Water Research 233 (2023) 119737

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

In-sewer decay and partitioning of *Campylobacter jejuni* and *Campylobacter coli* and implications for their wastewater surveillance

Shuxin Zhang [a], Jiahua Shi [b,c], Elipsha Sharma [a], Xuan Li [d], Shuhong Gao [e], Xu Zhou [e], Jake O'Brien [f], Lachlan Coin [g], Yanchen Liu [h], Muttucumaru Sivakumar [a], Faisal Hai [a], Guangming Jiang [a,b,*]

[a] School of Civil, Mining and Environmental Engineering, University of Wollongong, Australia
[b] Illawarra Health and Medical Research Institute (IHMRI), University of Wollongong, Wollongong, Australia
[c] School of Medical, Indigenous and Health Sciences, University of Wollongong, Australia
[d] Centre for Technology in Water and Wastewater, School of Civil and Environmental Engineering, University of Technology Sydney, Ultimo, NSW 2007, Australia
[e] State Key Laboratory of Urban Water Resource and Environment, School of Civil & Environmental Engineering, Harbin Institute of Technology (Shenzhen), Shenzhen 518055, China
[f] Queensland Alliance for Environmental Health Sciences (QAEHS), The University of Queensland, Brisbane, Australia
[g] Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, VIC, Australia
[h] State Key Joint Laboratory of Environment Simulation and Pollution Control, School of Environment, Tsinghua University, Beijing 100084, China

ARTICLE INFO

Keywords:
Campylobacter coli
Campylobacter jejuni
Wastewater
Decay
Sewer biofilm
Wastewater-based epidemiology

ABSTRACT

Campylobacter jejuni and *coli* are two main pathogenic species inducing diarrhoeal diseases in humans, which are responsible for the loss of 33 million lives each year. Current *Campylobacter* infections are mainly monitored by clinical surveillance which is often limited to individuals seeking treatment, resulting in under-reporting of disease prevalence and untimely indicators of community outbreaks. Wastewater-based epidemiology (WBE) has been developed and employed for the wastewater surveillance of pathogenic viruses and bacteria. Monitoring the temporal changes of pathogen concentration in wastewater allows the early detection of disease outbreaks in a community. However, studies investigating the WBE back-estimation of *Campylobacter* spp. are rare. Essential factors including the analytical recovery efficiency, the decay rate, the effect of in-sewer transport, and the correlation between the wastewater concentration and the infections in communities are lacking to support wastewater surveillance. This study carried out experiments to investigate the recovery of *Campylobacter jejuni* and *coli* from wastewater and the decay under different simulated sewer reactor conditions. It was found that the recovery of *Campylobacter* spp. from wastewater varied with their concentrations in wastewater and depended on the detection limit of quantification methods. The concentration reduction of *Campylobacter. jejuni* and *coli* in sewers followed a two-phase reduction model, and the faster concentration reduction during the first phase is mainly due to their partitioning onto sewer biofilms. The total decay of *Campylobacter. jejuni* and *coli* varied in different types of sewer reactors, i.e. rising main vs. gravity sewer. In addition, the sensitivity analysis for WBE back-estimation of *Campylobacter* suggested that the first-phase decay rate constant (k_1) and the turning time point (t_1) are determining factors and their impacts increased with the hydraulic retention time of wastewater.

1. Introduction

Outbreaks of infectious diseases cause losses in both human and animal lives and serious economic damage to societies, especially in urban areas with high population density. *Campylobacter* is one of the main pathogens causing bacterial gastroenteritis. *Campylobacter jejuni* (*C. jejuni*) and *Campylobacter coli* (*C. coli*) are the top two thermophilic *Campylobacter* species that are responsible for more than 95% of *Campylobacter*-induced illness worldwide (Dai et al., 2020). *C. jejuni* and *C. coli* widely exist in animal food products with a mean prevalence of 19.3% and 9.7%, respectively, while eggs, sausages, milk, and dairy products were found to have the lowest prevalence (Zbrun et al., 2020). The infection dose of *Campylobacter* spp. is as low as 500 cells (Epps et al., 2013). The high prevalence in the food and the low infectious dose

* Corresponding author at: School of Civil, Mining and Environmental Engineering, University of Wollongong, Australia.
E-mail address: gjiang@uow.edu.au (G. Jiang).

https://doi.org/10.1016/j.watres.2023.119737
Received 21 December 2022; Received in revised form 8 February 2023; Accepted 10 February 2023
Available online 12 February 2023

makes *Campylobacter* spp. the most common cause of human gastroenteritis in the world (Kaakoush et al., 2015). The disease surveillance for *Campylobacter* spp. has been reported by using food products and clinical samples (Joensen et al., 2021; Tong et al., 2021). However, the true incidence of gastroenteritis caused by *Campylobacter* is poorly understood, particularly in low- and middle-income countries. Studies in high-income countries estimate the annual incidence to be 4.4–9.3 per 1000 population (World Health Organization et al., 2013). Guillain-Barre syndrome (GBS) is one of the main sequelae of campylobacteriosis which is a serious illness, with about 20% requiring intensive care. The fatality rate in high-income countries is 3–10%. Globally, about one-third of GBS cases have been attributed to *Campylobacter* infection (World Health Organization et al., 2013). Traditional disease detection of *Campylobacter* infections relies on clinical diagnosis, which is untimely and only based on people who attend healthcare facilities for treatment. Thus, the clinical report usually leads to an underestimation of disease prevalence, and it is hard to achieve early warnings of public health threats (Seventer and Hochberg, 2017).

Wastewater-based epidemiology (WBE) is a relatively new method based on the analysis of chemicals and biomarkers in raw wastewater to obtain qualitative and quantitative data on the activities of residents in the sewer catchment area (Choi et al., 2018; Rousis et al., 2017; Sims and Kasprzyk, 2020). WBE-based disease surveillance can provide near real-time evidence to reveal that the infectious agent or its genetic component has entered the sewage system, sometimes even days before symptoms appear and often before an infected person comes into contact with a healthcare facility (Tiwari et al., 2023). Therefore, WBE is suggested as a promising pathway to provide early warning of disease outbreaks. In addition, in countries with limited resources, WBE is regarded as an attractive complementary approach since it is an inexpensive and non-invasive method of population surveillance compared to individual tests (Shrestha et al., 2021). With the ever-increasing public concerns about infectious diseases aroused by the COVID-19 pandemic, WBE-based disease surveillance was reported can achieve early warning of different human pathogens-induced outbreaks including both pathogenic viruses and bacteria (Abdeldayem et al., 2022; Anand et al., 2022; Riquelme et al., 2021; Zahedi et al., 2021). WBE back-estimation of SARS-CoV-2 prevalence in communities has been established and evaluated for the application of other pathogenic viruses (Guo et al., 2022; Li et al., 2022, 2021b).However, only a few studies were reported for the surveillance of *Campylobacter* prevalence based on wastewater (Bonetta et al., 2016; Hellein et al., 2011). There is a lack of systematic studies about the key WBE steps, including the recovery rate during detection, the decay rate in wastewater, the impact of sewer conditions, and the back-calculation of infections in communities.

According to the report of the Global Water Pathogen Project in 2017, significant data gaps exist in terms of the persistence of *Campylobacter* in wastewater and the environmental conditions that can affect the persistence (Orner et al., 2018). When evaluating microbial persistence in water bodies, the temperature, sunlight, dissolved oxygen (DO), soluble chemical oxygen demand (SCOD), nutrient availability, and salinity were found to be important environmental conditions (Orner et al., 2018). For wastewater-based epidemiology, the decay/-reduction of the biomarker concentration during the in-sewer transport is also essential for the WBE back-estimation (Li et al., 2021c). Sewer systems could be divided into two main types by the flow regimes, including rising mains (RM) and gravity sewers (GS). Rising main pipelines are used to transport wastewater to higher elevations and operate under anaerobic conditions due to the fully filled wastewater in pipelines. In contrast, gravity pipelines deliver wastewater to lower elevations by gravity and are usually partially filled with wastewater, thus containing both aerobic and anaerobic conditions (Hvitved et al., 2013). Previous studies have compared and reported the stability of biomarkers (e.g. licit drug and pharmaceutical biomarkers) in wastewater only and in laboratory-scale sewer biofilm reactors and demonstrated that the biomarkers' stability evaluated with the biofilm-free conditions cannot reflect sewer conditions (Choi et al., 2020; Li et al., 2018, 2021a). However, no such studies have been carried out for *Campylobacter* spp.

