Inhibition of Beer Volatiles Formation by Carbon Dioxide Pressure[1]

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ABSTRACT

The formation of total volatiles and yeast growth were inversely related to the CO_2 counterpressure applied during 100-liter fermentations. However, the rate of fermentation was relatively unaffected. Thus, 8 psig (22.7 psia) CO_2 counterpressure in a 22°C fermentation repressed both total yeast growth and total volatiles concentration to the levels present in a 15°C fermentation with 0 psig (14.7 psia) CO_2 counterpressure. The repression of individual volatile compounds, however, was not uniform. The influence of temperature, agitation, and CO_2 counterpressure upon the dissolved CO_2 concentration during active fermentation was quantified. It was shown that the degree of supersaturation (termed the supersaturation coefficient), which is the ratio of the dissolved CO_2 concentration during active fermentation to the CO_2 saturation level, was constant over the ranges of temperature and CO_2 counterpressure examined, at constant agitation. The supersaturation coefficient varied inversely and in a linear manner with agitation. At sufficiently high agitation, no CO_2 supersaturation existed during active fermentation, thus the supersaturation coefficient was 1.0.

Key words: *Agitation, Fermentation, Temperature, Yeast growth.*

The recent literature indicates that there is considerable interest in the use of CO_2 counterpressure to control the formation of volatiles during fermentation. Many authors reported that increased CO_2 pressure decreased the concentration of most volatiles, such as the higher alcohols and acetate esters (11,12,14,21). Some authors also reported increased free acids and their ethyl esters (14), as well as acetaldehyde (11,12) and pyruvate (2), with increased CO_2 pressure. Several papers described the use of CO_2 counterpressure to minimize flavor changes in high-temperature, accelerated fermentations (11,22,23,24). In fact, some European brewers are using variations of this technique commercially (10).

This paper describes our pilot studies of CO_2-pressurized, high-temperature fermentations, and also shows the quantitative relations among temperature, agitation, and CO_2 pressure upon the dissolved CO_2 concentration during fermentation.

EXPERIMENTAL

Fermentation Conditions

Pitched, aerated, commercial wort was aseptically transferred from a 1000-bbl production fermentor to a 100-liter pilot fermentor, 16–24 hr after filling. This allowed time for normal assimilation of dissolved oxygen, since anaerobic conditions were established by packing the autoclaved pilot fermentor with carbon dioxide prior to transfer.

The pilot fermentor, equipped with turbine-type impellers, allowed precise control of agitation, temperature, and carbon dioxide counterpressure over a wide range. These conditions were held constant throughout each fermentation.

Sample Preparation

Fermentations were aseptically sampled at intervals and centrifuged prior to analysis. The clarified samples were stored at −25°C until analyzed for vicinal diketones and carbohydrates.

Yeast Concentration

Yeast dry weights were determined after centrifugation as previously described (17).

Vicinal Diketones

Vicinal diketones and their 2-acetohydroxy acid precursors

(subsequently referred to as diacetyl) were measured by a method developed in our laboratory (18) which combines the best features of the Micro method of Owades and Jakovac (15) and the UV method of Canales and Martinez (4). The procedure of Inoue and Yamamoto (9) for the conversion of precursors to vicinal diketones prior to analysis (17) was modified by incubating at 65°, rather than 60°C, for 0.5 hr.

Carbohydrates

The liquid chromatographic method of Brobst *et al.* (3) for wort was slightly modified by using only 1/3 the flow rate.

Dissolved Carbon Dioxide

Dissolved carbon dioxide was measured during fermentation using a modification of the manometric/volumetric method of Heard (7).

A 50-ml gas-tight plastic syringe (Stylex or equivalent), lightly coated internally with silicone stop-cock lubricant, was fitted with a low dead-space valve ("Mininert," Precision Sampling Corp., No. 654050) and a 21-gauge hypodermic needle. This was used to withdraw a 10–20-ml sample through a rubber septum in a sampling port at the bottom of the fermentor. About 100 ml of beer was first washed out through a large bore (19 gauge) hypodermic needle to ensure that a representative sample was taken. The method and equations of Heard (7) were used to measure the dissolved carbon dioxide in the syringe sample, after correcting for the conical tip of the syringe barrel and plunger.

