NON-INVASIVE METHODS FOR MONITORING BIOFILM GROWTH IN INDUSTRIAL WATER SYSTEMS

S. G. GÓMEZ DE SARAVIA[1,2] and M. FERNÁNDEZ LORENZO DE MELE[1,3]

[1]*INIFTA. UNLP. C.C. 16, Suc. 4, 1900, La Plata. ARGENTINA.*
[1,2]*CIC.* [1,3]*CONICET.*
sgomez@inifta.unlp.edu.ar, mmele@inifta.unlp.edu.ar

Abstract. **Microbiological control in industrial environments is frequently restricted to monitor the number of planktonic (suspended) cells. However, this number is not often related with the microorganisms attached to the surfaces (sessile bacteria). The aim of this research work was to develop a method for monitoring biofilm growth and the biocidal efficacy through simple non-invasive ways. With this purpose, biofilms were formed on stainless steel coupons and after preset periods were removed and immersed in sterile solutions with and without biocide. The number of sessile cells attached on the metal surface and of planktonic cells that were shed from the biofilmed coupons (pcb) and grow in the initially sterile solutions were determined. Sessile cells were scrapped from the metal surface to be enumerated. The relation between the degree of growth of pcb and the reactivation capacity of the biofilm was evaluated. It could be observed that pcb growing in a nutrient non aggressive medium was related to the number of sessile cells that remain alive after the biocidal treatment. The early stages of the biofilm growth, the thickness of the biofilms and their microstructural characteristics before and after the biocidal treatment could be followed through optical microscopy using a non-invasive technique recently developed in the laboratory. Microscopic observations showed that the biofilm thickness varied to obliterate the unevenness of rough surfaces.**

Keywords. **Biofilm, Biocide, Biocidal Treatment, Optical Microscopy, *Pseudomonas fluorescens***

I. INTRODUCTION

Biofilm mode of microbial growth is predominant in aquatic environments and protects the microorganisms from adverse environmental conditions and from biological and chemical antimicrobial agents. It prevails in virtually all nutrient–sufficient aquatic systems independent of its type and geometry (Lappin-Scott and Costerton, 1989). Biofilms are dynamic structures, which consist of cells, their secreted matrix of insoluble extracellular polymers matrix (EPM) and inorganic materials entrapped in it. Planktonic cells are usually shed from established biofilms and lose their protection so that new fresh habitats can be colonized by new biofilms (Costerton *et al*., 1995).

During several decades biofilms were thought as uniform layers of cells embedded in an EPM without heterogeneities inside them. Traditional microscopic methods of sampling and culture did not allow examining the biofilm microstructure. Planktonic cells often represents only 0.1 % of bacteria in an ecosystem (Geesey *et al*, 1978). Phenotypic changes that bacteria undergo when they adhere to surfaces have been reported (Davies *et al*., 1993). The number of cells in the biofilm is frequently underestimated because the aggregates obtained by scrapping the sample produce only a single colony on the plates. Moreover, the biofilm population in many aquatic ecosystems may be viable but nonculturable (Colwell, 1984). Notwithstanding this, many researchers in microbiology still study microbial ecosystems by extrapolation from planktonic samples. The quantitative numerical analysis available must be tested against the direct microscopic examination. Thus, if we are to understand biofilm processes, living biofilms microscopy must be made.

Confocal Scanning Laser Microscope (CSLM) (Costerton *et al*., 1995) revealed the complex biofilm architecture of some ecosystems in which microcolonies are enclosed in EPM separated by water-filled channels. The discovery of convective flow within the water channels has revolutionized the concept of bacterial growth within biofilms. Through these channels the nutrients are carried throughout the biofilm like a primitive circulatory system (McFeters *et al*., 2000).

Unfortunately, some of the high-resolution techniques are not a dairy tool in many laboratories. On the other side, some microscopic techniques frequently used by researchers require extensive sample preparation ex situ including dehydratation, use of chemicals to fix the biofilm to the surface, use of fluorescent stains, coated by a conductive layer, etc. (Beech *et al*, 1996). Morever, some of them cannot be used for observations at real time.

Biofilms are the cause of several operational problems associated with industrial and drinking water systems (Geesey and Bryers, 2000). They originate not only economical looses but health diseases as well. The control of biofilms is a real challenge within engineered systems. Engineers are familiar with the effects of biofilms in heat exchangers, trickling filters and anaerobic digestors caused by biofouling formation