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Microbial risk assessment of drinking water based on hydrodynamic modelling of pathogen concentrations in source water

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Abstract

Norovirus contamination of drinking water sources is an important cause of waterborne disease outbreaks. Knowledge on pathogen concentrations in source water is needed to assess the ability of a drinking water treatment plant (DWTP) to provide safe drinking water. However, pathogen enumeration in source water samples is often not sufficient to describe the source water quality. In this study, the norovirus concentrations were characterised at the contamination source, i.e. in sewage discharges. Then, the transport of norovirus within the water source (the river Göta älv in Sweden) under different loading conditions was simulated using a hydrodynamic model. Based on the estimated concentrations in source water, the required reduction of norovirus at the DWTP was calculated using quantitative microbial risk assessment (QMRA). The required reduction was compared with the estimated treatment performance at the DWTP. The average estimated concentration in source water varied between 4.8×10^2 and 7.5×10^3 genome equivalents L^{-1} ; and the average required reduction by treatment was between 7.6 and 8.8 Log_{10} . The treatment performance at the DWTP was estimated to be adequate to deal with all tested loading conditions, but was heavily dependent on chlorine disinfection, with the risk of poor reduction by conventional treatment and slow sand filtration. To our knowledge, this is the first article to employ discharge-based QMRA, combined with hydrodynamic modelling, in the context of drinking water.

Key words: water quality modelling; norovirus; quantitative microbial risk assessment; QMRA; discharge-based QMRA.

1 Introduction

Gastrointestinal diseases related to the contamination of drinking water constitute a major threat to global human health (WHO, 2011). Although this problem is most dominantly present in developing countries, the microbial contamination of drinking water causes outbreaks in developed countries as well (e.g. Braeye et al., 2014; Craun et al., 2010; Larsson et al., 2014; Mac Kenzie et al., 1994). Water is an important transmission route of noroviruses, and human noroviruses are considered a significant cause of waterborne gastroenteritis (Mathijs et al., 2012).

To assess drinking water safety, the World Health Organization recommends a risk-based approach encompassing all steps of the drinking water supply system from the catchment to the consumer (WHO, 2011). In this light, Quantitative Microbial Risk Assessment (QMRA) is widely used to analyse and inform the management of the drinking water supply system (Medema and Smeets, 2009; Smeets et al., 2010). Generic QMRA tools have been developed (Pettersson and Stenström, 2007; Schijven et al., 2011) to perform QMRA for specific drinking water treatment plants (DWTPs) with different treatment trains and varying pathogen levels in the source water.

A major limitation of QMRA is constituted by a common lack of proper input data on the pathogen levels in source water. The monitoring of pathogen concentrations in source water is often too infrequent. However, pathogen concentrations in water sources are driven by upstream loading events and are highly variable, with peaks that can occur suddenly and may disappear within only a few hours (Åström et al., 2007; Westrell et al., 2006b). Thus, monitoring processes often fail to detect potentially hazardous peaks in pathogen concentrations. In addition, detection limits of analytical methods can be higher than pathogen concentrations relevant to public health. Therefore, monitoring data could provide misleading input for QMRA. This could negatively impact the safety of the produced drinking water and the health of consumers.

In order to address the limitation regarding the lack of source water quality data, the monitoring data can be supplemented by the results of hydrodynamic modelling (McBride et al., 2012). Hydrodynamic modelling has proven to be useful in order to describe the microbial water quality in water sources and to assess the influence of different conditions and events (e.g. Liu et al., 2006; Sokolova et al., 2013). Thus, a hydrodynamic model can be used to simulate the influence of upstream loading events on the source water quality at the intake of a DWTP.

The aim of this article was to quantify the impact of upstream loading events on the health risks for drinking water consumers. For this purpose, hydrodynamic modelling and QMRA approaches were combined. The norovirus concentrations in the source water during various upstream loading conditions were quantified using hydrodynamic modelling. Based on the modelling results, the required reduction of norovirus concentrations at the DWTP was calculated and compared with the estimated performance of the DWTP. To our knowledge, this article is the first to employ discharge-based QMRA, combined with hydrodynamic modelling, in the context of drinking water management.

2 Methods

2.1 Study area

The Överby DWTP produces drinking water for approximately 49 000 consumers in the municipality of Trollhättan in Sweden. The Överby DWTP draws water from the river Göta älv and employs the following treatment steps: coagulation/flocculation, rapid sand filtration, slow sand filtration and free chlorine disinfection.

Göta älv is a river that drains Lake Vänern into the strait Kattegat. The length of the river between the outflow from Lake Vänern and the mouth of the river is 93 km. The water flow in the river Göta älv is regulated by several hydropower stations. In this study we focus on the 11 km stretch of the river between the Vargön and Trollhättan hydropower stations. In 2011, the water flow in the studied part of the river varied between 138 and 898 m³/s, with an average of 575 m³/s.

The virus contamination at the intake can be caused by sewage discharges into the river from faecal sources located upstream the Överby DWTP: the Holmängen wastewater treatment plant (WWTP) and the sewage pumping stations. The Holmängen WWTP treats sewage from approximately 27 000 persons. The treatment at the Holmängen WWTP consists of the following treatment steps: rotating screens, aerated grit chambers, aeration tank, primary sedimentation tank, trickling filter, denitrification, flocculation tanks and sedimentation tanks. By-passes of untreated sewage are possible, but occur relatively seldom – approximately once a year. Untreated sewage often enters the river during the periods of heavy rain through emergency discharges from the pumping stations.

2.2 Quantification of norovirus concentrations in sewage

A norovirus monitoring campaign was undertaken as a part of the European Union project VISK (visk.nu). During the monitoring campaign, the sewage from the inflow and outflow of the Holmängen WWTP was sampled daily from 15 to 29 February 2012 (n=15). Each of these days, the sewage was collected over 24 hours using an automated sampler, which was flow rate driven. The sewage volume used for analysis was 50 mL. The sewage was analysed for norovirus genogroups I and II (GGI and GGII) using quantitative real-time PCR (qPCR) based on reported methods (Dienus et al., 2015; Kageyama et al., 2003; Nordgren et al., 2009; Shieh et al., 1995) as described in Appendix A. The GGI and GGII were chosen, since they constitute the most important norovirus genogroups in terms of human infection (Matthews et al., 2012). The qPCR analysis determined the concentration of norovirus genome equivalents.

