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Detection of biofilm formation by ultrasonic Coda Wave Interferometry

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ABSTRACT

The biofilm formation on food-contact-surfaces is a serious threat for public health and often results in huge human and economic losses. Thus, it is of importance to develop new strategies that allow the monitoring of the biofilm formation on food-contact-surfaces. Such strategies permit effective and timely countermeasures and, therefore, to avoid the microbiological risk and associated environmental impact due to cleaning processes. Therefore, this work investigated a non-invasive method for monitoring and detection of the biofilm formation using Coda Wave Interferometry (CWI) method. The principle of this method and the calculation of decorrelation coefficient based on the ultrasonic measurements were explained. The results underlined that our developed method is able to detect the early stage of the biofilm formation of *Staphylococcus aureus* on the stainless steel. Namely, it is shown that the bacterial cell kinetic is well captured by the evolution of the decorrelation coefficient as a function of incubation times. Overall, this work showed that this cost effective CWI method is a promising tool for the detection of early stage biofilm formation on food-contact-surfaces.

1. Introduction

The biofilms represent a great issue and have substantial implications in several process industries, such as food, drinking water, paper production, petroleum, nuclear power and marine industries Freeman, Lock, Marxsen and Jones (1990); Van Houdt and Michiels (2010); FRANK and Koffi (1990); Lens, O'Flaherty, Moran, Stoodley and Mahony (2003). In food sectors, the biofilm formation on food-contact surfaces is a major problem in terms of public health threats and economic losses Abdallah, Benoliel, Drider, Dhulster and Chihib (2014a). Such biofilms act as reservoirs of microorganisms which often lead to the dissemination of pathogens and, therefore, to foodborne infections. This fact was highlighted in several studies underlining that foodborne diseases are caused to a large extent by the biofilms formed on equipment surfaces Abdallah et al. (2014a). For example, it has been reported that more than 65% of all microbial infections in food area

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are caused by biofilms. According to the French institute of public health surveillance (InVS) report of 2016 InVs (2016), 1455 collective food poisoning have been reported in France, affecting 13997 persons, resulting in 634 hospitalizations and 3 deaths. In Europe, the report of the Global Burden of Foodborne Diseases underlined that more than 23 million Europeans become ill each year after consuming contaminated food leading to up to 5000 deaths Abdallah, Khelissa, Ibrahim, Benoliel, Heliot, Dhulster and Chihib (2015). Every year, up to 600 million people, or nearly 1 in 10 people in the world, become sick from eating contaminated food. These infections cause more than 420000 deaths. In addition to human losses, food-borne and health-related infections cause significant morbidity and economic losses. For example, it has been reported that the annual cost of nosocomial and foodborne infections in the United States is around $ 16.6 and $ 77.7 billion, respectively Abdallah et al. (2015).

In this regard, excessive cleaning and sanitization processes are constantly used in food industries in order to combat biofilms and to consistently meet hygienic manufacturing requirements Mussalli, Hecker, Padmarabhan, Kasper and Chow (1985). Such cleaning process often results in frequent process interruptions and related economic and environmental impacts. The environmental impact of cleaning and disinfection processes is mainly linked to the significant use of natural resources (ie water and energy), the use of chemicals (chloride, detergents, disinfectants) and the generation of CO_2 emissions and waste water Van Asselt, Vissers, Smit and De Jong (2005); MARTY (2001). Furthermore, such traditional sanitizing processes often fail in the eradication of biofilm and require additional enzymatic treatment in order to remove mature biofilms from food contact surfaces. In fact, the mature biofilms are known to be highly resistant to disinfecting/sanitizing agents and this is due to the interference of the self-produced exopolymeric matrix that hinders the diffusion of antimicrobials inside the biofilm. Thus, the biofilm early stage detection is of a vital importance to increase the likelihood of the biofilm eradication and to prevent the economic and environmental costs of cleaning processes. An efficient biofilm online detection/monitoring would allow the detection of the early stage of biofilm formation and to enable timely initiation of cleaning processes.

Along with issues caused by the biofilm formation or fouling on food-contact-surfaces, several works focused on the development of novel detection methods. These methods are usually based on agar plating or on metagenomic and metatranscriptomic studies Abdallah et al. (2014a). However, such methods are not effective at industrial level, which is due to the presence of viable but non-culturable (VBNC) cells in some biofilms and to the high cost of reagents and equipment. In addition, such methods do not provide online monitoring and may take hours or days in order to detect the biofilm formation of food-contact-surfaces. More recently, new methods, based on thermal pulse analysis, have been developed in attempt to detect the biofilm formation Fratamico, Annous and Guenther (2009). The principle of such methods is

based on implementation of sensors that measure the local thermal conductivity and heat variations due to the biofilm formation. However, these methods are somewhat limited because they are only able to detect thick deposits higher than few micrometers and, thus, cannot detect the early stage of biofilm formation Galiè, García-Gutiérrez, Miguélez, Villar and Lombó (2018). Other alternative technologies, such as the commercial quartz crystal microbalance (QCM) device analyzes, have been proposed Sprung, Wählisch, Hüttl, Seidel, Meyer and Wolf (2009); Olsson, Mitzel and Tufenkji (2015). The adhesion of biofilms or other deposits to a quartz crystal changes the vibration frequency of such a surface. However, these sensors suffer from many complications regarding the temperature, the flow velocity and the nutrient presence in the bulk, which affect the frequency values. In addition, these sensors are disadvantageous in terms of their high cost (tens of thousands of dollars), limiting their use to lab-based studies. In addition, such detection techniques are usually invasive and based on the introduction of an external perturbation in the system, which are often not suitable for the hygienic requirements in food industries Withers (1996). Finally, even though other techniques such as electrochemical impedance spectroscopy Dheilly, Linossier, Darchen, Hadjiev, Corbel and Alonso (2008) or electric resistance measurement Chen, Li, Lin and Ozkan (2004) have demonstrated their efficiency for biofilm and fouling monitoring, the measurements necessitate a particular setup and cannot be easily implemented online.

