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Drinking water pipeline: New PVC formulation anti-biofilm for the Moroccan industry

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- ✓ PVC pipeline
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Abstract

The traditional cade oil, the "*Katran*", which has been widely used for long time in the decoration of drinking ceramic pottery items produced in Morocco, it has been used also during this work, for its antibacterial and deworming effects, in the manufacture of anti-biofilm drinking water pipeline. The antibacterial effect has been confirmed by adhesion tests and by microscopic observations in SEM. The manufactured pipes have good mechanical properties. Infrared spectroscopy has revealed structural changes of the exposed groups in the surface of pipes. The contact angle allows detection of possible modification in hydrophobicity and acid-base character, which may explain some variability observed after adhesion test.

1. Introduction

Biofilms are deposits that are formed naturally and spontaneously in the drinking water distribution networks. Sometimes spectacular, such biofilms are often harmless. They require, however, to be controlled because they can harbor pathogenic germs. As a result of detachment, the microbiological quality of the drinking water can strongly deteriorate during its transport in the distribution networks. To combat these nuisances, the preventive strategy, which consists of reacting in the early stages of their formation, is more practical. Curative measures such as rehabilitation of networks seem to be enormously costly [1-2].

Historically, many materials have been used in pipelines to transport water from the distribution plant to the consumer. However, these are increasingly being replaced today by plastic pipes which have not ceased to gain popularity, and have become today the materials of predilection in many countries [1-2].

In this study, special attention has been paid to polyvinyl chloride (PVC) pipes. The objective aimed at the design of anti-adhesion material pipe by changing formulation level of the tube generated by the extrusion process. Knowing that PVC resins are never used alone, because their thermal stability at the transformation temperature is too low, it is common for additives to be added to them. Like most polymers, PVC tends to stick and degrade on the hot metal processing machines. Among the additives used there are lubricants and stabilizers [3]. We turned to the lubrication component given first of all the close connection with the bonding and adhesion phenomenon and also the fact that at the level of the tube formulation the latter naturally migrate towards the surface. The use of biologically active oil could inhibit the formation of bacterial biofilms at the pipe surfaces.It has been reported that cade oil (*Juniperus oxycedrus*) possesses antibiotic activities, including antifungal and antibacterial activities [4-7].

In this study, the percentage of the lubricant in PVC pipe formulation will be partially replaced by oil cade (at the rate of 10% in proportion of the lubricant). The objective is to design a new formula for anti-biofilms drinking water supply pipelines.

2. Materials and methods

2.1. Formulation and production of PVC pipe

The pipes tested were produced by extrusion process. About 100 kg of mixture of different components (Table 1) were mixed in a turbo-mixer for 5 minutes at 160 ° C., then kneaded until homogenized and cooled to about 40 °C., then sent to the extruder with a flow rate of 100 kg / hour. The extruder temperature varies between 175 °C. at the inlet, 185 °C. in the middle and 165 °C. at the exit of the die. The tube comes out continuously and cooled, then cut to the desired length. Table 1 below shows the various formulations thus used.

Figure 1: (a) Isolated breeding of oxycedrus tree [8]; (b) *Juniperus oxycedrus* leaves and fruits in an area near Marrakech [8]; (c) Jar with drawings made of Katran.

Table 1: Industrial formulation of PVC pipe (values are expressed in Kg). * Standard industrial formulation [9].

Components	**Role**	**T***	**F1**
Resin PVC	Bringcohesiontomaterial	90.00	90.00
Mineral loads	Improve certain mechanical properties,reduce the cost of the part	7.00	7.00
Stabilizing	Delay the degradation of polymer	2.00	2.00
TiO2	Dye and screen with UV	0.45	0.45
Carbon Black	Pigment	0.05	0.05
Lubricants	Avoid assembly and degradation on hot metal processing machinery, facilitate the implementation	**0.50**	**0.45**
Cade Oil	**New component**	0	**0.05**
	Total	100	100

The *Juniperus oxycedrus* shrub is the species used in the production of medicinal tar. It grows in the Mediterranean region (Figure 1). This species constitutes an important part of Moroccan forests in the Atlas Mountains; the common name in the region of Marrakech is "*Tîqqi*" [4-5]. Cade oil is obtained by carbonization (pyrolysis) of the trunk and the large branches of the old cardoons of the Middle Atlas of Morocco, according to a process known as *per ascensum*.This is presented in a clear, homogeneous and black liquid form with a reddish reflection.

