Virus elimination in activated sludge systems: from batch tests to mathematical modeling

Emma Haun, Katharina Ulbricht, Regina Nogueira and Karl-Heinz Rosenwinkel

ABSTRACT

A virus tool based on Activated Sludge Model No. 3 for modeling virus elimination in activated sludge systems was developed and calibrated with the results from laboratory-scale batch tests and from measurements in a municipal wastewater treatment plant (WWTP). The somatic coliphages were used as an indicator for human pathogenic enteric viruses. The extended model was used to simulate the virus concentration in batch tests and in a municipal full-scale WWTP under steady-state and dynamic conditions. The experimental and modeling results suggest that both adsorption and inactivation processes, modeled as reversible first-order reactions, contribute to virus elimination in activated sludge systems. The model should be a useful tool to estimate the number of viruses entering water bodies from the discharge of treated effluents.

Key words | activated sludge, adsorption, inactivation, mathematical modeling, virus removal

Emma Haun (corresponding author)
Katharina Ulbricht
Regina Nogueira
Karl-Heinz Rosenwinkel
Leibniz University of Hanover,
Institute for Sanitary Engineering and Waste Management (ISAH),
Welfengarten 1,
D-30167 Hanover,
Germany
E-mail: *haun@isah.uni-hannover.de*

INTRODUCTION

Apart from organic pollution, nutrients and trace elements, wastewater also contains pathogens such as bacteria and viruses. The activated sludge process is known to reduce the load of pathogens in the wastewater (Fleischer *et al.* 2000; Ottoson *et al.* 2006; Hewitt *et al.* 2011). Still, relatively high concentrations in the effluent of the biological treatment might pose a risk to public health (Carducci *et al.* 2009; Hata *et al.* 2013). The viruses adsorb on activated sludge flocs (Funderburg & Sorber 1985; Tanji *et al.* 2002; Nakajima *et al.* 2003) and are inactivated by the biocenosis in the biological treatment via active predation, protist grazing and virucidal bacterial extracellular compounds (Kim & Unno 1996; Deng *et al.* 2013). However, since some of the virus particles cannot be digested by microorganisms of the activated sludge and get excreted, active viruses might apparently be reactivated (Deng *et al.* 2013).

Modeling of virus transport and attenuation in soils is well documented. These models describe the interaction between viruses and inorganic particles as a first-order reversible adsorption process (Schijven & Hassanizadeh 2000). Using similar kinetics, Kim *et al.* (1995) has modeled virus elimination in activated sludge. Considering the increasing demand on water quality, mathematic models can be useful tools to estimate the number of viruses entering surface water bodies from the discharge of treated effluents.

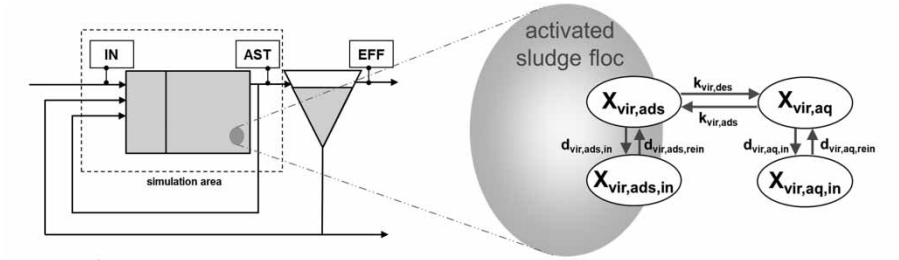
The aim of this paper is to upgrade the conventional Activated Sludge Model No. 3 (ASM3) with a virus tool that examines the processes influencing virus elimination in the activated sludge. The extended model was calibrated with laboratory-scale batch tests and full-scale measurement campaigns.