Acoustic detection techniques have been widely used to detect defects Tandon and Choudhury (1999); Donskoy, Sutin and Ekimov (2001). In the field of fouling detection on food-contact-surfaces, some classic acoustic monitoring techniques have been proposed using different kinds of direct acoustic ultrasonic waves (longitude wave, transverse wave, surface wave,...) Lohr and Rose (2003); Merheb, Nassar, Nongaillard, Delaplace and Leuliet (2007); da Silva, Wanzeller, Farias and Neto (2008); Withers (1994); Collier (2014). These techniques are mainly based on the measurement of reflection coefficient of transverse waves, the time of flight and attenuation of echoes. In the classic acoustic detection techniques, the sensitivity is generally limited by the wavelength of the direct acoustic wave. For thin layer detection, high frequency waves are required to achieve acceptable results. However, the increase of the frequency leads to the increase of attenuation of acoustic wave energy. The high attenuation reduces the signal-to-noise ratio (SNR) and leads to an inaccurate detection. In order to overcome such sensitivity limit without modifying the signal wavelength, the Coda Wave Interferometry (CWI), which is known to provide good performances, can be used. Coda waves have already been applied in seismic Snieder (2002); Zhou, Huang, Rutledge, Fehler, Daley and Majer (2010), volcano monitoring Matsumoto, Obara, Yoshimoto, Saito, Ito and Hasegawa (2001); Ratdomopurbo and Poupinet (1995), concrete detection Hilloulin, Zhang, Abraham, Loukili, Grondin, Durand and Tournat (2014); Planès and Larose (2013) and other domain BALAA, Le Duff, Plantier and El Guerjouma (2009),

but never used with the aim of fouling contamination detection. Furthermore, our previous work underlined that this technique is able to detect manually deposited wax on stainless steel Chen, Callens, Campistron, Moulin, Debreyne and Delaplace (2018). However, the application of such technique in the detection of biofouling materials, such as biofilms, has never been achieved.

In this paper, the non-destructive ultrasonic monitoring technique using coda waves is explored in order to monitor a biofilm formation on a stainless steel substrate. For this study, *Staphylococcus aureus*, a Gram-positive bacterium, is used for the biofilm formation. This bacterium is known to cause serious health issues in food sectors. The principle of this ultrasonic method and signal processing is described. In particular, the computation of the decorrelation coefficient, which is used as an indicator of the biofilm detection, is plotted as a function of incubation time. Then, the evolution of decorrelation coefficient is compared to the kinetic of the biofilm formation, which is followed by the bacterial cell enumeration using the conventional plate-counting technique.

2. Principle of ultrasound coda processing

In classical ultrasound techniques, echographic measurements use only direct ultrasound waves to detect and characterize changes in medium properties. These waves are the ones that propagate directly from the source to the sensor via a unique reflection at a given interface, and they consequently appear in the first part of the signal recording (basic illustration for our biofilm application is shown in Figure 1-left). In applications where these changes will only introduce very low property contrast, these direct echos will be very slightly affected, which will make the detection hazardous.

On the contrary, multiply-reflected and multiply-scattered wavepaquets will cumulate the effects introduced by the medium changes over several wave paths (Figure 1-right). These so-called "coda waves" will naturally appear later signal plot. Briefly, the medium is crossed one time by direct waves and several times by coda waves. This will result in a much higher sensitivity of the coda waves to small property changes (such as caused here by the biofilm formation and evolution) than the direct wavepackets.

Since coda corresponds to a superposition of waves propagating along various paths, it is difficult to analyze it using classical acoustic parameters such as time-of-flight, reflection and transmission coefficients. Instead, useful information will be extracted by quantifying coda signal changes or dissimilarities between two states of the medium. Ti that end, the decorrelation coefficient is used as an indicator of the dissimilarity between two recorded coda signals s_1 and s_2, respectively. It is computed in a given time-window $[t_0, t_1]$

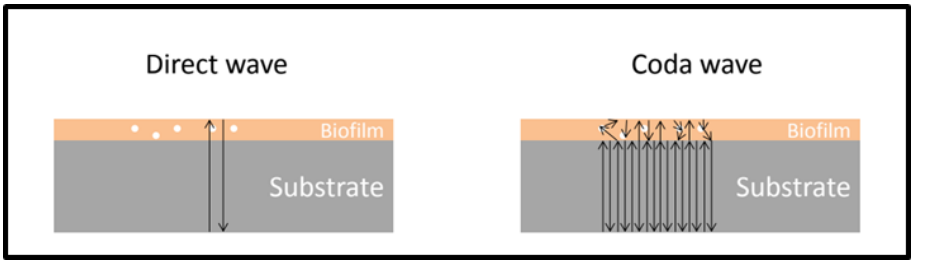

Figure 1: Schematic illustration of ultrasound wave propagation in the medium: direct waves (left) correspond to first reflected and backward propagated echoes, whereas coda waves (right) are multiply reflected and scattered inside the medium.

according to the following relation Zhang (2013):

$$D_{1,2} = 1 - \frac{\int_{t_0}^{t_1} s_1 s_2 dt}{\sqrt{\int_{t_0}^{t_1} s_1^2 dt \ \int_{t_0}^{t_1} s_2^2 dt}} \tag{1}$$

The second term in Eq. (1) corresponds to the normalized cross-correlation of s_1 and s_2 at zero lapse-time. Mathematically, the value of $D_{1,2}$ is between 0 and 2. Identical s_1 and s_2 signals lead to zero decorrelation coefficient and nonzero values are an indicator of waveform shapes and phases differences between signals. Hence, the value of $D_{1,2}$ will be directly related to the degrees of state changes in the medium. Therefore, in our application, its evolution during the monitoring procedure is expected to be linked to the biofilm formation on the substrate. Typical behaviors of early (direct) wave signals and coda signals will be shown in section 3.2, after presenting the experimental setup.