Beer Volatiles

Volatiles were determined by gas chromatography on clarified, fully fermented beer, using the carbon disulfide extraction method (16,20), modified as follows:

Column: 8% SP-1000 on Chromosorb G, 80–100 mesh.
Column temperature: 85°C for 6 min, then programmed at 4°C/min to 215°C.
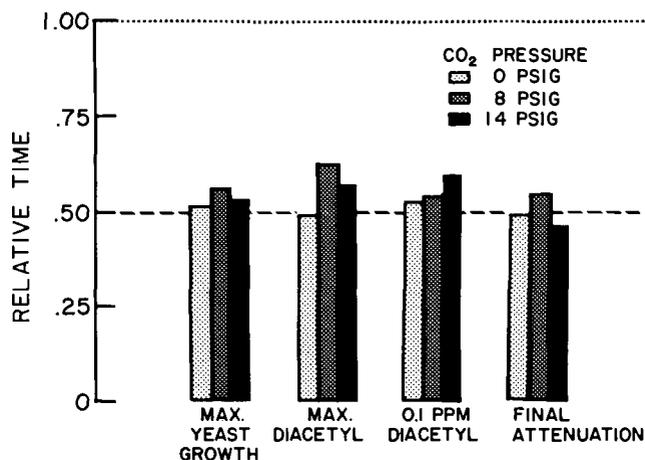Carrier gas: Helium at 25 ml/min.

Fig. 1. Time to reach various fermentation characteristics in 22°C fermentations, under different CO_2 counterpressures, relative to a 15°C fermentation with 0 psig (14.7 psia) CO_2 counterpressure. Bars indicate data from 22°C fermentations under 0, 8, and 14 psig CO_2 (14.7, 22.7, and 28.7 psia) in relation to the corresponding data from the 15°C fermentation, indicated by the dotted line at 1.0. The dashed line indicates the 0.5 level of relative time.
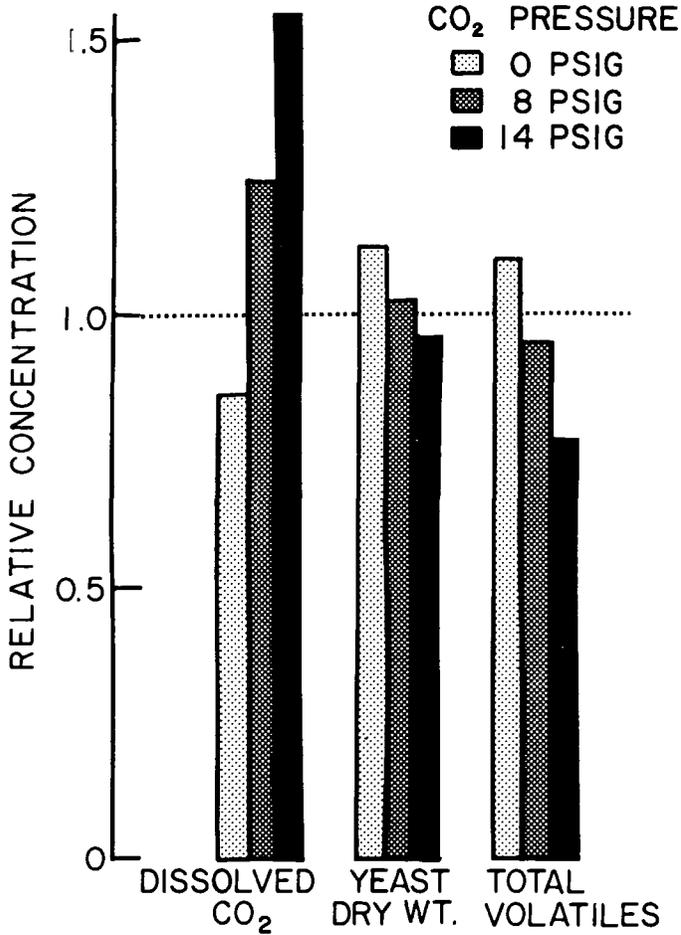
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Fig. 2. Fermentation characteristics in 22°C fermentations relative to a 15°C fermentation. The legend is the same as in Fig. 1.

Injection sample size: 4 μl.
Internal standard: 4-methyl-2-pentanol.

In a few fermentations, the headspace method of Hoff and Herwig (8) was used in addition to the carbon disulfide extraction method.

RESULTS AND DISCUSSION

A series of pilot fermentations was run at 22°C, at various levels of CO_2 counterpressure, and compared to fermentations run at 15°C with 0 psig (14.7 psia) counterpressure. Agitation, maintained at 33 rpm in all fermentations, was just sufficient to keep the yeast in suspension. Temperature, CO_2 pressure, and agitation were held constant throughout each fermentation.