MATERIALS AND METHODS

Mathematical modeling

A mathematical model for virus elimination in activated sludge systems – virus tool – was developed and integrated in the ASM3 (Gujer *et al.* 1999). Viruses were modeled as particles due to their negligible chemical oxygen demand (COD). Four fractions were considered (Figure 1). The fraction of free viruses in the aqueous phase ($X_{vir,aq}$) can adsorb on the surface of the flocs and generate the fraction of adsorbed viruses ($X_{vir,ads}$). The adsorption was modeled as a reversible first-order process, $k_{vir,ads}$ being the adsorption rate constant and $k_{vir,des}$ the desorption

doi: 10.2166/wst.2014.349



WWTP sampling points
IN-influent, **AST**-activated sludge tank, **EFF**-effluent

Processes	$X_{vir,aq}$	$X_{vir,ads}$	$X_{vir,aq,in}$	$X_{vir,ads,in}$	Process rate [PFU/(l·d)]
Adsorption of free viruses	- 1	1	0	0	$k_{vir,ads} \cdot X_{vir,aq}$
Desorption of adsorbed viruses	1	- 1	0	0	$k_{vir,des} \cdot X_{vir,ads}$
Inactivation of free viruses	- 1	0	1	0	$d_{vir,aq,in} \cdot X_{vir,aq}$
Reactivation of free viruses	1	0	- 1	0	$d_{vir,aq,rein} \cdot X_{vir,aq,in}$
Inactivation of adsorbed viruses	0	-1	0	1	$d_{vir,ads,in} \cdot X_{vir,ads}$
Reactivation of adsorbed viruses	0	1	0	- 1	$d_{vir,ads,rein} \cdot X_{vir,ads,in}$

Figure 1 | Proposed model for virus elimination by reversible adsorption and inactivation processes and stoichiometric matrix. (WWTP sampling points: IN – influent; AST – activated sludge plant; EFF – effluent.)

rate constant. Adsorbed and free viruses can both be inactivated by biological processes. The $X_{\mathrm{vir,aq}}$ and $X_{\mathrm{vir,ads}}$ upon inactivation generate $X_{\mathrm{vir,aq,in}}$, the fraction of inactivated viruses in the aqueous phase, and $X_{\mathrm{vir,ads,in}}$, the fraction of inactivated viruses adsorbed on the flocs, respectively. The inactivation was also modeled as a reversible first-order process, $d_{\mathrm{vir,aq,in}}$ being the inactivation rate constant of free viruses and $d_{\mathrm{vir,ads,in}}$ the inactivation rate constant of adsorbed viruses. The reactivation rate constants for free and adsorbed viruses, $d_{\mathrm{vir,aq,rein}}$ and $d_{\mathrm{vir,ads,rein}}$ respectively, account for the reversibility of the inactivation process. The growth of coliphages, used as indicator virus, was disregarded in the model because our previous results indicated a negligible contribution of this process due to the low concentration of *Escherichia coli* (host for the coliphages) in the activated sludge and the unfavorable environmental conditions (Ulbricht *et al.* 2014). Simulations were performed using SIMBA 5.2®. The model was calibrated with the results from the laboratory-scale batch tests with activated sludge and from measurements in a municipal wastewater treatment plant (WWTP).

Model evaluation statistics

Several error indices were used in model evaluation. These include measured (*Mm*) and simulated (*Ms*) mean values and their standard deviation (σ), the mean error ($ME = (Ms–Mm)/Ms$), the mean absolute error (*MAE*), the root mean square error (*RMSE*) and the Nash–Sutcliffe coefficient (*E*). *RMSE* and *MAE* values of 0 indicate a perfect fit. A fit is considered good when $ME < 10\%$, $MAE \leq RMSE < 0.5\ \sigma$ and *E* is close to 1 (Singh *et al.* 2005).

Indicator virus

The somatic coliphages (viruses that infect bacteria) were used as an indicator for human pathogenic enteric viruses due to the significant positive correlation between both parameters in the wastewater treatment process (Carducci *et al.* 2009; Selinka *et al.* 2011). They were determined according to ISO10705-2:2000 2001 as plaque-forming units (PFU).

Activated sludge system

The municipal WWTP under study (approximately 500,000 population equivalent) has a biological treatment for phosphorus and nitrogen removal. The sludge load is about 0.16 kgCOD/(kg mixed liquor suspended solids [MLSS] d) in winter and 0.25 kgCOD/(kgMLSS d) in summer; the sludge retention time (SRT) is about 13 d in winter and 11 d in summer. For additional inflow and operational data see Ulbricht *et al.* (2014).

