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Effects of pH and light exposure on the survival of bacteria and their ability to biodegrade organic compounds in clouds: Implications for microbial activity in acidic cloud water

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Abstract

Recent studies have reported that interactions between live bacteria and organic matter can potentially affect the carbon budget in clouds, which has important atmospheric and climate implications. However, bacteria in clouds are subject to a variety of atmospheric stressors, which can adversely affect their survival and energetic metabolism, and consequently their ability to biodegrade organic compounds. At present, the effects of cloud water pH and solar radiation on bacteria are not well understood. In this study, we investigated how cloud water pH (pH 3 to 6) and exposure to solar radiation impact the survival and energetic metabolism of two *Enterobacter* bacterial strains that were isolated from an aerosol sample collected in Hong Kong and their ability to biodegrade carboxylic acids. Experiments were conducted using simulated sunlight (wavelength 320 to 700 nm) and microcosms comprised of artificial cloud water that mimicked the pH and chemical composition of cloud water in Hong Kong, South China. Our results showed that the energetic metabolism and survival of both strains depended on the pH. Low survival rates were observed for both strains at pH < 4 regardless whether the strains were exposed to simulated sunlight. At pH 4 to 5, the energetic metabolism and survival of both strains were negatively impacted only when they were exposed to simulated sunlight. Organic compounds such as lipids and peptides were detected during exposure to simulated sunlight at pH 4 to 5. In contrast, there were minimal effects on the energetic metabolism and survival of both strains when they were exposed to simulated sunlight at pH > 5. The biodegradation of carboxylic acids was found to depend on the presence (or absence) of simulated sunlight and the pH of the artificial cloud water medium. Comparisons of the measured biodegradation rates to chemical reaction rates indicated that the concentrations of radical oxidants will also play important roles in dictating whether biodegradation processes can serve as a competitive sink for carboxylic acids in cloud water. Overall, this study provides new insights into how two common atmospheric stressors, cloud water pH and exposure to solar radiation, can influence the survival and energetic metabolism of bacteria, and consequently the roles that they play in cloud processes.

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1. Introduction

Clouds are an important medium for the aqueous-phase formation and transformation of organic and inorganic compounds. In addition to inorganic and organic compounds, clouds contain biological matter including biological debris (e.g., dead cells, cell fragments) and live microorganisms (e.g., bacteria, fungal spores) (Bauer et al., 2002; Jaenicke, 2005; Burrows et al., 2009). Live microorganisms are mainly emitted directly into the atmosphere from natural sources (Jaenicke, 2005; Möhler et al., 2007; Burrows et al., 2009; Attard et al., 2012; Hu et al., 2018). Once airborne, they can participate in a variety of atmospheric processes such as cloud formation, precipitation, ice nucleation, microbiological-chemical and microbiological-ecosystem interactions (Amato et al., 2005; Delort et al., 2010; Vaitilingom et al., 2010; Vaitilingom et al., 2013; Morris et al., 2014; Morris et al., 2017; Hu et al., 2018; Huang et al., 2021; Zhang et al., 2021). Bacteria are incorporated into clouds through nucleation and scavenging processes (Möhler et al., 2007). So far, only bacterial communities in clouds in some areas (e.g., Puy de Dôme in France, Mt. Tai in North China) have been extensively investigated. These studies showed that the bacterial communities in clouds are highly complex and diverse, and mainly originate from vegetation, soil, and water bodies (Vaïtilingom et al., 2012; Wei et al., 2017; Zhu et al., 2018). However, a significant fraction of the bacteria in clouds may be major allergens and/or pathogens that originate mainly from anthropogenic activities, and their concentrations usually increase during air pollution episodes (Wei et al., 2017; Peng et al., 2019). The cell concentrations of metabolically active bacteria in clouds typically range from about 10^2 to 10^5 cells mL^{-1} (Amato et al., 2005; Burrows et al., 2009; Amato et al., 2017). At present, our knowledge on bacterial communities in clouds are limited to the few areas that have been studied, and only to cultural bacteria which typically makes up about 1% of the entire bacteria community (Amato et al., 2005; Amato et al., 2017).

Previous studies have reported that the degradation of organic compounds as a result of microbiological-chemical interactions between live bacteria and organic matter can play an important role in influencing the carbon budget in clouds, which will have important atmospheric and climate implications (Delort et al., 2010; Vaitilingom et al., 2010; Vaitilingom et al., 2013; Ervens and Amato, 2020; Zhang et al., 2021). Many bacteria species isolated from

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cloud water have the enzymes needed to biodegrade organic compounds such as carboxylic acids, formaldehyde, methanol, phenolic compounds, and amino acids (Ariya et al., 2002; Husárová et al., 2011; Vaïtilingom et al., 2011; Jaber et al., 2020; Jaber et al., 2021). In addition to having the appropriate enzymes, the bacteria need to be metabolically active to biodegrade organic compounds. However, the bacteria are exposed to a variety of stressors that can negatively impact their survival and microbial activity in clouds. Joly et al. (2015) previously investigated the individual impacts of osmotic shocks, freeze-thaw cycles, and exposure to light and H_2O_2 on the survival of different bacterial strains in microcosms mimicking the Puy de Dôme. Osmotic shocks and freeze-thaw cycles reportedly had the greatest negative impacts on the survival of bacteria, while exposure to light and H_2O_2 had limited impacts on the survival of bacteria. However, there are other stressors that bacteria in clouds are commonly subjected to beyond the four stressors investigated by Joly et al. (2015). In addition, when combined together, the stressors may have synergistic negative impacts on the survival and microbial activity of bacteria in clouds. The potentially synergistic negative impacts that stressors have on the survival and microbial activity of bacteria in clouds have yet to be investigated. Some bacteria species respond to stressors by releasing organic compounds (e.g., proteins, pigments, lipids) as a defensive mechanism (Davey and O'toole, 2000; Delort et al., 2010; Flemming and Wingender, 2010; Vaïtilingom et al., 2012; Matulova et al., 2014). When bacteria species cannot withstand the stress, the resulting cellular damage and lysis will lead to the release of biological material. In addition, the ability of bacteria to biodegrade organic compounds in clouds will decrease if their metabolism and survival are negatively impacted.