3. Materials and methods

3.1. Biofilm formation assay

The biofilm formation was performed on the upper side of circular stainless steel slides (3) Figure 2 (diameter : 4.1 cm; thickness: 1 mm). The CWI sensor is attached to the lower side of the stainless steel slides in order to detect the biofilm formation (Figure 2). In order to obtain the formation of the biofilm, the circular slide of stainless steel is placed in a static reactor (Figure 2-A and B). The reactor consists of several assembled pieces of stainless steel and a rubber O-ring (Figure 2-(C)), as previously described by Abdallah et al. Abdallah et al. (2015). The circular base of this reactor (5) is made of stainless steel and can receive an O-ring (4) which is used to fit perfectly one circular test slide (3) (Figure 2-A). Then a stainless steel cylinder (2) was placed in order to form the well of the biofilm formation. A collar clamp (6) was used to provide tightness and a metal cover (1) was used to ensure the sterility of the closed system (Figure 2-C and

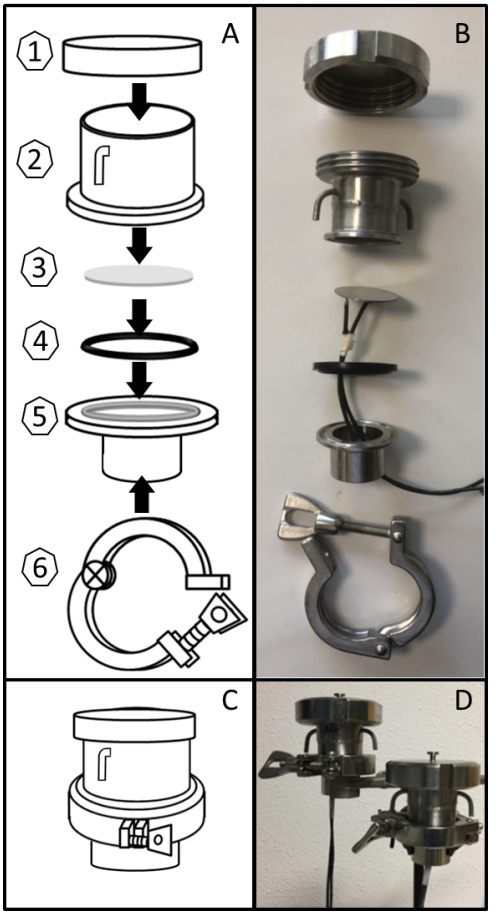

Figure 2: Description of the installation. A and B present schematics of the system assemblage; C and D present the assembled system: (1) metal cover; (2) stainless steel cylinder; (3) circular stainless steel slide; (4) rubber O-ring; (5) circular stainless steel base; (6) collar clamp.

D). Two similar reactors are used to evaluate the ability of the sensor for detecting the deposit formation (Figure 2-D). The first one is for monitoring the biofilm formation (marked as reactor 1), and the second one is used as a negative control or witness (marked as reactor 2).

Prior to their use, the reactors were cleaned and disinfected using ethanol 95% (v/v) to ensure sterility. The formation of biofilm was performed as described by Abdallah et al Abdallah et al. (2015). The first step of the biofilm formation was the bacterial adhesion to the surface. Then the formation of biofilm layers was triggered by adding the culture medium in the wells and placing reactors in an incubator with a controlled temperature. Biofilm formation was performed under the same conditions using separated reactors. Part of reactors was used to perform bacterial counting using the reference method (plate counting) and the other part was used to perform CWI monitoring. In details, step 1 was performed by the deposition of 5 mL of bacterial suspension (10^7 CFU/mL) in the well of reactor 1 and, then, incubated at 20°C for 1 h to allow the bacterial adhesion to the substrate. For the negative control (or witness), 5 ml of sterile physiological

Figure 3: Photo of biofilm formed on the substrate of reactor 1

saline water were used. After 1h of bacterial adhesion, step 2 was started. For that, the 5 mL of bacterial suspension were removed and the slides were washed twice using physiological saline water (8.5% of Nacl) in order to remove loosely attached cells. These 5 ml were removed in order to reduce the number of floating cells. Then 25 mL of Tryptone Soy Broth (TSB; Biokar Diagnostics, Pantin, France)) were deposited in each well. Thereafter, reactors are sealed by lids and incubated at controlled temperature of 30°C to allow the biofilm formation (Figure 3). The average thickness of biofilm layer, after 30 h, has been checked visually and is around 50 μm as measured by Abdallah et al. Abdallah et al. (2015). All experiments were done under sterile environment to avoid undesired contamination and to control as much as possible the biofilm growth.

The biofilm formation kinetics were investigated both with the plate count method and CWI monitoring, for comparison. For the classic plate count method, which will serve as the reference method, stainless steel slides were removed from the first part of reactors and placed in separated sterile containers Abdallah, Chataigne, Ferreira-Theret, Benoliel, Drider, Dhulster and Chihib (2014b). The bacterial cells were detached in 20 ml of phosphate buffer (100 mM) by vortexing for 30 s followed by a sonication at 37 kHz for 5 min. Then tenfold serial dilutions of each bacterial suspension were made in tryptone salt broth (TS; Biokar Diagnostics, Pantin, France). 100 μl of each dilution were spread onto tryptic soy agar plates (TSA; Biokar Diagnostics, Pantin, France). Agar plates were incubated at 37°C and the number of viable and culturable cells was counted after 0, 6, 12, 18, 24 and 30 h of incubation. The results are expressed in log CFU/cm^2 and represent the means of three independent experiments. The bacterial counts of bacterial cells after step 1 showed that the number of attached cells in reactor 1 was around 10^5 CFU/cm2. As expected, no adhered bacterial cells were found in reactor 2 (witness or control condition).

