

# Monitoring cell detachment by surfactants in a parallel plate flow chamber

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**Abstract** The efficacy of the surfactants SDS and CTAB in detaching *P. fluorescens* from glass surface was evaluated in a parallel plate flow chamber. This device enables “in situ” determinations of cells detachment following the application of surfactants under well controlled hydrodynamic conditions. The results showed that SDS was able to remove almost all adhering bacteria in a short period of time, whereas CTAB did not promote much cell desorption. On the contrary, this surfactant increased the adhesion strength between cells and glass. Both surfactants promoted different alterations of cell surface properties, which explains their dissimilar effectiveness as cleansing agents.

**Keywords** Surfactants; parallel plate flow chamber; detachment; hydrophobicity; surface charge

## Introduction

Surfactants have been intensively used in the disinfection of industrial equipment, especially in food industry (Zottola and Sashara, 1994) and membrane technologies (Flemming *et al.*, 1997). Although there is a great variety of commercial available surfactants, the most commonly used are anionic and cationic. Both types have a dual role in disinfection, they can inactivate leaving cells (McDonnell and Russell, 1999) or/and they alter the surface properties of the attachment substratum, thereby either preventing attachment (Campbell *et al.*, 1999) or promoting detachment of the adhering cells (Eginton *et al.*, 1998). The efficacy of surfactants in removing attached cells has been thoroughly evaluated, however most of the studies are based on static attachment and detachment assays. In this type of experiment hydrodynamic conditions are poorly controlled. It is well documented that shear rate influences cell detachment (Meinders and Busscher, 1993). So, to study the rate of cell detachment following the application of surfactants well controlled hydrodynamic condition should be created. Flow devices, designed to study microbial adhesion to substratum under carefully controlled hydrodynamic and mass transport conditions, constitute a powerful technique to test the efficacy of surfactants. In this study the rate of cell detachment after the application of a cationic (cetyltrimethyl ammonium bromide-CTAB) and an anionic surfactant (sodium dodecylsulfate-SDS) was studied in a parallel plate flow chamber. The results were interpreted according to the alterations of bacterial cell surface properties induced by the surfactants.

## Material and Methods

### Microbial cells and surfactants

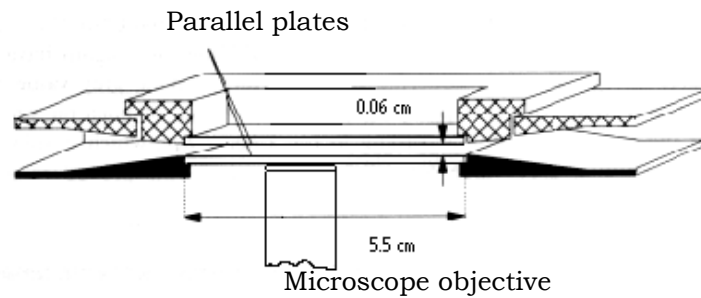
*Pseudomonas fluorescens* ATCC 13525, a Gram-negative aerobic bacterium, was used in this work.

A continuous pure culture of the *P. fluorescens* was grown in a 2 L glass fermenter, at  $27\pm 1^\circ\text{C}$ , suitably aerated and magnetically agitated. The fermenter was continuously fed with 0.05 L/h of a sterile nutrient solution consisting of 5 g glucose  $\text{L}^{-1}$ , 2.5 g peptone  $\text{L}^{-1}$ , 1.25 g yeast extract  $\text{L}^{-1}$  in phosphate buffer at pH 7. A suitable amount of *Pseudomonas fluorescens* culture was removed from the fermenter, centrifuged (5000 rpm, 5 min,  $4^\circ\text{C}$ ) and washed twice with sterile citrate buffer (pH 6.0; 0.05 M).

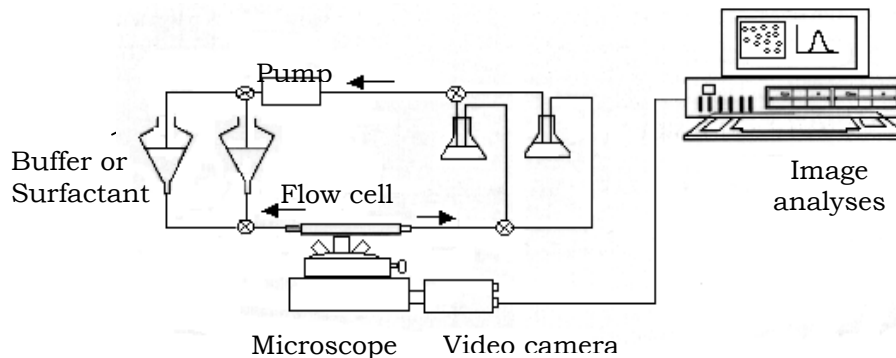
The surfactants used were sodium dodecylsulfate (SDS) and cetyltrimethyl ammonium bromide (CTAB), prepared in solutions with concentrations under their CMC; 1mM and 0.5 mM respectively.