To address the research gaps for the wastewater-based epidemiology of *Campylobacter*, a battery of experiments was carried out including the assessment of the recovery rate of *C. jejuni, C. coli*, and *C. sputorum* from wastewater at three different seeding levels, the concentration reduction of *C. jejuni* and *C. coli* in wastewater phase in different types of laboratory-scale sewer reactors, the adsorption and desorption of *C. jejuni* and *C. coli* in sewer biofilms, and their decay in the phases of wastewater and biofilms at room temperature. Our previously developed triplex qPCR assay for *C. jejuni* and *C. coli* quantification in wastewater with *C. sputorum* as the inhibition control reported was adopted to delineate the recovery, decay and adsorption/desorption behaviours of *C. jejuni* and *C. coli* in sewers. Furthermore, a sensitivity analysis was carried out on the decay parameters acquired in this study for the WBE back-estimation of *C. jejuni* and *C. coli*. The results of this study paved the road for wastewater surveillance of *Campylobacter*-induced illness and would help improve the accuracy of WBE back-estimation of *Campylobacter* spp. prevalence in communities. In addition, the wastewater monitoring of *Campylobacter* concentration changes could also provide a cost-effective methodology for its surveillance in low- and middle-income countries to achieve early warning and timely intervention of disease outbreaks.

2. Materials and methods

2.1. Bacterial cultures

Three *Campylobacter* species including *Campylobacter jejuni* subsp. *jejuni* (ATCC® 700,819™), the *Campylobacter coli* (ATCC® 33,559™), and the *Campylobacter sputorum* biovar *sputorum (*ATCC® 33,562™*)* were purchased from the American Type Culture Collection (ATCC). All three strains were incubated on Trypticase Soy Agar (TSA) with 5% Sheep Blood Agar Plates (Thermo Fisher Scientific, Australia) for 2–5 days at 42 °C under microaerophilic conditions (Anaerocult® C for microbiology for the generation of an oxygen-depleted and CO_2-enriched atmosphere in an anaerobic jar, Merck, Australia).

2.2. Wastewater samples

Wastewater samples collected from a local wastewater treatment plant (WWTP) in Wollongong, Australia were used for the wastewater seeding experiments and in-sewer decay batch tests. These wastewater samples were tested as negative or having a Ct value of more than 40 by PCR detection for all three campylobacter species of this study. These samples were sent to the laboratory within 30 min and kept in the refrigerator at 4 °C until further tests within one week. All of these wastewater samples have typical pH values of 6.8–7.1, sulfate levels of 10–45 mg-S/L, dissolved oxygen (DO) levels of around 0.7 mg/L, total suspended solids (TSS) levels of 200–600 mg/L, total chemical oxygen demand (TCOD) levels of 150–500 mg/L, and soluble chemical oxygen demand (COD) levels of 50–130 mg/L (Shi et al., 2022).

2.3. Evaluation of Campylobacter species recovery of wastewater

The *C. jejuni, C. coli*, and *C. sputorum* cultures were spiked into 1 mL of wastewater to get a series of positive wastewater mocks with gradient concentrations around 10^2, 10^4, and 10^6 cells/mL, respectively. *C. sputorum* (primarily as an animal pathogen) is mainly used as an analytical control while *C. jejuni* and *C. coli* were evaluated as human pathogens for their fate in sewers. Since only rough concentrations are required (log level) and the recovery was calculated based on qPCR results, the initial bacteria concentrations were determined with a cell density meter (Biochrom, C08000), and converted automatically in the cell density calculator (https://www.agilent.com/store/biocalculators/calcODBacterial.jsp). Five parallel mocks were extracted at each

concentration level. Then, the spiked wastewater mocks were centrifugated at 12,000 g for 5 min in the Lysing Matrix E tube of the FastDNA™ SPIN Kit for Soil (MP Bio, Australia). Then the supernatant was removed, and the solid particle was used for further DNA extraction. The DNA extraction was strictly conducted according to the instruction in the kit's manual. The final extracted DNA volume of the 1 mL wastewater sample was 50 μL. All the extracted DNA was stored at −80 °C for further analysis.

2.4. Laboratory-scale sewer system

To simulate real sewers, a laboratory-scale sewer reactor system (Fig. S1) was used in this study. The sewer reactor system has been supplied with domestic wastewater (collected from a WWTP in Wollongong, Australia) for biofilm cultivation since 2020. 90 L of residential wastewater was collected every two weeks and kept at 4 °C. This system was made up of two types of reactors: rising main (RM) reactors and gravity sewer (GS) reactors, each with an 80 mm diameter and a water height of 150 mm. Our previous studies demonstrated that the laboratory-scale sewer reactors can reflect real sewer conditions in terms of biofilms and their biological activities (Jiang et al., 2009; Thai et al., 2014). The sewer reactors were used extensively in evaluating the stability of various biomarker compounds like illicit drugs and pharmaceuticals (Choi et al., 2020; Li et al., 2019a, 2018). The working volume of wastewater in each reactor was around 0.75 L. Each reactor has a total biofilm area of around 0.05 m^2, including the reactor wall and carrier surface. As a result, the biofilm area to wastewater volume ratio (A/V) was approximately 70.9 m^2/m^3. A magnetic stirrer (MLS8, VELP Scientific, Italy) was employed to provide continuous mixing (250 rpm) to create a modest shear force (1.7 Pa) on the inner surface of the reactor wall and to prevent solids from sinking at the bottom. Batch tests were conducted in the RM_B and GS_B reactors to measure biofilm activity (Table S1), including the sulfate reduction rate and COD reduction rate, to ensure that the reactors had attained a steady state before the tests with *Campylobacter* (Jiang et al., 2009; Li et al., 2019a, 2018).

2.5. Sewer reactor experiments and sampling schemes

Four reactors, including the RM and GS sewage reactors (RM_B and GS_B) and two control reactors (RM_C and GS_C), were used for the in-sewer decay batch tests. The control reactors are empty reactors with the same construction as the biofilm reactors but without biofilms. Before each batch test, wastewater was continuously pumped into the sewer system for 5 min to refresh all the working wastewater in RM and GS sewer reactors. Simultaneously, 0.75 L of wastewater was added to the RM_C and GS_C control reactors for parallel testing. The temperature of the wastewater was around room temperature (around 15°C) since the sewer system was located in a lab without an air conditioner and the experiments were carried out during July 2022. This temperature is much lower than the optimal growth temperature of *Campylobacter* at 37–42°C (Davis and DiRita, 2008). There is negligible multiplication of *Campylobacter* during the test considering its doubling time around 2–7 h (Battersby et al., 2016). Then, a volume of 5 mL *C. jejuni* and *C. coli* mix bacteria culture was spiked into both control (RM_C and GS_C) and sewer reactors (RM_B and GS_B) and waited for 2 min to allow a thorough mixing in reactors. The concentration of *C. jejuni* and *C. coli* was around 10^7 - 10^8 cells/L at the beginning of batch tests.

The duration of one complete batch test was 36 h, including one 24-h decay/adsorption test followed by another 12-h desorption test. First, 1 mL wastewater was sampled from RM_B, GS_B, RM_C, and GS_C reactors at the time points of 0 h, 0.5 h, 1 h, 2 h, 4 h, 6 h, 8 h, 12 h, and 24 h. One bio-carrier (biofilm sample) was also collected at the time points of 0 h, 12 h, and 24 h for adsorption evaluation. Then, after the wastewater and biofilm samples of 24 h were collected, new wastewater without seeding was pumped into the sewer system for 5 min to refresh the seeding wastewater in sewer reactors (RM_B and GS_B). After that, 1 mL wastewater was sampled from RM_B and GS_B reactors at the time points of 0 h, 0.5 h, 1 h, 2 h, 4 h, 6 h, 8 h, and 12 h after the pumping event to evaluate the desorption of *C. jejuni* and *C. coli* from sewer biofilms. Meanwhile, one bio-carrier (biofilm sample) was also collected at the time points of 12 h for the final desorption evaluation. In conclusion, a total number of 17 wastewater samples and four biofilm samples (four bio-carriers) were collected for each sewage reactor (RM_B and GS_B) during one complete batch test within 36 h. For control reactors, nine wastewater samples were collected for each sewer reactor during one complete batch test to evaluate the decay of *C. jejuni* and *C. coli* in wastewater under biofilm-free conditions within 24 h.

