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FRESH WATER BACTERIAL AGGREGATE DEVELOPMENT: EFFECT OF DISSOLVED ORGANIC MATTER

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NWRI Contribution No. 91-75

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MANAGEMENT PERSPECTIVE

Existing information on the potential of suspended particles as carriers of environmental contaminants is extensive and focuses primarily on abiotic components. Bacteria, however, because of their large surface area and surface charge may also be carriers of aquatic contaminants. Bacteria often flocculate with other bacteria and/or inorganic sediment. This aggregation results in significant changes to the hydrodynamic properties of bacteria and subsequently may have significant effects on down stream contaminant transport and storage. This report provides information on how microbial aggregates are formed in aquatic environments by the utilization of available dissolved organic carbon (DOC) and how these aggregates concentrate environmental contaminants.

SOMMAIRE À L'INTENTION DE LA DIRECTION

Les données existantes sur la possibilité que des particules en suspension servent de véhicules aux contaminants de l'environnement sont considérables et portent principalement sur des composants abiotiques. Toutefois, en raison de leur grande surface et de leur charge superficielle, les bactéries peuvent également servir au transport de contaminants aquatiques. Les bactéries floculent souvent avec d'autres bactéries et (ou) des sédiments inorganiques. Cette agrégation porduit des modifications importantes des propriétés hydrodynamiques des bactéries, ce qui par la suite peut avoir des effets importants sur le transport et l'emmagasinage en aval des contaminants. Le présent rapport renseigne sur la formation d'agrégats microbiens dans les milieux aquatiques grâce à l'utilisation du carbone organique dissous disponible et sur la façon dont ces agrégats concentrent des contaminants de l'environnement.

ABSTRACT

Suspended sediment particles and bacteria because of their surface area and charge may play a role in the binding of aquatic contaminants. Little is known, as to what degree each of these factors play in the formation of suspended aggregates. Flocculation of particles can alter their hydrodynamic properties in aquatic environments and therefore may have significant implications for contaminant transport. In this study, we examine the role dissolved organic carbon plays in the production of bacterial aggregates as a first step to gaining a better understanding of bacterial-particle interaction and suspended particulate formation.

RÉSUMÉ

En raison de leur superficie et de leur charge superficielle, les particules de sédiment en suspension et les bactéries peuvent jouer un rôle au niveau de la fixation de contaminants aquatiques. Nous possédons peu de connaissances sur l'action de chacun de ces facteurs au niveau de la formation des agrégats en suspension. La floculation des particules peut modifier leurs propriétés hydrodynamiques dans les milieux aquatiques et peut donc avoir des effets importants sur le transport des contaminants. La présente étude porte sur le rôle du carbone organique dissous dans la production d'agrégats bactériens comme première étape en vue de mieux comprendre l'interaction entre les bactéries et les particules et la formation de particules en suspension.

INTRODUCTION

Bacteria have the highest surface area to volume ratio of all cells and, therefore, have broad interaction with their aqueous surroundings as well as their neighbouring cells (Beveridge, 1988). Thus, they have a high capacity to flocculate and to sorb contaminants onto their surface. Bacteria are also highly interactive with fine-grained solids such as clays which act as colonization sites as well as sources of nutrients and contaminant uptake (Walker et. al., 1989). The implications of heavy metal contaminant adsorption by biological particles has been outlined by Xue et. al., (1988).

Many researchers have traditionally focused on the physico-chemical factors which affect particle flocculation (Luckham and Vincent, 1983; Hunt, 1980, 1982; Krone, 1978; Sholkovitz, 1976). These studies have been primarily concerned with the marine environment where electrochemical flocculation is enhanced by suppression of the electrical double layer due to the salt content of the water. With the absence of salt ions in freshwater systems, aggregates were assumed to be less significant components of the suspended loads. Recent findings by Droppo and Ongley (1989) have, however, demonstrated that aggregates can be a significant component of the suspended material in freshwater fluvial systems.

Only recently has the role of bacterial organic matter been examined as a factor for aggregate development (Biddanda, 1985; Muchenheim et. al., 1989) and as a site for aquatic contaminant sorption (Geesey, 1982; Beveridge, 1989).

