Using modelling and field testing to learn about bulk-biofilm interactions in reactive sewer systems

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Abstract
In order to learn about bacterial activity and process mechanisms in a dynamic sewer environment, an interactive investigation method was used, which requires an intense combination of and a back-and-forth comparison between modelling tools and field tests. It focuses on usefulness and software engineering needs of model and experiment, instead of merely pursuing to match modelling results and experimental data. The whole study includes model calibration, scenario analysis and field experiments in a force main system and was accomplished in two investigation phases. A nitrate (NO_3) dosing test was carried out following model-based experimental design. The sewer system is very reactive. NO_3 dosing decreased volatile fatty acid and sulfide, as well as soluble organics and nutrients, and increased alkalinity and suspended solids. Model predictions and field measurements were carefully compared and analyzed. It was found that both biofilm and bulk-flow bacteria participate in sewer processes and it is their co-action that explains the onsite observations. Denitrification was prompted by NO_3 dosing. Bacteria in the sewer are able to switch their functions between fermentation and denitrification, but some adaptation time seems to be required. Based on the analysis, model improvements are suggested regarding the modelling of bacteria species and biofilm-bulk flow interactions.

Keywords
Sewer biochemical processes; modelling; field test; biofilm; chemical dosing

INTRODUCTION

The processes in sewer systems are not limited to physical transportation of sewage, but also include biochemical reactions. It is well known that hydrogen sulfide (H_2S) is produced via sulfate (SO_4) reduction and is the main reason of pipe corrosion (EPA, 1985, 1991; Hvitved-Jacobsen et al., 2013). Besides sulfur-containing species, there are bacteria, organics and nutrients present in sewers. The sewer environment can be aerobic, anoxic or anaerobic, depending on sewer characteristics and operational conditions. Therefore, in addition to the transformation of sulfur-containing species, in-sewer biochemical processes also include other reactions caused by bacterial activities and metabolism, like hydrolysis, volatile fatty acids (VFA) production by fermentation, nutrient uptake of ammonia (NH_4) and phosphate (PO_4), and organics degradation (Hvitved-Jacobsen et al., 2013). Bacteria live both in the biofilm that is attached to sewer walls and in the bulk flow that is flowing inside the pipe. Some researchers state that the biofilm is the driving force behind in-sewer processes (Jensen et al., 2016). However, bacteria can be detached from a biofilm to join the suspended biomass in the bulk flow of the sewer (Houhou et al., 2015). Given the plug-flow behavior of sewer systems, the contribution by bulk flow activity warrants attention. To investigate particular roles and mechanisms of biochemical processes in biofilm and bulk flow, a study that combines modelling and experimentation is needed.

Mathematical modelling has been demonstrated to be useful to study and predict odor generation, corrosion and other biochemical processes occurring in sewers (Jiang et al., 2009; Hvitved-Jacobsen et al., 2013; Mourato et al., 2003; Ramin et al, 2016; Sharma et al., 2008). It can assess process performance and guide control strategy design prior to operational implementation.

One important task in general modelling practice is the calibration and validation of a model, which is aimed at testing and tuning the model and making the modelling results agree with experimental data (the reality) as much as possible (Jiang et al., 2009; Vanrolleghem et al., 2003; Rieger et al., 2012; Sharma et al., 2008; Vollertsen et al., 2015). With that task accomplished, the model can be used to represent the real world, but it must be applied within boundaries. Guo and Vanrolleghem (2014) found that two different models could give similar calibration results under certain circumstances, but when temperature changes the difference between the models become visible.

The usefulness of modelling tools should not be limited to pursuing an ideal representation of reality. In fact, the difference between modelling results and measurement data is valuable too, because it can inspire new thoughts and learning. In most cases, a model is built by assembling findings and knowledge of previous researchers and studies (Henze et al., 2006; Hvitved-Jacobsen et al., 2013). Therefore, when measurement data (i.e. observations for a particular case) show a significant difference with modelling results (i.e. prediction according to vast expert experience), it is worthy to perform in-depth investigations and review both the model and the measurement data. In that sense, the model becomes a learning tool.

Therefore, an interactive method was used in this study, which involves an intense interaction between computer modelling and field experimentation. Instead of relying on only one of both methods or placing one in a more important place than the other, this study shows that model and field experiment can be used collaboratively to provide new insights on in-sewer biochemical processes. The specific goal of this study is to better understand the biochemical processes, especially fermentation and denitrification, when using nitrate (NO_3) as a chemical for VFA and H_2S control (Guo et al., 2018), and to learn about the interactions between biofilm and bulk-flow bacteria and their contributions to water quality changes.

METHODS

Modelling tool

The modelling tool used in this paper is a software platform that can handle different scales of systems under both dynamic and steady-state conditions with a graphical user interface (GUI) and provides flexibility in the selection of in-sewer process models (Guo et al., 2018). In this study, simulation was run under dynamic conditions and the SeweX model (Sharma et al., 2008) was selected. The SeweX model has gained wide application in practice to capture typical biochemical and physicochemical processes occurring in typical sewer environments or under chemical dosing strategies that are commonly used in sewer odor and corrosion control. The biochemical processes of the SeweX model include transformations of sulfur-containing species (like SO_4 reduction, H_2S oxidation, etc.), fermentation, hydrolysis, denitrification (when NO_3 is dosed), methane production, and bacterial growth and decay. The model also simulates chemical precipitation of iron, PO_4 and H_2S, chemical oxidation/reduction, and liquid-gas transfer of gaseous species like H_2S, methane, oxygen, etc.