Table 1
Biofilm biomasses as a function of incubation time

Time (h)	Biofilm biomass (Log CFU/cm^2)
0	5.1 ± 0.2
6	5.7 ± 0.1
12	6.2 ± 0.2
18	7.0 ± 0.1
24	7.9 ± 0.4
30	8.1 ± 0.3

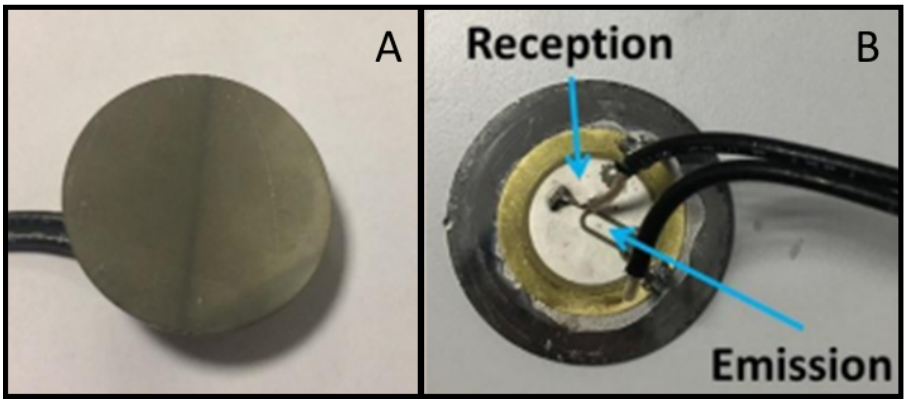

Figure 4: Piezoelectric transducer used for the acquisition. A and B present the upper and the lower faces, respectively.

3.2. Ultrasound acquisition

For the biofilm formation monitoring, biofilms were formed on stainless steel according the protocol described above. A continuous ultrasound monitoring is applied in order to detect the different steps of the biofilm formation in the reactors 1 and 2. The signal acquisition system consists of 5 components: a laptop PC with acquisition software (Matlab, instruments drivers), a waveform generator (Keysight 33600A Series), an acquisition board (PicoScope 5000 Series), an amplifier with internal high-pass filters (Eurosonics-Mistras Group, 1 kHz-50 MHz frequency range, 40 dB gain) and two transducers. The transducers used in this experiment are low-cost piezoelectric patches (Figure 4), consisting of two separate electroded parts for emission and reception. Their thickness resonance frequency is around 10 MHz. As mentioned above, they are glued on the lower side of the circular stainless steel substrate as described in Figure 2. This device was used to monitor the biofilm formation occurring on the upper side of the circular slides.

The excitation signal is one period of sinus at 10 MHz. The transducers convert the electrical signals to ultrasound waves through piezoelectric effect. After propagation and multiple reflections and scattering in the sample (see more detailed explanation below), parts of these waves are returned towards the transducer and converted back into electrical signals at the reception electrode. After high-pass filtering above 1 kHz (aimed at filtering out low frequency vibrations coming from the environment) and amplification, the received

signals are fed into the acquisition board with sampling frequency 125 MHz and recorded in the PC after averaging over 100 signal acquisitions. This acquisition process is then repeated every five minutes, from the beginning of biofilm formation and for a total monitoring duration of thirty hours. The biofilm formation on the surface will modify the substrate/biofilm interface properties and therefore lead to subsequent changes of reflected and back-scattered ultrasound signals. It is important to highlight here that even though bacterial arrangement in the biofilm constitutes a disordered medium, which results in complex ultrasound coda signals with intricated multi-path propagation, these signals are fully reproducible for a given biofilm state (*i. e.* a given incubation time). Indeed, since the ultrasound sensor is fixed relatively to the medium, and acquisition time of the signals is very small compared to the time scale of biofilm evolution, two signals recorded at the same state of the medium are identical.

The changes related to the biofilm evolution are monitored using the coda processing explained in section 2. To improve the signal-to-noise ratio, the signals are band-pass filtered with a certain frequency band $[f_1\ f_2]$ around the excitation frequency f_0, prior to decorrelation estimation computed from Eq. (1). For correct account of the frequency content, the width of time window $\Delta t = t_1 - t_0$ should satisfy the condition $\Delta f\,\Delta t \gg 1$ (where $\Delta f = f_2 - f_1$). In the following, as in other literature studies Payan, Garnier, Moysan and Johnson (2009); Snieder (2004); Chen et al. (2018), Δt will be chosen to be at least 10 periods of excitation signal. This will require the relative bandwidth to be larger than 10%.

To illustrate the effect of biofilm formation on the ultrasound signals, two recorded experimental signals are plotted on Figure 5. The blue one is the reference signal, measured at initial state (before biolfilm formation). Time $t = 0$ s correponds to the instant of emission of the incident ultrasound signal and this timebase reference is the same for all recordings. The red signal is recorded after 4 hours of biofilm formation. It can be seen that the signal parts corresponding to direct waves (the first part in the signals with strong amplitude) did not change with the variation of the surface state. However, in the coda part (next to the direct waves around 8 μs with weaker amplitude), there is a remarkable difference between the perturbated signal and the reference signal. Obviously, coda waves are more suitable for detection of the state change of medium than direct waves (Figure 5).