2.6. Sample processing, DNA extraction and the triplex qPCR assay for Campylobacter

The genomic template DNA of three *Campylobacter* species used for the positive control of the triplex qPCR test were extracted from each bacteria culture by using the GenElute™ Bacterial Genomic DNA Kits (Sigma-Aldrich, Australia). The DNA quality was assessed using the NanoDrop 2000c (Thermo Fisher Scientific, Australia), and the DNA concentration was determined using the Qubit 4.0 Fluorometer and the Qubit™ 1 × dsDNA High Sensitivity (HS) and Broad Range (BR) Assay Kits (0.1–120 ng; Thermo Fisher Scientific, Australia). The final concentration of each template DNA used in the qPCR assay was adjusted to 0.2 ng/μl and stored at −80 °C.

For the DNA extraction of wastewater samples, 1 mL of sample was transferred into the Lysing Matrix E tube of the FastDNA™ SPIN Kit and centrifugated at 12,000 g for 5 min. Then the supernatant was removed, and the DNA extraction of solid particles was strictly conducted according to the instructions in the kit's manual. The final extracted DNA volume of the 1 mL wastewater sample was 50 μL. For the DNA extraction of biofilm samples, one bio-carrier was vortexed and brushed in 5 mL of 4 °C phosphate-buffered saline (PBS, pH 7.4) for 5 min to detach the biofilm. Then, 1 mL of the liquid was used for the DNA extraction by following the same procedure for wastewater samples. All the extracted DNA was stored at −80 °C for further analysis.

The triplex qPCR assay, with further details reported in another study (under review),was adopted for all quantification tests in this study. Sequence information of three primer-probe sets and the thermal cycle protocol used were listed in Table S2 in the supplementary material. The final 20 μL qPCR system included 1 μL of *C. sputorum* genomic template (0.2 ng/μL) as the internal amplification control to exclude the presence of PCR inhibitors. Only *C. sputorum* assay results with a Ct value between 31 and 33 cycles (less than two-cycle alterations relative to positive control) were included. To eliminate false negative results, negative samples with a Ct value of *C. sputorum* assay outside of this range were tested again with a 10-fold dilution of the extracted DNA.

2.7. Data analysis of the C. jejuni and C. coli reduction

The temporal profiles of *C. jejuni* and *C. coli* concentrations in the wastewater of control or sewer reactors were analyzed using the monophasic and biphasic first-order decay kinetics because of their broad utility for evaluating microbial decay in wastewater Ahmed et al., 2020b; Hokajärvi et al., 2021). The variation of *C. jejuni* and *C. coli* concentration was linearized using the natural log (ln)-transformation of the calculated DNA concentration of each sampling point as shown in Eq. ((1) (Monophasic) and 2 (Biphasic), where C_t and C_0 are the concentrations (based on gene copies/mL) of targeted gene copies at time t and time 0, respectively. k (h^{-1}) is the decay rate constant of the monophasic first-order decay model. k_1 (h^{-1}) and k_2 (h^{-1}) are the decay rate constants of the first and second phases of the biphasic first-order decay model, respectively. t_1 (h) is the turning time point of the first and second phases. The monophasic and biphasic first-order decay rates constant with the associated 95% confidence interval (CI) were estimated using GraphPad Prism Version 9.0.0 (GraphPad Software, La

Jolla, CA, USA). The fitness was assessed by the coefficient of determination (R^2), root-mean-square error (RMSE), and the runs test. The time required to achieve a 90% (T_{90}) reduction of the targeted DNA was calculated by using k values according to Eq. (3).

$$\ln\left(\frac{C_t}{C_0}\right) = -k \times t \tag{1}$$

$$\ln\left(\frac{C_t}{C_0}\right) = -k_1 \times t, \text{ when } t \le t_1; \quad \ln\left(\frac{C_t}{C_0}\right) = -k_1 \times t - k_2 \times (t - t_1), \text{ when } t > t_1 \tag{2}$$

$$T_{90} = \frac{\ln(0.1)}{k} \tag{3}$$

2.8. Sensitivity analysis of parameters for WBE back-estimation of C. jejuni and C. coli

The WBE back-estimation equation Eqs. (4) and ((5)) was adopted in the sensitivity analysis (Guo et al., 2022; Li et al., 2022).

$$P_{\text{catchment}} = \frac{C_{\text{RNA}} \times e^{k_1 \times t} \times Q}{P_S \times Q_S \times C_S} = \frac{10^{R_C} \times e^{k_1 \times t} \times Q}{P_S \times Q_S \times 10^{R_S}}, \text{ when } t \le t_1 \tag{4}$$

$$P_{\text{catchment}} = \frac{C_{\text{RNA}} \times e^{k_1 \times t_1 + k_2 \times (t - t_1)} \times Q}{P_S \times Q_S \times C_S} = \frac{10^{R_C} \times e^{k_1 \times t_1 + k_2 \times (t - t_1)} \times Q}{P_S \times Q_S \times 10^{R_S}} \ldots \text{when } t \le t_1 \tag{5}$$

$P_{\text{catchment}}$ is the number of infected cases of *Campylobacter* within the sewer catchment; t is the hydraulic retention time (h); t_1 is the turning time point of k_1 and k_2; C_{RNA} is the concentration of *C. jejuni* or *C. coli* concentration in wastewater (gene copies/L); Q is the daily wastewater generated by each person (L/d·person); P_S is the shedding probability in stool from an infected person (%); Q_S is the daily shedding amount of stool of an individual (g/d·person), and C_S is the shedding concentration of *Campylobacter* in the stool (gene copies/g). R_C is the logarithmic concentration of target DNA in wastewater (log10, gene copies/L) and R_S is the logarithmic shedding concentration of target DNA in the stool (log10, gene copies/g). The Oracle Crystal Ball software was used to simulate the above WBE back-calculation model in different scenarios to determine the sensitivity of $P_{\text{catchment}}$ to the decay rate constants. The Monte Carlo method was adopted to sample data from the defined distributions of the parameters including k and hydraulic retention time. The program took 10,000 samples from the defined parameters to calculate predictions, thereby creating sensitivity maps and frequency distributions (Petterson et al., 2021).

3. Results and discussion

3.1. Campylobacter spp. recovery from wastewater

The *Campylobacter* spp. recovery of the sample processing and DNA extraction methods was assessed by using five parallel three-bacteria culture spiked wastewater mocks. The results are provided in the box plot of Fig. 1. The recovery was calculated by dividing the gene copies per mL calculated based on the qPCR results with the original seeding concentration (cells/mL). The results showed that *C. jejuni* recovered at concentrations of 10^6, 10^4, and 10^2 cells/mL with rates of 22.05 ± 9.76%, 10.65 ± 3.42%, and 65.41 ± 48.41%, respectively. The recovery of *C. jejuni* at 10^6 cells/mL was around 2.1 times that at 10^4 cells/mL. The exceptionally high recovery and large variation at the concentration of 10^2 cells/mL were probably due to the low concentration almost below the qPCR assay detection limit, which was determined as above 10^2 cells/mL in wastewater. The *C. coli* had a recovery of 15.76 ± 6.48%, 9.74 ± 2.44%, and 10.58 ± 3.96% at the concentration of 10^6, 10^4, and 10^2 cells/mL, respectively. The recovery of *C. coli* at 10^4 cells/mL was similar to that at 10^2 cells/mL, and the recovery at 10^6 cells/mL was around 1.5 timesthat at 10^4 and 10^2 cells/mL. For *C. sputorum*, no positive result was acquired at the 10^2 cells/mL seeding level. The recovery of *C. sputorum* at 10^6 and 10^4 cells/mL was 48.92 ± 12.07% and 25.43 ± 18.56%, respectively. The recovery of *C. sputorum* at 10^6 cells/mL was around 1.9 times that at 10^4 cells/mL. The results showed that the recovery varied for different *Campylobacter* species even within the same genus and also varied at different concentrations of the same species (Fig. 1). However, at the 10^4 cells/mL seeding level, the recovery rate of *C. jejuni* and *C. coli* are very close. The results at 10^4 and 10^6 cells/mL revealed that a 2-log variation of the pathogen concentration could induce a 1.5–2 times variation of the recovery within the same species. In addition, it seems that, when the pathogen concentration in wastewater is close to the LoQ/LoD, the recovery efficiency becomes unreliable because of the large deviation between replicate extractions. Therefore, the detection limit of the adopted methods is significant for acquiring accurate wastewater recovery for wastewater with low pathogen concentrations.

