

Parenterals and Injectables

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SANOFI Industrial Affairs

Biofilm : Reminders

They represent a major source of problems to maintain the quality of water for pharmaceutical use (WPU) (+ 60%, FDA 1991).

Biofilm: Appearance

- The adhesion of microorganisms is a natural process that occurs regardless of the nature of:
  - **Environment (suspending fluid)**
  - **Microorganism**
  - **Support material.**
- This bio-accession gives microorganisms advantages and competitiveness compared to microorganisms in suspension.
- This bio-accession led to the formation of a complex called: **Biofilm**

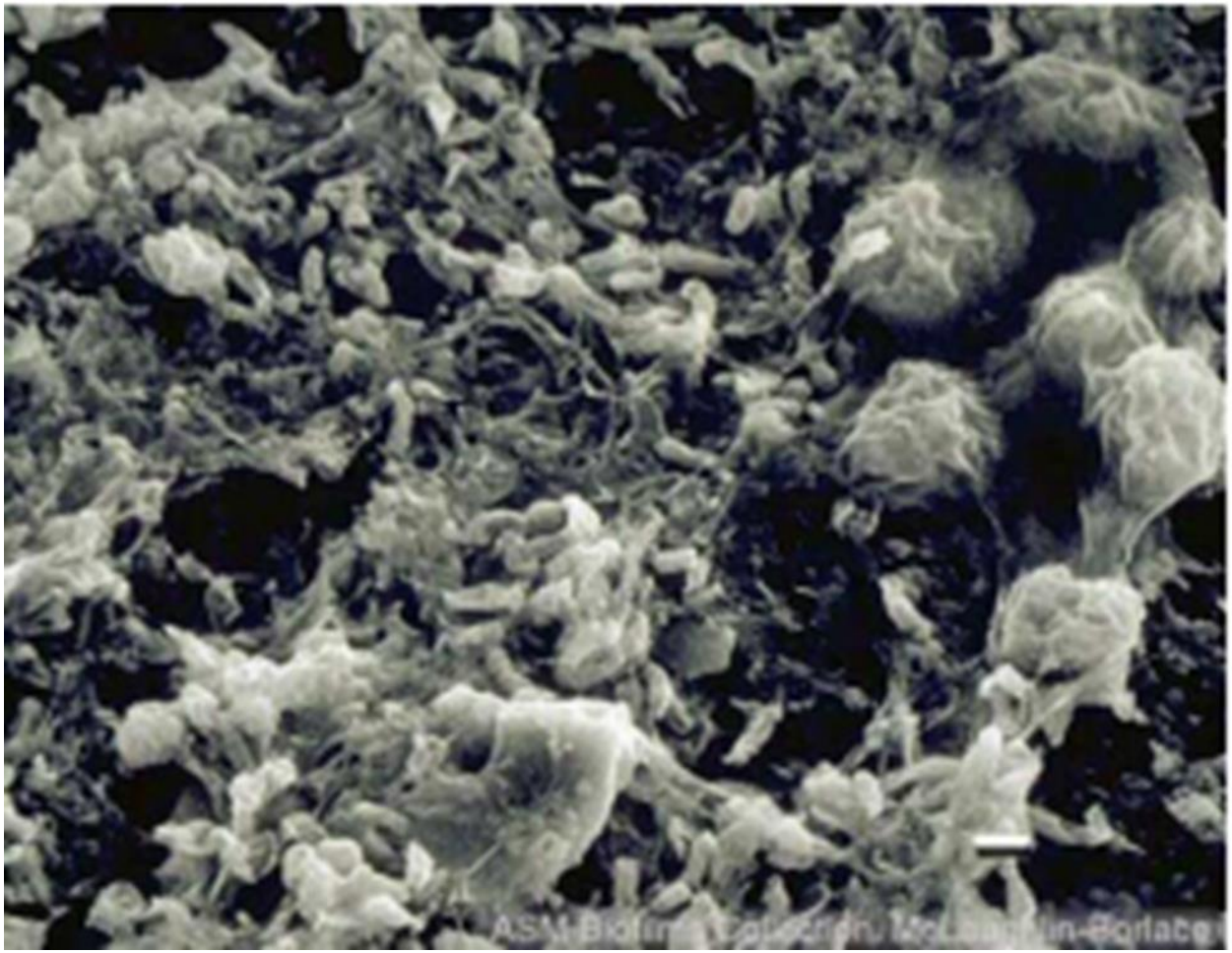
Biofilm: Appearance

- Biofilms are matrices consisting in particular of organic polymers of microbial origin containing a mostly heterogeneous population of microorganisms which may comprise bacteria, viruses, fungi, algae, protozoa adhering to each other and / or to the surfaces and interfaces (viruses, algae, protozoa play a minor role compared to the bacteria).