In this report we examine the nature and extent to which certain natural dissolved organic substances influence bacterial aggregate formation in river water devoid of inorganic particles. Furthermore, we discuss the role bacterial aggregates may play in contaminant adsorption.

Laboratory Methods

Laboratory controlled microcosm experiments were performed using three one litre glass containers and inorganic particle-free river water containing native bacteria. Inorganic particles were removed by settling and decanting off the surface waters. Algal exudates (Chlorella Sp. and Scenedesmus Sp.) were used as the source of dissolved organic carbon and were each added individually to their respective containers. The third container was used as a control and only contained the particle free water and native bacteria. The concentrations of DOC employed in our experiments (20 mg C/L) were slightly higher than those normally reported from freshwater environments. The average reported DOC concentration for over 500 Wisconsin lakes was 15.2 mg C/L (Wetzel, 1975). This experimental concentration of DOC was used to examine its effects on the production of bacteria and their subsequent incorporation into aggregates within the experimental time frame. The samples were gently agitated using a magnetic stirrer set at low speed to facilitate uniform mixing of bacteria without disrupting the integrity of the already formed aggregates. Ten and 25 ml of each suspension was removed at different time intervals for bacterial density determination and bacterial aggregate sizing, respectively.

Bacterial/Aggregate sizing

Bacterial aggregates were sized using the method of Droppo and Ongley (1990). This method requires settling chambers, an inverted microscope equipped with 35 mm camera and a translucent digitizer. A subsample of the suspension at each time interval was gently placed into a 25 mL settling chamber using a wide mouth pipette (5 mm diameter). It has been reported that pipetting with a tip greater than 3 mm in diameter minimizes aggregate breakup (Gibbs 1982). The suspended particulate matter was allowed to settle for 24 hrs. after which time the settling chamber was placed on an inverted microscope (Wild Leitz) where bacterial aggregates were photographed (as slides) at 100 times magnification. The slides were then rear projected onto a scriptel digitizer which has a resolution of 0.025 mm and an accuracy of +/- 0.64 mm. The aggregate's perimeters were traced to obtain surface area which were then converted into equivalent spherical diameters. Median floc sizes were then determined from the equivalent spherical diameter data set for each time interval of the experiment.

Bacterial Density Determination

Water samples (10 mL) from the experimental chambers were immediately preserved after collection with 0.1 mL of 37% formaldehyde. Bacterial counts were determined using a modification of the acridine orange direct microscopic counting procedure outlined by Rao et. al., (1984). In order to demonstrate that bacteria are entering into aggregation, we counted the number of singular free-floating bacteria (i.e. if aggregates are formed due to bacterial incorporation then the number of free-floating bacteria will decrease). A sub-sample of the initial suspension was gently filtered onto a 1.0 µm Nuclepore filter. As the majority of bacteria in freshwater are between 0.3 and 0.7 µm in diameter (Hobbie et. al., 1977), this procedure of filtering will allow the majority of free-floating bacteria to pass through the filter while the majority of the aggregated bacteria will be trapped by the filter. The bacterial suspension which passed through the 1.0 µm filter were immediately filtered through pre-stained (sudan black) 0.1 µm Nuclepore filters (Droppo, 1990). Although this method is not totally effective in separating all of the free-living bacteria from the aggregated bacteria, it provides a semi-quantitative estimate of bacterial populations.

Results and Discussion

Suspended aggregate production in aquatic environments has been attributed to physico-chemical flocculation (Kranck and Milligan, 1980). However, in experiments using bacteria-killed controls in marine environments, Biddanda (1985) has indicated that the presence of viable bacteria is essential for such aggregation processes. The effects of dissolved organic substances which are easily utilized by aquatic bacteria have been studied using labelled substances (Paerl, 1974). Paerl demonstrated using radioactive glucose and acetate that these compounds were accumulated by bacterial cells and filaments within 48 hours and with continued incubation, the localization of carbon was found to be greatest in bacteria. However, Paerl's study does not provide specific data with regard to the increase of bacteria due to carbon incorporation and the subsequent increased bacterial aggregate formation.

