

QUANTITATIVE ADSORPTION OF METHYLENE BLUE BY DEAD YEAST CELLS¹

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Quantitative studies of dye adsorption by bacteria have been made (Kennedy and Barbaro, 1953; Barbaro and Kennedy, 1954; Kennedy and Woodhour, 1956; Barbaro *et al.*, 1956) in order to measure the degree of gram-positiveness, and to study the influence of several factors on the quantity of dye retained by the microorganisms.

No attention has been given, however, to the applicability of the physicochemical adsorption law in the cases studied.

This report is divided into three parts. The first part shows that the adsorption of methylene blue by dead yeast cells follows the well known Freundlich law. The second part shows that it is possible to make very precise determinations of total yeast concentration by the measurement of the adsorption of methylene blue. The third part shows the possibility of determining the percentage of dead cells by a new colorimetric method.

Freundlich's law. The classic form of the physicochemical adsorption law (Freundlich's law) is (Glasstone, 1943):

$$\frac{x}{m} = kc^n \quad (1)$$

where x is the mass of the adsorbed substance, m is the mass of the adsorbing material, and c is the equilibrium concentration of the solution.

If we assume that Freundlich's law can be applied to the methylene blue adsorption by dead yeast cells, equation 1 will give

$$\frac{C_i - C_f}{C_f^n} = KPC \quad (2)$$

Where C_i is the initial methylene blue concentration, C_f is the methylene blue concentration at the equilibrium point, C is the total yeast cell concentration, and P is the percentage of dead yeast cells; K is a constant that probably

depends upon the temperature, the strain of microorganism, and the dye used.

MATERIALS AND METHODS

Saccharomyces cerevisiae (Standard Brands of Brazil, Inc., and Usina Itaiquara) was used in the experiments. The number of dead cells in the pressed yeast was determined by the methylene blue method (Jørgensen, 1948). Total yeast concentrations were measured in grams of dry matter per liter of suspension (White, 1954b).

Stock yeast suspensions for each experiment were prepared in the following way: a known mass of pressed yeast was mixed with distilled water and agitated for 20 min to disperse the aggregated cells; when a dead cell suspension was desired, the cells were killed by boiling for 10 min; after cooling, the suspension was diluted with distilled water to the desired volume in order to give a known total yeast concentration.

From the stock suspensions of dead or live cells, suitable volumes were pipetted and mixed together in order to prepare suspensions of different dead cells; methylene blue solution then was added to the mixtures and the volumes were diluted with distilled water in order to give known total cell concentrations. The mixtures were agitated at a given temperature for a given time, and then centrifuged at 4500 to 5000 rpm for 20 to 30 min to separate the cells.

The methylene blue concentrations were measured colorimetrically (Coleman Junior Spectrophotometer) at 440 μ .

When necessary, the number of living yeast cells was determined by the plating method (White, 1954a).

The numerical equations presented in this report were derived by the application of the least squares method to the experimental values.

RESULTS

The experiments carried out with suspensions of dead cells, show that Freundlich's law of

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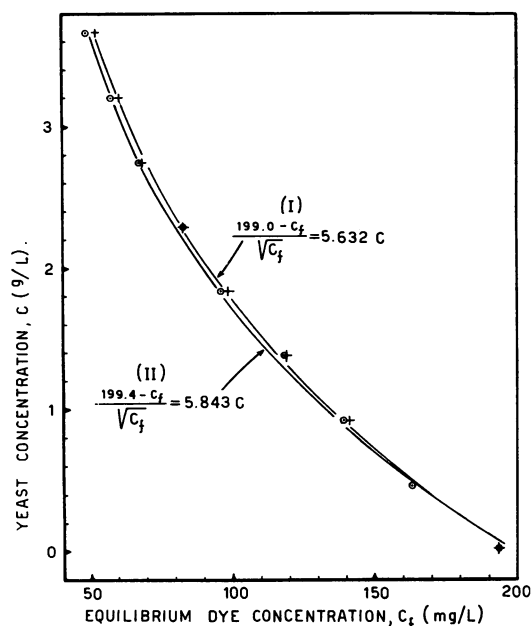


Figure 1. Relation between yeast concentration and equilibrium dye concentration. Experiments carried out with dead cell suspensions, at 30 C, with 30 min of agitation (Curve I: experimental points, +), and with 90 min of agitation (Curve II: experimental points, O).

