



BIOTECH - GERMANDE

H Y G I E N E - F O R M A T I O N - E V A L U A T I O N
R E C H E R C H E & D E V E L O P P E M E N T

COMPARISON OF DETERGENT ACTIVITY OF TWO TYPES OF CLEANERS ("BRUSH" AND DISPO'CLEAN CLEANER)

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ENGLISH TRANSLATION

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I: DESCRIPTION OF THE STUDY:

Title:	Comparison of the detergent activity of two types of cleaners ("brush" cleaner vs. Dispo'Clean cleaner) vis-à-vis PTFE tubes contaminated on their inner surface by a mono-bacterial biofilm with <i>Pseudomonas aeruginosa</i> .
Internal code:	Study no.: 196.ODO.03
Sponsor:	ODON life technologie Zone industrielle 14260 Aunay Contact: Mr Serge NOUDELBERG
Period of study:	4 February 2004 to 28 March 2004
Person responsible for the study:	Dr Lionel PINEAU (Microbiologist – Laboratory Director).
Tests carried out by:	Audrey RIBIOLLET (Test manager) Delphone AVON (Logistics manager)
Test laboratory:	BIOTECH-GERMANDE Laboratory Luminy Science Park 163 Avenue de Luminy – Box 927 13288 Marseille Cedex 9

II: PURPOSE OF THE STUDY

To compare the activity of 2 types of cleaners ("brush" cleaner vs. Dispo'Clean cleaner) vis-à-vis a complex stain of the biofilm type with *Pseuomonas aeruginosa* formed inside a PTFE tube with an inside diameter of 4 mm.

III: PRINCIPLE

PTFE tubes with an inside diameter of 4 mm are artificially contaminated by a monobacterial biofilm with *Pseudomonas aeruginosa* according to a defined experimental protocol. After a homogeneous, stable biofilm is obtained (approx. 10^8 bacteria/cm²), PTFE tube portions approximately 60 cm long (contaminated on their inner surface by the biofilm) are cut then subjected to the cleaning procedure.

The detergent activity of each cleaning procedure tested ("brush" cleaner and Dispo'Clean cleaner) is quantified by monitoring, after each passage of the cleaner (1 passage = 1 outward-return movement):

- ✓ the development of the number of viable bacteria adhering to the inside of the biofilm
- ✓ the development of the residual quantities of proteins and polysaccharides on the surface of the medium

In parallel with these tests a control procedure, including initial washing with 50 ml of sterile distilled water, then 5 successive irrigations with 50 ml of water from the bath is tested.

IV: EQUIPMENT AND METHOD:

a) Cleaners:

i) “Brush” cleaner (cf. photo no. 1):

Nature of the product:	Disposable gastr-Colo.
Reference of the product:	05-BR-ENDO
Batch number:	940084/3019
Manufacturer:	Odon Life-Technology
Delivery date:	04 February 2004
Brush delivery:	6 mm

ii) “Dispo’Clean” cleaner (cf. photo no. 2):

Nature of the product:	Disposable cleaner
Reference of the product:	NC
Batch number:	NC
Manufacturer:	Odon Life-Technology
Delivery date:	04 February 2004
Diameter:	4.2mm

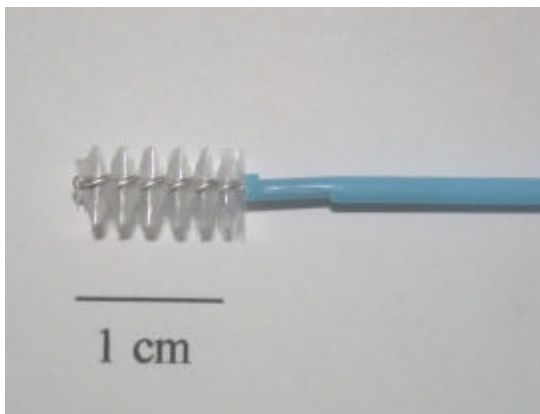


Photo no.1: “Brush” cleaner

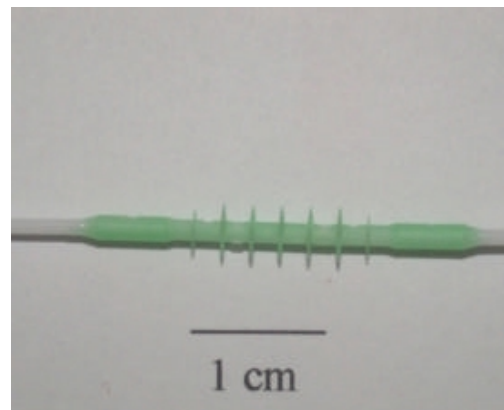


Photo no. 2: “Dispo’Clean” cleaner

b) Bacterial colony (culture):

Pseudomonas aeruginosa CIP A22

c)

Culture environments:

i) Maintenance environment and microorganism counting environment:

Trypticase soya: Biomérieux, 51044
Batch no.: 777959501

ii) Liquid atmosphere used for forming the biofilm:

Composition of the substrate used to supply the circuit and promote the formation of the biofilms:

Animated acids (DIFCO, 228820, batch no.: 144194)	0.1 g/l
Yeast extract (SIGMA, 7Y-1625, batch no.: 81K0354)	0.1 g/l
MgSO ₄ , 7H ₂ O (FLUKA, 61138, batch no.: 41094/1)	0.2 g/l
FeSO ₄ , 7H ₂ O (SIGMA, F-2387, batch no.: 49H3647)	0.0005 g/l
Anhydrous Na ₂ HPO ₄ (SIGMA, S-9763, batch no.: 42K0186)	1.25 g/l
KH ₂ PO ₄ (SIGMA, P-0662, batch no.: 29H0005)	0.5 g/l
Lactose (PROLABO, 29946294, batch no. D97G)	0.025 g/l

iii) Recuperation solution:

Lecithin (SIGMA, P-5394, batch no.: 128H8003)	2% (w/v)
Sodium thiosulphate (SIGMA, S-8503, batch no.: 41K0256)	0.5% w/v)
Tween 80 (SIGMA, P-1754, batch no.: 102K0034)	10% (v/v)
Histidine (SIGMA, H-8000, batch no.: 11K0894)	1% (w/v)
Trypticase-soya broth (Biomérieux, 51019, batch no.: 774802201)	q.s.p. 100 ml

Internal reference

810.1.7, 816.1.1

iv) Protein dosage:

UPTIMA kit	UP75860a
	Batch no.
	D05KL03

v) Polysaccharide dosage

Phosphate buffer	SIGMA, P-0662, batch no. 29H0005.
Phenol	SIGMA, P-4161, batch no.: 39H0910
H ₂ SO ₄	FLUKA, 84727, batch no.: 425763/1
Glucose	SIGMA, G-5767, batch no.: 51K0062

vi) Scanning electron microscope:

Manufacturer	MEB, field emission gun
Make	Jeol
Series	6320F
Specificity	Linked to an EDS analyser (TRACOR 5500 series 2)

vii) PTFE tube:

Manufacturer	Bioblock
Reference	82854
Batch no.	1130201

a) Formation of the biofilms / experimental assembly (Figure no. 1)

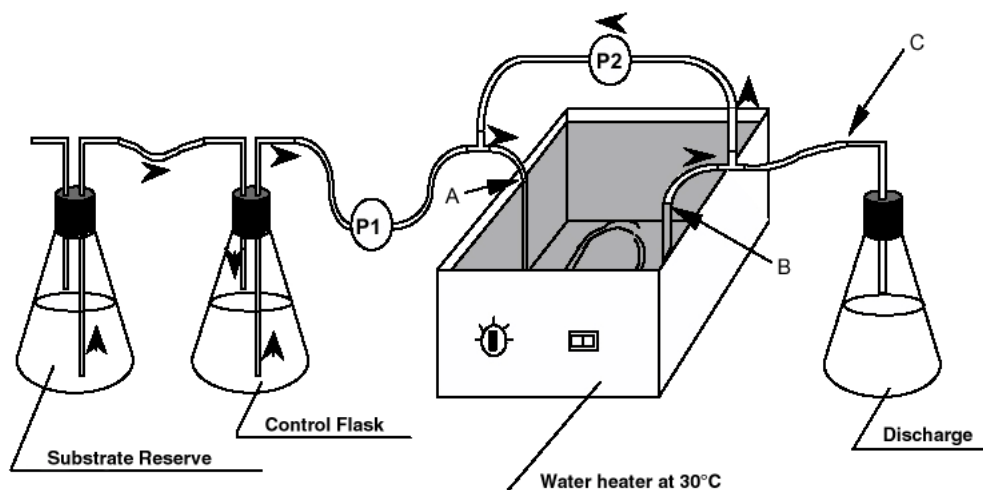


Figure 1: *Experimental device for the formation of biofilms:* P1: Substrate feed pump (2.5 to 3 ml/min), P2: Recirculation pump (100 ml/min), A-B: positioning zone for PTFE tube portions, B: Point of inoculation of the circuit.

i) **Experimental assembly:**

The two pumps used serve, respectively:

- to feed the circuit with substrate (pump no. 1). The pump flow is then regulated between 2.5 and 3 ml/min.
- to agitate the contents of the circuit (pump no. 2), and its speed of rotation is of the order of 40 rpm, i.e. 100 ml/min (laminar flow)

The PTFE tube portions 60 cm in length, intended to be used for the tests, are positioned inside the circuit (between points A and B).

