Evaluation of the risk of *Legionella spp.* development in sanitary installations (part 2)

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Abstract

In order to determine whether it is possible to reduce energy use for domestic hot water (DHW) production and distribution, without increasing the risk of *Legionella spp*. development in sanitary installations, a full-scale test facility was built. On a daily basis, a consumption profile corresponding to the DHW use of a single family was applied separately using two tap pipes, one corresponding to a kitchen and the other to a bathroom. *Legionella spp*. was cultivated in a separate water tank and then transferred into the test facility. The DHW production temperature was kept at 45°C with a periodical heating to 60°C for different durations and different frequencies. *Legionella spp.* concentrations were then measured, both in the water and in the biofilm.

Previously, we found that the thermal shocks at 60°C of the water storage tank for many hours without treatment of the bottom of the tank and the draw-offs pipes at the same time was not effective to control *Legionalla spp.* growth. The *Legionella spp.* concentrations reached 10^5 - 10^6 cfu/l within a couple of days after a thermal shock. During the second part of 2017, we have increased the thermal shocks to 65°C in the test facility for different duration, and with or without thermal disinfection of the draw-off pipes. The expansion vessel, installed on the cold water inlet of the DHW storage tank, seemed to be an important source of recontamination of the installation. After removing the vessel, and by applying a weekly thermal shock at 65°C in combination with a regular draw-off on each draw-off pipe during this thermal shock for at least 150 seconds, we were able to maintain the *Legionella spp.* concentration beneath the limit of 1000 cfu/l.

Keywords

Water supply, *Legionella spp.* development, domestic hot water (DHW), disinfection, biofilm, thermal shock

1 Introduction

An optimal design [1] of the drinking water system (hot and cold) aims to combine energy efficiency with a high hygienic quality of the water at the taps by avoiding for instance the development of *Legionella spp.*, a pathogenic bacteria, which can lead to a severe pneumonia.

Knowing that *Legionella spp.* bacteria grow between 25°C and about 45°C while it is decimated above 50°C [2,5], the aim of the study was to evaluate whether it is possible to produce and distribute the domestic hot water at temperatures within the growth range of the bacteria - i.e. energy efficient, but still comfortable in use- in combination with regular thermal shocks above 50°C in order to ensure hygienic quality.

While several authors reported studies on the influence of the temperature on the growth/death rate of *Legionella spp.* bacteria in laboratory conditions [2,5] or in a pilot installation [3,4], the full-scale test facility offers the opportunity to study the effect of multiple controlled thermal shocks on the survival of *Legionella spp*. At the BBRI, a full-scale test facility was built, consisting of a distribution loop of nearly 40 metres long, a 200 liters DHW storage tank ("test tank") maintained at 45°C, and 2 draw-off lines.

The test facility, the applied draw-off profile and the sampling protocol are described in our previous article.

2 Heat shock treatment in the test facility

Previously, we found that the thermal shocks at 60°C in the water storage tank for many hours without treatment of the bottom of the tank and the draw-offs pipes at the same time was not effective to control *Legionalla spp.* growth. The *Legionella spp.* concentrations reached 10^5 - 10^6 cfu/l within a couple of days after a thermal shock.

During the second part of 2017, we have increased the thermal shocks to 65°C in the test facility (see **table 1**).

Table 1 - Tested thermal shocks at 65°C.

Week	T production (tank)	T heating (thermal shock)	Duration	Frequency	Number of thermal shocks
26 (11/07)	45 °C	65 °C (setpoint = 68°C, with flow rate 1,3 l/min)	Warming up + 30 min	1x / week with extra circulation on tank.	1 shock
27 (18/07)	45 °C	65 °C (setpoint = 68°C, with flow rate 1,3 l/min)	Warming up + 1h	1x / week with extra circulation on tank.	1 shock