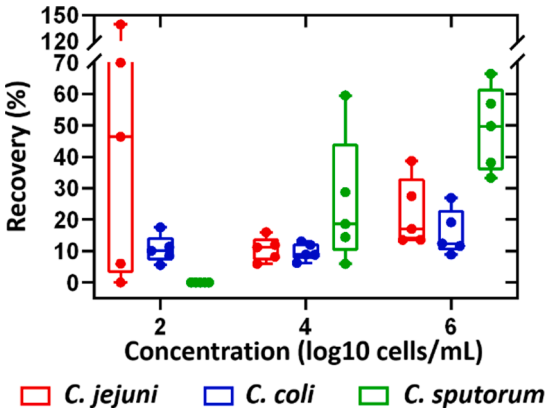

Fig. 1. Recovery of *Campylobacter* species from wastewater. The middle lines inside the box represent the median values. The top and bottom borders of the box represent the 75%ile and 25%ile of results, respectively. The top and bottom whiskers represent the maximum and minimum values of the result, respectively.

3.2. Concentration profiles of C. jejuni and C. coli in wastewater of sewer reactors

The concentration profiles of *C. jejuni* and *C. coli* in wastewater of different sewer reactors, both with (RM_B and GS_B) and without (RM_C and GS_C) biofilms, are shown in Fig. S2 (0 h to 24 h, before pumping new wastewater) and Fig. S3 (24 h to 36 h, after new pumping). The initial concentration of *C. jejuni* and *C. coli* in wastewater after seeding (at the time point of 0 h) was around 10^7 and 10^8 cells/L, respectively. In RM reactors (Fig. S2, A and C), for both *C. jejuni* and *C. coli*, the concentration reduction in wastewater in RM_C and RM_B reactors were similar. The *C. coli* concentration in both the RM_C and RM_B reactors reached a more than 1-log reduction within 7.5 h, which is 10 h faster than the 1-log reduction of *C. jejuni* in RM_C and RM_B reactors. In GS reactors (Fig. S2, B and D), the reduction of *C. jejuni* and *C. coli* in the GS_C reactor was similar to their reductions in the RM_C reactor. However, the concentration of both *C. jejuni* and *C. coli* had a significantly higher reduction in the GS_B reactor than that in the GS_C reactor. These results indicated that the conditions of the GS_B reactor enhanced the reduction of *C. jejuni* and *C. coli* concentration in wastewater, whereas no obvious effect was observed for the RM_B reactor conditions. After pumping new wastewater (24 h to 36 h, Fig. S3), the concentration of *C. jejuni* and *C. coli* in wastewater still displayed a declining trend and only a slight reduction of their concentration was observed after 12 h.

The larger fluctuation of the *C. jejuni* concentration in GS_B reactors might be because of the larger variation of recovery at this concentration (10^2 GC or cells/mL).

3.3. Reduction kinetics of C. jejuni and C. coli concentration in wastewater of sewer reactors

The monophasic first-order decay model was adopted to generate the reduction rate constant k, and the time required to achieve a 90% (T_{90}) reduction of the targeted DNA of *C. jejuni* and *C. coli*. The results were shown in Table S3 and Fig. S4. It is worth noticing that, although the monophasic first-order decay model enabled a reasonable R^2 (0.69–0.89) for the reduction of *C. jejuni* in RM_C, GS_C, and GS_B reactors, and for the reduction of *C. coli* in RM_C and GS_C reactors, the R^2 of *C. jejuni* reduction in RM_B and the R^2 of *C. coli* reduction in RM_B and GS_B were unreasonable. Furthermore, the fitted lines in Fig. S4 showed large deviations from the data, which suggested that the monophasic first-order decay model is insufficient to describe the reduction of *C. jejuni* and *C. coli* in wastewater of RM_B and GS_B reactors.

The biphasic first-order kinetic model was fitted to the concentration profiles as shown in Fig. 2, and the fitted parameters were listed in Table 1. All R^2 generated were equal to or above 0.96, which indicated its good fitness for representing the reduction kinetics of *C. jejuni* and *C. coli* concentration in wastewater of different sewer reactors. The k_1 value of *C. jejuni* in RM_B and GS_B reactors was around 2.3 and 4.1 times that in RM_C and GC_C reactors, respectively. After the turning time point, the k_2 value of RM_C, RM_B, GS_C, and GS_B reactors was more or less similar (0.05–0.06 h^{-1}) except for a higher level in GS_B (0.13 h^{-1}). The T_{90} of *C. jejuni* in RM_C, RM_B, and GS_C reactors were also similar, i.e., around 20.5–22.2 h, which were around 2.5 times that in the GS_B reactor. The results showed that sewer biofilms did not induce an obvious difference in the overall reduction kinetics, although the k_1 in the RM_B reactor was more than two times of that in the RM_C reactor. This is due to the much shorter first phase (t_1=2.73 h) in RM_B compared to that of RM_C (t_1=6.00 h). In contrast, for GS reactors, a four times faster reduction was observed in sewer reactors compared to the control reactor, due to higher values of both k_1 and k_2. In the case of *C. coli*, the k_1 value of *C. coli* in RM_B and GS_B reactors was around 1.5 and 3.9 times that in RM_C and GC_C reactors, respectively. Different from C. *jejuni*, the k_2 values of RM_C, RM_B, GS_C, and GS_B reactors were all around 0.07–0.09 h^{-1} although the biofilm reactors had slightly higher values. The turning time point t_1 of *C. coli* in RM_C, RM_B, GS_C, and GS_B reactors was 6.00 h, 3.64 h, 8.85 h, and 2.72 h, respectively, with the much shorter first phase in biofilm reactors. The faster decay kinetics in the first phase (k_1=1.04 h^{-1}) of GS_B was mainly responsible for the overall higher reduction. The T_{90} of *C. coli* in all reactors were 2.6–3.8 times faster than that of *C. jejuni*. This indicates the lower stability of *C. coli* than *C. jejuni* in sewers.

3.4. The total decay and the partitions of C. jejuni and C. coli in the wastewater and biofilm phases

The total gene number of *C. jejuni* and *C. coli* in the wastewater and biofilms phases of each reactor at the time point of 0 h, 12 h, and 24 h was calculated by supposing the total volume of wastewater was constantly as 0.75 L and the total area of sewer biofilm was constantly as 0.05m^2 (Fig. S5). The results showed that both the total *C. jejuni* and *C. coli* decay in the RM_B reactors (~0.53 log10 copies and ~1.1 log10 copies, the total gene number reduction in wastewater plus biofilm) were lower than the decay in the RM_C reactor (~1.08 log10 copies and ~1.57 log10 copies), and no obvious difference in the total *C. jejuni* and *C. coli* reduction in the wastewater phase was observed between the RM_C and RM_B reactors (Fig. S5 A and C, black and red curves). Different from the situation in RM reactors the total decay of *C. jejuni* and *C. coli* in the GS_B reactor (~1.21 log10 copies and ~ 1.63 log10 copies, total gene number in wastewater and biofilm phases) was faster than in the GS_C reactor (~ 1.05 log10 copies and ~ 1.51 log10 copies) in 24 h, which indicated that the conditions in the GS_B reactor enhanced the decay of *C. jejuni* and *C. coli*. Considering that there is no obvious difference in the total decay of *C. jejuni* and *C. coli* in the RM_C

