EFFECT OF DYES ON YEAST FERMENTATION AS INFLUENCED BY HYDROGEN-ION CONCENTRATION

By Esther Adams, instructor in biology, Trenton Junior College, and William J. Robbins, botanist, Missouri Agricultural Experiment Station

INTRODUCTION

The effect of hydrogen-ion concentration on the toxicity of various organic and inorganic compounds has been studied by a number of investigators. In general the results have shown that the toxicity of anions is increased by increasing the hydrogen-ion concentration and that of cations is increased by decreasing the hydrogen-ion concentration, provided the reaction does not affect the solubility of the compound studied. The earlier literature on this subject has been reviewed elsewhere (11). The present investigation is concerned with the influence of hydrogen-ion concentration upon the effect of certain acid and basic dyes on yeast fermentation. Von Euler and Florell (3) in studying the effect of dyes on yeast found that in the presence of 0.05 percent of the acid dye ponceau 3R, fermentation was checked a little more at pH 3.8 than at pH 6.1, and at a 0.5 percent concentration of the dye, fermentation was checked considerably more at pH 2.2 to 2.4 than at pH 6.1 to 6.3. Hydrogen-ion concentration had little effect upon the toxicity of the basic dye, methylene blue. No other investigations on the influence of hydrogen-ion concentration on the toxicity of dyes to yeast have been noted by the authors.

INVESTIGATION

PROCEDURE

The acid dyes, eosin (Schultz no. 512) and rose bengaw (Schultz no. 520); and the basic dyes, dahlia (Schultz no. 451), safranine (Schultz no. 584), and brilliant green (Schultz no. 428) were used (12). A suspension of yeast, Saccharomyces cerevisiae, was prepared by suspending a cake of Fleischmann's yeast in 50 cc of redistilled water. Twelve drops of this suspension were added to each fermentation tube (25 cc of solution). The fermentation tubes were incubated at 32° C. and the percentage of gas produced was measured at intervals by means of the Frost gasometer. Redistilled water and Pyrex glassware were used throughout.

In the first experiment performed, buffer mixtures were prepared by titrating \( \frac{N}{40} \) Na\(_2\)HPO\(_4\) with \( \frac{M}{10} \) H\(_3\)PO\(_4\) and diluting to constant volume so that the final solutions contained \( \frac{N}{50} \) sodium. To these buffer mixtures glucose sufficient to make the concentration 4 percent was added. A uniform dilution of the dye studied was added to the various buffer mixtures, which were placed in fermentation tubes, inoculated with yeast, incubated at 32° C., and the gas production noted. By following this procedure it was found that initial reactions of from pH 4.3 to pH 8.0 had little effect upon the amount of gas.
produced after 8, 20, and 34 hours in the buffer mixtures containing sugar but no dye. Some retardation of fermentation was observed at pH 3.1 and 3.6 and at pH 8.8. These results agree in general with those of Von Euler and Heintze (4, 5), who found little difference in fermentation at reactions of from pH 4.0 to pH 7.0, and with those of Hägglund and Rosengvist (6), who found that fermentation with yeast juice proceeded with practically the same velocity between pH 5.5 and pH 8.0.

If rose bengale at a dilution of 1 to 100 or 1 to 1,000 is added to the buffer mixtures containing glucose the results are as shown in figure 1. The toxicity of this acid dye is markedly increased at original pH values of 6.0 and lower. However, when this method of determining the influence of hydrogen-ion concentration on the toxicity of dyes to yeast is used, the production of carbon dioxide and other fermentation products affects the reaction of the buffer mixtures. Those solutions more alkaline than about pH 5.0 become more acid; whereas those solutions more acid than pH 5.0 are unaffected or become less acid. The original pH of the buffer mixtures does not represent, therefore, the reaction to which the yeast is subjected during the entire period of incubation.

To avoid this difficulty the following procedure was used: The yeast was exposed in 150 cc Erlenmeyer flasks for 24 hours at room temperature to the dye at various hydrogen-ion concentrations in the sodium phosphate buffer mixtures which contained no sugar. At the end of 24 hours the dye and buffer mixtures were decanted and replaced by a nutrient solution composed of KH$_2$PO$_4$, 0.1 g; NH$_4$NO$_3$, 0.1 g; autolyzed yeast, 5 cc of 1-percent extract; glucose, 60 g; redistilled water, 1,000 cc. The yeast and nutrient solution were transferred to fermentation tubes and incubated at 32° C. In this case the yeast was exposed to the dye at various hydrogen-ion concentrations, and since no fermentation took place during this exposure little change in reaction occurred. Fermentation by the treated yeast occurred at the same pH value, that of the nutrient solution, the pH of which was 5.9. By using this procedure the following results were secured.