3.3. Environmental temperature

Acoustic waves are sensitive and influenced by temperature changes, in particular when using codas. Therefore, a monitoring of temperature in the incubator is carried on using a thermometer at the same time as the ultrasound acquisition. Since the signal recording time is very short and the biofilm formation is very slow, the temperature change of sample and the growth of biofilm during one single acquisition can be

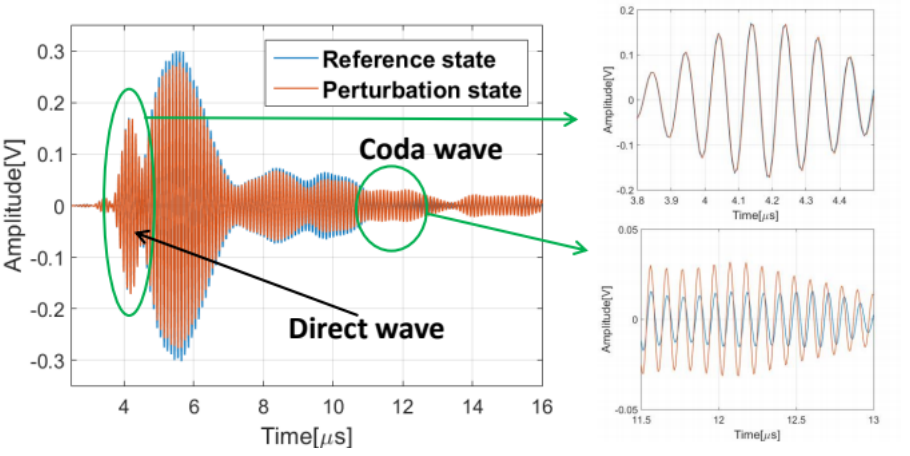

Figure 5: Two signals obtained in the experiment of monitoring the biofilm formation, representing two different sample states: Reference state (blue) and after 4 hours incubation (red)

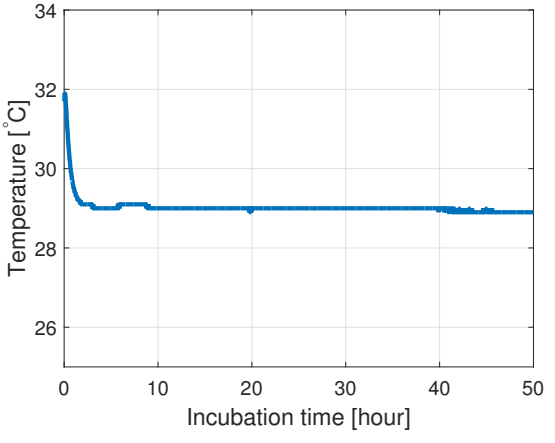

Figure 6: Evolution of the temperature in the incubator during the measurement.

neglected.

Temperature evolution measured during the biofilm formation in the reactor is shown in Figure 6-A. The curve shows that temperature inside the reactor stabilizes approximately after two hours of the beginning of incubation period. Therefore, the initial (or reference) state for the decorrelation coefficient estimation is defined at two hours of incubation time, in order to avoid the influence of temperature changes.

4. Results and discussion

Two series of signals are obtained from reactors 1 and 2 and are recorded from 2 hours of incubation time (taken as reference) with an interval time of 5 minutes. First, all these signals are numerically band-pass filtered between 9 and 11 MHz (relative bandwidth 20%) and then decorrelation coefficients between the

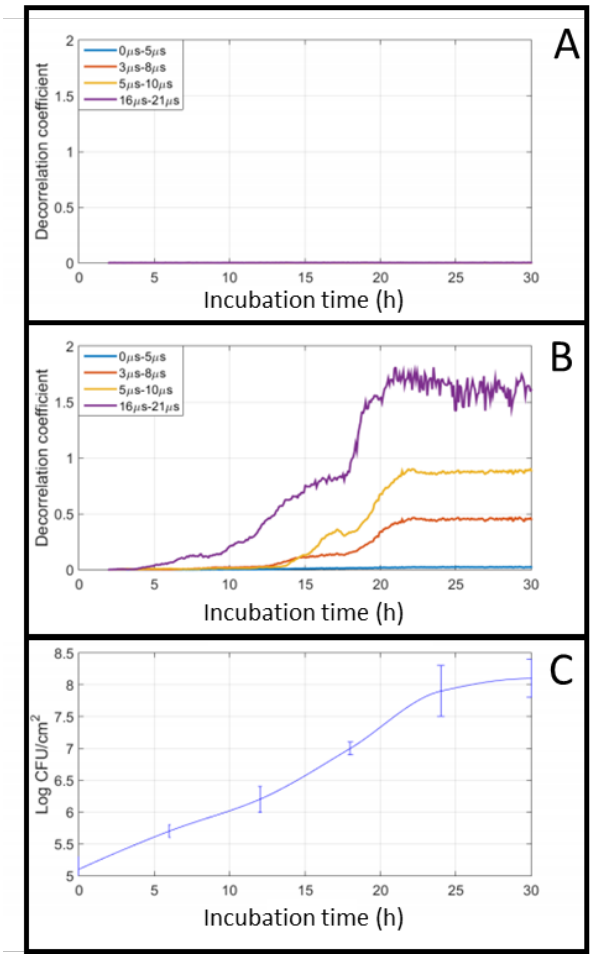

Figure 7: Decorrelation coefficient evolution as a function of incubation time for: A presents the control sample, B presents the biofilm formation sample, with different time windows: $0\ \mu s - 5\ \mu s, 3\ \mu s - 8\ \mu s, 5\ \mu s - 10\ \mu s, 16\ \mu s - 21\ \mu s$ (frequency band 9 MHz-11 MHz); C presents the kinetic of *S. aureus* biofilm formation on stainless steel substrate.

reference signal and every subsequent signal is computed using Eq. (1) for sliding time-windows of duration $\Delta t = 5\ \mu s$. This satisfies the conditions mentioned above on frequency bandwidth and time-window duration.