Laboratory-scale batch tests

Batch tests were executed to estimate the model's kinetic parameters (Figure 1). The reactors (2 l) were filled with activated sludge from the nitrification tank of the

WWTP, the pH was adjusted to 7.0 and the contents were aerated and mixed. The concentration of viruses in the activated sludge was determined both in the mixed liquor and the aqueous phase. The aqueous phase was separated by centrifugation of the samples at 4,000 *g* for 10 min at 4 °C (Ulbricht *et al.* 2014). The viruses in the aqueous phase represent the free non-adsorbed fraction ($X_{vir,aq}$) and the viruses adsorbed to activated sludge ($X_{vir,ads}$) were calculated as the difference between the viruses in the mixed liquor and the viruses in the aqueous phase.

Short-term tests

In the short-term tests at 20 °C, the concentration of biomass varied between 0 (control) and 6.7 gMLSS/l. The reactors were spiked with the somatic coliphage PhiX174 to obtain an initial $X_{vir,aq}$ around 10^7 PFU/l. Samples for the determination of $X_{vir,aq}$ were taken regularly during 2–4 h. To investigate the influence of easily degradable organic carbon on the elimination of viruses, a glucose solution was added to the reactors to obtain an initial food-to-microorganism ratio of 0.16 gCOD/gMLSS and the tests were done as previously described.

Long-term tests

In the long-term tests, the elimination of viruses in the activated sludge was studied over 60 d at 12 and 20 °C without initial spiking of coliphages. The initial biomass concentration was 3.7 gMLSS/l. To keep the biocenosis active, synthetic wastewater was dosed daily to obtain a food-to-microorganism ratio of 0.16 gCOD/(gMLSS d). Samples for the determination of $X_{vir,aq}$ and $X_{vir,ads}$ were taken daily.

Full-scale measurement campaigns

Full-scale measurement campaigns were done in summer and winter; for description see Ulbricht *et al.* (2014). The results obtained in summer under dry weather conditions (13 days between August 2011 and September 2011, average water temperature of 20 °C) were used to calculate the mass balance for viruses under dry weather conditions. In winter, a diurnal cycle of the concentration of viruses was recorded on 24 February 2011 (average water temperature of 12 °C). The sampling points are depicted in Figure 1.

Estimation of kinetic parameters from batch experiments

The elimination processes of viruses in activated sludge were described by a first-order reaction (Equation (1)). The adsorption (k) and inactivation (d) rate constants were determined by linear regression analysis.

$$\frac{dX_{vir}}{dt} = -(k \text{ or } d) \cdot X_{vir} \rightarrow \ln \frac{X_{vir}(t)}{X_{vir}(t_0)} = -(k \text{ or } d) \cdot t \quad (1)$$

Based on the concentration profiles obtained from the short-term tests ($X_{vir,aq}$, t), the adsorption rate constant ($k_{vir,ads}$) was estimated from the first part of the curve (0 to 1 h) corresponding to a sharp decrease of $X_{vir,aq}$. Afterwards, $X_{vir,aq}$ continues to decrease though very slowly, and from this second part of the curve (1 to 4 h) the inactivation rate constant of free viruses ($d_{vir,aq,in}$) was estimated.

Based on the concentration profiles obtained from the long-term tests for free ($X_{vir,aq}$, t) and adsorbed ($X_{vir,ads}$, t) viruses, the inactivation rate constants for free ($d_{vir,aq,in}$) and adsorbed ($d_{vir,ads,in}$) viruses were estimated from the first part of the curves (0–11 d at 20 °C and 0–20 d at 12 °C) corresponding to a decrease of $X_{vir,aq}$ and $X_{vir,ads}$, respectively. Afterwards, both curves attain a plateau (inactivation rate = reactivation rate) from which the reactivation rate constants for free and adsorbed viruses, $d_{vir,aq,rein}$ and $d_{vir,ads,rein}$, respectively, were estimated according to Equation (2) (Schijven & Hassanizadeh 2000)

$$d_{vir,aq,rein} = \frac{d_{vir,aq,in}}{\left(\frac{X_{vir,aq}(t_0)}{X_{vir,aq}(t_{eq})} - 1\right)} \quad \text{and}$$
$$d_{vir,ads,rein} = \frac{d_{vir,ads,in}}{\left(\frac{X_{vir,ads}(t_0)}{X_{vir,ads}(t_{eq})} - 1\right)} \quad (2)$$

RESULTS AND DISCUSSION

Laboratory-scale experiments results

The short-term tests without biomass (control) and with a biomass concentration up to 1.5 gMLSS/l showed a similar low reduction in the concentration of viruses. In contrast, in the tests with a biomass concentration higher than 1.9 gMLSS/l the viruses in the aqueous phase were reduced by two orders of magnitude (Figure 2 right).