Water for Pharmaceutical Use (WPU):
Biofilm: Setting up

- A transport phase of microbial cells to the receiving surface,
- An adhesion phase:
  - **Related to the physico-chemical interactions of Van der Waals type, acid-base (Lewis) and electrostatic between the surface and the various components in the presence,**
  - **Other mechanisms allow bacteria to adhere to surfaces by flagellas training and organelles or protein structures external to the cells.**
- A phase of colonization due to cell proliferation (easy), interactions between the different species and the production of exo-cellular substances.

Water for Pharmaceutical Use (WPU):
Biofilm: Installed



Water for Pharmaceutical Use (WPU):
Biofilm: How?

- The structure of the biofilm, called "glycocalyx" is a fibrous entanglement consisting of bacteria, polysaccharides and exo-cellular glycoproteins
- Microorganisms are very poorly fed with oxygen and nutrients which lead to express a "deprivation phenotype" reduced sensitivity
- This extracellular matrix is formed from polymeric materials including organic and inorganic components

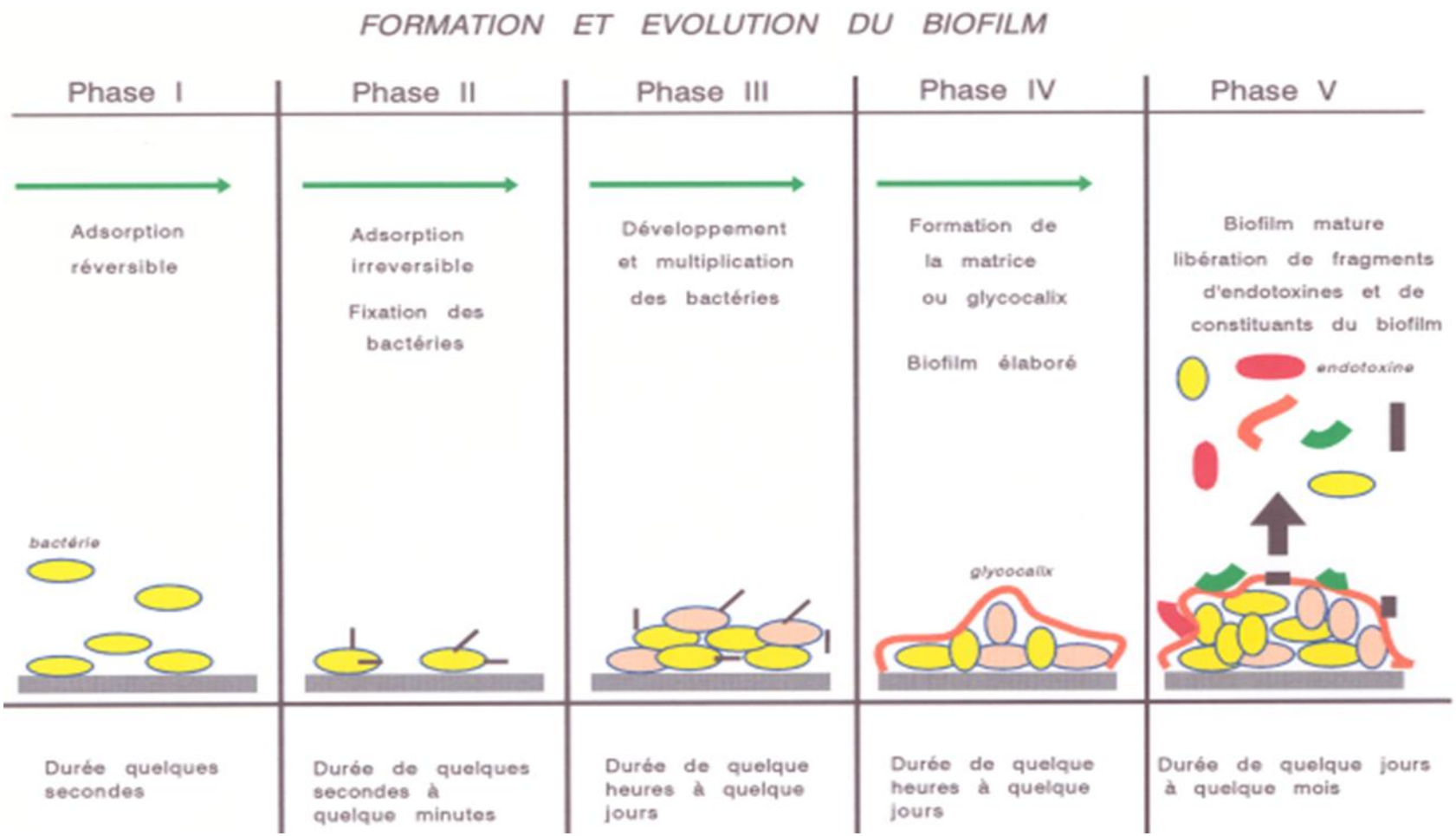
Water for Pharmaceutical Use (WPU):
Biofilm: How?

