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ORIGINAL PAPER

Growth kinetics of environmental *Legionella pneumophila* isolated from industrial wastewater

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Abstract

Wastewater treatment plants are environmental niches for *Legionella pneumophila*, the most commonly identified causative agent of severe pneumonia known as Legionnaire's disease. In the present study, *Legionella pneumophila*'s concentrations were monitored in an industrial wastewater treatment plant and environmental isolates were characterized concerning their growth kinetics with respect to temperature and their inhibition by organic acids and ammonium. The results of the monitoring study showed that *Legionella pneumophila* occurs in activated sludge tanks operated with very different sludge retention times, 2.5 days in a complete-mix reactor, and 10 days in a membrane bioreactor, indicating that this bacterium can grow at different rates, despite the same wastewater temperature of 35 °C. The morphology of *Legionella* cells is different in both reactors; in the membrane bioreactor, the bacteria grow in clusters, while in the complete-mix reactor, filaments predominate demonstrating a faster growth rate. *Legionella pneumophila* concentrations in the complete-mix reactor and in the membrane bioreactor were within the range 3×10^1 to 4.8×10^3 GU/mL and 3×10^2 to 4.7×10^3 GU/mL, respectively. Environmental *Legionella pneumophila* SG2–14 isolates showed distinct temperature preferences. The lowest growth rate was observed at 28 °C, and the highest 0.34 d^{-1} was obtained at 42 °C. The presence of high concentrations of organic acids and ammonium found in anaerobically pre-treated wastewater caused growth inhibition. Despite the increasing research efforts, the mechanisms governing the growth of *Legionella pneumophila* in wastewater treatment plants are still unclear. New innovative strategies to prevent the proliferation of this bacterium in wastewater are in demand.

Keywords *Legionella pneumophila* · Industrial wastewater · Activated sludge · Growth kinetics · Inhibition

Introduction

Legionella pneumophila (*L. pneumophila*) is a pathogenic bacteria found in nature as well as in man-made water systems. *L. pneumophila* was the first species among the genus *Legionella* to be identified after an outbreak of pneumonia in 1976 during the American Legion convention in Philadelphia, USA (Fields et al. 2002; WHO 2018). Legionellosis is caused by the inhalation of contaminated aerosolized water, and it can vary from a mild fever flu-like illness called Pontiac fever to a sometimes severe form of pneumonia, known as Legionnaires disease (LD) (US-EPA 2016). The genus *Legionella* includes nowadays more than 50 species and circa 70 serogroups, from which almost half of the species have been identified to be pathogenic (Borella et al. 2005; Fields et al. 2002; WHO 2018). *L. pneumophila* the most relevant and studied species within the genus is divided into 15 serogroups, from which *L. pneumophila* SG1 has been identified as the causative agent of more than 90% of confirmed LD outbreaks and sporadic cases (ECDC 2015; Fields et al. 2002). The United States Environmental Protection Agency (US EPA) has included *L. pneumophila* within the drinking water contaminant candidate list and regulatory determination 4 (CCL4) (US EPA 2016). Other non-*L. pneumophila* species have also been frequently isolated from infected patients, namely *L. micdadei*, *L.*

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bozemanii, *L. dumoffii*, and *L. longbeachae* (US-EPA 2016). Particularly, *L. longbeachae,* which has been mainly isolated from compost and potting mixes, is the main epidemic agent of Legionnaires disease cases in Australia and New Zealand since 1980 (Whiley and Bentham 2011). *Legionella* are often found in water as free-living bacteria or intracellularly inside protozoa (Scheikl et al. 2014). They are capable of colonizing different water systems with varying environmental conditions such as temperature, pH, oxygen concentration, and nutrients availability (Borella et al. 2005; Fliermans et al. 1981; Van Heijnsbergen et al. 2015). According to the literature, the key factors favouring growth and persistence of *Legionella* in drinking water distribution systems are temperature (25–40 °C) (Ohno et al. 2003), biofilm formation (Lau and Ashbolt 2009), and the presence of protozoa (Buse et al. 2012). The ability of *Legionella* to parasite and multiply inside several protozoa species including amoeba and ciliates is considered as one of the most important strategies to survive complex matrixes like biofilms within drinking water distribution systems (Buse and Ashbolt 2011; Rowbotham 1986; Taylor et al. 2009; Wadowsky et al. 1985).

