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The Effect of Light on Champagne Yeast Cell Growth and Ethanol Production Under Variable pH Conditions

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ABSTRACT

The effect of white light on unaerated growth of Baker's yeast and the accompanying ethanol production has been studied in a batch process at 27℃. Over the 80 hour period of the Champagne yeast process without pH control, the cell growth was inhibited by the fluorescent light. Another observed difference between the runs is that the drop and subsequent rise in redox potential occurred much sooner in the fermentation with light than in the fermentation without light. This preliminary study indicated that ethanol production can be enhanced by light as the cell concentration is repressed. The possible pathway shift of the sugar substrate toward ethanol and away from cells was manifested by another difference as well. As observed under the microscope, many of the yeast cells grown under light budded without dividing by the normal fission process as they did in the dark. Furthermore, the undivided and branched(light grown)cells did not agglutinate at the end of the fermentation process as did the distinct spherical (dark grown) cells.

INTRODUCTION

Previous studies in lighted fermentations have noted two interesting results: The yeast cell growth and extracellular protein secretion are both suppressed, (1), (3), (4). These results have been duplicated in the course of the present study. However, previous studies have not apparently examined the effect of light on yeast cell clumping or on the yeast cell morphology.

The affect of light on ethanol production by yeast apparently has also not been explored. Whereas there is considerable interest in accelerating the rate of ethanol formation in the champagne production process in Hungary (2), the means of acceleration is other than changing the light to the system. The present study was conducted to further explore the effect of lighted fermentations on yeast, and to determine the effect of light on ethanol production in the fermentation process, with the idea of applying it to this particular wine making process.

To help elucidate this study, the Oxidation-Reduction potential variable was also monitored. The Oxidation-Reduction (redox) potential is an easily measured quantity and can be continuously measured during the entire fermentation. The redox potential can be related to the concentration of yeast cells in the system as well as their group behavior(agglutination).

APPARATUS

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All initial conditions were identical for runs with or without light; the only change from run to run was the presence or absence of light from a thirteen watt fluorescent bulb (number F13T9/CW) placed in the center of an annulus through which the fermentation solution passed. Figure 1 illustrates the system. The fermentation medium flows from the double walled jacketed beaker(liquid reservoir) annular reactor, where it can be exposed to light at close contact and without significant heat buildup. The annular reactor is 24.5 centimeters long with a 5.3 centimeter inside diameter and a centimeter outside diameter. The annulus is 0.6 centimeters thick, allowing a 4.1 centimeters diameter in which to place the fluorescent bulb. About half of the fermentation mixture is in the beaker, with nearly half of the fermentation mixture in the annulus, at any given time. The annulus is of sufficient thinness to alow uniform lighting of the mixture passing by the outer wall. Water from constant temperature water bath flows through the beaker jacket to cool the fermentation mixture and keep it at a uniform temperature of 27℃. This cooling insures that the light effects seen in the system are due to only light and not to heat. The redox potential is measured on-line and continuously.

EXPERIMENTAL PROCEDURE

Before each run, all equipment was revised with a warm solution of sodium sulphite followed by a water rinse. The redox meter was standardized at zero volts by using a sodium sulphite solution(2%) with a known redox potential of zero. A Bausch & Lamb Spectronic 20 spectrophotometer was used to measure the optical density of the solution at 520 nanometers. The yeast cell concentration was then determined from a correlation between optical density and the yeast concentration. The blank was water. The percent ethanol was measured with a vinometer made by Wine King (West Germany), corrected for interferences from glucose and total protein. This will be dealt with further in the Results and Discussion section.