2.2. Biological models

The bacterial strains *Pseudomonas aeruginosa* ATCC27853 and *Escherichia coli* ATCC25922 are used as a biological model to carry out the adhesion tests. These are gram-negative bacilli commonly used for water quality control and the effectiveness of drinking water disinfection protocol [1]. Table 2 shows the microbiological characteristics of the strains used.

2.3. Preparation of bacterial suspension

For each bacterial culture, a pre-culture was prepared by planting the bacteria followed by overnight incubation at 37 °C. in *Luria-Bertani* liquid medium (LBL). Since it is always advantageous to carry out the tests using

bacteria in their exponential phase of growth, a second culture is then prepared from this first culture by adding sterile LBL culture medium in a ratio of 1/1, (v/v) which is incubated for 90 minutes at 37 °C. Then, the bacterial cells are recovered by centrifugation at 8400g for 15 min, washed twice with sterile mineral drinking water and finally diluted with this same water until a bacterial suspension of optical density has been got, at 405 nm, between 0.7 and 0.8.

Table 2: Microbiological characteristics of the strains used.

Strain	***P. aeruginosa***	***E. coli***
Gram	Negative	Negative
Mobility	Flagellae	Flagellae
Appearance	Mucoid	Non-mucoid
Exponential Phase Duration	~ 8 hours	~ 3 hours
Generation time	70 minutes	40 minutes

2.4. Preparation of the adhesion supports

From the produced PVC tubes, surfaces of 0.5 x 0.5 cm² are cut, which will be cleaned and disinfected to remove mineral and organic impurities from the surface [1-10].

2.5. Adhesion test

The adhesion tests consist of placing the supports in the horizontal contact with the bacterial suspension in the medium, which is a drinking water. The contact lasts about 120 min, then the bacterial suspension is removed, the surface is gently shaken by sterile drinking water to remove any bacteria that have not adhered and which are likely to distort the measurements of adhered cell density. Each surface is examined by observation (average of 3 enumerations) in MEB (Philips, XL30 Model) after the classical phase regarding the preparation of the relative sample.

2.6. Tests and mechanical testing

Tests have been performed in accordance with ISO 604: 2002 [11]. The Mechanical Compression Testing Machine is an electromechanical machine type 3R model RP 25 ATF (Recherche & Réalisations Rémy, France)

2.7. Infrared analysis

Spectral analysis in the infrared medium (MIR, between 4000 cm^{-1} to 400 cm^{-1}, 2.5 µm to 25 µm) in ATR mode have been performed on small pieces of pipelines by a spectrophotometer (Thermo Scientific-Nicolet 6700).

2.8. Contact angle

The contact angle allows detection of possible modification in hydrophobicity and acid-base character, which may explain some variability observed after adhesion test. Otherwise, any changes in bacterial adhesion will be attributed to the active functional group exposed to the surface and not to a change in the physicochemical properties of the surface of the pipes.

Water (W), Ethylene Glycol (EG) and di-iodomethane (DM) have been used as reference solvents for the physicochemical characterization by the contact angle. Table 3 presents the surface energy components of each of them [12-13].

Table 3: Characteristics of reference solvents used for contact angle measurements and surface energy components (expressed in mJ / m^2).

Solvent	γ^{total}	γ^{lw}	γ^{AB}	γ^{+}	$\gamma-$
water	72.8	21.8	51.0	25.5	25.5
Di-iodomethane	50.8	50.8	0	0	0
Ethylene-glycol	48	29	19	1.92	47

A 2 µl drop is formed at the end of a syringe to be automatically deposited on the surface of the sample to be tested. A digital image is immediately acquired using a CCD camera placed on a goniometer (Visiodrop-MCAT of GBX SC Instruments, France). Three measurements are made for each sample. From the mean value calculated, free surface energies are determined: total, dispersive and acid-base [1-10, 12-14].

3. Results and interpretation

3.1. Adhesion test

The following figure (Figure 2) summarizes the results of bacterial enumerations per area unit observed in MEB. Values represent an average of three observations.

