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Modeling the pH effect on sulfidogenesis in anaerobic sewer biofilm

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ARTICLE INFO

Article history:

Received 24 July 2013

Received in revised form

8 November 2013

Accepted 14 November 2013

Available online 22 November 2013

Keywords:

Inhibition

pH

Sewer biofilm

Modeling

Sulfide production

ABSTRACT

Wastewater pH affects the transfer of H₂S from liquid to gas and hence impacts on the extent of odour and corrosion. Sewage pH may change significantly in sewer networks due to, for example, the fermentation of organic compounds, particularly when the hydraulic retention time is long, the discharge of trade wastes that are organic-rich or with extreme pH, and the intentional addition of alkali to raise the sewage pH. Such a variation of pH is expected to affect sulfate reduction by sewer biofilms. In this work, experimental studies were carried out to reveal the detailed effects of pH on sulfidogenesis by anaerobic sewer biofilms developed in a laboratory sewer reactor fed with real sewage, and the potential causes for the inhibitory effects observed under both high and low pH conditions. The data clearly showed that the sewer biofilm had the highest sulfate reduction rate at around neutral pH (6.5–7.5), and the activity decreased when pH was higher or lower. The data also suggested that the inhibiting effect at a higher pH is likely due to the inhibitory effects of free ammonia, while at a lower pH it is more likely due to the pH itself. An inhibition model formulated based on the above hypotheses was able to adequately describe the measured sulfide production rates under different pH levels and different free ammonia concentrations, as well as data previously reported in literature. The calibrated model was used to simulate sulfide production in a sewer network receiving alkali dosage. The model adequately predicted sulfide production in the network, which could not be achieved with a model not considering the pH effect.

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1. Introduction

Anaerobic conditions in sewer biofilms, especially in forced mains, result in the generation of hydrogen sulfide, which is the result of the microbiological reduction of sulfate to sulfide by sewer biofilm and the microbial breakdown of sulfur-containing organics such as proteins. The generation and emission of hydrogen sulfide has since long been identified as

a major cause of corrosion and odor problems in a sewer system (Pomeroy and Parkhurst, 1977; Boon and Lister, 1975; Thistlethwayte, 1972; USEPA, 1974). Cost effective management of these problems is a major challenge for water utilities.

The modeling of sulfide formation in sewers has attracted considerable attention in the last few decades since it provides strong support to water utilities to achieve cost-effective sulfide management. Earlier models developed in 1970s were empirical in nature (Pomeroy and Parkhurst, 1977; Boon and

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<http://dx.doi.org/10.1016/j.watres.2013.11.019>

Lister, 1975; Thistlethwayte, 1972). Factors affecting H₂S production considered in these models are limited to a few, leading to inadequate sulfide prediction under many circumstances. For example, both the Boon and Lister (1975) and Pomeroy and Parkhurst (1977) models ignored the effect of sulfate concentration, rendering them inapplicable to situations where sulfate becomes a limiting substrate (Hutchinson and Hamilton, 2005). In recent years, a more comprehensive and detailed sewer model, named the Wastewater Aerobic/Anaerobic Transformations in Sewers (WATS) model, has been developed by Hvitved-Jacobsen and co-workers (Hvitved-Jacobsen, 2002). Both the anaerobic and aerobic processes involving multiple carbon and sulfur species are incorporated into the model. This model is a major step forward, compared with earlier models based on empirical expressions as more biological, chemical and physical processes have been included (Nielsen et al., 2005; Yongsiri et al., 2004). Sharma et al. (2008a) reported the development of the SeweX model, which has been widely applied in Australia to predict dynamic sulfide generation in sewer by taking into account the dynamics of sewer flow and wastewater composition, as well as the addition of various chemicals such as oxygen, calcium nitrate, ferrous or ferric chloride and magnesium hydroxide (Sharma et al., 2008b; 2012). A detailed biofilm model, which takes into account the growth and activity of the heterotrophic, autotrophic and sulfate reducing bacteria in sewer biofilms, has been recently developed by Jiang et al. (2009).