- It results from the production by germs members fibrous tangle (polysaccharides, glycoproteins).

- This results in a biofilm which is subsequently responsible for the release of micro-organisms, microbial derivatives such as peptidoglycans, endotoxins (MM 20 000 Daltons), proteins, polysaccharides (MW> 200,000 Daltons) and fragments and basic shapes from walls of bacteria G (MM 500-1 500 Daltons).

Water for Pharmaceutical Use (WPU):

Biofilm: For example



Water for Pharmaceutical Use (WPU):
Biofilm: Resistance

- This matrix protects microorganisms biocides attacks.
- In vitro experiments suggest that bacteria "packed" in biofilms can be 50 to 500 times more resistant to chemotherapy as identical original bacteria "free" state.
- Biofilm is the vector by release of colonization of microorganisms, bacterial by-products (endotoxins, bacterial wall G- ...)

Water for Pharmaceutical Use (WPU):
Biofilm: Resistance

- It makes it difficult or inefficient chemical disinfection.

- For example, it has been demonstrated that the ClO or ClOH fraction of sodium hypochlorite (according to the pH of the solution) is blocked on the surface of polymers, a biofilm constituents.

Water for Pharmaceutical Use (WPU):
Biofilm: Importance of the temperature

- The temperature has a major importance in the metabolism and the growth of microorganisms.
- Indeed, every body possesses an optimal temperature of growth rate.
- For the values above or below this value, the growth rate quickly decreases.

Water for Pharmaceutical Use (WPU):
Biofilm: Importance of the temperature

- It is thus possible to classify bacteria according to their behavior in front of temperatures of their environment:
  - **Compulsory psychrophilic, as Bacillus psychrophilic, develops only for temperatures inferior or equal in15°C.**
  - **Mesophilic(coli E.) develops in temperature between 20 - 45°C**
  - **Thermophilic, as Thermus aquaticus, lives in temperatures superior or equal 55°C**
  - **Hyper-thermophilic, as Pyrococcus sp. or Sulfolobus sp ., distinguish themselves from the previous ones for the record values of survival in extreme temperatures (superior or equal 90°C). Their optimal temperatures of growth vary between 80 -113°C.**