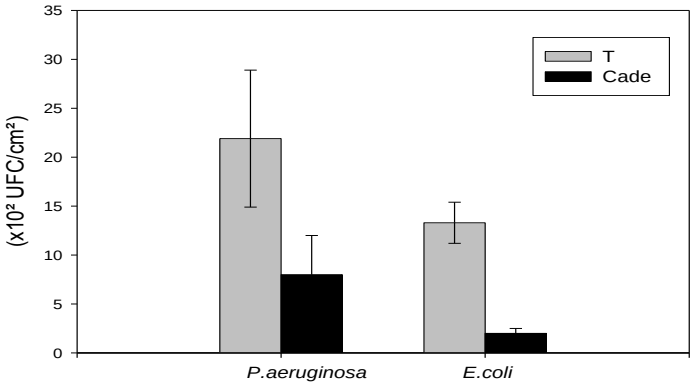

Figure 2: Bacterial enumeration.

It is noted that the adhesion decreases by integrating the cade oil in the formula of the tube that it is for *P. aeruginosa* or *E. coli*. In fact, we have recorded a respectively reduction of approximately 60% and 85% for F1. Figures 3 and 4 show examples of scanning electron microscopic observation photographs of *E. coli* and *P. aeruginosa* showing the effect on adhesion.

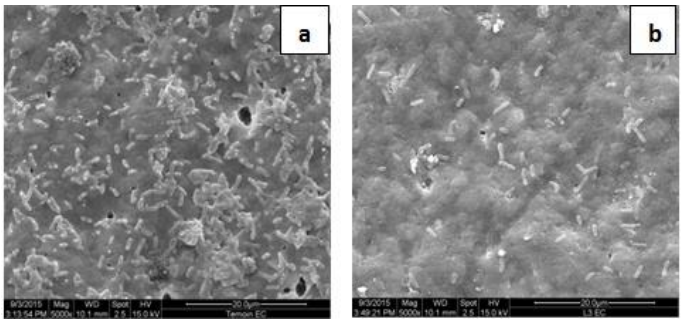

Figure 3: MEB photos after adhesion test of *E. Coli* after 2 hours of contact on the new surface of PVC (5K magnification). (a) Classical Formula T; (b) new formula F1.

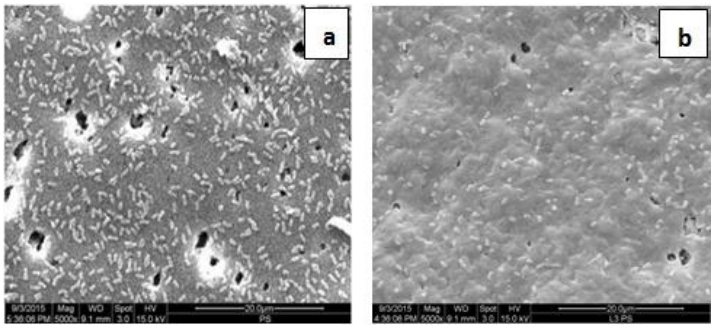

Figure 4: MEB photos after adhesion test of *P. aeruginosa* after 2 hours of contact on the new PVC surface (5K magnification). (a) Classical Formula (T); (b) F1.

3.2. Mechanical properties of the tube

Table 4 shows the results of the mechanical tests conducted.In its pure form, PVC is a material of better stiffness in the polymer family. However, it becomes fragile at low temperatures. Its modulus of Young (E), characterizing its stiffness, is between 0.35 and 2.5 GPa [15]. From the table, it is noted that T has the greatest stiffness because of its Young's module, which is relatively high. Poisson's ratio υ reinforces the observed remark. However, the mechanical properties of F1 remain within the required standards for drinking water pipes [11, 15].

Table 4: Some physical and mechanical properties of the PVC tube generated by extrusion process. D: diameter (mm); P: pressure (bar); E = Module of young; υ = Poisson's ratio; A = Elongation; R_r = Breaking strength; R_m = Maximum resistance.

