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Reduction Intensity of Sterile Bouillon: Dye Reduction Controlled by Electrode Measurements.*

CALVIN B. COULTER.

From the Department of Bacteriology, Columbia University.

The reduction potential which may be attained under anaerobic conditions in sterile bouillon has been reported¹ as reaching an apparent equilibrium at E_H —0.085 to 0.095 volt at pH 7.5.

Dubos² has found, however, that the reduction intensity of sterile broth is sufficient to bring about the reduction of dyes more positive in E_o value than indigo disulfonate, and at least the partial reduction of this dye. This corresponds to a potential considerably more negative than those reported for electrode measurement. Simultaneous measurement of dye reduction and electrode potential is necessary to explain the discrepancy and to throw light on the possible catalytic effect of the dyes. This is the subject of the present paper.

The procedure for measurement of the electrode potentials has been described elsewhere.¹ In the present work a quadrant electrometer was used instead of a galvanometer in order to minimize polarization of the electrodes. Sterility of the bouillon was maintained throughout. The bouillon represented various lots of the infusion medium used in this laboratory for growth of the streptococcus. It was adjusted to pH 7.6, buffered by phosphate in M/10 concentration and autoclaved immediately before use in each experiment. Determination of the final pH was made with the hydrogen electrode at the close of the experiment, in some cases.

The time-potential course of the bouillon without addition of dye was followed first for a period of one or more weeks. The potential was regarded as having attained its equilibrium value when no further negative drift was observed over a period of at least 48 hours. At this point a sufficient amount of the indicator dye in deaerated solution was added through an anaerobic burette to give a distinct color to the bouillon, and the subsequent course of the electrode potentials was followed until again a value was reached which remained constant for 48 hours or longer. Methylene blue, indigo tetra-, di-, and mono-sulphonate, obtained from the La Motte

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¹ Coulter, C. B., *J. Exp. Med.*, 1929, xlix, 711.

² Dubos, R., *J. Exp. Med.*, 1929, xlix, 507.

Chemical Co., were the dyes employed; their concentration in bouillon was between 0.0001 and 0.0002 M.

The apparent equilibrium potentials of the bouillon in this series, before the addition of the indicator, have fallen between E_H —0.110 and —0.0135 volt within the pH range of 7.2 to 7.45. After the addition of the dye, the final electrode potentials have attained E_H —0.135 to —0.150 volt, so that there is evident a distinctly more negative potential attained in the presence of the dye and apparently catalyzed by it. The oxidation reduction system of the bouillon itself, however, determines the behavior of the electrodes since the dyes of increasingly negative E_o values do not occasion a correspondingly greater change in the potential of the bouillon.

All the dyes were completely decolorized except mono-sulphonate and with this dye only a faint greenish tint was observed. The percentage reduction of each of the 4 dyes, calculated for the pH and redox potential found in the bouillon in the corresponding experiment is as follows:

M. B.	100% reduction	
Tetra-sulf.	98	"
Di-sulf.	34	"
	47	"
Mono-sulf.	8	"
	14	"

The slow precipitation of the mono-sulphonate may explain the very faint color present under conditions which should yield only 8 or 14% reduction. The case of the disulphonate is of more importance since the pioneer observations of Smith³ and those of Dubos record reduction of this dye by sterile bouillon. In our experiments the decolorization of the dye has been complete within 48 hours while the observed potentials even at the most negative value attained correspond to only partial reduction. Even under the most rigid protection from oxygen this dye appears to combine with a constituent of the bouillon: the blue color was not restored at the conclusion of the experiments by aeration or the addition of ferri-cyanide. Peculiarities noted by Clark in his study of the disulphonate led this author to suspect the formation of a compound between the reduced form of the dye and other substances. The conditions in our experiments were such as to favor the formation of such a compound.

³ Smith, T., *Cent. f. Bakt., Orig.*, 1896, **xix**, 181.