Field test

The field test was carried out in a force main system located in California, US. The force main conveys sewage from a pumping station (i.e. the influent of the force main) to the headworks of a

WWTP (i.e. the effluent of the force main). The length and the diameter of the force main pipe are 9093 m and 406 mm, respectively. The average flow rate of the force main is 2732 m^3/day, resulting in an average hydraulic retention time (HRT) of 11 hours, ranging between 6 and 15 hours.

Field measurements were carried out at both the pumping station and the headworks, by utilizing online monitoring instruments and grab sample analysis methods. An online VFA analyzer (AnaSense, Hach, Loveland, Colorado, US) was installed at the headworks to monitor hourly VFA and alkalinity changes under the baseline conditions, i.e. in the current situation without chemical dosing or any changes to the existing operation, and under NO_3 dosing. YSI probes were installed at both the pumping station and the headworks to record dissolved oxygen, pH, temperature and oxidation/reduction potential. Total suspended solid (TSS) was also measured at the pumping station and the headworks by TSS probes. Many water quality parameters, such as total chemical oxygen demand (tCOD), soluble COD (sCOD), NH_4, NO_3, H_2S, PO_4, total nitrogen, etc., were analyzed immediately on site by HACH methods. Besides that, grab samples were also preserved and used for ion chromatography (IC) analysis (Metrohm, Herisau, Switzerland), measuring sulfate (SO_4) and validating the online VFA data.

Interactive model-field test investigation
The main goal of this study was to get more insights on in-sewer biochemical process by testing and comparing model predictions against field measurements, especially regarding fermentation and denitrification processes when NO_3 is dosed at the pumping station for VFA and H_2S control. The whole study consists of two phases with different focuses. The relationship between the two phases is illustrated in Figure 1, showing the supportive relationship between modelling and field tests.

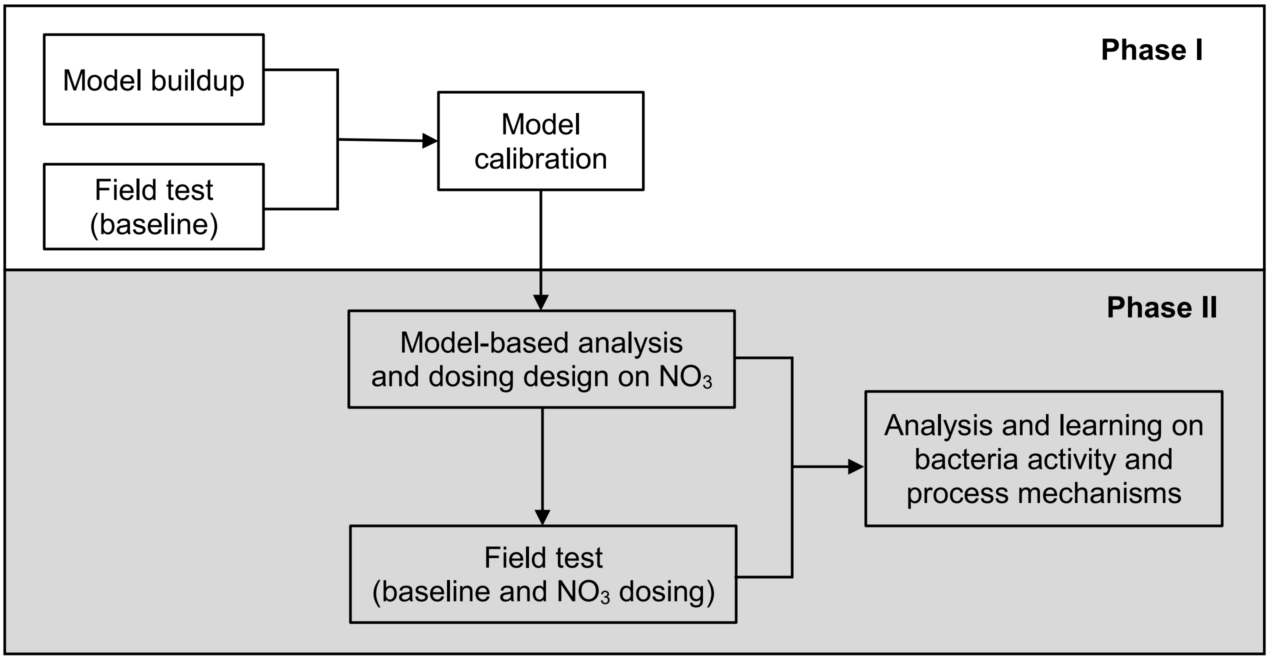

Figure 1 Scheme of investigation procedure

The aim of Phase I was to understand sewer performance under the baseline conditions and to calibrate the SeweX model. A data set of 24 hours was obtained from the Phase I field measurement. Phase II focused on model-based experimental design and a chemical dosing test. The aim was to test the model-designed NO_3 dosing strategy against field measurements and to acquire new knowledge regarding sewer biochemical processes and the bacterial activities by investigating the sewer response to changing sewer conditions. The Phase II field experiment was longer than the Phase I experiment, with two weeks of data being obtained.