The occurrence of *Legionella* in municipal and industrial wastewater treatment plants (WWTPs) is widespread and has been reported in several studies as discussed in the recent literature review by Caicedo et al. (2019). Most of the studies reported the growth of *L. pneumophila* in industrial WWTPs operating in a temperature range between 30 and 38 °C treating wastewater from wood production and paper mills (Allestam et al. 2006; Fykse et al. 2013; Kusnetsov et al. 2010), breweries (Maisa et al. 2015; Nogueira et al. 2016), and petrochemicals and food production (Lund et al. 2014; Nguyen et al. 2006). In Scandinavian countries, extensive research in WWTPs from the paper mill industry has shown that for temperatures equal to or higher than 30 °C in the aerobic biological treatment systems, *L. pneumophila* was present; otherwise, it could not be detected, showing a clear relationship between temperature and the occurrence of *L. pneumophila*. In industrial WWTPs, the combination of anaerobic–aerobic treatment processes is widely used. Monitoring of *Legionella* has been mainly done in aeration tanks where these bacteria can grow. The pre-anaerobic treatment of wastewater with the concomitant production of high concentrations of ammonium and organic acids might influence *Legionella's* growth in the aerobic post-treatment. Studies on the occurrence of *L. pneumophila* in industrial WWTPs rarely report either on the operational conditions in the treatment processes or on the presence of high concentrations of specific compounds that might interfere with *Legionella's* growth. A more comprehensive monitoring programme including microbiological as well as physicochemical and operational parameters can shed light on *Legionella's* growth strategy(-ies) in industrial WWTPs.

Identifying all the relevant factors that condition the growth and persistence of *L. pneumophila* in biological treatment systems is extremely challenging. However, comprehensive knowledge of the growth kinetics, inhibiting compounds, and interactions with protozoa are needed for a better understanding of *L. pneumophila* occurrence in WWTPs. Hence, this study done from August 2017 until June 2018 in Hannover, Germany, aimed to investigate: (1) the occurrence of *L. pneumophila* in an industrial WWTP with a combined anaerobic–aerobic treatment processes; (2) the spatial distribution of *Legionella* and protozoa within aerobic reactors; and (3) the growth kinetics *L. pneumophila* environmental isolates at different temperatures and inhibition by organic acids and ammonium.

Materials and methods

Sample collection

Samples were collected from an industrial treatment facility receiving wastewater from a food-processing industry. The industrial plant treats wastewater with a temperature range between 30 and 35 °C, an average chemical oxygen demand (COD) of 15.8 g/L, and an average total Kjeldahl nitrogen (KN) of 555 mg/L. The average sludge-loading rate was 0.65 kg COD kg VSS^{-1} d^{-1}. As depicted in Fig. 1, the industrial wastewater (process water), with high nutrient content, is anaerobically pretreated and then directed to a membrane bioreactor (MBR) that is operated at 2.5 days hydraulic retention time and 8–12 days sludge age. As also shown in Fig. 1, 20 − 25% of the process water is aerobically treated in a complete-mix reactor (CMR) operated at 2–2.5 days hydraulic retention time.

A total of 25 grab samples were collected between August and November 2017 from different process steps

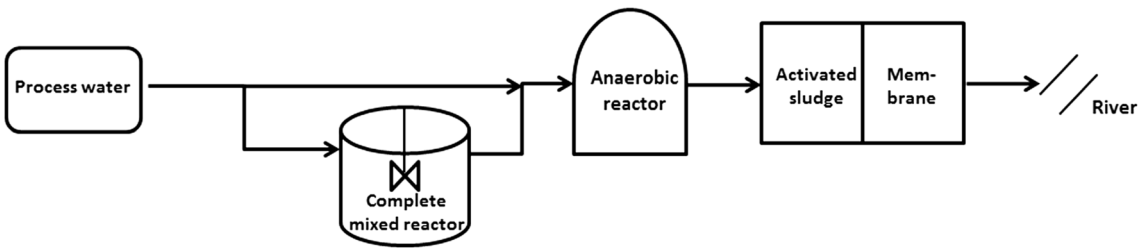

Fig. 1 Simplified diagram of the industrial WWTP

in the WWTP, namely 5 samples from process water, 10 samples from the mixed liquor in the CMR, 4 samples from the effluent of the anaerobic reactor, and 6 samples from the activated sludge tank in the MBR. For the quantification of *L. pneumophila* by the qPCR method, samples were collected in polyethylene containers and transported at room temperature within 24 h to the laboratory.