CO_2 Counterpressure and Fermentation Rates

As shown in Fig. 1, CO_2 pressure had essentially no effect upon the rate characteristics of the 22°C fermentations. The time required to reach either maximum yeast dry weight or maximum diacetyl concentration, or to assimilate either fermentable carbohydrates or diacetyl (to 0.10 μg/ml), was about one-half the time required in the 15°C fermentation, regardless of the degree of CO_2 counterpressure. It has been reported (11,12) that CO_2 counterpressure retards fermentation; however, in that reference (11) in which temperature and pressure conditions approximated those in our 22°C fermentations, there was little evidence of decrease in fermentation rate.

CO_2 Counterpressure and Yeast Growth

In earlier work (19), we demonstrated that total yeast growth is inversely related to CO_2 counterpressure and, more fundamentally, to dissolved CO_2 concentration. This inverse relation is shown in Fig. 2.

CO_2 Counterpressure and Volatiles Formation

Figure 2 also shows that the CO_2 inhibition of total volatiles was directly related to yeast growth inhibition. Note that 8 psig (22.7
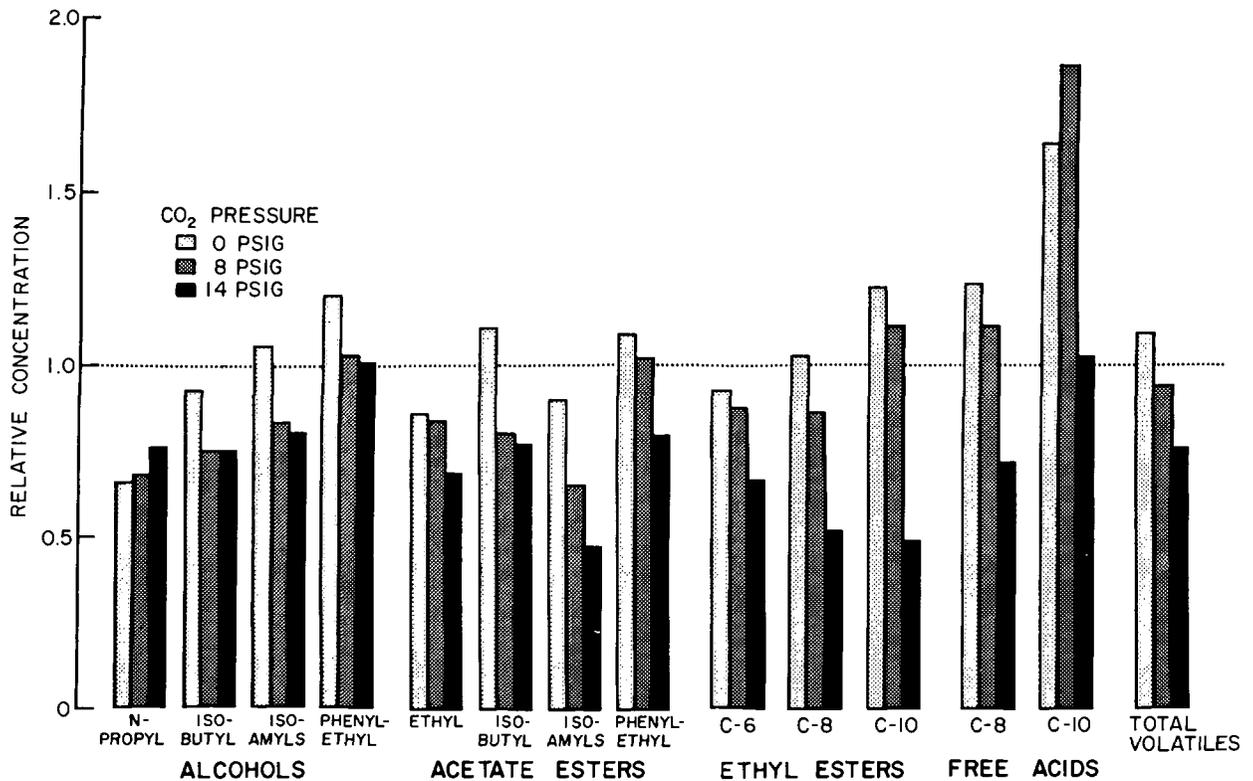

Fig. 3. Concentrations of volatile compounds in 22°C fermentations relative to a 15°C fermentation. The legend is the same as in Fig. 1.

