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Kinetics of Bacterial Colony Growth by Laser Induced Fluorescence[1]

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Abstract—The growth kinetics of an aerial bacterial colony on solid agar media was studied using laser induced fluorescence technique. Fluorescence quenching of Rhodamin B by the bacterial colony was utilized for the study. The lag phase, log phase, and stationary phase of growth curve of bacterial colony was identified by measuring peak fluorescence intensity of dye doped bacterial colony.

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1. INTRODUCTION

Microbial growth can be defined as an increase in cellular components. It leads to an increase in cell number when microorganisms reproduce by processes like budding or binary fission. Growth also results when cells simply become longer or larger [1]. Investigating colonial growth of micro organism is of considerable importance both in theoretical as well as applied research. There are many ways to measure microbial growth. The most obvious way to determine microbial number is through direct counting using different techniques such as microscopic counts, membrane filter technique, plate count etc. Microbial growth can also be determined by measuring cell mass. The most direct approach for measuring cell mass is the determination of microbial dry weight. However, no single technique is always the best, the most appropriate approach will depend on the experimental situation [1].

The present paper deals with an experimental work in which the growth kinetics of an aerial bacterial colony in a closed system was studied using Laser Induced Fluorescence (LIF) Technique. Laser Induced Fluorescence has proven to be a versatile tool for a myriad of applications. It is a powerful technique for studying molecular interactions in analytical chemistry, biochemistry, cell biology, physiology, nephrology, cardiology, photochemistry, and environmental science. As the theoretical underpinnings of fluorescence became more understood, a more powerful set of applications emerged that yield detailed information about complex molecules and their reaction pathways. For example the quantum efficiency of fluorophores can change as a function of variations in the local environment of the fluorophore molecule like viscosity, temperature, refractive index, pH, calcium and oxygen concentration, electric field, etc. Measuring fluorescence quantum efficiency is one of the experimental techniques to characterize biological samples. Rex et al. [2] utilized laser induced fluorescence to determine NADH in experimental neuroscience using an optical fiber probe. Giorgadze et al. [3] measured degree of abnormality of tissue with the help of LIF. There are many examples in biological applications where LIF technique is applied. In bacteriological studies, the LIF has been shown to be a very sensitive analytical tool to distinguish between the two species of bacteria [4].

Study on growth kinetics of bacterial colony using LIF was carried out by doping Rhodamin B dye in culture medium. Rhodamin B is an appropriate dye for doping because of its high fluorescence quantum efficiency [5, 6]. It was found that the concentration of Rhodamin B at 10^{-4} M was appropriate to give sufficient fluorescence intensity. Higher concentration of the dye effects bacterial growth negatively.

2. SAMPLE PREPARATION

Nutrient agar medium containing 0.3×10^{-4} Rhodamin B dye was used for the study. The nutrient agar were exposed to air for a short duration and then incubated at 25°C at room temperature. The colonies developed on the surface were observed and a circular colony with regular margin was selected for studying the growth kinetics. Figure 1 shows digital photographic images of the dye doped aerial bacterial colony formed on agar plate.

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Fig. 1. Aerial bacterial colony formed on dye doped nutrient agar medium.

3. EXPERIMENTAL SETUP

The selected pure culture was streaked on to fresh nutrient agar plate containing Rhodamin B and the intensity of fluorescent emission was measured. Diode pumped solid state laser of 532 nm (5 mW) with a spot size of 3 mm was used as an excitation source. The power of the laser source was reduced using neutral density filters. The laser was irradiated on to the colony in such a way that it excites the entire colony. A multimode plastic fiber having a core diameter of 980 μm was used to collect the fluorescent emission, which is placed at an angle of 42° with excitation source. The other end of the fiber is coupled to the slit of 0.25 m monochromator-PMT assembly. The size variation of the growing colonies was measured with the help of an eyepiece micrometer of a binocular magnifier. The schematic diagram of the experimental setup is shown in Fig. 2.