Quantification of *Legionella*

The qPCR method was used for the specific detection and quantification of *L. pneumophila* in wastewater and sludge samples. For this type of samples, the qPCR method gives better results compared to the cultivation method according to the literature (Caicedo et al. 2019; Lund et al. 2014; Medema et al. 2004). The culture method is considered to underestimate *Legionella*'s concentration in wastewater/sludge samples because of the overgrowth of *Legionella* by background flora, the sample pre-treatment conditions that can affect *Legionella*'s cultivability, and the limitation to detect viable but non-culturable *Legionella.*

DNA from the samples was extracted by using the extraction kit QIAamp Fast DNA Stool Mini Kit DNA (Qiagen, Germany) and stored at − 20 °C for subsequent qPCR analysis as described in Caicedo et al. (2016). The mericon Quant *L. pneumophila* Kit (Qiagen) was used for the detection and quantification of *L. pneumophila* in water. Specifically, serial dilutions of DNA standards with a concentration range of 25.000–25 copies per reaction were amplified to generate standard curves. The obtained standard curves were applied to determine the number of copies of the targeted genes in the DNA-extracted samples. To identify potential qPCR inhibitors in the extracted DNA, the mericon PCR assays include internal control, which was co-amplified in each qPCR reaction with target DNA. qPCR amplification curves and *L. pneumophila* concentrations were calculated by the Rotor-Gene Q-series system software version 2.3.1 (Qiagen). The assay detects 6 GU (genomic units) of *L. pneumophila* DNA in a reaction (limit of detection of the qPCR) and 60 GU in 1 mL sample (limit of detection of the method including the DNA extraction). The limit of quantification of the qPCR is 12 GU in a reaction, and the limit of quantification of the method is 120 GU in a 1 mL sample. The qPCR results are expressed as GU/mL.

Isolation of *L. pneumophila* from activated sludge

To obtain *L. pneumophila* environmental isolates from activated sludge samples taken from the MBR, the standard method ISO 11731:2017 "*Water quality and Enumeration of Legionella*" was applied. Samples were pre-treated with HCl (pH 2.2) for 5 min and heated up to 50 °C for 30 min to suppress the growth of background flora. Afterwards, to isolate *Legionella*, samples were serially diluted and plated on GVPC agar (this medium contains the antibiotics glycine, vancomycin, polymyxin B, and cycloheximide). The GVPC agar plates were incubated at 37 °C for 7–10 days. Suspected *Legionella* colonies were sub-cultured on blood agar plates. A serogroup typing of the isolates that did not grow on blood agar plates was done by using the *Legionella* latex test (Oxoid, UK). This test allows the identification of *L. pneumophila* serogroup 1, serogroups 2–14, and *Legionella* species (*Legionella longbeachae* serogroups 1 and 2, *Legionella bozemanii* serogroup 1, *Legionella dumofii*, *Legionella gormanii*, *Legionella jordanis*, *Legionella micdadei,* and *Legionella anisa*.

Fluorescent in situ hybridization (FISH)

Samples from the CMR and MBR were analysed by fluorescence in situ hybridization (FISH) with 16S rRNA oligonucleotide probes. The detection of *Legionella* and protozoa was carried out by applying the following 16S rRNA oligonucleotide probes: LEG705 specific for *Legionella* spp. (Manz et al. 1995), LEGPNE specific for *L. pneumophila* (Grimm et al. 1998), EUK516 targeting eukaryotes (Amann et al. 1990), HART498 for *Hartmanella* spp., NAEG1088 for *Naegleria* spp., and GSP for *Acanthamoeba* spp. (Grimm 2000). The hybridization was done in fixed samples (4% paraformaldehyde) according to Manz et al. (1995) and Nogueira et al. (2002). Probe-conferred fluorescence was detected with a Zeiss observer microscope equipped with ApoTome 2. Images were processed with the software ZEN-Pro delivered with the equipment.