28 (26/07)	45 °C	65 °C (setpoint = 68°C, with flow rate 1,3 l/min)	Warming up + 4 x 30 min (for taps disinfection)	1x / week with extra circulation on tank. 4 x 30 minutes thermal disinfection of the sampling taps and draw-off pipes in the 'circulation' direction	1 shock
29 (31/07)	45 °C	65 °C (setpoint = 68°C, with flow rate **4,4** l/min)	Warming up + 1 h	7x / week with extra circulation on tank	7 shocks
30 08/08 removing of the expansion vessel (09/08 shock)	45 °C	65 °C (setpoint = 68°C, with flow rate 4,4 l/min ;	Warming up + 4 x 30 min (for taps disinfection)	1x / week with extra circulation on tank. 4 x 30 min thermal disinfection of the sampling taps and draw-off pipes in the 'circulation' order	1 shock
31 - 34 (18/08) - (01/09) (08/09)	45 °C	65 °C (setpoint = **65**°C with flow rate 4,4 l/min ;	8 h	1x / week with extra circulation on tank. + automatic scheduled draw-offs	3 shocks (no shock during the second week)
35 (14/09)	45 °C	65 °C (65°C with flow rate 4,4 l/min	24 h	1x / week with extra circulation on tank. + automatic scheduled draw-offs (kitchen on 13:45 = 30 s)	1 shock
36 (21/09)	45 °C	65 °C (65°C with flow rate 4,4 l/min	24 h	1x / week with extra circulation on tank. + automatic draw-offs (kitchen on 13:45 = **90** s)	1 shock
37 (28/09) & 38 (05/10)	45 °C	65 °C (65°C with flow rate 4,4 l/min	24 h	1x / week with extra circulation on tank. + automatic draw-offs (kitchen on 13:45=**120** s)	2 shocks
39 to 48 (12/10) - - - - - (23/11) (30/11) (07/12) (14/12)	45 °C	65 °C (65°C with flow rate 4,4 l/min	24 h	1x / week with extra circulation on tank. + automatic draw-offs (kitchen on 13:45=**150** s)	1 shock, then no schocks during 5 weeks + 4 shocks

3 Results

3.1 Efficiency of the thermal shocks on the *Legionella spp.* concentration in water

In order to reach 65°C at the end of the circulation pipe, the temperature setpoint of the DHW storage tank during the thermal shocks was first set on 68°C with a flow rate of 1,3 l/min (from 11/07/2017 to 07/08/2017). Despite this higher setpoint in the main part of the facility during 3 shocks (weeks 26 to 28), no complete disinfection of the test facility was reached, even after 7 daily thermal shocks (week 29).

From week 29 onwards, the circulation flow rate in the loop was increased to 4,4, l/min in order to reduce the thermal gradient along the loop (deltaT = 1,3°C). After 7 consecutive daily thermal shocks in week 29 (temperature setpoint at 68°C for 1 hour during the night, circulation flowrate at 4,4 l/min) *Legionella spp.* concentrations reached 10^5 cfu/l in the depart of the circulation loop already a few hours after the thermal shocks.

As we suspected *Legionella* growth in the expansion vessel, situated on the cold water supply of the DHW storage tank (see Figure 1), samples of the water in the connexion pipe between the expansion vessel and the return circulation pipe were analysed.

Figure 1 - Global views of the expansion vessel on the inlet connexion of the test tank.

Table 2 shows the concentration of *Legionella spp*. found in this part of the installation.

As the concentration in the water from the connexion pipe between the expansion vessel ant the circulation return pipe was high, we decided to remove the expansion vessel from the installation in order to avoid reinoculation of the test facility through the expansion vessel.

Table 2 - *Legionella spp.* concentration on 08/08.

Water Sample	Concentration in *Legionella sp* [cfu/l]
Water from the Depart of the distribution pipe	1.00E+05
Water from the return Circulation pipe	2.40E+01
Water from the connexion pipe between the expansion vessel and the return circulation pipe	1.40E+04

On 09/08 (the day just after the removal of the expansion vessel), a new thermal shock was applied in combination with a thermal treatment of the 2 draw-offs pipes (30 minutes of draw-off > 60°C). It was the first time that the samples collected in the next morning were all below the limit of 1000 cfu/l (see **table 3**).

Table 3 - *Legionella spp.* concentration on 10/08

Water samples (1st liter)	***Legionnella spp.* concentration [cfu/l]**
Depart pipe of the circulation loop	77
T-piece to the kitchen draw-off pipe	30
Kitchen faucet	below detection limit
T-piece to the shower draw-off pipe	140
Shower	below detection limit
Return pipe of the circulation loop	below detection limit

After two weeks without shock, our next step was to apply a thermal shock of 65°C during the normal consumption profile in order limit manual intervention on the test facility during the shock.

During this shock, the temperature setpoint was 65°C during 24 hours, the circulation flowrate was 4,4 l/min, and the automatic consumption profile was applied. Only for the kitchen draw-off pipe, this shock was not effective. Due to this short tapping duration (30 seconds) in this pipe, the maximum temperature reached at the kitchen faucet was only 61,8 °C, and 60°C was only exceeded during 13 seconds.

