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# Light Mediated Yeast Cell Growth and Metabolism

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## ABSTRACT

In this paper the effect of light on non-aerated Baker's Yeast (*Saccharomyces cerevisiae*) production and the protein excretion to the extracellular fluid is studied. Previous results in our laboratory indicate that at pH=5 and T−32℃ yeast may be affected by light, but those differences seem to be within statistical variation of the data. In this paper, cell and extracellular protein concentrations along with redox potential are monitored for batch fermentations in the presence and absence of light at pH levels of 3 and 5 and at 31℃, in order to explore whether possible light effects can be more readily discerned at lower pH values. Yeast particle size distributions are also determined over the course of fermentation using a particle counter in order to add one more measuring tool to our usual cell and total protein measurements. An apparently noticeable difference in the redox potential is observed between the light and the dark runs for early times for the pH=3 runs. The particle size distributions show differences in the particle diameters between light and dark runs at pH=3, but those differences fall within one standard deviation of the mean particle diameters.

## INTRODUCTION

The objective of this work is to explore the effect of light on Baker's Yeast, *Saccharomyces cerevisiae*. The yeast's response to light and dark at a constant pH was measured over the course of a batch fermentation process in terms of: 1) the cell concentration, 2) the extracellular protein level, 3) The Redox Potential of the extracellular medium, and 4) the yeast cell particle size distributions. If significant light effects can be found, light could potentially be used as a cheap and efficient means of controlling and monitoring yeast development and extracellular protein production during the cellular growth process.

Preliminary work regarding light effects on nonaerated yeast cell growth, extracellular protein production, and redox potentials was previously reported by Hojnicki et al.(1) and Boero et al.(2). Here, the study replicates the previous observations at pH 5 and explores the effect of light at a more "stressed" pH value, pH=3. In addition, changing yeast cell size distributions are measured using a particle counter for the first time in conjunction with this study of light effects.

In the work by Hojnicki et al.(1), the fermentations were nonaerated, and the pH, starting at 5, was not controlled. It was revealed that for early times cell, concentrations were slightly lower in the absence of light than

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in the prsence of light. However, for later times, the light may have repressed the cell growth as compared to the dark run growth. These early results seemed to be corroborated by the work of Boero et al.(2) at pH 5, but they observed significant scatter in the data.

Hojnicki et al.(1) observed that extracellular protein levels in an uncontrolled pH run (starting at pH 5) were significantly higher in the dark run than in the light run, especially after 7 hours. Boero et al.(2) also observed higher protein levels for dark runs at pH 5 than for lighted runs at later times, but, again, the large scatter in the data obscured the differences. Since yeast fermentations drop in pH over the time course, the exploration of a lower pH domain which was reached in Hojnicki's experiments (e.g. pH=3) may help clarify these earlier results.

Kjaergaard (3) notes that "it is evident that the redox potential is a parameter that can provide valuable information about the status of microbial cultures."Boero et al. (2) demonstrated that the redox potential drops by over 350 millivolts over the course of a fermentation, and that light and dark runs may, perhaps, be distinguished by small differences in their redox potential time trajectories. Their data at pH 5 suggest that light runs may reach a slightly lower change in redox levels relative to the initial redox value than the dark runs.

## MATERIALS AND METHODS

### Microorganism

*Saccharomyces cerevisiae* was used in all the experiments in the form of Fleischmann's Active Dry Yeast. The presumed expiration date on the yeast packets was given as 9/27/90, (Batch C). Packages with this same expiration date were used in all of the experiments. Runs 3 and 4 (pH 5) were conducted before this date, and runs 1 and 2 (pH 3) were conducted within three months after that date. Since the yeast packets were kept refrigerated, it was assumed that their activity had not diminished significantly when they were used in the later experiments.