Our results indicated that the total bacterial densities were substantially enhanced by the available organic exudate of algal origin. An order of magnitude increase in bacterial numbers for Chlorella sp. was observed within 96 hrs. (7.0x 10^5 to 6.0x 10^6 counts per mL). There was no significant difference in increase in bacterial numbers between the exudates from the two algal species. In the control, where no dissolved organic carbon was added, the bacterial counts remained relatively constant at 10^5 counts per mL (Fig. 1a). The increase in bacterial numbers was accompanied by an increase in median bacterial aggregate size (Fig. 1b and Fig. 3). This suggests that the bacterial response to the added organic material contributed to the production of larger sized aggregates. An increase from approximately 8 µm to 16 µm in the median aggregate size was observed after 144 hours (Fig. 1b) McCoy et. al., (1981) indicated that the glue like properties of extracelluar exudates hold bacteria together as biofilm so firmly that they can only be detached by excessively strong shear forces. This would suggest that the minimum operational perturbations that were encountered during our study would have little effect on the integrity of bacterial aggregates.

Not all of the bacteria were observed to be incorporated into aggregates. However, the numbers of non-aggregated or free living bacteria decreased with time indicating their subsequent entry into the aggregation process (Fig. 2). Free living bacterial counts at the beginning were relatively high at 60 x 10^6 or 6.0 x 10^7 per mL and declined to 9 x 10^6 per mL by 144 hours.

The reduction of non-aggregated bacteria and the accompanying increase in aggregate size may have implications for contaminant transport within freshwater fluvial systems. While the bacterial content was small relative to the amount of inorganic sediment present, the aggregation ability of bacteria demonstrated here indicate that they may likely interact with inorganic particles to form organic/inorganic aggregate complexes (Walker et. al., 1989). Whether or not this interaction will increase the effective size and density of the particles and therefore their settling properties needs to be investigated. Furthermore our recent studies (unpublished data) on the association of copper as a model contaminant with laboratory induced and native bacterial aggregates clearly indicate that the contaminant is bound within the negatively charged polymer matrix of the bacterial aggregates. Thus, bacteria and bacterial aggregates have potential implications for the adsorption and transport of contaminants. Further research will examine the role bacteria and bacteria-aggregates play in the binding of aquatic contaminants in freshwater systems.