Cloud water acidity is another stressor that bacteria are subjected to in clouds. There has been limited study on the impact of cloud water pH on the survival and microbial activity of bacteria in clouds. However, some studies have reported that the cloud water pH influences the diversity and composition of bacterial communities (Amato et al., 2005; Peng et al., 2019). For instance, spore-forming bacteria were abundant in pH 4.9 cloud water at Puy de Dôme, while more diverse and higher concentrations of non-spore-forming bacteria were observed in pH 5.8 cloud water (Amato et al., 2005). The pH of cloud water typically lies between 3 and 6 (Pye et al., 2020), with a global mean of around pH 5.2 (Shah et al., 2020). Areas with high

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inputs of sulfuric acid and/or nitric acid combined with low inputs of ammonia, dust, and sea salt, especially in parts of East Asia, have moderately acidic to highly acidic cloud water (pH < 5) (Li et al., 2020; Pye et al., 2020; Shah et al., 2020; Qu and Han, 2021). To the best of our knowledge, there has been no studies on how moderately acidic to highly acidic cloud water affects the survival and microbial activity of bacteria. The effects of light exposure on the survival and microbial activity of bacteria are also ambiguous. Some studies reported that exposure to UVA and visible light will lead to the formation of intracellular reactive oxidative species, which can damage important cell components and cause cell death (Anglada et al., 2015). However, exposure to light reportedly did not impact the survival rates of bacterial strains from *Pseudomonas syringae*, *Arthrobacter* sp., and *Sphingomonas* sp. (Joly et al., 2015). While it is possible that exposure to acidic cloud water and light have a synergistic effect on the survival and microbial activity of bacteria, previous laboratory investigations were mainly performed in microcosms with the pH set between 5 to 7 to mimic cloud water in areas that have high inputs of ammonia, dust, and sea salt, such as the Puy de Dôme (Vaïtilingom et al., 2011; Joly et al., 2015; Jaber et al., 2021; Jaber et al., 2020).

This study investigates how cloud water pH and exposure to solar radiation affect the survival and energetic metabolism of bacteria and their ability to biodegrade organic compounds in clouds. We designed a series of laboratory experiments in microcosms containing artificial cloud water that mimicked the pH and chemical composition of atmospheric cloud water collected at the Tai Mo Shan station in Hong Kong, South China. South China is a region with moderately acidic to highly acidic cloud water due to its higher concentrations of acidic ions (e.g., SO_4^{2-}, NO_3^-) compared to alkaline ions (e.g., NH_4^+, Ca^{2+}) (Li et al., 2020; Qu and Han, 2021). Different pH (pH 3.3 to 5.9) and irradiation (illuminated vs. dark) conditions were employed in the experiments, during which we analyzed the biological material and organic compounds in the artificial cloud water medium at different reaction time points. Since cloud water bacterial isolates from the Tai Mo Shan station are not available, two *Enterobacter* bacterial strains that were isolated from an aerosol sample in Hong Kong were used as model bacteria in this study. In general, our current knowledge of the diversity and composition of bacteria communities in cloud water in Hong Kong and South

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China is very limited due to the scarcity of characterization studies conducted in this region. Results from a previous study suggested that *Enterobacter* was one of the bacteria species in cloud water collected at the Nanling Mountain station in South China (Peng et al., 2019). *Enterobacter* bacteria is pathogenic, and they originate mainly from anthropogenic activities. *Enterobacter* bacteria has been detected in urban aerosols in different parts of the world, including South China (Chen et al., 2012; Després et al., 2012; Ding et al., 2015; Zhou et al., 2018; Prokof'eva et al., 2021). In addition, the enrichment of *Enterobacter* bacteria in the atmosphere during air pollution episodes has been reported in parts of Asia, America, and Europe (Romano et al., 2019; Ruiz-Gil et al., 2020; Romano et al., 2021). Since carboxylic acids are ubiquitous in clouds (Tsai and Kuo, 2013; Löflund et al., 2002; Sun et al., 2016; Li et al., 2020) and can be biodegraded by most bacteria (Vaitilingom et al., 2010; Vaïtilingom et al., 2011), we chose seven carboxylic acids that are commonly detected in clouds (formic acid, acetic acid, oxalic acid, maleaic acid, malonic acid, glutaric acid, and methanesulfonic acid) as model organic compounds for our investigations of how cloud water pH and light exposure affect the ability of bacteria to biodegrade organic compounds in clouds.

2. Methods

2.1. Strain isolation and whole genome sequencing

Two new strains (B0910 and pf0910) belonging to *Enterobacter* species were isolated from an aerosol sample collected in Hong Kong using repeated plating on Luria broth (LB) agar. The genomes of the two strains were sequenced using a GridION sequencer (Oxford Nanopore Technologies) by following the manufacturer's workflow. Genome assembly and the downstream genomic analyses are described in detail in Section S1. Based on genome comparison, *E. hormaechei* B0910 is most similar to *Enterobacter hormaechei* subsp. *hoffmannii* DSM 14563 (Average Nucleotide Identity (ANI) = 98.92) and *E. hormaechei* pf0910 to *Enterobacter hormaechei* subsp. *steigerwaltii* DSM 16691 (ANI = 98.73) (Figure S1). *E. hormaechei* B0910 has a chromosome (4.69 Mbp) with 4875 coding sequences (CDSs) and a single plasmid (373 Kbp) with 383 CDSs. *E. hormaechei* pf0910 strain has a chromosome (4.78 Mbp) with 5072 CDSs and two plasmids of 281 Kbp (344 CDSs) and 73 Kbp (79 CDSs).

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2.2. General experimental approach

To simulate cloud water conditions in Hong Kong, artificial cloud water containing major organic and inorganic ions in cloud water previously collected at the Tai Mo Shan station (TMS; 22°24'N, 114°16'E, 957 m a.s.l.) were used in each experiment. Organic (acetic acid, formic acid, oxalic acid, pyruvic acid) and inorganic (magnesium chloride, calcium chloride, potassium chloride, sodium chloride, ammonium sulfate, ammonium nitrate, sodium hydroxide and hydrochloric acid) compounds were used to prepare the artificial cloud water. Experiments were performed using a Rayonet photoreactor (RPR-200, Southern New England Ultraviolet Company). We followed the method employed in previous studies (George et al., 2015; Huang et al., 2018; Misovich et al., 2021) and used eight lamps with outputs centered at different wavelengths to roughly simulate the range of solar radiation wavelengths (320 to 700 nm) inside the photoreactor. Figure S2 shows the resulting photon flux inside the photoreactor. The temperature (25 ℃) during the experiment was regulated by a fan located at the bottom of the photoreactor.

The two strains were grown in LB broth at 37 ℃ to stationary phase. The culture was then centrifuged at 6000 rpm for 10 min at 4 ℃ and the cell pellets were rinsed with artificial cloud water (Table S1) three times. For investigations of the time evolution in the survival and energetic metabolism of bacteria at different pH under illuminated vs. dark conditions (Section 2.2), the cells were re-suspended in artificial cloud water to an initial concentration of ~10^5 cells mL^{-1}. For investigations of the biodegradation of carboxylic acids by bacteria at different pH under illuminated vs. dark conditions (Section 2.3), the cells were re-suspended in artificial cloud water to an initial concentration of ~10^6 cells mL^{-1}. A calibration curve was used to convert between optical density and bacterial cell concentration.

Quartz tubes containing bacterial cells suspended in artificial cloud water (5 mL) were placed on a rotating vial rack in the middle of the photoreactor. The quartz tubes for the dark control experiments were wrapped in aluminum foil and placed inside the photoreactor. The pH of the artificial cloud water did not change significantly during the experiments. Aliquots of the solutions were taken at every hour over 12 hours for various offline chemical analyses.

