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ORIENTATIONAL ORDER IN PSEUDOMONAS BACTERIAL COLONY FORMATION

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ABSTRACT

Fresh water *Pseudomonas* bacteria colonies were grown on glass plates in a biofilm reactor with flow and without flow. In the colony grown with flow, at the center of the colony, bacteria were aligned parallel to each other. At the edges of the colony, the bacteria were disordered in orientation. The colony grown in static water didn't show any order. Bacteria orientataional angle distribution and interbacterial separation distance distribution, are presented. The orientational order at the colony centre is understood to be induced by the shear caused by the flow. At the edges, no order existed presumably due to the reversible adhesion phase of the bacteria. The interbacterial closest approach distance was too large to be explained by Derjaquin-landau-Verweey-Overbeek (DLVO) potential.

Key words: *Pseudomonas*, Bacterial Colony, Orientational Order, DLVO potential.

INTRODUCTION

Bacteria are known to have net negative surface charge due to dissociable acidic groups on the surface (Savage and Fletcher 1988). *Pseudomonas* bacteria form a 2 - dimensional colony on a glass plate (Korber et al 1989). This bacterium is a round edged rod of about 1.3um diameter. The growth kinetics of microcolonies of Pseudomonas bacteria, attached to the wall of a continuos flow slide culture, have been studied (korber et al 1989, Caldwell and Lawrence 1986, 1989). The growth was flow dependent when the glucose concentration was low, but flow independent otherwise. Caldwell and Lawrence (1989) observed various phase in the adhesion process of *Pseudomonas* bacteria like reversible adhesion and irreversible adhesion. They reported cell division and the longitudinal and lateral movement of the daughter cell in the initial stages of *Pseudomonas* microcolony formation. They observed a considerable amount of orientation of the long axis of bacteria in the flow direction. In their investigation on the effect of flow rate on the biofilm formation, Santos et al (1991) have conjectured that *Pseudomonas* bacteria would align in the direction of flow since this would offer least resistance. DLVO potential has been applied to explain the intercolloidal particle interactions (Verweey and Overbeek, 1948), as well as intervirus interactions (Kupe and Beams, 1979). But, controversy exists over the applicability of this potential to bacteria

(Marshall and Cruickshank, 1973, Fletcher, 1987 and Uyen et al, 1988). In the present work, we report ordered 2-dimensional microcolony of fresh water *Pseudomonas* bacteria. We have grown the colony on the surface of a glass slide immersed in a biofilm reactor in which pond water, rich in *Pseudomonas* bacteria, flows. The photomicrographs of 2-D colony grown were studied with an image analyzer. We present the angle distribution of the long axes of the bacteria and the interbacteria transverse separation distance distribution and discuss the results.

EXPERIMENTAL

The *Pseudomonas* bacteria were grown in a biofilm reactor (60 x 25 x 11 cm) made of perspex. Fresh water from a pond was passed through the biofilm reactor at the flow rate of 7.4 ml/min. The quality of the water is given in Table 1.

Table1. Pond water quality.

Temperature	30.5 °C
pH	8.24
Conductivity	325 uS/cm
Total Suspended Matter	2.6 mg/l
Phenopthalien Alkalinity	12.6 mg/l
Total Alkalinity	108 mg/l
Hardness	78 mg/l
Dissolved Oxygen	5.6 mg/l
Chloride	32 mg/l
Sulphate	5.6 mg/l

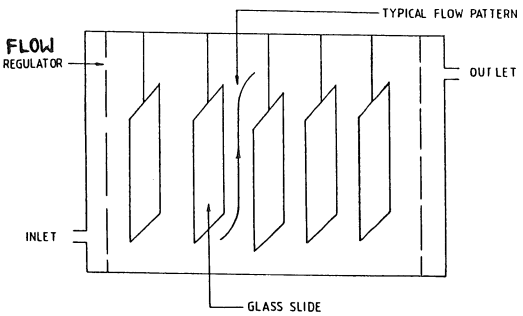

Fig. 1. Schematic representation of biofilm reactor.