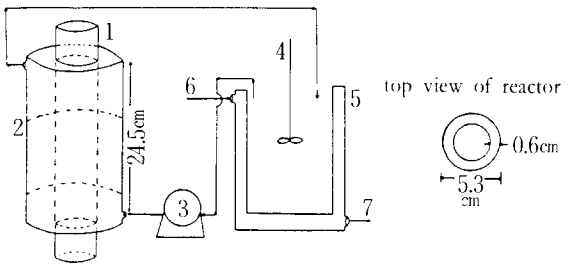

Fig. 1. Apparatus.
1. Fluorescent bulb
2. Reactor 3. Pump
4. Stirrer 5. Beaker
6. Cooling water in
7. Cooling water out

The vinometer is a small capillary tube approximately 12 centimeters long. The top is a narrow funnel that receives the wine solution to be tested. The remainder of the vinometer is a long capillary tube that has gradations corresponding to percent ethanol. The vinometer works on the principle of capillary action. It is very important to remove all traces of the cleaning agent before measuring the capillary action of the wine. If the wine itself is measured, the method is not only rapid but fairly accurate. Since proteins and glucose can cause the vinometer to give high readings of ethanol, the readings are corrected for these two interfering substances. The vinometer is first placed top up and filled with methanol to clean out any residue in the capillary section. The capilary is then blown clean of methanol. The wine is added to the vinometer. After three drops of wine fall from the device, the vinometer is inverted and the top used for a stand. If no bubbles have formed in the capillary, then the reading can be made. The lowest point to which the wine falls in the capillary corresponds to the percent ethanol on the vinometer. The vinometer is then cleaned and the procedure repeated. The results obtained are reproducible within 0.2 ethanol percentage points.

The fermentation medium consists of:

- 54ml of Alexander's Sun Country Grape Concentrate (68° brix, or weight percent sugar)
- 650ml of water (tap)
- 75 grams of corn sugar (dextrose/maltase)
- 2 grams Red Star California Champagne Yeast produced by Universal Foods.

The total mixture was 750ml; therefore the initial yeast concentration was 2.67 g / l. The initial redox potential was 15 ±5 millivolts and the temperature was controlled with a water bath at 27℃. The mixture was pumped between the reservoir and reactor and back again to the

reservoir at a rate of approximately 475ml / min.

Both short and long time runs were made in the study. The "short" runs were made for 24 hours while the " long" ones were made for 80 hours. The 24 hour runs were more closely monitored during the duration of the experiment. The 80 hour runs were performed to check the long time effects of light on the fermentation, particularly the formation of ethanol to make the champagne. Each hour, cell concentrations and percent ethanol were measured, along with the redox potential. Initial experiments showed that the pH varied only slightly in a thirty hour period, falling from an initial pH of 4.2 to a pH of 3.5. The pH is allowed to vary in commercial wine production hence the pH was not controlled in these experiments. None of the fermentations was aerated.

RESULTS AND DISCUSSION

The redox potential and the yeast cell activity were found to be related to one another. Ethanol was also found to be produced more quickly in lighted runs. The ethanol results were obtained using the vinometer, therefore, they should be considered to be tentatively contingent upon verification by more accurate ethanol measurement methods.

Several factors affect the redox potential in the yeast fermentation systems. Other studies have shown yeast cell concentrations to affect redox potentials (1). Previous studies (3),(4) have also shown that light suppresses yeast cell growth. This has also been observed for the unaerated conditions for producing champagne, as shown in Figure 2. Therefore, lighted fermentations may have different redox curves than nonlighted fermentations. Figures 3 and 4 show an inverse relationship between the cell concentration on the redox in both the dark and light situations. For dark trials the redox falls as the cell concentration rises and rises as the cell concentration falls. For lighted trials, the same relationship is again seen, except the rise in redox occurred around 15 hours, whereas in the dark run that rise occurred over 50 hours. Although a smooth curve has been drawn through the scattered points for the sake of simplicity, th scatter may offer additional information. Just when the cell concentration oscillates, the redox potential oscillates mirroring the movement of the cell concentration curve. A rapid and