The obtained qPCR data (Table 1) (Dienus et al., 2015) were used to characterise the probability density functions for norovirus GGI and GGII in raw and treated sewage from the Holmängen WWTP. The virus concentrations were assumed to follow a gamma distribution with parameters ρ (shape) and λ (scale):

Equation 1

$$f(c|\lambda, \rho) = \frac{e^{-\frac{c}{\lambda}} \lambda^{-\rho} c^{-1+\rho}}{\Gamma[\rho]}$$

The gamma distribution was then used to calculate the likelihood function for each dataset of reported concentrations (c_1, c_2, \dots, c_{15}):

Equation 2

$$L(\lambda, \rho | c_i) = \prod_{i=1}^{15} f(\lambda, \rho | c_i)$$

The likelihood functions were optimised (in Mathematica 8.0) to obtain maximum likelihood estimators of ρ and λ for each of the four datasets.

Table 1 Summary of reported norovirus GGI and GGII concentrations (genome equivalents L⁻¹) in raw and treated sewage from the Holmängen WWTP during daily sampling in February 2012

	Sewage	n (detects)	Min	Max	Mean
GGI	Raw	15(15)	8.0×10 ⁴	1.7×10 ⁶	6.9×10 ⁵
	Treated	15(15)	1.2×10 ⁵	4.5×10 ⁵	2.8×10 ⁵
GGII	Raw	15(15)	4.2×10 ⁵	4.8×10 ⁶	2.7×10 ⁶
	Treated	15(15)	8.9×10 ⁴	1.7×10 ⁶	1.0×10 ⁶

2.3 Hydrodynamic modelling

To simulate the transport of norovirus within the river Göta älv, a hydrodynamic model was set up. A three-dimensional time-dependent hydrodynamic model was used – MIKE 3 FM (MIKE Powered by DHI software package). This hydrodynamic model is based on the numerical solution of three-dimensional incompressible Reynolds averaged Navier-Stokes equations invoking the assumptions of Boussinesq and of hydrostatic pressure (DHI, 2011). The model consists of continuity and momentum equations and is closed by a turbulent closure scheme (DHI, 2011). The water density was assumed to be homogenous (barotropic formulation).

The modelling domain was approximated with prisms (triangles in horizontal plane) using a flexible mesh approach. The length of the triangles' sides varied from approximately 40 to 90 m. In vertical direction, the river was divided into 10 layers with a thickness that could vary depending on the depth and water surface elevation in the river (sigma-layers).

The initial conditions in the river were defined by the water surface elevation. The upstream and downstream boundary conditions were defined by the water flow through the Vargön hydropower station and the water surface elevation at the Trollhättan hydropower station (data provided by the hydropower company Vattenfall), respectively. On the land boundary, the normal component of velocity was set to zero. The horizontal and vertical eddy viscosities were simulated using Smagorinsky and Log law formulations, respectively (DHI, 2011). The bed resistance was described by a constant roughness height of 0.05 m. The model was run using default parameterisation (DHI, 2011).

The hydrodynamic model was applied to simulate the impact of sewage discharges on the norovirus GGI and GGII concentration at the water intake. Variability exists in both sewage discharges and river flow conditions. To represent the overall range of variability, the selected scenarios regarding sewage

discharges were simulated for conditions of low (138 m³/s), average (575 m³/s) and high (898 m³/s) water flow in the river. The following scenarios were selected:

- Baseline conditions
 - Regular discharge from the Holmängen WWTP – discharge of treated sewage
 - Continuous discharge of average magnitude (0.2 m³/s)
 - Continuous discharge of peak magnitude (0.67 m³/s)
- Loading events
 - By-pass at the Holmängen WWTP – discharge of raw sewage
 - 1 day pulse discharge of average magnitude (0.2 m³/s)
 - 1 day pulse discharge of peak magnitude (0.67 m³/s)
 - 1 hour pulse discharge of average magnitude (0.2 m³/s)
 - 1 hour pulse discharge of peak magnitude (0.67 m³/s)
 - Pumping station overflow – discharge of raw sewage
 - 3 hour pulse discharge of 550 m³
 - 1 hour pulse discharge of 550 m³

Norovirus contamination was simulated as a passive substance, i.e. the decay and settling of norovirus in the water source were not taken into account.

The Holmängen WWTP is located approximately 9.5 km upstream the Överby DWTP. Since the model only covers the part of the river between the hydropower stations, discharges from the Holmängen WWTP were assumed to enter the river on the upper boundary of the model (the Vargön hydropower station), i.e. approximately 6 km upstream the Överby DWTP and approximately 3.5 km closer to the Överby DWTP than in reality.

In the municipality of Vänersborg there is a number of pumping stations from which overflows may occur. Based on the proximity of these pumping stations to the river Göta älv and the data on the total volumes of overflows from each pumping station during year 2011, several pumping stations could be potentially relevant for the water quality in the river Göta älv. For the purpose of this study, one pumping station, located approximately 3 km upstream the Överby DWTP was selected.

Using the modelling results and the quantified concentrations in the sewage, the peak norovirus concentrations in the source water at the intake were calculated for each loading event.

2.4 Quantitative microbial risk assessment

The hydrodynamic model provided a time series of concentration at the water intake in response to each simulated contamination condition. For each peak contamination level, Equation 3 was used to find the required Log₁₀ reduction to achieve the health target. The health target suggested by the World Health Organization for drinking water was used: 1×10⁻⁶ DALY (disability adjusted life years) per person per annum (WHO, 2011), which can be translated to 2.74×10⁻⁹ DALY per person per day. By complying with the daily target, an even stricter requirement than the annual target is fulfilled. Equation 3

$$\text{Health target} = \text{DALY} \times (1 - e^{(-r \times C \times V \times 10^{-LR})})$$

where:

- *DALY* is the DALY weight per infection (7.16×10^{-4}) calculated using data on the population structure of Sweden in 2010 and data on age dependency of health outcomes, duration and severity factors for viral infections (reanalysis of data given by Kemmeren et al. (2006)).
- *r* is the coefficient of the exponential dose-response function. The infectivity of norovirus was described by the exact beta-Poisson model ($\alpha=0.04$, $\beta=0.055$; infectivity parameter values fitted to combined dataset, dose in genome equivalents L^{-1}) (Teunis et al., 2008). The exact beta-Poisson model can be simplified to the exponential model at low doses with $r=\alpha/(\alpha+\beta)=0.42$ (i.e. the expected value of the beta distribution).
- *C* is the estimated norovirus concentration (GGI+GGII) in source water (genome equivalents L^{-1}). It is known from previous studies that only a small fraction of virus particles is infectious (Hewitt et al., 2011). On the other hand, the virus enrichment process and extraction of viral RNA followed by cDNA synthesis result in detection of only a small fraction of virus particles. To avoid underestimation of the health risks, in this study it was thus assumed that all virus particles detected by qPCR were infectious.
- *V* is the exposure volume (L). The mean of the volume of unheated water consumed in Sweden as described by a Log-Normal distribution ($\mu=-0.299$, $\sigma=0.57$) (Westrell et al., 2006a) was 0.74 L.
- *LR* is the treatment performance of the DWTP (Log_{10} reduction).