Figure 7-A and B show the evolution of the decorrelation coefficient as a function of incubation time for the control and the biofilm formation samples, respectively. In both cases, decorrelation values which are computed in four time-windows, are shown: $0\ \mu s - 5\ \mu s, 3\ \mu s - 8\ \mu s, 5\ \mu s - 10\ \mu s, 16\ \mu s - 21\ \mu s$.

In the negative control reactor (Figure 7-A), the decorrelation coefficients are close to 0 for any incubation time and selected time window. This indicates the absence of any significant change from the initial surface property state. This is coherent with the absence of biofilm formation, checked with naked eyes, on the negative control substrate. In addition, these results confirm that no apparent variable factor, especially the temperature, other than the biofilm formation on substrate influence the propagation of acoustic waves.

On the contrary, the results in Figure 7-B show that the decorrelation coefficients of biofilm monitoring

sample increased significantly as the incubation time increased . It also shows that the sensitivity of the state change detection depends on the time windows. The first window ($0\ \mu s - 5\ \mu s$) is not sensitive at all since the decorrelation coefficients are close to 0. On the contrary and for the other time windows, corresponding to later parts of the coda, significant increases of the decorrelation coefficient can be observed. Finally, the decorrelation coefficients become stable after about 22 hours, which is probably due to the reaching of quasi-steady state of the biofilm formation.

In order to verify the correlation between the evolution curves in Fig. 7-B and the biofilm formation on substrate, the kinetic of *S. aureus* biofilm formation on the stainless steel was investigated by enumeration of biofilm cells on agar plates, as explained in Sec. 3.1. The results are presented in Fig. 7-C, as mean values and the standard error to the mean (SEM). Data analysis was performed using Sigma Plot 11.0 (Systat Software, USA), using one-way ANOVA (Tukey's method) to determine the significance of differences. These results showed that the counts of the initial adhered bacteria were in the range of 5 log CFU/cm^2 (Table 1). They also showed that the biofilm biomasses increased with the increase of the incubation time and reached the steady state around 24 h of incubation time. In fact, the biofilm biomasses were stable and in the range of 8 log CFU/cm^2 (Figure 7-C). The biomass evolution could be compared with the decorrelation coefficient (Figure 7-B). Indeed, the data of the bacterial cell enumeration are well described by the decorrelation coefficient evolution.

Changes in the liquid nutrient medium during the bacterial growth could be another factor affecting the decorrelation coefficient. However, as soon as a biofilm is forming at the substrate-medium interface, the reflection coefficient will be essentially affected by the cell deposit on the substrate surface and its interfacial adhesion properties. Therefore, we are confident that the decorrelation coefficient is directly and essentially associated to the biofilm evolution, which the good matching between curves of Figure 7-B and Figure 7-C tends to confirm. These data proved that this method has the potential to detect and monitor the early stage of the biofilm formation on a substrate in a non-invasive way, and apparently with an accuracy comparable with more classical and more invasive methods, though this point will have to be investigated in more details in future works.

Finally, we will conclude this discussion by a short focus on temperature influence. As explained, the coda decorrelation results shown above correspond to constant temperature conditions (stabilization after two hours of incubation time). We present now in Figure 8-A the evolution of the decorrelation coefficient for the control sample during the first five hours of incubation time. For convenience temperature recorded at the same times is plotted again on Figure 8-B.

As expected, the decorrelation coefficient varies when temperature changes, even in the absence of biofilm

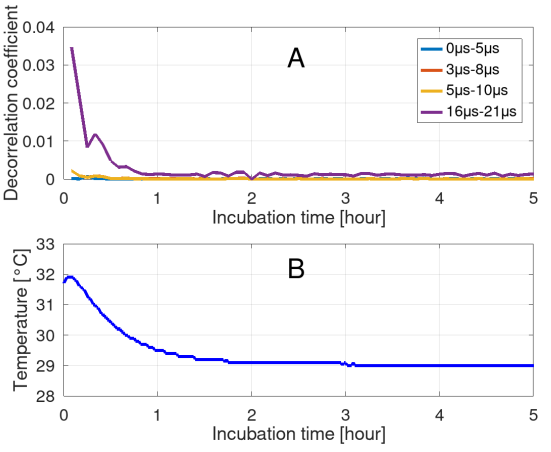

Figure 8: Influence of temperature on coda decorrelation. A: evolution of the decorrelation coefficient for the control sample. B: evolution of the temperature in the incubator during the measurement.

evolution. This validates the choice of waiting after two hours of incubation to avoid any temperature-induced decorrelation during biofilm monitorig. However we can also see that these variations are relatively moderate compared to those induced by the biofilm (Fig. 7-B). Quantitatively, for a temperature change of approximately 3 °C, the relative variation of the decorrelation coefficient is of the order of 2%, relatively to the full-scale of biofilm-induced variation. This means that even a variation of a few °C would only introduce a small error in the biofilm monitoring decorrelation curves. This is clearly encouraging for realistic application cases, though this point will obviously necessitate further studies.

5. Conclusion

The detection of biofilm formation on surfaces is of critical importance to sectors that directly affect human health. In fact, biofilm represents a reservoir of pathogens which results in serious human infections. Thus, an efficient biofilm monitoring on surfaces, commonly used in food and medical sectors, is needed in order to reduce the microbiological risks and associated human and economic losses. In this context, our work described a monitoring method which is based on Coda Wave Interferometry to detect the biofilm formation on stainless steel.