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Colony Forming Unit (CFU) counts on LB agar at 37 °C for 16 hours was also performed to determine the culturable bacterial cell concentrations, which was used to calculate the bacteria survival rates. The ADP/ATP ratios were measured using an assay kit (EnzyLight™, BioAssay Systems) and a biolumineter (SpectraMax M2e) to determine changes in the bacteria energetic metabolism. All the experiments and measurements were performed in triplicates

2.3. Investigations of the survival and energetic metabolism of bacteria at different pH under illuminated vs. dark conditions

Six pH conditions (pH 3.3, 4.3, 4.5, 4.7, 5.2 and 5.9) were chosen for this set of experiments, which were performed under both dark and illuminated conditions. The six pH conditions investigated fall within the range of pH values for cloud water previously measured at Tai Mo Shan (pH 3.0 to 5.9) (Li et al., 2020). The pH of the artificial cloud water used to suspend the bacterial cells was adjusted using sodium hydroxide and hydrochloric acid. Table S1 shows the resulting concentrations of organic and inorganic ions in the artificial cloud water used in these experiments, which are similar to those in cloud water collected at Tai Mo Shan by Li et al. (2020).

During some experiments, aliquots of the solutions were taken at time points 0 h, 2 h, 4 h, 8 h, and 12 h and analyzed by ultra-performance liquid chromatography-mass spectrometry (UPLC-MS). Each aliquot of solution was first passed through a 0.22 μm filter to remove intact bacterial cells. Water-insoluble and water-soluble biological material and organic compounds were then extracted from these filtered solutions using the method described in Section S2. 200 μL of the extract was then transferred into glass vial inserts for UPLC-MS analysis. Non-targeted UPLC-MS analysis was performed using an ultrahigh performance liquid chromatography system (ExionLC AD system, Sciex) coupled to a high-resolution quadrupole-time-of-flight mass spectrometer (TripleTOF 6600 system, Sciex) equipped with electrospray ionization (ESI). Chromatographic separation was performed on a Kinetex HILIC LC column (100 × 2.1 mm, 2.6 μm, 100 Å, Phenomenex) using positive ESI mode. Since very low signals were obtained for negative ESI mode, we did not use it for our analysis. Details about the UPLC-MS operation, data processing, and statistical analysis can be found in Section S3.

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209 **2.4. Investigations of the biodegradation of carboxylic acids at different pH under**
210 **illuminated vs. dark conditions**

211 The biodegradation of seven carboxylic acids (formic acid, acetic acid, oxalic acid,
212 maleaic acid, malonic acid, glutaric acid, and methanesulfonic acid (MSA)) that were mixed
213 together were measured at pH 4.3 and pH 5.9 under both dark and illuminated conditions. The
214 concentrations for each of the forementioned carboxylic acids in cloud water and rain water
215 typically fall within the range of 1 to 10 μM (Tsai and Kuo, 2013; Löflund et al., 2002; Sun et
216 al., 2016; Li et al., 2020). Due to the detection limits of the IC system used to measure the
217 carboxylic acids, the concentration for each carboxylic acid was set to 50 μM (Table S2), which
218 is around 10 times higher than the concentrations typically measured in cloud water. The
219 concentrations of inorganic ions in the artificial cloud water were also increased by 10 times.
220 Previous studies have reported that the same biodegradation rates will be obtained as long as
221 the concentration ratio of the chemical compounds to bacterial cells is constant (Vaitilingom
222 et al., 2010; Jaber et al., 2020; Jaber et al., 2021). Hence, the bacteria concentration used was
223 set to 10^6 cells mL^{-1} to maintain the same concentration ratio of the carboxylic acids to bacterial
224 cells. Table S2 shows the resulting concentrations of the organic and inorganic ions in the
225 artificial cloud water used in these experiments.

226 During each experiment, aliquots of the solutions were taken every 2 hours over 12
227 hours. The carboxylic acid concentrations in each filtered aliquot of solution were measured
228 by ion chromatography (IC) using a Dionex ICS-1100 (ThermoFisher Scientific) system.
229 Details of the IC operation can be found in Section S4. To calculate the initial biodegradation
230 rate, the time evolution of each carboxylic acid concentration over 12 h was plotted and fitted
231 with the following equation (Vaïtilingom et al., 2011; Jaber et al., 2020; Jaber et al., 2021):

232 $$\ln\left(\frac{C}{C_0}\right) = f(t) = -k \times t \quad (1)$$

233 where k (s^{-1}) is the rate constant obtained from the exponential fit to the decay of the
234 carboxylic acid. The following equation was used to calculate the biodegradation rate per
235 bacteria cell (R):

236 $$\mathrm{R} = \frac{k \times C_0}{[Cell]_{experiment}}, (mol\ cell^{-1} s^{-1}) \quad (2)$$

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where C_0 $(mol \cdot L^{-1})$ is the initial concentration of the carboxylic acid, $[Cell]_{experiment}$ $(cell \cdot L^{-1})$ is the concentration of bacterial cells in the experiment. Control experiments were performed using solutions that contained carboxylic acids but no bacterial cells. The carboxylic acids did not degrade in these control experiments.

3. Results and discussion

3.1. Impact of pH on the survival and energetic metabolism of bacteria under illuminated and dark conditions

Figure 1 shows the survival rates and ADP/ATP ratios of the *E. hormaechei* B0910 and *E. hormaechei* pf0910 strains over time under illuminated and dark conditions at different artificial cloud water pH. The ADP/ATP ratio is used an indicator of the bacteria's metabolic activity with normal functioning cells usually maintaining a constant ADP/ATP ratio. This is because whenever there is a decrease in intracellular ATP production, its degradation product ADP will be resynthesized to form ATP to maintain intracellular ATP concentrations. However, when there is a disruption in the metabolism of ATP production, ATP cannot be resynthesized from ADP even though ATP is still converted to ADP, which will cause the ADP/ATP ratio to increase.

The artificial cloud water pH clearly had a significant effect on the survival rates and ADP/ATP ratios of the two strains. At pH 3.3, the concentrations of viable cells decreased to zero after 20 minutes regardless whether the strains were exposed to light. For pH 4.3, 4.5 and 4.7, the survival and ADP/ATP ratios of the two strains depended on whether they were exposed to light. There were no significant changes in the survival rates and ADP/ATP ratios for both strains under dark conditions. In contrast, the concentrations of viable cells for both strains gradually decreased when they were exposed to light. The ADP/ATP ratios for both strains also increased over time. The survival rates and ADP/ATP ratios were the lowest and highest, respectively, at pH 4.3 after 12 h of illumination. There were no significant changes in the survival rates and ADP/ATP ratios of both strains at pH 5.2 and 5.9 under illuminated and dark conditions.