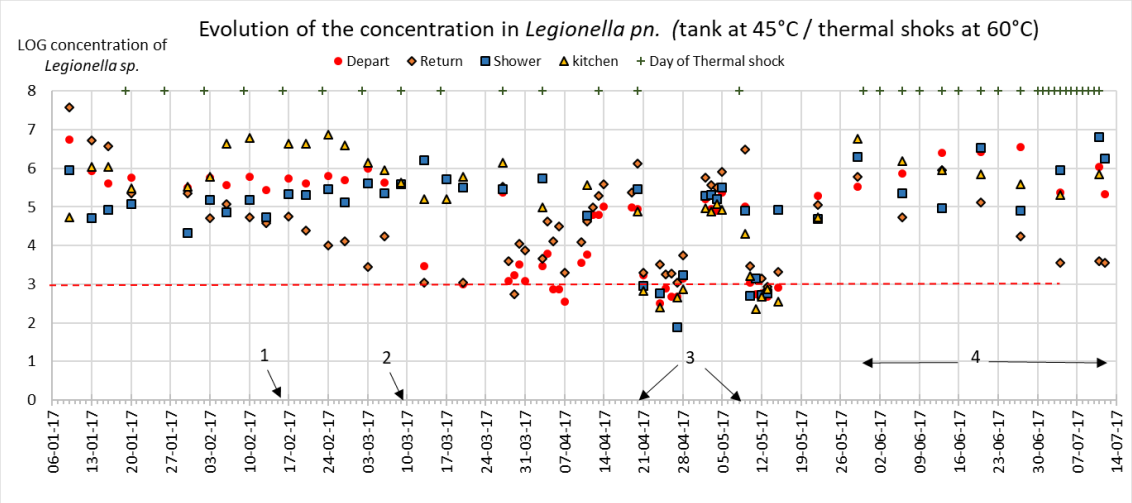
During the following weeks (36 to 39), the draw-off duration in the kitchen was progressively increased (90 s, 120 s, 150 s). The draw-off duration of 150 s seemed to be satisfying to maintain de *Legionella spp.* concentration below the limit of 1000 cfu/l.

After the *5* weeks without thermal shocks (from 12/10 until 23/11), the concentration of the *Legionella spp.* reached $10^5 – 10^6$ cfu/l again and the last thermal shock was then re-applied in order to confirm the result.

We concluded that it was possible to maintain the concentration of the *Legionella spp.* below the limit of 1000 cfu/l, in this test facility, with a weekly thermal treatment of 65°C for 24 hours, assuring the use of all faucets during the shock and this for at least 150 seconds.

We also tested a temperature shock of 70°C during 4 minutes over the circulation loop, and even 7 daily shocks of 1h at 70°C. These shocks affected the circulation loop, but did not eradicate *Legionella spp.* from the installation.

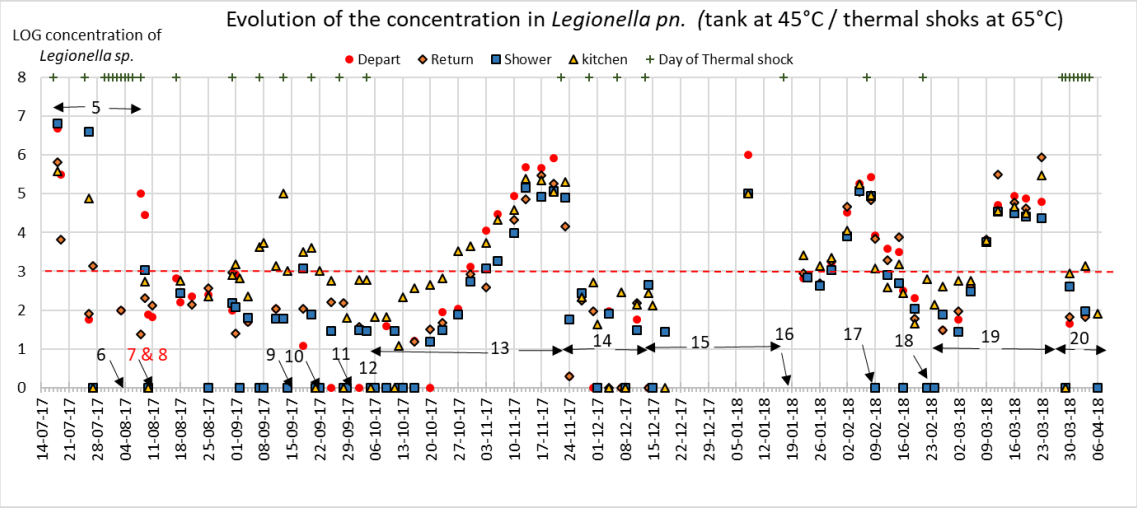
Figures 2a and 2b show the evolution of the *Legionella spp.* concentration in the test facility maintained at 45°C, with different protocols of thermal shocks at 60°C and 65°C.