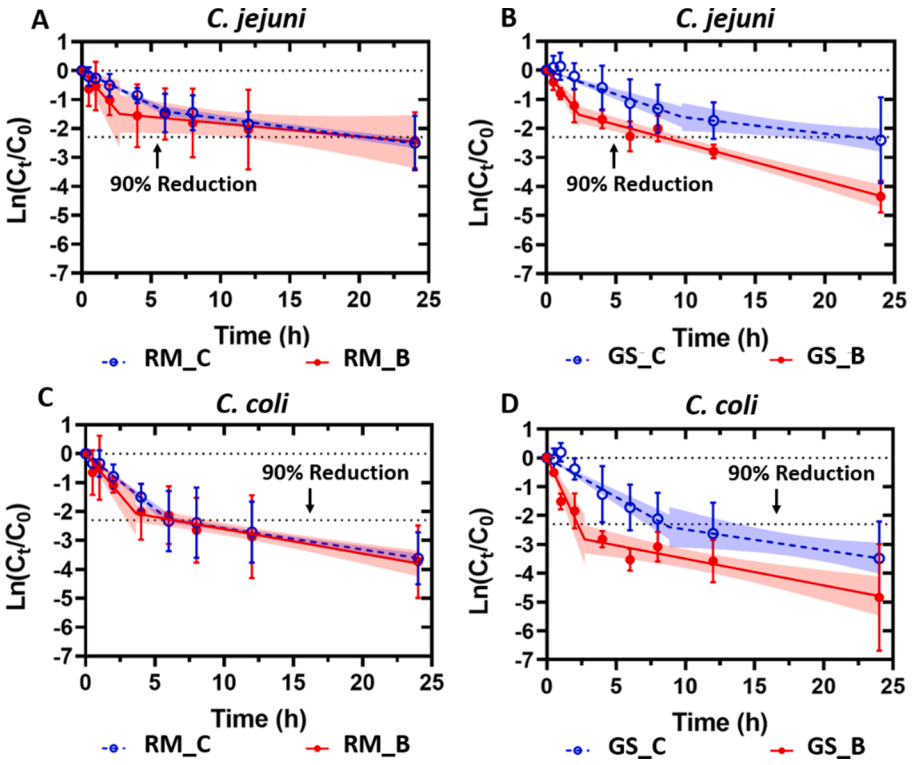

Fig. 2. The reduction kinetics of *C. jejuni* and *C. coli* in wastewater of different types of control and sewer reactors. Lines are fitted with the biphasic first-order kinetic model. The results of three parallel batch tests were presented as mean ± SD. The color bands represent the 95% confidence bands of each line.

Table 1
Reduction rate k_1 (h^{-1}) and k_2 (h^{-1}), phase turning time point t_1 (h) and T_{90} values (h) of *C. jejuni* and *C. coli* in sewer reactors based on the biphasic first-order decay model.

Reactors		RM_C	RM_B	GS_C	GS_B
C. jejuni	k_1 (h−1) [95% CI]	0.24 [0.21–0.27]	0.55 [0.37–0.92]	0.16 [0.13–0.22]	0.65 [0.47–1.16]
	k_2 (h−1) [95% CI]	0.06 [0.05–0.07]	0.05 [0.02–0.07]	0.06 [0–0.1]	0.13 [0.10–0.16]
	t_1 (h) [95% CI]	6.00 [4.97–7.07]	2.73 [1.34–4.48]	9.75 [4.44-NA]	2.33 [1.02–3.72]
	R^2	0.99	0.96	0.98	0.99
	RMSE	0.06	0.15	0.19	0.15
	Runs test	NS, p = 0.93	NS, p = 0.89	NS, p = 0.13	NS, p = 0.71
	T_{90} (h) [95% CI]	20.52 [18.62–23.14]	20.51 [15.49–35.8]	22.23 [17.09–48.91]	8.35 [6.68–9.82]
C. coli	k_1 (h−1) [95% CI]	0.38 [0.36–0.42]	0.57 [0.42–0.78]	0.27 [0.22–0.37]	1.04 [0.71–1.35]
	k_2 (h−1) [95% CI]	0.07 [0.06–0.09]	0.08 [0.05–0.11]	0.07 [0.01–0.13]	0.09 [0.05–0.14]
	t_1 (h) [95% CI]	6.00 [5.29–6.48]	3.64 [2.40–5.71]	8.85 [4.32-NA]	2.72 [1.27–4.40]
	R^2	0.99	0.98	0.98	0.97
	RMSE	0.06	0.17	0.19	0.26
	Runs test	0.97	0.43	0.4	0.93
	T_{90} (h) [95% CI]	6.3 [5.58–7.67]	6.43 [2.97–9.01]	8.5 [7.20–13.95]	2.22 [1.70–2.72]

and RM_B reactors (Fig. S5 A and C), it is reasonable to infer that the faster reduction in sewage reactors compared to control reactors during the first reduction phase is mainly caused by the adsorption of sewer biofilms and the decay of adsorbed *Campylobacter* was similar to those in wastewater. In addition, after the turning time point, the decay rate constant of the second phase (k_2) in both the RM_C and RM_B reactors is between 0.05 h^{-1} and 0.08 h^{-1}. Based on the above observations, the *C. jejuni* and *C. coli* concentration reduction in the wastewater phase of the sewer reactors could be divided into two steps: (i) the biofilm adsorption dominated fast concentration reduction; (ii) the decay in the wastewater phase after achieving an adsorption equilibrium. In GS reactors, a higher total decay of *C. jejuni* and *C. coli* was observed in sewer reactors compared to the control reactors (Fig. S5 B and D). However, in RM reactors, the total decay of *C. jejuni* and *C. coli* in sewer biofilm reactors was similar to or even slightly lower than that in control reactors (Fig. S5 A and C). These results revealed that the RM and GS sewer environment may have aggravating and alleviating effects, respectively, on the decay of *C. jejuni* and *C. coli*.

Environmental factors including temperature, sunlight, dissolved oxygen (DO), soluble chemical oxygen demand (SCOD), nutrient availability, and salinity have also been reported to be significant to the *Campylobacter* spp. persistence in environmental water (Orner et al., 2018). This study used the same raw wastewater in reactors wrapped with foil at the same room temperature for all batch tests. The most significant difference between the control and the sewer reactors is the absence or presence of different sewer biofilms, i.e., anaerobic biofilms in RM and both aerobic and anaerobic biofilms in GS reactors (Jin et al., 2018; Li et al., 2019b; O'Brien et al., 2019; Shi et al., 2022). The effects of temperature, sunlight and wastewater characteristics were not considered. In addition, studies have reported the survival of *Campylobacter. jejuni* is directly affected by the oxygen concentrations of its surrounding environments (Kim et al., 2015). Therefore, the difference in the ambient oxygen concentrations in RM_B and GS_B reactors might be the most possible reason for the different decay kinetics of *C. jejuni* and *C. coil*. Furthermore, the components of sewer biofilms such as the extracellular polymeric substances (EPS) and the DNA enzymes are also potential factors in the adsorption and decay of *C. jejuni* and *C. coli* (Li et al., 2019b). It is worth noticing that, during the real in-sewer transport of these pathogens, the wastewater cannot stay at the same pipe location for such a long time (24 h). Therefore, the concentration reduction of *C. jejuni* and *C. coli* in wastewater caused by the adsorption into sewer biofilms might be even higher in real sewers than under the experimental conditions of this study.

3.5. The adsorption/desorption of C. jejuni and C. coli into sewer biofilms

According to the author's assumption, an adsorption equilibrium could be achieved after the turning time point, thus it is reasonable to consider that, at the time point of 12 h, 24 h, and 36 h of one batch test, the RM_B and GS_B reactor were in adsorption equilibrium status. Therefore, the adsorption isotherms of *C. jejuni* and *C. coli* on the biofilm of different sewer reactors were generated based on the *C. jejuni* and *C. coli* concentration in wastewater and in biofilm phases at the time of 12 h, 24 h, and 36 h to provide an insight into the adsorption capacity of different biofilms for *C. jejuni* and *C. coli* (Fig. 3) (Borkowski et al., 2015; Lupascu et al., 2022). Based on the slope of the fitted lines, it seems that the biofilm in the RM_B reactor (slope = 1.2) has a slightly higher adsorption capacity for *C. jejuni* than the biofilm of the GS_B reactor (slope = 1). In contrast, the biofilm in the GS_B reactor (slope = 0.67) has a slightly higher adsorption capacity for *C. coli* than the biofilm of the RM_B reactor (slope = 0.41). In addition, both the biofilm of RM_B and GS_B reactors showed an obviously higher adsorption capacity of *C. jejuni* than of *C. coli*. Exclude the constant condition of pH and the temperature of the wastewater used in this study, the reasons for causing these differences might include the structure and components of the biofilms and the different bacteria sizes and surface characters (Borkowski et al., 2015; Li et al., 2019b). However, this conclusion was only based on three data points, to obtain a clear view of the adsorption capacity of different sewer biofilms for *C. jejuni* and *C. coli*, further detailed studies should be conducted.