4. RESULTS AND DISCUSSION

4.1. Effect of Growth of Bacterial Colony on Fluorescence

The fluorescence spectra of bacterial colony sample marked by Rhodamin B was studied at different days (Fig. 3). As seen in this figure the intensity of emission was reduced with number of days due to fluorescence quenching by bacterial colony. So it is clear that fluorescence of dye is strongly quenched by bacterial colony. This indicates that the cultured bacterium was a gram positive bacterium and it was confirmed with gram staining method. The quenching effect of dye by gram positive bacterium is due to its thick cell wall. It consists of a thick layer of peptidoglycan embedded with teichoic acids. The peptidoglycan is the binding site of dye and the thick layer blocks the dye from further penetrating into the cell. The quenching effect of the dye is due to the effect of teichoic acid [4].

4.2. Growth Kinetics of Bacterial Colony as Inferred from Fluorescent Emission Intensity

4.2.1. Radial growth of bacterial colony as a function of time. To study the growth dynamics of bacterial colony, radius of the colony vs time is plotted (Fig. 4). Radius measurement started after 12 h of incubation. From Fig. 4 it is clear that after 25 h of growth, the colony radius R appeared to increase linearly with time. Radial growth rate of the colony (K_r) is found to be 0.083 mm/h. The value of K_r remains constant as long

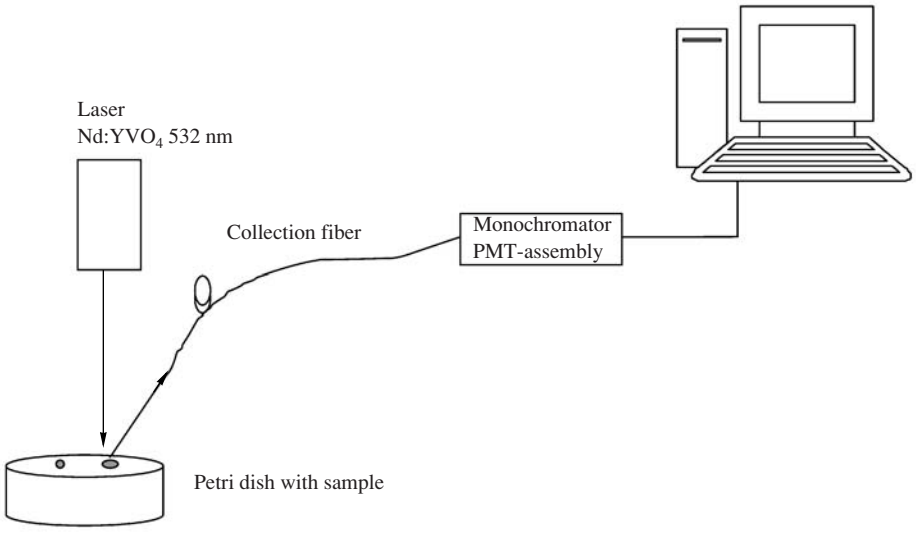

Fig. 2. Experimental setup to study the growth kinetics of bacterial colony by using laser induced fluorescence technique.

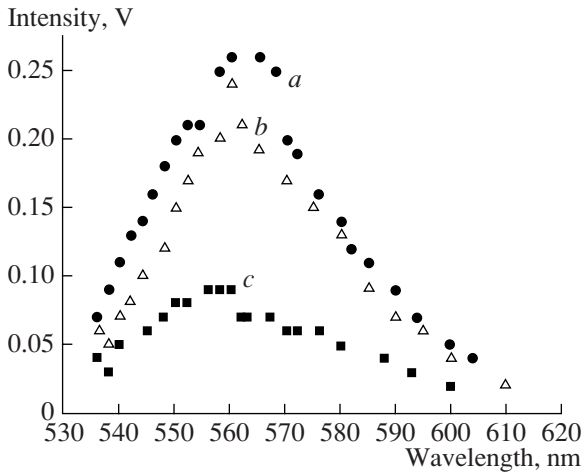

Fig. 3. Fluorescence spectra of bacterial colony marked by Rhodamin B showing quenching effect. (*a*, *b*, *c* represents fluorescence spectra corresponding 4th, 5th, 6th day).

as the growth condition in the peripheral zone do not change. A plot of area of bacterial colony against time is shown in Fig. 5. Here area increases exponentially after 25 h of growth. Rate of exponential growth is calculated to be 0.039 mm^2/h by theoretical fit. This indicates the exponential growth of bacterial population associated during this period to exponential phase of colony growth. After 85 h, the growth was terminated. At initial stage also the value of K_r is found to be very small. The shape of the bacterial growth curves depends on medium composition and inoculums density [7].