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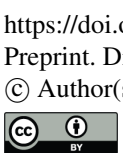

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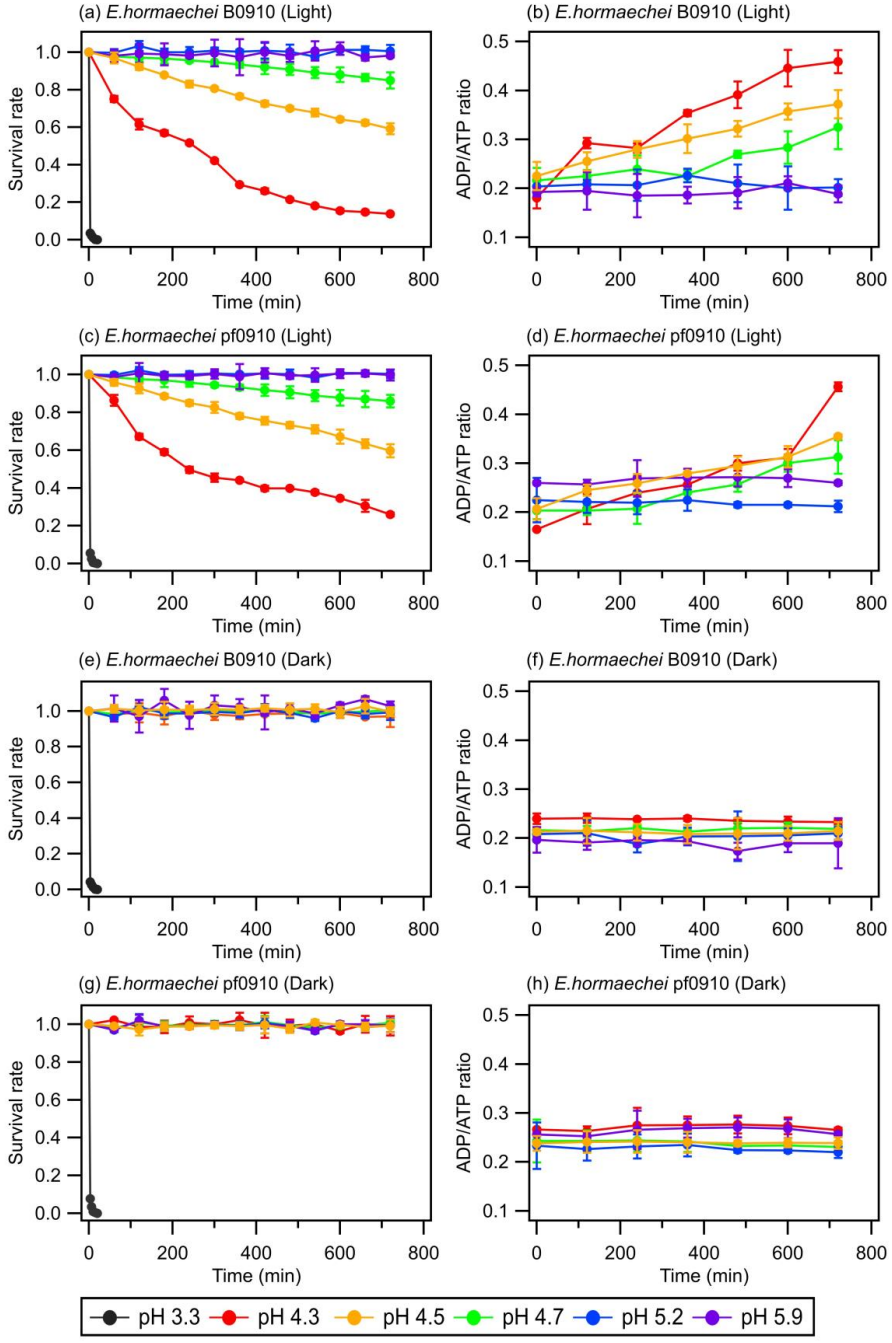

Figure 1. Survival rates and ADP/ATP ratios of the *E. hormaechei* B0910 and *E. hormaechei* pf0910 strains at pH 3.3 to pH 5.9 under illuminated and dark conditions over time. The

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survival rate is defined as the number concentration of culturable viable cells divided by the initial number concentration of culturable viable cells at time point 0 min. Error bars represent one standard deviation from the mean of biological triplicates.

Figure 1 clearly shows that the artificial cloud water pH and exposure to light can have a synergistic effect on the survival and energetic metabolism of *E. hormaechei* B0910 and *E. hormaechei* pf0910. Based on these results, both strains will likely survive during the daytime and nighttime in pH > 5 cloud water. However, cloud water pH will play an important role in dictating the fraction of the bacteria that will survive in the daytime at pH 4 to 5. A low pH environment can lower the internal pH of cells, which affects essential pH-dependent biological and cellular functions such as decreased enzymatic activity, compromised cellular processes (e.g., central metabolic pathways, ATP production), and protein denaturation in cells (Bearson et al., 1997; Lund et al., 2014). Our results indicated that both strains will likely survive in pH 4 to 5 cloud water at night. However, being in cloud water at pH 4 to 5 will likely negatively impact the ability of cells to tolerate sunlight, which will affect their survival during the daytime. Both strains will likely not survive in pH < 4 cloud water during the daytime and nighttime.

3.2. Compounds released by bacteria under acidic and illuminated conditions

Some bacteria species adapt to sunlight exposure and acidic environments by deploying adaptation strategies and defensive mechanisms such as undergoing DNA repair, aggregation-promoting, and pigmentation mechanisms (Bearson et al., 1997; Davey and O'toole, 2000; Delort et al., 2010; Flemming and Wingender, 2010; Vaïtilingom et al., 2012; Matulova et al., 2014; Guan and Liu, 2020). Some of these adaptation strategies and defensive mechanisms will cause the bacteria to release organic compounds into cloud water (Davey and O'toole, 2000; Delort et al., 2010; Flemming and Wingender, 2010; Vaïtilingom et al., 2012; Matulova et al., 2014). In addition, bacterial cellular damage and lysis will lead to the release of biological material and organic compounds. To investigate the compounds released by *E. hormaechei* B0910 and *E. hormaechei* pf0910 during exposure to light and acidic environments, we used UPLC-MS to analyze the solutions in experiments where pH 4.3 and pH 5.9 artificial cloud

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water were used. The UPLC-MS measurements revealed that cell lysis led to the production of water-soluble and water-insoluble compounds when the two strains were exposed to light at pH 4.3. The quantities of these compounds changed with light exposure time. In contrast, no water-soluble and water-insoluble compounds were detected in the solutions of the two strains under dark conditions at pH 4.3, and under dark and illuminated conditions at pH 5.9. This suggested that these two strains did not release organic compounds and the cells remained intact under these conditions. It is also possible that these two strains released organic compounds as an adaption strategy and/or defensive mechanism but the concentrations of these compounds were below the detection limits of our UPLC-MS instrument.

Principal component analysis (PCA) with 95% confidence ellipse was applied to the UPLC-MS data of the detected water-soluble and water-insoluble compounds to identify discriminations between samples with different light exposure times. In each PCA plot (Figure 2), samples with the same light exposure time clustered together. While there was slight overlap between some of the clusters in the PCA plots, the clusters were mostly separated from one another. Partial least squares discrimination analysis (PLS-DA) was applied to the UPLC-MS data to identify water-soluble and water-insoluble compounds that showed significant changes in their relative abundances during exposure to light. 259 water-soluble compounds and 215 water-insoluble compounds were identified for *E. hormaechei* B0910 (Figure S3), while 209 water-soluble compounds and 251 water-insoluble compounds were identified for *E. hormaechei* pf0910 (Figure S4). We identified the molecular formulas and chemical structures of 78 water-soluble compounds and 144 water-insoluble compounds released by *E. hormaechei* B0910, and 118 water-soluble compounds and 114 water-insoluble compounds released by *E. hormaechei* pf0910. These identified compounds were subsequently classified into different classes based on their chemical functionalities.

