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A technique to investigate some aspects of the growth kinetics of bacterial colonies on agar media

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Abstract : A simple theoretical model for the growth kinetics of a bacterial colony on agar medium is derived. An optical densitometry scanning technique for the study of the growth of micro-organisms in agar media is presented (the principle of the technique is discussed and an instrument employing the technique is described). The operational features of the instrument and some 'experimental data' obtained with it is also presented. This experimental data was used to test the theoretical model derived. A few potential applications of the technique which can lead to new fields of investigation are mentioned.

1. Introduction

The optical densitometry technique for studying the growth of micro-organisms under varying environmental conditions is largely limited to liquid media. The application of this technique to solid media (e.g. growth of bacterial colonies on agar media) has not (to the knowledge of the authors) been reported.

The quantitative study of the growth of micro-organisms on solid media provides certain types of information that cannot be obtained from liquid media studies and hence could lead to new areas of investigation. In a liquid medium, for example, the cells are usually distributed homogeneously and the growth characteristics of the micro-organisms are the same in different parts of the culture with the result that only a gross picture emerges. On solid media, however, colonies of micro-organisms show characteristic morphological patterns, like the differential distribution of particle density within a bacterial colony and differing growth rates at different regions of the colony.

The techniques of studying colony growth on solid media which are presently available are mainly limited to observations (using microscopes) of shapes and sizes of colonies and their variations with time. The instrument described in this paper provides a quantitative method for the time variation studies of spatial particle density distributions within a colony as well as variations of shape and size of the colony under investigation.

2. Principle of the technique

The intensity of a light beam passing through a medium at a point where the particle density is N, is attenuated from I_0 to I according to the well known law in optics :—

$$I = I_0 \exp(-\alpha N), \quad \alpha = \text{a constant} \tag{1}$$

The fractional light absorption F is given by

$$F = \frac{I_0 - I}{I_0} = 1 - \exp(-\alpha N) \tag{2}$$

Hence

$$N = \frac{1}{\alpha} \ln \frac{1}{1 - F} \tag{3}$$

Where N is the partical density at the region under investigation at time t.

If the probability of cell division per unit time is $P(t)$ and the number of cells at time t is N and at time $t + dt$ is $N + dN$,

then $$P(t)\,dt = \frac{dN}{N} \tag{4}$$

(since the division of a single cell increases the population by one, i.e. its own disappearance and two new cells forming). Due to the environmental resistance to growth (e.g : reduction of nutrients, etc.) the probability of cell division could be expected to decrease as the colony grows and hence the probability of cell division would be both a function of the spatial position within the colony and also of time. Let us *assume* the time dependance to have the form of exponential decay,

$$P(t) = P(0)e^{-\lambda t} \tag{5}$$

Where λ is a constant depending on the nature of the bacteria, the medium and other environmental factors.

Eqns : (4) and (5) yield

$$\int_{N_S}^{N} \frac{dN}{N} = P(0) \int_{\infty}^{t} e^{-\lambda t}\,dt$$

where Ns represents the saturation state of the population.

$$\ln \frac{N}{N_s} = -\frac{P(0)}{\lambda} e^{-\lambda t}$$

let $N_s - N = n$

$$\ln \frac{N}{N_s} = \ln \left(1 - \frac{n}{N_s}\right) = - \left\{\frac{n}{N_s}\right\} - \frac{1}{2}\left\{\frac{n}{N_s}\right\}^2 - \frac{1}{3}\left\{\frac{n}{N_s}\right\}^3 \ldots\ldots\ldots\ldots$$

Since n is always less than N_s and as saturation is approached

$$\frac{n}{N_s} < < 1 \left(\text{as } t \to \infty, \quad N \to N_s\right)$$

$$\text{In } \frac{N}{N_s} \cong - \frac{n}{N_s} = \frac{N - N_s}{N_s}$$

$$\frac{N - N_s}{N_s} = - \frac{P(0)}{\lambda} e^{-\lambda t}$$

$$\text{ie. } N = N_s \left(1 - \frac{P(0)}{\lambda} e^{-\lambda t}\right) \tag{6}$$

Equations (3) and (6) yield,

$$\lambda t = \ln \left\{ \frac{\ln \frac{1}{1 - F_s}}{\ln \frac{1 - F}{1 - F^s}} \right\} \tag{7}$$

A plot of $\left\{ \frac{\ln \frac{1}{1 - F_s}}{\ln \frac{1 - F}{1 - F_s}} \right\}$ with respect to time t on semi-log

graph paper would yield a straight line whose slope with the t axis is λ.