### The parallel plate flow chamber

The parallel plate flow chamber used in these experiments is depicted in Figure 1. This device has been extensively described by Sjollem *et al.* (1989). Briefly, the chamber consists of a nickel-coated brass with dimensions  $16\times 8\times 1.8$  cm. The bottom and top plates with dimensions of  $5.5\times 3.8$  cm are made of glass. A Teflon spacer is placed in between the two plates yielding a separation distance of 0.06 cm. The device is mounted in an inverted microscope (Nikon, Diaphot 300). The images are acquired in a CCD camera (Sonny, AVC-D5CE) connected to the microscope and coupled to an image analyser (Image Proplus 3.0; Media Cybernetics, Maryland) installed in a 166 MHz personal computer. With this set-up direct and “in situ” observations of the attachment and detachment processes are possible (Figure 2)



**Figure 1** Schematic representation of the parallel plate flow chamber used



**Figure 2** Schematic representation of the experimental set-up used

### Attachment and Detachment assays

A *Pseudomonas fluorescens* suspension ( $6 \times 10^6$  cells/cm<sup>3</sup> prepared in citrate buffer pH 6.0; 0.05M) was circulated through the parallel plate flow chamber at a flow rate of 0.11 ml/s until a certain surface coverage was achieved. Thereafter the flow was switched to citrate buffer (pH 6.0; 0.05M) to remove all unattached bacteria. After 30 minutes of buffer circulation the surfactant was mixed with the buffer until a final concentration of 0.5 mM for CTAB and 1mM for SDS. The surfactant solution was then circulated at the same flow rate. Images were grabbed every 15 seconds and the data obtained was expressed as number of cells attached per cm<sup>2</sup>.

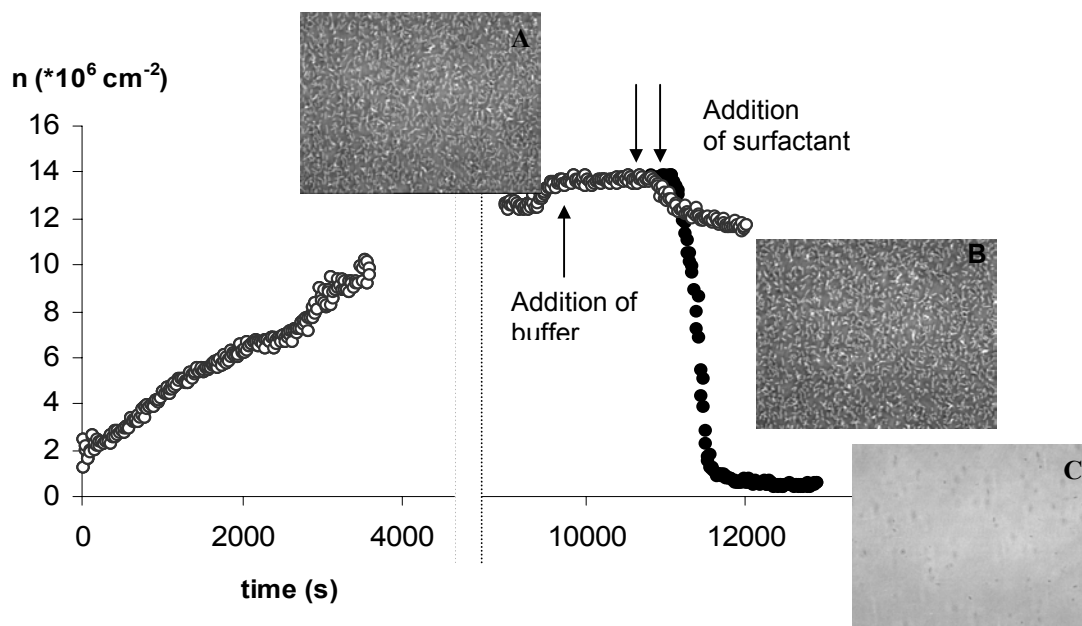
### Determination of cell surface properties

*Pseudomonas fluorescens* prepared as described above were suspended in the solutions of surfactants prepared with citrate buffer (0.1 mM for SDS and 0.5 mM for CTAB) until a final concentration of  $6 \times 10^6$  cells/cm<sup>3</sup>. These suspensions were incubated at room temperature during 30 minutes after which the surface properties of the cells were determined. Cell surface hydrophobicity was determined as described elsewhere (Azeredo *et al.*, 1998). Zeta potentials were measured in a Zeta-Meter 3.0+.

