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A SIMPLE FLOW CELL FOR MONITORING BIOFILM FORMATION IN LABORATORY AND INDUSTRIAL CONDITIONS

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Table of Contents:

ABSTRACT

This work proposes and discusses a simple flow cell reactor that provides a means to monitoring biofilm growth by periodical removing biofilm-attached slides for off-line, non-destructive and destructive biofilm analysis without the stoppage of the flow. With this flow cell, biofilm growth and respiratory activity can be easily followed, either in well defined laboratory conditions or in an industrial environment. The reproducible and typical biofilm development curves obtained validated this flow cell and confirmed its potential for different biofilm-related studies, which can include biocidal treatment.

KEYWORDS

Biofilm development; flow cell reactor; hydrodynamic conditions; pulp and paper mills.

INTRODUCTION

In the last few years, attempts have been made to develop laboratory biofilm reactors which minimise variability in experimental conditions and enable real-time evaluation of biofilm build up (Mittelman *et al.*, 1992). Laboratory biofilm research usually involves sophisticated techniques as confocal laser microscopy (CLSM), electrochemical and piezoelectric techniques, which are not practicable in industrial environments. It is recognised that the most trustworthy industrial data were obtained with less advanced methods, either via continuous, on-line fouling monitoring techniques or indirect, off-line techniques, the latter generally including the removal of test surfaces for analysis (Jones and Bradshaw, 1996).

Most laboratory biofilm studies have been done in fixed-support biofilm reactors such as the Roto torque and the Robbins device where the flow must be stopped for slide removal, and started again after closing the system, hence exposing the remaining biofilm to sudden hydrodynamics changes (i.e., non-uniform conditions for biofilm growth).

The present work introduces a simple design for a flow cell reactor, which operates in vertical position, in a continuous recycle mode, and provides controlled environmental conditions for the study of biofilm formation. It allows the study of biofilm processes under selected velocities, in such a way that different hydrodynamic conditions can be tested. This flow cell also permits visual surveillance of biofilm formation and periodical sampling by removing slides without stopping the flow, thus, diminishing the contamination and the disturbance of the biofilm. This flow cell reactor was used to study biofilm formation in a laboratory application and in an industrial setting through the circulation of real process water from the white water circuit of a paper mill.

MATERIALS AND METHODS

Flow Cell Reactor

The flow cell reactor used in this work (Figure 1) consists of a semi-circular perspex duct (45 cm of length and 2.02 cm of hydraulic diameter) with seven apertures on its flat wall, to suitably fit the several adhesion plates. These plates (0.8 x 2.0 cm) are made of stainless steel (ASI 316)

glued to rectangular pieces of perspex that properly fit in the apertures of the flow cell. Their upper faces, where the biofilm is formed, are in contact with the bacterial suspension or with industrial process water that circulates in the semi-circular duct. Each plate can be removed separately in such a way that it does not disturb the biofilm formed on the others and without stopping the flow. This can be managed because the flow cell has on its round surface several outlets ports above the slides that permit the continuous flow of the fluid while the slide is removed.