The technique for measuring F for a bacterial colony growing on agar involved allowing a narrow beam of light (thickness of the beam $\lesssim$ 1/10 diameter of the colony under investigation) to fall on a photodetector, after traversing through the point on the colony where the particle density was being estimated. When operating on the linear part of the photodetector characteristic, the photocurrent (i) is proportional to the light intensity (I)

and $$F = \frac{i_s - i}{i_s} \tag{8}$$

In order to obtain the particle distribution within a colony, the colony was moved across so that the beam scanned along a diameter of the colony. The photodetector current was fed into a pen chart recorder which traced out the current corresponding to the spatial distribution of the particle density along the colony.

The study of the time variation of the spatial F distribution was achieved using an automatic periodic scanning device.

3. Instrument

Fig. 1 shows the densitometer cum automatic scanning system. A projection system is incorporated into the instrument so that the colonies that are being scanned could be traced out in two dimensions. In Fig. 2 details of the rotating disc and the glass cell for containing the culture medium are shown. Conventional petri-dishes were not used as their surfaces are not optically homogeneous.

Fig. 3 gives the electrical circuits for the illuminator and detector of the instrument to be operated automatically and with the minimum consumption of recorder chart paper.

4. Procedure

The glass cells to be used in the experiment were sterilised in an autoclave and sterile medium of 1.5% Oxide Nutrient Agar was poured into these cells to fill upto half their thickness. The medium was allowed to set and measured amounts from a very dilute suspension of *Escherichia coli* in phosphate buffer was inoculated with a micropipette onto a central spot on the surface of the medium.

The glass cell thus prepared was placed over an aperture on the rotating disc (Fig. 2) so that the light beam passed through the inoculation point. The surface the medium on which the bacterial suspension was inoculated faced downwards. This was a precaution against water vapour condensing on the lid of the glass cell.

The width of the light beam was adjusted to a fraction of a millimeter. The scanner was made to operate continuously for 48 hours. This represented a scan per each of the eight cells once every hour. At the end of this period, scanning was done for two hours every 12 hours for about two weeks, using an automatic switching device. A calibration spot on one of the glass cells (which did not change with time) was used for monitoring the constancy of the overall densitometer sensitivity.

5. Results

Some samples of chart recorder tracings made in the process of scanning the colony during the growth is shown in Fig. 4. Fig. 5 shows the time variation of the particle density at the centre of the colony and the diameter of the colony. Fig. 6 shows the plot as indicated in equation (8). For the particular example considered $\lambda = 0.17$ day^{-1}.