Model calibration. The calibration used water quality data obtained at the influent and the effluent of the force main system during the Phase I field test. VFA is produced by fermentation of organics in the sewer and H_2S is produced through the SO_4 reduction process. Both processes are related to the presence of anaerobic conditions. Therefore, NO_3 dosing, which is used for controlling H_2S, also allows VFA control. NO_3 decreases VFA production through two pathways. One pathway is to reduce sCOD through denitrification process, whereas the other is to inhibit fermentation process by anoxic condition. However, the Phase I data, which were collected under anaerobic conditions (baseline), do not inform regarding the activity and potential amount of denitrifiers present in the force main.

The model was calibrated for two conditions, respectively with and without denitrifiers growing under NO_3 dosing. For the modelling without denitrifier growth, the bacterial denitrifying reactions were turned off in the SeweX model. For the modelling with denitrifier growth under NO_3 dosing, the fermenting bacteria modelled in SeweX are considered capable of denitrification and vice versa, based on the fact that some fermenting bacteria are facultative and can live under both anaerobic and anoxic conditions (Gerardi, 2003; Müller et al., 2019; Liu et al., 2015).

Model-based analysis of denitrifier activity. In order to differentiate the two conditions regarding denitrifier activity, a model analysis was run to predict the expected onsite observations under those two conditions (Figure 2). If there is little amount or low-activity of denitrifiers under NO_3 dosing, a step change in NO_3 should be observed at the headworks when NO_3 is dosed at the pumping station. The time when the step change of NO_3 occurs at the headworks depends on the hydraulic travelling time from the pumping station to the headworks. Conversely, no NO_3 will be measured at the headworks if the denitrifiers are active and consume all dosed NO_3 before the wastewater reaches the headworks.

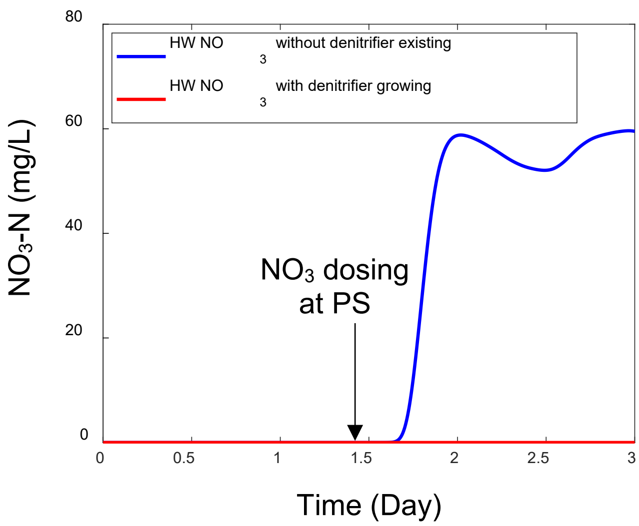

Figure 2 Modelling results under two opposite hypothesis regarding the denitrifier activity in the force main (PS: pumping station; HW: headworks; NO_3 dosing starts at 10:00 am on Day 1)

Modelling and field test of NO_3 dosing. A model-based scenario analysis was performed with the calibrated model of Phase I, in order to estimate the required NO_3 dosing at the pumping station based on the prediction of the desired VFA and H_2S reductions. The amount of required NO_3 dosing depends on the denitrifier activity, which could not really be deduced from the Phase I data. However, if there is no denitrification activity, only a small amount of NO_3 should suffice to inhibit fermentation. On the contrary, more NO_3 will be needed in case denitrifier activity turns out to be high, because NO_3 will be continuously consumed. Therefore, a scenario analysis was carried out under the condition that denitrifiers grow under NO_3 dosing.

The NO_3 dosing rate is calculated by multiplying the total daily dosing amount with a daily dosing profile defined on an hourly basis (Figure 3). The NO_3 dosing profile is flow-paced according to the force main flow rate, in order to maintain the NO_3 concentration around a certain level at the pumping station (Figure 3). In total 11 scenarios were run, including one baseline scenario and ten NO_3 dosing scenarios whose average NO_3-N concentrations at the pumping station are 10, 20, 30, …, and 100 ppm respectively (i.e. changing by an increment of 10 ppm). The preferred NO_3 dosing was selected from this scenario analysis and recommended for the field test of the Phase II.

The Phase II field test started from baseline condition, i.e. no chemical dosing, and then the NO_3 dosing pump was activated at the pumping station based on the suggestion from the scenario analysis. Two levels of NO_3 dosing rates were tested on site and field measurement data were compared with model predictions. The NO_3 dosing solution contains 122 g NO_3-N/L (i.e. 3.5 lb NO_3-O/gal in US unit).

