Biofilms can form under stressful conditions and offer protection against mechanical and chemical disruptions, hence providing a refuge for pathogens. One of the possible waterborne colonisers in drinking water distribution system biofilms is *Legionella pneumophila*, which is still responsible for many outbreaks of Legionellosis worldwide. The aim of this work was to study the influence of important operational parameters, including shear stress, carbon concentration and temperature, on persistence of this pathogen in drinking water biofilms. The biofilm studies were carried out using a two-stage chemostat system. The outflow culture of the first vessel fed three secondary chemostats in parallel and under different conditions of shear stress and carbon concentration. After 10 days the chemostats attained steady conditions and PVC coupons were then immersed to allow biofilm formation. The coupons were removed at different times (up to 32 days) and scraped with sterile glass beads. Planktonic and sessile cells were quantified by standard cultivation techniques (R2A and BCYE agar) and SYTO9 staining. In addition, a specific 16S rRNA peptide nucleic acid probe (PNA) was used to quantify the total numbers of *L. pneumophila* in the biofilm. The conditions described above were tested at two different temperatures, 15 and 20°C. Concerning sessile cells, the number of total fluorescently labelled bacteria and *L. pneumophila* were similar for the three conditions studied at both temperatures. However more cultivable cells were seen when chemostats were operated with high shear and high carbon concentrations, particularly the latter. This might indicate the existence of Viable But Non Cultivable *L. pneumophila* that can recover cultivability under certain conditions. For example, the total of fluorescently labelled *L. pneumophila* was higher at 15°C. In conclusion, temperature was the most important factor influencing the formation of biofilm and persistence of *L. pneumophila*. Carbon concentration also had a considerable influence on the number of total cells in water and biofilms. Shear stress played only a minor role in the numbers of planktonic and sessile cells present.

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INFLUENCE OF CARBON CONCENTRATION, SHEAR STRESS AND TEMPERATURE ON SURVIVAL OF LEGIONELLA PNEUMOPHILA IN DRINKING WATER BIOFILMS

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