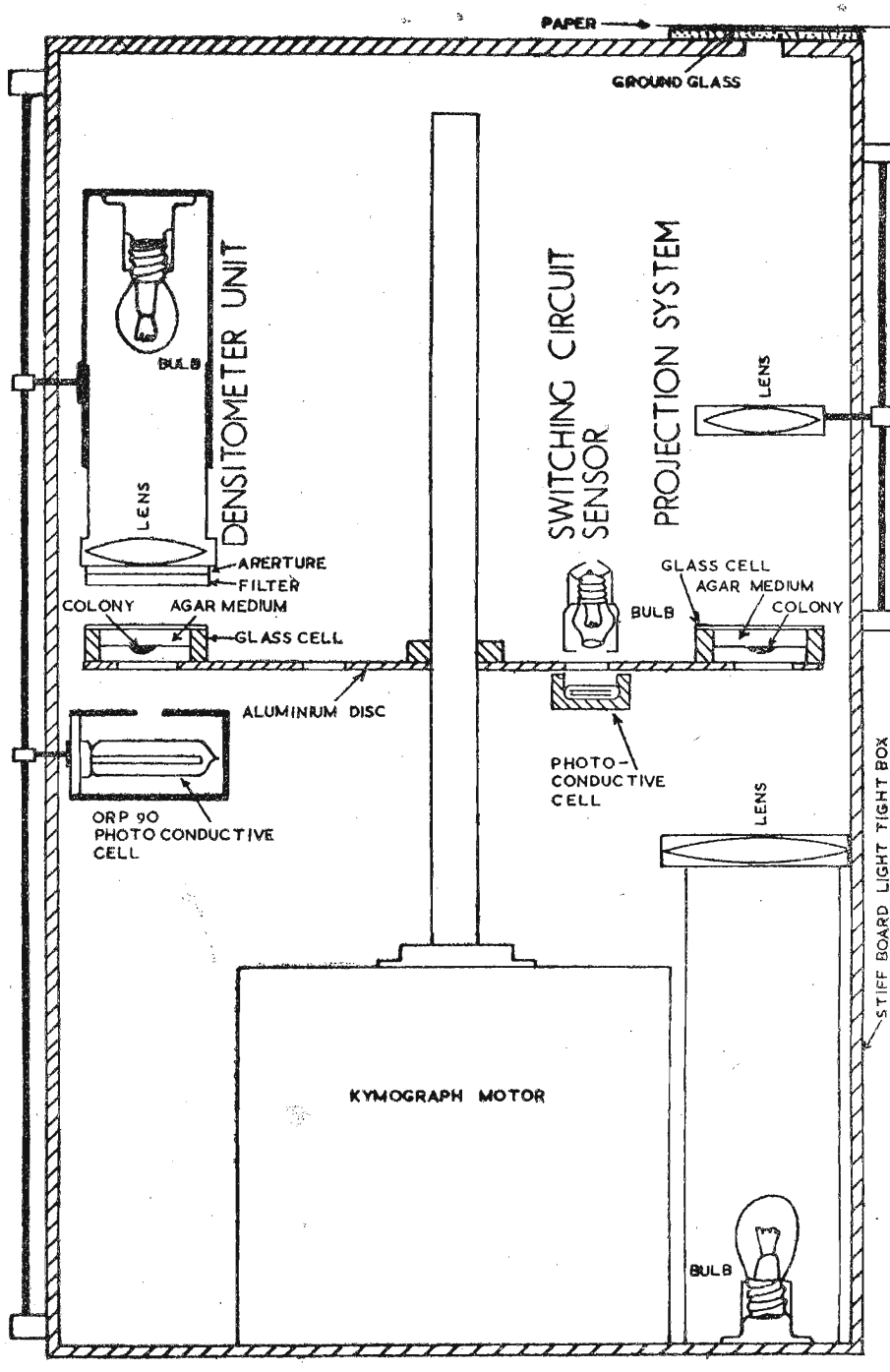

Fig.1 - Densitometer, scanning and projection system

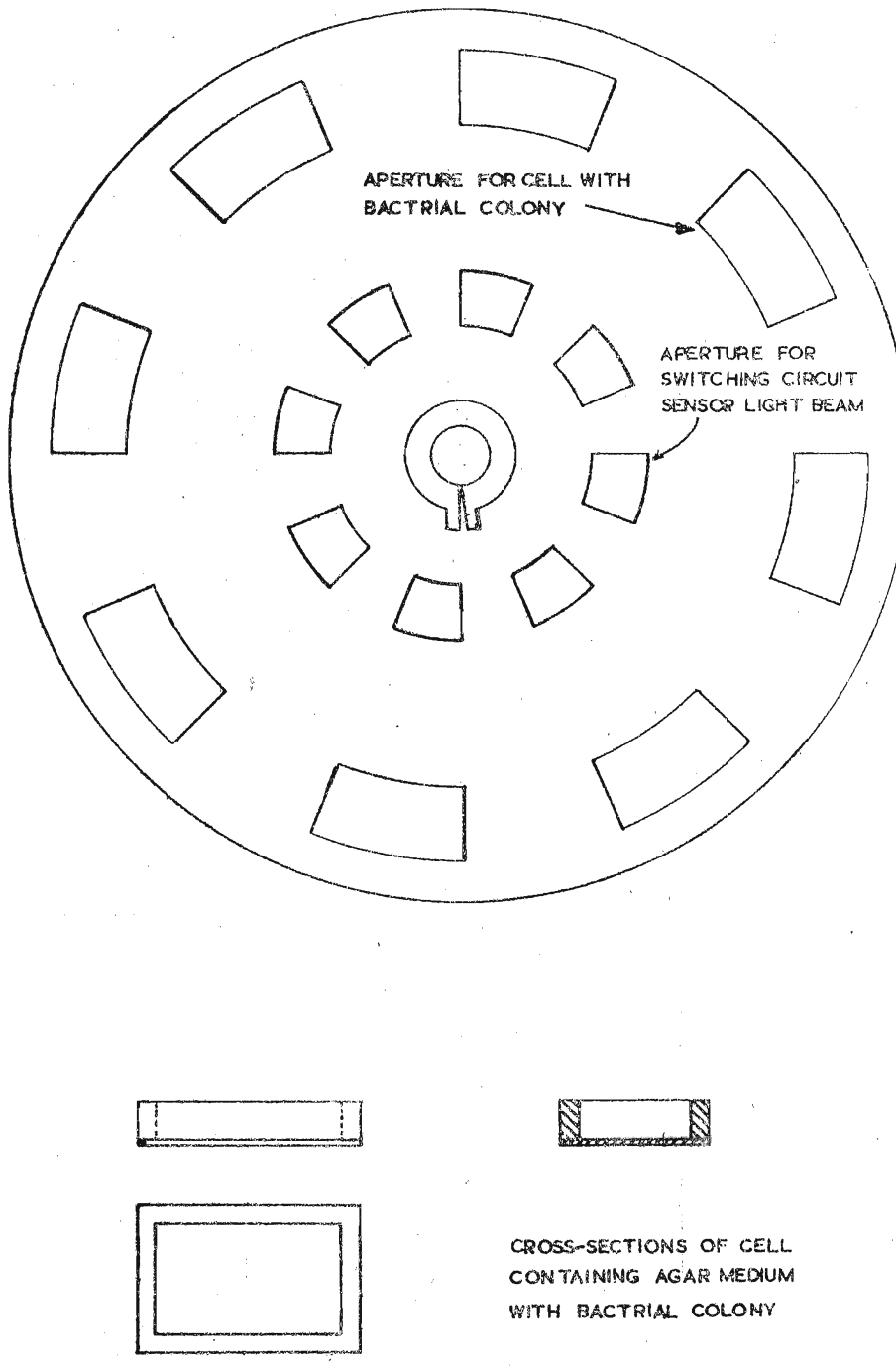