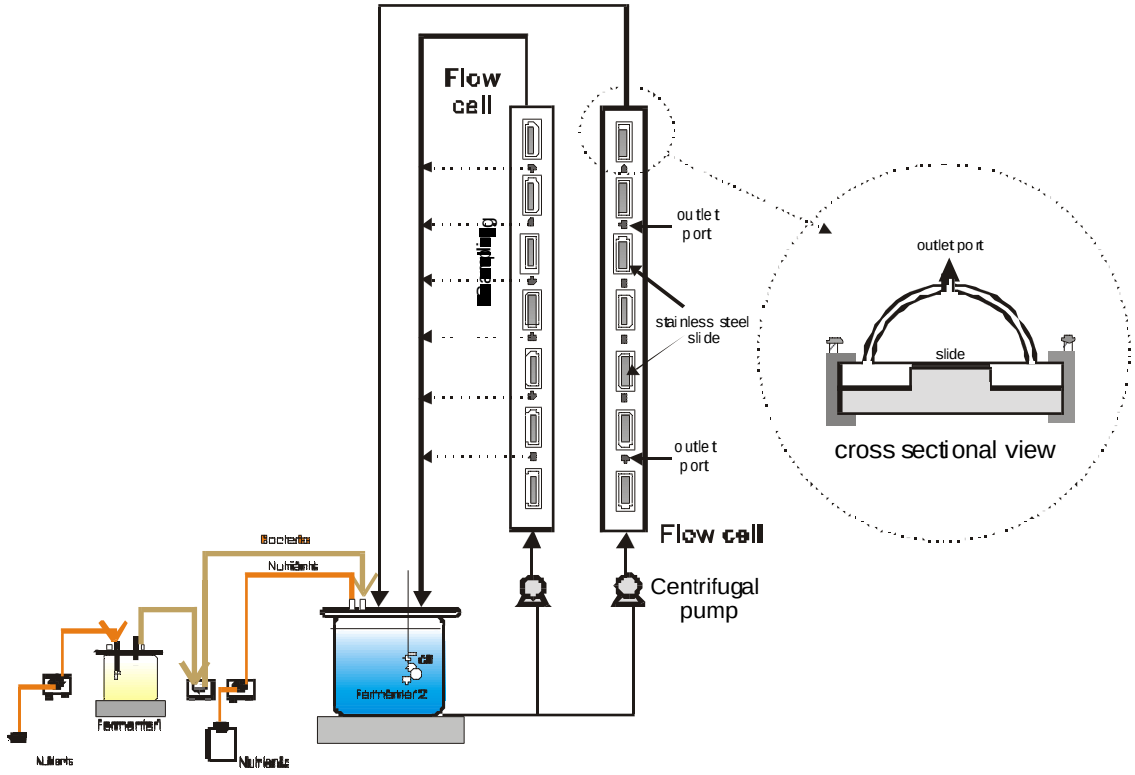

Figure 1. Simplified flow diagram of the experimental system.

Microorganism and Cell Growth

The microorganism used to produce biofilm in the laboratory tests was the Gram-negative aerobic bacteria *Pseudomonas fluorescens*. A pure culture of *P. fluorescens* was grown in a 1 L glass vessel (fermenter 1), aerated and agitated, continuously fed with a sterile nutrient solution consisting of 5 g glucose L^{-1}, 2.5 g peptone L^{-1} and 1.25 g yeast extract L^{-1}, in phosphate buffer at pH 7. This culture was used to continuously inoculate a 3 L vessel (fermenter 2) containing sterile water. The fermenter was fed with the medium described above and the bacterial culture was diluted with filtrated tap water in order to obtain a suspension with $6x10^7$ cells mL^{-1} and 20 mg L^{-1} of glucose.

Reactor Operation

Biofilms were formed on the stainless steel plates placed inside two similar flow cell reactors. One of them was used to promote laminar flows (*Re*=2000, *u*=0.21 ms^{-1}) and the other one turbulent flows (*Re*=5500, *u*=0.56 ms^{-1}). The flow cell reactors were inoculated by recirculating the bacterial suspension, coming from the fermenter 2 (Figure 1).

In the industrial assays, biofilms were formed by the circulation of real process water, coming through a side stream from the “white water” loop of a paper mill.

Every two days, one slide of each flow cell was carefully removed and replaced with a clean slide and the biofilm attached on the slide was collected for further analysis. During the removal of the slide, the water came out of the flow cell through the outlet port immediately below that slide.

Biofilm Properties

In the laboratory tests, the biofilm accumulation was followed by measuring the thickness along time. Thereafter, the biofilm covering the metal plates was totally scraped into a respirometric device (Yellow Spring Biological Oxygen Monitor, Model 53) to evaluate its activity through oxygen consumption as described by Nogueira *et al*. (1998). The biofilm formation in the paper mill assays was assessed by quantification of the biomass attached to the slides, along the time. In both cases, laboratory and industrial tests, some of the biofilm-coated slides were analysed by scanning electron microscopy (SEM).

RESULTS AND DISCUSSION

The first series of experiments was performed in well defined laboratory conditions. The results (Figure 2a) indicate typical biofilm growth curves, although the increase in the flow rate did not seem to cause a significant difference in the accumulation of the biofilm. Nevertheless, it must be emphasised that the higher thicknesses were obtained at lower velocities, this fact being more expressive for longer biofilm formation times (> 7 d). In general, higher flow rates give rise to biofilms strongly adhered to the surface. This may explain the more stable trend of the biofilm growth curve obtained under turbulent flow. The plateau thickness of this biofilm is about 20 μm.

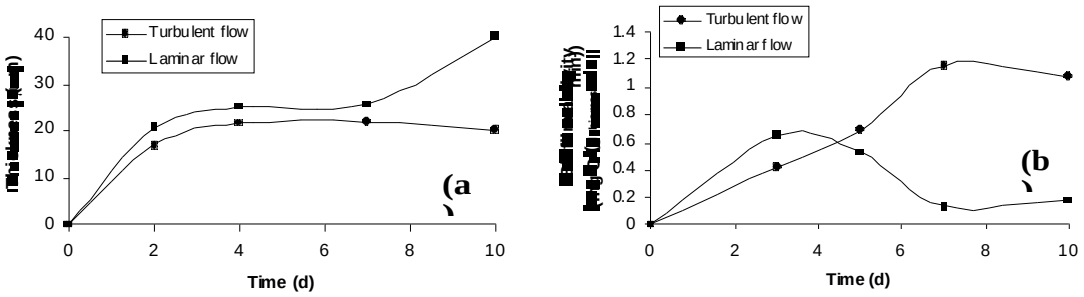

Figure 2. Biofilm thickness values (a) and respiratory activity (b) of biofilms formed under different flow conditions, as a function of time (laboratory tests).