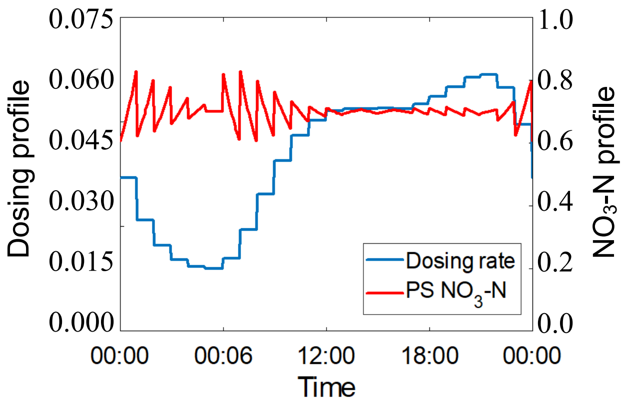

Figure 3 Dosing profile of NO_3 solution and consequential concentration profile of NO_3-N at the pumping station (PS). Under each scenario, the final NO_3-N at PS is the product of multiplying the target average NO_3-N with the NO_3-N profile, and the final dosing rate is the product of the daily total dosing amount with the dosing profile.

RESULTS

Model calibration

Figure 4 shows the model calibration results based on the Phase I data. The model essentially gives the same simulation results for the situation with and without denitrifier growth. In other words, the Phase I data cannot inform regarding the amount and the activity of denitrifiers.

Scenario analysis

Figure 5 presents the scenario analysis result with denitrifier growth under different NO_3 dosing. It compares VFA, H_2S and other key biochemical variables at the force main effluent. The selection of the dosing rate to be applied in the Phase II tests is based on considerations regarding VFA and H_2S removal and NO_3 overdosing. From Figure 5, it can be concluded that the NO_3-N concentration at the pumping station should be maintained between 50 ppm and 90 ppm (i.e. the green zone of Figure 5) in order to achieve VFA and H_2S removal and avoid NO_3 overdose (i.e. 0 NO_3-N at the effluent).

Therefore, the Phase II field experiment was run with 70 ppm NO_3-N dosing as the starting point (i.e. 1.51 m^3 NO_3-solution/day). Given the uncertainty regarding the model under denitrifier growth conditions, this value was continuously monitored and tuned on site (see below).

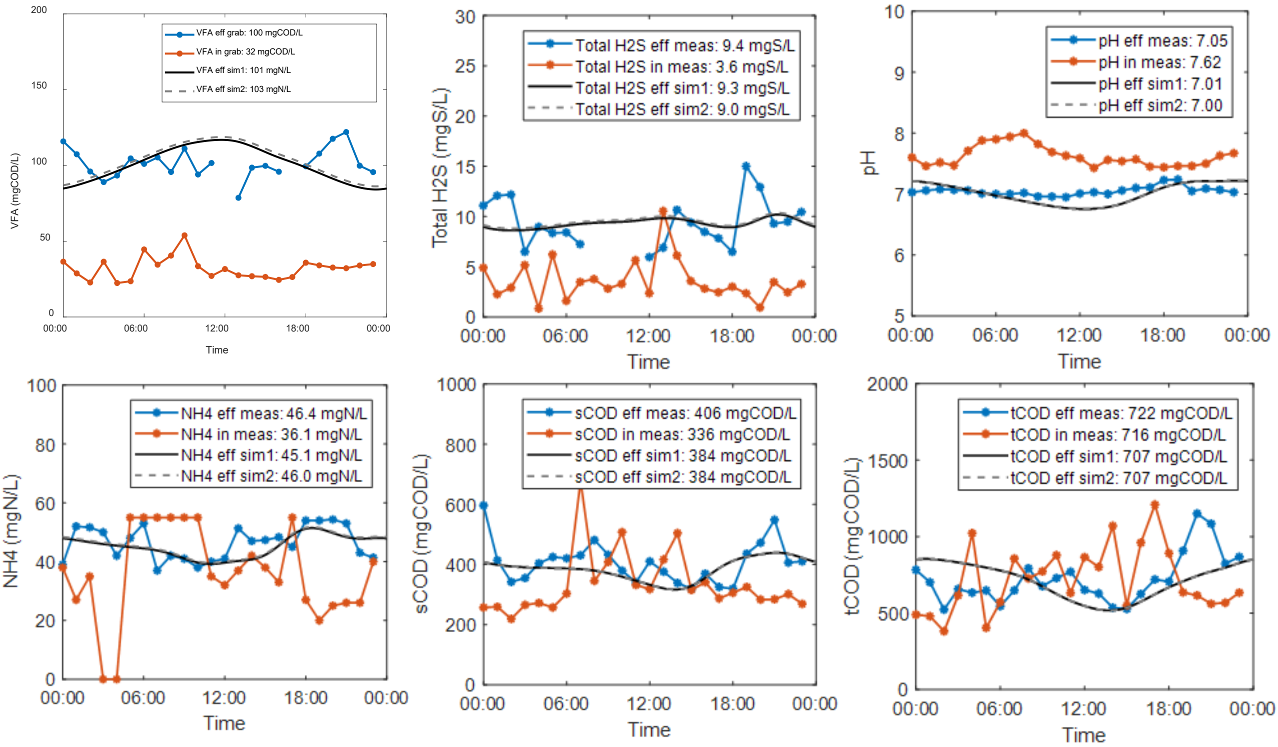

Figure 4 Comparison of model calibration with (sim1) and without denitrifier (sim2) growth (eff: effluent, in: influent)