Fig. 2 - The scanner, rotating disc and a glass cell

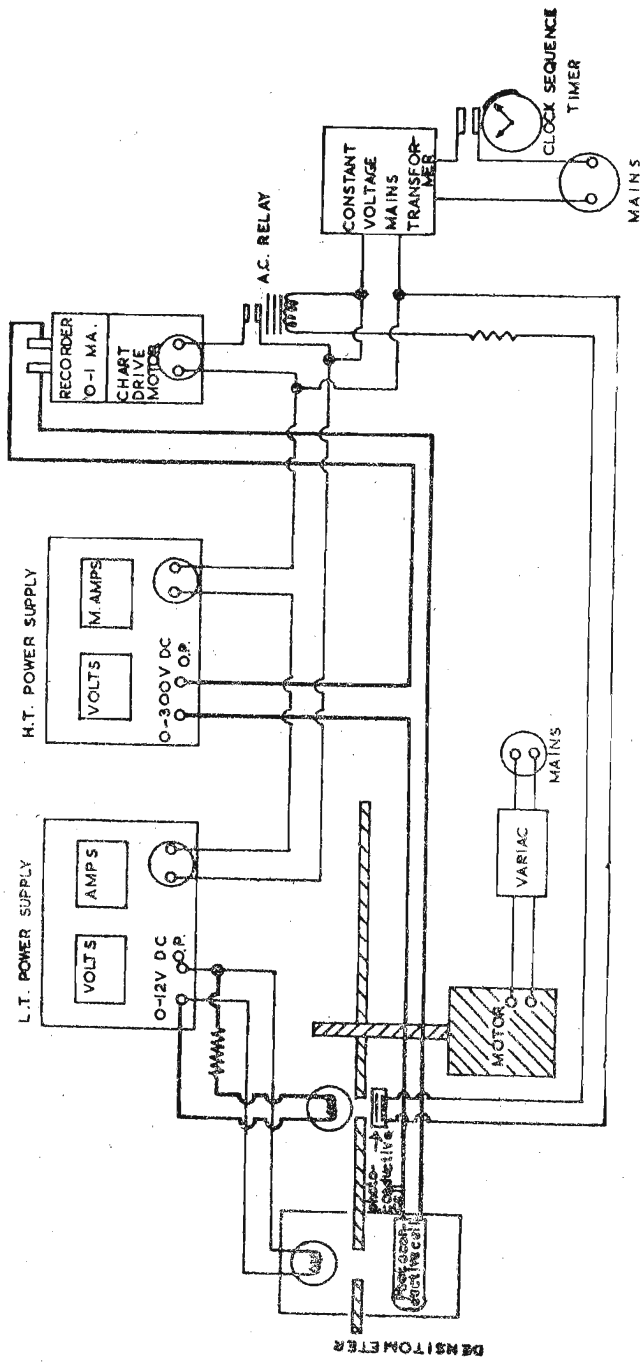

Fig. 3 - Electrical circuits for densitometer and the automatic scanning system