The entire experimental assembly shown in Figure 1 is placed under a laminar flow hood.

ii) **Inoculation of the circuit:**

After filling the circuit with the culture atmosphere, the flows of pumps 1 and 2 are regulated. The circuit is then inoculated by injecting at point B 5 to 10 ml of a bacterial suspension containing approx. 10^8 cells per ml (pump no. 1 stopped). The volume of the inoculum is calculated so that a concentration of microorganisms of the order of 10^7 bacterial per ml is obtained within the circuit.

Once the inoculum has been added the circuit is kept under agitation for some twenty minutes (pump no. 1 stopped, pump no. running), in order to distribute the bacteria in the inoculum throughout the circuit. After this homogenisation phase the circuit is again fed with substrate by pump no. 1).

iii) **Sampling from the PTFE tube portions**

After about 72 hours of incubation pumps 1 and 2 in the biofilm formation circuit are immediately stopped and the PTFE tube portions that are to be used for the tests are removed from the circuit after their outer surface has been disinfected with a sterile compress soaked in alcohol at 95°.

b) Determination of the detergent activity vis-à-vis biofilms:

The PTFE tube portions sampled (V-a-iii) are immersed in a tank containing 1 litre of sterile distilled water and subjected to the tested procedure. Three cleaning procedures are tested.

- ✓ “Dispo’Clean” procedure: immersion of the test tube portion in the sterile distilled water, irrigation of the test tube with 50 ml of sterile distilled water and cleaning (5 passages = 5 outward-return movements) with the Dispo’Clean cleaner.
- ✓ “Brush” procedure: immersion of the test tube portion in the sterile distilled water, irrigation of the test tube with 50 ml of sterile distilled water and cleaning (5 passages = 5 outward-return movements) with the Odon cleaner (brush 6 mm in diameter).
- ✓ Procedure 3: immersion of the test tube portion in the sterile distilled water, irrigation of the test tube with 50 ml of sterile distilled water, followed by 5 successive irrigations with 50 ml of water from the heater (control).

For procedures 1 and 21, after each outward-return movement, the visible impurities are removed from the cleaner and the tube is washed with 50 ml of water taken from the tank in order to discharge all the impurities released from the biofilm but still present in the tube.

Before immersion in the sterile water tank (initial level of contamination), and after each “cleaning + washing” sequence (or after each irrigation of the duct for the control procedure), 4 tube portions (each approx. 2 cm long) are sampled then analysed:

- two portions are used for counting the viable bacteria still fixed to the inner surface of the PTFE tube (V-b-i),
- two portions for dosing the residual quantities of proteins and polysaccharides on the inner surface of the PTFE tube (V-b-ii and V-b-iii).

- i) Determination of the number of viable bacteria fixed on the inner surface of the PTFE tube:

Each portion of the PTFE tube sampled (approx. 2 cm long) is immersed in 15 ml of recuperation solution and cut longitudinally into four identical sections. 5 grams of sterile sand are added to the tube containing the PTFE tube portion and the 15 ml of recuperation solution, and the whole is then subjected to Vortex type agitation (Vortex 2, shake 5, Scientific Industries, Bioblock, FRANCE) for exactly 12 minute. The purpose of this operation is to return to suspension the residual microorganisms of the biofilm.

From these 15 ml of neutraliser (parent suspension) a series of $1/10^{\text{th}}$ dilutions is carried out and 2×1 ml of each of these dilutions is cultured by inclusion in a trypticase soya medium (Biomérieux). After incubation of 24 to 48 hours at 37°C in aerobiosis, the colonies appearing on the Petri dishes are counted and the results expressed in the number of viable bacteria per cm^2 of PTFE tube (N_t).

- ii) Polysaccharide dosage: method of Dubois et al. (1956):

This dosage is based on the formation of furfuralic compounds by heating of neutral oses in a concentrated sulphuric atmosphere which, with the phenol, gives compounds with a yellow coloration dosed by spectrophotometry at an optical density of 490 nm.

After each “cleaning + washing” sequence (or after each irrigation in the case of the control procedure), two portions of the PTFE tube subjected to the test are sampled and each immersed in 3 ml of sterile distilled water in which the inner surface of the tube is scraped. After each “cleaning + washing” sequence the residual concentration of polysaccharides per cm^2 of the PTFE tube ($[\text{Pol.test}]_m$) is determined from the 3 ml of sterile distilled water in which the test tube portion is scraped.

- (iii) Protein dosage: method of Lowry et al. (1951):

The micro BC colorimetric method is based on the reduction of Cu^{2+} to Cu by the peptidic bonds of the proteins and formation of a purple soluble complex after reaction between the Cu and bicinchoninic acid.

The dosing is carried out on the same solutions as those previously prepared for polysaccharide dosing.

For each “cleaning + washing” sequence (or after each irrigation in the case of the control procedure), the residual concentration of proteins per cm^2 of PTFE tube ($[\text{Prot.test}]_n$) is determined from the 3 ml of sterile distilled water for scrapping the test tube portion.

- (iv) Observation in scanning electron microscopy:

In order to confirm or invalidate the results of the bacterial counts and protein and polysaccharide doses, an analysis under the scanning electron microscope of the inner surface of each PTFE tube tested is carried out before immersion in the tank of sterile distilled water, then after 3 and 5 outward and return movements.

Each portion of the tube sampled is then:

- Fixed by immersion in a 2.5% glutaraldehyde solution
- Dehydrated by successive immersions in a series of 30, 50 and 75% ethanol solutions
- Metallised in fine gold.
- Examined under a scanning electron microscope.

		Number of Cleaner Passages						
		0	1	2	3	4	5	
CONTROL PROCEDURE	Fixed Bacteria (log ₁₀ nb.UFC/cm ²)	\overline{x}	8,34	8,21	8,34	8,42	8,15	8,12
		?	0,02	0,17	0,18	0,02	0,12	0,01
	[Proteins] (μg/cm ²)	\overline{x}	46,04	40,03	40,45	30,79	31,16	28,60
		?	5,18	0,37	1,26	0,75	1,49	2,19
	[Polysaccharides] (μg/cm ²)	\overline{x}	16,52	6,79	6,64	6,79	4,46	1,93
		?	1,98	0,63	0,87	1,19	0,04	0,91
BRUSH CLEANER	Fixed Bacteria (log ₁₀ nb.UFC/cm ²)	\overline{x}	7,97	7,38	6,92	6,55	6,18	6,16
		?	0,21	0,31	0,15	0,28	0,03	0,02
	[Proteins] (μg/cm ²)	\overline{x}	34,48	3,64	1,17	2,10	1,45	0,75
		?	1,82	0,19	0,33	0,23	0,79	0,47
	[Polysaccharides] (μg/cm ²)	\overline{x}	12,77	6,28	3,27	2,21	0,00	0,00
		?	0,75	0,67	0,20	0,55	-	-
DISPO'CLEAN CLEANER	Fixed Bacteria (log ₁₀ nb.UFC/cm ²)	\overline{x}	8,10	6,82	6,47	6,42	6,32	6,21
		?	0,04	0,24	0,12	0,11	0,03	0,04
	[Proteins] (μg/cm ²)	\overline{x}	45,81	2,75	1,59	2,66	1,26	1,87
		?	0,93	0,89	0,19	0,7	0,14	0,19
	[Polysaccharides] (μg/cm ²)	\overline{x}	10,95	2,52	1,34	1,18	0,71	0,00
		?	0,51	0,71	0,08	0,08	0,71	-

\bar{x} = mean, ? = standard deviation

Table No. 1: Activity of 2 cleaning procedures (“brush” cleaner vs. Dispo’Clean cleaner) vis-à-vis PTFE tubes contaminated by a biofilm with *Pseudomonas aeruginosa* CIP A22:

Development of the number of viable bacteria (log.) present within the biofilm, the residual concentrations of proteins ([prot.test]) and polysaccharides ([poly.control]) on the inner surface of the PTFE tube, as a function of the number of passages of the cleaner and related to a control procedure.