Cleaned glass slides were vertically suspended in the biofilm reactor in such a way that a flow is maintained along the vertically suspended in the biofilm reactor in such a way that a flow is maintained along the vertical length of the glass slide (Figure 1). The flow direction was determined by injecting agar particles at the inlet and observing their flow near the glass slide. Pseudomonas colonies were also developed on cleaned glass slides in the same biofilm reactor filled with the same pond water without flow. After 18 hours of exposure the glass slides were removed, fixed, gram-stained and observed under microscope (Nikon Optiphot Photo-microscope) using 100 X oil immersion objective. Pseudomonas bacteria grown on selective agar medium, were smeared on the slide in a single stroke, fiixed, gram-stained and observed under the microscope as above.

The microphotographs of the various regions of the bacterial colony were imaged using video camera (Andrex, Denmark) and grabbed in a image analyzer)developed in - house). The coordinate ends of each bacteria (assumed a head (X_1, Y_1) and (tail X_2, Y_2)) were read from the image. The orientation angle distribution, n (θ), of the bacteria and interbacteria separation distance distribution, m(d), were obtained. Here, n(θ) and m(d) are the number of bacteria with the orientation angle, θ and the interbacteria separation distance, d respectively.

RESULTS AND DISCUSSION

Fig.2 shows the micro photograph of the centre of the Pseudomonas clolony grown in fresh water biofilm reactor with flow. The bacteria have covered the surface of the glass slide in a single layer. At few places on the slide there were no bacteria over a surface area of about 2 to 5 mm2. The reason around this void is refered to as edge of the colony, while regions distant from the void area were considered as centre of the colony. Form Fig.2 it was estimated that the size of the bacteria varied from 1.3 to 1.7 um in length and 0.5 to 0.7 um in diameter. The variations in the size is the result of various stages in the cell division process. It was observed that the bacteria are aligned almost parallel to each other with well defined inter bacteria separation distance. The long axes of the bacterial were found to be aligned in the direction of the flow.

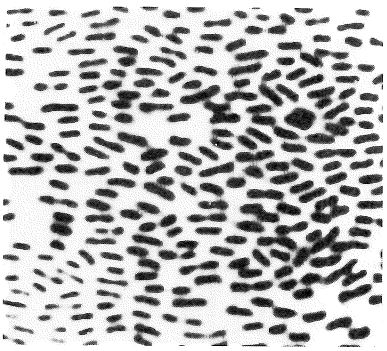

Fig. 2. Photomicrograph of the centre of the ordered Pseudomonas colony grown in biofilm reactor.

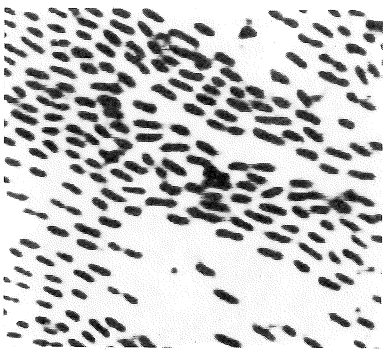

Fig.3. Photomicrograph of the cultured Pseudomonas bacteria after streaking on a glass surface.

Caldwell and Lawrence (1989) reported the mechanisms involved in bacterial adhesion and multiplication. Pseudomonas bacteria swim actively with in the hydro dynamic boundary layers (upto about 0.2 um distance from the surface) with same speed upstream and down stream. They approach the surface and make contact at an angle of about 40 to 45°. Once adhered, they rotate about the contact point and move across the surface. They can detach from the surface to emigrate away or to reattach in a nearby place. This is called the reversible adhesion phase. They further reported that when the rotation about the contact point slows down, the bacteria attach to the surface irreversible. This is called the irreversible adhesion phase. We conjecture that the bacteria irreversible attach at the the centre of the colony, while at the edges they are reversible attach since they may be the daughter cells or the new members of the growing colony.