TABLE I
Correlation between GLC Methods

Compound	CO$_2$ Counterpressure (psig) during Fermentation[a]	Ratio[b]	
		Headspace	CS$_2$ Extract
n-Propyl alcohol	0	1.34	0.85
	15	1.05	0.59
Isobutyl alcohol	0	0.93	0.83
	15	0.65	0.62
Isoamyl alcohols	0	0.94	0.92
	15	0.62	0.71
Ethyl acetate	0	1.14	0.99
	15	0.58	0.64
Isoamyl acetates	0	0.88	0.87
	15	0.41	0.44

[a]Fermentation temperature = 22°C; agitation = 33 rpm.
[b]Concentration of the compound in the 22°C fermentation relative to its concentration in a 14°C fermentation at 0 psig CO$_2$ counterpressure and 33 rpm agitation.
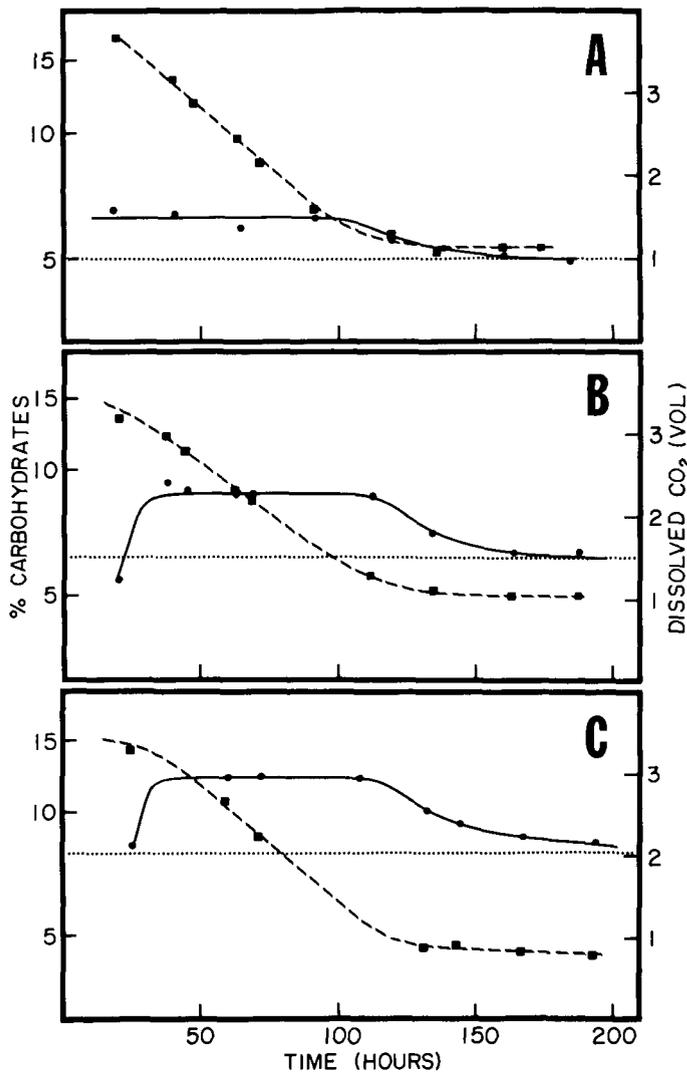

Fig. 4. Dissolved CO$_2$ concentrations in 15°C fermentations under the following CO$_2$ counterpressures: A, 0 psig; B, 8 psig; C, 14 psig. Data points for dissolved CO$_2$ (_____) are indicated by circles. Data points for carbohydrate concentration (- - - -) are indicated by squares. Concentration is expressed as % (wt/vol) on a logarithmic scale. The saturation concentration for dissolved CO$_2$ is indicated by a dotted line. Dissolved CO$_2$ concentration is expressed as volumes of CO$_2$ reduced to standard conditions (0°C and 760 mm) per volume of beer.

psia) CO$_2$ counterpressure decreased the total yeast growth and volatiles concentration in the 22°C fermentation to approximately the levels in the 15°C fermentation with 0 psig (14.7 psia) CO$_2$ counterpressure, while 14 psig (28.7 psia) over-repressed both yeast growth and volatiles formation. Total volatiles is defined as the sum of the areas of 13 component peaks separated by GLC from the fermented beer.

Although the concentration of total volatiles varied directly with temperature, the individual volatile components did not vary as consistently, as shown in Fig. 3. The unexpected decrease of several alcohols and esters at elevated fermentation temperatures was confirmed in other similar fermentations in which volatiles were determined by both carbon disulfide extraction and headspace gas chromatographic methods. Although the two methods detect many different volatile compounds, those compounds common to both procedures showed agreement except n-propanol (see Table 1). We have found that the carbon disulfide method lacks accuracy and precision for n-propanol, probably because it is only slightly soluble in carbon disulfide.