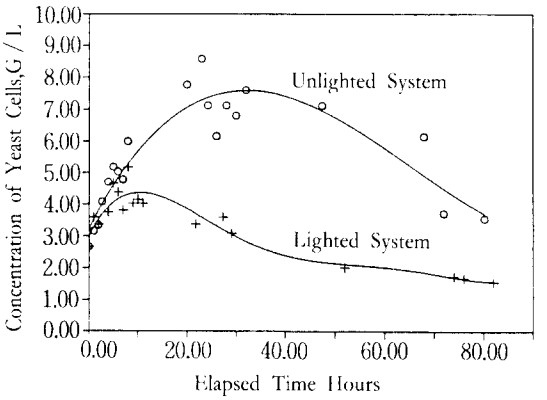

Fig. 2. Yeast Cell Concentrations in Fermentation Systems.

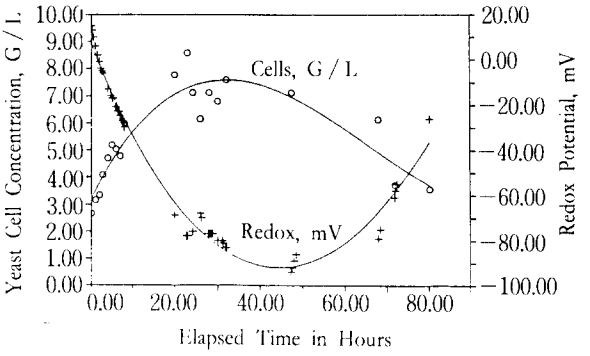

Fig. 3. Relating Cell Concentration With Redox Potential Unlighted System.

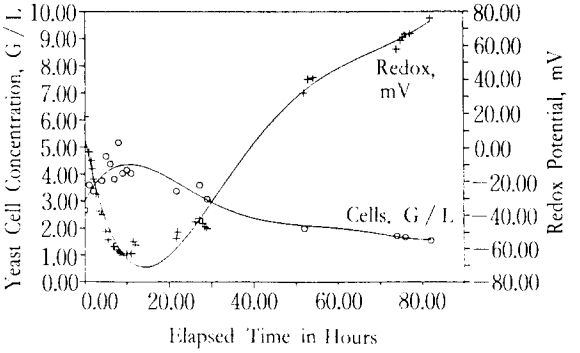

Fig. 4. Relating Cell Concentration With Redox Potential Lighted System.

direct response, therefore, exists, between the redox and the cell concentration.

Figure 5 compares the two redox curves. Also included on the graph are illustrations of the yeast cells as viewed under a microscope, both curves follow the same pattern of decline and then rise. Besides using sooner that the dark system, the lighted run redox reaches a low of about −70mV, whereas the dark redox levels drop to a low value of about −95mV.

The previously mentioned yeast cell suppression due to light can account for the change in magnitude in the two redox curves. Yeast cells carry on their outer membrane a negative charge potential relative to the inside of the cell. Because lighted fermentations suppress yeast cell growth (as shown in Figure 2), the redox curve for a lighted fermentation will not reach as low a point as the redox curve in a nonlighted fermentation. However, the two curves rise for two different reasons. As Figure 5 shows, yeast cells at time zero appear approximately spherical and are randomly distributed throughout the medium. The cell diagram at the bottom of the figure shows how yeast cells appear in a nonlighted system at approximately forty hours into the fermentation. The cells still have their spherical shapes, but agglutination (or flocculation) has occurred and all the cells are sticking together. As the cells clump together, the negative potentials of the cells in the middle are massed by the outer yeast cells and the overall potential is more positive. Also, these clumps of cells absorb and scatter light more effectively so that yeast cell optical densities for the dark system, which are determined by light absorption and light scattering, may be enhanced, relative to free floating yeast cells. As the upper microscopic diagram of cells at 40 hours in Figure 5 illustrates, agglutination is not present in lighted fermentations. The redox potential still rises with time, but for another reason than cell flocculation. Microscopic studies of the yeast cells from lighted fermentations show more probological changes in the cells. Some shapes have changed from spherical to rod-like structures. Remnants of cells that have lysed can also be seen under the microscope. As light causes cells to deform and lyse, the cell spills its contents into the fermentation medium. The cell membrane carries a nagative potential relative to the inside of the cell. Accordingly, the inner contents of the cell have a positive potential relative to the outer membranes. Thus, as the cell lyses, the redox potential rises becoming more positive.

