on separate days.

RESULTS AND DISCUSSION

The decreases in specific gravity of the stationary, shaken, and rapid fermentations are shown in Fig. 1. These data are compatible with the log phase of yeast growth (Fig. 2). The rate of yeast growth in the rapid fermentation was about three times that of the stationary fermentation during the first day. There was little difference between the stationary and shaken cultures in terms of specific gravity change or in yeast dry weight during the first day; however, there was a large difference in both parameters of these cultures at 2 days. Yeast growth reached a maximum after 4 days in the stationary and shaker fermentations and after 3 days in the rapid fermentation. The amount of log-phase yeast growth obtained from the rapid fermentation was about four times that of stationary fermentation. By comparison, the shaker fermentation showed a 2.4-fold increase over the stationary fermentation. The relative increase in dry yeast matter obtained was greatest with the normal inoculum on the shaker. Shaking produced a 13-fold increase in yeast harvest, whereas the stationary method produced only a fivefold increase (Fig. 2). This is in contrast to Cook's observation with S. carlsbergensis that shaking made little difference in the quantity of yeast produced (6).

The lag and postexponential phase in the rapid fermentation did not exist or was too short to be detected with the sampling intervals used (Fig. 2). It has been speculated that the lack of a lag phase with large inocula is due to carryover of metabolites, possibly ribonucleic acids or their precursors, which increase during the lag phase and are necessary for cell growth and multiplication (15).

Very little difference in the yeast growth, or in its chemical analysis, was produced by a yeast concentration 4 to 10 times the normal inoculum (Table 1). A relatively smaller increase in yeast production occurred with the larger inocula. Increase in nitrogen with an inoculum of 6 to 10 times normal may possibly be from yeast autolysis. The acidity of the beers increased slightly, as did the biomass, when up to four times the normal inoculum was employed. Beyond that it remained essentially constant.

As expected, the pattern of disappearance of reducing sugars from wort during fermentation (Fig. 3) was closely related to the decrease in specific gravity (Fig. 1), increase in production

![Fig. 1. Changes in specific gravity during wort fermentation. Symbols: O, stationary fermentation; ●, shaken fermentation; ▲, rapid fermentation.](image1)

![Fig. 2. Changes in yeast dry weight during wort fermentation. Symbols: O, stationary fermentation; ●, shaken fermentation; ▲, rapid fermentation.](image2)
### Table 1. Changes in beer analyses and yeast growth during fermentation (3 days) with varying yeast inoculation

| Inoculum (× normal) | pH  | Yeast biomass dry wt (mg/ml) | Sp gr of beer | Alcohol (% by wt) | Total nitrogen (μmol/ml) | Formol nitrogen (μmol/ml) | Reducing sugar (% by wt) | Dextrin (%) |
|---------------------|-----|-----------------------------|---------------|------------------|----------------------------|----------------------------|--------------------------|-------------|
| 1                   | 4.20| 5.76                        | 1.02088       | 2.82             | 47.11                      | 6.29                      | 2.60                     | 1.77        |
| 2                   | 3.95| 7.78                        | 1.01434       | 3.34             | 36.71                      | 3.57                      | 1.70                     | 1.75        |
| 3                   | 3.85| 8.33                        | 1.01159       | 3.69             | 37.14                      | 3.71                      | 1.52                     | 2.01        |
| 4                   | 3.80| 8.46                        | 1.01097       | 3.77             | 35.71                      | 3.64                      | 1.32                     | 1.80        |
| 5                   | 3.80| 8.57                        | 1.01056       | 3.82             | 33.57                      | 3.57                      | 1.32                     | 1.91        |
| 6                   | 3.85| 8.68                        | 1.01020       | 3.86             | 42.14                      | 3.71                      | 1.27                     | 1.97        |
| 7                   | 3.80| 8.97                        | 1.01075       | 3.85             | 41.43                      | 3.86                      | 1.27                     | 1.98        |
| 8                   | 3.85| 9.42                        | 1.01081       | 3.85             | 41.43                      | 3.86                      | 1.31                     | 1.89        |
| 9                   | 3.85| 9.72                        | 1.01083       | 3.86             | 41.43                      | 3.86                      | 1.29                     | 1.93        |
| 10                  | 3.86| 10.41                       | 1.01096       | 3.85             | 42.14                      | 4.07                      | 1.15                     | 2.10        |

**Fig. 3.** Changes in reducing sugar during wort fermentation. Symbols: ○, stationary fermentation; ●, shaken fermentation; ▲, rapid fermentation.

of alcohol (Fig. 6), and the increase in yeast growth early in fermentation (Fig. 2). Utilization of reducing sugar during fermentation reached a maximum after 3 days in the rapid fermentation and after 5 days in the shaker fermentation, whereas the stationary fermentation had not reached a maximum at 7 days. Reducing sugar utilization and yeast growth reached their maxima much more rapidly in the rapid fermentation, and from this point on the disappearance of reducing sugar became insignificant.