The values of the concentrations of viable bacteria and the quantities of proteins and polysaccharides still present on the surface of the test media, as a function of the number of passages, for the two types of cleaner ("brush" cleaner and "Dispo'Clean" cleaner), and for the control procedure, are shown in Table no. 1. Figures 1, 2 and 3 indicate for the three procedures tested respectively, the developments of the number of viable bacteria on the surface of the test medium (Figure no.1) and the developments of the residual concentrations of proteins (Figure no. 2) and polysaccharides (Figure no. 3).

The analysis of the developments of the number of viable bacteria present on the surface of the PTFE tubes (cf. Table 1 and Figure no. 1) shows that in the case of the control procedure the water circulation has no influence on the number of adhering viable bacteria. Therefore, after 6 successive irrigations with 50 ml of water, the number of viable bacteria is always of the order of $1.3 \cdot 10^8$ UFC/cm² before treatment.

Within the framework of the two procedures involving cleaning of the test tube, a considerable reduction in the number of adhering bacteria as a function of the number of passages is observed. For the two procedures tested the number of viable bacteria per unit of area is therefore reduced from approx. 10^8 UFC/cm² to $1.6 \cdot 10^6$ UFC/cm² (approximately 6.2 logarithmic units) after 5 passages (5 outward-return movements).

A first analysis of Figure no. 1 shows that the curves plotting the development of the number of viable bacterial on the surface of the medium against the number of passages tends toward a minimum value (approx. 10^6 UFC/cm²) below which it seems impossible to descend, even by increasing the number of passages.

Although the number of residual bacteria is identical for the two procedures, even after 5 passages, the profiles of the curves plotting the development of the number of viable bacterial on the surface of the medium against the number of passages are different. For "brush" type cleaners a relatively linear, slow reduction in the number of viable bacteria present on the medium is observed, and the minimum value is not reached until 4 cleaner passages. In the case of the procedure in which "Dispo'Clean" cleaners are used, the reduction in the number of variable bacteria on the medium takes place faster and the minimum value is reached almost immediately after the first passage of the cleaner.

These initial results appear to indicate that the two cleaning procedures tested give rise to a maximum reduction in the number of adhering viable bacteria within the biofilm of approximately 2 logarithmic units. This optimum efficiency is reached immediately after the first passage in the case of the "Dispo'Clean" cleaners, whilst 4 passages are required in the case of the "brush" type cleaners to reach the threshold value.

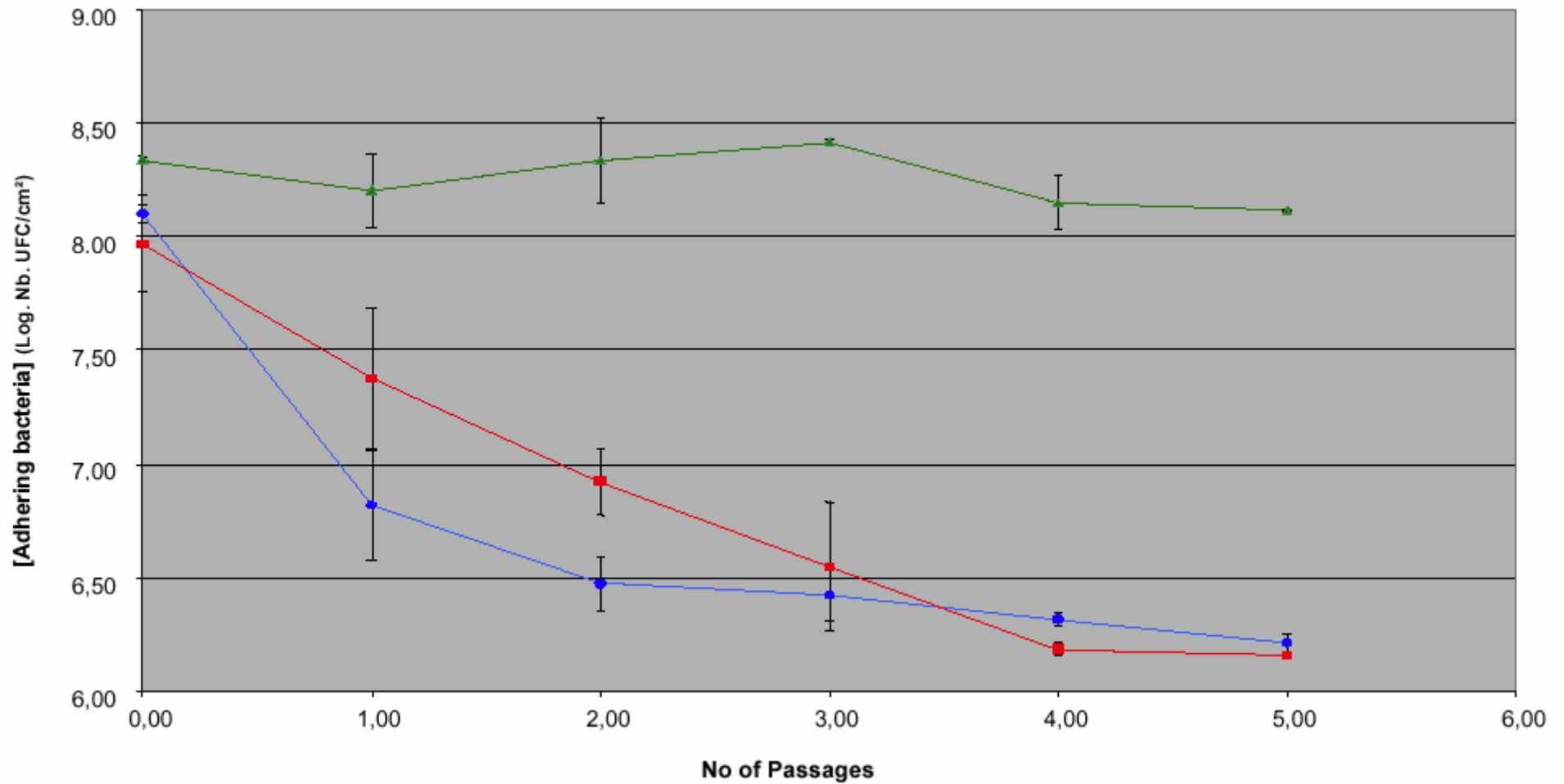


Figure 1: Activity of 2 cleaning procedures (“brush” cleaner vs. Dispo’Clean cleaner) vis-à-vis Teflon tube contaminated by a biofilm with *Pseudomonas aeruginosa* CIP A22: Development of the number of adhering viable bacteria on the inner surface of the Teflon tube plotted against the number of passages of the cleaner for each procedure tested [“brush” cleaner (■) “Dispo’Clean” cleaner (●)] in relation to the control procedure (▲).

The analysis of the development of the residual quantities of proteins on the surface of the medium (cf. table 1 and Figure no. 2) confirms the minimal effect of the water circulation in the PTFE tube artificially contaminated by a biofilm with *Pseudomonas aeruginosa*. In the case of the control procedure, although a slight reduction in the residual concentration of proteins is observed on the surface of the medium as a function of the number of volumes of water circulated in the tube, over 60% of the initial quantities of proteins is still present on the medium surface after circulation of 6 x 50 ml of water.

The results obtained for the two procedures involving cleaning of the test surfaces are essentially identical, with a fast, major reduction in the residual protein concentrations from the first passage, followed by stabilisation to values lower than 3 $\mu\text{g}/\text{cm}^2$. The reductions in the initial quantities of proteins are then approximately 90% for the “brush” cleaners, and 94% for the “Dispo'Clean” cleaners after a single passage.

For the two cleaning procedures a reduction in the residual concentrations of polysaccharides is also observed on the medium surface, and after 5 procedures no residual trace of carbohydrates is observed on the surface of the medium. Just as in the case of monitoring the bacterial contamination, the profile of the curves plotting the development of the residual quantities of polysaccharides on the medium surface against the number of passages of the cleaner appears to indicate that the “Dispo'Clean” cleaner gives rise to a faster reduction of the different constituents of the biofilm. Thus if, after a single passage of the “Dispo'Clean” cleaner, almost 77% of the initial quantities of polysaccharides has been eliminated, 2 to 3 passages will be required to achieve the same rate of reduction with the “brush” cleaner. Finally, if it is considered that the bacterial biofilm is a homogeneous mixture of bacteria, proteins and polysaccharides, the measured rates of reduction of the initial quantities of proteins and polysaccharides (approx. 90% after 5 passages) are compatible with the values of the logarithmic reductions of the adhering bacteria flora, since a reduction of 2 logarithmic units corresponds in fact to a reduction of 99%.