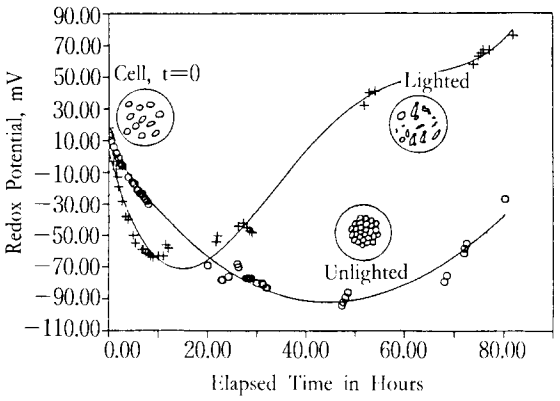

Fig. 5. Redox Potential Comparisons For Lighted and Unlighted Fermentations.

Previous studies(4) have shown that light suppresses the extracellular protein found in the fermentation broth. Several proteins have been associated with agglutins. Therefore, the suppression of proteins may also be linked to the lack of agglutination by such agglutins, seen in lighted systems. Finally, because cell concentration, cell lysing, and cell agglutination can affect the redox, the redox measurement can be used as a measure of certain cell activities.

Lighted fermentations also affect the production rate of ethanol. Figure 6 illustrates the difference in the ethanol formation for lighted and nonlighted fermentations. While the ethanol production rate is not greatly enhanced for the lighted systems, the increase over non-lighted systems does exist nonetheless. Stated more conservatively, light does not adversely affect ethanol production. The maximum ethanol percentage attainable for the system seems to be reached about ten hours faster in a lighted fermentation than in a nonlighted fermentation.

Figure 6 reports the actual ethanol reading obtained with the ethanol measuring device, the vinometer. Since the vinometer is sensitive to the effects of protein and glucose in the system, the data in Figure 6 need to be corrected. Glucose is assumed to be exhausted in ten hours in the lighted system and is assumed to be consumed in twenty-five hours in the nonlighted system(5). Protein levels are assumed to be thirty percent lower in the lighted

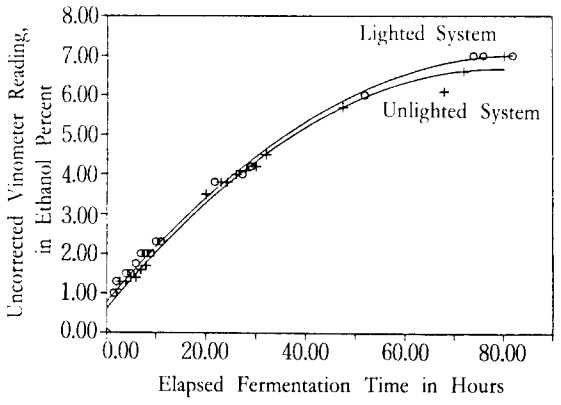

Fig. 6. Comparison of Uncorrected Vinometer Readings.

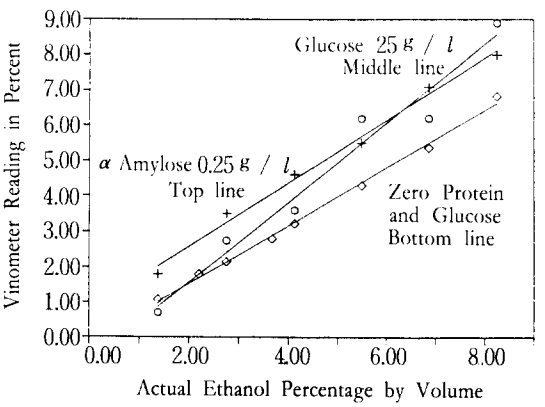

Fig. 7. Vinometer Calibration.

