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The Influence of Relative Dye and Cell Concentrations on the Adsorption Method for the Measurement of the Specific Areas of Microorganisms*

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Abstract

The experimental study of methylene blue and crystal violet adsorption by dead microorganisms (*Saccharomyces cerevisiae*, *Sarcina lutea*, *Serratia marcescens* and *Bacillus subtilis*) leads to the conclusion that unimolecular or multimolecular layers of dyes can be formed, depending on the relative values of the cell and dye concentrations.

Obviously, the adsorption method for specific cell area measurement proposed by Borzani[9~10] can be applied only when unimolecular layers are formed.

When the experimental conditions lead to multimolecular layer adsorption, the applicability of the physico-chemical laws of Freundlich and Lagmuir is only formal. In such cases, the results obtained until now do not make if passible to test the applicability of other adsorption laws.

Introduction

The main purpose of the experimental work carried out in this laboratory on the adsorption of dyes by cells[1~8] is to provide precise methods for the measurement of the specific areas of cells[9~10], and for the evaluation of the percentage of dead cells in a given culture[11~13], in order to try a new approach to the study of the kinetics and scale-up of fermentation processes. Such methods are based on the hypothesis that uniform unimolecular layers are formed when dyes are adsorbed by microorganisms. It is, then, necessary to know the influence of experimental conditions on the adsorption phenomena, in order to be sure that unimolecular layers of the dyes are fixed by the cell surface. In this respect, the purpose of this paper is to show the influence of cell and dye concentrations.

The adsorption laws of Freundlich and of Langmuir, already mentioned in previous reports, are considered in this paper. They may be stated as follows:

$$\text{Freundlich:}\quad (C_i - C_f)/C_f^n = KPC$$

$$\text{Langmuir :}\quad PC/(C_i - C_f) = (b/a) + (1/a)(1/C_f)$$

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where C_i is the initial dye concentration, C_f is the dye concentration at equilibrium, C is the cell concentration, and $100\,P$ is the percentage of dead cells (in our experiments, with 100% dead cells, $P=1$); K, n, a and b are experimental coefficients.

Materials and Methods

Saccharomyces cerevisiae (prepared as pressed yeast by Standard Brands of Brazil, Inc.), *Sarcina lutea*, *Serratia marcescens* and *Bacillus subtilis* (grown on glucose agar) were used in these experiments.

Stock cell suspension for each experiment was prepared as follows. A certain amount of microorganisms was mixed with distilled water, agitated for 30 min to disperse the aggregated cells, and heated (120°C for 15 min) to kill the cells. The dead cells were then washed twice with distilled water. A known mass of the washed microorganisms was suspendend in distilled water in order to give a known cell concentration.

Total cell concentrations were measured in grams of dry matter per liter of suspension.

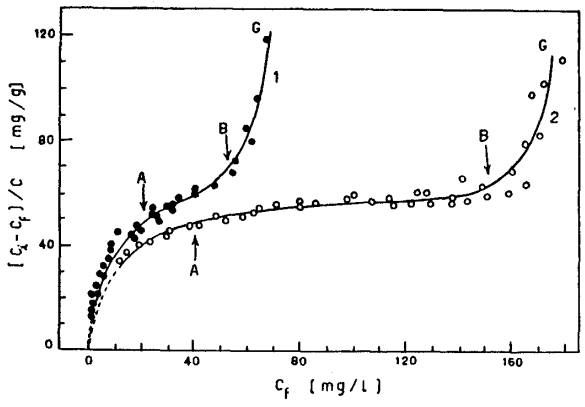
From the stock suspension, suitable volumes were pipetted, dye solution was added, and the volumes were diluted with distilled water to give convenient concentrations of dye and cells.

The mixture were agitated for 60 min to attain equilibrium and centrifuged at 10,000 rpm (9,000 g) for 30 min to separate the cells. Dye concentrations were measured colorimetrically.

The applicability of the physico-chemical laws was verified the least square method.

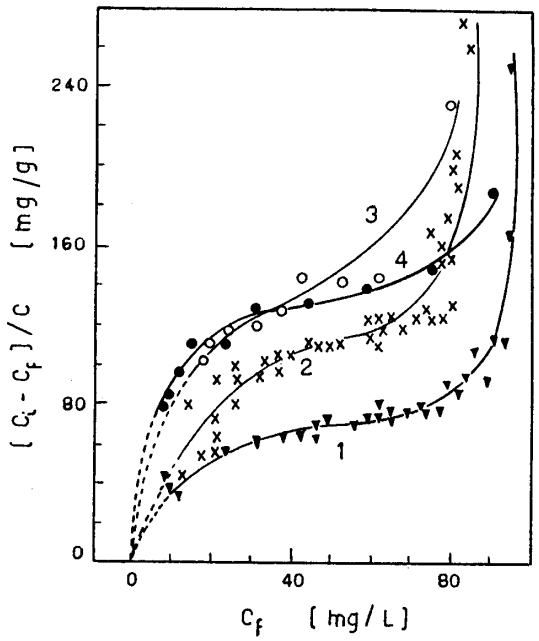
Results and Discussion

Figs. 1, 2 and 3 show several experimental results. The S-shaped curves represented these figures indicate that multimolecular layers can be formed when the ratio between the dye concentration and the cell concentration increases.