### Results and Discussion

Figure 3 presents the attachment and detachment kinetics of *Pseudomonas fluorescens* from glass after the application of SDS and CTAB. During the period of buffer circulation of the buffer no significant decrease in cell number was observed. After SDS application almost all attached cells were removed from the surface and the maximum rate of detachment was in average 294 cells s<sup>-1</sup>mm<sup>-2</sup>. This surfactant has been proved to be effective in the inhibition of cell attachment to surfaces (Campbell *et al.*, 1999), however its efficiency in removing attached cells is still not clear. Campbell *et al* (1999) did not find a significant effect of SDS in detaching cells from hydrophylic reverse osmosis membranes. Considering CTAB, this cationic surfactant did not promote a significant cleansing of the surface observed in image B of Figure 3. Although the maximum rate of cell detachment was in average 30 cells s<sup>-1</sup>mm<sup>-2</sup> (approximately 10 times lower that that of SDS), after a short period of time this surfactant was no longer able to remove adhering cells (Figure 3).

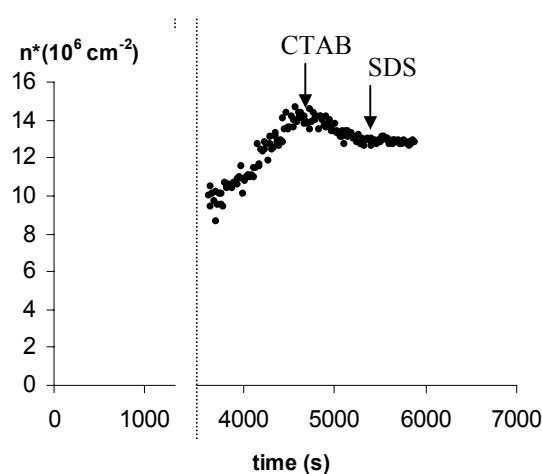
CTAB is a cationic surfactant, so it binds by chemisorption to bacterial cell surfaces, due to the electrostatic attraction, influencing the zeta potential of the cell (Neu, 1996). In fact, cells treated with CTAB become positively charged (Table 1). Accordingly, a great detachment was not observed, probably due to the increase of the electrostatic attraction to glass surface. In fact, CTAB cemented the cells rather than removing them from the surface. This is well elucidated in Figure 4 in which SDS was not able to detach cells after the application of CTAB.



**Figure 3** Kinetics of *P. fluorescens* attachment and detachment after the application of CTAB (○) and SDS (●) (n stands for number of cells per cm<sup>2</sup>) and respective images (A: surface covered with cells prior to the application of surfactants; B: surface after the application of CTAB; C: surface after the application of SDS).

**Table 1** Modification of the surface properties of *P. fluorescens* by SDS and CTAB

Cells	Hydrophobicity ( $\Delta G_{iwi}$ ; mJ/m <sup>2</sup> )	Zeta potential (mV)
<i>P. fluorescens</i>	+24.8	-16.1
<i>P. fluorescens</i> + SDS	+2.4	-22.4
<i>P. fluorescens</i> + CTAB	+33.4	+33.4



**Figure 4** Kinetics of *P. fluorescens* attachment and detachment after the application of CTAB followed by SDS (n stands for number of cells per cm<sup>2</sup>).

The efficacy of SDS in removing attached cells can be explained by the modification of cell surface properties induced by this surfactant. From Table 1 it can be seen that cells become less hydrophilic (the free energy of hydrophobic interaction  $\Delta G_{\text{wi}}$  is less negative) and more negatively charged after being treated with SDS. The alteration in cell surface hydrophobicity can be explained by the fact that SDS anchors to the cell surface through its polar part, exposing the apolar part. This is supported by the fact that the free energy of attraction of the cells to the polar part of the molecule ( $=-85.6 \text{ mJ/m}^2$ ) is greater than that to the apolar part of the molecule ( $=-47.9 \text{ mJ/m}^2$ ). This decrease in cell surface polarity promoted a decrease of the free energy of attraction of cells to glass (of about  $28.3 \text{ mJ/m}^2$ ). On the other hand, the increase in the negative cell zeta potential could also contribute to an increase in the electrostatic repulsion towards the glass surface.

## Conclusions

Studies of the cleansing effect of surfactants can be done in a parallel plate flow, which enables “in situ” and real time observation of cell detachment, under well-controlled hydrodynamic conditions.

SDS showed a good cleansing capability of glass surface due to the alterations of the surface properties of cell walls induced by this surfactant. CTAB interacted with the cells surfaces of *P. fluorescens*, turned them positively charged and thus strengthened the interaction with glass. Thus CTAB in spite of removing bacteria cements them. This highlights that changes in the attachment strength following treatment can have serious implications on the effectiveness of the surface cleansing and disinfection.

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