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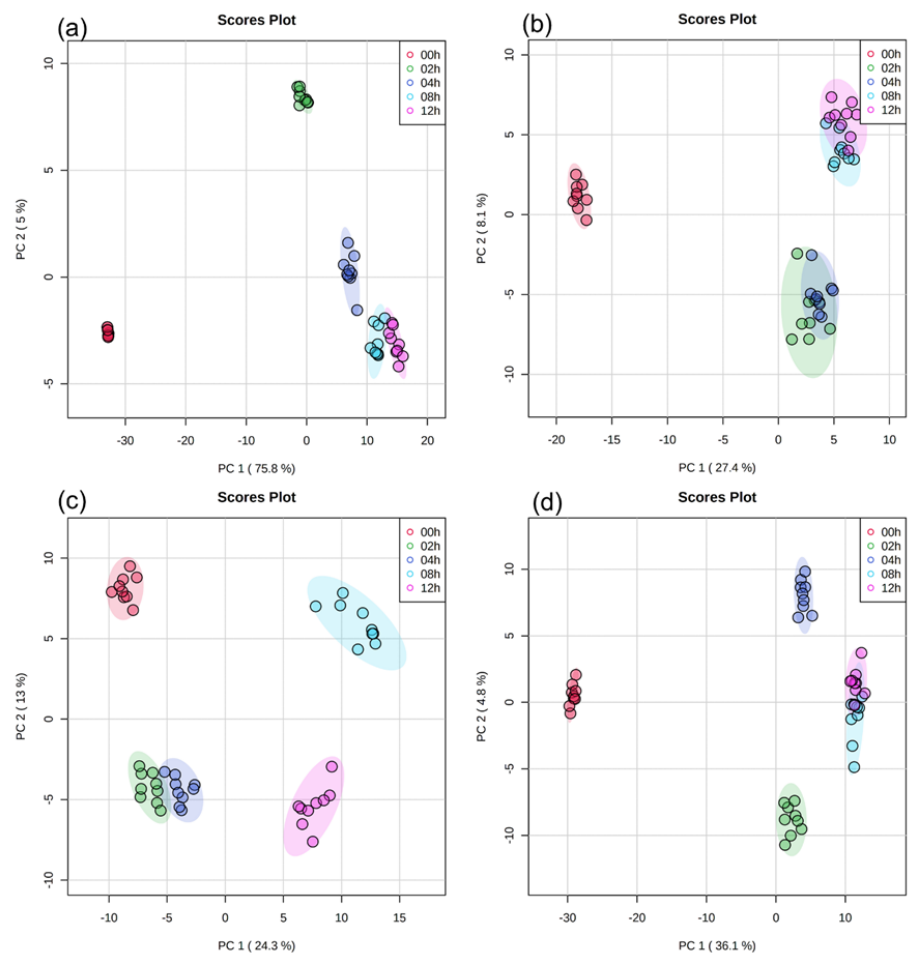

Figure 2. PCA results of UPLC-MS data: (a) water-soluble compounds and (b) water-insoluble compounds from *E. hormaechei* B0910, and (c) water-soluble compounds and (d) water-insoluble compounds from *E. hormaechei* pf0910 during exposure to light at pH 4.3. Each cluster representing a different light exposure time (i.e., 0 h, 2 h, 4 h, 8 h, and 12 h) has nine points since three samples were taken at each light exposure time, and UPLC-MS analysis was performed in triplicate for each sample.

Figures 3 and S5 show the time evolution of the UPLC-MS total ion chromatograph (TIC) signals of the different classes of water-soluble and water-insoluble compounds released by *E. hormaechei* B0910 and *E. hormaechei* pf0910 over time, respectively. The UPLC-MS TIC signals of the classes of water-soluble and water-insoluble compounds released by the two

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strains increased with light exposure time. The increase in the UPLC-MS TIC signals coincided with the decrease in the bacteria survival rate and the increase in the ADP/ATP ratio. Even though the heatmaps showed that some of the compounds had noticeable changes in their relative abundances during exposure to light (Figures S3 and S4), the relative abundances of the different classes of compounds contributed to the total TIC at each time point did not change substantially (Figures S6 and S7).

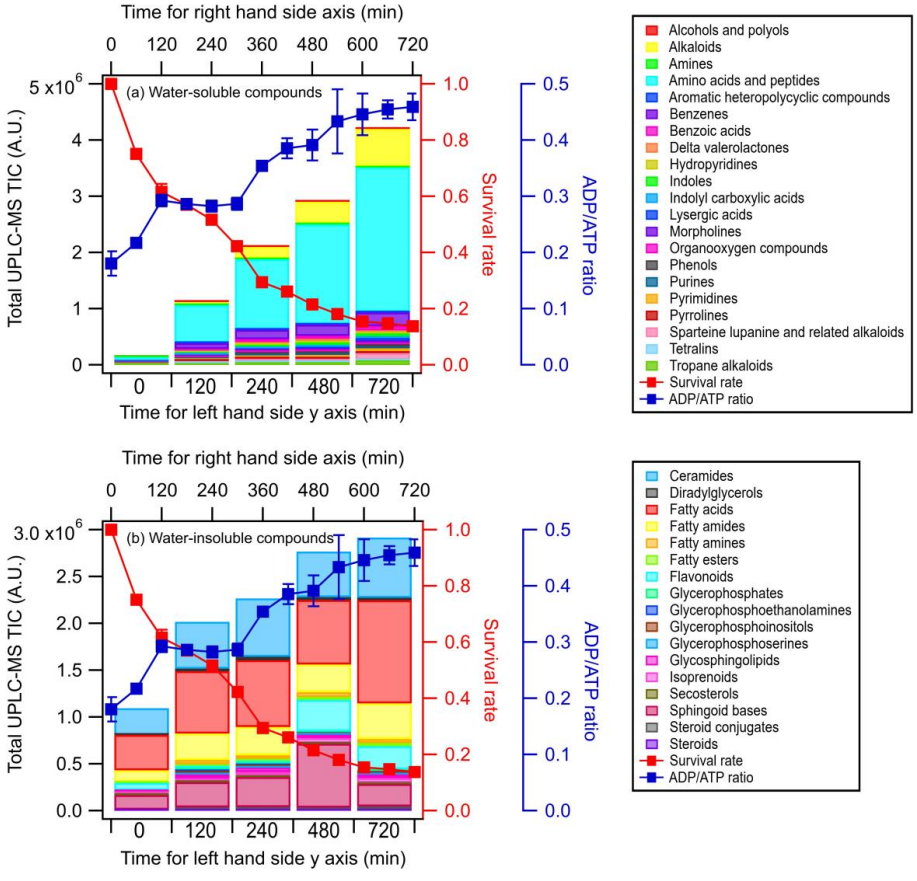

Figure 3. Time evolution of the UPLC-MS total ion chromatograph (TIC) signals of (a) water-soluble compounds, and (b) water-insoluble compounds from *E. hormaechei* B0910 during exposure to light at pH 4.3 over time. These compounds are classified based on their chemical functionality. Also shown are the time evolution of the survival rate and ADP/ATP ratio of *E. hormaechei* B0910.