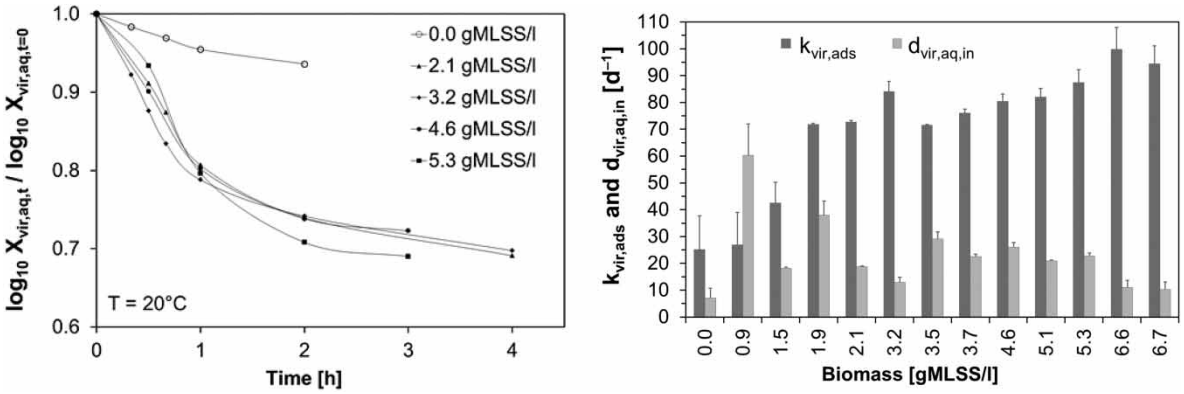


Figure 2 | Concentration profiles of coliphages in the aqueous phase (left) and adsorption- ($k_{vir,ads}$) and inactivation-rate constants ($d_{vir,aq,in}$) of coliphages in short-term tests (right).

The concentration profiles of viruses have two phases: an initial sharp decrease probably due to adsorption of the viruses on the flocs is followed by a slow decrease which might be attributed to inactivation by biological processes in the activated sludge (Figure 2 left). This behavior is consistent with results from previous studies (Usrael 1980; Kim *et al.* 1995). An average $k_{vir,ads}$ of 78.2 d^{-1} and an average $d_{vir,aq,in}$ of 18.2 d^{-1}, both with a standard deviation less than 5 d^{-1}, were determined by linear regression analysis, for a biomass concentration between 1.9 and 5.3 gMLSS/l (Figure 2 right). Contrary to earlier findings (Usrael 1980; Kim *et al.* 1995), we found no apparent influence of the biomass concentration in the range usually present in WWTPs in the adsorption/inactivation rate constants. The addition of glucose as a source of easily degradable organic carbon to the short-term tests had no effect on the adsorption and inactivation of viruses for the investigated food-to-microorganism ratio of 0.16 gCOD/gMLSS.

In the long-term tests, both free and adsorbed viruses were reduced by one order of magnitude at 12 °C and up to three orders of magnitude at 20 °C (Figure 3 middle and right). The decrease of the virus concentration happened in two phases at both temperatures: an initial decrease probably due to inactivation by biological processes is followed by a plateau with a nearly constant concentration (inactivation = reactivation). At 20 °C, the first phase lasted 25 d and the inactivation rate constants of free ($d_{vir,aq,in}$) and adsorbed ($d_{vir,ads,in}$) viruses were 0.25 and 0.20 d^{-1}, respectively. At 12 °C, the first phase lasted only 11 d, $d_{vir,aq,in}$ and $d_{vir,ads,in}$ being 0.24 and 0.22 d^{-1}, respectively. In the second stage, at 20 °C, the reactivation rate constants (Equation (2)) of free ($d_{vir,aq,rein}$) and adsorbed ($d_{vir,ads,rein}$) viruses were 0.0006 and 0.0008 d^{-1}, respectively. At 12 °C, $d_{vir,aq,rein}$ and $d_{vir,ads,rein}$ were 0.009 d^{-1} for free viruses and 0.008 d^{-1} for adsorbed viruses. According to these results, the inactivation rate constants are not significantly influenced by temperature. In contrast, the reactivation rate constants at 12 °C were circa 10 times higher than those at 20 °C.

