Analysis of engineered mixed culture biofilms: use of soft X-ray microscopy

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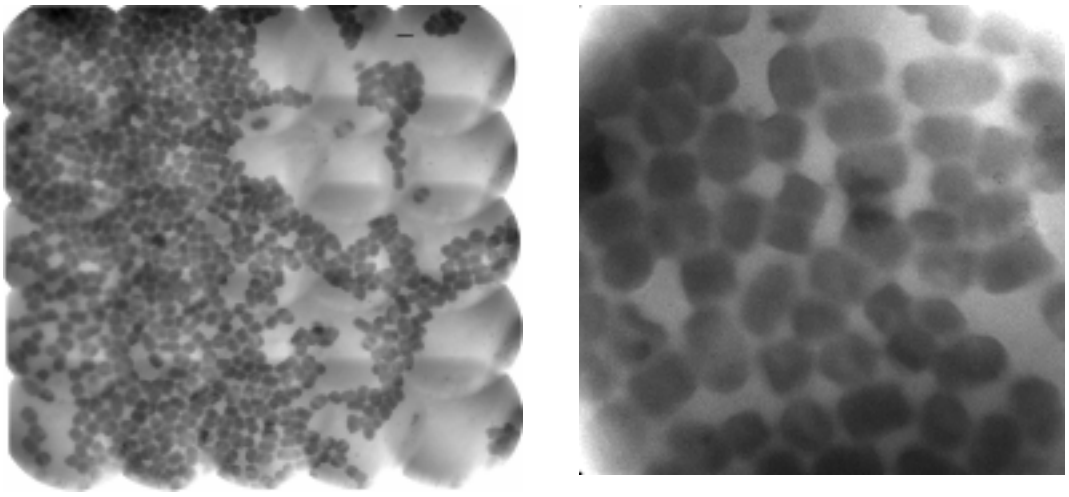
INTRODUCTION

Using biofilms for biotechnological applications

Biofilms are frequently studied for the economic damage that they cause. Their impact is felt in diverse areas, from biofouling of oil drilling rigs and heat exchangers to infections in medical implants. However, the attributes of biofilms that are problematic in uncontrolled situations are potentially useful biotechnological features, if the biofilm is populated with beneficial microorganisms. Decontamination of toxic waste using bacteria is an area that could benefit from biofilms. The production of useful bioproducts by metabolically engineered bacteria is another area where biofilms could be valuable.

Using biofilms to enhance bioremediation

Bioremediation is a "green" technology that takes advantage of the enzymatic capabilities of bacteria to decontaminate chemical pollution in the environment. Numerous case studies document the effectiveness of bioremediation for the cleanup of environmental pollutants including crude oil, jet fuel, volatile organic compounds, and chlorinated solvents (Hinchee, 1995a; Hinchee, 1995b). Biofilms are already in use in some wastewater treatment processes, and could play a meaningful role in detoxifying a broad range of industrial and environmental pollutants.



(a) (b)

Figure 1. (a) Tiled image of *Escherichia coli* biofilm. Bar at top right = 1 micron.
(b) Close- up of biofilm; 2400x magnification.

Features of the XM-1 soft X-ray microscope that benefit biofilm research

Biofilms have a complex three-dimensional structure and are almost always hydrated. It is often difficult to image them at high magnification, for example, by electron microscopy, because the sample preparation dehydrates the biofilm, altering its structure. A unique feature of soft X-ray microscopy that is beneficial for the analysis of biofilms is the ability to image samples while they are still hydrated. Another feature of the XM-1 soft X-ray microscope that is good for biofilm research is its tiling feature. Using this tool, large areas of biofilms can be scanned and regions of special interest can be located for later examination at high magnification (Fig. 1).

CURRENT RESEARCH

Engineered biofilms for bioremediation of mixed wastes.

Industrial wastes sites often contain mixtures of contaminants. For example, trichloroethylene (TCE) and chromium are common contaminants of groundwater that co-occur near former semiconductor manufacturing sites. A separate microorganism may be required to detoxify each component of the waste. Biofilms can help maintain mixed cultures of bacteria with different growth kinetics, since the cells are immobilized (Eliashberg and Keasling, 1998).

We are currently investigating the hypothesis that the distribution of bacteria in engineered biofilms can influence their efficiency for mixed waste detoxification. Recent images of a dual-species biofilm that detoxifies mixed phenol-chromium waste revealed broad areas of surface colonization by the *E. coli-Pseudomonas* coculture. An interlocking structure of irreversibly attached cells dominated by *Pseudomonas* was observed (Fig. 2). A notable feature of the colonization was the relatively high percentage of unoccupied area between the cells attached to the substratum.

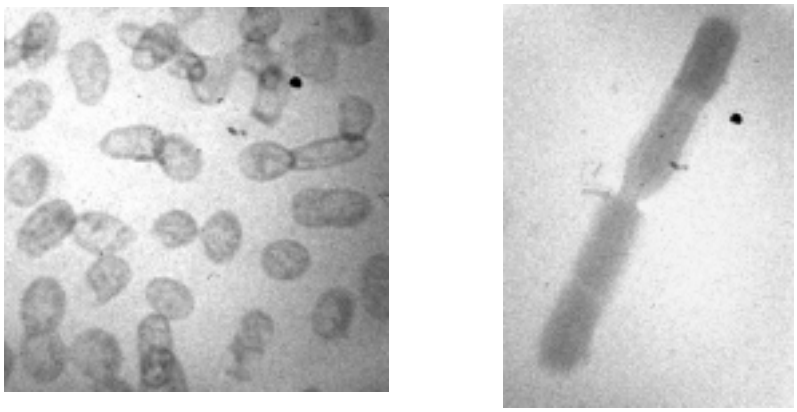


Figure 2. (a) Dual species biofilm. Note the overlap of cells. (b) *Escherichia coli* 33456 grown in rich media containing 150 μg mL^{-1} K_2CrO_4. 2400x magnification.

CITATIONS

1. Hinchee, R.E., Leeson, A., & Semprini, L., eds. (1995a). Bioremediation of chlorinated solvents. Battelle Press, Columbus.

2. Hinchee, R.E., Kittel, J.A. & Reisinger, H.J. (1995b). Applied bioremediation of petroleum hydrocarbons. Battelle Press, Columbus.

3. Eliashberg, N. & Keasling, J. D. (1998). Population dynamics and species spatial organization in simulated multispecies biofilms. *J. Theor. Biol.* In review.

PUBLICATIONS BASED ON DATA COLLECTED AT THE XM-1

Gilbert, E.S., A. Khlebnikov, W. Meyer-Ilse and J.D. Keasling. Use of soft X-ray microscopy for the analysis of early-stage biofilm formation. Water Science and Technology, in press. 4 manuscript pages.

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