Growth characterization of *L. pneumophila* environmental and clinical isolates

A total of six environmental isolates obtained from activated sludge, and two clinical isolates [*Legionella longbeachae* (strain type DSM 25315) and *Legionella pneumophila* Bellingham (strain type DSM 25214)] were characterized concerning their growth kinetics at six different temperatures: 28 °C, 30 °C, 32 °C, 35 °C 37 °C, and 42 °C. The clinical isolates were obtained from the Leibniz Institute DSMZ—German Collection of Microorganisms and Cell Cultures.

The growth assays were performed following the methodology described by Sharaby et al. (2017). Briefly, all growth assays were done with yeast extract broth (YEB) medium prepared by adding 10 g of yeast extract (BactoTM 212750), 10 g of ACES *N*-(2-acetamido)-2-aminoethanesulfonic acid (Sigma A-3594), 0.4 g of cysteine-HCl (Sigma C-7477), and 0.25 g of Fe-pyrophosphate (Sigma p-6526) into 1000 mL of sterile distilled water. The pH of the YEB medium was adjusted to 6.9, and afterwards, the medium was sterilized

by filtration through a 0.22-µm filter. The YEB medium was diluted 1:1 with sterile distilled water and inoculated with the environmental and clinical isolates. The initial *Legionella* concentration was adjusted to an optical density ($OD_{620\,nm}$) of 0.1, which corresponds to a concentration of approximately 10^7 cells/mL. A volume of 100 µL from each *Legionella* suspension was added to a sterile flat bottom 96-well microplate (3370 Corning USA) and incubated in a microplate reader (Infinite F200 Tecan) until all isolates reached the stationary phase of growth at all desired temperatures. Each isolate was incubated in eight wells for each measurement, and sixteen wells with YEB medium without *Legionella* were incubated for blank values and negative controls. No evidence of cross-contamination was seen in any experiments. Cell growth was measured spectrophotometrically at a wavelength of 620 nm.

The obtained data were processed according to Zwietering et al. (1990). In brief, average values were calculated for each isolate, from which average blank values were subtracted to assure that the $OD_{620\,nm}$ obtained corresponded only to bacterial growth. From these values, the natural logarithm of the fraction: optical density at the time (N_t) and the optical density at an initial time (N_0) were calculated. The obtained values of ln (N_t/N_0) of all environmental isolates were averaged and represented in one growth curve for each temperature. Data analysis was performed by applying the package Grofit of the software R as described by Kahm et al. (2010). Model fitting of the measured growth curves was done applying four well-known bacterial growth models: Logistic, Gompertz, Gompertz exponential, and Richards (Supplementary Material Table 1). The obtained models were then fitted to the bacterial growth curves using the nonlinear least-square fitting method (Sharaby et al. 2017). Lengths of lag phase (λ), maximal specific growth rates (μ), and maximal cell densities (A) were derived from the best-fitted model at each temperature.

Inhibition of *L. pneumophila* by organic acids and ammonium

The influence of ammonium and organic acids on the growth of *Legionella* was evaluated following the aforementioned methodology at three different temperatures: 28 °C, 35 °C, and 42 °C. The YEB medium was prepared by adding 15 mg/L ammonium, 2200 mg/L acetic acid, 800 mg/L propionic acid, 150 mg/L iso-butyric acid, 1200 mg/L butyric acid, 100 mg/L iso-valeric acid, 300 mg/L valeric acid, and 40 mg/L hexanoic acid. This YEB medium with organic acids and ammonium will be referred to as supplemented YEB medium. The used ammonium and organic acids concentrations were chosen to mimic the concentrations found in anaerobically treated wastewater from an industrial WWTP, where *L. pneumophila* was not detected (Data not shown). The pH of the YEB medium containing organic acids and ammonium was also adjusted to 6.9, and the medium was sterilized by filtration through a 0.22-µm pore size filter.