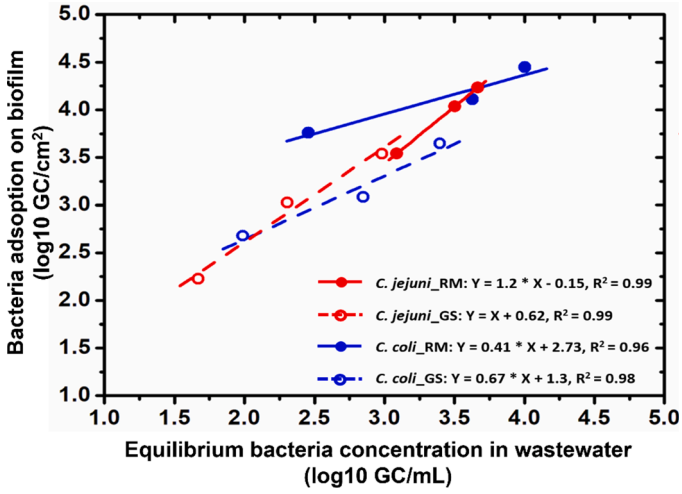

Fig. 3. Adsorption isotherms of *C. jejuni* and *C. coli* on the biofilm of different sewer reactors. The data points were the mean concentrations of the wastewater and biofilm samples collected in three repeated batches at 12, 24, and 36 h, respectively.

3.6. Sensitivity analysis for WBE back-estimation of C. jejuni and C. coli

To evaluate the application of the parameters acquired in this study in the WBE back-estimation of *C. jejuni* and *C. coli* prevalence, their sensitivity for inducing the variance of WBE back-estimation was investigated based on the WBE back-estimation equation. The shedding concentration of *Campylobacter* spp. in human stool specimens has been estimated to be 10^6–10^9 CFU/mL (Buss et al., 2019). Therefore, the shedding concentration (R_S) used in the sensitivity analysis of this study was defined and fixed as 7.5 log10 gene copies/g. According to the meta-analysis of the *Campylobacter* spp. prevalence in wastewater investigated by another study (under review), the *Campylobacter* concentration in wastewater (R_C) used for sensitivity analysis was defined as 4.31 ± 0.39 (95% confidence interval) log10 gene copies/L with normal distribution. The shedding probability in stool from an infected person (P_S,%) was assumed as 100% with no prior information reported for *Campylobacter* spp. The daily wastewater generated by each person (Q) was assumed to be 250L/d·person. The daily stool shedding amount of each person (Q_S) was assumed as 250 g/d·person (Ahmed et al., 2020a; Guo et al., 2022; Li et al., 2022, 2021b; Miura et al., 2021). Other parameters including the k_1, k_2, and t_1 with the 95% CI acquired in Section 3.3 were also defined with normal distribution in this sensitivity analysis.

The results showed that (Fig. 4), under the analysis conditions defined in this study, when hydraulic retention time ($t = 1$ h) was lower than the turning time point (t_1), the campylobacter concentration in wastewater (R_C) was the dominating parameter that contributed 80% - 99% variance to the WBE back-estimation of both *C. jejuni* and *C. coli* in all types of reactors. The k_1 was the second parameter that contributed 4% - 19.7% variance in the biofilm reactors. When the hydraulic retention time equalled the turning time point ($t = t_1$), the sensitivity of k_1 increased especially for the RM_B and GS_B reactors, which became the dominating parameter that contributed more than 50% variance of the WBE back-estimation of *C. jejuni*. When the hydraulic retention time was higher than t_1 ($t = 12$ h), the sensitivity of t_1 had an obvious increase for both *C. jejuni* and *C. coli* in all reactors except for the RM_C. For *C. coli* in RM_B and GS_B, t_1 became the dominating parameter that contributed 40.9% and 56.1% variance, respectively. When the hydraulic retention time was up to 24 h, the sensitivity of R_C decreased to below 50% in all types of reactors except for the RM_C. In total, with the increase of the hydraulic retention time, the sensitivity of R_C kept decreasing. In contrast, the sensitivity of decay-related parameters (k_1, t_1, and k_2) increased and contributed the most variance to the WBE back-estimation of both *C. jejuni* and *C. coli*, especially in biofilm reactors. Previous studies have reported that the human stool shedding concentration and the concentration in wastewater of the target human pathogens are the top two sensitive parameters for inducing the variance of WBE back-estimation (Guo et al., 2022; Li et al., 2021b, 2021c). This observation is based on very limited data on pathogen shedding and low accuracy in analysing pathogen concentrations in wastewater. The sensitivity of the decay rate constant is usually reported as nil because of the lack of information on their in-sewer decay (Guo et al., 2022). However, our study revealed that the sensitivity of decay-related parameters can induce significant variance to the WBE back-estimation of both *C. jejuni* and *C. coli*, thus should be further studied to improve the accuracy.

4. Conclusions

This study conducted a series of experiments to comprehensively investigate (i) the recovery efficiency of *Campylobacter* species from wastewater at different concentrations, (ii) the decay of *C. jejuni* and *C. coli* in wastewater of laboratory-scale sewer biofilm reactors, (iii) the adsorption and desorption of *C. jejuni* and *C. coli* in sewer biofilm reactors, (iv) the parameter sensitivity of the WBE back-estimation of *C. jejuni* and *C. coli*. The key conclusions are:

- The recovery efficiency of *Campylobacter* spp. increased with their concentrations in wastewater, while the *C. jejuni* and *C. coli* have a very similar recovery of around 10% at 10^4 cells/mL.
- The decay of *C. jejuni* and *C. coli* in the gravity sewer reactor is faster than that in the rising main sewer reactor, and the presence of dissolved oxygen might play an important role in aggravating the decay of *C. jejuni* and *C. coli*. *C. coli* generally decayed faster than *C. jejuni* in wastewater under all conditions.

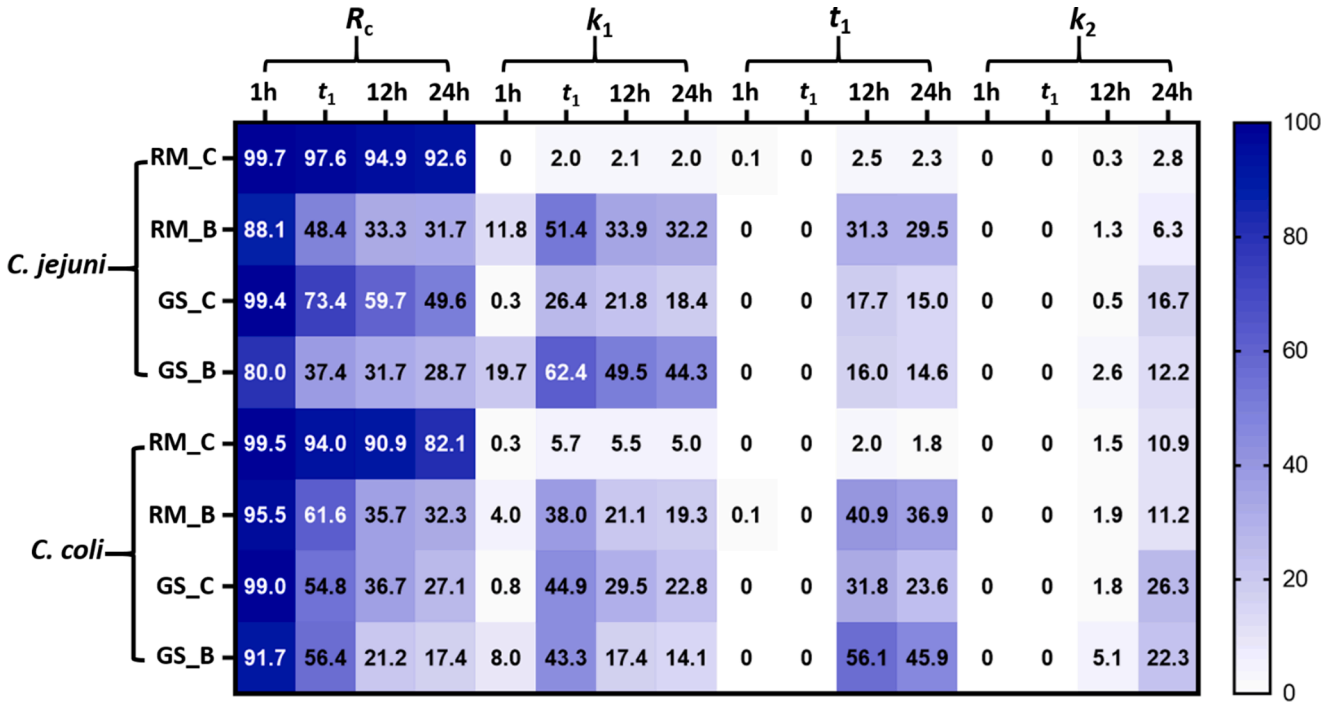

Fig. 4. Sensitivity (%) of WBE back-estimation to *C. jejuni* and *C. coli* decay parameters (R_C, k_1, k_2, and t_1) (Assuming all parameters as normal distributions; The hydraulic retention time $t = 1$ h, t_1, 12 and 24 h, $Q = 250$ L/ d·person, $Q_S = 250$ g/d·person, $Ps = 100\%$, R_S =7.5 log10 gene copies/g).