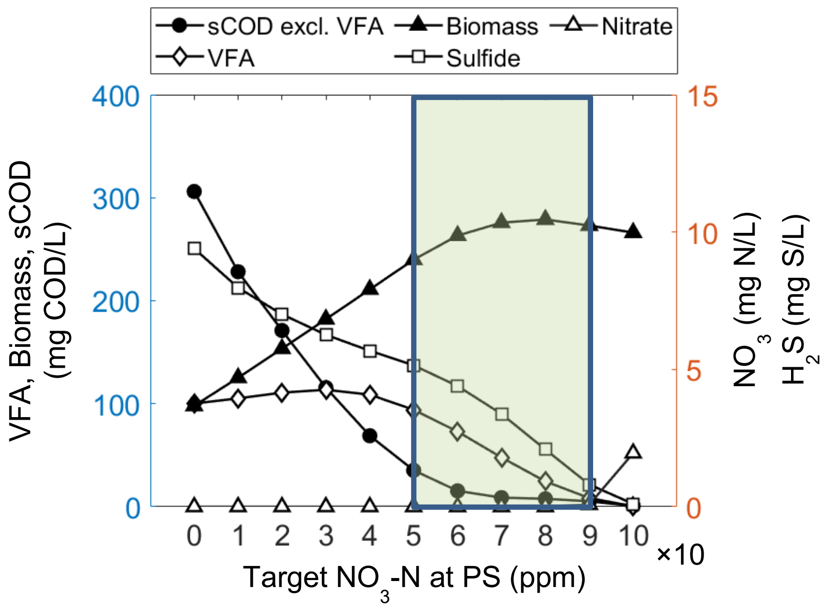

Figure 5 Scenario analysis of nitrate dosing (PS: pumping station)

Model predictions and field measurements

The Phase II field experiment started from baseline monitoring, and then changed to the 70 ppm NO_3-N dosing strategies using the dosing profile and amount recommended by the model. Water quality, especially VFA and H_2S, was carefully monitored at the headworks. As experiment continued, more VFA reduction was observed than the model predicted, so the NO_3 dosing was reduced to a median level (50 ppm NO_3-N at pumping station, i.e. 1.08 m^3 NO_3-solution/day). By the end of the field test, NO_3 dosing was turned off to monitor how the system would return to baseline.

In general, the field test showed more reduction on VFA than the model predicted (Table 1). Besides that, a transition period was observed when NO_3 dosing started. As shown in Figure 6, a peak in NO_3 concentration was observed at the beginning of the NO_3 dosing at the force main effluent, arriving at the headworks according the hydraulic travelling time (8.4 hours). After 18 hours, the concentration had decreased again to the background level, indicating enhanced denitrification. A similar trend was recorded for VFA and H_2S, whose concentrations initially remained high before showing significant reductions.

By comparing Figure 6 with the pre-test model analysis (Figure 2), it can be seen that the observation on site is a combination of the two possible conditions regarding denitrifier activity and growth. At the beginning of the NO_3 dosing, the denitrification activity was insufficient to consume all the dosed NO_3, presenting a curve of NO_3 similar to the blue line in Figure 2. Only after 18 hours, the denitrifiers had grown sufficiently to reduce the NO_3 concentration to the background level (the red line in Figure 2). The 18 hours can be considered the time for the bacteria to adapt to the NO_3 presence and grow in sufficient numbers. Consequently, VFA and H_2S showed a similar transient.

The result also indicates that when implementing dosing strategies on site, it would be better to start dosing at a small rate and increase it gradually to reach the desired outcome, in order to give time for bacteria to adapt and avoid overdosing at the initial stage.

Table 1 Comparison of model and Phase II field measurements in terms of VFA and H_2S reduction.

	Model prediction	Field measurement
VFA reduction (%)		
High NO_3 dosing rate (70 ppm scenario)	50	90
Median NO_3 dosing rate (50 ppm scenario)	10	65
H_2S reduction (%)		
High NO_3 dosing rate (70 ppm scenario)	65	85
Median NO_3 dosing rate (50 ppm scenario)	45	40

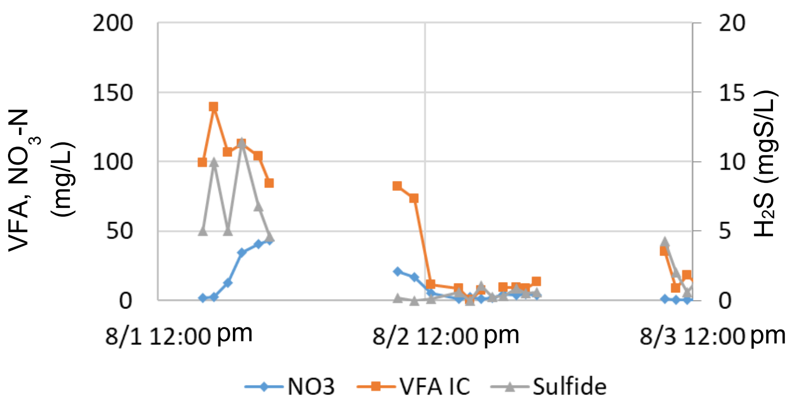

Figure 6 Phase II measurement data of NO_3, VFA and H_2S at the force main effluent after NO_3 solution was added (Note: NO_3 dosing started at 10:00 am on August 1st).