Results and discussion

Monitoring of *L. pneumophila* occurrence in an industrial WWTP

The concentrations of *L. pneumophila* determined with the qPCR method in samples taken from process water, CMR, anaerobic reactor, and MBR are depicted in Fig. 2. In 4 out of 5 process water samples, the concentration of *L. pneumophila* was below the LOD, suggesting sporadic contamination. The concentration of *L. pneumophila* in activated sludge samples from the CMR was in the range from < 30 to 2×10^4 GU/mL, and in the MBR, the concentration varied from 3×10^2 to 4×10^3 GU/mL. It is worthy to notice that *L. pneumophila* can grow in aerated reactors operating with considerably different sludge retention time (2–2.5 days for the CMR and 10–12 days for the MBR) showing the versatility of this bacterium. In 2 out of 4 effluent samples from the anaerobic reactor, *L. pneumophila*'s concentration was above the LOD of the qPCR method (9×10^2 and 1×10^3 GU/mL). This result suggests that the anaerobic reactor does not continuously inoculate the MBR with *L. pneumophila*. In an extensive study done in the Netherlands, similar concentrations of *L. pneumophila* (10^1 to 4×10^4 GU/mL) have been reported in aerobic reactors within industrial WWTPs from different industrial sectors (Lund et al. 2014). The study of Ma et al. (2015) showed that a MBR treating restaurant wastewater could efficiently remove pathogenic bacteria including *Legionella*, as well as their hosts (e.g. amoebae) from effluents. However, the application of MBRs should also consider that *Legionella* could accumulate in the

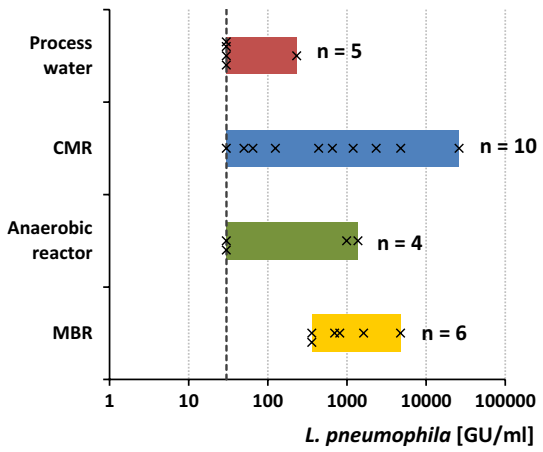

Fig. 2 Concentration of *L. pneumophila* at different sampling points. The dashed line represents the limit of detection of the qPCR method and the black crosses the measured values

aerated tanks due to membrane retention, and the generation of contaminated aerosols could increase and reach vicinity areas. To minimize the risk of *Legionella*-aerosols dispersion, additional measures like covering the aeration tanks, or the application of submerged aeration should be considered.

Spatial arrangement of *Legionella* and protozoa in activated sludge flocs

The spatial arrangement of *Legionella* and protozoa in activated sludge samples taken from the CMR and MBR was investigated by fluorescence in situ hybridization with specific 16S rRNA oligonucleotide probes for *Legionella* and protozoa combined with fluorescence microscopy. *Legionella* were mainly detected as clusters in the activated sludge from the MBR (Fig. 3a), while in the CMR they appeared mainly in form from very long filaments (Fig. 3b). It has been reported in the literature that filamentation is a morphological adaptation to environmental stress (Justice et al. 2008; Young 2006). The short sludge retention time (2–2.5 days) in the CMR can be seen as a stressing factor for *Legionella,* which in response adapted their cellular morphology to facilitate rapid growth, preventing washout from the reactor.

Most protozoa detected with the eukaryotes' specific probe were not infected with *Legionella* in activated sludge samples from both reactors. FISH with specific probes for *Acanthamoeba* spp., *Hatmanella* spp., and *Naegleria* spp. gave positive results; however, these specific protozoa were rare in both samples. Similar results have been reported by Caicedo et al. (2018). *Legionella* spp. clusters were also detected in association with rotifers (Fig. 3c, d); however, it cannot be concluded from the microscopic analysis if *Legionella* were contained intracellularly or attached to the surface of the rotifer. The co-occurrence of amoeba, rotifers, and *Legionella* has also been described in biofilms from cooling towers by Taylor et al. (2013). The fact that several water and wastewater systems have been confirmed as sources of *Legionella* (Van Heijnsbergen et al. 2015) suggests that *Legionella* can adapt to different host populations, from which many are still poorly studied or identified.