2.5 Evaluation of the drinking water treatment plant performance

The Överby DWTP consists of three process barriers: (i) coagulation/flocculation, sedimentation and rapid sand filtration (together referred to as conventional treatment), (ii) slow sand filtration and (iii) free chlorine disinfection. The performance of the Överby DWTP was evaluated based on routine monitoring data and reported values in the literature.

As part of the routine monitoring program undertaken at the Överby DWTP (Table 2), samples were collected and analysed for *E. coli* in source water (weekly samples between 29 September 2008 – 2 October 2012; $n=212$), after rapid sand filtration and after slow sand filtration (monthly samples each from 1 December 2008 – 4 September 2012 ($n=44$)). Results were reported in colony forming units (CFU) per 100 mL and included left (<) (all datasets) and right (>) (source water only) censored outcomes.

The removal of *E. coli* across rapid sand filtration and slow sand filtration was quantified by constructing a statistical model appropriate for describing microbial counts following the approach presented by Teunis et al. (1999) and modified for the left and right censoring of the current dataset (see also Appendix B). Variable and constant fraction removal models were compared to obtain the best estimate of the Log_{10} reduction by filter line for each barrier.

The reduction of norovirus by free chlorine disinfection was estimated based on the mean Ct values for the disinfection contact chamber at the Överby DWTP and literature data on the sensitivity of norovirus surrogates to free chlorine disinfection.

Table 2 Summary of reported historical *E. coli* data from the Överby DWTP monitoring program

Summary of reported <i>E. coli</i> data				
Source water (29 September 2008 – 2 October 2012)				
Detects (total n=212)	175			
Right censored (> variable limit, maximum 100 CFU 100 mL ⁻¹)	39			
Mean ^a (CFU 100 mL ⁻¹)	21			
Post rapid sand filtration (1 December 2008 – 4 September 2012)				
	Filter 1	Filter 2	Filter 3	Filter 4
Detects (total n=44)	6	7	8	1
Max (CFU 100 mL ⁻¹)	3	12	5	1
Post slow sand filtration (1 December 2008 – 4 September 2012)				
	Filter 1	Filter 2	Filter 3	Filter 4
Detects (total n=44)	1	1	1	1
Max (CFU 100 mL ⁻¹)	1	3	1	2

^a Arithmetic mean with left censored data equal to the detection limit and right censored data equal to the maximum detection.

3 Results and Discussion

In this study, the pathogen concentrations at the water intake were estimated using the pathogen concentrations characterised at the contamination source and hydrodynamic modelling. Based on the estimated concentrations in source water and the health target, the required levels of water treatment at the DWTP were calculated using QMRA. In this way, we implement discharge-based QMRA, as suggested by McBride et al. (2013), and integrate it with hydrodynamic modelling, as recommended by McBride et al. (2012). The novelty of this work lies in the combination of these two approaches in the context of drinking water management.

3.1 Norovirus concentrations in sewage

The norovirus GGI and GGII concentrations in raw and treated sewage were described by gamma distributions (Figure 1). The expected values of norovirus concentration in raw and treated sewage, and the parameters of the gamma distributions are summarised in Table 3. The mean norovirus reduction at the Holmängen WWTP was 0.39 Log₁₀ for GGI and 0.41 Log₁₀ for GGII.

Thus, despite the treatment train at the Holmängen WWTP, norovirus reduction was poor, and the norovirus concentration in treated sewage was approximately 10⁵ – 10⁶ genome equivalents L⁻¹ (Table 3). Moreover, since the sewage at the Holmängen WWTP is not disinfected, the viruses discharged into the river with treated sewage are likely to be as infectious as in raw sewage.

Quantitative predictions of infectious norovirus particles using qPCR analysis are subjected to a number of uncertainties (Hewitt et al., 2011; Rutjes et al., 2009; Thebault et al., 2013). First of all, only a small fraction of the virus particles is infectious (Hewitt et al., 2011). Furthermore, uncertainties are associated with the efficiency of virus enrichment procedure, the RNA extraction, cDNA synthesis and the qPCR. These uncertainties are outside of the scope of this article and are discussed elsewhere (Dienus et al., 2015).

Since the norovirus concentrations in sewage were quantified using samples collected in February, these concentrations represent a specific period rather than a full year. The incidence of norovirus gastroenteritis in the population is generally the highest during wintertime (Pérez-Sautu et al., 2012; Rohayem, 2009; Verhoef et al., 2008). Consequently, the selected period is associated with high virus loading and represents the time of the highest risks for drinking water consumers. Therefore, looking only at this period may result in overestimation of the annual risk.