- The adsorption of *C. jejuni* and *C. coli* onto sewer biofilms is a significant factor that can enhance their concentration reduction in the wastewater phase. This overall reduction can be described by biphasic first-order kinetics.
- The decay parameters induce significant variance to the WBE back-estimation of *C. jejuni* and *C. coli* prevalence, especially for sewer catchments with long hydraulic retention time.

The recovery and decay parameters obtained in this study can significantly improve the precision of WBE back-estimation of *Campylobacter* prevalence in communities. The further development of wastewater surveillance as a supplement to the clinical surveillance could achieve early warning of outbreaks, particularly in low- and middle-income countries. In future studies, considering the environmental temperature could have a certain effect on the multiplication of target pathogens and the biological activities of biofilm communities, more experiments should be carried out to evaluate the in-sewer decay and partition of *Campylobacter* spp. under different environment temperatures to map the seasonal variation of its decay and partition and further improve the WBE-based back estimation of *Campylobacter* spp. prevalence in communities and relevant environments.

CRediT authorship contribution statement

Shuxin Zhang: Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Jiahua Shi:** Establishment (main) and maintenance of the laboratory-scale sewer reactor system. **Elipsha Sharma:** Wastewater collection and maintenance of the laboratory-scale sewer reactor system. **Xuan Li:** Establishment (secondary) of the laboratory-scale sewer reactor system. **Shuhong Gao:** Writing – review. **Xu Zhou:** Writing – review. **Jake W. O'Brien:** Writing – review. **Lachlan Coin:** Writing – review. **Yanchen Liu:** Writing – review. **Muttucumaru Sivakumar:** Writing – review & editing. **Faisal Hai:** Writing – review. **Guangming Jiang:** Conceptualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Guangming Jiang reports financial support was provided by ARC Discovery project.

Data availability

Data will be made available on request.

Acknowledgments

This research was supported by the ARC Discovery project (DP190100385). Shuxin Zhang receives the support from a University of Wollongong PhD scholarship. Jake W. O'Brien is the recipient of an NHMRC Emerging Leadership Fellowship (EL1 2009209). The Queensland Alliance for Environmental Health Sciences (QAEHS), The University of Queensland, gratefully acknowledges the financial support of Queensland Health.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2023.119737.

References

Abdeldayem, O.M., Dabbish, A.M., Habashy, M.M., Mostafa, M.K., Elhefnawy, M., Amin, L., Al-Sakkari, E.G., Ragab, A., Rene, E.R., 2022. Viral outbreaks detection and surveillance using wastewater-based epidemiology, viral air sampling, and machine learning techniques: a comprehensive review and outlook. Sci. Total Environ. 803, 149834.

Ahmed, W., Angel, N., Edson, J., Bibby, K., Bivins, A., O'Brien, J.W., Choi, P.M., Kitajima, M., Simpson, S.L., Li, J., Tscharke, B., Verhagen, R., Smith, W.J.M., Zaugg, J., Dierens, L., Hugenholtz, P., Thomas, K.V., Mueller, J.F., 2020a. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: a proof of concept for the wastewater surveillance of COVID-19 in the community. Sci. Total Environ. 728, 138764.

Ahmed, W., Bertsch, P.M., Bibby, K., Haramoto, E., Hewitt, J., Huygens, F., Gyawali, P., Korajkic, A., Riddell, S., Sherchan, S.P., Simpson, S.L., Sirikanchana, K., Symonds, E. M., Verhagen, R., Vasan, S.S., Kitajima, M., Bivins, A., 2020b. Decay of SARS-CoV-2 and surrogate murine hepatitis virus RNA in untreated wastewater to inform application in wastewater-based epidemiology. Environ. Res. 191, 110092.

Anand, U., Li, X., Sunita, K., Lokhandwala, S., Gautam, P., Suresh, S., Sarma, H., Vellingiri, B., Dey, A., Bontempi, E., Jiang, G., 2022. SARS-CoV-2 and other pathogens in municipal wastewater, landfill leachate, and solid waste: a review about virus surveillance, infectivity, and inactivation. Environ. Res. 203, 111839.

Battersby, T., Walsh, D., Whyte, P., Bolton, D.J., 2016. *Campylobacter* growth rates in four different matrices: broiler caecal material, live birds, Bolton broth, and brain heart infusion broth. Infect. Ecol. Epidemiol. 6, 31217.

Bonetta, S., Pignata, C., Lorenzi, E., De Ceglia, M., Meucci, L., Bonetta, S., Gilli, G., Carraro, E., 2016. Detection of pathogenic *Campylobacter, E. coli* O157:H7 and *Salmonella* spp. in wastewater by PCR assay. Environ. Sci. Pollut. Res. 23 (15), 15302–15309.

Borkowski, A., Szala, M., Cłapa, T., 2015. Adsorption Studies of the Gram-Negative Bacteria onto Nanostructured Silicon Carbide. Appl. Biochem. Biotechnol. 175 (3), 1448–1459.

Buss, J.E., Cresse, M., Doyle, S., Buchan, B.W., Craft, D.W., Young, S., 2019. *Campylobacter* culture fails to correctly detect *Campylobacter* in 30% of positive patient stool specimens compared to non-cultural methods. Eur. J. Clin. Microbiol. Infect. Dis. 38 (6), 1087–1093.

Choi, P.M., Li, J., Gao, J., O'Brien, J.W., Thomas, K.V., Thai, P.K., Jiang, G., Mueller, J.F., 2020. Considerations for assessing stability of wastewater-based epidemiology biomarkers using biofilm-free and sewer reactor tests. Sci. Total Environ. 709, 136228.

Choi, P.M., Tscharke, B.J., Donner, E., O'Brien, J.W., Grant, S.C., Kaserzon, S.L., Mackie, R., O'Malley, E., Crosbie, N.D., Thomas, K.V., Mueller, J.F., 2018. Wastewater-based epidemiology biomarkers: past, present and future. TrAC Trends Anal. Chem. 105, 453–469.

Dai, L., Sahin, O., Grover, M., Zhang, Q., 2020. New and alternative strategies for the prevention, control, and treatment of antibiotic-resistant *Campylobacter*. Transl. Res. 223, 76–88.

Davis, L., DiRita, V., 2008. Growth and Laboratory Maintenance of *Campylobacter jejuni*. Curr. Protoc. Microbiol. 10 (1), 8A.1.1–8A.1.7.

Epps, S.V.R., Harvey, R.B., Hume, M.E., Phillips, T.D., Anderson, R.C., Nisbet, D.J., 2013. Foodborne *Campylobacter*: infections, metabolism, pathogenesis and reservoirs. Int. J. Environ. Res. Public Health 10 (12), 6292–6304.

Guo, Y., Li, J., O'Brien, J., Sivakumar, M., Jiang, G., 2022. Back-estimation of norovirus infections through wastewater-based epidemiology: a systematic review and parameter sensitivity. Water Res. 219, 118610.

Hellein, K.N., Battie, C., Tauchman, E., Lund, D., Oyarzabal, O.A., Lepo, J.E., 2011. Culture-based indicators of fecal contamination and molecular microbial indicators rarely correlate with *Campylobacter* spp. in recreational waters. J. Water Health 9 (4), 695–707.

Hokajärvi, A.M., Rytkönen, A., Tiwari, A., Kauppinen, A., Oikarinen, S., Lehto, K.M., Kankaanpää, A., Gunnar, T., Al-Hello, H., Blomqvist, S., Miettinen, I.T., Savolainen-Kopra, C., Pitkänen, T., 2021. The detection and stability of the SARS-CoV-2 RNA biomarkers in wastewater influent in Helsinki, Finland. Sci. Total Environ. 770, 145274.

Hvitved, T., Vollertsen, J., Nielsen, A.H., 2013. Sewer Processes: Microbial and Chemical Process Engineering of Sewer Networks. CRC Press.