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To better understand the compounds released by the two strains, the O/C and H/C elemental ratios of the identified compounds were used to construct Van Krevelen (VK) diagrams. Regions of the VK diagrams were assigned to eight chemical classes based on the combined O/C and H/C ratios: lipids, unsaturated hydrocarbons, condensed aromatic structures, peptides, lignin, tannin, amino sugars, and carbohydrates (Table S3) (Bianco et al., 2018; Laszakovits and Mackay, 2022). Figures S8 and S9 show the VK diagrams for water-soluble and water-insoluble compounds released by *E. hormaechei* B0910, respectively, while Figures S10 and S11 show the VK diagrams for water-soluble and water-insoluble compounds released by *E. hormaechei* pf0910, respectively. Majority of the water-soluble and water-insoluble compounds released from both strains (50% to 60%) were assigned as lipids based on their O/C and H/C ratios. This was unsurprising since lipids are the main component of cell membranes so large quantities of lipids are expected from the lysed cells. The second most abundant compound class was peptides (10% to 20%), which were likely formed from biological and/or chemical modifications of proteins. The two least abundant compound classes were amino sugars and carbohydrates. This was somewhat surprising since amino sugars and carbohydrates form important constituents of cells. It is possible that these compounds were biologically and/or chemically modified to form other compounds (e.g., exopolymeric substances) during exposure to light (Matulova et al., 2014). In addition, the extraction procedure employed (Section S2) may not have extracted these compounds effectively for analysis. These compounds may also have been poorly separated in UPLC and/or inefficiently ionized by ESI.

3.3. Impact of pH on the biodegradation of carboxylic acids by bacteria under illuminated and dark conditions

The biodegradation of seven carboxylic acids (i.e., formic acid, acetic acid, oxalic acid, maleic acid, malonic acid, glutaric acid and MSA) that were mixed together were measured under dark and illuminated conditions at pH 4.3 and pH 5.9. Only some of the seven carboxylic acids were biodegraded by the two strains. *E. hormaechei* B0910 biodegraded formate and oxalate under dark and illuminated conditions at pH 4.3 and pH 5.9, and biodegraded malonate and maleate only under dark conditions at pH 4.3 and pH 5.9. In contrast, *E. hormaechei* pf0910

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biodegraded only formate and oxalate under dark and illuminated conditions at pH 4.3 and pH 5.9. Biodegradation was not observed for acetate, MSA, and glutarate. Section S5 and Table S4 discuss the enzymes and metabolic mechanisms associated with the biodegradation of carboxylic acids by the two strains.

Figure 4 summarizes the measured biodegradation rates of the carboxylic acids for the two strains under dark and illuminated conditions at pH 4.3 and pH 5.9. The measured biodegradation rates were around 10^{-19} to 10^{-18} mol cell^{-1} s^{-1}, which were on the same order of magnitude as the bacterial strains isolated from cloud water and implemented into cloud models (Vaitilingom et al., 2010; Vaïtilingom et al., 2011; Fankhauser et al., 2019). Although both strains were affiliated to *E. hormaechei*, the artificial cloud water pH and exposure to light impacted their biodegradation of carboxylic acids differently. The rates at which formate and oxalate were biodegraded by *E. hormaechei* B0910 had the following order: dark conditions at pH 5.9 > illuminated conditions at pH 5.9 > dark conditions at pH 4.3 > illuminated conditions at pH 4.3. This order was different for *E. hormaechei* pf0910: dark conditions at pH 5.9 > dark conditions at pH 4.3 > illuminated conditions at pH 5.9 > illuminated conditions at pH 4.3. Despite the effects that the artificial cloud water pH and exposure to light had on the formate and oxalate biodegradation, the fastest and slowest biodegradation rates only differed by a factor of 1.4 to 3.7. Figure S12 compares the biodegradation rates measured at pH 4.3 vs. pH 5.9, and under illuminated vs. dark conditions. For the effect of artificial cloud water pH on the biodegradation of carboxylic acids by *E. hormaechei* B0910, the differences in the biodegradation rates were statistically significant for the four acids. Conversely, the decreases in the biodegradation rates of formate and oxalate as a result of light exposure were statistically significant at pH 5.9. For the effect of artificial cloud water pH on the biodegradation of carboxylic acids by *E. hormaechei* pf0910, only the difference in the dark biodegradation of oxalate was statistically significant. In contrast, light exposure reduced the formate biodegradation rates significantly at both pH 4.3 and pH 5.9, and the oxalate biodegradation rate significantly at pH 5.9.

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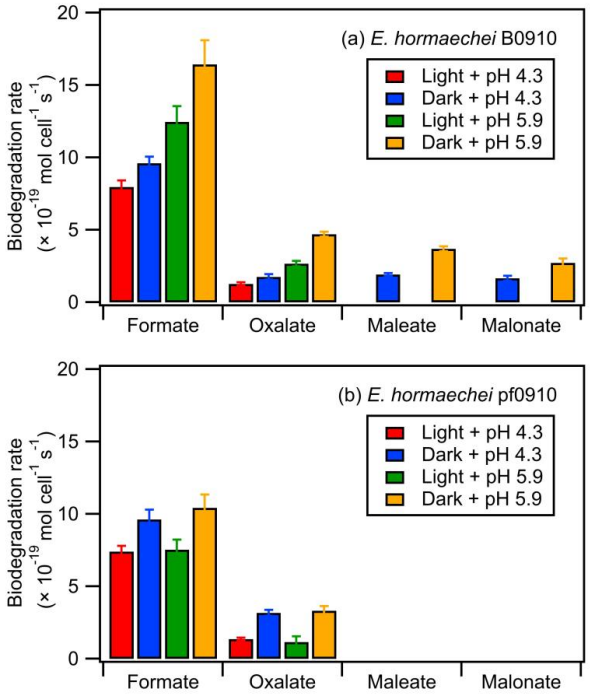

Figure 4. Biodegradation rates of oxalate, maleate, and malonate by (a) *E. hormaechei* B0910 and (b) *E. hormaechei* pf0910 under light and dark conditions at pH 4.3 and pH 5.9. Error bars represent one standard deviation from the mean biodegradation rate.

The survival rates and ADP/ATP ratios of both strains were also monitored during the biodegradation experiments (Figure S13). There were no significant changes in the survival rates and ADP/ATP ratios of both strains during the biodegradation process under dark conditions at pH 4.3, as well as under dark and illuminated conditions at pH 5.9. In contrast, the concentrations of viable cells gradually decreased until only 48% and 60% of the initial concentrations of viable cells remained at 12 h for *E. hormaechei* B0910 and *E. hormaechei* pf0910, respectively, during exposure to light at pH 4.3. The ADP/ATP ratios for both strains also increased during this time period.