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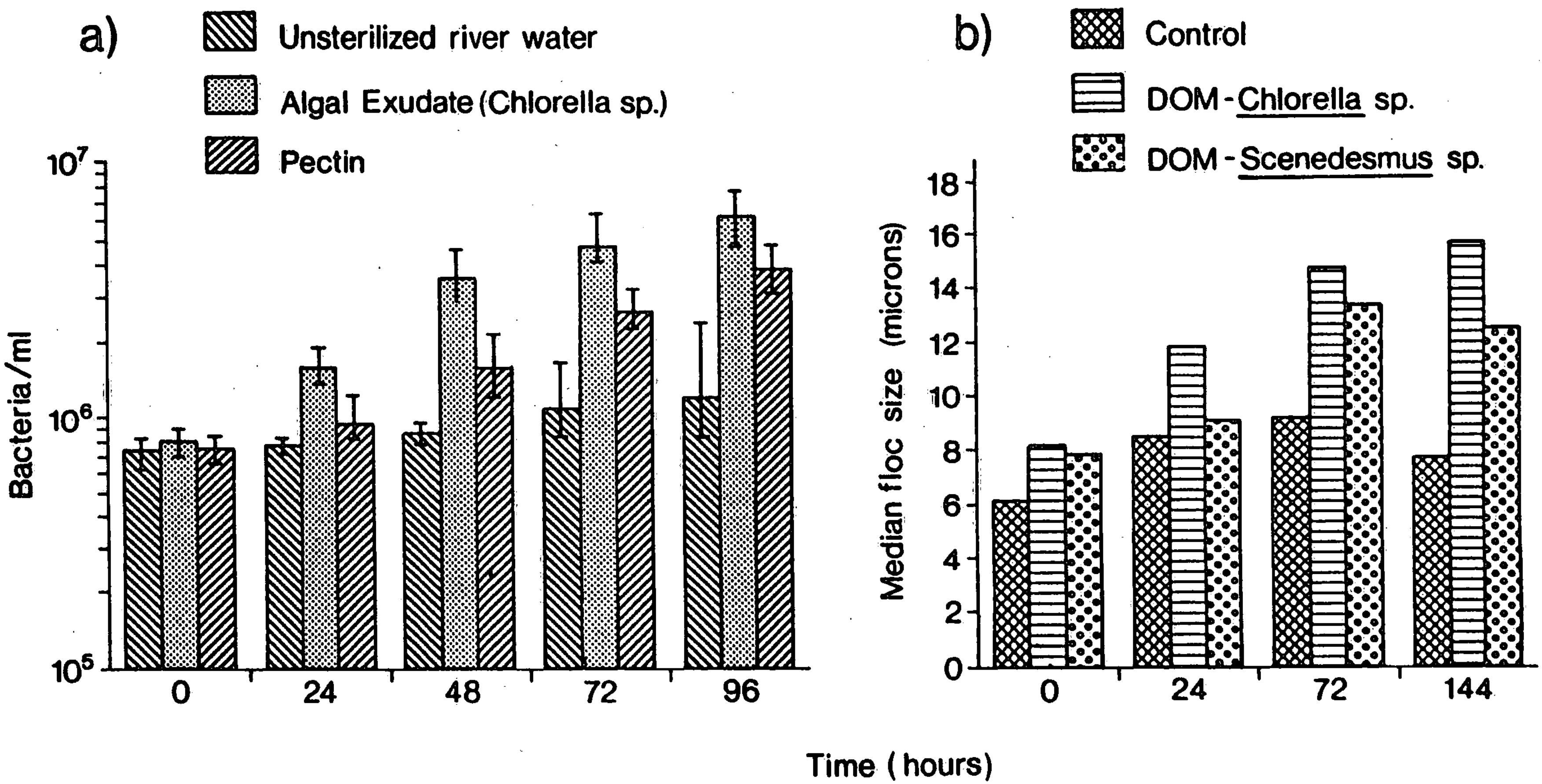


Figure 1 Effect of dissolved organic matter (DOM) on the production of a) bacteria and b) bacterial flocs.