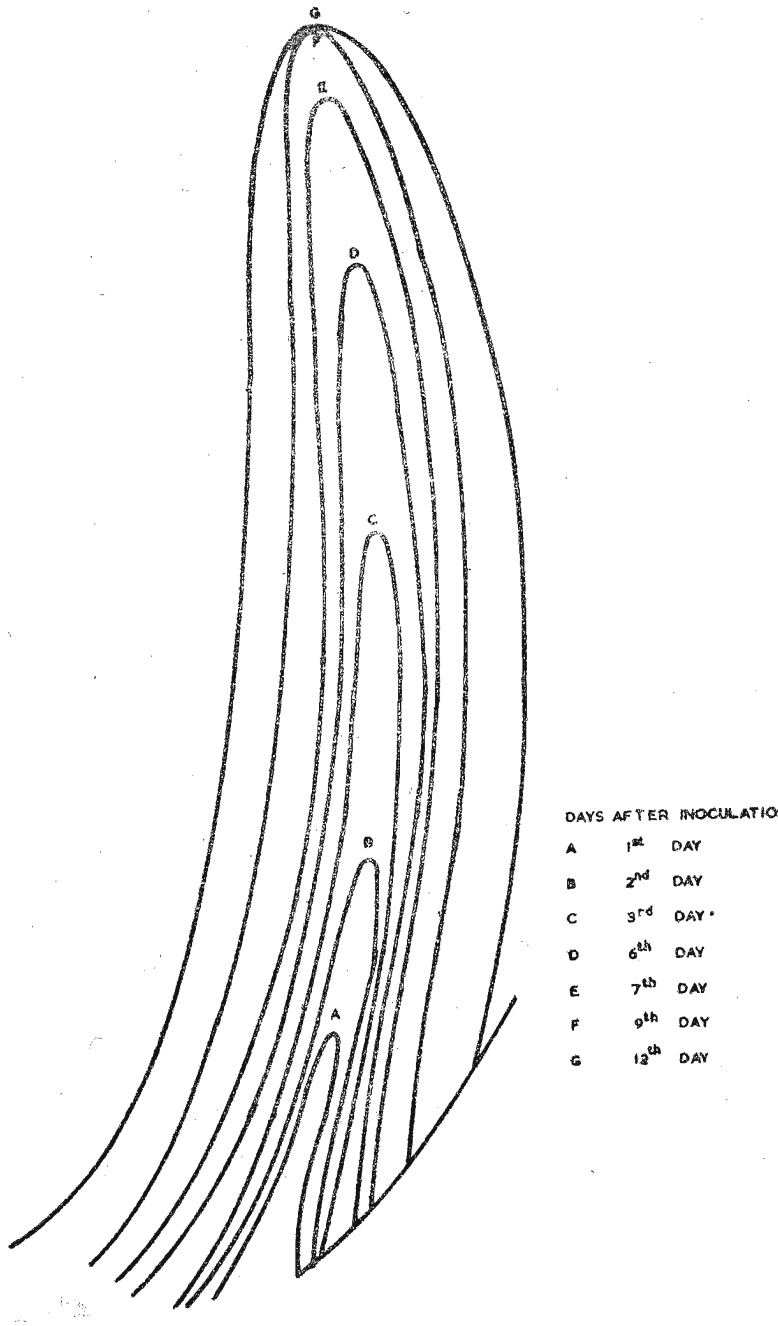

Fig. 4 – Pen chart recorder tracings on scanning
Note: Chart recorder pen introduces a curvature