Growth kinetics of *L. pneumophila* environmental isolates

The growth curves of six *L. pneumophila* SG2–14 isolates obtained from activated sludge were determined in batch

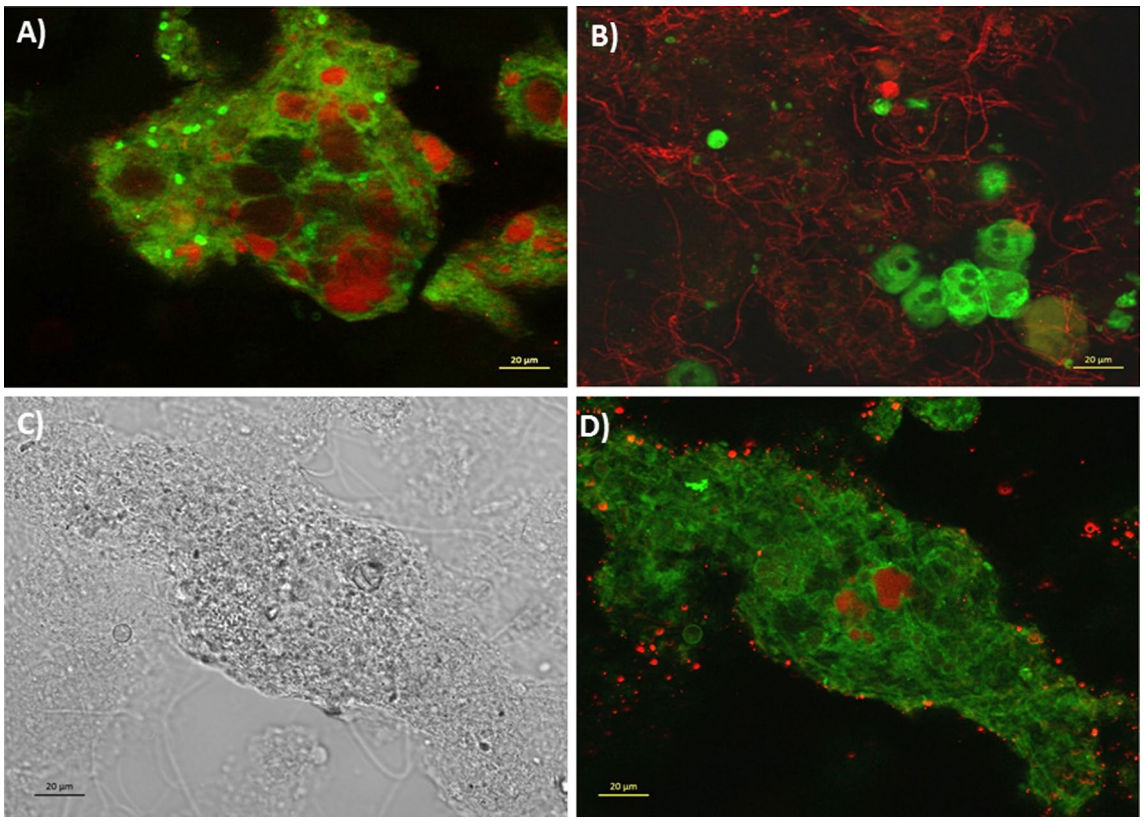

Fig. 3 Spatial distribution of *Legionella* and protozoa in activated sludge investigated with FISH with probes for *Legionella* spp. (LEG705) and eukaryotes (EUK516): **a** *Legionella* spp. clusters (red) and protozoa (green) in MBR; **b** *Legionella* spp. forming filaments and clusters (red) and protozoa (green) in CMR; **c** light microscopy micrograph showing presumably a rotifer and **d** rotifer (green) and *Legionella* spp. clusters (red)