Water quality changes under NO_3 dosing
Besides their impact on the VFA and H_2S decrease, NO_3 dosing also affected other biochemical variables. sCOD was decreased by about 50% compared to the baseline, which further supports that denitrification is prompted by NO_3 dosing. Nutrient uptake was also observed, i.e. NH_4 was reduced by about 20% and PO_4 by about 50%, and the TSS probe showed an increase in TSS at the headworks. One explanation for the nutrient decrease and the TSS increase is that more bacteria can be grown through the denitrification process than through fermentation. Thanks to denitrification, a proton consuming process, alkalinity was increased by about 50%. All those observations indicate that the sewer is a reactive bioreactor and its processes and products can be managed via controlled chemical addition.

DISCUSSION

Biofilm and bulk-flow bacteria
Biofilm and bulk-flow bacteria co-exist in the sewer. The bacterial community of biofilm is functional for in-sewer biochemical processes (Jensen et al., 2016; Liu et al., 2015). However, the ongoing interaction of biofilm and bulk flow (Houhou et al., 2015) and the HRT of sewer system indicate that the bacterial activity in bulk flow also needs to be studied. In order to shed some light on the question regarding biofilm and bulk-flow bacteria, field observations from the Phase II study were carefully compared against the two statements regarding bacteria and bulk flow bacteria contributions. The adaptation time of the bacteria under NO_3 dosing revealed that the interaction of biofilm and bulk flow bacteria may be important in leading to the observed outcome, as explained below.

First, if the observation on site would be a result only driven by biofilm processes, the transition period would be longer than the observed 18 hours for the biofilm to switch its roles and structure in terms of denitrification. Indeed, this biofilm transformation normally takes weeks or months to accomplish (Auguet et al., 2019; Habouzit et al., 2014; Weissbrodt et al., 2013). Moreover, unlike biofilm reactors used in wastewater treatment, where the specific surface areas provided for the biofilm to grow are usually between 100-1000 m^2/m^3 (Hosono et al., 1980; IWA Task Group on Biofilm Modeling, 2006), the specific surface area of the studied force main is only 10 m^2/m^3, much smaller than that. Therefore, it is challenging to ask for the force main biofilm to denitrify all the dosed NO_3 within the hydraulic travelling time (6-15 hours).

On the other hand, if the observation on site was caused only by bulk flow bacteria, the system should have reached stable results faster, i.e. it would show either no NO_3 or a repeatable NO_3 pattern once the NO_3 dosing arrived at the headworks. In other words, no transient period would have been observed. Therefore, the most plausible explanation at this stage is that both biofilm and bulk flow bacteria influence the in-sewer biochemical processes and that their interaction and exchange caused the transient period.

Denitrifiers and fermenting bacteria
In order to explain the difference between model prediction and measurement data regarding VFA, the settings and structure of the SeweX model were reviewed. In the SeweX model, a bacterial component can easily switch its role between denitrification and fermentation, and the bacteria grow faster and accumulate to a larger extent under anoxic conditions than under anaerobic conditions. On the other hand, as long as NO_3 is not overdosed, the force main pipe will have two zones, i.e. an anoxic zone close to the dosing point followed by an anaerobic zone when all NO_3 is denitrified. In the SeweX model, after the large amount of bulk flow bacteria growing under anoxic zone enter the subsequent anaerobic zone, they can act immediately in fermentation. However, in reality, bacteria

need time to adapt to new environments, as can be seen from Figure 6. The hydraulic travelling time within the anaerobic zone (about 3-8 hours) is possibly insufficient for the needed adaption time (about 18 hours). This could be the reason behind the observation that the model prediction for VFA is higher than the field measurement.

Possible pathways

Based on the analysis of the modelling results and the field measurement data, one hypothetical explanation was given regarding the bacterial activity and the process mechanism under NO_3 dosing. When NO_3 dosing just starts, only a small amount of denitrifiers may be present in the biofilm (most likely at the top of biofilm). They could be facultative anaerobes that can also perform denitrification or they could be a small amount of existing denitrifiers (Auguet et al., 2015; Gerardi, 2003; Müller et al., 2019; Liu et al., 2015). As NO_3 dosing continues, their population increases steadily, because NO_3 and organic substrates are present in excess in the sewer. It was found that there is an increase of nitrate reduction bacteria in the biofilm under NO_3 dosing (Auguet et al., 2015). Under the shear effect of the sewage flow, a part of the biofilm detaches and enters the bulk flow. These suspended denitrifiers grow quickly and accumulate quickly as they travel downstream with the sewer flow, behaving like a plug-flow reactor. In other words, the biofilm provides a bacterial seed and the conditions in the bulk flow allow amplifying this microbial community. To some extent, the characteristics of the bulk flow suspension reflect those of the biofilm, because there is a continuous interaction and exchange between them (Houhou et al., 2015). The exchange and adaptation time between biofilm and bulk flow bacteria result in the transition period (i.e. adaptation time) under NO_3 dosing.