Figures 4 and 5 illustrate the disappearance from the medium of formol and total nitrogen, respectively. There appeared to be little or no lag phase in the uptake of formol nitrogen by any of the three types of culture, although there were obvious differences in the rates of uptake. There was also no lag phase in the uptake of total nitrogen by the rapid fermentation, but both the stationary and shaker cultures exhibited a lag. The large inoculum and improved availability of substrates and oxygen during the early hours of culture in the rapid fermentation permitted an immediate and rapid uptake of larger peptides and other nitrogenous compounds as well as the amino acids and smaller peptides.
As might be expected, the production of alcohol (Fig. 6) coincides very closely with the disappearance of reducing sugar (Fig. 3). The amount of alcohol production in the stationary fermentation (7 days) compared favorably with that produced in 4 days in the shaken culture and that produced in slightly over 2 days in rapid fermentation.

To determine the effects of scaling up the rapid fermentation by approximately 20-fold, a comparison was made between a normal stationary microbrew and one in which five times the normal inoculum was used with stirring (Table 2). Stationary and rapid fermentations (100 ml) were also run for comparison. There was generally good agreement in the beer analyses and yeast growth between both types of fermentations after 3 days, although the rapid microbrew was somewhat slower in maltose, formol, and total nitrogen uptake. The beer analyses and yeast growth in the stationary microbrew and stationary (100 ml) fermentation were comparable in pH, alcohol production, and utilization of reducing sugar, but the decreases in specific gravity, utilization of wort formol, and total nitrogen were higher in the stationary microbrew fermentation after 3 and 7 days. Yeast growth was also greater in the stationary microbrew after 3 days. The beer produced by these fermentations contained relatively low carbonation and somewhat more initial haze than either the stationary or the shaken fermentation products. Neither of these

![Fig. 5. Changes in total nitrogen during wort fermentation. Symbols: ○, stationary fermentation; ●, shaken fermentation; ▲, rapid fermentation.](image1)

![Fig. 6. Changes in production of alcohol during wort fermentation. Symbols: ○, stationary fermentation; ●, shaken fermentation; ▲, rapid fermentation.](image2)

| Fermentation | Period of incubation (days) | Yeast biomass dry wt (mg/ml) | pH | Sp gr of beer | Alcohol (% by wt) | Total nitrogen (µmol/ml) | Formol nitrogen (µmol/ml) | Reducing sugar (% by wt) | Dextrin (% by wt) |
|--------------|----------------------------|-----------------------------|----|--------------|-----------------|-----------------------|-------------------------|--------------------------|-------------------|
| Stationary   | 3                          | 2.80                        | 4.2| 1.02675      | 1.95            | 47.57                 | 6.57                    | 4.44                     | 1.95              |
| Microbrew    | 7                          | 3.08                        | 4.1| 1.01149      | 3.35            | 43.34                 | 3.47                    | 1.27                     | 1.95              |
| Stationary   | 3                          | 2.14                        | 4.4| 1.03161      | 1.84            | 52.86                 | 11.26                   | 4.98                     | 2.05              |
| (100-ml)     | 7                          | 2.97                        | 4.3| 1.01478      | 3.30            | 46.00                 | 6.54                    | 1.55                     | 1.82              |
| Microbrew    | 3                          | 7.62                        | 3.95| 1.01046     | 3.83            | 39.98                 | 2.97                    | 1.52                     | 1.93              |
| Rapid (100-ml)| 3                          | 8.1                         | 3.85| 1.01097     | 3.82            | 34.29                 | 2.50                    | 0.94                     | 1.66              |
properties was of serious consequence, since they could be normalized by carbonation and chill proofing. The aroma and taste of the rapidly microbrewed beer were judged as acceptable or better than those of the stationary, microbrewed, control beer.

The results described here show that fermentation time with *S. carlsbergensis* at 12 C can be reduced by about 4 days by use of five times the normal pitching rate and stirring. This is in general agreement with the data of Hudson (11), who found that four times the normal pitching rate with strains of *S. cerevisiae* at 18 C reduced fermentation time by about one-half.

Fermentations of the type described here provide a convenient tool for evaluating on a small scale the effects of wort composition on fermentation efficiency and beer properties. The results obtained with rapid microbrews prepared with larger inocula and stirring suggest an advantage in the use of rapid fermentations for pilot brewing studies.

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