SENSIRION
THE SENSOR COMPANY

Formation, Prevention and Removal of Biofilms

Application Note for Liquid Flow Sensors

Preface

Sensirion's liquid flow sensors are based on a microthermal measurement principle. The key element is an integrated digital CMOSens® microchip bonded to the outside of the sensor's flow channel, which measures the flow rate precisely. The sensor element is media-isolated by means of the wall of a straight flow channel.

Any deposition or layer of any substance which was not present during the in-factory calibration at Sensirion, will negatively impact the sensors' sensitivity and consequentially overall performance. A biofilm is one possible reason for such reduced sensor performance.

This application note shall give users of Sensirion's liquid flow sensors a basic understanding of the formation, the prevention and the removal of a biofilm.

Important Notice

The information provided in this document reflects the state of Sensirion's current understanding and the information available to Sensirion. However, compatibility of wetted materials with cleaning detergents cannot be guaranteed and it is in the user's responsibility and best interest to check material compatibility beforehand.

Contents

1 Introduction and motivational background

Sensirion's liquid flow sensors are based on a microthermal measurement principle (see Figure 1). The key element is an integrated digital CMOSens® microchip bonded to the outside of the sensor's flow channel, which measures the flow rate precisely. Owing to its position on the outer wall of the capillary, the sensor element is media-isolated from the liquid inside the straight and unobstructed flow channel. Depending on the specific sensor model, this very thin wall is made of a polymer (e.g. LCP), high grade stainless steel or glass. Additionally, each liquid flow sensor from Sensirion features one or more factory calibrations stored in the microchip's memory.

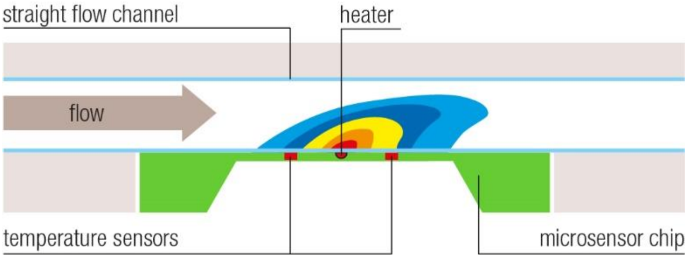

Figure 1: Microthermal measurement principle. Blue lines indicate the flow channel walls made from plastic, stainless steel or glass.

The factory calibration takes the whole liquid flow sensor interface (CMOSens® microchip, capillary wall and material and the liquid inside the flow channel) into account to produce a linearized digital flow signal. Therefore, any change to this interface, be it through a complete or partial deposition layer or through abrasion of the capillary wall material, will directly impact the heat transfer into the medium and consequentially the sensor's sensitivity and overall performance.
Water or aqueous solutions are prone to the formation of biofilm, which leads to the above described decrease in sensitivity and sensor performance.

This application note aims to give users of Sensirion's liquid flow sensors a basic understanding of the formation, the prevention and the removal of a biofilm if it occurs inside the sensor's flow channel.

2 Definition and formation of a biofilm

Biofilms are made up of a complex matrix of microorganisms in which cells stick to each other and to a surface. They can form on various surfaces and can be prevalent in natural, industrial, and hospital settings. Biofilms may include a single species or a diverse group of microorganisms. In general, they consist of bacteria and a matrix of proteins (e.g. adhesive pili), polysaccharides, extracellular DNA and enzymes, which are collectively also referred to as "extracellular polymeric substances".
Structurally, a biofilm may also be considered a hydrogel, which is a complex polymer that contains up to 90% water. Biofilms are not just biological slime layers but biological systems; the constituents organize themselves into a coordinated functional community.

The formation of a biofilm in systems carrying water takes place in several steps (see Figure 2) and is influenced by initial contamination, temperature, water parameters, the availability of nutrients and the way in which the water system is operated. It starts with the attachment of free-floating microorganisms to a surface. The first free-floating cell of a biofilm may adhere to the surface initially by the weak van der Waals forces and hydrophobic effects. If the cells are not immediately separated from the surface, they can anchor themselves more permanently using cell adhesion structures such as pili.
This bond is initially reversible. Subsequently, the bacteria form a microcolony through cell division and production of an extracellular matrix, which stabilizes and matures in the course of time and thus complicates its removal. The biofilm can subsequently release bacterial cells or biofilm parts again, which adhere to another surface in the fluidic system. This final stage of the biofilm formation is called dispersion.