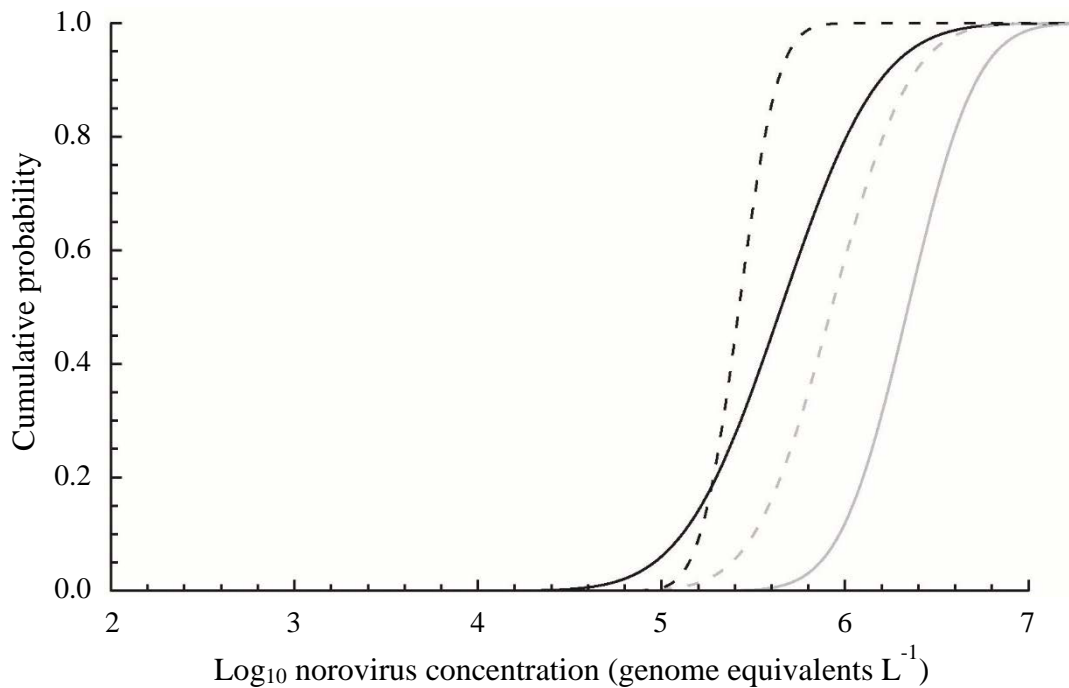


Figure 1 Concentration of norovirus GGI (black colour) and GGII (grey colour) in raw (continuous line) and treated (dotted line) sewage at the Holmängen WWTP. The cumulative density function shows the variability in the sewage concentration.

Table 3 Maximum likelihood gamma distribution parameters fitted to reported norovirus GGI and GGII concentrations (genome equivalents L^{-1}) in raw and treated sewage from the Holmängen WWTP during February 2012

Sewage	GGI			GGII		
	ρ	λ	$E(c)^a$	ρ	λ	$E(c)^a$
Raw	1.3	5.2×10^5	6.8×10^5	2.9	9.3×10^5	2.7×10^6
Treated	7.7	3.7×10^4	2.8×10^5	2.7	3.8×10^5	1.0×10^6

^a Expected value of concentration ($\lambda\rho$)

3.2 Estimated norovirus concentrations in source water and required norovirus reduction by treatment

The estimated peak norovirus concentrations at the water intake under baseline conditions (regular discharge of treated sewage) are illustrated in Figure 2. According to the modelling results, the regular discharge from the Holmängen WWTP is an important source of viruses in the source water (Figure 2, Table 4).

The results for the loading events are summarised in Table 4. The loading events, when the pumping station overflows and by-passes at the WWTP are triggered by rainfall and surface runoff, lead to increased virus concentrations in the source water. Interestingly, the estimated concentrations at the water intake under baseline conditions were similar to the concentrations caused by loading events (Table 4). Of these events, the one-day-long overflow of untreated sewage resulted in the highest concentration at the water intake (Table 4). The transport time in the river for this study area was short. After the occurrence of an event, it took between 2 and 40 hours before the peak concentration arrived at the water intake (Table 4), depending on the type of event and the river flow conditions. It may be possible to avoid drawing water from the river during such events, if these events would be registered in time.

In this study, the hydrodynamic model provided a conservative estimation of the virus concentrations at the water intake, since the decay and settling of viruses in the water source were not taken into account. Norovirus is highly resistant to environmental degradation in water (Bae and Schwab, 2008; Ngazoa et al., 2008; Seitz et al., 2011). For example, Bae and Schwab (2008) reported the nucleic acid decay of the prototype human norovirus to be $0.08 \pm 0.02 \text{ Log}_{10}$ per day in surface water at 25 °C. Therefore, given that (i) the water temperature in the river Göta älv is on average 8 °C and does not exceed 23 °C, and that (ii) in our study, the transport times in the river are on a scale of hours rather than days (Table 4), the overestimation of the norovirus concentrations at the water intake, due to the assumption of no decay, can be neglected. Viruses can adsorb on the surface of particles (Bellou et al., 2015; Wong et al., 2012) that can settle or resuspend (Xagorarakis et al., 2014). The settling of viruses was not accounted for due to (i) the complexity of this process and the involved uncertainty summarised by Hipsey et al. (2008), and, (ii) similarly to decay, the fact that settling is likely to have a minor influence on the results of this study, due to the short transport times in the water source (Table 4).

The level of water treatment required to cope with the negative effects of the tested scenarios on the source water quality (Table 4) was calculated using QMRA. The average required reduction of norovirus at the Överby DWTP varied for different scenarios between 7.6 and 8.8 Log_{10} (Table 4).

The results of the hydrodynamic modelling provided information regarding the relative magnitude and, thus, the importance of upstream loading events, as well as information on the time between the occurrence of an event and the arrival of the peak concentration at the water intake. In this way, the modelling results provided information for managing the operation of the Överby DWTP in terms of the required level of treatment and the response time to upstream incidents. The modelling results provided information about the impact of different contamination sources. This information can be used to apply mitigation measures at the contamination source (in the catchment) to protect the water quality in the drinking water source.