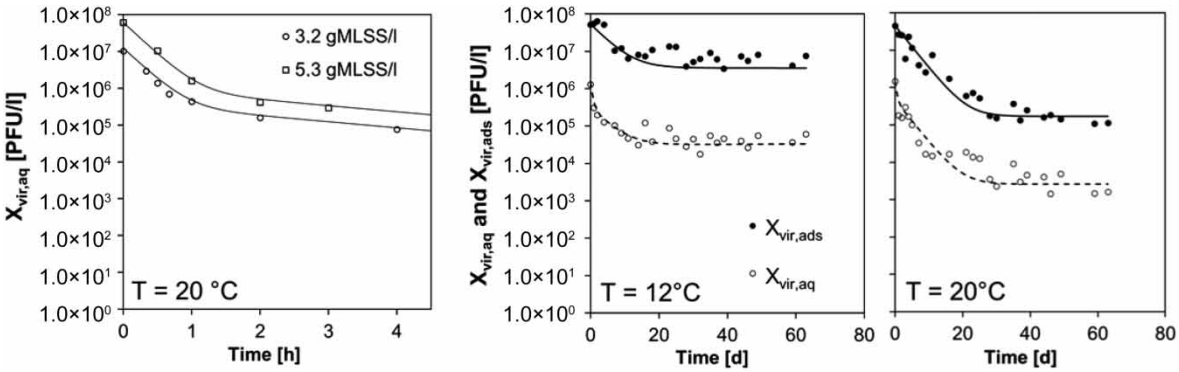


Figure 3 | Experimental and simulated results of the short-term tests at 20 °C (left); long-term tests at 12 °C (middle) and 20 °C (right). The markers are the experimental data and the lines the model results.

Modeling of the experimental results

The kinetic parameters used in the simulations are given in Table 1. In the long-term tests designed to study the inactivation and reactivation of free and adsorbed viruses, adsorption plays a minor role compared to the short-term tests where viruses were initially spiked. As shown in Table 1, the values of the adsorption/desorption rate constants of long-term tests are considerably lower than the ones of the short-term tests. The experimentally determined values for the inactivation and reactivation parameters in long-term tests were not significantly different from the calibrated values.

Model predictions of $X_{vir,aq}$ and $X_{vir,ads}$ in short- and long-term tests fit the measured values at 20 °C well (Figure 3), according to the statistical criteria presented above (for $X_{vir,aq}$ in the short-term test with 3.2 gMLSS/l: $ME = 5.8\%$ and $E = 1.00$; in the long-term test: $ME = 7.2\%$ and $E = 0.96$ for $X_{vir,aq}$, and $ME = -1.7\%$ and $E = 0.41$ for $X_{vir,ads}$). In the long-term test at 12 °C, the model undersimulated $X_{vir,ads}$ for a time higher than 15 d, resulting in a poor fit according to the statistical criteria ($ME = -55\%$, $E = 0.74$). A decrease in biomass concentration observed in the batch test along the 60 days could have influenced the experimental results and accounted for the gap between measured and modeled data.

The diurnal cycles of $X_{vir,aq}$ and $X_{vir,ads}$ in the activated sludge tank (AST) in winter were simulated with two different kinetic parameter sets (Table 1, Figure 4). A sensitivity analysis of the model parameters indicated that $X_{vir,aq}$ is affected by the hydraulic retention time and the adsorption rate constant ($k_{vir,ads}$). Consequently, a higher $k_{vir,ads}$ (parameter set (a)) contributes to a lower fluctuation in $X_{vir,aq}$ and hence to a better adjustment of the model to the experimental results (for $X_{vir,aq}$: $ME = 3.7\%$ and $E = 0.24$; for $X_{vir,ads}$: $ME = -1.7\%$ and $E = 0.41$). Furthermore, $X_{vir,ads}$ is affected by the SRT and the inactivation rate constants. With the parameter set (b), $d_{vir,aq,in}$ is 10 times higher than

Table 1 | Kinetic parameters used in the model (*)