### Medium

Medium C described by Maxon and Johnson (4) was used as the prescription for the synthetic medium of vitamins and minerals. One hundred grams per liter of glucose was added to the medium. This solution was inoculated with 1.4 g of yeast per 700mL broth solution to give a concentration of 2 g/L at the beginning of each run, except for Run 1 which was inoculated with 2.0 g of yeast per 700mL broth to give a 2.86 g/L cell concentration. The medium was autoclaved (vitamins and sugar separately) prior to the addition of the yeast, for 15 minutes at 15 psig to create an aseptic condition. The two control runs, at pH=3, to check the effect of light on the redox probe, were conducted as the other runs, except no yeast was added.

### Apparatus

Figure 1 is a schematic depiction of the apparatus. The principal components are 1) the broth reservoir, 2) the polymethyl methacrylate shell and tube light reactor, 3) the constant temperature bath, 4) the redox probe shielded from the light by both the reactor shell and its own cover and external to the reactor at a distance of 15cm from the fluorescent bulb, and 5) the pH meter. A Cole Parmer Chemcadet pH/redox meter was used with a P186 Chemtrix Electrode (containing a silver/silver chloride internal reference) to measure the redox potential. The zero redox reading was standardized with the recommended 20 g/L $Na_2(SO_3)$ solution. A Beckman Zeromatic II pH meter was used to measure the pH.

The broth reservoir was a double walled glass beaker with a 500mL capacity. The constant temperature bath circulated heated water between the two walls of the reservoir, and maintained the contents at a temperature of 31℃ ± 2℃. A magnetic mixer at the bottom of the reservoir was used to prevent the yeast from settling. A peristaltic pump drove broth through the light reactor from the reservoir. About 500mL of the ca. 700mL broth solution remained in the reservoir during the fermentation process and about 200mL was in the reactor shell and connecting tubing.

Broth entered from the bottom of the light reactor, circulated between the shell and tube, exited from the top, and returned to the reservoir. No external aeration was introduced in the reactor. An F13T8/CW Sylvania 13 watt fluorescent lamp was inserted into the tube of the reactor during light runs. The tube opening was covered with aluminum foil during dark runs. The outer wall of the reactor was wrapped in aluminum foil to minimize the yeast's exposure to room lighting for all runs. A redox