Water for Pharmaceutical Use (WPU):
Biofilm: Importance of the temperature

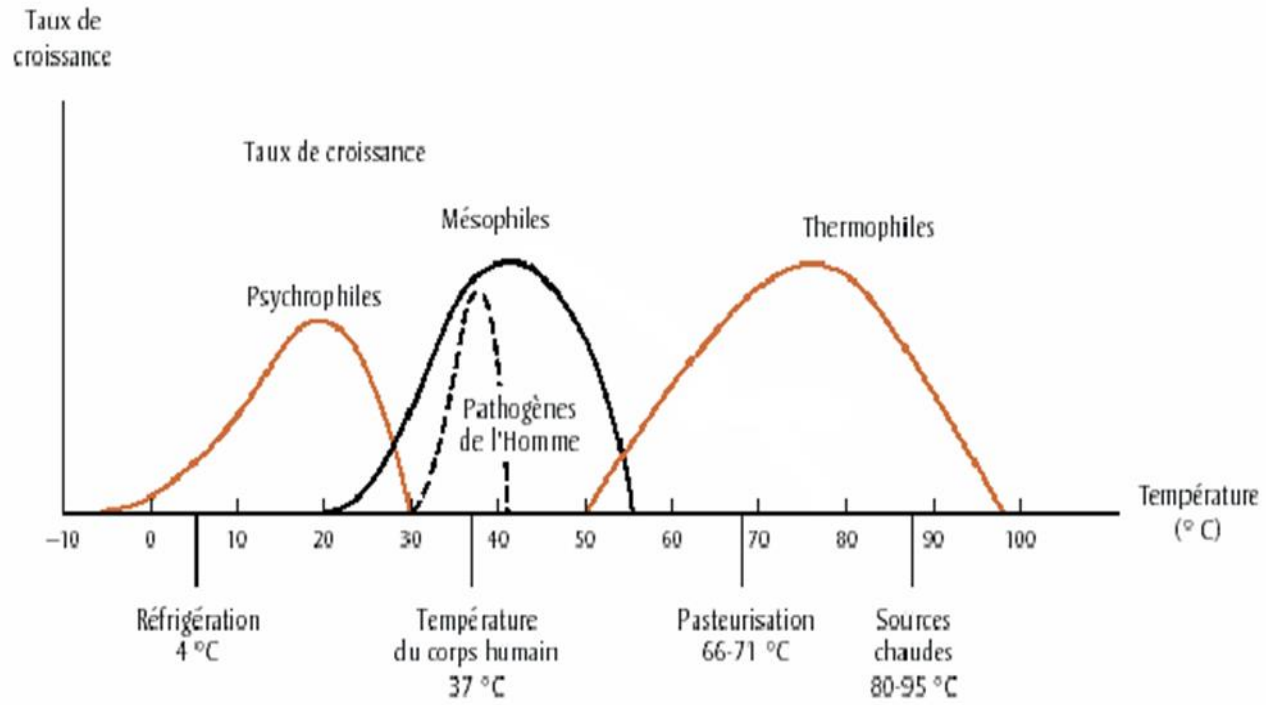
- You see in these examples that the temperature of 20°C is a little bit particular temperature because it is often the starting point of the important growths of mesophilic (all the human pathogenic origins are mesophilic!!). It is one of reasons why, during bacterial identification of the mesophilic florae, we chose 20-22°C or 30-35°C.

- For that reason it's better to maintain the loops of BPW in a temperature between 15 and 18°C and not in 20°C.

Water for Pharmaceutical Use (WPU):
Biofilm: Importance of the temperature

- Curve of growth of microorganisms according to the temperature

Figure 2. Courbes de croissance des microorganismes psychrophiles, mésophiles et thermophiles selon la température