Parameters [d^{-1}]	Short-term tests 20 °C	Long-term tests 12 °C	Long-term tests 20 °C	Full-scale steady-state 20 °C	Full-scale diurnal cycle 12 °C (a)	Full-scale diurnal cycle 12 °C (b)
$k_{vir,ads}$	79	**0.85**	**0.92**	*90*	**64**	**25**
$k_{vir,des}$	**1**	**0.01**	**0.02**	*0.9*	**0.5**	**0.2**
$d_{vir,aq,in}$	18	*1.0*	*1.3*	18	**0.7**	**7**
$d_{vir,aq,rein}$	*0.0008*	*0.01*	*0.0008*	*0.0008*	*0.01*	*0.01*
$d_{vir,ads,in}$	**9**	*0.2*	*0.25*	**1.06**	**0.7**	**0.8**
$d_{vir,ads,rein}$	*0.001*	*0.015*	*0.001*	*0.001*	*0.015*	*0.015*

(*) The distinct formats used to depict the data correspond to the different methods by which they were obtained: underline: experimentally determined in short-term tests; *italic*: experimentally determined in long-term tests and further calibrated; bold: curve fitting of the model to the experimental results.

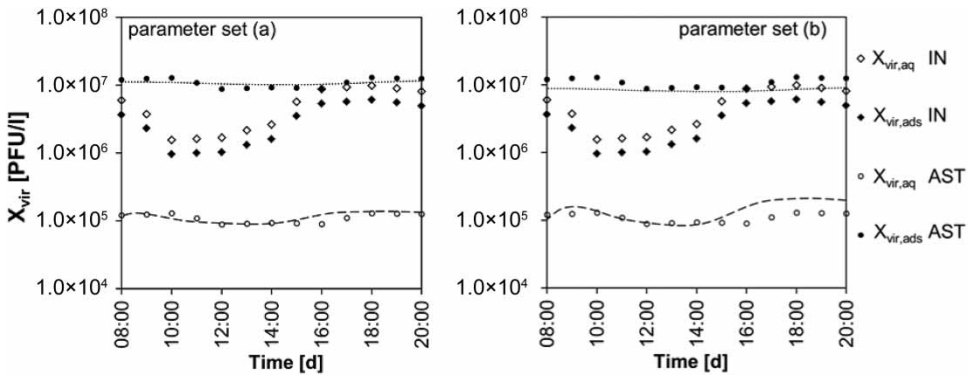


Figure 4 | Experimental (marks) and simulated (lines) values of $X_{vir,aq}$ and $X_{vir,ads}$ in the influent and AST of the full-scale WWTP (water temperature about 12 °C) with two sets of kinetic parameters (Table 1).

with parameter set (a), leading to a lower concentration of $X_{vir,ads}$ (for $X_{vir,aq}$: $ME = 22.2\%$ and $E = -8.04$; for $X_{vir,ads}$: $ME = -29.6\%$, $E = -1.97$).

Next, the extended model was used to simulate the effects of different scenarios on the steady-state concentration of viruses in the AST.

(1) Standard: actual conditions in the full-scale AST (kinetic parameters presented in Table 1 under 'Full-scale steady-state' column): $X_{vir,aq}/X_{vir,ads} = 0.62/0.38$ in the influent, SRT = 11 d.
(2) No reactivation: $d_{vir,aq,rein} = d_{vir,ads,rein} = 0$.
(3) Only free viruses in the influent: $X_{vir,ads} = 0$.
(4) Only adsorbed viruses in the influent: $X_{vir,aq} = 0$.
(5) Low SRT: 9 d.
(6) High SRT: 21 d.

The model – scenario (1) – was able to reproduce the concentrations of COD and N (data not shown) as well as $X_{vir,aq}$ and $X_{vir,ads}$ (compare AST_M and scenario (1) in Figure 5(a)). As shown in Table 1, the calibrated value of the adsorption rate constant ($k_{vir,ads}$) is higher for the full-scale plant than for the short-term test. We cannot account for these results; it can only be suggested that the batch tests, despite being a useful tool to obtain an estimation of the parameters, are an oversimplification of the processes in a full-scale plant.