	T	**F1**
Color	Dark gray	Dark gray
Exterior diameter (mm)	32	32
Nominale pressure (bar)	16	16
Interior diameter (mm)	28	28
E (GPa)	1.1220	0.8770
R_m (MPa)	4	5
R_r (MPa)	5.9	4
A (%)	4.69	9.27
υ	0.4	0.1

3.3. Infrared spectral analysis

The following figure 5 shows the infrared spectra collected for the pieces of pipes tested (a) and the spectra deduced by derivation for pre-visualizing the variability zones (b).

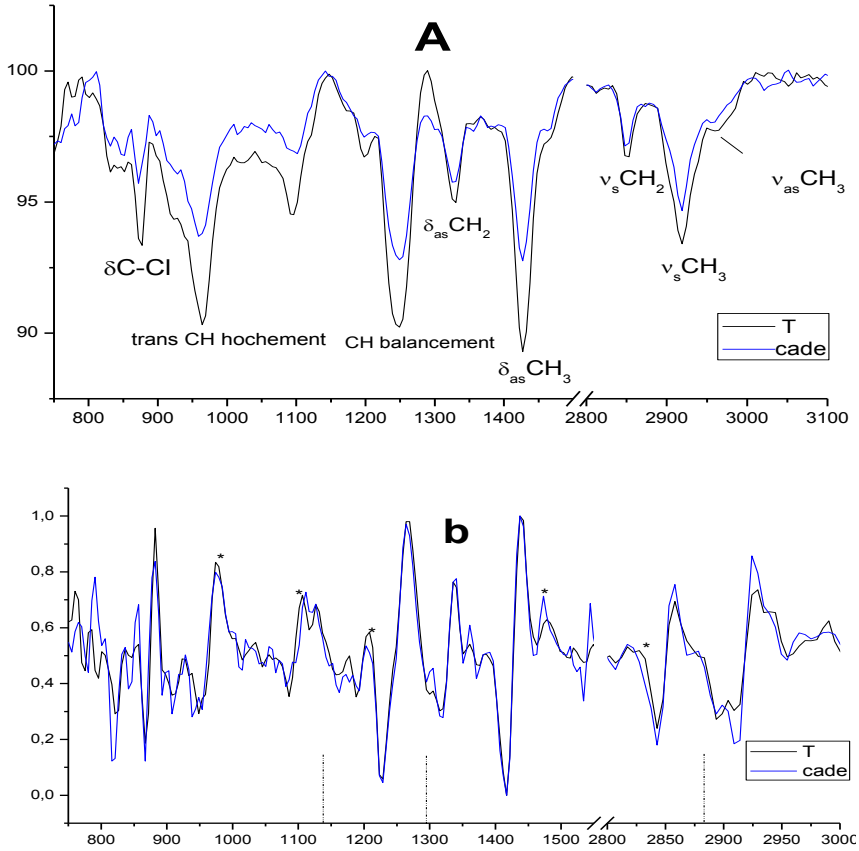

Figure 5: (a) the infrared spectra collected for the pieces of pipes tested (b) and the spectra deduced by derivation for pre-visualizing the variability zones.

By comparing the two spectra, it is observed that the zone between 1000 and 1350 cm^{-1} exhibits several spectral delocalisations.This zone for which the cade may manifest several vibrations modes, namely: ph-O-C elongation (Figure 6). The same is observed in other places in about 2850cm^{-1}.

This analysis has been carried out mainly to prove that cade oil is always present in the plastic and integrates the tube structure. Some parts of the plastic extrusion process impose a temperature rise until 185 °C, while the cade oil has an evaporation temperature around 400°C [7].

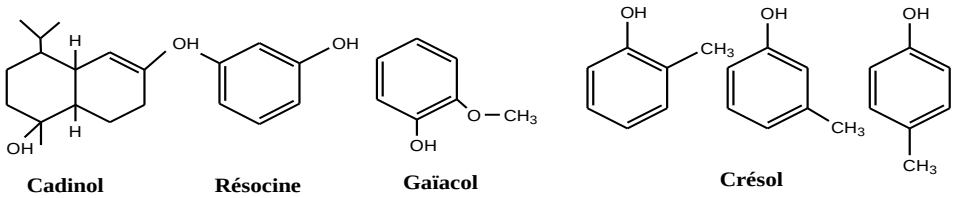

Figure 6: Structure of some major compounds of the cade oil [15].