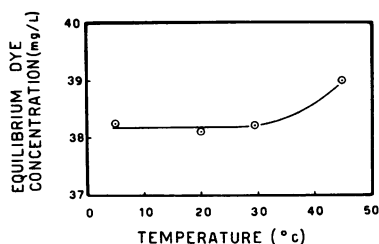


Figure 2. Influence of temperature on the equilibrium dye concentration. Each point is the average of two measurements. Initial dye concentration = 114.8 mg per L. Yeast concentration = 3.22 g per L.

physicochemical adsorption is obeyed if the total yeast concentration is not greater than about 3.5 g per L, and if the initial methylene blue concentration is 100 to 200 mg per L. Figure 1 shows the results of a typical experiment, and also that the results obtained with 30 min and with 90 min of agitation are practically the same. Figure 2 shows that the variation of temperature in the interval 5 to 30 C does not affect the results. Three experiments were carried out with

TABLE 1
Experimental error affecting measurements with dead cell suspensions

Yeast Concentration, C	Initial Dye Concentration, C _i	Equilibrium Dye Concentration, C _f *	Per Cent Standard Deviation of:	
			Measured C _f values	Calculated C values
g/L	mg/L	mg/L		
3.13	167.4	69.4	1.7	2.2
1.55	152.7	94.9	1.7	3.9
0.308	189.7	167.0	1.3	9.7

* Average of eight independent determinations.

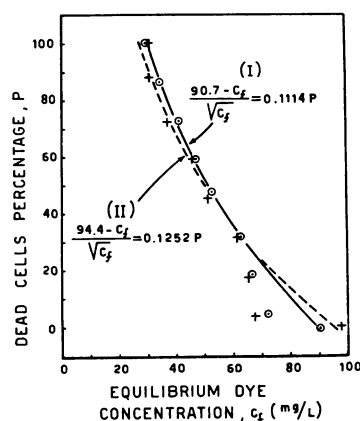


Figure 3. Relation between the percentage of dead yeast cells and the equilibrium dye concentration. Curve I (experimental points, O): initial dye concentration = 90.3 mg per L; yeast concentration = 3.12 g per L; temperature = 20 C; time of dyeing = 4 min. Curve II (experimental points, +): initial dye concentration = 97.9 mg per L; yeast concentration = 3.17 g per L; temperature = 20 C; time of dyeing = 30 min.

different total yeast concentrations in order to evaluate the experimental error; eight independent measurements were made from each suspension, giving the values shown in table 1.

Figure 3 shows the results obtained in typical experiments carried out with mixtures of dead and live yeast cells. In these cases Freundlich's law is followed only if the percentage of dead cells is greater than about 40 per cent. The fact that the live cells also adsorb the methylene blue, as shown in figure 4, explains the observed deviations from the adsorption law. Three experiments were carried out with different percentages of dead cells in order to evaluate the experimental error; eight independent measure-

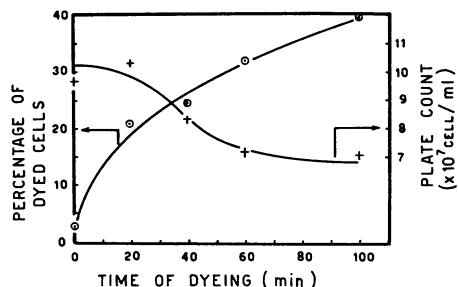


Figure 4. Variation of plate count and of the percentage of dyed cells with the time of dyeing. Experiments carried out with suspensions of initially live cells. Yeast concentration = 3.2 g per L. Initial dye concentration = 200 mg per L. Temperature = 20 C.

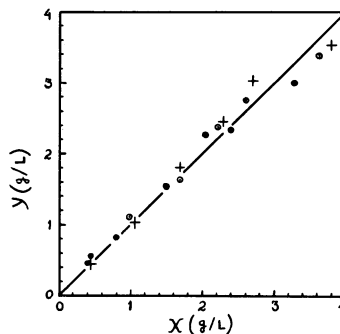


Figure 5. Relation between the yeast concentration calculated by the colorimetric method (Y) and the real yeast concentration (X).

TABLE 2

Experimental error affecting measurements with mixtures of dead and live cells

Dead Cells Quantity, P	Yeast Concentration, C	Initial Dye Concentration, C_i	Equilibrium Dye Concentration, C_f^*	Per Cent Standard Deviation of:	
				Measured C_f values	Calculated P values
%	g/L	mg/L	mg/L		
53.1	3.11	159.0	96.4	0.83	1.7
71.3	3.09	160.1	74.0	0.40	0.56
90.5	3.04	158.4	55.1	1.3	1.3

* Average of eight independent determinations.

ments were made from each suspension, giving the results of table 2.

The results obtained show that the adsorption of methylene blue by dead yeast cells follows the well known Freundlich law of the physicochemical adsorption with $n = 0.5$. The deviations observed when the experiments were made with mixtures of dead and live cells can be easily explained because the dye is also adsorbed by the live cells.