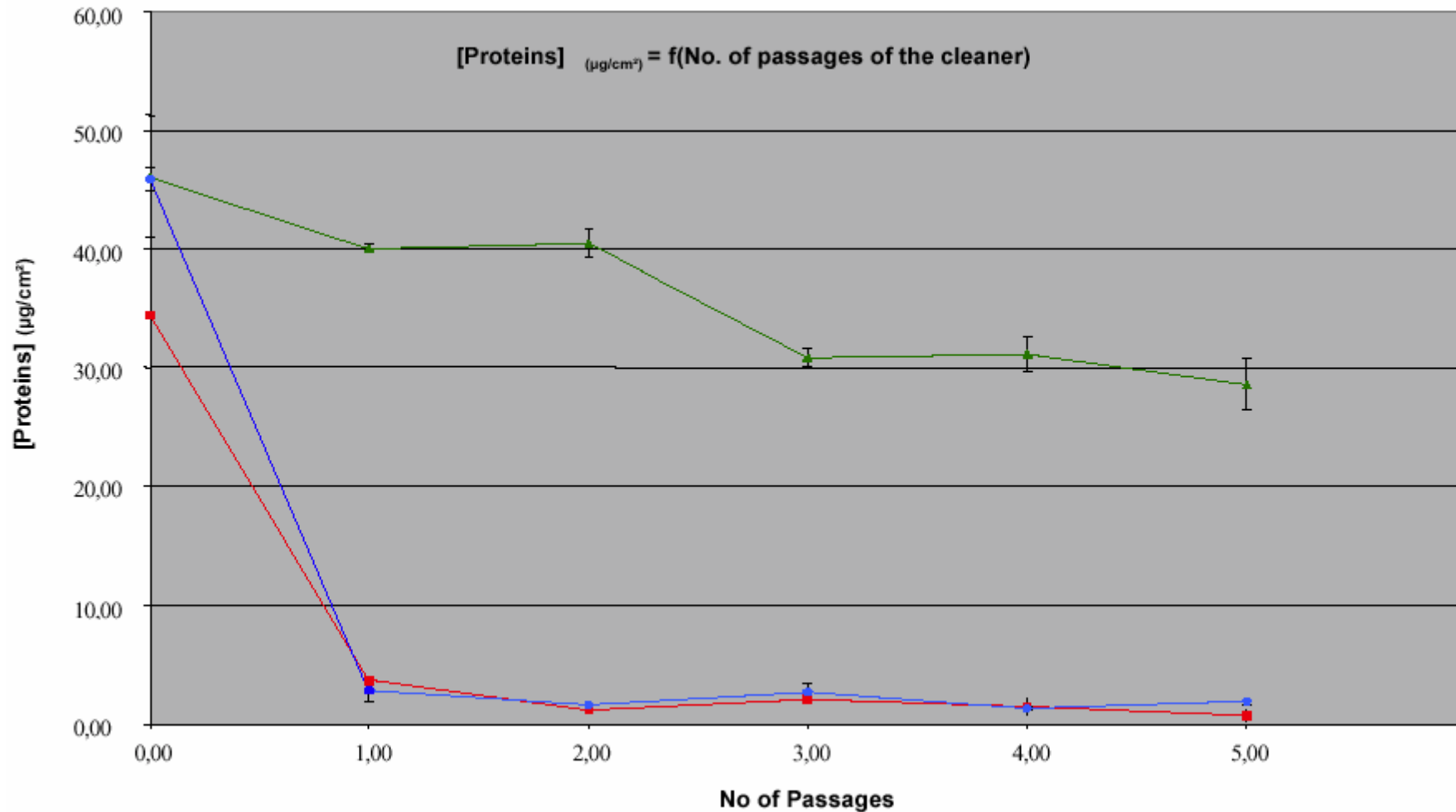


Figure 2: Activity of 2 cleaning procedures (“brush” cleaner vs. Dispo’Clean cleaner) vis-à-vis PTFE tube contaminated by a biofilm with *Pseudomonas aeruginosa* CIP A22: Development of the residual concentrations of proteins on the inner surface of the PTFE tube plotted against the number of passages of the cleaner for each procedure tested [“brush” cleaner (■) “Dispo’Clean” cleaner (●)] in relation to the control procedure (▲).

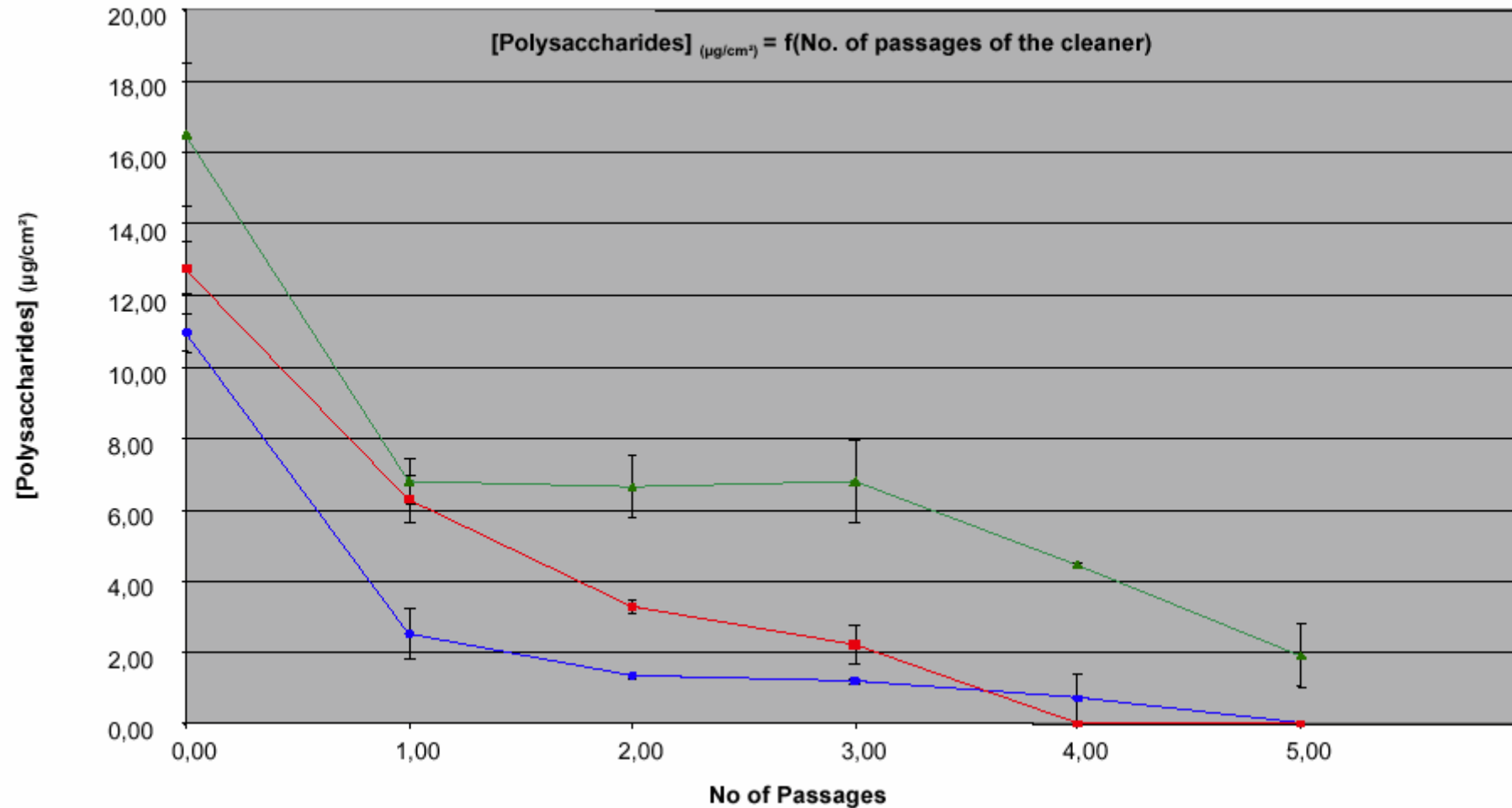


Figure 4: Activity of 2 cleaning procedures (“brush” cleaner vs. Dispo’Clean cleaner) vis-à-vis Teflon tube contaminated by a biofilm with *Pseudomonas aeruginosa* CIP A22: Development of the residual concentrations of polysaccharides on the inner surface of the Teflon tube plotted against the number of passages of the cleaner for each procedure tested [“brush” cleaner (■) “Dispo’Clean” cleaner (●)] in relation to the control procedure (▲).