This decrease in concentration at elevated fermentation temperatures is not without precedent; there are reports that several alcohols (6) and esters (13,14) reach maximum concentrations at a fermentation temperature between 15° and 20°C, then decrease.

While increased CO$_2$ counterpressure during fermentation decreased the concentrations of almost all volatile compounds examined, the decrease was not always proportional (see Fig. 3). Also, decanoic acid was exceptional in that it reached a maximum concentration at 8 psig CO$_2$, then decreased. Norstedt et al. (14) reported such peaking at intermediate CO$_2$ counterpressures for certain free acids and ethyl esters.

Effect of Some Fermentation Parameters upon Dissolved CO$_2$

It is probable that the repressive effect of CO$_2$ upon yeast growth and volatiles formation is fundamentally related to its concentration in solution (14,19). The ease with which fermenting beer becomes supersaturated with CO$_2$ (5,19) complicates the relationship of temperature, agitation, and CO$_2$ pressure to the
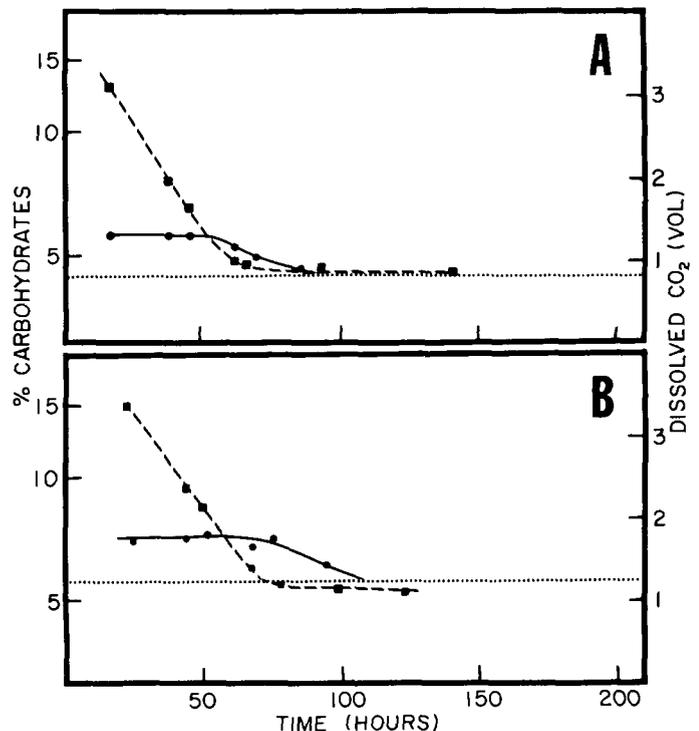

Fig. 5. Dissolved CO$_2$ concentrations in 22°C fermentations under the following CO$_2$ counterpressures: A, 0 psig; B, 8 psig. The legend is the same as in Fig. 4.
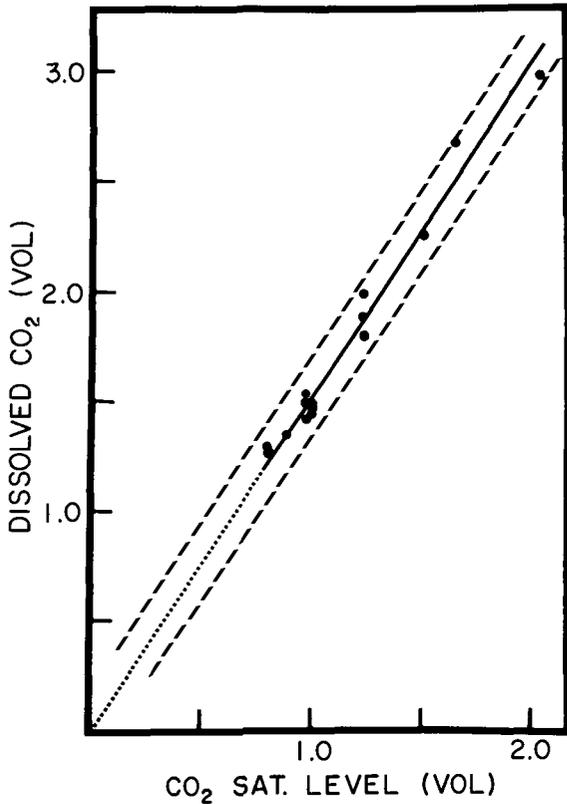
Fig. 6. Dissolved CO_2 concentration during active fermentation vs. the corresponding saturation concentration, for 15 pilot fermentations agitated at 33 rpm (●). The regression line (———) has been extrapolated to zero (...) and has a correlation coefficient of 0.990 on 14 degrees of freedom. The dashed lines show the 95% confidence limits of the regression line.

concentration of dissolved CO_2. We have quantitatively related these parameters to the dissolved CO_2 concentration, making it possible to estimate their influence.