Sharma et al. (2013) recently reported that the sewage pH varies considerably both temporarily and spatially during its transport in sewers, and extended the SeweX model to predict pH variation in sewers. Several field measurements carried out by these authors revealed that pH of raw sewage discharged from households varies between 7.2 and 8.5, with the peak pH observed at around 8:00 AM and lowest pH around mid-night and early morning. This variation of pH was postulated to be linked to human activities as a strong correlation was observed between pH levels and the ammonia and phosphate concentrations believed to be due to the discharge of urine and feces (Sharma et al., 2013). During the transport of sewage in sewers, fermentation of organic matter occurs. The production of volatile fatty acids (VFAs) generates protons resulting in pH decrease, the extent of which depends upon the hydraulic retention time (HRT), pipe surface to volume (A/V) ratio and the wastewater composition. pH variation due to both of the above causes has a time scale of hours.

Temporal pH variation is also expected in sewers receiving the discharge of industrial wastewater with pH that is different from that in sewage. Brewery wastewater for example has a pH of 4.0–6.5 (Sudarjanto et al., 2011; Feng et al., 2008), while dairy wastewater has a pH of 8.0–11.0 (Ince, 1998). Some of these industrial wastewaters also have very high organic contents causing large production of VFAs, resulting in further lowering of the wastewater pH during its transportation (Sudarjanto et al., 2011). In some cases, alkali such as Mg(OH)₂ is deliberately added to sewers to raise pH to 8.5–9.0, to reduce the molecular H₂S fraction of the total dissolved sulfide and hence its liquid to gas mass transfer (Ganigué et al., 2011).

pH is an important parameter because it not only affects the emission of hydrogen sulfide through influencing the chemical equilibrium between H₂S and HS⁻, but also impacts on the sewer biofilm activities (Gutierrez et al., 2009). Among other possible influences, the change in pH causes disruption of cell homeostasis due to passive transport of free acids or bases across the cell membrane and their subsequent dissociation inside cells (Henderson, 1971). Since the concentrations of free acids or bases relative to the corresponding ionic compounds are strongly pH dependent, pH can be considered as the cause of inhibition (Batstone et al., 2002). Key free acids or bases include free organic acids such as acetic acid with pKa of 4.8 at 25 °C, free ammonia with pKa of 9.3 at 25 °C, and dissolved hydrogen sulfide with pKa of 7.0, also at 25 °C (Lide, 1999; Sillén and Martell, 1971).

A number of models have been suggested for incorporating the inhibiting effects of pH, free organic acids, free ammonia and H₂S into the anaerobic digestion models, as summarized in Table 1. The application of these models has so far been limited to anaerobic digestion where relatively high substrate levels prevail. Also, these models have mainly been applied to fermentation, acetogenic, acetoclastic, and hydrogenotrophic processes (Angelidaki et al., 1993; Siegrist et al., 2002) rather than the sulfidogenesis process. Fedorovich et al. (2003) incorporated their H₂S inhibition and Angelidaki's pH inhibition models (Angelidaki et al., 1993) to sulfate reduction in an extended anaerobic digestion model. However, this work has been limited to a narrow range of pH (7.0–8.0). To the best

Table 1 – Summary of models taking into account the potential inhibitory effects of pH, free acids and bases.