**EOSIN**

Sodium phosphate buffer mixtures with the sodium constant at $\frac{N}{50}$ and ranging from pH 3.2 to pH 8.4 were prepared. Fifty cc quantities
of the buffer mixtures were placed, with sufficient eosin to make the dilution 1 to 5,000, in 150 cc Erlenmeyer flasks. Twenty-four drops of the standard yeast suspension were added to each flask. After 24 hours at room temperature the yeast had settled to the bottom of the flasks. The supernatant liquid was decanted and the color of the yeast examined. At pH 3.2, 3.6, and 3.9 the yeast was quite red; at pH 4.5, 5.0, and 5.5 lighter red; at pH 6.0, 6.5, 6.9, 7.5, 7.9, and 8.4, faint red with a slight gradation of color; and at pH 8.4 it was lightest in color. Forty cubic centimeters of the nutrient solution containing glucose was then added to each flask and the flask was shaken until all the yeast was in suspension. The contents of each flask were divided between two fermentation tubes, thus making duplicates for each reaction in the series. The tubes were incubated at 32° C. and gas production was measured at intervals. The results at the end of 4½ and 7 hours are shown in figure 2. The toxicity of eosin is greater in the acid solutions, the effect becoming noticeable in solutions more acid than pH 5.0. After 7 hours the fermentation tubes containing yeast exposed to eosin at hydrogen-ion concentrations of from pH 5.0 to pH 7.9 were entirely filled with gas while those containing yeast which had been exposed to eosin at pH 3.2 and 3.5 showed almost no gas production. As was noted with the acid dye, rose bengale, the toxicity of eosin increases with increasing hydrogen-ion concentration. This greater toxicity is correlated with the amount of dye absorbed by the yeast, the greater amounts of dye being absorbed in the more acid solutions.

SAFRANINE

Using the same procedure as before, the yeast was exposed to the basic dye safranine, at a dilution of 1 to 25,000. When the buffer mixtures were decanted the yeast was colored as follows: At pH 3.1 and 3.6, light red; at pH 4.3, 5.1, and 6.0, gradually deeper red; at pH 6.5, 7.1, 8.0, and 8.8, deep red. The production of gas in the nutrient solution after 8 and 20 hours' incubation is shown in figure 3. The basic dye safranine is more injurious with decreasing hydrogen-ion concentration, the injurious effect under the conditions of the experiment becoming noticeable at reactions more alkaline than pH 6.0. Observations of the fermentation tubes at the end of 20 hours showed that at pH 3.1, 3.6, 4.3, 5.1, and 6.0 the yeast was grow
and the contents of the tubes were cloudy as a result; little or no growth was evident at pH 6.5, 7.1, 8.0, and 8.8. Dilutions of safranine, 1 to 50,000, 1 to 100,000, and 1 to 500,000, also were used. Safranine at these dilutions for the period of exposure employed was not particularly toxic to yeast, and no differences in gas production by yeast exposed to the dye at various hydrogen-ion concentrations were noted.

**DAHLIA**

The basic dye dahlia was used at a dilution of 1 to 50,000. When the buffer mixtures were decanted, the yeast was deeply stained at all reactions. The gas production in the nutrient solution was observed after 8 and 20 hours; the results after 20 hours’ incubation are shown in figure 4. The effect of hydrogen-ion concentration on the toxicity of dahlia to yeast is slight, much less than on that of safranine, eosin, or rose bengale. Dahlia is somewhat less toxic at pH 3.2 and 3.6 than at more alkaline reactions. Similar results were secured with dahlia at a dilution of 1 to 100,000.

**BRILLIANT GREEN**

The basic dye brilliant green was used at dilutions of 1 to 10,000 and 1 to 5,000. When the dye and buffer mixtures were decanted from the yeast exposed to brilliant green at a dilution of 1 to 10,000, the yeast was stained as follows: At pH 3.1, light green; at pH 3.6 and 4.3, deeper green; at pH 5.1, deepest green; at pH 6.3, lighter green, about the same color as yeast from pH 4.3; at pH 6.5, lighter green; at pH 7.1, 7.5, 8.0, and 8.8, successively lighter in color. The production of gas in the nutrient solution after 5 and 9½ hours by the yeast exposed to brilliant green, 1 to 10,000, is shown in figure 5. The absorption of the dye by the yeast at the various hydrogen-ion concentrations and the production of gas by the treated yeast, which
is correlated with the amount of dye absorbed, do not agree with the results anticipated for the influence of hydrogen-ion concentration on the effects of a basic dye. Dye absorption and injury increased as the acidity decreased from pH 3.1 to pH 5.1. Further decrease in acidity resulted in a decrease of dye absorption and of toxicity. The resulting curves for gas production show 2 maxima with a minimum at pH 5.1 between the 2 maxima.

The gas production, after 5 and 9½ hours, by yeast exposed for 24 hours to brilliant green, 1 to 5,000, at various hydrogen-ion concentrations is shown in figure 6. The results are similar to those secured with brilliant green, 1 to 10,000. Marked injury is evidenced at somewhat more acid reactions than with the 1 to 10,000 dilution and the reduction in toxicity in the more alkaline reactions of the series is less marked.