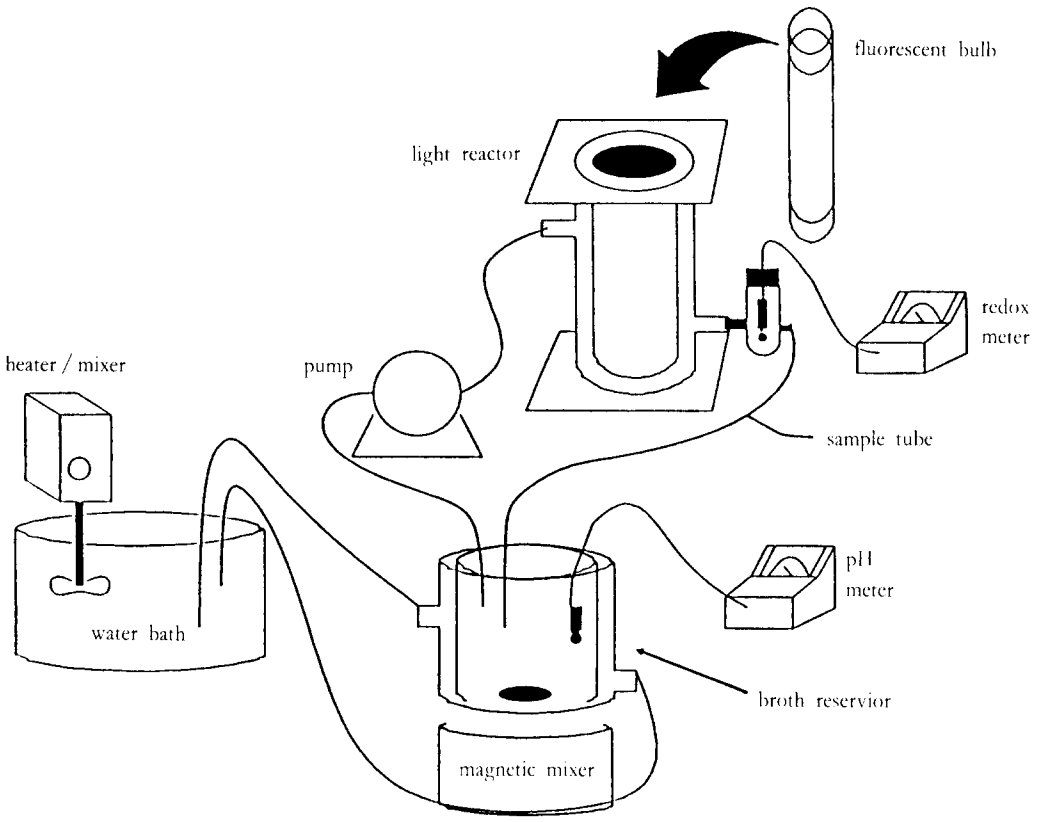


Fig. 1. Experimental apparatus.

probe measured the oxidation reduction potential of the broth leaving the light reactor, (it is shown at the "bottom" of Figure 1; in fact, however, this outlet was at the top for the runs described in this paper) and the pH was measured by an online probe inserted into the preservoir. The redox probe drift, determined by placing the redox probe in the calibrating solution at the end of each run was no more than 45mV at 24−26 hours. The pH was maintained manually at 3±.3 or 5±.3, by titrating with dilute HCl or $NH_4OH$.

## Experimental Procedure

Each experimental run lasted approximately 24 hours. The redox probe was zeroed at the start of the run using a 20 g / L solution of sodium sulfite in water. The fermentation was closely monitored for the first eight to ten hours, and for the last two to three hours. During these times the redox potential was recorded approximately every fifteen minutes, and a broth sample was taken every hour. Cell concentrations were determined from optical density measurements with a Bausch and Lomb Spectronic 20 spectrophotometer, and total extracellular protein concentrations were determined using the Bradford Coomassie Blue dye binding assay with that spectrophotometer. Every two hours a portion of the sample was processed in an Elzone particle counter to determine the particle size distribution of the yeast cells for the pH 3 runs.

## Cell Concentration Measurement

A cell concentration calibration curve was developed using a series of known concentrations of Baker's yeast, as measured in grams of dry yeast per liter of water. The optical density(OD) of each standard sample was measured at a wavelength of 595nm using a Bausch and Lomb Co

Spectronic 20 spectrophotometer, with water as the blank. The data were used to develop the standard curve fitted to a polynomial having the equation:

$$\text{cell conc} = 0.7607(\text{OD})^2 + 0.459(\text{OD}) + 0.01673 \quad (1)$$

This cell concentration equation was developed, (and hence, is applicable) over the dry yeast range of 0 to 1 g / L. Samples taken over the course of a run were diluted by a factor of ten with deionized water to keep the optical density, (OD), (=absorbance on the spectrophotometer) measurements within the Beer's law range (absorbance ≦.4), the "linear"portion of Equation 1. The OD of the sample was measured and converted into cellular concentration units using Equation 1.

## Total Extracellular Protein Measurement-The Bradford Assay

Extracellular protein concentrations were measured with the Bradford Coomassie Blue dye binding assay (5), (6), (7). A total extracellular protein standard curve from 0 to 100mg / L was developed using fungal $\alpha$-amylase (Miles Laboratories, Inc., Catalog No. 31-001 -1; from *A. oryzae*). This protein was previously shown, by Franklin et al.(7), to have a similar standard curve to sonicated yeast proteins. The extracellular fermentation broth samples were assayed as follows; 0.6mL of protein sample was added to 2.4mL of Coomassie Blue, and the optical density was measured on the spectrophotometer at 595nm. This particular variation of the Bradford Method is referred to as the "Micro Method". Zeroed optical density readings were obtained by subtracting the optical density readings of each standard sample from the optical density of a deionized water blank. The spectrophotometer could not simply be zeroed with a dye and water blank, because the dye slowly reacted with the water and, consequently, the dye blank optical density decayed over the fermentation time period. The data were fitted to a linear equation for protein concentration in mg / L, to generate a calibration curve with optical density (OD):