Description	Model equation	Reference
Rate of biological activity	$k = k_{\max} \cdot I_1 \cdot I_2 \cdot I_3 \cdot I_4$	
Empirical upper and lower pH inhibition	$I_1 = \frac{1 + 2 \times 10^{0.5(\text{pH}_{\text{UL}} - \text{pH}_{\text{LL}})}}{1 + 10^{(\text{pH} - \text{pH}_{\text{UL}})} + 10^{(\text{pH}_{\text{LL}} - \text{pH})}}$	Angelidaki et al. (1993)
Empirical lower pH inhibition only	$I_1 = \exp \left[-3 \left(\frac{\text{pH} - \text{pH}_{\text{UL}}^+}{\text{pH}_{\text{UL}}^+ - \text{pH}_{\text{LL}}^+} \right)^2 \right]$	Ramsay (1997)
Non-competitive pH inhibition – strong	$I_1 = \frac{1}{1 + \left(\frac{S_{\text{H}}}{K_{\text{I,H}}} \right)^2}$	Siegrist et al. (2002)
Competitive inhibition due to free acid (acetate)	$I_2 = \frac{1}{1 + \left(\frac{K_{\text{I,AO}}}{S_{\text{AO}}} \right)^2}$	Angelidaki et al. (1993)
Non-competitive inhibition (applicable to free ammonia inhibition)	$I_3 = \frac{1}{1 + \frac{S_{\text{FA}}}{K_{\text{I,FA}}}}$	Angelidaki et al. (1993), Ahring and Westermann (1988), Siegrist et al. (2002)
Non-competitive inhibition – strong (applicable to free ammonia inhibition)	$I_3 = \frac{1}{1 + \left(\frac{S_{\text{FA}}}{K_{\text{I,FA}}} \right)^2}$	Siegrist et al. (2002)
Inhibition due to H ₂ S	$I_4 = \frac{1}{1 + \frac{S_{\text{H}_2\text{S}}}{K_{\text{I,H}_2\text{S}}}}$	Fedorovich et al. (2003)

knowledge of the authors, the effect of pH on sulfide production by anaerobic sewer biofilms has not been studied to date.

In sewers, the consideration of the impact of pH is generally limited to the modeling of chemical and physical processes such as chemical oxidation of sulfide (Nielsen et al., 2003) and emission of H₂S (Yongsiri et al., 2003). The impact of pH on biochemical processes has been neglected to date. Indeed, there is a lack of data that describes the detailed effects of pH on the activity of sewer biofilm. Recently, there have been some attempts to investigate the impact of high pH on sulfide production in a laboratory sewer system (Gutierrez et al., 2009). The study revealed 30% reduction in sulfide production at pH 8.6 and 50% reduction at pH 9.0. However, the data are still scarce and the dependency of the biological activity of sewer biofilm on pH in both acidic and basic ranges remains largely unknown.

The aim of this study is to develop a model to predict the impact of pH on sulfate reduction by sewer biofilm for a wide range of pH. For that end, the sulfate-reducing rate of a laboratory sewer biofilm reactor was measured in the pH range of 4.0–9.0. Additional experiments were carried out to identify the potential causes of the inhibition observed. A mathematical model was then proposed and calibrated to describe the inhibitory effects. Finally, the model was shown to adequately predict sulfide production in a real sewer network receiving alkali addition.

2. Materials and methods

2.1. Laboratory sewer reactor setup and operation

A laboratory reactor representing a section of a sewer pipe was used to grow sewer biofilm. Fig. 1 shows a schematic of the experimental setup. The reactor was made up of a Perspex™ cylinder 80 mm in diameter and 150 mm in height. Internal surface area of the reactor was 0.06 m² and the volume of the reactor was 1.0 L. The reactor was sealed with rubber O-rings at the top and bottom to make it air-tight. Plastic Kaldnes K1 carriers (circular, 1 cm diameter/length; Anox Kaldnes, Norway) were placed on three steel rods inserted into the reactor to provide an additional surface area of 0.0033 m²/rod. Three lateral ports, one for pH sensor and the other two for collecting wastewater samples were provided. The reactor lid was connected to a 70 mL container filled with

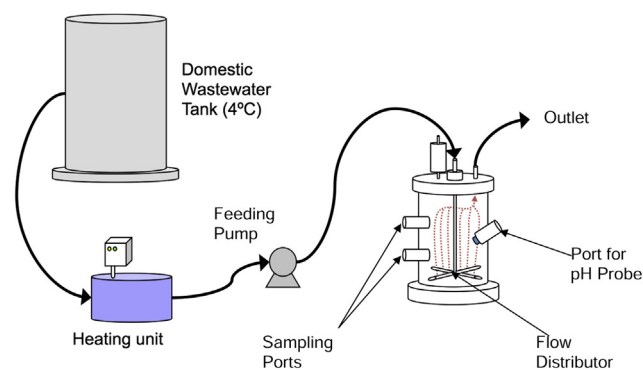


Fig. 1 – A schematic of the experimental setup.

the same wastewater as in the reactor so as to prevent vacuum pressure during sampling. The whole outside reactor surface was covered with aluminum foil to protect the biofilm from being exposed to light.