An analysis of the observations made in scanning electron microscopy partially confirms the results obtained, particularly the absence of any appreciable effect of the water circulation on the bacteria present in the biofilm (photos 3.1 to 3.3). The only notable effect associated with the water circulation appears to be better visualisation of the bacterial entities of the biofilm after 3 and 5 volumes of water (washing phenomenon), which could be the cause of the phenomenon of a reduction in the residual concentrations of carbohydrates on the surface of the medium. An analysis of the surface condition of the test media after 3 passages of the “brush” and “Dispo’Clean” cleaners reveals, for both procedures, absence of visible bacterial contamination and the continued presence of a light deposit on the surface of the test tube. This light deposit appears to be distributed over the entire surface in the case of the “brush” cleaners (spreading phenomenon), whilst the “Dispo’Clean” cleaner appears to result in scraping of the surface, with an accumulation of residual contamination (stains) across longitudinal streaks.

Contrary to the results of the bacterial counts, which appeared to indicate that a residual bacterial contamination was still present on the surface of the medium, the observations under the MEB (scanning electron microscope) show that after 3 successive passages of one or other of the cleaners, no viable bacterium is detected on the surface of the medium. One of the hypotheses that may explain these contradictory results is that the two cleaning procedures result in the release of the biofilm and suspension of the bacterial cells in the water contained in the soaking bath, and that in the absence of a biocidal agent the bacteria in suspension recontaminate the surfaces (inner and outer) of the test media.

Allowing for the fact that the portions of PTFE 60 cm long used for the tests contain approximately $2 \cdot 10^8$ UFC/cm², the number of bacteria potentially responded in the litre of sterile distilled water present in the tank may be estimated at $1.5 \cdot 10^{10}$. The concentration of microorganisms in the tank is then approx. $1.5 \cdot 10^7$ UFC/ml. The continued presence of 200 µl of this solution on the surface of the 2 cm of PTFE tube sampled is therefore sufficient to explain the results of the bacterial counts obtained. Moreover, the results of the bacterial counts show that the differences in efficiency between the two procedures are mainly visible after 1 and 2 passages, whereas after 3 passages (1st observation under the MEB (scanning electron microscope)) the efficiency levels reached are more or less the same for the two procedures.

Finally, it should be noted that no trace of deterioration in the material was observed on the surfaces treated with the Dispo’Clean cleaner.