Jiang, G., Sharma, K.R., Guisasola, A., Keller, J., Yuan, Z., 2009. Sulfur transformation in rising main sewers receiving nitrate dosage. Water Res. 43 (17), 4430–4440.

Jin, P., Shi, X., Sun, G., Yang, L., Cai, Y., Wang, X.C., 2018. Co-Variation between distribution of microbial communities and biological metabolization of organics in urban sewer systems. Environ. Sci. Technol. 52 (3), 1270–1279.

Joensen, K.G., Schjørring, S., Gantzhorn, M.R., Vester, C.T., Nielsen, H.L., Engberg, J.H., Holt, H.M., Ethelberg, S., Müller, L., Sandø, G., 2021. Whole genome sequencing data used for surveillance of *Campylobacter* infections: detection of a large continuous outbreak, Denmark, 2019. Eurosurveillance 26 (22), 2001396.

Kaakoush, N.O., Castaño-Rodríguez, N., Mitchell Hazel, M., Man Si, M., 2015. Global Epidemiology of *Campylobacter* Infection. Clin. Microbiol. Rev. 28 (3), 687–720.

Kim, J.-C., Oh, E., Kim, J., Jeon, B., 2015. Regulation of oxidative stress resistance in *Campylobacter jejuni*, a microaerophilic foodborne pathogen. Front .Microbiol. 6.

Li, J., Gao, J., Thai, P.K., Shypanski, A., Nieradzik, L., Mueller, J.F., Yuan, Z., Jiang, G., 2019a. Experimental investigation and modeling of the transformation of illicit drugs in a pilot-scale sewer system. Environ. Sci. Technol. 53 (8), 4556–4565.

Li, J., Gao, J., Thai, P.K., Sun, X., Mueller, J.F., Yuan, Z., Jiang, G., 2018. Stability of illicit drugs as biomarkers in sewers: from lab to reality. Environ. Sci. Technol. 52 (3), 1561–1570.

Li, J., Gao, J., Zheng, Q., Thai, P.K., Duan, H., Mueller, J.F., Yuan, Z., Jiang, G., 2021a. Effects of pH, temperature, suspended solids, and biological activity on transformation of illicit drug and pharmaceutical biomarkers in sewers. Environ. Sci. Technol. 55 (13), 8771–8782.

Li, W., Zheng, T., Ma, Y., Liu, J., 2019b. Current status and future prospects of sewer biofilms: their structure, influencing factors, and substance transformations. Sci. Total Environ. 695, 133815.

Li, X., Kulandaivelu, J., Guo, Y., Zhang, S., Shi, J., O'Brien, J., Arora, S., Kumar, M., Sherchan, S.P., Honda, R., Jackson, G., Luby, S.P., Jiang, G., 2022. SARS-CoV-2 shedding sources in wastewater and implications for wastewater-based epidemiology. J. Hazard. Mater. 432, 128667.

Li, X., Kulandaivelu, J., Zhang, S., Shi, J., Sivakumar, M., Mueller, J., Luby, S., Ahmed, W., Coin, L., Jiang, G., 2021b. Data-driven estimation of COVID-19 community prevalence through wastewater-based epidemiology. Sci. Total Environ. 789, 147947.

Li, X., Zhang, S., Shi, J., Luby, S.P., Jiang, G., 2021c. Uncertainties in estimating SARS-CoV-2 prevalence by wastewater-based epidemiology. Chem. Eng. J. 415, 129039.

Lupascu, L., Petuhov, O., Timbaliuc, N., Lupascu, T., 2022. Study of the Adsorption of Bacillus subtilis and Bacillus cereus Bacteria on Enterosorbent Obtained from Apricot Kernels. C J. Carbon Res.

Miura, F., Kitajima, M., Omori, R., 2021. Duration of SARS-CoV-2 viral shedding in faeces as a parameter for wastewater-based epidemiology: *re*-analysis of patient data using a shedding dynamics model. Sci. Total Environ. 769, 144549.

O'Brien, J.W., Choi, P.M., Li, J., Thai, P.K., Jiang, G., Tscharke, B.J., Mueller, J.F., Thomas, K.V., 2019. Evaluating the stability of three oxidative stress biomarkers under sewer conditions and potential impact for use in wastewater-based epidemiology. Water Res. 166, 115068.

Orner, K., Naughton, C., Stenstrom, T.A., Mihelcic, J.R. and Verbyla, M.E. 2018. Water and sanitation for the 21st century: health and microbiological aspects of excreta and wastewater management (Global Water Pathogen Project).

Petterson, S., Li, Q., Ashbolt, N., 2021. Screening Level Risk Assessment (SLRA) of human health risks from faecal pathogens associated with a Natural Swimming Pond (NSP). Water Res. 188, 116501.

Riquelme, M.V., Garner, E., Gupta, S., Metch, J., Zhu, N., Blair, M.F., Arango-Argoty, G., Maile-Moskowitz, A., Li, A., Flach, C.F., Aga, D.S., Nambi, I., Larsson, D.G.J., Bürgmann, H., Zhang, T., Pruden, A. and Vikesland, P.J. 2021. Wastewater based epidemiology enabled surveillance of antibiotic resistance. medRxiv, 2021.2006.2001.21258164.

Rousis, N.I., Gracia-Lor, E., Zuccato, E., Bade, R., Baz-Lomba, J.A., Castrignanò, E., Causanilles, A., Covaci, A., de Voogt, P., Hernàndez, F., Kasprzyk-Hordern, B., Kinyua, J., McCall, A.-K., Plósz, B.G., Ramin, P., Ryu, Y., Thomas, K.V., van Nuijs, A., Yang, Z., Castiglioni, S., 2017. Wastewater-based epidemiology to assess pan-European pesticide exposure. Water Res. 121, 270–279.

Seventer, J.M.V. and Hochberg, N.S. (2017) International Encyclopedia of Public Health (2nd Ed.). Quah, S.R. (ed), pp. 22–39, Academic Press, Oxford.

Shi, J., Li, X., Zhang, S., Sharma, E., Sivakumar, M., Sherchan, S.P., Jiang, G., 2022. Enhanced decay of coronaviruses in sewers with domestic wastewater. Sci. Total Environ. 813, 151919.

Shrestha, S., Yoshinaga, E., Chapagain, S.K., Mohan, G., Gasparatos, A. and Fukushi, K. 2021 Wastewater-based epidemiology for cost-effective mass surveillance of COVID-19 in Low- and middle-income countries: challenges and opportunities.

Sims, N., Kasprzyk, H.B., 2020. Future perspectives of wastewater-based epidemiology: monitoring infectious disease spread and resistance to the community level. Environ. Int. 139, 105689.

Thai, P.K., Jiang, G., Gernjak, W., Yuan, Z., Lai, F.Y., Mueller, J.F., 2014. Effects of sewer conditions on the degradation of selected illicit drug residues in wastewater. Water Res. 48, 538–547.

Tiwari, A., Adhikari, S., Kaya, D., Islam, M.A., Malla, B., Sherchan, S.P., Al-Mustapha, A. I., Kumar, M., Aggarwal, S., Bhattacharya, P., Bibby, K., Halden, R.U., Bivins, A., Haramoto, E., Oikarinen, S., Heikinheimo, A., Pitkänen, T., 2023. Monkeypox outbreak: wastewater and environmental surveillance perspective. Sci. Total Environ. 856, 159166.

Tong, S., Ma, L., Ronholm, J., Hsiao, W., Lu, X., 2021. Whole genome sequencing of *Campylobacter* in agri-food surveillance. Curr. Opin. Food Sci. 39, 130–139.

World Health Organization, Food, Agriculture Organization of the United, N. and World Organisation for Animal, H., 2013. The Global View of *Campylobacteriosis*: Report of an Expert consultation, Utrecht, Netherlands, 9-11 July 2012. World Health Organization, Geneva.

Zahedi, A., Monis, P., Deere, D., Ryan, U., 2021. Wastewater-based epidemiology—Surveillance and early detection of waterborne pathogens with a focus on SARS-CoV-2, *Cryptosporidium* and *Giardia*. Parasitol. Res. 120 (12), 4167–4188.

Zbrun, M.V., Rossler, E., Romero-Scharpen, A., Soto, L.P., Berisvil, A., Zimmermann, J. A., Fusari, M.L., Signorini, M.L., Frizzo, L.S., 2020. Worldwide meta-analysis of the prevalence of *Campylobacter* in animal food products. Res. Vet. Sci. 132, 69–77.