The reactor was fed intermittently with domestic wastewater using a peristaltic pump (Masterflex® model 7520-47). A pumping pattern consisting of 16 pumping events of varying intervals each day, similar to that of the UC09 rising main (Gold Coast, Australia) as detailed in Guisasola et al. (2008), was applied. The pump flow rate was maintained at 0.5 L/h with each pumping event lasting for 2 min. The hydraulic residence time (HRT) in the reactor varied between 1 and 9 h with an average of 3 h. The reactor contents were continuously mixed using a magnetic stirrer (Heindolph® model MR 3000R) throughout the operation. The mixing conditions corresponded to a Reynolds number of 6400, which indicated turbulent flow conditions in the reactor. Calculation of shear stress to the biofilm on reactor walls, which is main contributor to overall biofilm activity, showed an average value of 1.6 Pa. The shear condition in the reactor was therefore similar to that normally observed in rising main sewers while a pump is running.

2.2. Wastewater characteristics

The reactor was fed with wastewater collected weekly from the Roberston Park Pump Station (Indooroopilly, Brisbane, Australia), which mainly receives domestic wastewater from the surrounding area. Immediately after the collection, wastewater was transported to laboratory and was stored at 4 °C in a cold room to minimize the biological activity so that its composition remains stable. Composition of the wastewater is shown in Table 2. The wastewater was preheated to 20 °C before being pumped into the reactor (Fig. 1).

2.3. Batch experiments to investigate the impact of pH

The reactor was operated for 6 months before the experiments reported below were conducted. The reactor reached pseudo steady state performance, as evidenced by the stable sulfate reduction rates measured over time.

A number of batch tests were conducted to investigate the short-term effect of pH on the sulfate reduction rate by the sewer biofilm. For each test, 5 L wastewater was collected from the storage tank and heated to the room temperature (20 °C). The pH was then adjusted to a pre-designed level (3.9, 4.8, 5.0, 5.5, 6.3, 6.7, 7.0, 7.1, 8.0, 8.5 and 9.0) using either a 1 M NaOH or a 1 M HCl solution as applicable. Batch tests were conducted at each of these initial pH levels, one test at each pH level except 7.1 and 8.5, where duplicate tests were conducted making 13 batch tests in total. The pH-adjusted wastewater was then pumped through the sewer reactor at a flow rate of 0.5 L/min. A batch experiment was started when the pump was turned off. Liquid samples were collected every 30 min over a period of 2 h for the analysis of dissolved inorganic sulfur species, VFAs and ammonium using the methods described in 2.6. In each of the batch tests, the change in pH during the test was monitored and the pH was maintained at the intended level by the manual addition of NaOH solution. Sulfate reduction rate was calculated by linear regression of

Table 2 – Characteristics of domestic wastewater used in this study.

pH	Sulfide (mg S/L)	Sulfate (mg S/L)	Sulfite and thiosulfate (mg S/L)	Ammonium (mg N/L)	Volatile fatty acids (mg COD/L)
6.5–7.5	<3	10–25	<1	50–60	40–60

the collected sulfate data. All the batch experiments were conducted at $20 \text{ }^\circ\text{C} \pm 1 \text{ }^\circ\text{C}$.

2.4. Batch experiments to investigate the impact of free ammonia

Four additional batch tests were performed to study the impact of free ammonia on sulfide production. In each experiment, the wastewater pH was adjusted to a constant level of 8.5 at the beginning of the test with manual addition of NaOH, which was followed by the addition of a known volume of a concentrated ammonium solution (10 g N-NH₄⁺/L), prepared with the ammonium chloride salt. The added initial ammonium concentrations in the tests were 80, 99, 153, and 223 mg N/L respectively, with the true initial ammonium concentrations being approximately 60 mg N/L due to the presence of ammonium in sewage. The free ammonia concentration in each test was estimated using the following equation (Anthonisen et al., 1976).