Effect of Temperature and CO_2 Counterpressure upon Dissolved CO_2

Pilot fermentations were run at both 15° and 22°C, under various levels of CO_2 counterpressure. Agitation was maintained at 33 rpm. This agitation in the 100-liter fermentor approximates that created by evolving CO_2 in commercial batch fermentations (19). Both dissolved CO_2 and carbohydrate concentrations were measured throughout the fermentations. Figure 4 shows the influence of CO_2 counterpressure upon the dissolved CO_2 concentration in 15°C fermentations. The fermenting beer rapidly became supersaturated with the evolving CO_2, and this supersaturated condition was maintained throughout active fermentation. When the fermentable carbohydrates were about 90% assimilated, the beer began to lose its supersaturation and approached the normal CO_2 saturation concentration. This saturation condition is a function of both the temperature and CO_2 counterpressure and can be determined from the literature (1). Note that the CO_2 concentrations both during active fermentation and at saturation increased with increasing counterpressure. However,

TABLE II
Agitation Levels in Pilot Fermentations

Impeller Speed[a]	Power Input[b]
33	0.018
70	0.18
100	0.56
150	1.8
320	18

[a]Expressed as revolutions/min.
[b]Expressed as horsepower expended in mixing per 1000 gal.
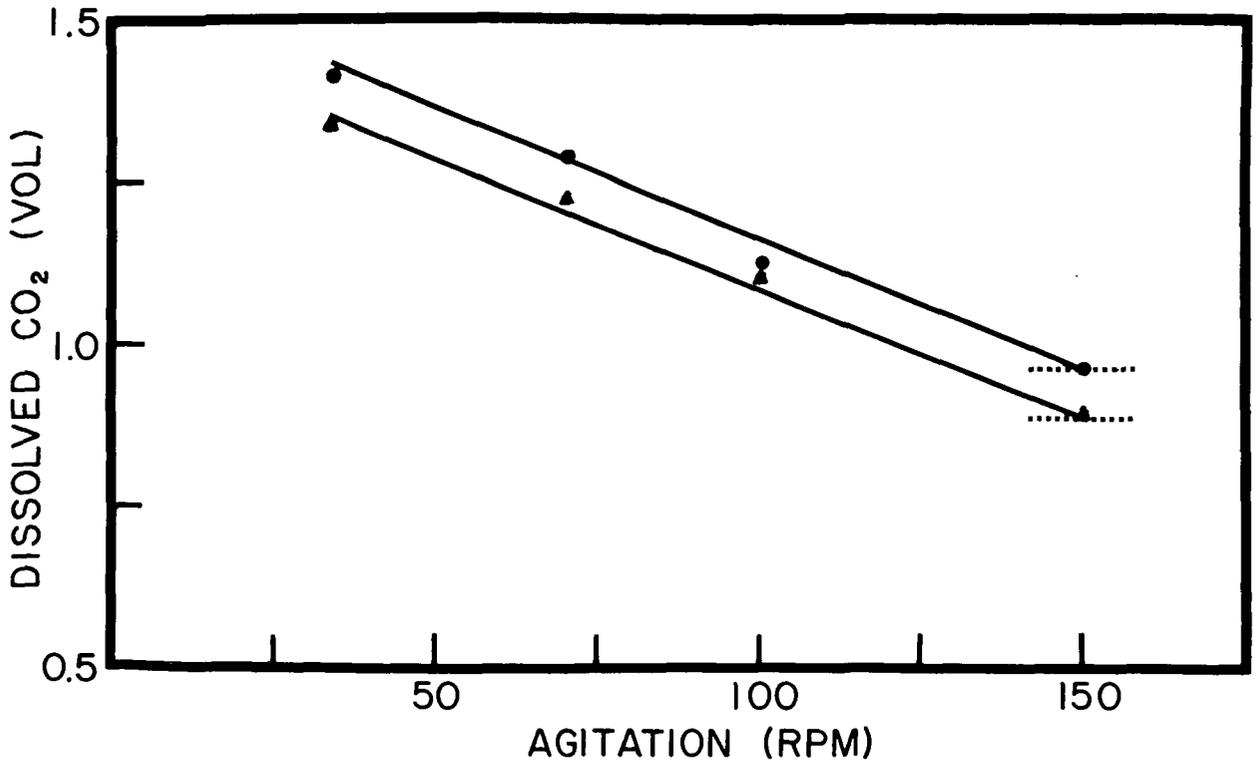