Colorimetric method for the determination of total yeast concentration. The observed applicability of Freundlich's law to the methylene blue adsorption by dead yeast cells, makes it possible to establish a new method for the determination of the total yeast concentration. This new method is as follows: the yeast suspension is boiled to kill the cells ($P = 100$ per cent); then, after cooling, is mixed with methylene blue solution, and the mixture is diluted with distilled water to a known volume in order to give a methylene

blue concentration between 100 and 200 mg per L and a total yeast concentration not greater than 3.5 g per L; the mixture is agitated for about 10 min at a temperature of 10 to 25 C, then centrifuged at 4500 to 5000 rpm for 20 to 30 min to separate the cells, and the equilibrium dye concentration is measured. A previous determination of the K value (equation 2) can easily be made with a yeast suspension of known concentration. Equation 2 permits calculation of the yeast concentration of the unknown suspension.

Figure 5 shows typical results obtained in experiments carried out to test the colorimetric method described above.

Colorimetric method for the determination of percentage of dead yeast cells. A new colorimetric method for the measurement of the percentage of dead yeast can also be derived from the results obtained in the first part of this report.

This colorimetric method is as follows: the original yeast suspension is divided into two equal volumes; one of these volumes is boiled to kill the cells, and after cooling, the two volumes are mixed together (if P is the percentage of dead cells in the original suspension, $50 + P/2$ will be the percentage of dead cells in the final suspension; the deviations from Freundlich's law observed when the percentage of dead cells is smaller than 40 per cent are then avoided); methylene blue solution is added to the yeast suspension and the mixture is diluted with distilled water to a known volume in order to give a total yeast concentration of about 3 g per L, and a dye concentration of about 100 mg per L; the mixture is agitated 5 min at 10 to 25 C, then centrifuged at 4500 to 5000 rpm for 20 to

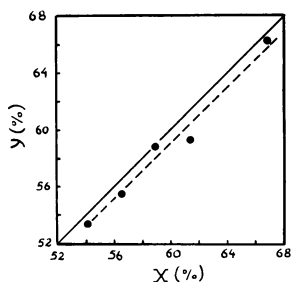


Figure 6. Relation between the percentage of dead cells determined by the proposed method (Y) and the percentage of dead cells determined microscopically (X). Experimental equation ($Y = 0.988X - 0.2$) and theoretical equation ($Y = X$) are represented.

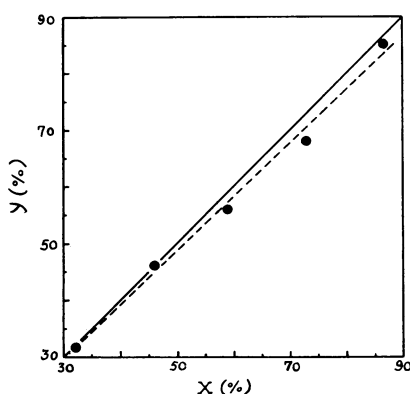


Figure 7. Relation between the percentage of dead cells determined by the proposed method (Y) and the percentage of dead cells determined microscopically (X). Experimental equation ($Y = 0.943X + 1.4$) and theoretical equation ($Y = X$) are represented.

30 min to separate the cells, and the equilibrium dye concentration is measured. Equation 2 then permits calculation of the percentage of dead yeast cells.

Figures 6 and 7 show the results obtained in typical experiments carried out to test the described colorimetric method.

DISCUSSION

The value of K , experimentally determined by the application of Freundlich's law to the methylene blue adsorption by dead yeast cells, seems to be a logical and probably the best measure of the specific adsorption of the dye by a

given strain of yeast. Experiments are being undertaken to verify the applicability of Freundlich's law to the adsorption of crystal violet by bacteria; the values of K , in these cases, will be probably the best measures of the degree of gram-positiveness of the microorganisms.

The proposed colorimetric method for the determination of total yeast concentration is a very simple and precise method with the advantage that it can be applied even with small volumes of a suspension.

The new method for the determination of the percentage of dead yeast cells certainly is more precise than the classic microscopic counting method, and is also a simpler one. The differences observed between the theoretical and the experimental equations (figures 6 and 7) can be easily explained because the error of the microscopic determination of dead cells is a very large one.

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SUMMARY

It was observed that the methylene blue adsorption by dead yeast cells follows Freundlich's law. Deviations were observed when mixtures of dead and live cells were used, because the live cells also adsorb the dye. The experimental errors were measured. New methods were described for determining the total yeast concentration and the percentage of dead cells in a suspension.

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