$$[\text{NH}_3] = \frac{[\text{NH}_{\text{total}}] \times 10^{\text{pH}}}{\frac{1}{K_a} + 10^{\text{pH}}} \text{ where } \frac{1}{K_a} = \exp\left(\frac{6334}{273 + T}\right) \quad (1)$$

where [NH₃] is the free ammonia concentration (mg N/L), [NH_{total}] is the total ammonia (ammonium plus ammonia) concentration (mg N/L), K_a is the equilibrium constant of the NH₄⁺/NH₃ pair (K_a = 10^{-9.24} at 25 °C) and T is the temperature (°C).

Batch experiments were conducted and the sulfate reduction rates determined, as explained in the previous section.

2.5. Batch experiments to investigate the impact of hydrogen sulfide

Batch tests were conducted in an anaerobic laboratory sewer reactor during various stages of biofilm development to monitor the change in the sulfide concentration after fresh wastewater was fed. Due to the gradual change in the activity of the biofilm, the dissolved sulfide concentration at the end of the measurement period (typically of about 45 min) varied. During each of the batch tests conducted, pH was monitored continuously, while dissolved sulfide concentration was measured using an S::CAN UV-VIS spectro: lyzer sensor (Messtechnik GmbH, Austria) as described in Sutherland-Stacey et al. (2008). As the measurement was done once in every two minutes, the collected data allowed calculation of sulfide production rate as a function of time, and hence the H₂S concentration (calculated from the measured total dissolved sulfide and pH) by considering 4 data points at a time.

2.6. Chemical analysis

Wastewater samples were withdrawn from the reactor through a sampling port using sterile 10 mL syringes. The samples were immediately filtered using 0.22 μm filters (Milipore, Millex GP) and stored in vials suitable for the analytical instrument in an icebox. The samples were analyzed for various species using the methods outlined in Table 3.

2.7. Model

A model describing the impact of pH on sulfate reduction was developed using the data collected in this study, as well as

Table 3 – Details of the analytical methods used.

Species	Sample preparation and preservation	Analytical method/material	Reference
Dissolved sulfur species (SO ₄ ²⁻ , HS ⁻ , S ₂ O ₃ ²⁻ , SO ₃ ²⁻)	0.5 mL of sulfide anti-oxidant buffer (SAOB) + 1.5 mL filtered wastewater sample in 2.0 mL IC vials, samples stored at 4-C until analysis was done	Ion chromatograph equipped with an UV and conductivity detector (Dionex® ICS-2000)	Keller-Lehmann et al. (2006), Gutierrez et al. (2008)
Volatile Fatty Acids (VFAs)	0.9 mL of filtered wastewater + 0.1 mL of formic acid injected in gas chromatography vials. Stored at 4-C.	Perkin-Elmer Autosystem gas chromatography with a polar capillary column DB-FFAP at 140 °C. The injector and flame ionization detector (FID) were operated at 220 and 250 °C respectively. High purity helium was used as carrier gas (17 ml/min).	
Ammonium concentration	5 mL of filtered wastewater injected in a 10 mL sterile vial. Stored at 4-C.	Flow injection analysis (FIA)	

long-term data previously reported in literature (Gutierrez et al., 2009). A number of possibilities based on different inhibitory mechanisms were examined. Various combinations of models describing the combined effects of pH and related inhibiting species (i.e. NH_3 , H_2S and HAC) were evaluated, and the combination that described the experimental data with minimum sum of squared errors was selected. The model parameters were determined from the experimental data using the Nelder-Mead Simplex minimization algorithm. The confidence intervals of parameter estimates were determined using the Fisher Information Matrix (FIM) as described in Dochain and Vanrolleghem (2001). The covariance matrix (COV), standard errors (σ) and confidence intervals were estimated as follows:

$$\text{COV}(\theta_0) = \text{FIM}^{-1} \quad (2)$$

$$\sigma(\theta_i) = \sqrt{\text{COV}(i, i)} \quad (3)$$

$$\text{CI} = \theta \pm t_{\alpha, N-P} \cdot \sigma(\theta_i) \quad (4)$$

where, θ is the parameter value, i is the index of the parameter, t is the Student-t distribution value for the confidence level α , N is the number of data sets and P the number of parameters.