Water for Pharmaceutical Use (WPU):
Biofilm: Importance of the temperature

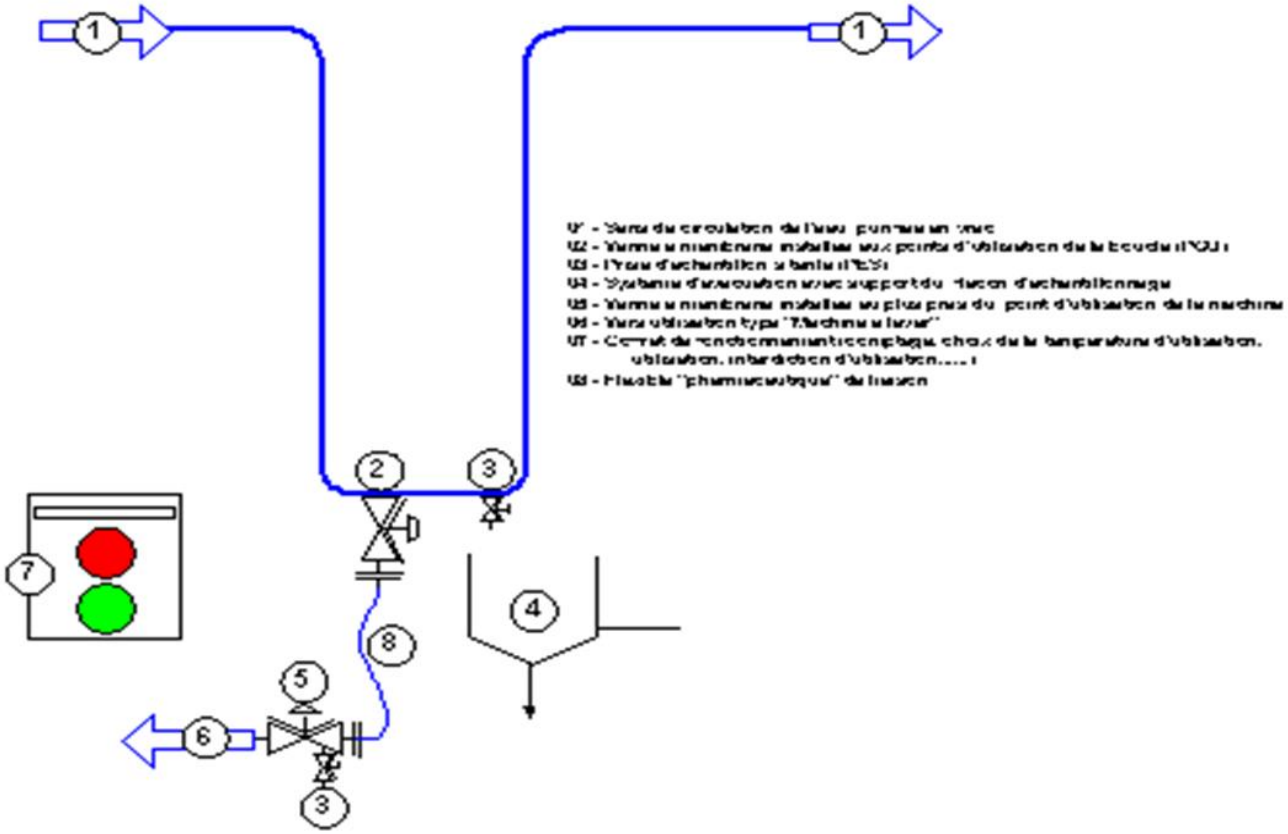
- Cold loop:
  - **Biofilm settles very quickly in cold loops maintained 15/18 °C (BPW or BWFI) and very rarely in a loop continuously maintained at a temperature greater than or equal to 70 ° C (BPW or BWFI).**
  - **Cold loops are most affected by the biofilm: A periodic thermal or chemical disinfection (70-80°C) is not effective on a biofilm**

Water for Pharmaceutical Use (WPU):
Biofilm

- The conditions allowing the biofilm to settle are:
  - **Temperature above 20 ° C.**
  - **Bacteria**
  - **Nutrient.**
- Therefore control these parameters:
  - **The temperature of the loop should never be higher than 18 ° C**
  - **Using a bulk purified water producer producing quality having less than 10 CFU / ml, and a TOC below 50 ppb**
- Pharmacopoeias asked to produce the BPW consistent and maintain the quality when we distribute.