experiments performed at six different temperatures in the range of 28–42 °C. The respective kinetic parameters were derived from the best-fitted model described in the "Growth characterization of *L. pneumophila* environmental and clinical isolates" section and are depicted in Fig. 4. All isolates multiplied at the studied temperatures (Supplementary Material, Fig. I and Fig. II), being 0.34 h^{-1} the highest growth rate observed at 42 °C. The length of the lag phase drastically decreased from 6.95 to 0.67 h for temperatures above 30 °C. The maximal cell density did not differ much between the different temperatures, being the lowest value obtained at 28 °C (2.9) and the highest at 35 °C (3.5). The growth curves were obtained using a cultivation medium (YEB medium), which allows *L. pneumophila* to multiply and reach a stationary phase within 72 h. In activated sludge tanks *L. pneumophila* will probably grow with a lower growth rate due to several factors including wastewater composition and the presence of other bacteria and protozoa. Thus, further studies aiming to determine the growth kinetics of *L. pneumophila* isolates in wastewater, the competition between *Legionella* and other bacteria in the activated sludge and the interaction with protozoa are necessary for a comprehensive understanding of the conditions that support their growth in WWTPs.

The growth kinetic parameters of *L. pneumophila* Bellingham and *L. longbeachae* are also depicted in Fig. 4 for comparison with the *L. pneumophila* SG2–14 environmental isolates. The growth rates of both clinical isolates were similar to the median values obtained with the environmental isolates for temperatures in the range of 28–42 °C. At 42 °C, *L. longbeachae* grew faster than the other bacteria. A similar decreasing tendency of the length of the lag phase with increasing temperature was observed for both clinical and environmental isolates. The shortest lag phase obtained for *L. longbeachae* at 30 °C can be considered as an exception. The maximal cell densities of the clinical isolates were equal or below the maximal cell densities of the environmental isolates (Fig. 4) with two exceptions observed at 28 °C and 42 °C. These two clinical isolates were chosen for the determination of growth kinetics because they have also been reported to be detected in WWTPs. *L. pneumophila* Bellingham was detected in wastewater and sludge samples obtained from the biological treatment of paper mill wastewater with a temperature around 35 °C (Kusnetsov et al. 2010). *L. longbeachae* has been mainly isolated from potting mixes and compost samples (Whiley and Bentham 2011). The study of Lund et al. (2014) showed that *L. longbeachae* also occurred in the biological treatment of petrochemical wastewater with a temperature ranging from 25 to 35 °C.

Sharaby et al. (2017) reported that for a temperature below 30 °C, *L. pneumophila* environmental isolates, obtained from a drinking water distribution system, grew faster than clinical isolates of *L. pneumophila*. This might be explained by physiological differences between clinical and environmental