Legend :

1	Extra recirculation on the DHW storage tank during the thermal shock (since 16/02/2017)
2	Systematic disinfection of the sampling valves with Alcool 70° for 2 min. (since 09/03/2017)
3	Thermal disinfection includes draw-offs pipes on 20/04/2017 and 08/05/2017
4	Thermal disinfection of the loop (1 hour@60°C) during the night : 2x/week then 1x/day from 30/05/2017 to 10/07/2017

Figure 2a - Evolution of the *Legionella spp.* concentration in the test facility maintained at 45°C, with different protocols of thermal shocks at 60°C.



Legend :

1	Extra recirculation on the DHW storage tank during the thermal shock (since 16/02/2017)
2	Systematic disinfection of the sampling valves with alcohol 70° for 2 min. (since 09/03/2017)
3	Thermal disinfection includes draw-offs pipes on 20/04/2017 and 08/05/2017

4	Thermal disinfection of the loop (1 hour@60°C) during the night : 2x/week then 1x/day from 30/05/2017 to 10/07/2017
5	Temporary transitional regime 45°C / thermal shocks @65°C (T° setpoint at 68°C) during the night (from 11/07/2017 to 07/08/2017)
6	Circulation flow set on 4,4 l/min since 31/07/17 (while previously set on 1,3 l/min)
7	Disassembling of the expansion vessel (get off/ away) since 08/08/2017
8	Thermal disinfection includes 30 min disinfection of the draw-off pipes on 09/08/2017
9	Automatic kitchen draw-off on 13:45 set on 30 second (initial value) during the thermal shock on 14/09/2017
10	Automatic kitchen draw-off on 13:45 set on 90 second during the thermal shock on 21/09/2017
11	Automatic kitchen draw-off on 13:45 set on 120 sec during the thermal shock on 28/09/2017
12	Automatic kitchen draw-off on 13:45 set on 150 second during the thermal shocks since 05/10/2017
13	Period of 5 weeks without any disinfection (concentration in *Legionella spp.* below limit)
14	Same as 12. with thermal shock 1x/ week (23/11 ; 30/11; 07/12 and 14/12)
15	Period of 5 weeks without any disinfection (from 15/12 to 18/01/2018)
16	Same as 12 (kitchen draw-off on 13:45 set on 150 second during the thermal shock)
*	20/01/2018 : Leakage on the circulation pump and dismounting of the thermal insulation beneath the tank (25/01) --> 3 weeks without any disinfection
17	Same as 12 but without thermal insulation beneath the storage tank (8/02)
18	Same as 12 but with new thermal insulation beneath the storage tank (22/02)
19	Period of 5 weeks without any disinfection (from 23/02 to 28/03/2018)
20	29/03/2018 : 1 thermal shock on 70°C/ 4 min during the day and then daily shocks on 70°C /1 h during the night (from 30/03 to 06/04/2018)

Figure 2b - Evolution of the *Legionella spp.* concentration in the test facility maintained at 45°C with different protocols of thermal shock at 65°C.

3.2 Efficiency of the thermal shocks on the fixed *Legionella sp.* (biofilm)

On the return pipe, a section of pipe DN25 : ø 25 x 2,5 mm (length = 0,8 m) was inserted with 20 pieces of PE-x pipe (diam. 12 x 1,2 mm) into. The length of each PE-x pipe measure nearly 29,7 mm, so the total developed surface is ~ 20 cm². The DHW can flow through this littles pieces and the biofilm can grow on it. Before and after a disinfection shock, we can stop the loop, close the valves and take one of this piece for biofilm sample analyses.

After several thermal shocks at 60°C (13x) a tube was collected from the return pipe. To recover the biofilm, it is placed in a sterile funnel with 50 ml of sterile water and sonicated. This solution was used to analyse for the presence of *Legionella* bacteria and ATP.