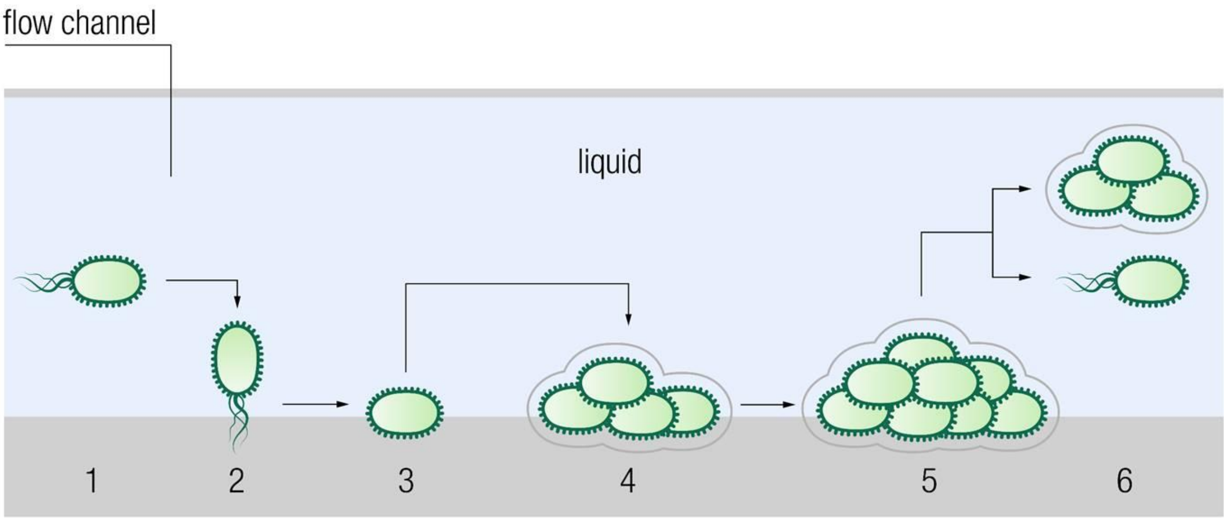

Figure 2: Stepwise formation of a biofilm: (1) a free-floating bacterial cell (2) attaches reversibly to a surface, (3) the adhesion increases and the attachment becomes harder to reverse, (4) microcolonies begin to form, (5) the biofilm matures and becomes a three-dimensional structure, and finally (6) cells break off from the mature structure to form new biofilms.

3 Prevention of biofilm formation inside Sensirion's liquid flow sensors

The prevention of biofilm is a concern in many industries from medical to industrial environments. It may occur in all components and on all wetted surfaces of a fluidic system (pumps, valves, tubes, etc), not only inside the flow sensor. Below, a few possibilities for test setups, which have been proven effective in the past for evaluating Sensirion's liquid flow sensors are listed:

- If possible, use a sterile medium, e.g. sterile water, for your test measurements. Ensure that the medium reservoir is closed off to the environment while testing is ongoing to avoid the entry of microorganisms through the air.
- If sterile water is not available, you may also use DI or RO water and add biofilm remover[1]. A concentration of 0.25 – 0.5% is recommended. Please note, that the usage of DI or RO water alone may not prevent the formation of a biofilm, as such media may still contain bacterial cells.
- Use e.g. an alcohol – water mixture to thoroughly clean your fluidic system on a regular basis after usage. A 70 % IPA water mixture has been proven to be most effective for this purpose.
- After ending your measurements, make sure to always store the sensor with a clean and dry flow channel. To do so, always drain the fluid, flush with suitable solvents or cleaning agents and blow out with dry air or allow to air dry.
  Caution: Remember to always check the solvents for compatibility with the flow sensor's wetted materials! For example, do not use acetone with the sensors of the LD20 series. For further details, please consult the Application Notes *Cleaning and Clean Handling* as well as *Chemical Resistance* and the respective sensor's datasheet.

As mentioned above, such preventive actions should be applied not only to the sensor but at best to the whole fluidic system to keep the complete system clean and prevent carry over from other parts of the system into the sensor's flow channel.

[1] Recommended biofilm removers include for example *Terg-a-zyme®* and *IO-Biofilmentferner (supplied by Oetzel Ingenieursbüro)*.