At the centre of the colony (Fig.2) the alignment would have occured, due to the shear cause by the flow during reversible adhesion phase. In order to support the view, we have smeared the agar cultured pseudomonas bacteria on the surface of the slide in a single sweep (Fig. 3). Long axis of the bacteria is align along the smear direction suggesting that the shear on the bacteria causes it to align. Fig.4 shows the angle distribution, $n(\theta)$, of the long axes of the bacteria obtained from Fig.2 using an image analyzer. The peak angle 90° corespondes to the vertical direction whioch coincides with the direction. This distribution is obtained after averaging over about 900 bacteria. Full width at half maximum (FWHM) of the distribution is 25. Fig.5 shows the distribution m(d), of the inter bacteria transverse separation distance d, obtianed from Fig.2 by averaging over 200 bacterial pairs. The average separation is around 0.9 um, the cross-sectional diameter of the bacteria is ~0.6 um and average surface to surface distance between the bacteria is around 0.3 um. This value matches with separation measure in

the reported micro photographs of Pseudomonas (Caldwell and Lawrence, 1989). FWHM of the distribution is around 0.06um.

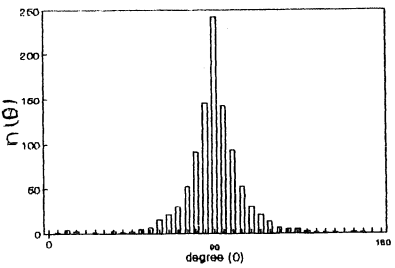

Fig. 4. Orientational angle distribution n(θ) distance obtained for the centre of the colony.

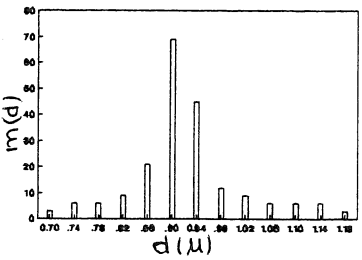

Fig. 5. Interbacteria transverse separation distribution m(d) obtained for the centre of the colony.

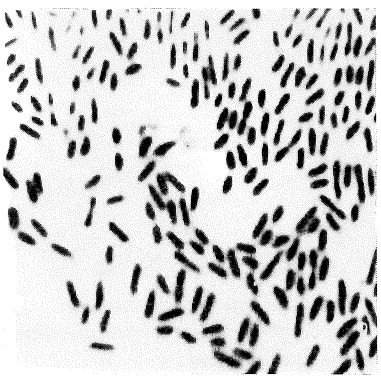

Fig. 6. Photomicrograph of the edge of the Pseudomonas colony grown in the biofilm reactor.

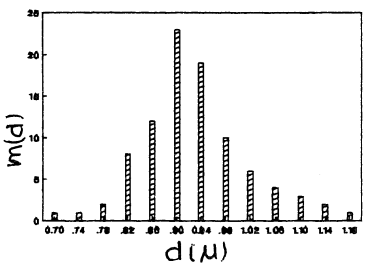

Fig. 7. Interbacteria transverse separation distance distribution m(d) obtained for the edge of the colony.

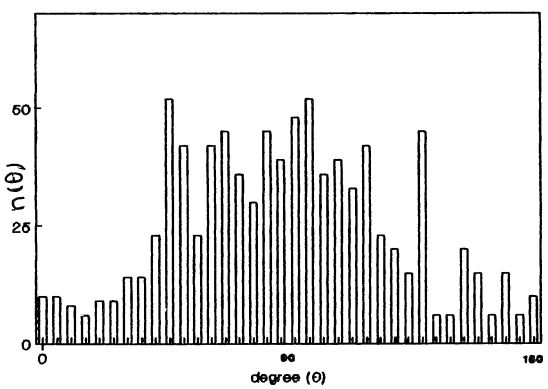

Fig. 8. Orientational angle distribution n(θ) obtained for the edge of the colony .

Fig.6 shows the photo micrograph taken at the edge of the colony. The orientaion of the bacteria is not as dominant as the same at the centre of the colony (Fig.7). This may be because the bacteria are in the reversible adhesion phase where they attach temporarily and rotate. The

micro graphs of the colony grown without flow did not show any orientational order. This result is consistant with the conjecture that the orientaional order is induced by the flow.