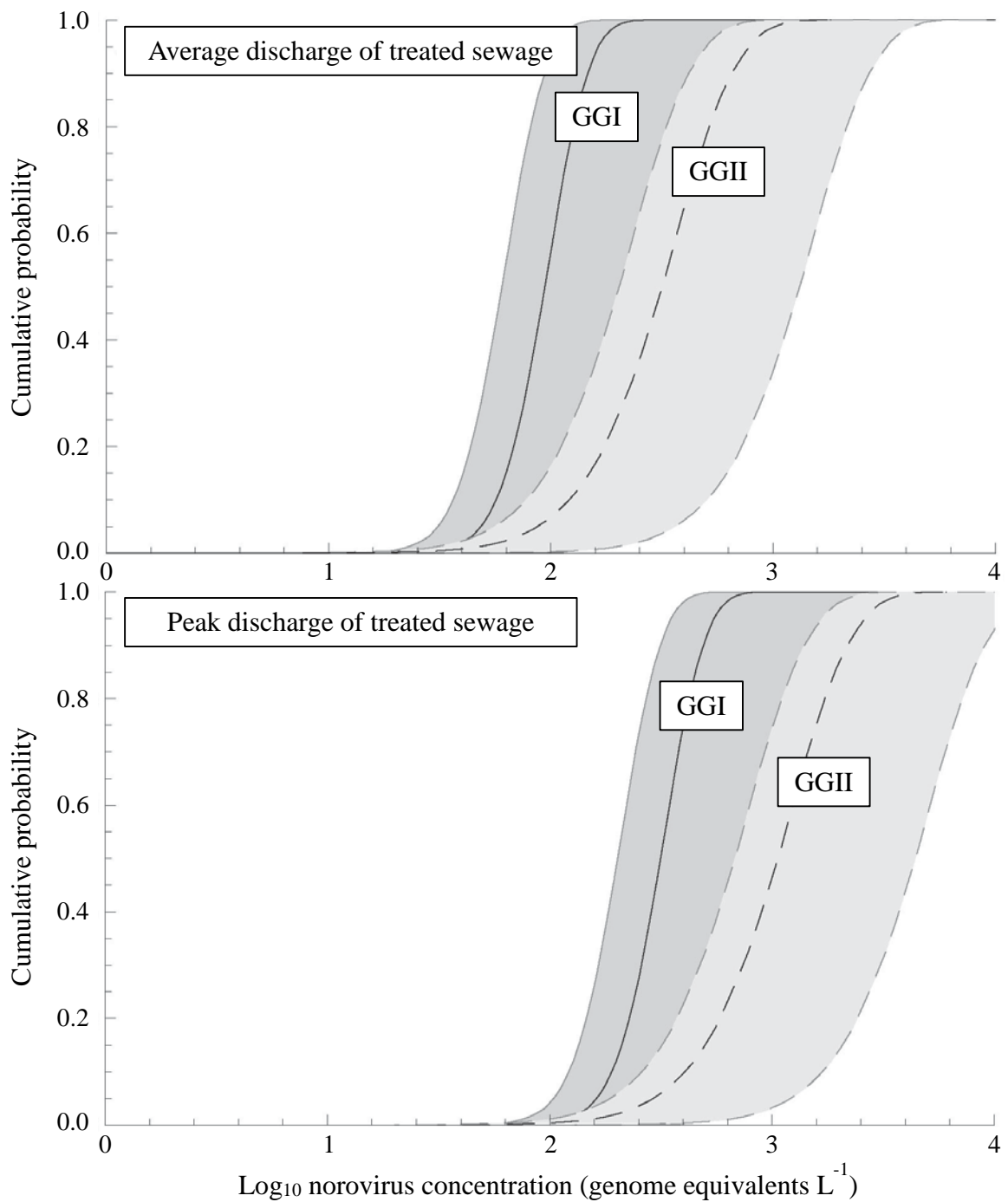


Figure 2 Modelling results: concentration of norovirus GGI and GGII at the intake of the Överby DWTP due to regular discharge (baseline conditions) from the Holmängen WWTP. The cumulative density function shows the variability in the sewage concentration, the shaded range indicates the variability due to the river flow conditions. The left hand boundary is for high flow conditions; the right hand boundary is for low flow conditions.

Table 4 Estimated influence of upstream discharges on the total norovirus (GGI and GGII) concentration at the intake of the Överby DWTP and the required norovirus reduction by treatment

	Discharge magnitude	Peak concentration at intake ^a [genome equivalents·L ⁻¹]	Required reduction ^a [Log ₁₀]	Time to arrival at intake ^b [days, hours, min]	
Baseline	Discharge WWTP treated sewage continuous	Mean 8.7×10 ² (3.3×10 ³)	7.9 (8.4)		
		Peak 2.9×10 ³ (1.1×10 ⁴)	8.4 (9.0)		
Events	By-pass WWTP raw sewage	Mean 2.2×10 ³ (2.9×10 ⁴)	8.3 (9.4)	(0,13,0),	
	1 day pulse	Peak 7.5×10 ³ (8.6×10 ⁴)	8.8 (9.8)	(1,16,10)	
	By-pass WWTP raw sewage	Mean 7.0×10 ² (1.6×10 ³)	7.8 (8.1)	(0,4,20),	
	1 hour pulse	Peak 2.4×10 ³ (5.4×10 ³)	8.3 (8.6)	(0,23,20)	
	Pumping station overflow	3 hour 550 m ³	4.8×10 ² (1.7×10 ³)	7.6 (8.1)	(0,3,10), (0,11,50)
	raw sewage	1 hour 550 m ³	8.3×10 ² (2.1×10 ³)	7.8 (8.2)	(0,2,20), (0,10,45)

^a Mean (Upper 95th percentile). The variability is due to the variability in the sewage concentration, in flow conditions in the river and due to underlying assumptions for QMRA.

^b Time from the event to arrival of the peak concentration at the intake. Range indicates the minimum and maximum travel times under different river flow conditions.

3.3 Expected norovirus reduction at the drinking water treatment plant

3.3.1 Conventional treatment

For conventional treatment with rapid sand filtration, the estimated reduction of *E. coli* varied between filtration lines from 1.8 to 3.2 Log₁₀ (Table 5, Appendix B). When data for all filtration lines were combined, the overall reduction across the conventional barrier was best fit by the model allowing for (beta distributed) variability in removal. The expected value of the beta distribution was 2.0 Log₁₀ reduction. Hijnen and Medema (2010) reviewed six studies regarding the reduction of bacteria by full-scale conventional treatment processes. The average reported reduction varied between studies from 1.3 to 3.4, with a mean elimination capacity (MEC – weighted average defined within the review giving consideration to quality and representativity of the data) of 2.1 Log₁₀. This variability in reported values from the literature is of a similar range to the variability observed between treatment lines at the Överby DWTP. There is no reported correlation between *E. coli* and virus reduction across conventional treatment. However, the comparison with published studies may provide a reference point for how well the Överby DWTP is performing in comparison to other DWTPs.

In the absence of information on virus reduction for the Överby DWTP, and given the consistency between *E. coli* reduction at the Överby DWTP and the literature data on bacteria reduction, the reported range of virus reduction from the literature was considered applicable for the Överby DWTP. Hijnen and Medema (2010) reviewed seven studies concerning virus reduction across conventional treatment. The authors reported that the reduction of virus was on average 1 Log₁₀ higher than of bacteria and varied from 1.2 to 5.3 Log₁₀ with a MEC of 3.0. Thus, norovirus reduction at the Överby DWTP was assumed to be on average 3.0 Log₁₀ and vary between 1.2 and 5.3 Log₁₀.

Table 5 Estimated Log₁₀ reduction of *E. coli* by filtration line and for combined effluent for rapid sand filtration and slow sand filtration treatment barriers at the Överby DWTP

Rapid sand filtration	Filtration line				Combined effluent ^a
	1	2	3	4	
Log ₁₀ reduction	2.2	1.8	1.9	3.2	2.0

Slow sand filtration	Filtration line				Combined effluent
	1	2	3	4	
Log ₁₀ reduction	1.1	0.6	1.1	0.8	0.9

^a Expected value of the beta distribution ($-Log_{10} \left(\frac{\alpha}{\alpha+\beta} \right)$) describing variable reduction efficacy (see Appendix B)

3.3.2 Slow sand filtration

For slow sand filtration, the reduction of *E. coli* varied between filtration lines from 0.6 to 1.1 Log₁₀ (Table 5, Appendix B). The ability to demonstrate the reduction was limited by a low number of detects before slow sand filtration, and hence only a low reduction could be observed.