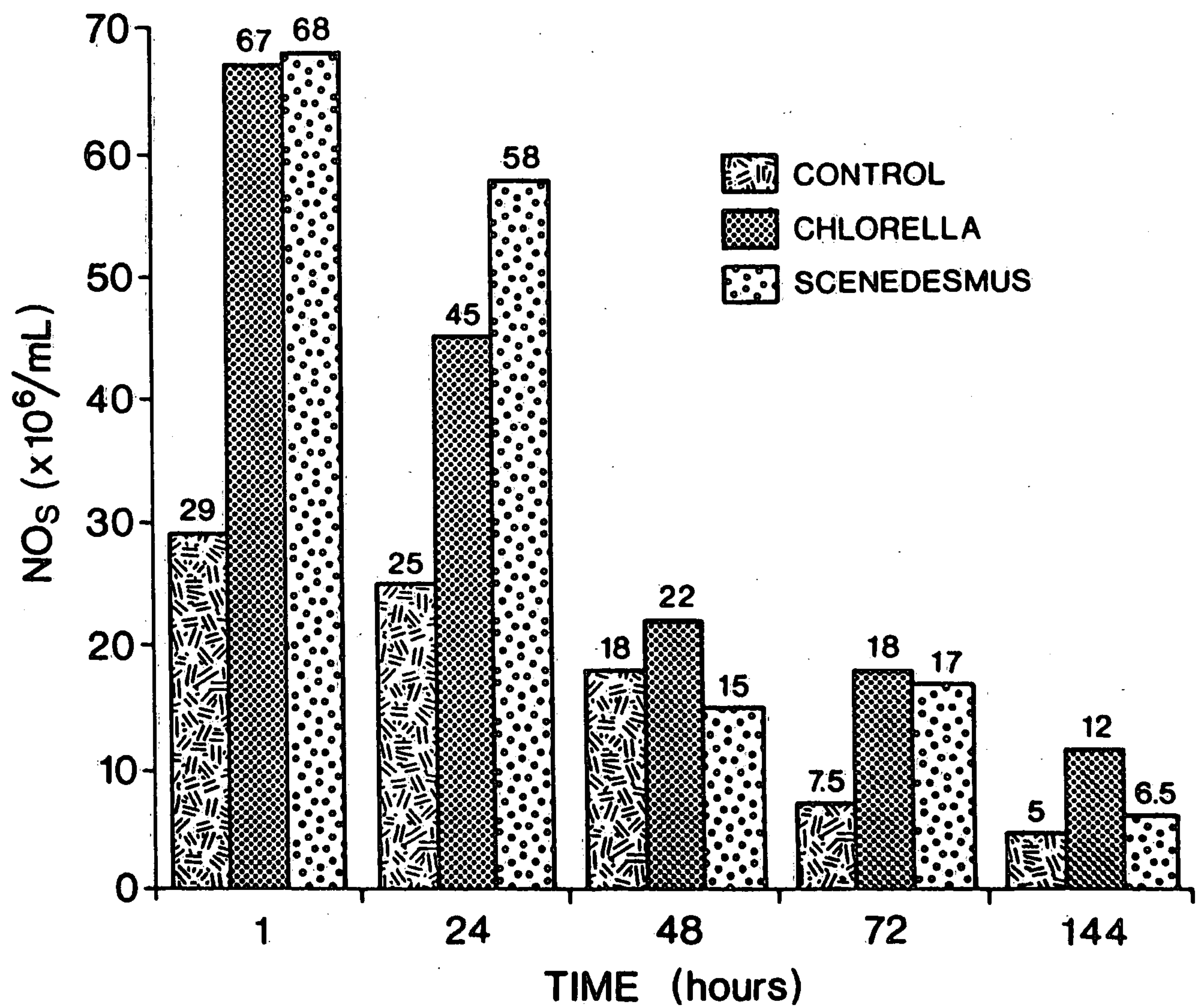
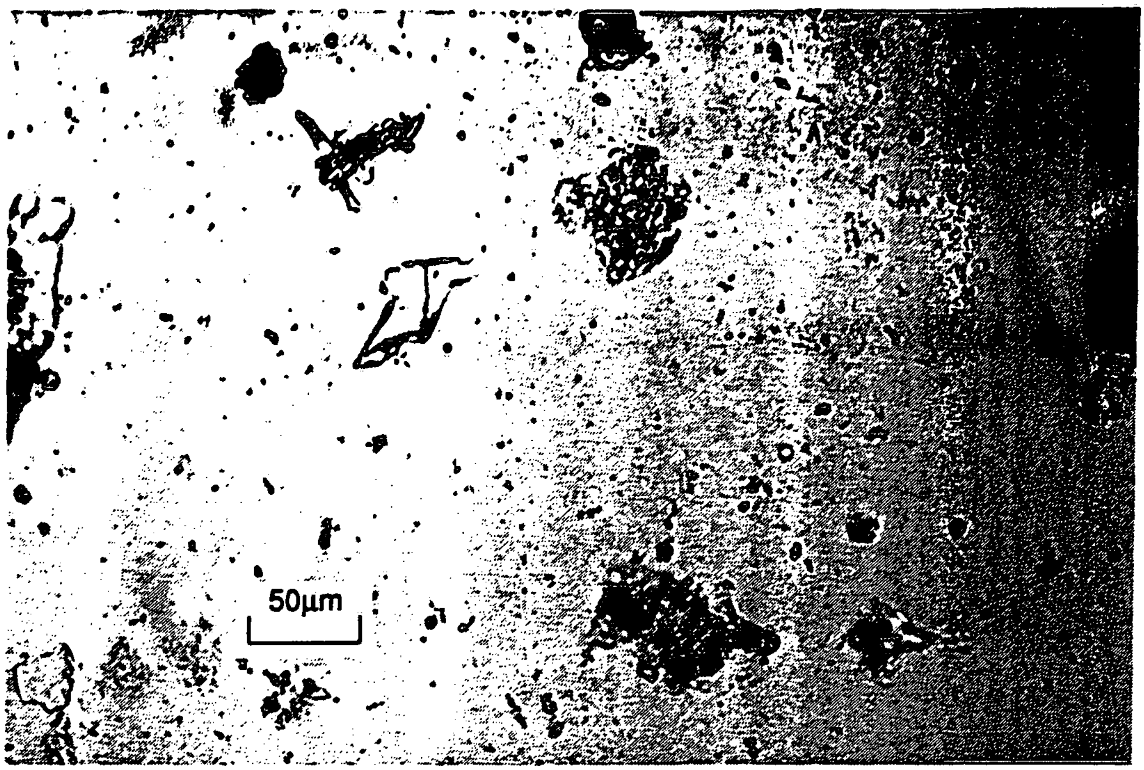
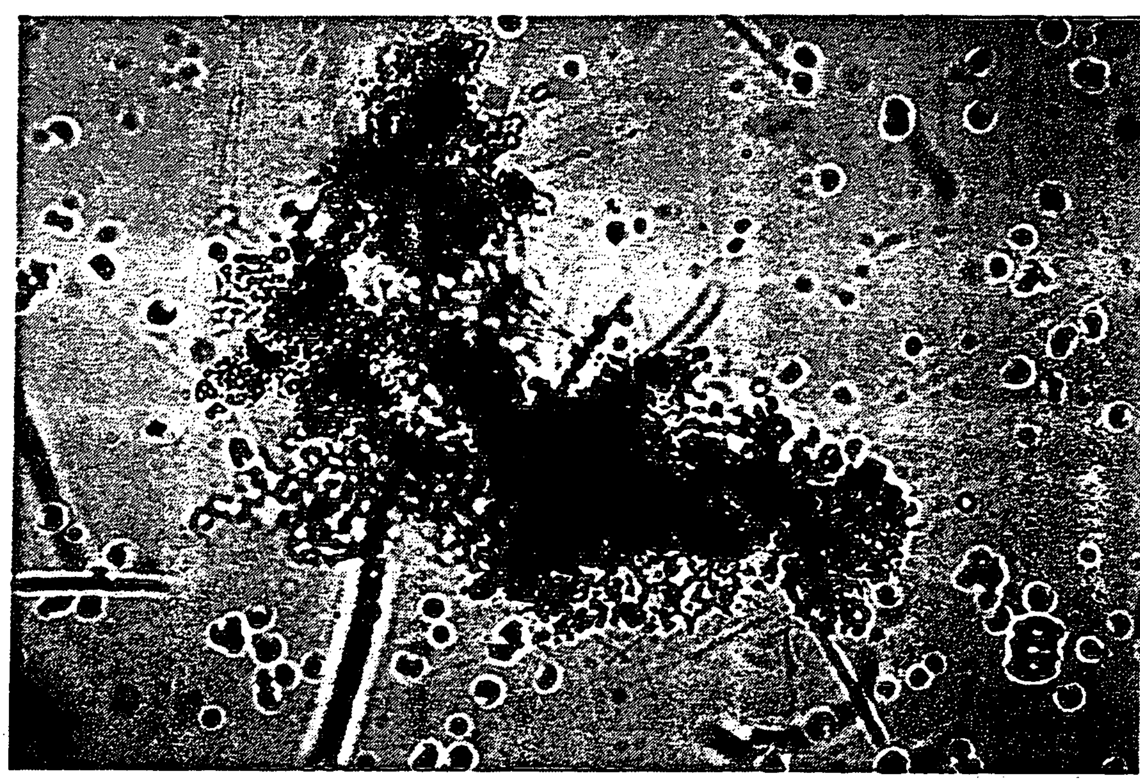


Figure 2 FREE FLOATING BACTERIA



A



B

Figure 3. Representative Floc Size: A 0hr; B 144hrs.

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