CONTROL PROCEDURE

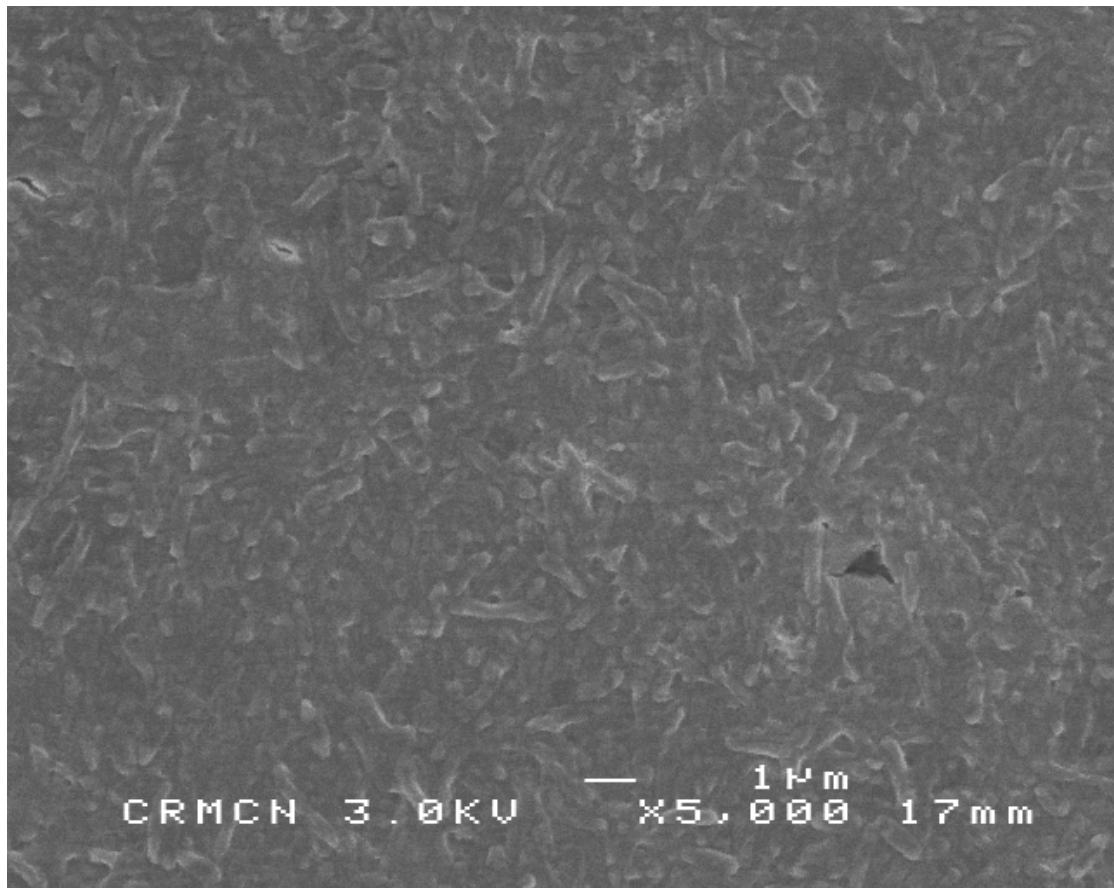


Photo 3.1: Before treatment

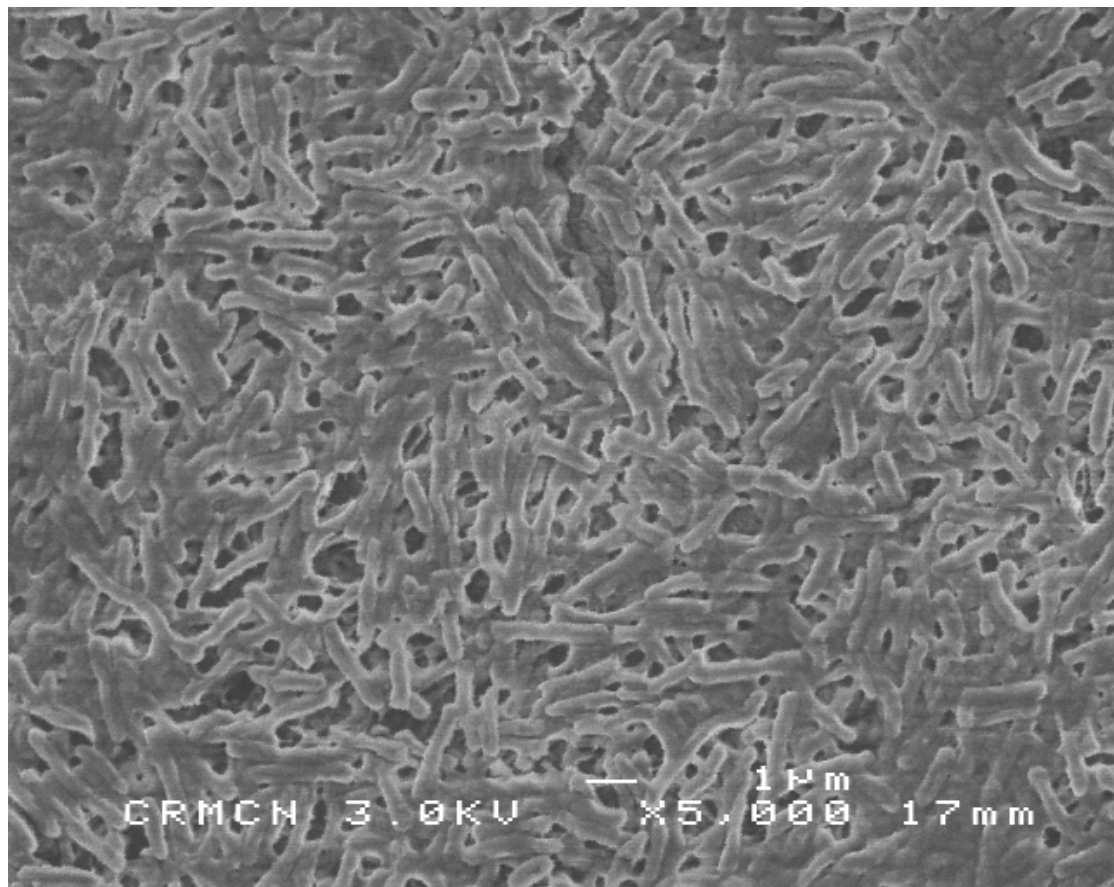


Photo 3.2: After 3 passages

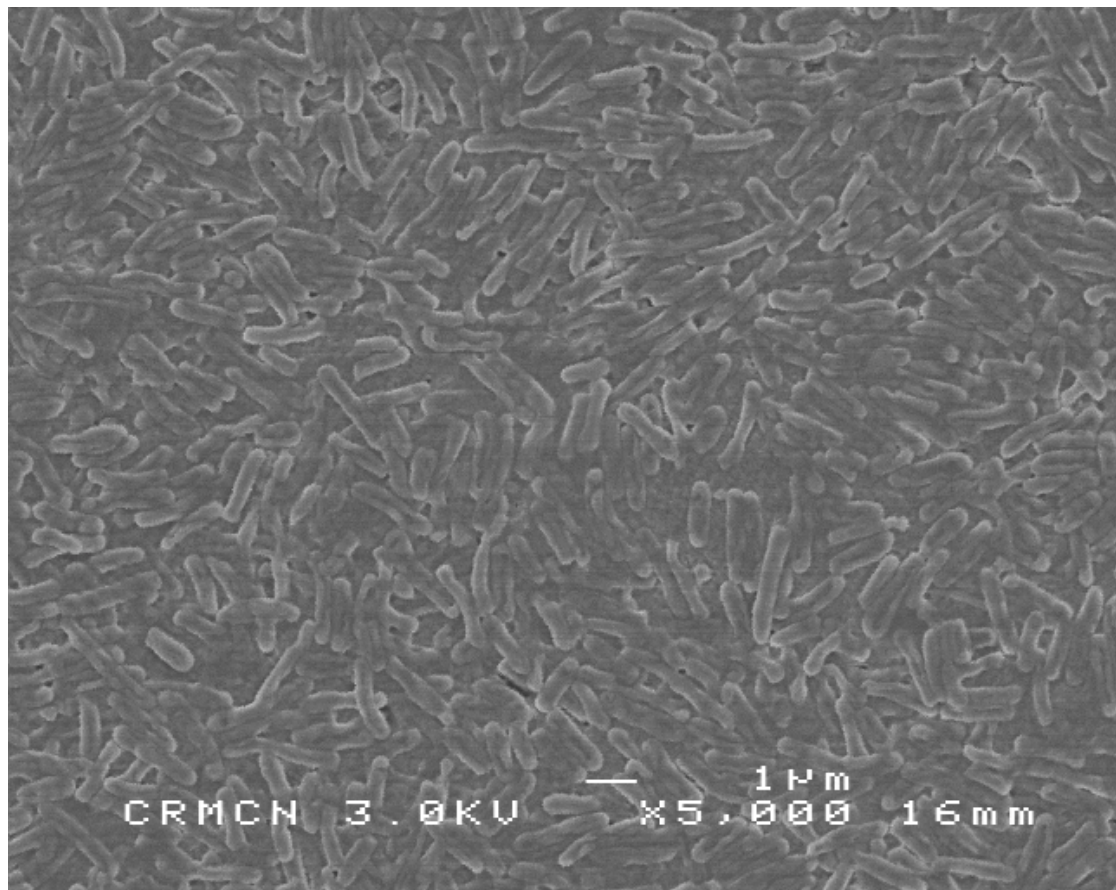


Photo 3.3: After 5 passages

“DISPO’CLEAN” CLEANER

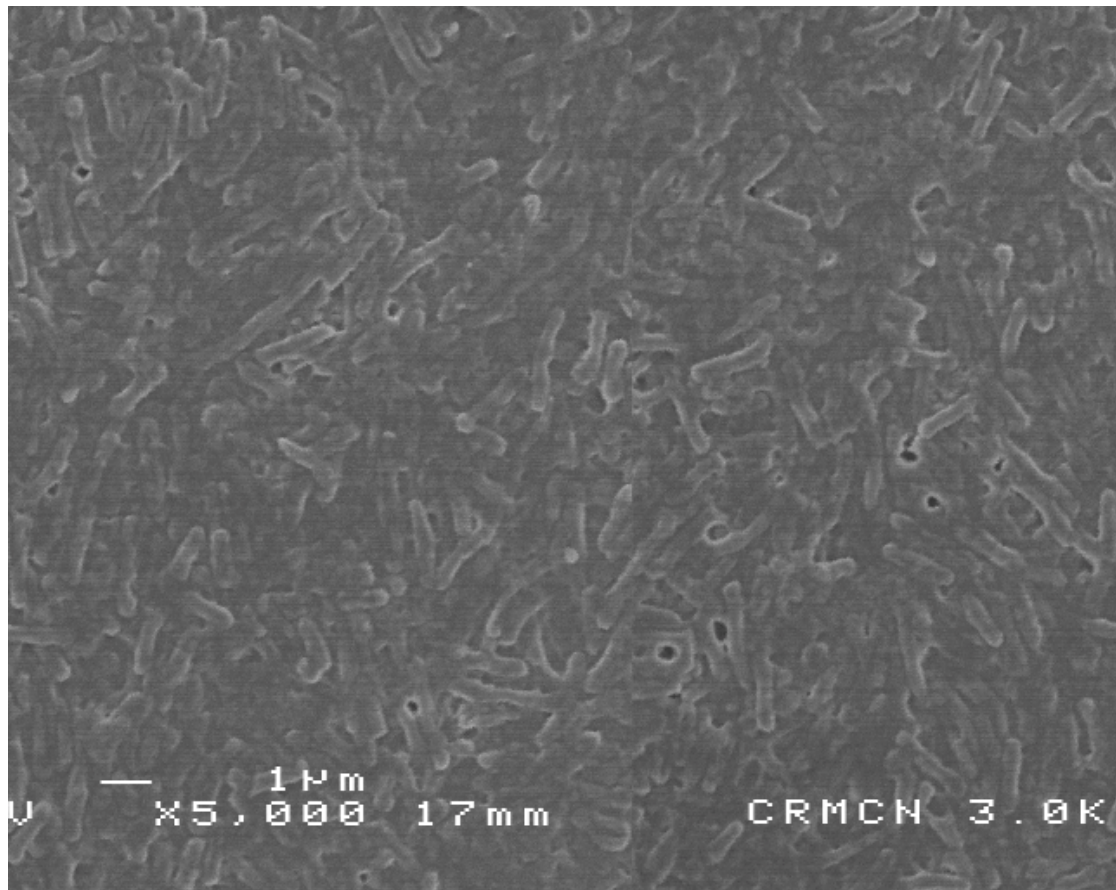


Photo 4.1: Before treatment

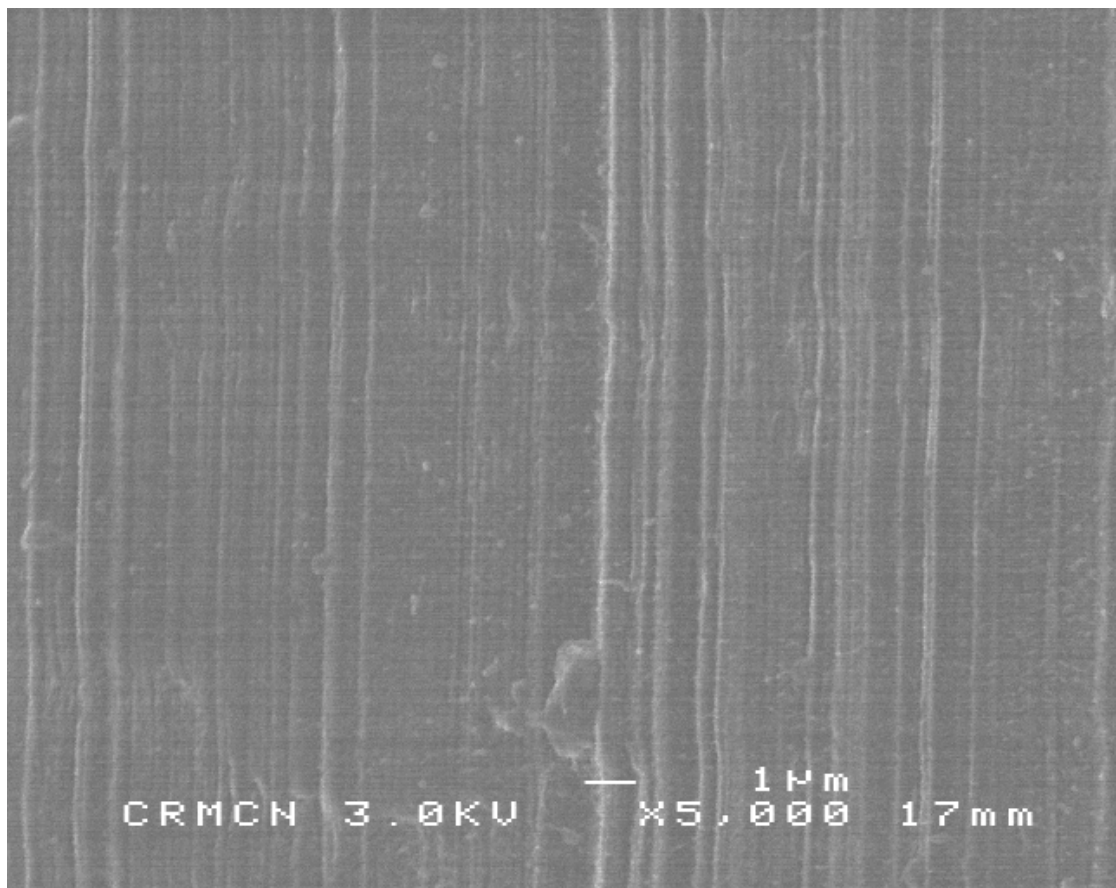


Photo 4.2: After 3 passages

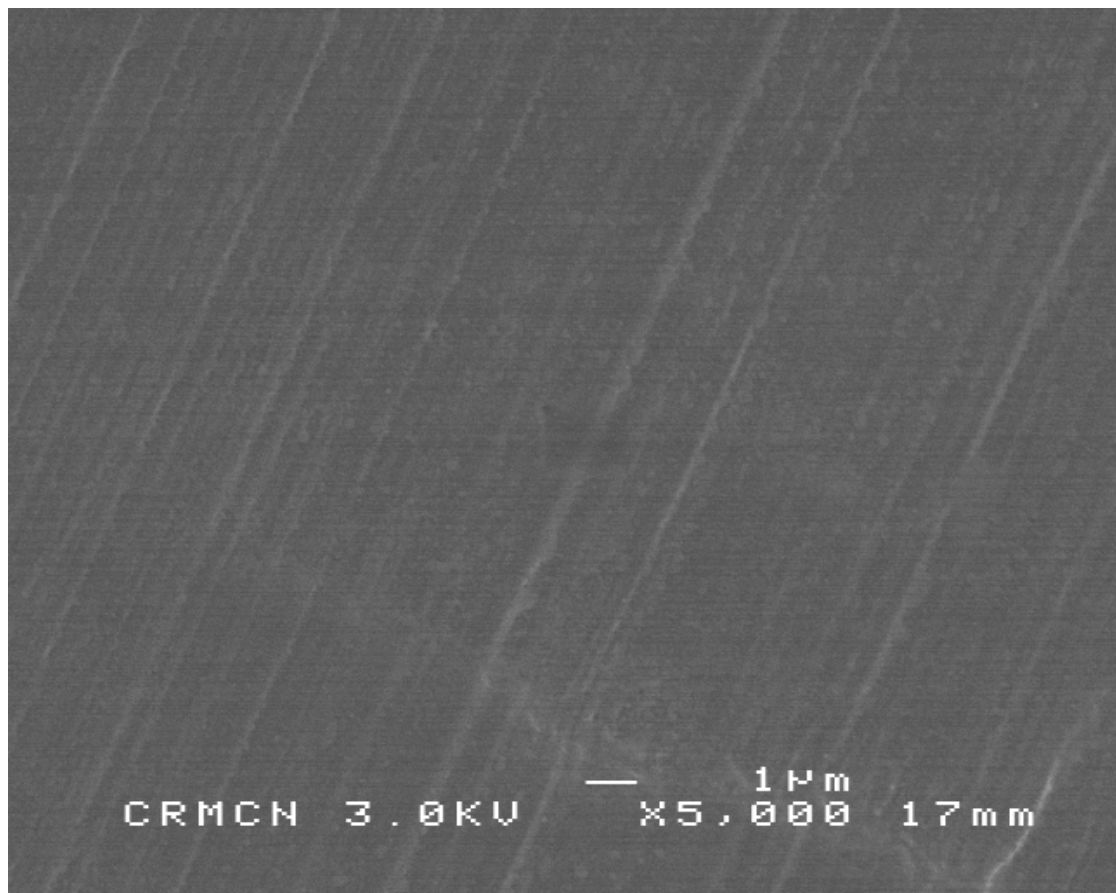


Photo 4.3: After 5 passages

“BRUSH” CLEANER

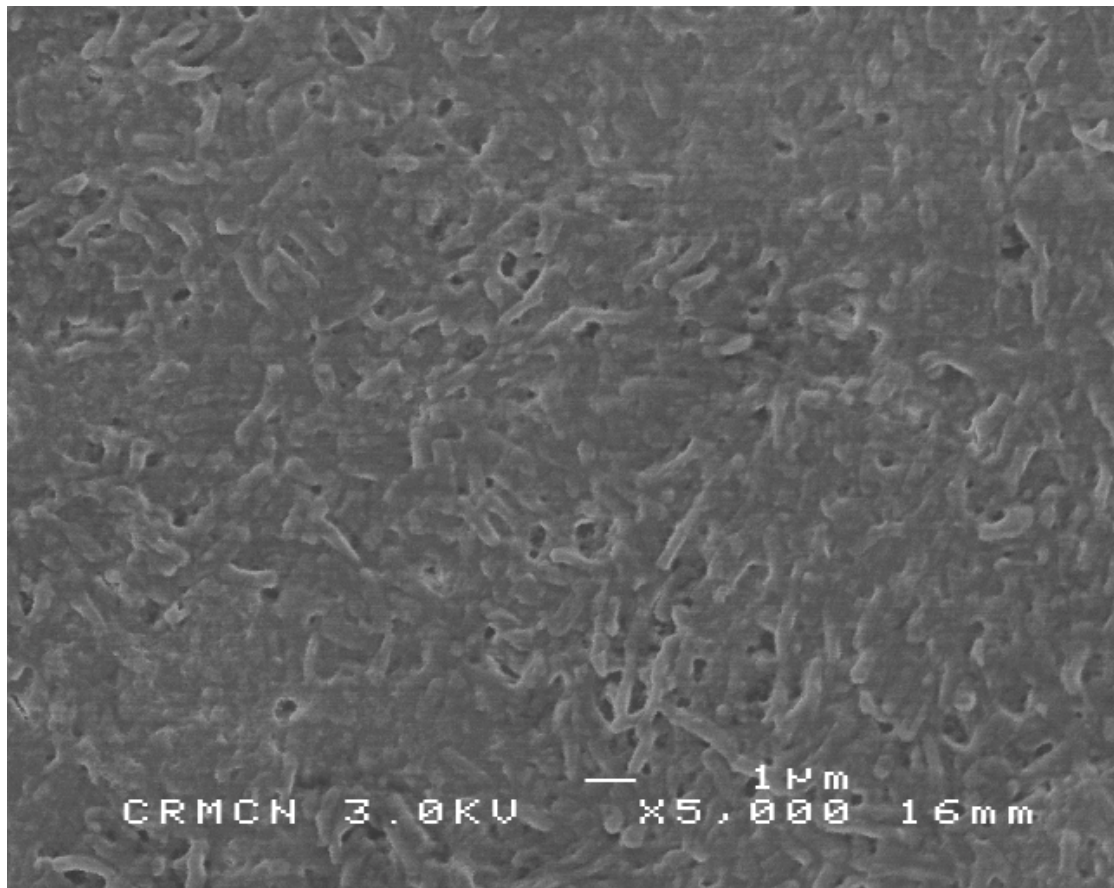


Photo 5.1: Before treatment

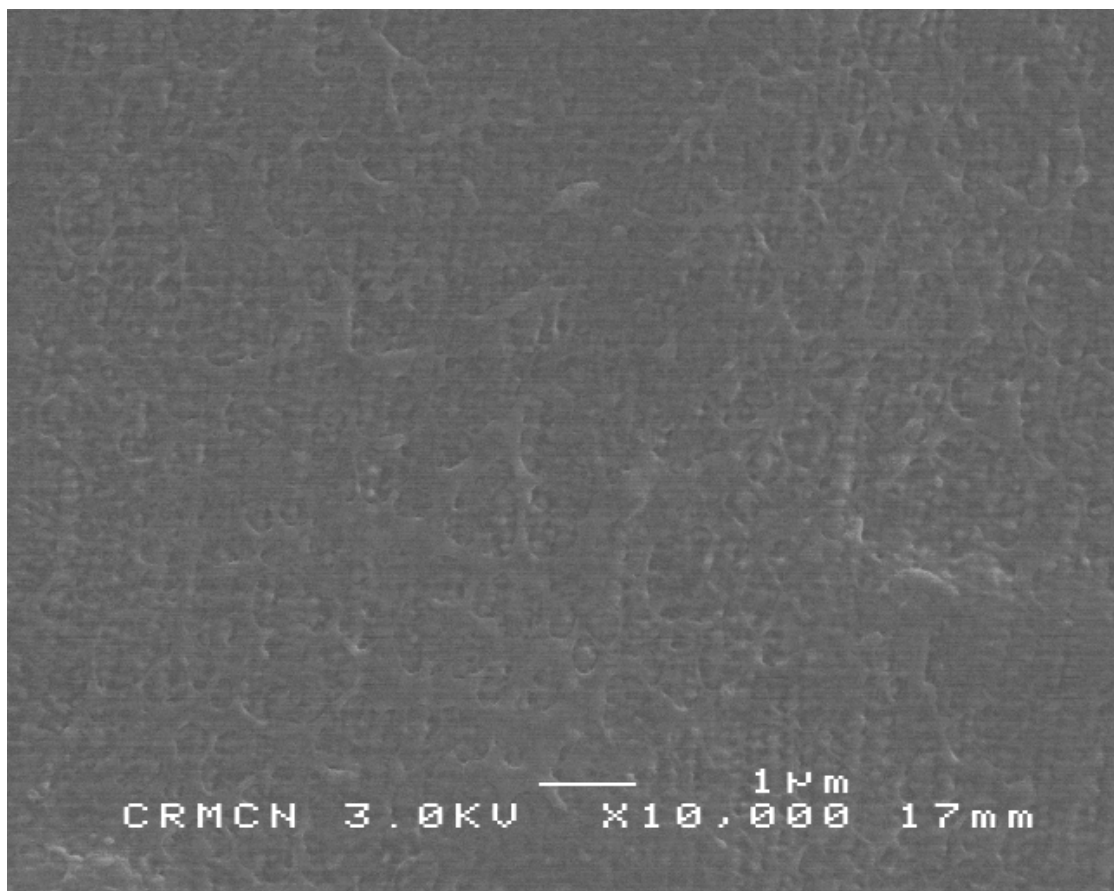


Photo 5.2: After 3 passages

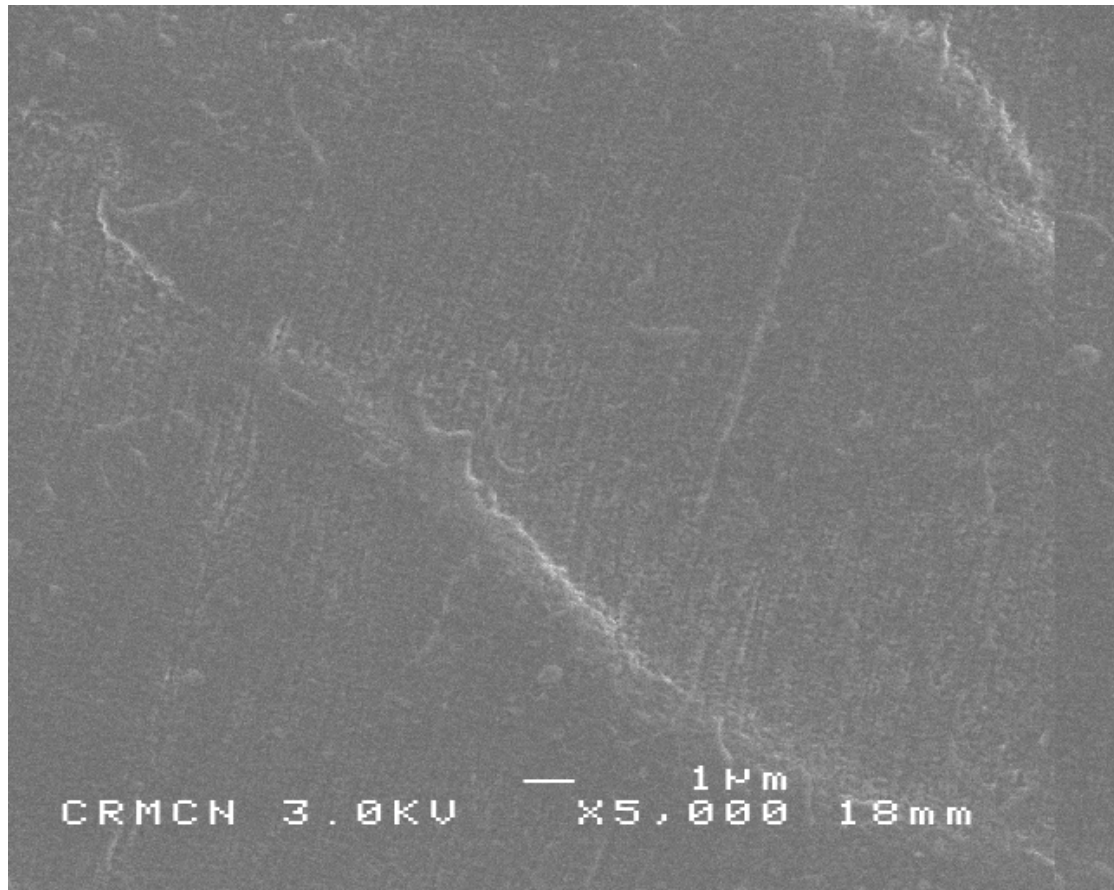


Photo 5.3: After 5 passages

Photos nos. 3 to 5: Observations under the scanning electron microscope of the different surfaces after application of the procedures tested. A: Control procedure. B: “Dispo’Clean” cleaner. C: “Brush” cleaner.



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R E C H E R C H E & D E V E L O P P E M E N T

VII : CONCLUSIONS :