3.4. Contact angle

It is generally accepted that non-specific interactions play an important role in the initial adhesion of planktonic bacteria to a support. The forces involved consist of Lifshitz-van der Waals (LW), electrostatic (EL) and acid-base (AB) interactions [1]. Using the acid-base approach established by Van Oss *et al*. [14], and which constitutes the DLVO extended approach (XDLVO) for measuring interactions LW and AB, the components of the acidic, basic and dispersive surface tension can be determined from the measurement of contact angles using three reference solvents [1,14]. The results of measurement of contact angle and energy characterization of the various surfaces developed are summarized in the following table (table 5).

Table 5: Contact angle (± 2°) and surface energy components (± 1 mJ / m^2) of the different materials tested. γ^{total}: total energy; γ^{lw}: dispersive component; γ^{AB}: polar component; γ^{+}: electron acceptor component γ-: electron donor component.

	T	**F1**
Di-iodométhane	58.3	41.8
Water	56.3	43.6
Ethylene glycol	44.7	32.8
γ^{total}	36.2	42
γ^{Lw}	29.5	38.7
γ^{AB}	6.6	3.3
γ^{+}	0.4	0.1
γ^{-}	31.3	41.2

From these results, compared with the control, it is noted that the formulation F1 has an increase in the apolar component γ^{Lw}. On the other hand, with respect to the polar component, it is noted that this one dropped remarkably for F1; it went from 6.6 to 3.3.

Discussion

The formation of biofilms passes through several stages; Firstly by attachment, consolidation achieved by the synthesis of extracellular compounds and finally the colonization and formation of a mature biofilm. In this study, we are interested in fighting biofilms to act upstream of the process leading to their formation.

The initial adhesion is the pre-consolidation step leading to the formation of a mature biofilms. This stage can be considered as a key step in the formation of biofilms. It is generally non-specific and of short duration (5 to 10 hours) [16]. It is agreed that this step involves interactions of a physicochemical nature (electrostatic and electrodynamic interactions), and depends on the nature of the support [10-16].

From a microscopic point of view after the adhesion test, the results obtained show, first of all, that in general the initial adhesion of *P. aeruginosa* is relatively greater than *E. coli*. These results are in agreement with the work of several authors and have been explained by the different membrane nature between the two bacterial strains. Furthermore, the more hydrophobic membrane properties of the strain, the greater its adhesion on solid surfaces [16 -18]. Boutaleb *et al*. 2007 [1] have stressed that the adhesion of *P. aeruosgina* exceeds, depending on the medium, that of *E. coli* by a variable factor ranging from 4 to 100 times more. The same strains, under drinking water conditions, have been evaluated and *P. aeruginosa* was more hydrophobic. If only the hydrophobicity is considered, according to the previous works, the apolar component of F1 is larger than the control, so it is more hydrophobic, yet the adhesion to this support is considerably less than the control.

Thus, the new formula allows a reduction in the adhesion of the strains, by the presence of active groups (antifungal and antibacterial) [6-7] and not because of a modification of physicochemical properties of the

surface. The IR results confirm the presence of a structural modification at the level of the exposed groups on the internal face of the PVC tube. The change of the environment of structural groups had led to a relocation of IR spectrum.

Our hypotheses are consistent with those obtained in several studies, which have evaluated the presence of natural substances and extracts on the formation of biofilm. Lahaye *et al*. 2016 [19] have noted the evolution of a filamentous fungal biofilm towards a positive bacterial biofilm after a treatment of the pipes by essential oils. This study has been carried out in model farms of pig breeding. Similar results have been obtained by Bazargani *et al*. 2016 [20] in the presence of an essential oil which acts by causing morphological damage to the bacterial cells, affecting the cells motility, and considerably modifying the structure of the biofilm formed.

Conclusions

This study proposes a promising way of preventive and less dangerous strategy to combat the formation of biofilms in drinking water pipelines. A new formulation of the PVC tube has been studied. This study incorporates cade oil with 0.05%, known for its disinfecting and antiparasitic properties; its use is highly appreciated in drinking pottery, and is an integral part of the local culture. The new pipelines have the desired effect Proven by the adhesion tests. Colonisation has decreased by integrating the cade oil in the formula of the tube by approximately 60% for *P. aeruginosa* and 85% for *E. coli*.

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