$$\text{Conc} = (119.12)\ \text{OD} \quad (2)$$

After measuring the OD of the diluted (10 fold) broth sample to determine cell concentration using Equation 1, the sample was centrifuged for five minutes. The supernatant was carefully poured off and assayed for total protein in the same manner as the standard samples. The resulting optical density value was then converted into an extracellular protein concentration using Equation 2.

## Size Distribution

Yeast size distributions were determined at various times with an Elzone 280PC-H particle counter, manufactured by Particle Data Inc.(Elmhurst, IL.) The data were observed and recorded using an Elzone 280 PC interface operating on an IBM 286 compatible computer. This procedure was performed three times for each broth sample, and the results were averaged. Samples were prepared as follows: 10$\mu$L of sample was added to 20mL of saline solution, shaken, and then placed in the particle counter. The data were then recorded and analyzed using the computer.

# RESULTS AND DISCUSSION

## Cell Concentration

The cell concentration vs. time data for typical light and dark runs at pH's of 3 and 5, respectively, can be summarized by the following equation:

$$(X) = At + B, \text{ for } 0 \leqq \pm \leqq 10 \text{ hrs}, \quad (3)$$

where the A and B coefficient values are given in Table 1.

All of the curves follow the typical sigmoidal growth cycle pattern. The cells experience nearly linear growth for the first ten hours, increasing in concentration from ca. 2 g / L to ca. 5 g / L. They then move into a stationary phase, measured at ca. 24 hours.

Since the data exhibit so much scatter, they are analyzed using standard statistical methods over the linear (first 10 hours) range.

At each pH, the growth curves for light and dark are almost identical. The pH 3 results seem to suggest some growth enhancement due to light, however, that effect is primarily due to the inadvertent inoculation with 2.8 g / L yeast cells(run 1) rather than the usual 2 g / L (run 2). When this 40% higher cell level is taken into account, the curves almost overlap. Assuming that the final cell levels are proportional to the initial cell levels, the cellular yield is constant throughout the fermentation process. There is some observable difference in final concentrations for the two pH's. The late time cell concentrations for the pH 5 runs seem to be lower than those for the pH 3 runs, and, as shown in Table 1, the cell levels for the runs with light are higher than the runs without light.

Since the values for coefficient A (slope of Equation 3) are equal (within one standard deviation) as listed in Table 1, the early time growth rates do not seem to be significantly affected by light at each pH.

Additional measurements are clearly called for to clarify whether light has a significant and reproducible effect on yeast cell growth.

## Protein Concentrations

The total extracellular protein concentration vs. time trajectories for the pH 3 and pH 5 cases, respectively, are summarized using the following equation for early times:

$$(P)=Ct+D, \text{ for } 0 \leqq t \pm 10 \text{ hrs}, \quad (4)$$

where the C and D coefficient values are given in Table 2.

All the protein concentration data show a good deal of scatter, as quantified by the very large standard deviation, $\sigma$, for each rate term C. For early times, virtually all the concentrations are below 50mg / L (.05 g / L). At

**Table 1. Coefficients for the linearized equation (equation 3) describing the early cellular time course**

| Run Number | Condition | Parameter A | Parameter B | Correlation Coefficient $R^2$ | Standard Deviation, $\sigma$, for Parameter A | Final Cell Concentration @ 24-26 Hours, mg / L |
|---|---|---|---|---|---|---|
| 1 | pH=3 (Light) | 0.00718 | 1.71 | 0.90 | 0.0011 | 9.57 |
| 2 | pH=3 (No Light) | 0.00588 | 1.48 | 0.99 | 0.00025 | 8.38 |
| 3 | pH=5 (Light) | 0.00627 | 0.890 | 0.94 | 0.00055 | 5.94 |
| 4 | pH=5 (No Light) | 0.00557 | 1.03 | 0.97 | 0.00032 | 4.74 |

