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Combining hydrodynamic and enzymatic treatments to improve multi-species thick biofilm removal

Y. Pechaud [a,b,c], C.E. Marcato-Romain [d], E. Girbal-Neuhauser [d], I. Queinnec [e], Y. Bessiere [a,b,c], E. Paul [a,b,c,*]

[a] Université de Toulouse, INSA, UPS, INP; LISBP, 135 Avenue de Rangueil, F-31077 Toulouse, France
[b] INRA, UMR792 Ingénierie des Systèmes Biologiques et des Procédés, F-31400 Toulouse, France
[c] CNRS, UMR5504, F-31400 Toulouse, France
[d] Université de Toulouse, UPS, LBAE, Laboratoire de Biotechnologie Appliquée à l'Agro-alimentaire et à l'Environnement, EA 4565, Institut Universitaire de Technologie, 24 rue d'Embaquès 32000 Auch, France
[e] CNRS, LAAS, 7 avenue du Colonel Roche, F-31400 Toulouse, France

HIGHLIGHTS

- Biofilms were grown under well defined conditions.
- In response to hydrodynamic treatments, both biofilm detachment and compaction occur.
- Enzymatic treatments applied alone do not induce detachment of biofilm.
- Combined treatments greatly improve biofilm removal.

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ABSTRACT

The high water recycling ratio used in some industries can cause nutrients to accumulate in the system, leading to thick, rapidly growing biofilms inducing detrimental effects. Nowadays, chemical treatments are widely used but the increasingly restrictive environmental regulations induce the necessity to take the environmental aspect into account in the choice of a suitable treatment. With this in mind, this study analyses a method for controlling thick, dense biofilm development by applying synergistic actions having low environmental impact, i.e., an enzymatic and a mechanical (shear stress) treatment well suited to industrial water networks. For that purpose, biofilms were grown on plastic plates set in a Couette–Taylor reactor (CTR) that was inoculated using a "white water" sampled from a paper industry network. Development of the biofilms was controlled by applying a constant continuous feeding with a high COD/N ratio of 20, a well-defined shear stress and an external aeration, those conditions leading to a well-controlled G-value and a $\gamma'_{S/O2}$ value close to 1, and consequently, to the development of thick mainly aerobic heterotrophic biofilms representative of that encountered in high loaded industrial water networks. Optimal operating conditions for biofilm treatments were determined considering the penetration time for the enzymatic treatment taking into account both internal and external mass transport (using Biot number calculation). In addition, an optimal shear stress increment of 2.5 Pa was selected in order to maximise biofilm detachment while avoiding compression phenomena due to the mechanical treatment. The combined treatment led to an increase of 80% in biofilm mass removal (COD) compared to the enzymatic treatment alone and removed a large part of the basal layer of the biofilm, 80% reduction being observed in the support coverage.

1. Introduction

The closing of water circuits has been largely applied in industry to reduce water consumption. Consequently, an increase in nutrient concentrations has been observed in pipes, leading to the proliferation of very aged, thick, dense biofilms called slimes (Blanco et al., 1996). These biofilms lead to detrimental effects affecting productivity, safety, product quality and, consequently, product cost (Jass and Walker, 2000). Controlling biofilm development in industrial pipes is therefore a crucial issue (Rochex et al., 2008). Currently, strong oxidizing agents such as chlorine and bromide or organic biocides such as isothiazolones are used, which cause high levels of hazardous by-products (Torres et al., 2011). These biocides and their corresponding by-products present a health risk for operators, lead to wastewater treatment

* Corresponding author at: Université de Toulouse, INSA, LISBP, 135 Avenue de Rangueil, F-31077 Toulouse, France.
E-mail address: paul@insa-toulouse.fr (E. Paul).

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plant failures and can be found in the receiving water, causing environmental health risks. The use of dangerous biocides must be reduced (European Community Regulation on chemicals REACH (EC 1907/2006)) and alternative substances or strategies respecting the environment and compatible with the context of production must be proposed to industries (Xavier et al., 2005).

Biofilms are described as heterogeneous porous media composed of microorganisms embedded in an extracellular polymeric matrix (Van Loosdrecht et al., 2002) which plays an essential role in the cohesiveness and physical properties of the biological structure. Cohesion stratification is observed through the thickness of the biofilm (Coufort et al., 2007; Zhang and Bishop, 1994a) and increases with biofilm age (Ohashi et al., 1999). These physical properties partly explain the high resistance of biofilms to biocides. Consequently, in a nutrient-rich environment, controlling the biofilm growth by means of bactericides or bacteriostatic substances requires intensive treatment consuming large amounts of hazardous substances. It has thus been pointed out that the development of technologies for detaching and dispersing biofilms rather than killing the microorganisms should be the primary desired outcome (Jones et al., 2011).