Fig. 7. Dissolved CO_2 concentration during active fermentation in pilot runs at 33, 70, 100, 150, and 320 rpm. Data points for 15° (●) and 18°C (▲) are shown. Dissolved CO_2 concentrations for fermentations at 320 rpm are the same as those at 150 rpm. CO_2 saturation concentrations are shown (. . .) for each fermentation temperature.
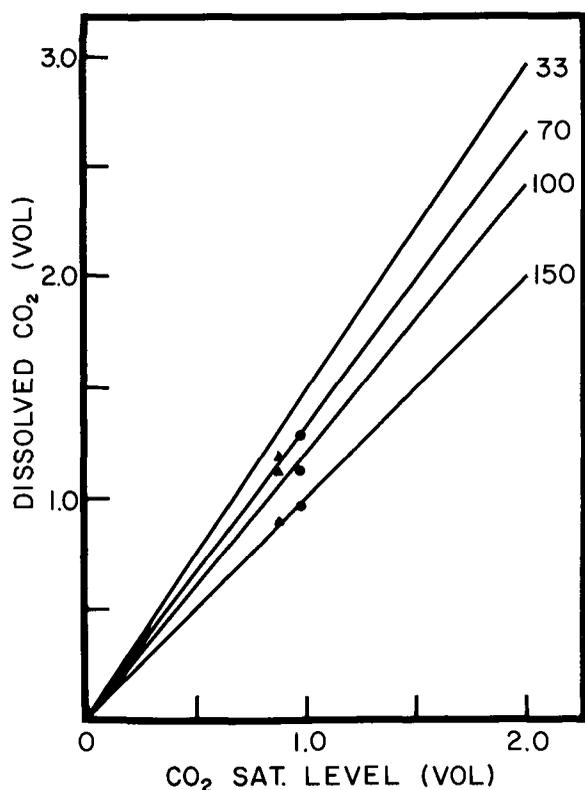
Fig. 8. Dissolved CO_2 concentration during active fermentation *vs.* the corresponding saturation concentration at different agitation levels (expressed as rpm). The data from the fermentations run at 15° (●) and 18°C (▲) were used to estimate these lines, except the line for 33 rpm, which is taken from Fig. 6.

their ratio is constant; the CO_2 level during active fermentation was 1.5 times the saturation level at all counterpressures. Total fermentation time was about the same in all these 15°C fermentations, regardless of the amount of CO_2 counterpressure.

Figure 5 shows the influence of CO_2 counterpressure upon the dissolved CO_2 concentration in 22°C fermentations. Again, the level of CO_2 supersaturation remained constant until the fermentable carbohydrates were nearly all assimilated, then decreased to approach the saturation level. Also, the ratio of the concentration of CO_2 during active fermentation to saturation was 1.5, though both CO_2 levels were lower than in the corresponding 15°C fermentation. The 22°C fermentations proceeded about twice as fast as the 15°C fermentations.

Figure 6 demonstrates this constant relation between the CO_2 concentration during active fermentation and the corresponding saturation level in 15 fermentations with various combinations of temperature and CO_2 counterpressure, all agitated at 33 rpm. The slope of the regression line is 1.50, which means that for all fermentations agitated at 33 rpm, independent of temperature or CO_2 counterpressure, the level of dissolved CO_2 during active fermentation is 1.5 times the corresponding saturation level. We term this slope the *supersaturation coefficient*.