**Table 2. Coefficients for the linearized equation (equation 4) describing the early cellular time course**

| Run Number | Condition | Parameter C | Parameter D | Correlation Coefficient $R^2$ | Standard Deviation, $\sigma$, for Parameter A | Final Cell Concentration @ 24-26 Hours, mg / L |
|---|---|---|---|---|---|---|
| 1 | pH=3 (Light) | $4.39\times10^{-6}$ | 0.0226 | 0.00327 | $3.42\times10^{-5}$ | 156 |
| 2 | pH=3 (No Light) | $8.89\times10^{-6}$ | −0.00551 | 0.0229 | $2.19\times10^{-5}$ | 77 |
| 3 | pH=5 (Light) | $-6.1\times10^{-5}$ | 0.261 | 0.112 | $6.05\times10^{-5}$ | 464 |
| 4 | pH=5 (No Light) | $9.09\times10^{-5}$ | 0.238 | 0.136 | $8.09\times10^{-5}$ | 263 |

the final time of 24–26 hours the protein levels rise 3 to 5 fold, with the lighted runs having twice the concentration as the non-lighted runs, at each pH level. Except for the final protein values, no clear effect on extracellular protein levels due to light can be ascribed to these data. Other measurements are therefore needed to help clarify whether light has an effect on yeast cell exoprotein production.

## Redox Potential

Figures 2 and 3 present the change, or delta, in redox potential vs. time data for light and dark runs at pH's of 3 and 5, respectively. The delta redox potential is difined as the difference between the redox potential measured during the run at time, t, and the initial redox potential measured as soon as the probe was introduced to the broth, within 5 minutes of adding the yeast and before the broth is circulated through the light reactor, i. e.

$$\Delta(R.P.)=R.P.\Big|_{t}-R.P.\Big|_{t=o} \quad (5)$$

The delta redox potential was used to allow comparisons for different runs with different initial (reference) points.

The initial redox potentials for each run were as follows: At pH 3: run 1, 144 mV; run 2, 259 mV; and at pH 5: run 3, 193 mV; and run 4, 213 mV. The initial redox potentials for the pH=5 runs are nearly equal (203±10mV), and the differences between the two pH 3 runs seems to reflect the 43% difference in initial cell levels. Figures 2 and 3 demonstrate the first clear differences between light and dark runs at a given pH. Note that all of the runs depicted on these figures start at ΔR.P. =0. The light run at pH 3 seems to show a greater extreme of high and low delta redox values than the counterpart case at a pH of 5. The actual differences between the light and dark redox profiles are not as prominent at pH 5. The pH 3 data exhibit a rapid rise in redox over the first hour, not seen in the pH 5 data. However, as seen in both figures, at early times after the pH 3 curves ceased rising, the slope of the falling redox curve for light became noticeably steeper than for the dark runs. Changing the pH from 5 to 3 seems to exacerbate the difference between the light and dark curves, particularly during the first three hours.

All the curves follow the same general "U" pattern. The delta redox potential falls steeply for the first 10 to 12 hours (after the initial rise for pH 3). It then moves into a more gradual decline, and it begins to head back up again at approximately twenty hours. The redox potential here is a qualitative measurement when applied to the complex extracellular medium, but it does strongly suggest significant differences between light and dark runs, particularly at early times. Furthermore, the general trends seen in Figures 2 and 3 can be associated with specific changes in the yeast cell environment.