Figure 6 shows the typical growth curve of microbes grown in a batch culture or closed system. Here the growth of microorganism is plotted as the logarithm of the number of viable cells versus the incubation time. Since the area of the bacterial colony is proportional to the number of cells in colony it is possible to compare Figs. 6 and 5. By comparing these two plots it is clear that the lag phase represents initial points in Fig. 4. During the period of 25 to 85 h of growth, the area increases exponentially representing exponential or log phase of growth curve. It is clear from Figs. 5 and 6 that bacterial colony enters in to stationary phase after 85 h of growth.

4.2.2. Peak fluorescence intensity vs time plot. Figure 7 shows peak fluorescence intensity vs time plot of growth of growing bacterial colony. From the figure it is clear that the fluorescence intensity decreases as bacteria grows. The rate of reduction in fluorescence intensity was negligible at initial stage (up to 25 h). By comparing with Figs. 5 and 6 it is clear that, this stage represents lag phase of bacterial growth curve. Although cell division does not take place right away and there is no net increase in mass, the cell is synthesizing new components. The lag phase varies considerably in length with the condition of the micro organism and the nature of the medium. After 25 h of growth the intensity decreases exponentially. The rate of decay of fluorescence is found to be 0.034 V/h by theoretical fit. During this period area of the colony increases exponentially with a rate of 0.039 V/h. It is clear that quenching of the fluorescence of dye by bacterial colony is proportional to the increase in growth rate of the bacterial colony. This can be identified as the log phase or exponential phase of growth curve. In this phase micro organisms are growing and dividing at the maximal rate. Since microorganism are dividing and doubling in number at regular intervals and their rate of growth is constant. The intensity of the fluorescence is constant after 85 h of growth. This stage represents the stationary phase of growth curve. Bacterial cells enter in to stationary phase mainly because of nutrient depletion and accumulation of toxic waste products. The rea-

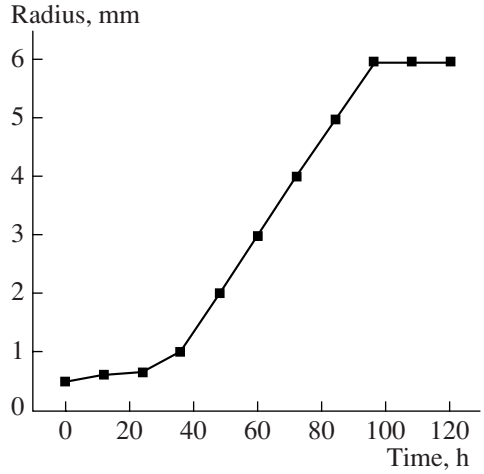

Fig. 4. Radial growth of bacterial colony vs. time.

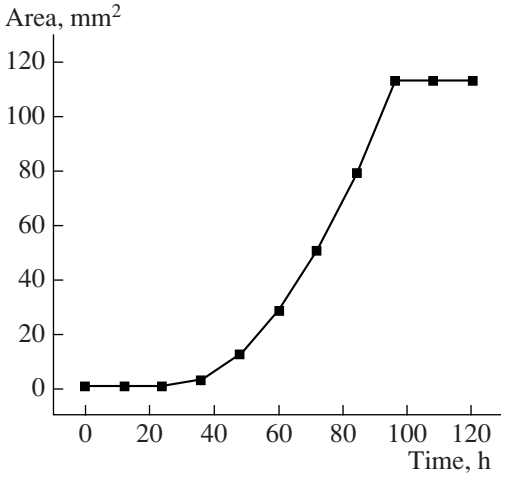

Fig. 5. Area of the bacterial colony vs. time.