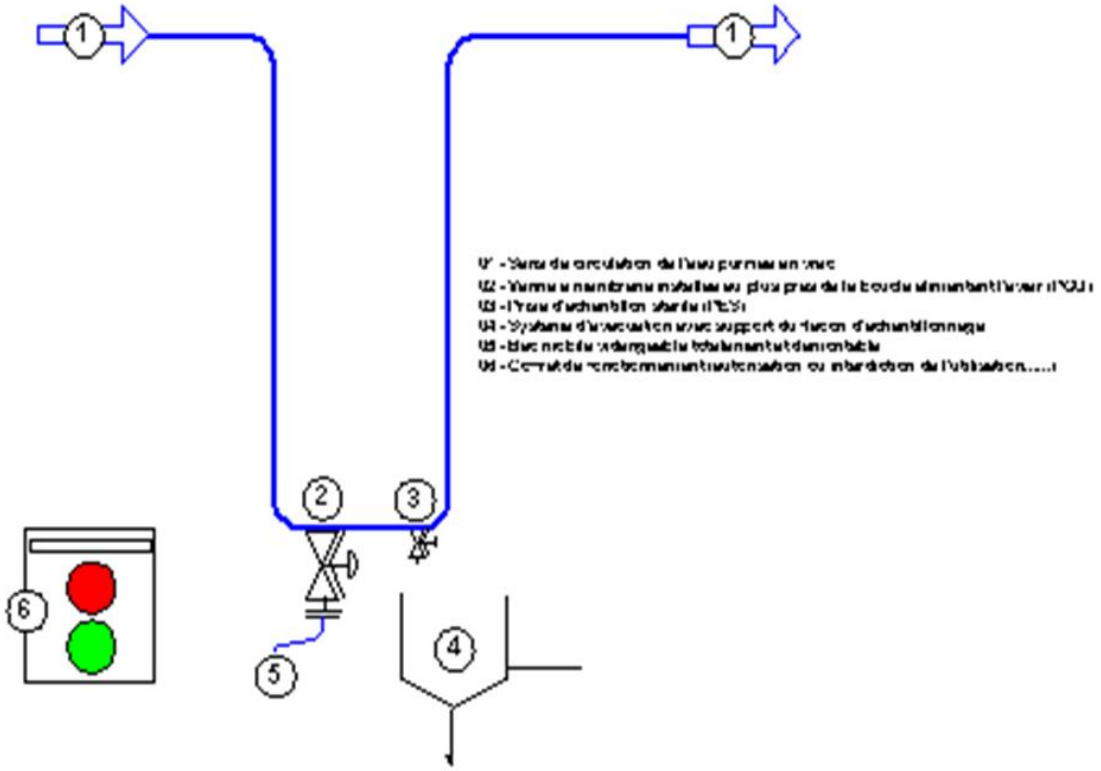
Water for Pharmaceutical Use (WPU):
Biofilm: Point Of Use (POU)

- POU and Sampling valve: Where are Installed (distribution loop)?