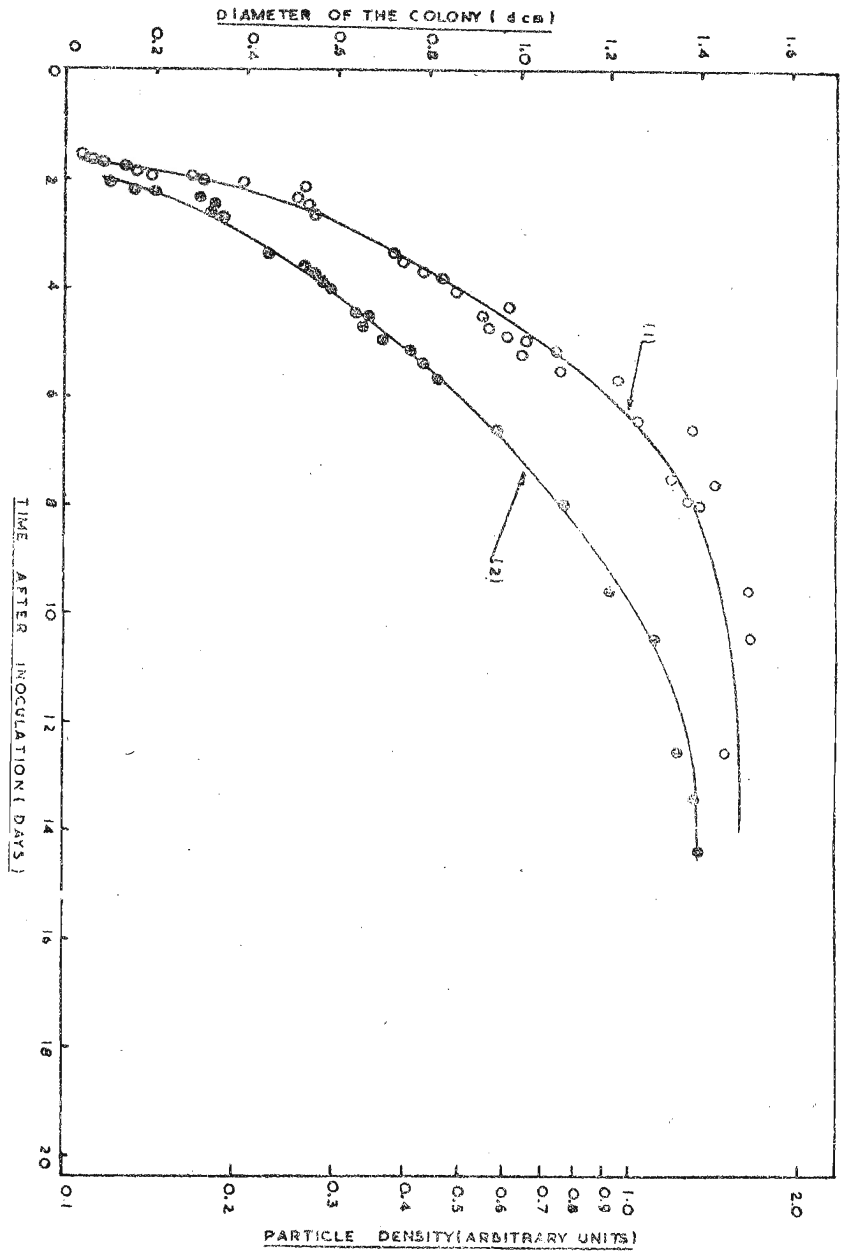

Fig. 5 1 CHANGE OF PARTICLE DENSITY (N) WITH TIME (t) NEAR THE CENTER OF THE COLONY.