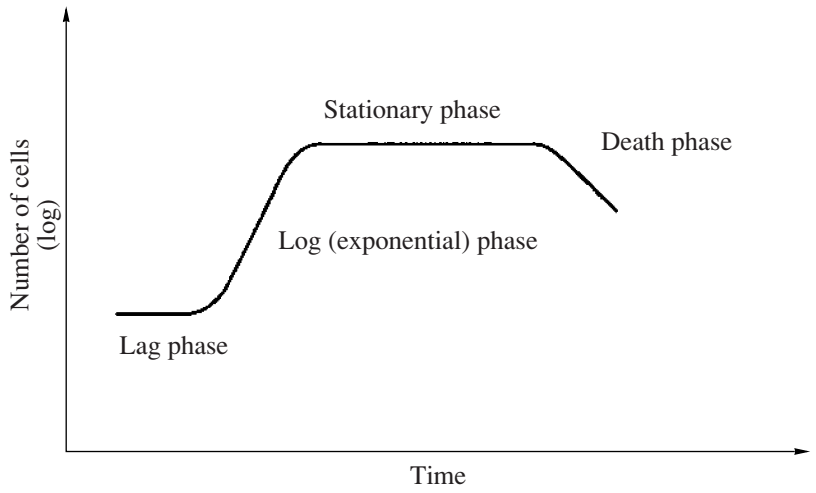

Fig. 6. Microbial growth curve in a closed system.

son for quenching of fluorescence is due to thick layer of bacterial cell wall as mentioned earlier. Quenching effect of dye is a measure of growth of gram positive bacterial cells.

5. THEORY OF GROWTH KINETICS OF BACTERIAL COLONY

The cultivation of bacteria on the surface of a solid nutrient agar medium is a general experimental technique. But no satisfactory quantitative theory was so far proposed to describe the development of bacterial colonies.

To study the growth dynamics of bacteria, it is necessary to know the rate of development of microbial growth and size of the colony. Pirt (1975) developed a model that describes the growth of a bacterial colony on solid homogeneous surface [8].

In the present study, the growth dynamics of the bacterial colony was determined by analyzing fluorescence intensity of dye which is doped in culture medium. From the study it is found that quenching of the fluorescence is in exponential manner in log phase.

Since the concentration of the cell of bacterial colony lies in the fluorescence quenching region we can write

$$N(t) \propto \frac{1}{I(t)}, \tag{1}$$

where $N(t)$, $I(t)$ are total number of bacteria, fluorescence intensity of dye doped bacterial colony at time t respectively.

At log phase $N(t) = N_0 e^{\mu t}$ [1] by assuming that the specific growth rate μ remains constant.

So growth equation can be written in terms of $I(t)$ as follows

$$\frac{dI}{dt} = -\mu I, \tag{2}$$

which gives fluorescence intensity of dye doped bacterial colony at given time t.

$$I = I_0 e^{-\mu t}, \tag{3}$$

which describes growth dynamics of bacterial colony during exponential phase.

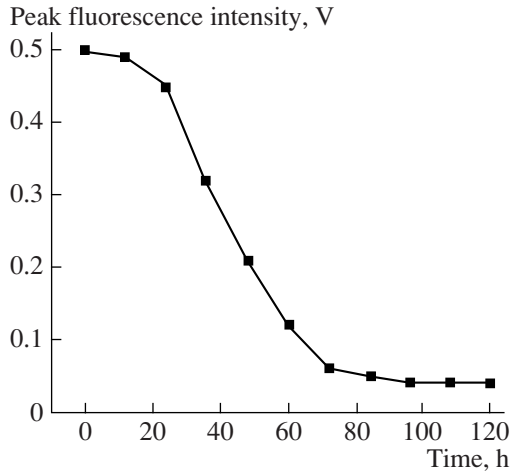

Fig. 7. Peak fluorescence intensity of dye doped bacterial colony vs. time plot.

6. CONCLUSIONS

Growth dynamics of an aerial gram positive bacterial colony on nutrient Agar medium is studied using laser induced fluorescence technique. This technique has been shown to be a useful technique for obtaining information about the different growth phase of the bacterial colony. Quenching effect of dye by bacterial colony can be effectively used to analyze growth kinetics of bacterial colony. Quenching effect of fluorescence indicates that cultured bacteria were gram positive. The rate of quenching of fluorescence of dye from bacterial colony was proportional to rate of increase in area of the bacterial colony which in turn indicates that the rate of quenching of the fluorescence was proportional to rate of growth of bacteria.

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