Hijnen and Medema (2010) reviewed nine studies related to the reduction of viruses by slow sand filtration, but only one study was conducted at full scale, and reported rates were low and variable (0.6 – 4.0 Log₁₀ with MEC=2.2). Another study analysed data from full scale seeding experiments using MS2 bacteriophages, and reported that median Log₁₀ reduction varied between experiments from 0.5 to 3.8 (Teunis et al., 2009).

In the absence of information on virus reduction by the slow sand filters at the Överby DWTP, the reported range from the literature (Hijnen and Medema, 2010) was considered applicable to describe norovirus reduction at the Överby DWTP. Thus, norovirus reduction at the Överby DWTP was assumed to be on average 2.2 Log₁₀ and vary between 0.6 and 4.0 Log₁₀.

3.3.3 Free chlorine disinfection

The chlorine disinfection contact chamber at the Överby DWTP has a mean hydraulic residence time of 86.4 minutes. The average of daily free chlorine measurements (n=386) within the chamber was 0.1 mg L⁻¹. The mean Ct across the contact chamber is therefore on average 8.64 mg L⁻¹ min⁻¹. Since it is so far not possible to cultivate human norovirus under laboratory conditions, surrogates, such as feline calicivirus and murine norovirus, have to be used (Bae and Schwab, 2008). Studies investigating the sensitivity of norovirus surrogates to free chlorine disinfection have reported that a Ct of 0.32 and 0.31 mg L⁻¹ min⁻¹ was sufficient for 4 Log₁₀ inactivation of feline calicivirus (Thurston-Enriquez et al., 2003) and murine norovirus (Cromeans et al., 2010; Kahler et al., 2010; Lim et al., 2010),

respectively. Assuming linear inactivation, this susceptibility would imply a very low probability of norovirus survival across the contactor at the Överby DWTP.

However, short circuiting of the contactor can lead to small portions of flow receiving very low dosage of chlorine, which may translate to thousands of litres of water with low virus inactivation distributed to the consumers. Therefore, achieving high reduction and safety for the consumers via the chlorine disinfection step is dependent upon good hydraulic behaviour of the contactor (Petterson and Stenström, 2015).

3.3.4 Overall norovirus reduction

The estimated overall mean reduction implies that the Överby DWTP complies with the target of 10^{-6} DALY per person per annum under all modelled loading conditions. However, variability in treatment performance is a critical factor to be considered. The overall reduction is heavily dependent upon effective chlorine disinfection, with the potential for very poor reduction by conventional treatment and slow sand filtration. Were this poor reduction by conventional treatment and/or slow sand filtration to coincide with shielding and possible short circuiting of the disinfection contactor, the treatment performance would be considerably reduced. Moreover, the treatment processes are interdependent, i.e. poor coagulation is likely to cause filter breakthrough and decreased reduction by chlorination. Understanding more of site-specific treatment performance, dependent on such factors as e.g. temperature, schmutzdecke age, sand grain size, characteristics of the disinfection contactor, would be beneficial. The estimation of the performance of different treatment barriers indicated which improvements within the DWTP can be made.

4 Conclusions

The outcomes of this study have important implications for understanding and managing the norovirus risks associated with the Överby DWTP:

- The regular discharge of treated sewage from the Holmängen WWTP is an important source of norovirus loading at the water intake. Measures to reduce the norovirus concentration in the treated sewage could provide an effective reduction in norovirus loading to the river under baseline conditions.
- The hydrodynamic modelling results provided information regarding the reaction time available between an upstream event and the norovirus load reaching the water intake. Notification strategies between the WWTP and the DWTP would allow for (i) selective abstraction to avoid the most contaminated source water, and/or (ii) proactive monitoring of coagulant dosage to ensure an optimal plant operation.
- Given what is known about norovirus loading in the river Göta älv and the expected performance of the treatment, the Överby DWTP is able to provide sufficient removal of viruses to meet the target of 10^{-6} DALY per person per annum.
- Meeting safe drinking water targets at the Överby DWTP is strongly dependent upon effective free chlorine disinfection. Limited information was available regarding the hydraulic behaviour of the disinfection contactor. Hence, the hydraulic behaviour of the contactor needs to be characterised to ensure a suitable minimum residence time.

It can be concluded that hydrodynamic models of water sources combined with QMRA provide valuable decision support for risk management, because they can be used:

- to simulate the effects of short term events that may be important for driving downstream risks, but cannot be captured by monitoring water quality at the water intake;
- to provide information on the relative importance of different sources and loading events for surface waters that are influenced by several upstream sources.

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Appendix A: Methodology for norovirus analysis

The description of the methodology for norovirus (NoV) analysis in sewage is based on Dienus et al. (2015).

Enrichment from sewage

Sewage samples were processed the same day as they were sampled. 50 mL of each sample was spiked with 5 µL murine norovirus 1 (MNV-1) solution and centrifuged at $4\ 300 \times g$ for 30 min to remove debris. 40 mL of the supernatant and 10 mL of sterile PEG solution (100 g PEG-8 000, 6 g NaCl in 250 mL water at pH 7.2) were mixed and slowly rotated overnight at 4 °C (Shieh et al., 1995). The mixture was then centrifuged at $10\ 000 \times g$ for 90 min at 4 °C, and the supernatant was removed. The pellet was dissolved in RLT buffer in a total volume of 300 µL. For process control, 50 mL tap water was handled in the same way as the samples.

Extraction of RNA and reverse transcription

Total RNA extraction was done in a Biorobot EZ1 (Qiagen) with the EZ1 Virus mini kit v.2.0 (Qiagen), according to the manufacturer's protocol. The sample volume was 300 µL, and the purified RNA was eluted in 60 µL. Potentially inhibiting components were eliminated by the OneStep™ PCR Inhibitor Removal Kit (Zymo Research, Irvine, CA). Purified total RNA was stored at -80 °C until further processing.

First strand cDNA synthesis was done in a total volume of 40 µL, containing 20 µL purified RNA, 8 µL RNase free water, 8 µL 5 × Reaction mix, and 4 µL Maxima™ First Strand cDNA Synthesis RT-qPCR enzyme mix (Thermo Scientific, Stockholm, Sweden) with random N6 primer. Synthesis of cDNA was done at 25 °C for 10 min, followed by 50 °C for 15 min. The reaction was stopped at 85 °C for 5 min, and then the synthesis was cooled to 4 °C. Until further processing, cDNA was stored at -20 °C. RNase free water was used as a negative control. Reverse transcription step inhibition control was tested with Alien Reference RNA VIC QRT-PCR Detection Kit (Agilent, La Jolla, CA), according to the manufacturer's instructions.