Generally, bacteria interact with the surface of the substrate as well as among themselves. This interaction might include elctrostatic forces, kinetomatic forces, specific forces, (Savage and Fletcher, 1988), Uyen et al. 1988). DLVO potential named after Derjaguin, Landau, Verwey and Overbeek (Verwey and Overdeek, 1948) as been invoked partly to sucessfully understand the adhesion behaviour of bacteria on to substrates(Marshall and Cruikshank, 1973; Fletcher 1987 and Uyen et al. 1988). This has not been applied to understand ht e inter bacteria interaction. Here we examined the applicablity of DLVO potential to understand the closest approach distance exhibited by the *Pseudomonas* bacteria. Suppose the DLVO potential is the dominant one in determining the positional order. There will be a potential well and a barrier between two bacteria as the inter bacteria separation decreases continuously. The bacteria would be trapped in the potential well. The thermal energy of the bacteria is responsible for the closest approach between them. At the closest approach distance, the increase in the DLVO potential energy from the well minimum should be of the order of K_BT, the thermal energy($4x10^{-4}$ ergs). Here K is the Boltzmann constant and T is the temperature. This closest approach distance has been measured from Fig.2 as 0.2 um.

The DLVO potential between two bacteria is given by (Uyen et all 1988):

$$U_T = U_R + U_A \tag{1}$$

where

$$U_R = \frac{(\varepsilon U_0^2 a)}{2} \ln\left(1 + e^{-KH}\right) \tag{2}$$

is the elecctrostatic repulsion between two bacteria of surface to surface separation H, effective radius a, bacteria surface potential U_o in a medium with a dielectric constant ε and inverse Debye screening length K, given by

$$K^2 = \frac{4\pi nq^2}{\varepsilon K_B T} \tag{3}$$

where q is the electronic charge, n is the number concentration of the ionic impurity in the medium. U_A is the London-van der Waals attractive part given by

$$U_A = \left(-\frac{A}{6}\right)\left[\frac{2a^2}{(H+2a)^2 - 4a^2} + \frac{2a^2}{(H+2a)^2} + \frac{\ln\left[(H+2a)^2 - 4a^2\right]}{(H+2a)^2}\right] \tag{4}$$

where A is the Hamaker constant. The effective radius of the bacteria is obtained form its volume as 0.5 um. K is calculated as one 1.67 $x10^6$ cm^{-1} from the conductivity of the fresh water used to grow the bacteria assuming that the conductivity due to NaCl type salt. For U = -25mV (Caldwell and Lawrence, 1989) and A = 5 x 10^{-15} ergs (Caldwell and Lawrence, 1989). U_T is obtained from equation.(1). At H = 0.2 um, U is -1.5 x 10^{-15} ergs, which is higher than the potential well minimum value by $3.5x10^{-15}$ ergs. This increase in potential energy amounts to only 9% of the thermal energy. Hence, thermal energy can push the bacteria closer than H = 0.2 um. Thus DLVO potential is not strong enough to keep the bacteria at the closest approach of 0.2 um. Only at H = 0.03 um, the increase in potential energy from the well minimum equals to thermal energy. This amounts to two bacteria almost touching at the magnification employed here. But, no bacteria was found to be present at such a low separation distance in the present study as well as in the photo micro graphs presented by Caldwell and Lawrence (1986 & 1989). Hence DLVO potential is not dominant in inter bacteria interaction. Specific forces due to some

structures extending from the bacteria for into the suspension (0.1 um distance) might be responsible as suggested by Uyen et al (1988).

CONCLUSION

Orientational and transverse positional order in the pseudomonas bacetria colony as been investigated. The orientational order is understood to be induced by the shear cost by the flow.Edge of the colony is disordered, since it is in the reversible phase of adhesion. For the work is needed to understand the role of specific forces in the transverse positional order.

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