La comparaison de l'activité détersive de deux types d'écouvillons à usage unique (écouvillon "brosse" vs. écouvillon Dispo'Clean) vis-à-vis de tubes PTFE contaminés sur leur surface interne par un biofilm monobactérien à *Pseudomonas aeruginosa* montre que dans les conditions opératoires décrites les deux procédures d'écouvillonnage testées permettaient d'atteindre après 5 passages, un même niveau d'efficacité, avec une mise en suspension de la totalité des bactéries initialement présentes au sein du biofilm et une réduction de plus de 95% des quantités initiales de protéines et polysaccharides.

Une analyse plus précise des courbes traçant l'évolution des constituants du biofilm en fonction du nombre de passage de l'écouvillon, semble toutefois indiquer que la procédure d'écouvillonnage utilisant l'écouvillon "Dispo'Clean" permet d'atteindre plus rapidement un niveau d'efficacité donné. Ainsi, pour atteindre le niveau d'efficacité obtenu après deux passages de l'écouvillon "Dispo'Clean" (environ 86% de l'activité maximale observée), 3 à 4 passages de l'écouvillon brosse ont été nécessaires.

The comparison of the cleaning efficacy of two single use brushes (standard brush vs. Dispo' Clean brush) against PTFE tubes contaminated on their internal surface by a *Pseudomonas aeruginosa* biofilm shows that in the test conditions described, the two cleaning procedures reach after 5 passages, the same efficacy level, with a detachment of all fixed bacteria initially present within the biofilm and a reduction of more than 95% of the initial proteins and carbohydrates quantities.

A more precise analysis of the graphs representing the evolution of the biofilm components according to the number of brushings, seems however to indicate that the cleaning procedure using the "Dispo' Clean" brush reaches a same defined efficacy level more quickly. For example, the efficacy level obtained after 3 to 4 passages of the standard brush is reached with only one passage with the "Dispo' Clean" brush.

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