Detection and quantification by TaqMan real-time PCR

Quantitative real-time PCR was done as a duplex PCR in an ABI 7500 FAST PCR system (Applied Biosystems). Each sample was tested in duplicate for NoV GGI and GGII. The following thermal cycling was used: 95 °C for 10 min, followed by 50 cycles of 95 °C for 15 s and 56 °C for 60 s (data acquisition FAM/JOE). For the quantification of NoV, 2 µL of cDNA was used as a template in a total reaction volume of 25 µL, containing 200 nM of each primer (NV-G1-fwd1b, NV-G1-rev, NV-G2-fwd and COG2R), and 200 nM of each TaqMan probe (Kageyama et al., 2003; Nordgren et al., 2008). 0.2 µL of ROX (50 µM) and 12.5 µL Maxima™ Probe qPCR Master Mix (2X) (Thermo Scientific) were used. A pUC57 plasmid with two inserts (Genscript, Piscataway, NJ), which include the regions of the ORF1-ORF2 junction for NoV GGI and GGII, was used as a standard for quantification. The insert for NoV GGI was like Genebank NCBI JX023285.1 position 5286 to 5383, and for NoV GGII it was like AF414417.1 position 485 to 592. Quantification standards were amplified in duplicate with tenfold dilution series of the plasmid ranging from 10⁶ to 10 copies per reaction. The limit of quantification was 15 000 genome equivalents L⁻¹, and the limit of detection was extrapolated to 4 500 genome equivalents L⁻¹.

For the amplification of MNV-1 (used as process control of the virus enrichment and RNA purification) and of the tick borne encephalitis virus (TBEV) (used as process control of the PCR performance), the following primers and probes were used: MNV fwd, MNV rev, F-TBEV 1 SC, R-TBEV 1 SC, MNV-probe, TBEV-probe SC (Persson, 2013; Schwaiger and Cassinotti, 2003). The reaction volume was 25 µL, containing 300 nM of MNV primer, 100 nM of MNV probe, 200 nM of TBEV primer and probe, 2 µL of a 1:100 dilution of TBEV cDNA synthesis, 0.2 µL ROX (50 µM), and 12.5 µL Maxima™ Probe qPCR Master Mix (2X) (Thermo Scientific). The thermal cycling conditions were as follows: 95 °C for 10 min, followed by 45 cycles of 95 °C for 15 s and 60 °C for 60 s (data acquisition FAM/JOE). The individual filtration performance on a per sample basis was estimated from the quantification cycle value (C_p) value of MNV-1 in the sample.

Appendix B: Quantifying *E. coli* reduction at the drinking water treatment plant

Statistical models

Statistical models presented by Teunis et al. (1999) for describing microbial fractions from count data have been modified and applied to describe the counts of *E. coli* before and after the treatment barriers at the Överby drinking water treatment plant (DWTP).

E. coli concentration before treatment

The entire sample of *E. coli* measurements (n=212) in the source water was used to describe the source water concentration. Concentration before treatment was assumed to follow a gamma distribution, therefore, counts (*n* colonies in volume *v*) of *E. coli* before treatment were assumed to follow a negative binomial distribution:

Equation B.1

$$f(n|\lambda, \rho, v) = \frac{\lambda^{-\rho} v^n \left(\frac{1}{\lambda} + v\right)^{-n-\rho} \Gamma[n + \rho]}{n! \Gamma[\rho]}$$

where $\Gamma[x]$ is the Euler gamma function.

For a given dataset of m counts (n_i) in a constant sample volume v , the likelihood function (the function describing the distribution of parameter values given the data) is given by:

Equation B.2

$$L(\lambda, \rho, |n_i, v) = \prod_{i=1}^m f(\lambda, \rho | n_i, v)$$

The values of λ and ρ that maximise the likelihood function are termed the maximum likelihood estimators (MLE) of the parameters.

A minor modification to the models presented by Teunis et al. (1999) was applied to account for left and right censored data. For left censored data (<1), the count was assumed to be zero and hence the contribution to the likelihood was given by:

Equation B.3

$$f(\lambda, \rho | 0, 0.1)$$

For right censored data ($>x$), the contribution to the likelihood was given by:

Equation B.4

$$(1 - \sum_{i=0}^x f(\lambda, \rho | i, 0.1))$$

E. coli concentration after treatment

The concentration of *E. coli* after treatment was assumed to be given by πc where c is the concentration before treatment, and π is the probability that an organism is able to pass the treatment barrier. The counts (k colonies in volume v) of *E. coli* following treatment were therefore assumed to follow a negative binomial distribution:

Equation B.5

$$h(k | \lambda, \rho, v, \pi) = \frac{\lambda^{-\rho} (\pi v)^k \left(\frac{1}{\lambda} + \pi v\right)^{-k-\rho} \Gamma[k + \rho]}{k! \Gamma[\rho]}$$

Constant removal

If the performance of the treatment barrier is assumed to be constant, then the likelihood function for the input (m counts before) and output (l counts after) samples is given by:

Equation B.6

$$L(\lambda, \rho, \pi | n_i, k_j) = \prod_{i=1}^m \{f(\lambda, \rho | n_i, 0.1)\}^{\delta_i} \cdot \left\{ (1 - \sum_{\gamma=1}^{n_i} f(\lambda, \rho | \gamma, 0.1)) \right\}^{1-\delta_i} \cdot \prod_{j=1}^l [h(\lambda, \rho, \pi | k_j, 0.1)]$$

where $\delta=1$ for exact counts, and 0 for right censored counts. For left censored data, the count is zero and hence the likelihood is given by $f(\lambda, \rho | 0, 0.1)$.

Variable removal

If the removal is assumed to vary according to a Beta distribution $\pi \sim \text{Beta}(\alpha, \beta)$, then the counts (k) of *E. coli* after treatment are assumed to follow the distribution:

Equation B.7

$$g(k | \lambda, \rho, \alpha, \beta, v) = \int_{\pi=0}^1 \frac{\lambda^{-\rho} (\pi v)^k \left(\frac{1}{\lambda} + \pi v\right)^{-k-\rho} \Gamma[k + \rho]}{k! \Gamma[\rho]} \cdot \frac{\Gamma(\alpha + \beta)}{\Gamma(\alpha)\Gamma(\beta)} \pi^{\alpha-1} (1 - \pi)^{\beta-1} d\pi$$

Equation B.8

$$g(k|\lambda, \rho, \alpha, \beta, v) = \frac{\lambda^k v^k \Gamma[\beta] \Gamma[\alpha + k] \Gamma[k + \rho] \text{H2F1}[\alpha + k, k + \rho, \alpha + \beta + k, -\lambda v]}{\text{Beta}[\alpha, \beta] k! \text{Gamma}[\rho]}$$

where H2F1 is a Hypergeometric Function.