The behavior of brilliant green is due in part to the precipitation of the dye in the more alkaline solutions. When sufficient dye to make a dilution of 1 to 5,000 was added to buffer mixtures in test tubes a precipitate was observed at pH 5.5, 6.0, 6.5, 6.9, 7.5, 7.9, and 8.4.

The effect of hydrogen-ion concentration upon the toxicity of brilliant green to yeast is the result of the influence of reaction on the toxicity of the basic dye, which is increased by decreasing hydrogen-ion concentration, and the result of the influence of reaction on the solubility of the dye, which is decreased by decreasing hydrogen-ion
As the hydrogen-ion concentration is decreased from pH 3.1, the basic dye, brilliant green, becomes more toxic. In the vicinity of pH 5.5, however, the dye is precipitated. As a result further decrease in hydrogen-ion concentration decreases the toxicity of the dye. This probably explains the results reported by Stearn and Stearn (16, pp. 349-350) on the influence of hydrogen-ion concentration upon the toxicity of brilliant green to bacteria.

**DISCUSSION**

The experiments reported in this paper show that hydrogen-ion concentration is a factor which cannot be ignored in a study of the toxicity of compounds to living organisms. When hydrogen-ion concentration does not affect the solubility of the dye within the range of reactions studied, increasing hydrogen-ion concentration increases the toxicity of acid dyes, and decreasing hydrogen-ion concentration increases that of basic dyes. Since for the acid dyes the toxic ion is the anion and for the basic dye it is the cation, the influence of hydrogen-ion concentration upon the toxicity of acid and basic dyes agrees with the generality stated at the beginning of this paper, namely, that the toxicity of anions is increased by increasing hydrogen-ion concentration and that of cations is increased by decreasing hydrogen-ion concentration. The degree to which hydrogen-ion concentration affects toxicity varies with the dye, as is shown by the differences found between dahlia and the other dyes used.

The effect of hydrogen-ion concentration upon toxicity has been variously ascribed to its influence upon the organism by changing its permeability (1) or the combining capacity of its constituents for anions and cations (11, 13, 14, 15), or some other property of the cell (10, 7); to its influence upon the toxic substance by affecting its surface tension (17, 18), dissociation (1), solubility (9), partition coefficient (9), or formation of free acid or free base (2, 8); to a combination of effects upon the cell and upon the toxic substance (1). Until further evidence is at hand the simplest and most reasonable explanation for the effect of hydrogen-ion concentration upon the toxicity of acid and basic dyes is to assume that the most toxic form of the dye is the free base or free acid and that hydrogen-ion concentration affects the toxicity through its influence upon the production of free base and free acid.

**SUMMARY**

The influence of hydrogen-ion concentrations, ranging from about pH 3.0 to about pH 8.0, on the effect of rose bengale, eosin, safranine, dahlia, and brilliant green on yeast fermentation was studied.

Under the conditions of the experiments the toxicity of rose bengale and of eosin was greater in the more acid solutions, the effect becoming noticeable in solutions more acid than pH 6.0 and pH 5.0, respectively; the toxicity of safranine was greater in the less acid solutions, the injurious effect becoming noticeable at reactions more alkaline than pH 6.0.

The toxicity of dahlia was least in the most acid solutions used. However, the influence of hydrogen-ion concentration on the toxicity of this dye was not marked.

The toxicity of brilliant green under the conditions of the experiments increased with decrease in hydrogen-ion concentration up to about pH 5.5, where precipitation of the dye occurred.
In general increasing hydrogen-ion concentration increased the toxicity of the acid dyes, and decreasing hydrogen-ion concentration increased that of the basic dyes.

LITERATURE CITED

(1) Bonacorsi, L.

(2) Crane, M. M.

(3) Eulier, H. v., and Florell, N.

(4) ——— and Heintze, S.
    1919. über die pH empfindlichkeit der hefegärung. Arkiv Kemi, Min. och Geol. 7, no. 21, 21 pp.

(5) ——— and Heintze, S.

(6) Hägglund, E., and Rosengvist, T.

(7) Kligler, I. J.

(8) Michaelis, L., and Dernby, K. G.

(9) Pribram, E.

(10) Prowazek, S. V.
    1910. giftwirkung und protozoenplasma. Arch. Protistenk. 18: [221]-244, illus.

(11) Robbins, W. J.
    1926. the isoelectric point for plant tissues and its importance in absorption and toxicity. Mo. Univ. Studies 1: 2-60.

(12) Schultz, G., and Julius, P.

(13) Scott, I. T.

(14) Simon, C. E., and Wood, M. A.

(15) Stearn, A. E., and Stearn, E. W.
    1924. the chemical mechanism of bacterial behaviour. iii. the problem of bacteriosis. Jour. Bact. 9: 491-570, illus.

(16) Stearn, E. W., and Stearn, A. E.

(17) Traube, J.

(18) ———