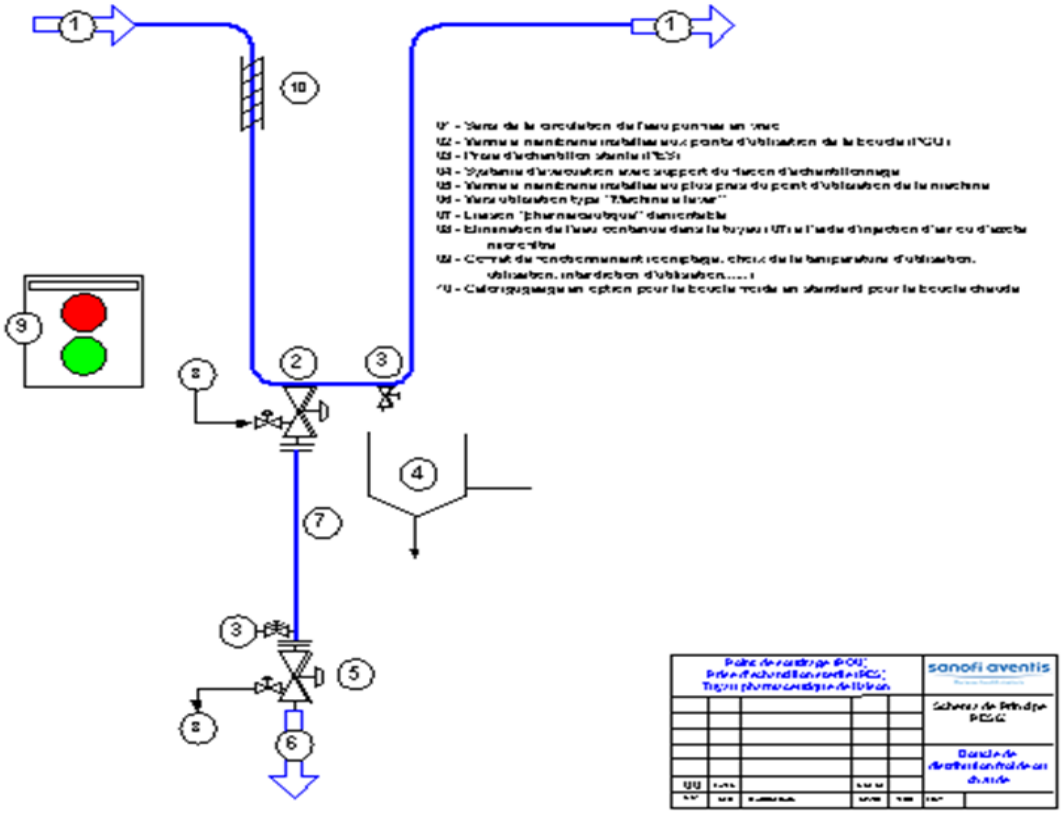
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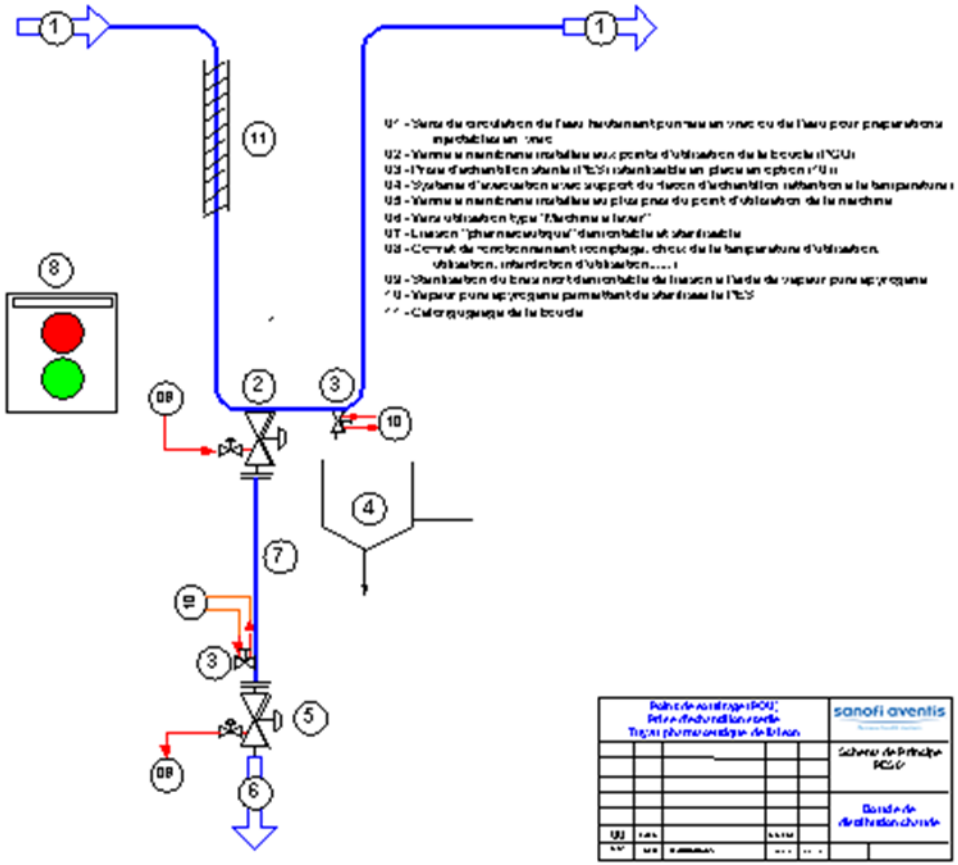
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Water for Pharmaceutical Use (WPU):
Biofilm: Point Of Use (POU)

- POU and Sampling valve: Where are Installed (distribution loop)?