2.8. Model application

A field study was conducted on the Tugun Elanora sewer system in Gold Coast (Figure S1, Supplementary Information), Australia to evaluate the impacts of magnesium hydroxide dosing (and hence the raised pH) on sulfide production. The sewer system consists of 13.9 km rising mains with pipe diameters varying from 100 mm to 600 mm. The system collects wastewater from 13 pump stations and the average daily flow during the study period was approximately 15 ML. The network receives $\text{Mg}(\text{OH})_2$ dosing at 1705 kg per day in order to elevate sewage pH to 7.8–9.0 and thus reduces H_2S emission.

To evaluate the effect of pH elevation on sulfide production in the network, pH and dissolved sulfide levels at the end of the sewer system were measured continuously using an online pH meter (MiniChem) and an online UV–VIS spectrolyzer (Sutherland-Stacey et al., 2008), respectively, several weeks prior to and after the commencement of the $\text{Mg}(\text{OH})_2$ dosing.

The inhibition model developed and calibrated using the laboratory study data was incorporated into the SeweX model. The SeweX model (Sharma et al., 2008a, 2008b) is a dynamic sewer model, which takes into account the hydraulics of the sewer flow and predicts the changes in wastewater composition in spatial and temporal scales. The model describes biological, chemical and physical processes that occur in the sewer system, and can be used to investigate temporal and spatial variations of sulfide production in rising and gravity main sewers. Furthermore, the model is capable of predicting changes in wastewater composition including pH with or without chemical dosing (Sharma et al., 2013).

The SeweX model enhanced with the pH inhibition component was employed to model sulfide production in the Tugun Elanora sewer network for the cases with and without magnesium hydroxide dosing. All the pump stations of the

sewer network were equipped with constant speed pumps controlled by water levels in the wet well. The supervisory control and data acquisition (SCADA) data could be obtained from all the pump stations except two. The data provided the exact timings when the pumps were turned on and off. However, the data was collected on different dates for different pump stations. Drawdown tests were conducted to all the major pump stations to verify the pumping rates. For others, the flow rates were obtained from the communication with the operators. For the pump stations for which no such data was available, the pumping pattern was assumed to be the same as the one with similar daily flow. Pump flow rate from SCADA and drawdown tests was estimated as follows:

$$Q_{\text{in}}(t_1 - t_0) = V \quad (5)$$

$$Q_{\text{in}}(t_2 - t_1) + V = Q_{\text{pump}}(t_2 - t_1) \quad (6)$$

$$Q_{\text{pump}} = V \cdot \frac{(t_2 - t_0)}{(t_1 - t_0) \cdot (t_2 - t_1)} \quad (7)$$

where Q_{in} is the average sewage flow rate into the wet well between t_0 and t_1 ; Q_{pump} is the constant pump flow rate when the pump is turned on; V is volume of water in the wet well that is pumped out in each pump cycle calculated from water levels and the dimensions of the wet well; t_0 is the time when pump stopped during the previous pump cycle, t_1 is the time when the pump started in the current cycle, and t_2 is time when pump stopped during the current pump cycle.

The pH, VFA and sulfate levels in the wastewater entering the network were measured through grab sampling on an hourly basis and offline analysis employing the methods summarized in Table 3. Other wastewater composition parameters were assumed according to typical domestic wastewater for this region (Sharma et al., 2008b). The buffering capacity of the wastewater was determined by using an online titration device fitted with a dosing pump and a pH sensor, as previously described in Sharma et al. (2013). The model-predicted sulfide results were then compared with the measured data to evaluate the accuracy of the model.

3. Results and discussion

3.1. Impact of pH on sulfate reduction

Batch experiments were conducted at various pH and ammonia levels and the data collected from these tests was used to calculate the sulfate reduction rates. As an example, the sulfate profile during one batch test is shown in Fig. 2. The rate of sulfate reduction, which is the same as the rate of sulfide production, was calculated through linear regression.