The likelihood function for the input (m counts) and output (l counts) samples is given by:

Equation B.9

$$L(\lambda, \rho, \alpha, \beta | n, k) = \prod_{i=1}^m \{f(\lambda, \mu | n_i, 0.1)\}^{\delta_i} \cdot \left(1 - \sum_{j=1}^{n_i} f(\lambda, \rho | j, 0.1)\right)^{1-\delta_i} \cdot \prod_{i=1}^l [g(\lambda, \rho, \alpha, \beta | k, 0.1)]$$

where $\delta=1$ for exact counts, and 0 for right censored counts. For left censored data, the count is assumed to be zero and hence the likelihood is given by $f(\lambda, \rho | 0, 0.1)$.

The likelihood functions were constructed in Mathematica 8.0 and optimised to obtain MLE of all parameters.

Model fitting and results

Historical monitoring *E. coli* data were available for the source water; following each rapid sand filter; and following each slow sand filter. The above statistical models were fitted to the *E. coli* data to characterise rapid sand filtration and slow sand filtration. The datasets used to characterise the *E. coli* concentration before and after these treatment barriers are summarised in Table B.1.

Rapid sand filtration

MLE for parameters of the constant and the variable removal models fitted to datasets before and after rapid sand filtration are summarised in Table B.2. The estimated removal varied between 1.78 and 3.15 Log_{10} . The variable model could not be fitted across Filter 4. The reduction in the deviance by applying the variable model only produced a significant improvement in fit for Filter 2 ($14.58 > \chi^2_{0.95,1}=3.84$), Filters 1 and 3 were adequately fit by the constant removal model. When all outflows were combined, the variable removal model produced a significant improvement in fit ($22.72 > \chi^2_{0.95,1}=3.84$). The maximum likelihood beta distribution describing removal across all filters is illustrated (with 95% credible interval) in Figure B.1.

Slow sand filtration

MLE for the model parameters for the constant removal model are summarised in Table B.2. In addition, due to the small number of detects in the finished water, it was not meaningful to fit the variable removal model to the dataset. The Log_{10} reduction varied between filters from 0.64 to 1.11. Only a low level of removal could be demonstrated by the data since the inflow concentration was low.

Table B.1 *E. coli* data before and after treatment barriers at the Överby DWTP for quantifying reduction

Process characterised	Data before	Data after
Rapid sand filtration	Counts in source water (n=212)	Counts after each filter (4×n=44) Combined outflow (n=176)
Slow sand filtration	Combined counts in rapid sand filtration effluent (n=176)	Counts after each filter (4×n=44) Combined outflow (n=176)

Table B.2 Maximum likelihood estimators of all parameters and deviance for constant and variable removal models fitted to the datasets from the outflow of each individual filter, and the combined outflow

	Filter 1	Filter 2	Filter 3	Filter 4	Combined outflow: all filters
Rapid sand filtration					
Constant Removal					
λ	872	963	888	860	1055
ρ	0.37	0.34	0.36	0.37	0.32
$\text{Log}_{10}(\pi)$	-2.15	-1.78	-1.90	-3.15	-2.06
Deviance	1392.59	1413.10	1412.28	1352.92	1559.15
Variable removal					
λ	861	859	861	-	860
ρ	0.37	0.37	0.37	-	0.37
α	0.89	0.086	0.62	-	0.19
β	122.20	4.28	44.95	-	19.29
Deviance	1392.07	1398.52	1411.11	-	1536.43
Slow sand filtration					
Constant Removal					
λ	40.15	45.03	40.15	42.75	46.67
ρ	0.074	0.066	0.074	0.069	0.063
$\text{Log}_{10}(\pi)$	-1.10	-0.64	-1.11	-0.81	-0.88
Deviance	202.96	208.83	202.96	206.51	243.03

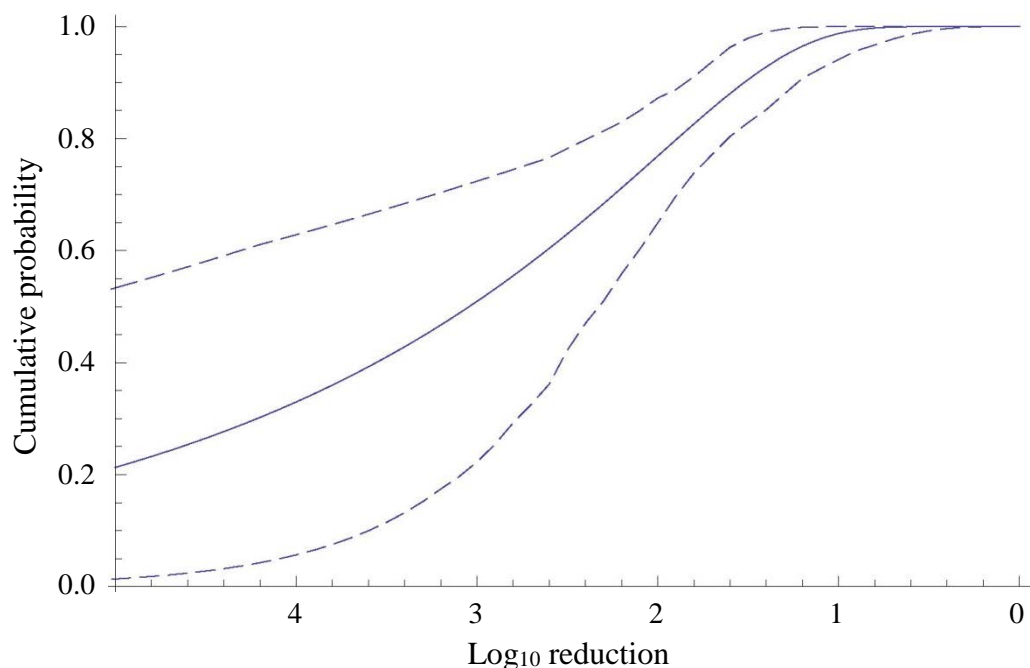


Figure B.1 Beta distribution cumulative density function for removal of *E. coli* by rapid sand filtration at the Överby DWTP (combined result for all four filters). Maximum likelihood (solid line) and 95 % credible interval (dashed lines).

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