The results from the ATP measurements showed that the bacterial flora on the tube was not affected by the different heat shocks (mean value 5.5 +- std dev 0.5 log cell counts/ml; pg/ml ATP converted to log cell counts per ml). No correlation was found between the total bacterial flora and *Legionella spp.* in the biofilm. Thermal shocks on a weekly bases showed a progressive decrease of the *Legionella spp.* concentration on the tubes (up to a reduction of 3.3 log), but after a period of 15 days without any thermal shock *Legionella spp.* bacteria recovered to the initial concentration in the biofilm.

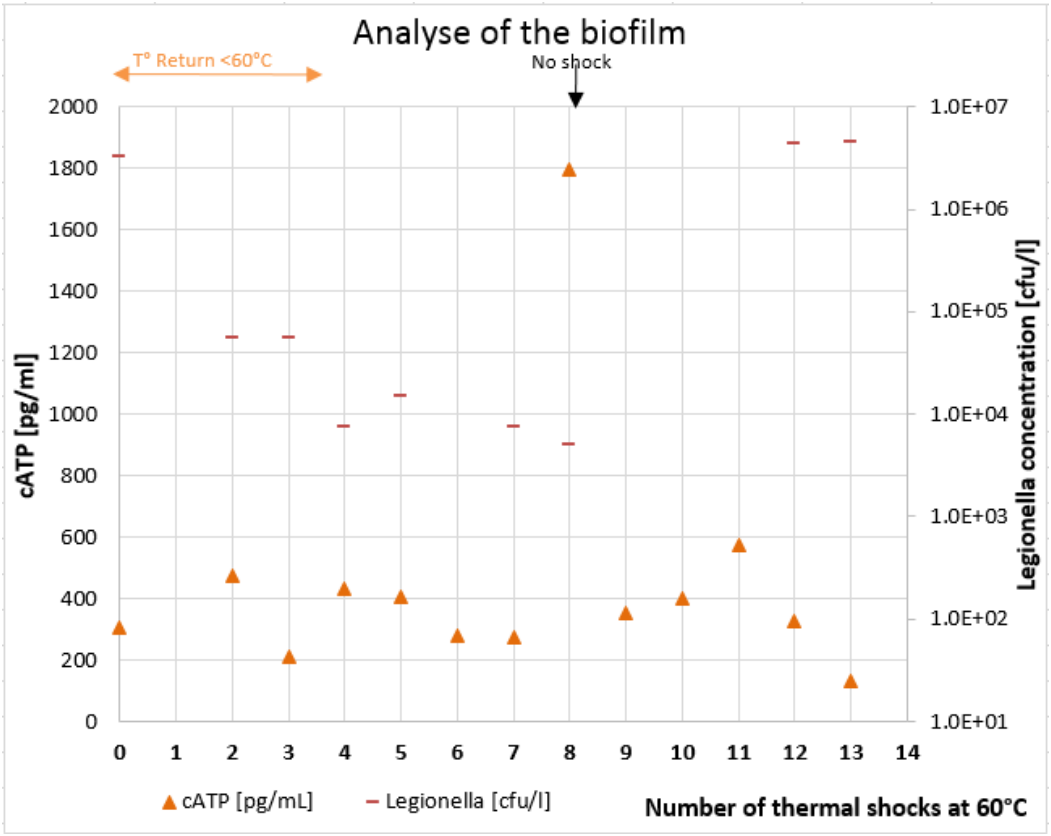


Figure x – Evolution of the cATP and *Legionella spp.* population into the biofilm after the different thermal shocks at 60°C.

4 Conclusions

In this test facility, applying different types of thermal shocks at 60°C (up to 2h) on a contaminated installation with a DHW production temperature of 45°C was not sufficient to keep *Legionella spp.* concentrations beneath 1000 cfu/l, in both the water tank and the circulation loop. Even daily shocks at 60°C were insufficient After the thermal shocks the Legionella concentrations reached 10^5 - 10^6 cfu/l within a couple of days.

The expansion vessel, installed on the cold water inlet of the DHW production, proved to be an important source of recontamination of the installation after a thermal shock.

Applying a weekly thermal shock of 24h at 65°C, in combination with regular draw-off during this shock on both draw-off pipes (of minimum 150s in this test facility), lead to stable *Legionella spp.* concentrations <1000 cfu/l.

Daily heat shocks at 70°C with a short duration on the circulation loop did not eradicate *Legionella spp.* from the whole test facility.

5 References

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6 Presentation of Author(s)

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