A simple analysis was performed to determine whether the measured biodegradation rates are competitive with aqueous-phase chemical reactions in transforming carboxylic acids

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412 in cloud water during the daytime and nighttime. Our approach of considering daytime and
413 nighttime processes separately was different from the approach used by previous studies, which
414 determined the relative contributions of bacterial activity and chemical reactions on the
415 degradation of organic compounds by only considering dark biodegradation processes and ·OH
416 photochemical reactions (Vaïtilingom et al., 2011; Jaber et al., 2020; Jaber et al., 2021). Here,
417 biodegradation rates that were measured under illuminated conditions were used for the
418 daytime scenario, while biodegradation rates that were measured under dark conditions were
419 used for the nighttime scenario. We used the average of biodegradation rates measured for the
420 two strains for our calculations. Formate, oxalate, and malonate were chosen for our analysis
421 since their ·OH and NO_3· reaction rate constants were available in the literature. ·OH and NO_3·
422 are the main tropospheric aqueous-phase free radicals during the daytime and nighttime,
423 respectively (Herrmann et al., 2010). The average measured biodegradation rates of formate,
424 oxalate, and malonate were first converted to biodegradation rate constants. These
425 biodegradation rate constants and the corresponding ·OH and NO_3· reaction rate constants
426 provided by the literature (Table 1) were subsequently used for calculations of the
427 biodegradation rates and chemical reaction rates in cloud water (Section S6). A constant
428 bacteria concentration of 8×10^7 cell L^{-1} was assumed in our calculations, which was the same
429 bacteria concentration used in previous studies (Vaïtilingom et al., 2011; Jaber et al., 2020;
430 Jaber et al., 2021). The rates of oxidation by ·OH and NO_3· chemical reactions will depend on
431 their respective concentrations. Hence, we used the average ·OH and NO_3· concentrations
432 reported by Herrmann et al. (2010) for remote, marine, and urban environments in our
433 calculations (Table S5) (Herrmann et al., 2010).

434 **Table 1.** Rate constants used to estimate the loss rates by biodegradation and chemical reactions
435 (i.e., ·OH oxidation (daytime) and NO_3· (nighttime)).

		Rate constant (Daytime)		
Reaction		Formic	Oxalic	Reference
Chemical	$k_{OH,Acid}$ $(L\ mol^{-1}\ s^{-1})$	2.40×10^9	1.60×10^8	(Ervens et al., 2003)
Biodegradation	$k_{cell,acid}$ (pH ~4) $(L\ cell^{-1} s^{-1})$	1.53×10^{-13}	2.65×10^{-15}	This study

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	$k_{cell,acid}$ (pH ~5) ($L\ cell^{-1}s^{-1}$)	1.92×10^{-13}	2.36×10^{-14}		This study

436

Rate constant (Nighttime)					
Reaction		Formate	Oxalate	Malonate	Reference
Chemical	$k_{NO_3,Acid}$ ($L\ mol^{-1}\ s^{-1}$)	4.20×10^{7}	4.40×10^{7}	5.60×10^{6}	(Herrmann et al., 2010)
Biodegradation	$k_{cell,acid}$ (pH ~4) ($L\ cell^{-1}s^{-1}$)	1.92×10^{-13}	5.18×10^{-15}	2.81×10^{-15}	This study
	$k_{cell,acid}$ (pH ~5) ($L\ cell^{-1}s^{-1}$)	2.59×10^{-13}	7.80×10^{-14}	4.55×10^{-14}	This study

437 Calculations were performed for a variety of remote, marine, and urban environments
438 with different formate, oxalate, and malonate concentrations that were previously reported in
439 the literature (Table S6). Figure 5 shows the predicted relative contributions of bacterial
440 activity vs. ·OH/NO_3· chemistry in remote, marine, and urban environments. ·OH
441 photochemistry will make a larger contribution to the daytime degradation of formate and
442 oxalate in remote and marine environments due to the high ·OH concentrations in these
443 environments (2.2×10^{-14} M and 2×10^{-12} M, respectively). In contrast, bacterial activity will
444 play a bigger role in the daytime degradation of formate in urban environments due to their
445 lower ·OH concentrations (3.5×10^{-15} M). However, ·OH photochemistry will play a larger
446 role in the daytime degradation of oxalate in urban environments due to the slow oxalate
447 biodegradation rates. The low nighttime NO_3· concentrations in remote and marine
448 environments (5.1×10^{-15} M and 6.9×10^{-15} M, respectively) will result in bacterial activity
449 playing a bigger role in the nighttime degradation of formate, oxalate, and malonate in these
450 two environments. In urban environments, bacterial activity will play a bigger role in the
451 nighttime degradation of formate, but the nighttime degradation of oxalate and malonate will
452 be dominated by NO_3· chemistry due to the slow biodegradation rates of oxalate and malonate.
453 Overall, our analysis indicated that the measured biodegradation rates can be competitive with
454 aqueous-phase chemical reactions in transforming carboxylic acids in cloud water, but it will
455 depend on the carboxylic acid, cloud water pH, radical oxidant concentration, and time of day
456 (i.e., daytime vs. nighttime).

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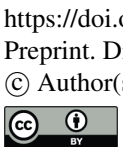

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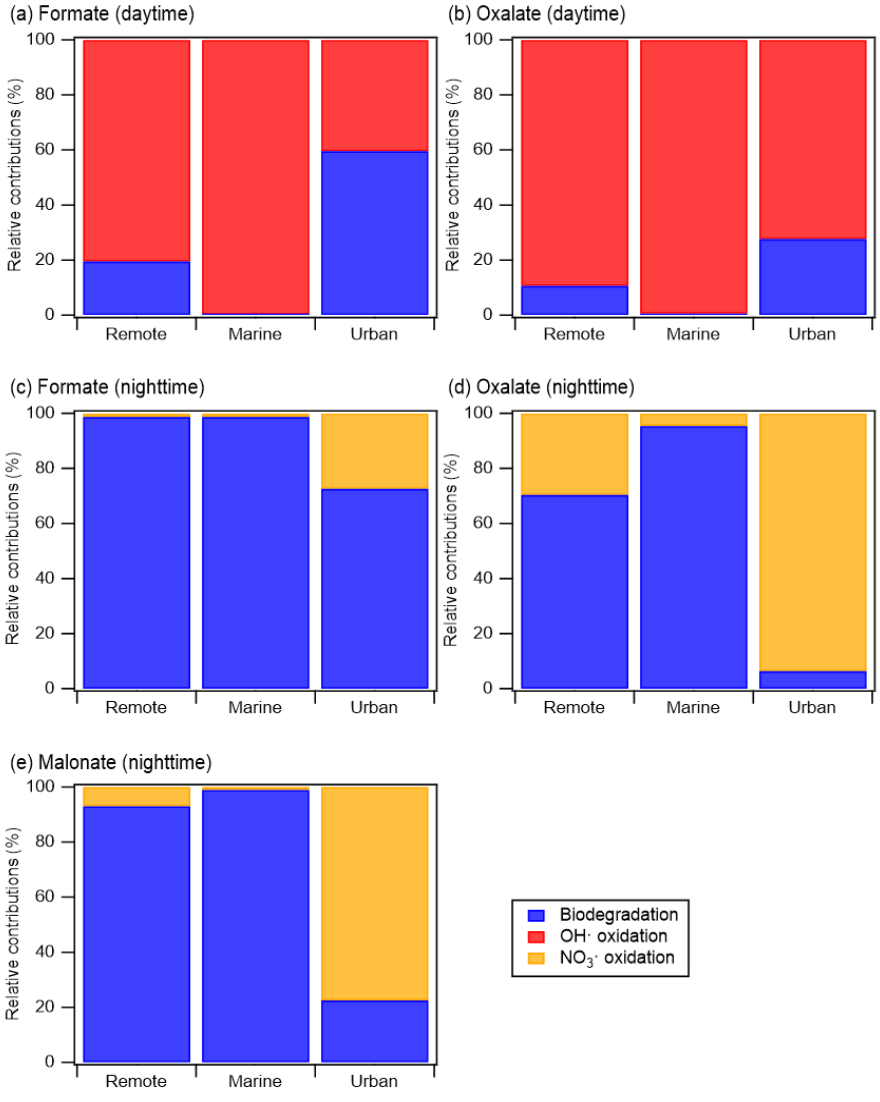