Effect of Agitation upon Dissolved CO_2

Fermentations were run at five levels of agitation between 33 and 320 rpm and at 15° and 18°C. CO_2 counterpressure was 0 psig. Agitation levels together with corresponding power inputs are shown in Table II. The power values are useful for equating agitation levels in different fermentors or for scale-up to production equipment. The relation between the level of agitation and the concentration of dissolved CO_2 during active fermentation is shown in Fig. 7. The regression lines for both the 15° and 18°C fermentations show that there is an inverse linear relation between CO_2 concentration and agitation level. Note that there was no CO_2 supersaturation during active fermentation at agitation levels of 150 rpm or greater.
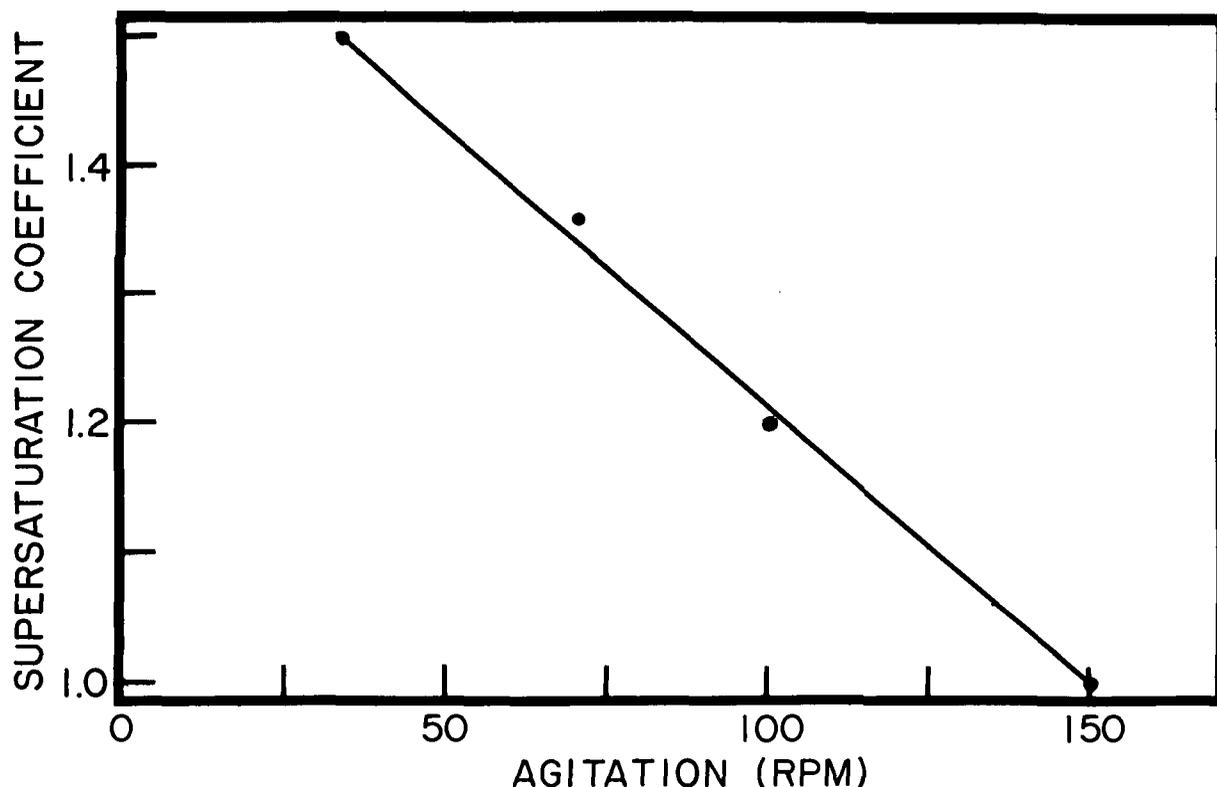

Fig. 9. Relation between supersaturation coefficients and agitation levels. Supersaturation coefficients are equivalent to the slopes of the lines from the various agitation levels shown on Fig. 8. The correlation coefficient of the regression line is 0.998 on 3 degrees of freedom.

When dissolved CO_2 during active fermentation is plotted against saturation at each agitation level, the family of lines shown in Fig. 8 is obtained. The uppermost line is the one shown in Fig. 6; it is based on 15 fermentations at 33 rpm and has a slope, or supersaturation coefficient, of 1.50. The bottom line is the plot of fermentations at 150 rpm or more, where no supersaturation existed during active fermentation. Therefore, the supersaturation coefficient is 1.0. The intermediate lines represent data from fermentations at 70 and 100 rpm and have intermediate supersaturation coefficients. Two assumptions have been made in plotting these lines—first, that the data plot as straight lines, and second, that the extrapolated lines pass through the origin. Both of these conditions were true for the fermentations at 33 rpm; consequently, we consider them to be reasonable assumptions over the range of our experimental conditions. The supersaturation coefficients of these fermentations vary inversely with agitation. Hence, if we plot these coefficients vs. agitation, we obtain Fig. 9. It is evident that the inverse relation between agitation and supersaturation during active fermentation is linear, under these experimental conditions.

The advantage of increasing fermentation temperature to decrease total fermentation time is obvious. However, this change is accompanied by marked changes in the volatile components of the beer produced. Perhaps the most significant aspect of this work is the concept that a suitable combination of increased temperature and CO_2 counterpressure makes it possible to decrease fermentation time with minimal changes in the total volatiles production. The quantitative relations developed in this work could facilitate the extension of these concepts to commercial practice. Obviously, tank geometry, with its attendant influence upon agitation and hydrostatic pressure, would need to be considered.

Acknowledgments

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