For example, a plot of redox potential vs. glucose concentration at pH=5 and 32℃ is nearly linearly correlated by the equation:

$$R.P.=\text{Redox Potential}=\frac{(1.8mV)}{g/L}\ (\text{Glucose})+20\ mV$$

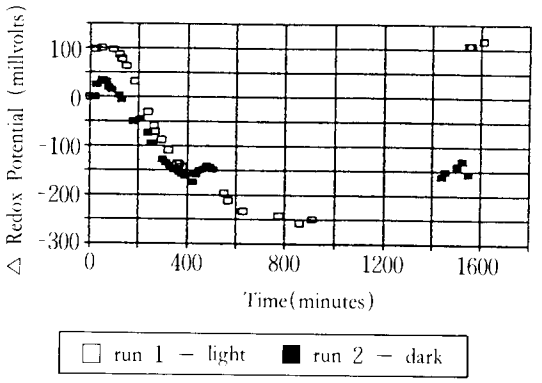


Fig. 2. Effect of light on delta redox potential at pH 3.

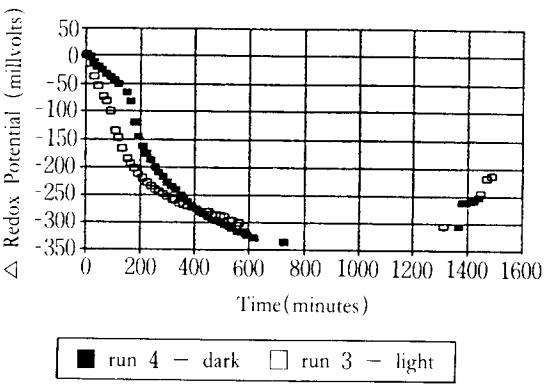


Fig. 3. Effect of light on delta redox potential at pH 5.

over the glucose concentration range of 0 to 100 g / L. This can also be expressed as $\Delta(R.P.)=\frac{(1.8mV)}{g/L}$ (Glucose)$-180$mV, or $\Delta(R.P.)=0$ at $t=0$ and $\Delta(R.P.)=-180$ mV when the glucose concentration becomes negligible (around 600−720 min. from our previous experience).

This glucose phenomenon seems to physically account for the early time redox potential drop as the glucose is taken up by much of the yeast cells during fermentation. This glucose effect on redox potential may, therefore, account for about 50% of the drop in redox potential for the pH=5 fermentation runs (Figure 3) by the 600 −700 min. times.

The middle portion of the graph may reflect the increasing(unmeasured here) ethanol concentrations in the unaerated broth. As the ethanol content increases, the redox continues to drop more slowly. Finally, and particularly for the pH 3 runs, as the cells reach late times, they may lyse and release intracellular salts, sugars, as well as proteins. These added extracellular salts and sugars may act to cause the extracellular redox potential to rise back up to the early time levels.

To determine whether the light affected the redox probe, even in the absence of yeast, control runs were conducted at pH=3 and T=32℃ for the fermentaton media along in the reactor system. The drift in the redox probe reading, Δ(R.P.), was 40 mV over 3 hours for both the light and no light cases. The positive drift response was about 20 mV in the first half hour, and another 20 mV in the next hour, coming in asymptotically to the +40mV point for both the dark and light cases. When another (newer) redox / pH meter was used, the drift was reduced to 5 mV indicating that there was negligible drift in the probe itself. Typical drift readings over the course of a 24 hour fermentation run (as determined by reintroducing the redox probe into the calibrating solution) was +45 mV. These controls, seem to indicate that light itself had a negligible effect in the redox probe readings. The difference due to light, therefore, is attributed primarily to the different