2 CHANGE OF DIAMETER (d) OF THE COLONY WITH TIME (t)

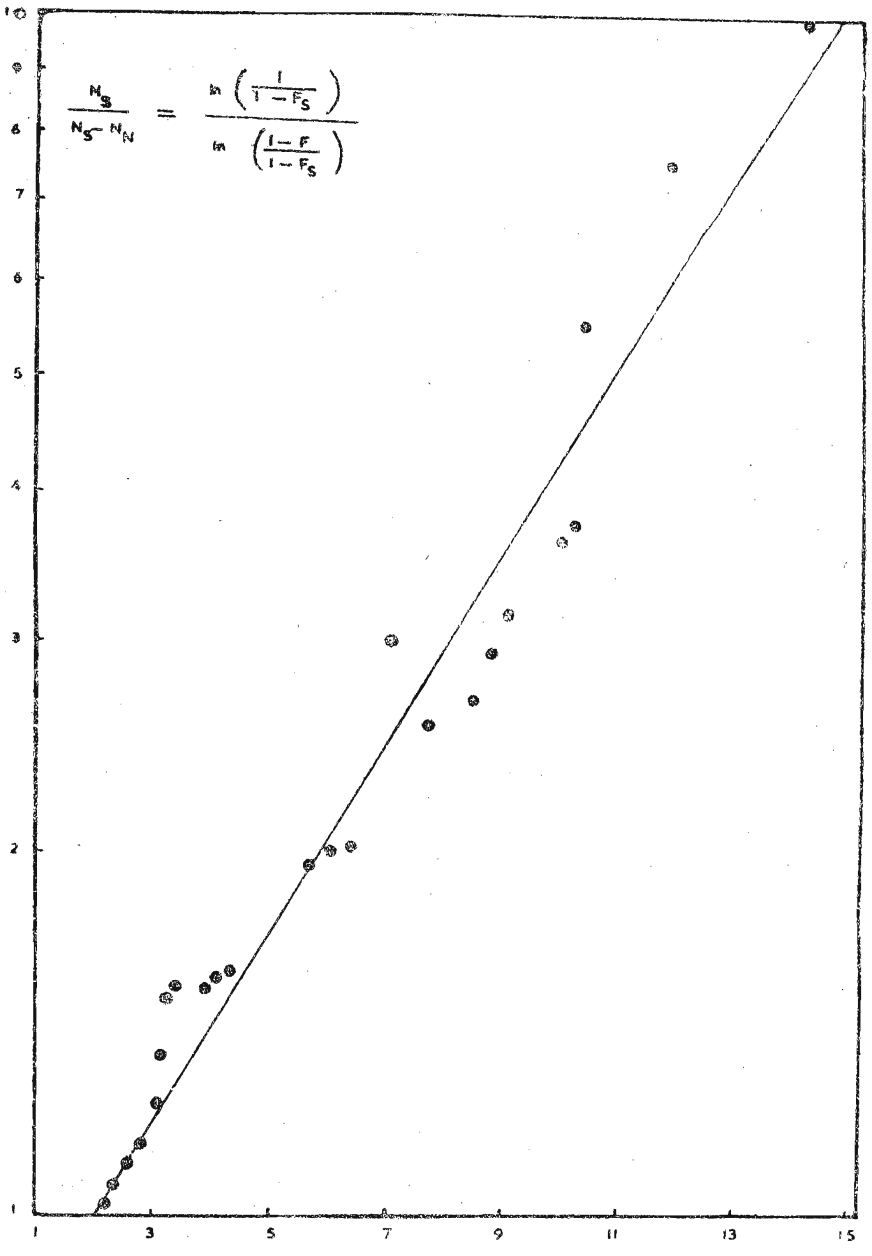

Fig. 6 CHANGE OF THE RECIPROCAL OF THE FRACTIONAL DEVIATION FROM SATURATION $\frac{N_S}{N_S - N}$ WITH TIME (t)

The paper is confined purely to a discussion of the basic technique. The instrument described is inexpensive but possesses a capacity for further sophistication if the problem investigated so demands. It could also be used without modification as an electrophoresis or chromatographic spot scanner and in other applications of densitometry.

The growth constant would be a useful quantitative index of growth. This index could be measured under varying environments and for different organisms.

This technique has potential application in a large variety of studies a few of which are the following :

(1) Kinetic studies of growth of bacterial and fungal colonies.

(2) Differentiation studies of fungal and bacterial colonies.

(3) Growth patterns of colonies in different types of media.

(4) Response of colony growth to different substances e.g. antibiotics, inhibitors, etc.

(5) Growth of bacteriophage plaques in bacterial cultures.

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