To better compare the different scenarios, the logarithm of the difference between the output ($X_{vir,aq}$ and $X_{vir,ads}$) of scenario (1) and the output of scenarios (2)–(6) was calculated and depicted in Figure 5(b). The reactivation of free and adsorbed viruses significantly affects the concentration of viruses in the AST: $X_{vir,aq}$ and $X_{vir,ads}$ are about 4.0 and 6.0 logPFU/l higher in scenario (1) compared to scenario (2), respectively (Figure 5(b), (1) and (2)). The distribution of viruses between the flocs and the aqueous phase in the influent also affects the quality of the effluent: a higher influent $X_{vir,aq}$ produces a higher $X_{vir,aq}$ and a lower $X_{vir,ads}$ in the AST (scenario (3)). Conversely, a higher influent $X_{vir,ads}$ produces a lower $X_{vir,aq}$ and a higher $X_{vir,ads}$ in the AST (scenario (4)). These results suggest that it is important to evaluate the distribution of viruses in the influent between the adsorbed and aqueous phase to better calibrate the model. Finally, increasing the SRT produces an effluent with a lower quality: $X_{vir,aq}$ and $X_{vir,ads}$ are about 4.6 and 6.7 logPFU/l higher in scenario (6) compared to scenario (1), respectively. The opposite effect was observed for scenario (5). These simulation results suggest that there is an optimal SRT for the elimination of viruses. At the optimum, the rate of the inactivation of viruses equals the rate of enrichment through the recirculation of sludge.

The virus tool describes the elimination of viruses in the activated sludge by reversible adsorption and inactivation

(a)

Viruses	Measured data			Simulated data for scenarios (1) to (6) in AST					
log_{10}PFU/l	IN	AST_M	EFF	(1)	(2)	(3)	(4)	(5)	(6)
$X_{vir,aq}$	8.00	6.104	6.104	6.104	6.100	6.128	6.061	6.098	6.118
$X_{vir,ads}$	7.80	8.091	~0	8.091	8.087	8.061	8.137	8.085	8.108

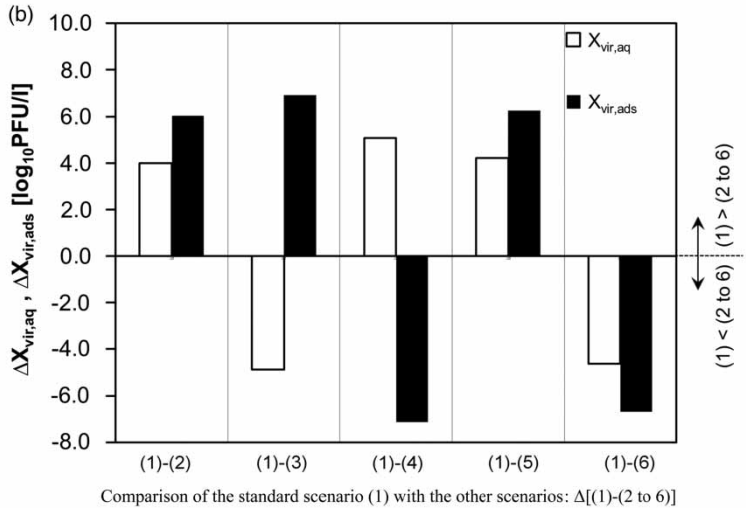


Figure 5 | (a) Measured and simulated concentrations $X_{vir,aq}$ and $X_{vir,ads}$ (b) relative effect of the different scenarios in relation to the standard scenario on $X_{vir,aq}$ and $X_{vir,ads}$ in the AST.

processes. The results of the modeling study suggest that the concentration of free viruses in the effluent of the AST is mainly affected by the adsorption rate and the hydraulic retention time, followed by the inactivation/reactivation rates. Furthermore, an appropriate regulation of the SRT can improve elimination of viruses in the AST.

CONCLUSIONS

In this study a mathematical model describing virus elimination in biological systems – virus tool – was developed and integrated in the ASM3. The virus tool considers six kinetic processes describing the adsorption and desorption of viruses to activated sludge flocs as well as the inactivation and reactivation of free and adsorbed viruses.

The extended model was used to simulate the fate of viruses in batch tests as well as in a municipal full-scale WWTP. Currently, the virus tool provides the three crucial factors that influence the elimination of viruses. These are the concentration of viruses, the hydraulic retention time and the SRT in the AST. The extended model should be a useful tool to estimate the number of viruses entering surface water after passing the WWTP. Further investigations of the influence of the temperature and the sludge load and the contribution of free and adsorbed viruses to the elimination kinetics are necessary.

REFERENCES

Carducci, A., Battistini, R., Rovini, E. & Verani, M. 2009 Viral removal by wastewater treatment: monitoring of indicators and pathogens. *Food and Environmental Virology* **1**, 85–91.