C =cell concentration.
C_i=initial dye concentration.
C_f=dye concentration at equilibrium.
Curve 1: C_i=75 mg/l; cell concentrations in g/l varied from 5.168 to 1.236 in interval OA, from 1.236 to 0.325 in interval AB, and from 0.325 to 0 in interval BG.
Curve 2: C_i=188 mg/l; cell concentrations in g/l varied from 5.306 to 3.947 in interval OA, from 3.947 to 1.281 in interval AB, and from 1.281 to 0 in interval BG.

Fig. 1. Adsorption of methylene blue (curve 1) and crystal violet (curve 2) by dead yeast cells.



C =cell concentration.
C_i=initial dye concentration.
C_f=dye concentration at equilibrium.

Curve 1: Methylene blue and *Sarcina lutea* C_i=104 mg/l; C=0.038 to 2.031 g/l.

Curve 2: Crystal violet and *Sarcina lutea* C_i=89 mg/l; C=0.025 to 1.723 g/l.

Curve 3: Crystal violet and *Serratia marcescens* C_i=88 mg/l; C=0.037 to 0.711 g/l.

Curve 4: Methylene blue and *Serratia marcescens* C_i=107 mg/l; C=0.065 to 1.235 g/l.

Fig. 2. Adsorption of dyes by dead bacterial cells.

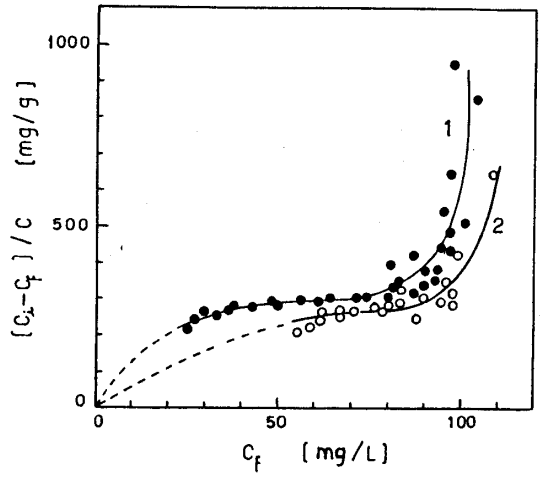


Fig. 3. Adsorption of methylene blue (curve 1) and crystal violet (curve 2) by dead cells of *Bacillus subtilis*.

C =cell concentration.
C_i=initial dye concentration.
C_f=dye concentration at equilibrium.

Curve 1: C_i=106 mg/l; C=0.0042 to 0.383 g/l.

Curve 2: C_i=113 mg/l; C=0.0068 to 0.264 g/l.

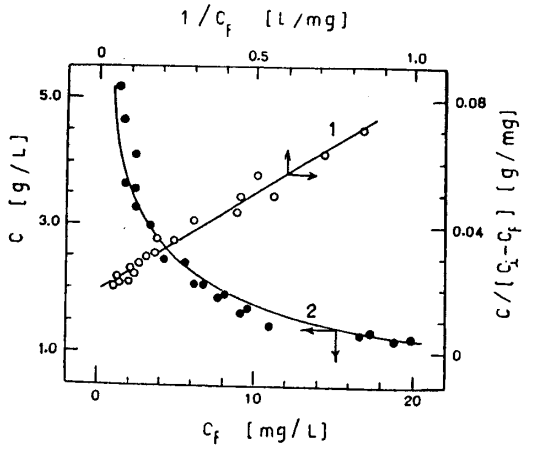


Fig. 4. Applicability of the laws of Langmuir (curve 1) and of Freundlich (curve 2) to the adsorption of methylene blue by dead yeast cells.

C =cell concentration=1.236 to 5.168 g/l.
C_i=initial dye concentration=75m g/l.
C_f=dye concentration at equilibrium.

Curve 1: equation $C/(75-C_f)=0.0200+0.0609/C_f$.

Curve 2: equation $(75-C_f)/C_f^{0.337}=16.69\,C$.

From the origin to point A (Fig. 1) the curves show typical unimolecular layer adsorption; the adsorption laws, especially the Langmuir isotherm, can be applied (Fig. 4), and the specific area of the cells can be calculated.

The interval between points A and B (Fig. 1) indicates the beginning of multi-layer adsorption.