The principle of this monitoring technique is to compare the acoustic signals received at different instants. Decorrelation coefficient of multiply-reflected or scattered (coda) waves is used as an indicator of state changes in the samples. The method described herein presents several advantages over conventional methods based on optical, heat transfer, pressure drop, fluid dynamic gauging, direct weighing and thickness measurements. Indeed, conventional methods are usually invasive. On the contrary, the method described in this paper is non-invasive, cheap and easy to apply in food and medical environments. In addition, this

method is highly sensitive in the detection of the early stage of the biofilm growth. A bacterial biomass of 5 log CFU/cm^2 appears to be enough to trigger a significant evolution of decorrelation coefficient (Figure 7). Indeed, a 0.1 unit has been recorded and represents at least 5 % of signal variation of the value of decorrelation coefficient which theoretically varies between 0 and 2 units.

Our results show that this technique has the potential for performing continuous real-time biofilm detection and monitoring in sealed opaque equipment. In addition, this method interestingly succeeded to detect the early stage of biofilm formation. In fact, the decorrelation coefficient started increasing from a bacterial concentration of 10^5 CFU/cm^2.

This technique has some limitations which can be easily addressed. First, it allows only a local monitoring of biofilm formation (i.e. a small monitoring area of food contact surface). The use of multiple, judiciously distributed sensors could overcome this issue. Second, the technique is sensitive to temperature changes since this will affect the ultrasound coda properties. Though this is not a problem in applications where thermostatic control is ensured, this aspect could limit the extension of application range in cases where significant temperature changes cannot be avoided. A possible way to mitigate the impact of temperature changes and to improve the accuracy of detection, could be the use of multiple reference signals, recorded at different temperatures. Thus, by measuring the temperature simultaneously with the ultrasound codas, decorrelation coefficients related recorded temperature could be computed. This work is currently under progress. Finally, further studies will have to be conducted in order to quantify precisely the influence of the liquid medium properties.

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References

Abdallah, M., Benoliel, C., Drider, D., Dhulster, P., Chihib, N.E., 2014a. Biofilm formation and persistence on abiotic surfaces in the context of food and medical environments. Archives of Microbiology 196, 453–472. doi:10.1007/s00203-014-0983-1.

Abdallah, M., Chataigne, G., Ferreira-Theret, P., Benoliel, C., Drider, D., Dhulster, P., Chihib, N.E., 2014b. Effect of growth temperature, surface type and incubation time on the resistance of staphylococcus aureus biofilms to disinfectants.

Applied Microbiology and Biotechnology 98, 2597–2607. URL: https://doi.org/10.1007/s00253-013-5479-4, doi:10.1007/s00253-013-5479-4.

Abdallah, M., Khelissa, O., Ibrahim, A., Benoliel, C., Heliot, L., Dhulster, P., Chihib, N.E., 2015. Impact of growth temperature and surface type on the resistance of Pseudomonas aeruginosa and Staphylococcus aureus biofilms to disinfectants. International Journal of Food Microbiology 214, 38–47. doi:10.1016/j.ijfoodmicro.2015.07.022.

BALAA, E., Le Duff, A., Plantier, G., El Guerjouma, R., 2009. Interférométrie par onde de coda: Effet de la température sur la propagation d'ondes acoustiques dans une plaque d'aluminium, in: XXIIe Colloque GRETSI (Traitement Du Signal et Des Images), Dijon (FRA), 8-11 Septembre 2009, GRETSI, Groupe d'Etudes du Traitement du Signal et des Images.

Chen, B., Callens, D., Campistron, P., Moulin, E., Debreyne, P., Delaplace, G., 2018. Monitoring cleaning cycles of fouled ducts using ultrasonic Coda Wave Interferometry (CWI). Ultrasonics 96, 263–260. doi:10.1016/j.ultras.2018.12.011.

Chen, X.D., Li, D.X., Lin, S.X., Ozkan, N., 2004. On-line fouling/cleaning detection by measuring electric resistance equipment development and application to milk fouling detection and chemical cleaning monitoring. Journal of Food Engineering 61, 181–189. doi:10.1016/S0260-8774(03)00085-2.

Collier, N., 2014. Développement d'un Outil Ultrasonore de Caractérisation Des Propriétés d'adhésion de Milieux Modèles Avec Application Aux Dépôts Laitiers. Ph.D. thesis. Lille 1.

Dheilly, A., Linossier, I., Darchen, A., Hadjiev, D., Corbel, C., Alonso, V., 2008. Monitoring of microbial adhesion and biofilm growth using electrochemical impedancemetry. Appl. Microbiol. Biotechnol. 79, 157–164.

Donskoy, D., Sutin, A., Ekimov, A., 2001. Nonlinear acoustic interaction on contact interfaces and its use for nondestructive testing. NDT & E International 34, 231–238. doi:10.1016/S0963-8695(00)00063-3.

FRANK, J.F., Koffi, R.A., 1990. Surface-adherent growth of Listeria monocytogenes is associated with increased resistance to surfactant sanitizers and heat. Journal of food protection 53, 550–554.

Fratamico, P.M., Annous, B.A., Guenther, N.W., 2009. Biofilms in the Food and Beverage Industries. Woodhead Publishing.

Freeman, C., Lock, M.A., Marxsen, J., Jones, S.E., 1990. Inhibitory effects of high molecular weight dissolved organic matter upon metabolic processes in biofilms from contrasting rivers and streams. Freshwater Biology 24, 159–166.