Deng, L., Krauss, S., Feichtmayer, J., Hofmann, R., Arndt, H. & Griebler, C. 2013 Grazing of heterotrophic flagellates on viruses is driven by feeding behaviour. *Environmental Microbiology Reports* **6** (4), 325–330.

Fleischer, J., Schlafmann, K., Otchwemah, R. & Botzenhart, K. 2000 Elimination of enteroviruses, other enteric viruses, F-specific coliphages, somatic coliphages and *E. coli* in four sewage treatment plants of southern Germany. *Journal of Water Supply: Research and Technology – AQUA* **49** (3), 127–138.

Funderburg, S. W. & Sorber, C. A. 1985 Coliphages as indicators of enteric viruses in activated sludge. *Water Research* **19** (5), 547–555.

Gujer, W., Henze, M., Mino, T. & Van Loosdrecht, M. 1999 Activated sludge model no. 3. *Water Science and Technology* **39** (12), 183–193.

Hata, A., Kitajima, M. & Katayama, H. 2013 Occurrence and reduction of human viruses, F-specific RNA coliphage genogroups and microbial indicators at a full-scale wastewater treatment plant in Japan. *Journal of Applied Microbiology* **114** (2), 545–554.

Hewitt, J., Leonard, M., Greening, G. E. & Lewis, G. D. 2011 Influence of wastewater treatment process and the population size on human virus profiles in wastewater. *Water Research* **45** (18), 6267–6276.

ISO10705-2:2000 2001 *Water Quality – Detection and Enumeration of Bacteriophages. Part 2: Enumeration of Somatic Coliphages*. European Committee for Standardization, Brussels, Belgium.

Kim, T. D. & Unno, H. 1996 The roles of microbes in the removal and inactivation of viruses in a biological wastewater treatment system. *Water Science and Technology* **33** (10–11), 243–250.

Kim, T. D., Shiragami, N. & Unno, H. 1995 Development of a model describing virus removal process in an activated sludge basin. *Journal of Chemical Engineering of Japan* **28** (3), 257–262.

Nakajima, M., Uwatoko, K., Kobayashi, K., Pan, I. J., Iwabuchi, N. & Sunairi, M. 2003 Adhesion and releasing of Poliovirus to activated sludge of wastewater purifying plants. *Water Science and Technology* **47** (9), 117–121.

Ottoson, J., Hansen, A., Westrell, T., Johansen, K., Norder, H. & Stenström, T. A. 2006 Removal of noro- and enteroviruses, *Giardia* cysts, *Cryptosporidium* oocysts, and fecal indicators at four secondary wastewater treatment plants in Sweden. *Water Environment Research* **78** (8), 828–834.

Schijven, J. F. & Hassanizadeh, S. M. 2000 Removal of viruses by soil passage: overview of modeling, processes, and parameters. *Critical Reviews in Environmental Science and Technology* **30** (1), 49–127.

Selinka, H.-C., Botzenhart, K., Feuerpfeil, I., Puchert, W., Schmoll, O., Szewzyk, R. & Willmitzer, H. 2011 Nachweis von Viren im Rohwasser als Grundlage einer Risikoabschätzung (Detection of viruses in raw water as a basic tool for risk assessment). *Bundesgesundheitsblatt* **54**, 496–504.

Singh, J., Knapp, H. V. & Demissie, M. 2005 Hydrologic modeling of the Iroquois River watershed using HSPF and SWAT. *Journal of the American Water Resources Association* **41** (2), 343–360.

Tanji, Y., Mizoguchi, K., Yoichi, M., Morita, M., Hori, K. & Unno, H. 2002 Fate of coliphage in a wastewater treatment process. *Journal of Bioscience and Bioengineering* **94** (2), 172–174.

Ulbricht, K., Selinka, H.-C., Wolter, S., Rosenwinkel, K.-H. & Nogueira, R. 2014 A mass balance approach to the fate of viruses in a municipal wastewater treatment plant during summer and winter seasons. *Water Science and Technology* **69** (2), 364–370.

Usrael, G. 1980 *Faktoren, die die Inaktivierung von Viren beim Belebungsverfahren beeinflussen (Factors Affecting the Inactivation of Viruses in the Activated Sludge Process)*. Institute of Water Quality and Landschaftswasserbau (river engineering with respect to the landscape), Vienna University of Technology, Vienna.

First received 29 April 2014; accepted in revised form 24 July 2014. Available online 7 August 2014