The typical multimolecular layer adsorpion curve begins at point B (Fig. 1). It must be emphasized that, in this last case, the adsorption laws apply only in a formal manner, since $n>1$ in Freundlich's equation, and $(b/a)<0$ in Langmuir's isotherm (Figs. 5 and 6).

The formal applicability of the adsorption laws was already observed in previ-

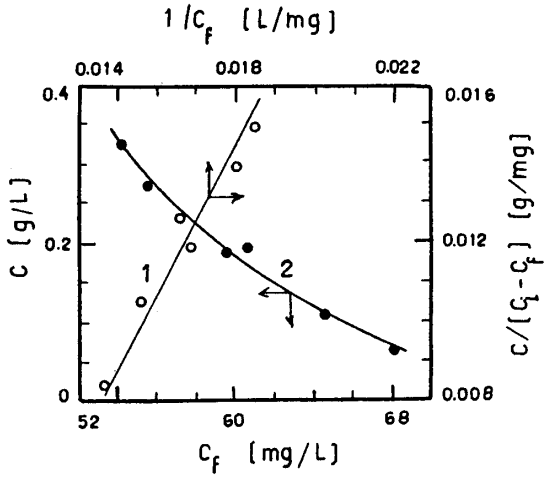


Fig. 5. Formal applicability of the laws of Langmuir (curve 1) and of Freundlich (curve 2) to the adsorption of methylene blue by dead yeast cells.

C =cell concentration$\leq$0.325 g/l.
C_i=initial dye concentration=75 mg/l.
C_f=dye concentration at equilibrium.
Curve 1: equation $C/(75-C_f)$
$=-0.0198+1.906/C_f$.
Curve 2: equation $(75-C_f)/C_f^{2.284}$
$=7.315\times10^{-3}\,C$.

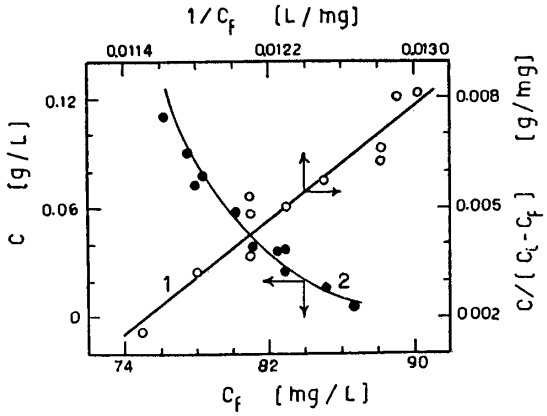
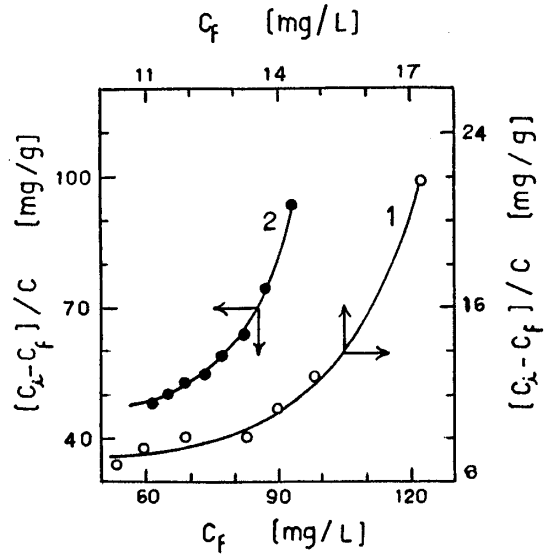


Fig. 6. Formal applicability of the laws of Langmuir (curve 1) and of Freundlich (curve 2) to the adsorption of crystal violet by dead cells of *Sarcina lutea*.

C =cell concentration$\leq$0.111 g/l.
C_i=initial dye concentration=89 mg/l.
C_f=dye concentration at equilibrium.
Curve 1: equation $C/(89-C_f)$
$=-0.0429+3.900/C_f$.
Curve 2: equation $(89-C_f)/10_f^{10.77}$
$=0.5728\times10^{-12}\,C$.



C =cell concentration.
C_i=initial dye concentration.
C_f=dye concentration at equilibrium
Curve 1: C_i=20.4 mg/l;
C=0.141 to 1.125 g/l.
Curve 2: C_i=103.3 mg/l;
C=0.108 to 0.863 g/l.

Fig. 7. Adsorption of methylene blue by live cells of *Saccharomyces cerevisiae* (curve 1) and by dead cells of *Penicillium chrysogenum* (curve 2).

ously published papers regarding the uptake of methylene blue by dead *Penicillium chrysogenum*[4] and by live cells of *Saccharomyces cerevisiae*[8].

No interpretation was then presented, but it may be observed now (Fig. 7) that, even in those cases, multilayer dye adsorption was responsible for the purely formal applicability of Langmuir's law and the consequent impossibility of calculating the specific cell surface.

Acknowledgement

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