The SEM micrographs of a 10 d biofilm revealed that flow conditions affect the morphology and composition of biofilms. In Figure 3a (turbulent flow) a more visible EPS (exopolymer) slimy matrix involving the cells attached to the slide is seen. This matrix plays an important role in protecting bacteria from external hostile conditions such as harmful substances and hydrodynamic stresses (Christensen, 1990). This may also contribute to explain the stability of the biofilm thickness values obtained under turbulent flow (Figure 2a). In Figure 3b the EPS layer is not evident (individual cells are easily identified).

Figure 3a

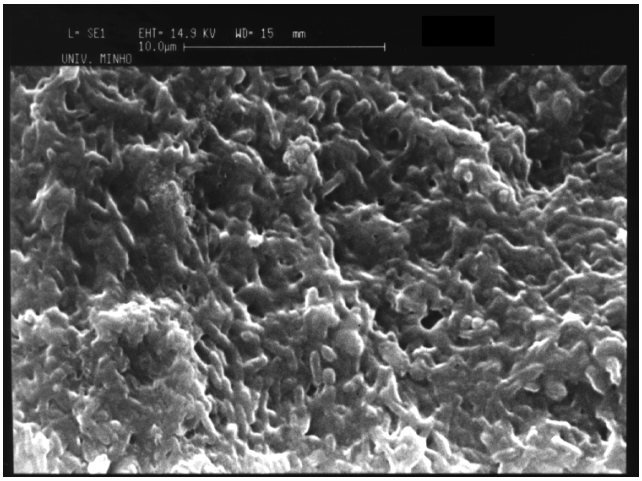

Figure3b

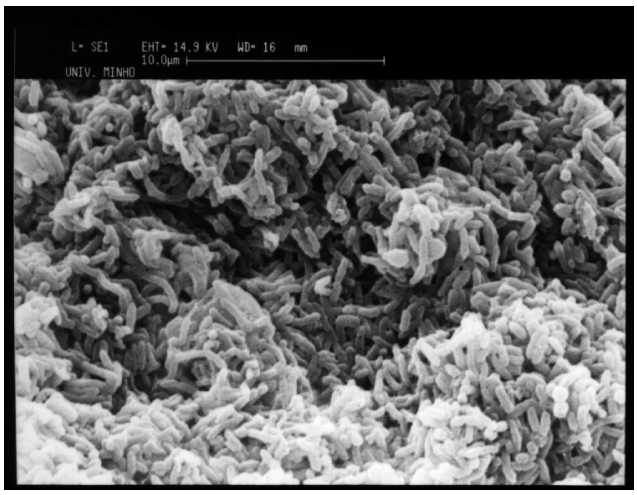

Figure 3. SEM micrographs (3900X) of laboratory biofilms formed on stainless steel slides by *Pseudomonas fluorescens* during ten days: (a) turbulent flow; (b) laminar flow.

In order to study biofilm activity, the respiration rates of the bacteria entrapped in the matrix were measured (Figure 2b). In laminar flow, the fluctuations in biofilm activity are more

pronounced than in turbulent flow, due to possible sloughing off of biofilm mass. This fact is not significant in the biofim formed under turbulent flow on account of the better defined protective EPS matrix.

The second phase of this work comprised the use of the flow cells to develop biofilms in industrial conditions through the circulation of white water of a paper mill. The results (Figure 4), once more, describe typical biofilm growth curves for both laminar and turbulent flows, the latter presenting a more stable trend than the one obtained under laminar flow.

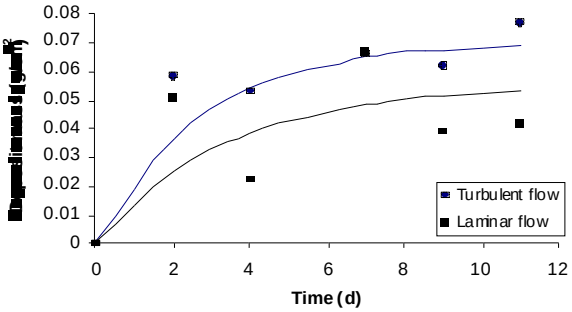

Figure 4. Deposit mass deposited on the steel surfaces, as a function of time (industrial tests).

CONCLUSIONS

The flow cell here proposed proved to be very useful in monitoring biofilm development in laboratory and industrial conditions, since each biofilm-covered slide could be easily removed for off-line, non-destructive and destructive biofilm analysis. The parameters monitored during biofilm growth (accumulation, respiratory activity and morphology), in laboratory well defined conditions or in industrial environments, clearly outlined typical and reproducible biofilm growth curves. The versatility of this flow cell should be emphasised since it can be easily adapted or redesigned according to the technique to be used for biofilm characterisation, as it happens with specific samples for CLSM or SEM. The procedures for the adaptation to the new conditions are basically the scale up (or scale down) of both the flow cells and the slides. Any kind of material can be used for the manufacturing of the slides.

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