The force main pipe has an anoxic zone and an anaerobic zone under non-excessive NO_3 dosing. Similar to the adaptation from fermentation to denitrification, time is also required to reverse from denitrification to fermentation, even if some of the bulk flow denitrifiers grown in the anoxic zone are potentially capable of fermentation. Therefore, when they travel through the anaerobic zone, they cannot immediately ferment. This could be an important reason for the differences observed between modelling results and measurements of VFA.

Note, however, that the fermenting bacteria in the biofilm of the anaerobic zone are still fully functional. Still, compared to the baseline conditions, less COD is available for fermentation and SO_4 reduction in the anaerobic zone, which results in a decrease of VFA and H_2S production.

While this investigation was carried out under NO_3 dosing, the findings from this study could be extended to and tested against other situations and used in a general sense to reflect on in-sewer biochemical process dynamics. Sedimentation may also have influence on in-sewer biochemical processes, which requires further study in the future.

Implication on model improvement

It is suggested that the model should consider the adaptation time needed for bacteria to switch functions between denitrification and fermentation. The model could for instance include two separate groups of bacteria, i.e. fermenting bacteria and denitrifiers, and set an adaptation rate between the two. The current SeweX model uses a fixed description of biofilm activity and productivity. In order to catch the biofilm and bulk flow interactions and better simulate biofilm activity and productivity under different conditions, a dynamic biofilm description is required for the model, which could be derived from literature (IWA Task Group on Biofilm Modeling, 2006; Jiang et al., 2009; Rauch et al., 1999).

CONCLUSION

An interactive investigation method involving modelling and field testing was used to uncover mechanisms and knowledge about in-sewer biochemical process. The key findings are summarized below:

Onsite measurements during a full-scale NO_3 dosing test showed that bacteria need time to adapt to the new sewer conditions. The adaptation was expressed in terms of a transient period observed at the beginning of the NO_3 dosing, and was explained by an interaction and dependency between biofilm and bulk-flow bacteria. The adaptation may be also present when bacteria change their functions between denitrification and fermentation.

Both biofilm and bulk-flow bacteria contribute significantly to in-sewer biochemical processes. Their co-action leads to the field observations. A possible explanation was given regarding the change and adaptation of sewer bacteria and the processes they induce in a new sewer environment. When NO_3 is dosed, the change of the in-sewer process starts from the biofilm bacteria, which form a bacterial seed to the bulk flow, and the bulk flow is like an amplifier for bacterial growth and accumulation as a plug-flow reactor. Consequently, water quality changes.

Both modelling and field testing are useful tools to allow hypothesis testing and improve the understanding on and management of sewer systems. A model-based experimental design was proposed to differentiate the possible models and provide insights on sewer process. The differences between model predictions and field measurements should be considered a very valuable information source for knowledge acquisition. Based on the analysis, model improvements suggested for the SeweX model include (i) the addition of fermenting bacteria and denitrifier as two species with an adaptation rate between them and (ii) to model dynamic changes and interactions between biofilm and bulk flow bacteria.

Sewer is reactive, and its processes can be managed. NO_3 dosing can mitigate fermentation and SO_4 reduction processes, while prompt and enhance denitrification in the sewer, resulting in decreased VFA and H_2S concentrations, as well as lower sCOD, NH_4 and PO_4 and increased alkalinity and TSS, thus performing part of the wastewater treatment. Based on the study, it is suggested that chemical dosing should start from a low rate and increase to a desired level, in order to give time for bacteria to adapt and to avoid overdose at the initial stage of the dosing strategy implementation.

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REFERENCES

Auguet, O., Pijuan, M., Batista, J., Borrego, C.M. & Gutierrez, O. (2019). Changes in microbial biofilm communities during colonization of sewer systems. *Applied and Environmental Microbiology*, **81**(20), 7271–7280.

Auguet, O., Pijuan, M., Guasch-Balcells, H., Borrego, C.M. & Gutierrez, O. (2015). Implications of Downstream Nitrate Dosage in anaerobic sewers to control sulfide and methane emissions. *Water Research*, **68**, 522–532.

EPA (1985). *Design Manual: Odor and Corrosion Control in Sanitary Sewerage Systems and Treatment Plants*, Report EPA/625/1-85/018, United States Environmental Protection Agency, US.

EPA (1991). *Hydrogen Sulfide Corrosion: Its Consequences, Detection and Control*, Report 832S91100, United States Environmental Protection Agency, US.

Gerardi (2003). *The Microbiology of Anaerobic Digesters*. John Wiley & Sons, Inc., Publication, US.