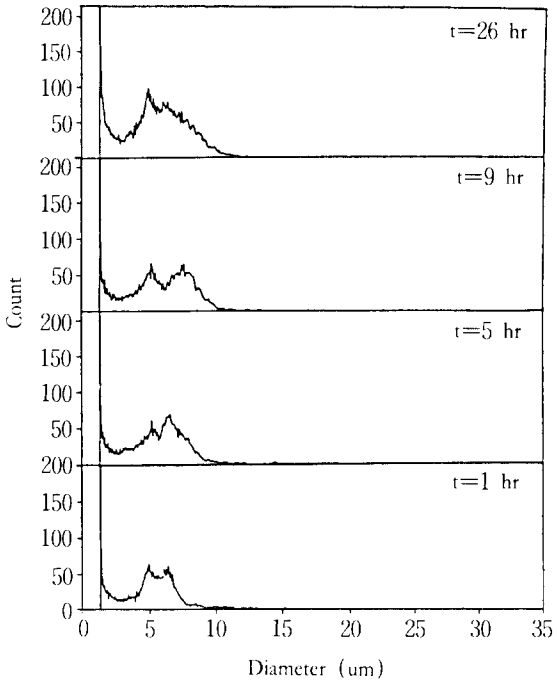


Fig. 4. Particle size distribution Run 1 (Light) at pH 3.

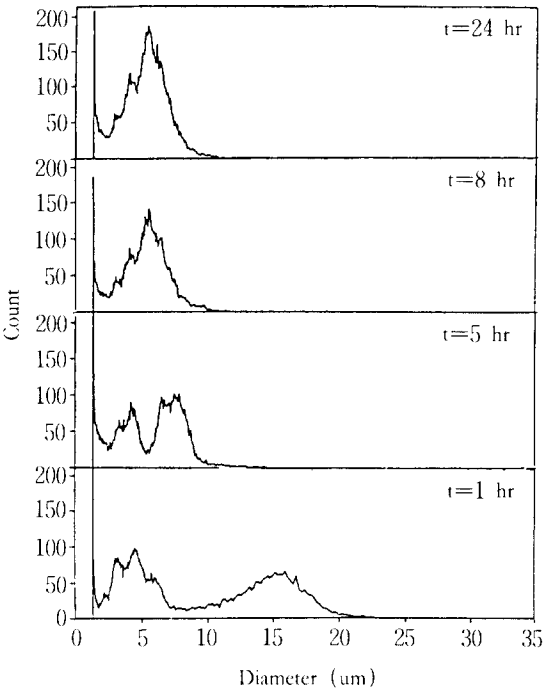


Fig. 5. Particle size distribution Run 2 (Dark) at pH 3.

Table 3. Statistical analysis of the yeast size distribution data in figures 4 and 5

| Point Number | Time 〈Hrs.〉 | RUN 1 Avg D 〈μm〉 | RUN 2 Avg D 〈μm〉 | RUN 1 Std Dev 〈μm〉 | RUN 2 Std Dev 〈μm〉 | RUN 1 Mode 〈μm〉 | RUN 2 Mode 〈μm〉 | RUN 1 Median 〈μm〉 | RUN 2 Median 〈μm〉 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 6.6559 | 4.6973 | 4.7165 | 1.8254 | 4.48 | 4.96 | 4.5768 | 4.8005 |
| 2 | 5 | 4.9984 | 4.9677 | 2.2450 | 2.0108 | 7.43 | 6.55 | 4.4335 | 5.0251 |
| 3 | 8.5 | 4.8130 | 5.1747 | 1.6218 | 2.1880 | 5.48 | 5.21 | 4.8748 | 5.0500 |
| 4 | 25 | 4.7449 | 4.9394 | 1.5948 | 2.0954 | 5.42 | 4.96 | 4.8020 | 4.8555 |

1=dark 2=light

metabolites in the fermentation broth resulting from the different response of the yeast to light over the course of the fermentation).

The most significant differences due to light seem to be for the first three hours of the pH = 3 runs (Figure 2), where the redox probe drift is the same and can therefore be neglected. It is emphasized here that while the t = 0 hour point for the light run is difficult to see on Figure 2, it is at zero as is the initial dark run point.

## Size Distribution

Figures 4 and 5 show size distributions for yeast cells for light and dark runs at a pH of 3. There are observable differences between the light and dark runs, but it is not obvious how to characterize them. For the light fermentation (run 1) there seems to be a leftward shift of the initial time 5 micron diameter peak to a diameter of ca. 4 microns, at the end of the run. That peak, while not so distinct as in the light run, seems to shift from an initial 5 microns to ca. 6 microns at the end of the run. This seems to indicate that, for a pH of 3 at late times, the cells may be smaller in the light than in the dark runs.