Water for Pharmaceutical Use (WPU):
Biofilm: Sampling Valve

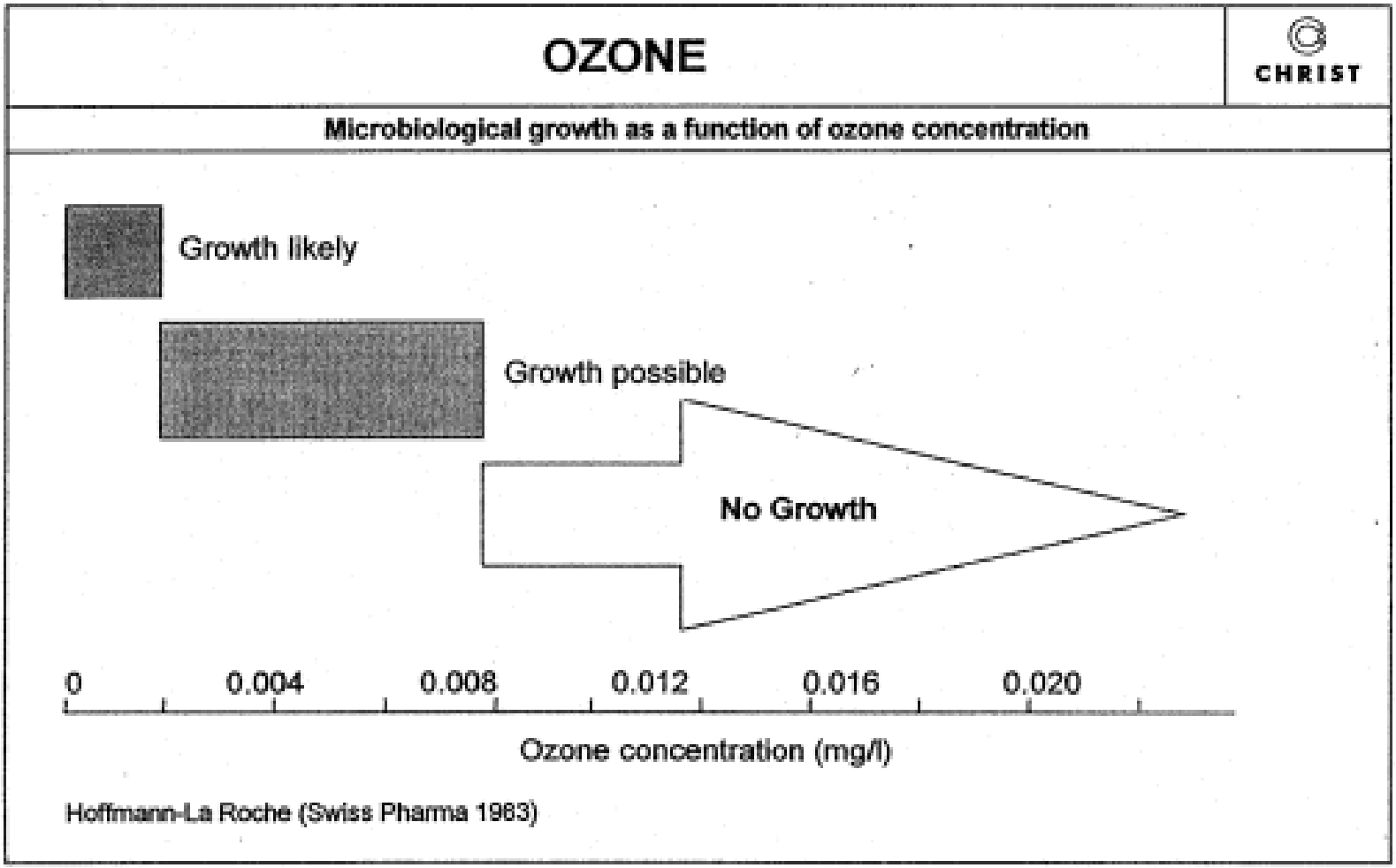




Water for Pharmaceutical Use (WPU):
Biofilm: Micro-Ozonation

- Strong oxidizing power (2.08 V)
- Disinfecting,
- Destruction of the envelope of the bacteria, viruses,
- Destruction of bacterial endotoxins,
- Prompt and effective action,
- TOC reduction in CO2,
- Short half-life in ultra pure water, less than 20 minutes at pH 7 and 25 ° C,
- Prevents biofilm formation,
- Total and rapid elimination

Water for Pharmaceutical Use (WPU):
Biofilm: Micro-Ozonation



Water for Pharmaceutical Use (WPU):
Biofilm: Conclusion

To prevent the onset of biofilm, it must:

- BPW superior quality demand pharmacopoeia for example:
  - **10 CFU / ml**
  - **50 ppb TOC**
- Use the technique of micro-ozonation.
- Write procedures for all POU.
- Control any back contamination.
- Control the temperature distribution in the loop 15 to 18°C

Water for Pharmaceutical Use (WPU):
Biofilm: Conclusion

- How can we detect the presence of a biofilm:
  - **By an increase in the TOC and endotoxin.**
  - **Contaminated POU are never the same.**
  - **Biofilm can hatch due to a water hammer, work on the loop ....**
- How can we eliminate:
  - **For the Cold loops: by chemical cleaning with caustic soda for BPW loops and sanitization by micro-ozonation.**
  - **For the Hot loops: By a thermal disinfection at 121 ° C**
- Comment:
  - **Good manufacturing practice asks to maintain the BWFI in a minimal permanent temperature of 70°C. This temperature avoids any appearance of Biofilm in the loop.**

Thank You