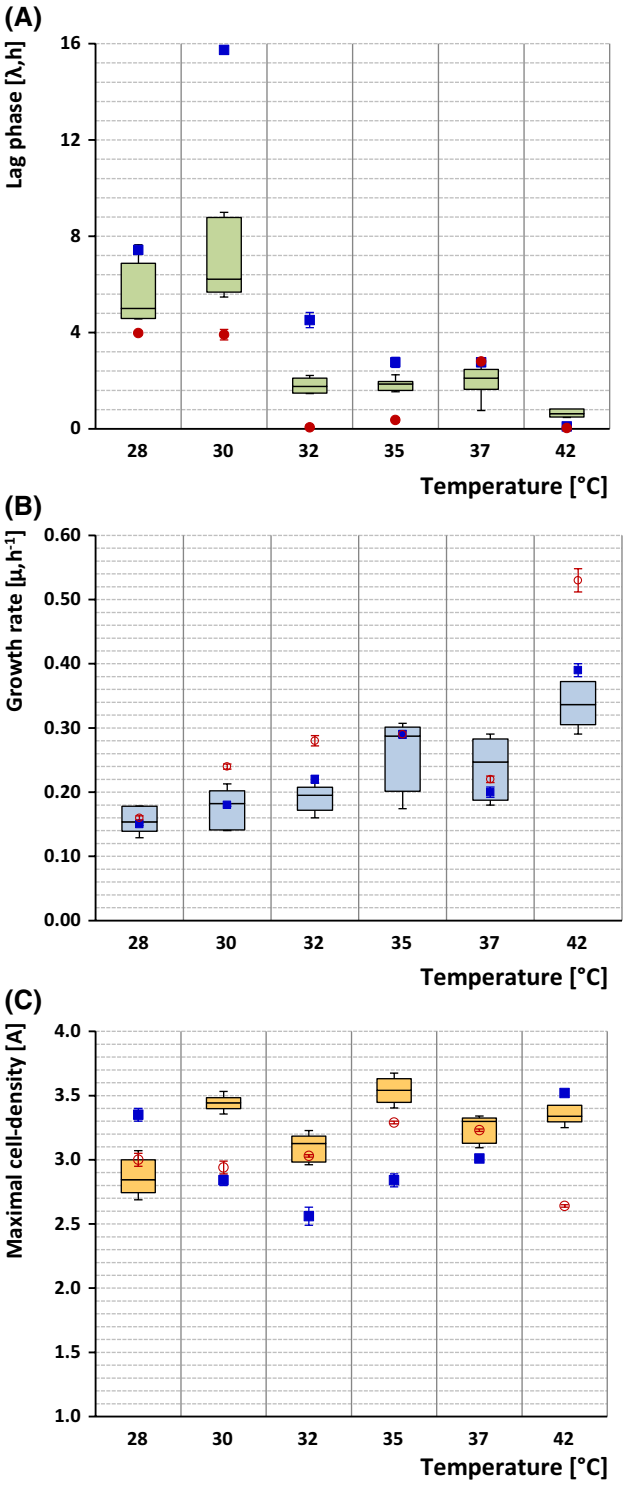

Fig. 4 Kinetic growth parameters obtained from the best-fitted model for environmental and clinical isolates at different temperatures: **a** lag phase length; **b** specific growth rate; **c** maximal cell density. Blue squares represent values for *L. pneumophila* Bellingham, and red circles represent values for *L. longbeachae*

isolates. In our study, both types of isolates grew faster at higher temperatures, which may be partly by the fact that the

environmental isolates were obtained from biological treatment systems operated at a higher temperature.

Inhibition of *L. pneumophila* growth in the presence of organic acids and ammonium

The monitoring results ("Monitoring of *L. pneumophila* occurrence in an industrial WWTP" section) indicate that *L. pneumophila*'s concentration in the effluent of the anaerobic treatment is lower than in the CMR. Based on these results, we tested the hypothesis that organic acids and ammonium produced in the anaerobic treatment can inhibit the growth of *Legionella*. The experimental results clearly showed that none of the *L. pneumophila* SG2–14 environmental isolates and clinical isolates could grow in the presence of a mixture of organic acids and ammonium at all tested temperatures (Supplementary Material Fig. II).

Organic acids have been extensively used in the food industry due to their antibacterial and antifungal activities (Bushell et al. 2019; Ricke 2003). This antimicrobial activity has been in part explained by the diffusion of the undissociated fraction of the organic acids across the cell membrane and dissociation inside the cell with several negative implications to the cell functioning (Bushell et al. 2019); however, the effect of organic acids can vary among bacterial species and strains (King et al. 2010). The study of Warren and Miller (1979) reported growth inhibition of *L. pneumophila* by several acids, namely acetic, citric, and pyruvic acids. As mentioned in the "Inhibition of *L. pneumophila* by organic acids and ammonium" section, the composition and concentrations of the mixture of organic acids and ammonium tested in the presented study are found in pre-anaerobically treated industrial wastewater. To validate the results obtained in the present study, further experiments with pre-anaerobically treated wastewater are needed. Additional experiments should also consider different concentrations of organic acids and ammonium to determine the minimum inhibitory concentrations, inhibition kinetic parameters, and the effect of pH on inhibition by different acids.

Conclusion

The monitoring study showed that *L. pneumophila* occurs in the WWTP treating nutrient-rich industrial wastewater, whereby the highest concentrations were found in the aerated activated sludge tanks. The morphological characteristics of *Legionella*'s growth might have been determined by the sludge retention time in the aerated tanks. Dense clusters of *Legionella* were detected at a high retention time, while long filaments prevailed at short sludge retention time. This morphological diversity allows *Legionella* to grow at very different specific growth rates.

The specific growth rate of *L. pneumophila* SG2–14 environmental isolates, obtained from activated sludge, increased with temperature in a range from 28 to 42 °C, and the maximum growth was observed at 42 °C. High concentrations of organic acids and ammonium inhibited the growth of this bacterium in the temperature range investigated in this study. Growth inhibition of *L. pneumophila* in WWTP is wanted, and knowledge about the inhibition kinetics will contribute to the development of new strategies to prevent the growth of *Legionella* in WWTPs.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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