Statistical analysis of the data in Figures 4 and 5 is summarized in Table 3. The Average Particle Diameter as a function of time is plotted in Figure 6, confirming the observation of the drop in average yeast size as the fermentation proceeded for the Run 1(light run) and the rise in average yeast size for Run 2(dark run). Unfortunately, the standard deviations of these yeast average diameters are so large (given in Table 3) that no strong statistical statement can be made at each time point distinguishing these diameters. Similarly, the Mode Particle Diameter as a function of time in exhibited in Figure 7. In this case, Run 1 (light run) has a higher diameter than Run 2 (dark run), following the early time point. The median values, also given in Table 3, consistently

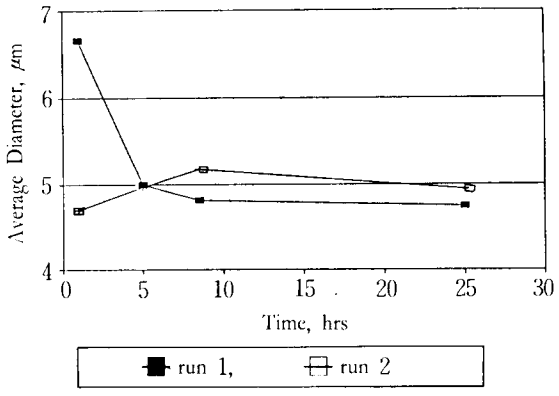


Fig. 6. Average particle diameter over the fermentation time course.

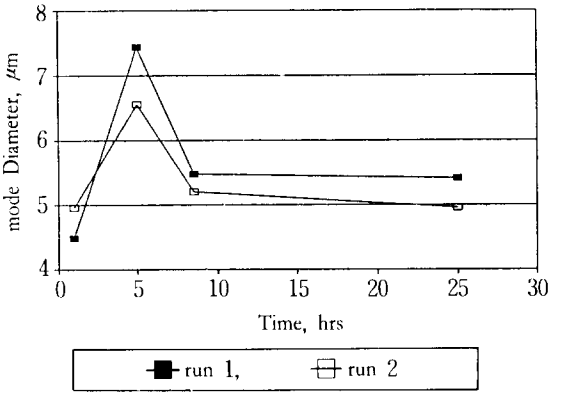


Fig. 7. Mode particle diameter over the fermentation time course.

show the diameters for the lighted runs to be less than the non-lighted run at every point, again exhibiting another way of distinguishing the light from the dark run, but with the resulting diameters closer together.

## CONCLUSIONS AND RECOMMENDATIONS

Differences in measured yeast cell growth and extracellular protein concentration profiles do not seem to be statistically different for the lighted and non-lighted Baker's yeast batch fermentations for the constant pH cases of 3 and 5.

Since the Bradford Coomassie Blue Protein Assay, employed in this study, only detects total extracellular protein concentrations, a method which measures greater resolution, such as gel electrophoresis, may perhaps reveal differences between light and dark runs through differences in individual protein levels.

The differences in the redox probe measurements for light and dark runs, particularly for early times at pH 3, suggest that the yeast cells do undergo changes in metabolite in response to the light. Perhaps light alters the cell wall or membrane permeability to the early time uptake of glucose, a metabolite which strongly affects the redox potential in the 0 to 100 g / L concentration range.

In general, the redox probe can be used to monitor the general progress of a fermentation, and it may reveal subtle changes due to variables not usually monitored, such as light, particularly in a lengthy industrial application.

For the fixed pH 3 runs, there appears to be an increase in the average particle diameter during the course of the fermentation for the dark run, and a decrease in the light run, although at any given time the diameters are within one standard deviation of each other. Because the effect of light on yeast leads to differences which fall within statistical variation, finer measurements and more time course measurements of early times are suggested for future studies to clarify and confirm the differences observed in this early study.

## ACKNOWLEDGEMENT

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