system than in the nonlighted one at any point in time, based on previous studies(4). The original vinometer readings, used in Figure 6, are corrected according to how much protein and glucose are in the fermentation medium at the time of the reading. Figure 7 is used for this purpose. The corrected results are graphed in Figure 8. Figure 7 is a calibration curve constructed to determine the effect of protein and glucose on the readings obtained with the vinometer. To construct this curve, the total protein concentration was held constant at an average system value of 250 micrograms / ml while the ethanol percentage was varied and vinometer readings were taken. α-amylase was used to characterize the total protein. Likewise, glucose was held constant at an average system concentration of 25 g / l and ethanol percentage was again varied. The corrected values are graphed in Figure 8. The effect of protein, which is more concentrated in nonlighted systems, is to reduce the uncorrected percentage of ethanol readings to be made. Because glucose may be used up more slowly in the nonlighted system than it is in the lighted system, the ethanol readings in the lighted system may be higher than the nonlighted ethanol percentages, after the glucose correction. While these vinometer corrections are improvement over the uncorrected readings, the values still do not account for other system impurities. However, the corrections indicate that the difference in ethanol production appear to be more significant. The percent difference between the two trajectories is 20 percent at most points and it may reach as high as 30 percent.

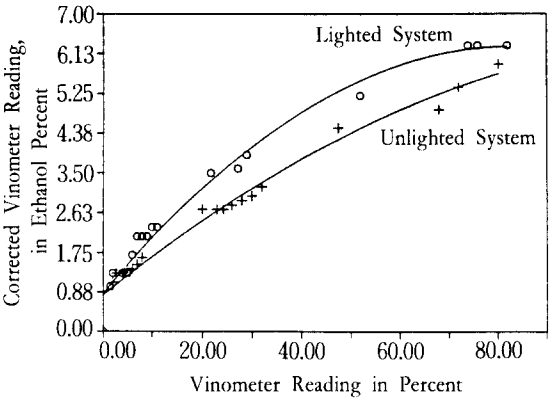

Fig. 8. Comparison of Corrected Vinometer Readings.

The increased production rate of ethanol seems to be contradictory to the suppression of cell growth in lighted fermentations. One possible explanation reconciling this possible contradiction does exist, however. As already shown in Figure 2, the yeast cell concentration in lighted fermentations is lower than the yeast concentration in nonlighted ones. At approximately eight hours into the lighted fermentation, the yeast cell concentration oscillates for about six hours. Previous observations have led researchers to believe that the glucose supply for the fermentation is spent around the time of this oscillation (5). The corresponding oscillation for the nonlighted fermentation does not occur until twenty hours of fermentation have elapsed. If the oscillation indicates the complete usage

of the fermentation sugars, (say, by cell lysis followed by growth and/or agglutination followed by dispersion of cells) then the lighted fermentations may have converted glucose into intermediates before the yeast cell growth is altered. The intermediates may then be converted into ethanol as the fermentation progresses. Another possible explanation is that the lysed cells components in the lighted system may still act to convert sugars into ethanol, in the same manner as whole cells(but with less cell membrane diffusional restrictions).

CONCLUSIONS

Several conclusions can be tentatively drawn from the results of this study regarding the potential, the cell activity, and the ethanol concentration:

- Illuminated fermentations have qualitatively different redox-time profiles than dark fermentations.
- The cell concentration is qualitatively inversely proportional to the redox potential in both the lighted and the nonlighted fermentations.
- The rate of ethanol production in lighted fermentations is greater than in dark fermentations, indicating that sunlight entering an open fermentor may reduce the fermentation time(neglecting evaporation losses).
- The redox potential is a useful tool for monitoring the cell activity in both light and dark fermentations.
- Yeast cell agglutination is inhibited in lighted fermentations.

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