Galiè, S., García-Gutiérrez, C., Miguélez, E.M., Villar, C.J., Lombó, F., 2018. Biofilms in the food industry: Health aspects and control methods. Frontiers in microbiology 9, 898.

Hilloulin, B., Zhang, Y., Abraham, O., Loukili, A., Grondin, F., Durand, O., Tournat, V., 2014. Small crack detection in cementitious materials using nonlinear coda wave modulation. NDT & E International 68, 98–104. doi:10.1016/j.ndteint.2014.08.010.

InVs, 2016. Surveillance des toxi-infections alimentaires collectives, in: Données de la déclaration obligatoire.

Lens, P., O'Flaherty, V., Moran, A.P., Stoodley, P., Mahony, T., 2003. Biofilms in Medicine, Industry and Environmental Biotechnology. IWA Publishing.

Lohr, K.R., Rose, J.L., 2003. Ultrasonic guided wave and acoustic impact methods for pipe fouling detection. Journal of Food Engineering 56, 315–324. doi:10.1016/S0260-8774(02)00156-5.

MARTY, P., 2001. Maîtrise de la consommation d'eau dans les industries agro-alimentaires: Dossier eau. Industries alimentaires et agricoles 118, 35–39.

Matsumoto, S., Obara, K., Yoshimoto, K., Saito, T., Ito, A., Hasegawa, A., 2001. Temporal change in P-wave scatterer distribution associated with the M6.1 earthquake near Iwate volcano, northeastern Japan. Geophysical Journal International

145, 48–58. doi:10.1111/j.1365-246X.2001.00339.x.

Merheb, B., Nassar, G., Nongaillard, B., Delaplace, G., Leuliet, J.C., 2007. Design and performance of a low-frequency non-intrusive acoustic technique for monitoring fouling in plate heat exchangers. Journal of Food Engineering 82, 518–527. doi:10.1016/j.jfoodeng.2007.03.022.

Mussalli, Y.G., Hecker, G., Padmarabhan, M., Kasper, J., Chow, W., 1985. Targeted chlorination, in: Condenser Biofouling Control Symposium: The State of the Art. Lake Buena Vista Florida. EPRI Report, 27pp.

Olsson, A.L., Mitzel, M.R., Tufenkji, N., 2015. QCM-D for non-destructive real-time assessment of Pseudomonas aeruginosa biofilm attachment to the substratum during biofilm growth. Colloids and Surfaces B: Biointerfaces 136, 928–934.

Payan, C., Garnier, V., Moysan, J., Johnson, P.A., 2009. Determination of third order elastic constants in a complex solid applying coda wave interferometry. Applied Physics Letters 94, 011904. doi:10.1063/1.3064129.

Planès, T., Larose, E., 2013. A review of ultrasonic Coda Wave Interferometry in concrete. Cement and Concrete Research 53, 248–255. doi:10.1016/j.cemconres.2013.07.009.

Ratdomopurbo, A., Poupinet, G., 1995. Monitoring a temporal change of seismic velocity in a volcano: Application to the 1992 eruption of Mt. Merapi (Indonesia). Geophysical Research Letters 22, 775–778. doi:10.1029/95GL00302.

da Silva, J.J., Wanzeller, M.G., Farias, P.d.A., Neto, J.S.d.R., 2008. Development of Circuits for Excitation and Reception in Ultrasonic Transducers for Generation of Guided Waves in Hollow Cylinders for Fouling Detection. IEEE Transactions on Instrumentation and Measurement 57, 1149–1153. doi:10.1109/TIM.2007.915480.

Snieder, R., 2002. Coda wave interferometry and the equilibration of energy in elastic media. Physical Review E 66, 046615. doi:10.1103/PhysRevE.66.046615.

Snieder, R., 2004. Extracting the Green's function from the correlation of coda waves: A derivation based on stationary phase. Physical Review E 69, 046610. doi:10.1103/PhysRevE.69.046610.

Sprung, C., Wählisch, D., Hüttl, R., Seidel, J., Meyer, A., Wolf, G., 2009. Detection and monitoring of biofilm formation in water treatment systems by quartz crystal microbalance sensors. Water Science and Technology 59, 543–548.

Tandon, N., Choudhury, A., 1999. A review of vibration and acoustic measurement methods for the detection of defects in rolling element bearings. Tribology International 32, 469–480. doi:10.1016/S0301-679X(99)00077-8.

Van Asselt, A.J., Vissers, M.M.M., Smit, F., De Jong, P., 2005. In-line control of fouling, in: Proceedings of Heat Exchanger Fouling and Cleaning-Challenges and Opportunities, Engineering Conferences International, Kloster Irsee, Germany.

Van Houdt, R., Michiels, C., 2010. Biofilm formation and the food industry, a focus on the bacterial outer surface. Journal of Applied Microbiology 109, 1117–1131. doi:10.1111/j.1365-2672.2010.04756.x.

Withers, P., 1994. Ultrasonic sensor for the detection of fouling in UHT processing plants. Food Control 5, 67–72. doi:10.1016/0956-7135(94)90088-4.

Withers, P.M., 1996. Ultrasonic, acoustic and optical techniques for the non-invasive detection of fouling in food processing equipment. Trends in Food Science & Technology 7, 293–298. doi:10.1016/0924-2244(96)10031-5.

Zhang, Y., 2013. Contrôle de Santé Des Matériaux et Structures Par Analyse de La Coda Ultrasonore. Ph.D. thesis. Université du Maine.

Zhou, R., Huang, L., Rutledge, J.T., Fehler, M., Daley, T.M., Majer, E.L., 2010. Coda-wave interferometry analysis of time-lapse VSP data for monitoring geological carbon sequestration. International Journal of Greenhouse Gas Control 4, 679–686. doi:10.1016/j.ijggc.2010.01.010.