Guo L., Walton J., Tik S., Scott Z., Sharma K.R, Boiocchi R., Yuan Z., Elbeshbishy E., Santoro D. & Vanrolleghem P.A. (2018). Model-based management and control of the bioreactions in a collection system. In: *Proceedings*

WEF's 91st Annual Technical Exhibition and Conference (WEFTEC2018), New Orleans, US.

Guo, L. & Vanrolleghem P.A. (2014). Calibration and validation of an activated sludge model for greenhouse gases no. 1 (ASMG1): prediction of temperature-dependent N2O emission dynamics. *Bioprocess and Biosystems Engineering*, **37**(2), 151–163.

Habouzit, F., Hamelin, J., Santa-Catalina, G., Steyer, J.-P. & Bernet, N. (2014). Biofilm development during the start-up period of anaerobic biofilm reactors: the biofilm Archaea community is highly dependent on the support material. *Microbial Biotechnology*, **7**(3), 257–264.

Henze, M., Gujer, W., Mino T. & van Loosedrecht, M. (2006). *Activated Sludge Models ASM1, ASM2, ASM2d and ASM3*. IWA Publishing, UK.

Hosono, Y., Kubota, H. & Miyaji, Y. (1980). Characteristic evaluation of trickling filter process. *Water Research*, **14**(6), 581–590.

Houhou, J., Lartiges, B.S., Mustin, C., Ghanbaja, J. & Khalil, Z. (2015). Bacterial biofilms and suspended matter in the sewer system: examination at various scales. *International Journal of Science and Research*, **6**(7), 1134–1142.

Hvitved-Jacobsen, T., Vollertsen, J. & Nielsen, A.H. (2013). *Sewer Processes: Microbial and Chemical Process Engineering of Sewer Networks*, 2nd Edition. CRC press, US.

IWA Task Group on Biofilm Modeling (2006). *Mathematical Modeling of Biofilms*. IWA Publishing, UK.

Jiang, F., Leung, D.H., Li, S., Chen, G.-H., Okabe, S., & van Loosdrecht, M.C.M., 2009. Biofilm model for prediction of pollutant transformation in sewers. *Water Research*, **43**(13), 3187–3198.

Jensen, H., Biggs, C.A. & Karunakaran E. (2016). The importance of sewer biofilms. *WIREs Water*, **3**(4), 487-494.

Liu, Y., Dong, Q. & Shi, H. (2015). Distribution and population structure characteristics of microorganisms in urban sewage system. *Applied Microbiology and Biotechnology*, **99**(18), 7723–7734.

Mourato, S., Matos, J., Almeida, M. & Hvitved-Jacobsen, T. (2003). Modelling in-sewer pollutant degradation processes in the Costa do Estoril sewer system. *Water Science & Technology*, **47**(4), 93–100.

Müller, M., Mentel, M., van Hellemond, J.J. Henze, K., Woehle, C., Gould, S.B., Yu, R.-Y., van der Giezen, M., Tielens, A.G.M. & Martin, W.F. (2019). Biochemistry and Evolution of Anaerobic Energy Metabolism in Eukaryotes. *Microbiology and Molecular Biology Reviews*, **76**(2), 444–495.

Ramin, P., Brock, L.A., Polesel, F., Causanilles, A., Emke, E., de Voogt, P. & Plósz, B.Gy. (2016). Transformation and sorption of illicit drug biomarkers in sewer systems: understanding the role of suspended solids in raw wastewater. *Environmental Science & Technology*, **50**(24), 13397–13408.

Sharma, K. R., Yuan, Z., de Haas, D., Hamilton, G., Corrie, S. & Keller, J. (2008). Dynamics and dynamic modelling of H_2S production in sewer systems. *Water Research*, **42**(10/11), 2527–2538.

Rauch, W., Vanhooren, H. & Vanrolleghem, P.A. (1999) A simplified mixed-culture biofilm model. *Water Research*, **33**(9), 2148–2162.

Rieger, L., Gillot, S., Langergraber, G., Ohtsuki, T., Shaw, A. & Takács, I. (2012). Good modelling practice – realizing the full benefits of wastewater treatment modelling. *WATER21*, October, pp. 28–29.

Vanrolleghem, P.A., Insel, G, Petersen, B., Sin, G., De Pauw, D., Nopens, I., Dovermann, H., Weijers, S. & Gernaey, K. (2003). A comprehensive model calibration procedure for activated sludge models. In: *Proceedings WEF's 76th Annual Technical Exhibition and Conference (WEFTEC2003)*. Los Angeles, US.

Vollertsen, J, Revilla, N., Hvitved-Jacobsen, T., & Nielsen, A.H. (2015). Modeling sulfides, pH and hydrogen sulfide gas in the sewers of San Francisco. *Water Environment Research*, **87**(11), 1980–1989.

Weissbrodt, D.G. Shani, N. & Holliger, C. (2014). Linking bacterial population dynamics and nutrient removal in the granular sludge biofilm ecosystem engineered for wastewater treatment. FEMS Microbiology Ecology, 88(3), 579–595.