Enzymatic treatment has received special attention because these molecules can be efficient in decreasing the biofilm cohesion by destroying the physical integrity of the matrix while having no identified negative impact on the environment (Lequette et al., 2010; Nemoto et al., 2000; Simoes et al., 2010; Torres et al., 2011). Hydrolytic enzymes are generally used and the type of hydrolase must be adapted to the biofilm composition (Torres et al., 2011; Marcato-Romain et al., 2012). Moreover, biofilm treatment efficiency depends on the capacity of enzymes to fully penetrate the biofilm (Xavier et al., 2005). External mass transfer in the boundary layer and internal mass transfer into the biofilm structure must therefore be considered (Stewart, 1998). The former depends both on the hydrodynamic conditions and biofilm morphological parameters such as roughness (Eberl et al., 2000). The latter depends on the biofilm physicochemical properties, such as density, porosity and tortuosity (Fan et al., 1990; Zhang and Bishop, 1994b), and also on the physicochemical properties of the diffusing molecule (Stewart, 1998). For large, charged, hydrophobic molecules, the effective diffusion coefficient in biofilms may be reduced by a factor of more than 10 compared to the diffusion coefficient in water (Sandt et al., 2007; Marcotte et al., 2004; Zhang et al., 2011). It is therefore necessary to determine the penetration time for the enzyme used in order to be sure of reaching the basal layer.

Another way to induce significant biofilm detachment is the use of various physical treatments such as ultrasound treatments (Rediske et al., 2000), thermal shocks, or mechanical treatments using pigs or shear stress induced by the fluid hydrodynamics (Eguia et al., 2008). In terms of hydrodynamic treatments, the effect of uniform shear stress, flow inversion and non-uniform distribution of shear stresses has been studied (Coufort et al., 2007; Ochoa et al., 2007; Talvy et al., 2011). However, for, well established biofilms (more than one month), hydrodynamic shears applied alone do not promote sufficient removal since a residual, very cohesive basal layer still remains (Coufort et al., 2007; Derlon et al., 2008; Mohle et al., 2007).

On the other hand, for all treatments currently applied, once the treating action is over, rapid regrowth is observed because of the fast cell growth kinetics (Stewart et al., 1996). It is thus necessary to find solutions that promote significant detachment amplitude including the basal layer. For that purpose, a synergistic approach combining hydrodynamics and treatments using chemicals (oxidizing agents and surfactants) could be used as described by Simoes et al. (2005b). On mono-strain biofilms, these authors showed that, by combining surfactant and shear stress treatment, a 40% increase in biofilm removal was obtained compared to the chemical treatment alone.

Because of the complexity of the biofilm structure, a mainly trial and error approach is often used to determine optimal conditions for biofilm detachment, even though the prediction of biofilm detachment in response to specific treatments is a crucial issue for minimizing treatment costs. Moreover, a problem that is recurrent in the literature and remains unsolved is the difficulty of comparing the efficiency of a given treatment on various biofilms since the biofilms are developed in growth conditions that are insufficiently controlled in terms of both nature of substrate and growth limitations. For comparisons to be valid, two main parameters must be determined and controlled during biofilm development: (i) the parameter $\gamma_{S/O2}$, which compares the penetration of organic substrate to that of oxygen into the biofilm and was proposed to estimate the limiting component (Elenter et al., 2007), (ii) the growth ratio G (Picioreanu et al., 2000), which compares the characteristic times for mass transport and conversion processes.

The present work focuses on biofilms developed under well controlled aerobic conditions (shear stress, substrate loading rates, G ratio, $\gamma_{S/O2}$). Its primary objective is to study the ability of treatments to remove multispecies, thick, dense, heterotrophic biofilms. Two treatments were applied alone and in combination: (1) an enzymatic treatment to reduce the biofilm cohesiveness by hydrolyzing actions and (2) a hydrodynamic treatment to promote detachment by inducing internal shear stresses without involving biofilm compression. The choice of the appropriate enzymes according to biofilm composition and structure has been described by Marcato-Romain et al. (2012) who recommend the use of Savinase®, a commercially available protease formulation. The determination of the required contact time for enzymatic treatment will be presented in the Material and Methods section. The present results will thus successively consider: (i) the identification of the optimal shear stress increment to be used for maximizing reduction of such biofilms; (ii) the evaluation of the synergistic effect of the enzymatic action plus the hydrodynamic effect to promote massive detachment including the biofilm basal layer. Moreover, the efficiency of the treatment with the non-consumed enzyme (penetration linked only to effective diffusion time scale) is compared with a treatment using NaClO, which is consumed by the oxidizing reaction with biofilm components (penetration linked both to diffusion and reaction processes time scales).

2. Material and methods

2.1. Biofilm development in Couette–Taylor reactor (CTR)

A CTR, named CTR 1, was used for biofilm growth under controlled shear stress conditions (see Fig. 1). It consisted of two concentric cylinders with an annular flow generated between the fixed external cylinder and the rotating inner cylinder. The size of the gap between the inner and outer cylinders was 15 mm. Rectangular polyethylene plates (100×50 mm) placed on the inside face of the external cylinder were used to develop biofilms. Inoculation was performed by maintaining a diluted solution of "white water", sampled from the primary water network of a paper plant, in the CTR for 24 h. This solution contained a complex mixture of microorganisms. Indeed, by a CE-SSCP analysis, another research laboratory (INRA-LBE Narbonne, France) has observed that the inoculum was composed by several dominant and subdominant microbial species. Then, the liquid was removed from the reactor and a constant substrate loading rate of 10 g COD m^{-2} d^{-1} was applied. A high COD/N ratio (20) was chosen to reproduce the high carbon content and low nitrogen content observed in many industries such as the paper industry (Rochex and Lebeault, 2007). Moreover, to reproduce situations observed in industrial water networks,