4 Removal of a biofilm inside Sensirion's liquid flow sensors

4.1 Identification of a biofilm inside Sensirion's liquid flow sensors

Typical signs of an existing biofilm on the flow channel's wall are symmetric measurement deviations over the whole measurement range of the sensor (in the negative as well as in the positive flow direction). The observed deviations become larger for higher flow rates. Also, the observed measurement deviations are typically negative due to the reduced sensitivity of the sensor. They may also increase over time with progressing biofilm growth (changes can typically be observed on a day-to-day basis not within hours).
The sensor's offset, when measured in water at zero flow, is however not significantly impacted by the existence of a biofilm due to the water-based nature of a biofilm. This can also be a means for differentiating a biofilm from other depositions on the internals of the flow channel like salts or minerals. See Figure 3 below for a visualization of the described sensor performance.

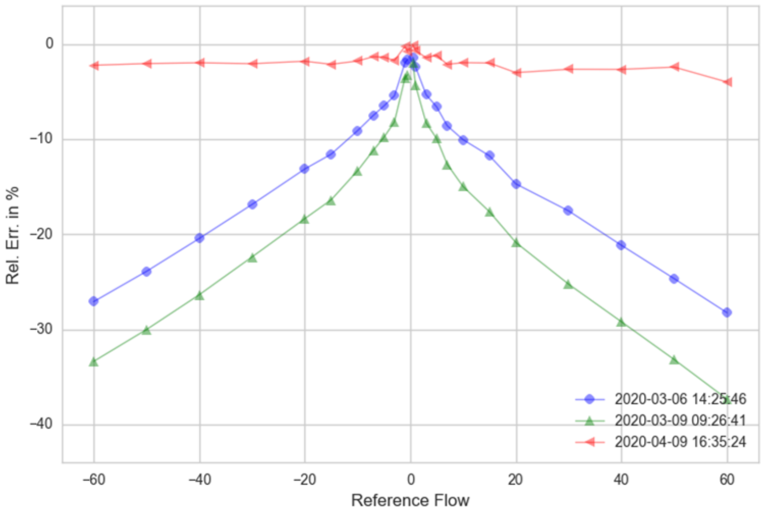

Figure 3: Typical relative error plot for a flow sensor with biofilm having formed on the flow channel's wall (here SLF3S-1300F sensor was used, x-axis in ml/min). Blue line shows first control measurement to confirm suspected biofilm growth; Green line shows further performance degradation after sensor was stored wet for 3 days; Red line shows full recovery after soaking of flow channel with biofilm remover over an extensive time period.

Note: The biofilm matrix features a sponge-like structure. Therefore, if the sensor was stored dry such that the biofilm dried out, too, the sensor's performance might be within specification first, but then rapidly declines as the biofilm soaks up the water again. Again, this can be a means of differentiating between the existence of a biofilm and other deposits on the inside of the flow channel.

4.2 Removal

The further the biofilm formation has advanced, the more and more difficult its removal becomes.
Various factors influence the effect of biocidal substances on the biofilm and determine the effectiveness of the cleaning process:

- Concentration of the active substance used for cleaning,
- Duration and nature of the exposure,
- Maturity of the biofilm,
- Whether it is a monomicrobial or polymicrobial biofilm,
- Type of material on which the biofilm has formed.

Regarding the first two bullet points, a few tips are listed below which have proven successful in the past when cleaning the flow channel of Sensirion's liquid flow sensors:

- First rehydrate the biofilm, in case the sensor and consequentially the biofilm have been stored dry.
- Use enzymatic cleaning agents (e.g. *Terg-a-zyme®)* and/or peroxide solutions (H2O2 or peracetic acid) at low concentrations. Do not use such cleaning agents simultaneously, as peroxide solutions may also destroy the enzymes.
- Use the chosen biocide at the recommended concentration to soak the sensor's internals for some hours or even overnight in case of highly resistant biofilms.
- Flush the biocide-water mixture back and forth through the sensor, i.e. move it in the negative and positive flow direction with the help of e.g. a syringe pump. The mechanical action of the fluid may help to loosen the biofilm. You may also use flow rates much above the sensor's specified flow range to achieve higher shear forces on the flow channel's wall, however, respect the maximum recommended operating pressure of the sensor.

Note: The removal of the biofilm may not always be successful and the replacement of the sensor may become necessary. Also, it is convenient to use the measurement setup or the fluidic system the sensor is installed in for the cleaning procedure as this will clean the remaining system simultaneously.

You can confirm the successful (or partial) removal of the biofilm by following the instructions in the Application Note *Basic Performance Check*. It provides guidelines on how to run a simple and quick test of a liquid flow sensor's accuracy. For sure, the original measurement system can also be used if available.

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