457

458 **Figure 5.** Predicted relative contributions of bacterial activity and chemical reaction (i.e., ·OH
459 oxidation (daytime) and NO_3· (nighttime)) to the degradation of carboxylic compounds in
460 remote, marine, and urban areas. This figure is based on estimated loss rates shown in Table
461 S6.

462 **4. Conclusions and implications**

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In this study, we investigated how cloud water pH and exposure to solar radiation impact the survival and energetic metabolism of bacteria and their ability to biodegrade carboxylic acids in clouds. Laboratory experiments were performed using artificial solar radiation and artificial cloud water that mimicked the pH and composition of cloud water previously collected in South China, which is a region with fairly acidic cloud water (pH 3 to 5.9). Using two *E. hormaechei* strains that were isolated from an aerosol sample in Hong Kong, we observed that the energetic metabolism and survival of both strains depended on the artificial cloud water pH. Low survival rates were observed for both strains at pH < 4 regardless whether the strains were exposed to light. At pH 4 to 5, the energetic metabolism and survival of both strains were only negatively impacted when they were exposed to light. In contrast, there were minimal effects on the energetic metabolism and survival of both strains when they were exposed to simulated sunlight at pH > 5. In addition, the biodegradation of carboxylic acids depended on the presence (or absence) of light and the artificial cloud water pH. The measured biodegradation rates were around 10^{-19} to 10^{-18} mol cell^{-1} s^{-1}, which were on the same order of magnitude as the bacterial strains isolated from cloud water and implemented into cloud models (Vaitilingom et al., 2010; Vaïtilingom et al., 2011; Fankhauser et al., 2019). Our analysis indicated that the carboxylic acid, cloud water pH, radical oxidant concentration, and the time of day will determine whether the measured biodegradation rates will be competitive with aqueous-phase chemical reactions in transforming carboxylic acids in cloud water.

This study has two important implications for our understanding of bacteria in clouds. First, this study underscores the importance of accounting for cloud water pH when simulating cloud processes involving metabolically active bacteria in atmospheric models, including microbiological-chemical interactions between live bacteria and organic matter. Results from this study imply that there is a minimum cloud water pH threshold at which the bacteria will survive and thrive in during the daytime and/or nighttime. The pH of cloud water typically lies between 3 and 6 (Pye et al., 2020). Regions with high inputs of sulfuric acid and/or nitric acid combined with low inputs of ammonia, dust, and sea salt, such as South China, will have moderately acidic to highly acidic cloud water (Li et al., 2020; Pye et al., 2020; Shah et al.,

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2020; Qu and Han, 2021). Most of the bacteria in the atmosphere are neutrophiles that generally survive and thrive in less acidic environments. Hence, even though our study focuses on two *Enterobacter* strains, we hypothesize that cloud water pH will also affect the ability of other neutrophilic bacteria species to survive and remain metabolically active. Second, results from this study imply that it is important to consider the potential synergistic negative impacts that different stressors have on the survival and microbial activity of bacteria in clouds. Much of our current knowledge on the effect of different stressors (osmotic shocks, freeze-thaw cycles, and exposure to light and H_2O_2) on the survival of bacteria in clouds originate from a previous study by Joly et al. (2015) who investigated the impacts of these four stressors individually. However, as demonstrated in this study, when combined together, some stressors (in this case, cloud water pH and exposure to sunlight) can have synergistic negative impacts on the survival and microbial activity of bacteria in clouds.

While this study builds on our existing knowledge of how different stressors will impact the survival and energetic metabolism of bacteria and their ability to biodegrade organic matter in clouds, there are a number of caveats that should be noted. First, we were limited to using bacterial strains isolated from an aerosol sample in this study due to the unavailability of bacteria isolates from cloud water in South China. Thus, if available, this work could be extended to bacteria isolates from cloud water in South China in the future to determine the pH conditions at which these isolates can survive and participate in microbiological-chemical interactions during the daytime and/or nighttime. The effect of cloud water pH on bacteria species that are reportedly common in cloud water (e.g., *Sphingomonadales*, *Rhodospirillales*, *Rhizobiales*, *Burkholderiales*, *Pseudomonadales* (Vaïtilingom et al., 2012; Zhu et al., 2018; Peng et al., 2019)) should also be investigated. Second, all the experiments in this study were conducted at 25 °C, which may be more representative of warmer regions during the summer (e.g., Hong Kong and parts of South China). Several studies have reported slower biodegradation rates at lower temperatures (Ariya et al., 2002; Vaitilingom et al., 2010; Husárová et al., 2011; Vaïtilingom et al., 2011), which suggest that cloud water temperature may influence the survival and energetic metabolism of bacteria. Third, the photon intensity in the photoreactor was kept constant in all the experiments. However, sunlight intensity will

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change throughout the day in the atmosphere. Fourth, this study does not consider how the presence of aqueous-phase oxidants (e.g., ·OH in the daytime, NO_3· in the nighttime) will impact the survival and energetic metabolism of bacteria in clouds. Hence, the effects of temperature, light intensity, and oxidants on the impact the survival and energetic metabolism of bacteria and their ability to biodegrade organic matter in clouds should be investigated in future studies.

Data availability: The data used in this publication is available to the community and can be accessed on request to the corresponding author (theodora.nah@cityu.edu.hk), or at: https://doi.org/10.5281/zenodo.7045510 (Liu et al., 2022).

Author contributions: Y.L., P.L., and T.N. designed the study. Y.L. conducted the experiments. Y.L., C.K.L., and Z.S. performed the data analysis. Y.L. and T.N. wrote the manuscript with contributions from all co-authors.

Competing interests: One of the authors is a member of the editorial board of *Atmospheric Chemistry and Physics*. The authors also have no other competing interests to declare.

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