Fig. 3 shows changes in sulfate-reduction rate as a result of the pH change. Two sets of data are presented: (1) the data collected in this study where short term change of pH was applied; and (2) data from a previous study by Gutierrez et al. (2009) in which, the sulfate reduction rates were measured after several months of adaptation of the sewer biofilm to elevated pH levels. Gutierrez et al. (2009) used the same reactor set-up as the one used in this study, wastewater

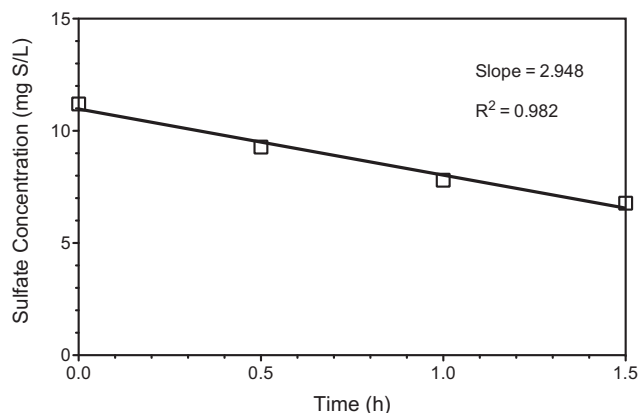


Fig. 2 – Sulfate concentration profile during a batch test (pH = 8.5, Ammonia + ammonium concentration = 99 mg N/L), and calculation of sulfate reduction rate.

collected from the same source as the current study was used, and reactor was operated in a similar manner. The rates of sulfide production are therefore expected to be comparable in the two cases.

The sulfate reduction rate varied with pH reaching the maximum level (5.8–6.6 mg S/L.h) near neutral pH (6.5–7.5). There was a gradual decrease in the sulfate reduction rate with the shift of pH away from the neutral range. The results demonstrated that pH outside the range of 6.5–7.5 has inhibiting effects on the sulfate reducing activity of sewer biofilms, with 50% inhibition achieved at approximately pH 4.0 and pH 9.0, respectively. The dependency of biological activity on pH is well documented in literature and the extent of inhibition is shown to depend on several factors including the type of microbial culture, the type of growth substrates, and the type of reactor (suspended growth or biofilm), and hence cannot be generalized. The information available in literature can't therefore be applied directly to sewer biofilms

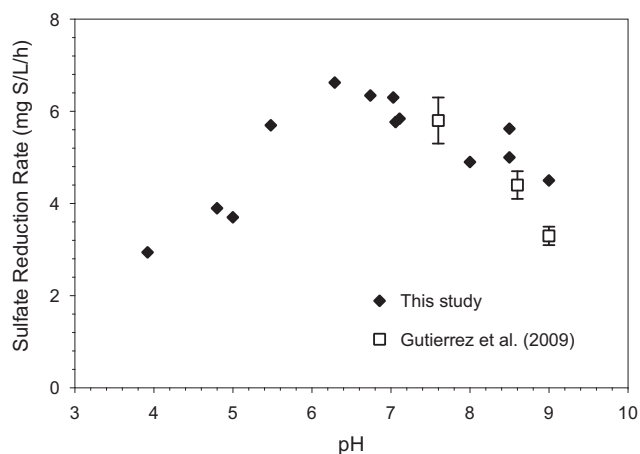


Fig. 3 – Variation of sulfate-reduction rate by anaerobic sewer biofilm with pH. Data obtained in this study are based on batch tests; data from Gutierrez et al. (2009) are based on long-term average rates of biofilm adapted to the specific pH.

without experimental verification. However, the fact that Fig. 3 resembles the typical behavior of a biological system as a response to pH change (O'Flaherty et al., 1998) suggests that the inhibitory effects of pH on sewer biofilm activities are not dissimilar to those observed in other systems.

3.2. Investigation of causes for the inhibition of sulfidogenesis

As the change in pH is associated with the changes in relative amounts of free acids such as H_2S_{aq} and HAc (at lower pH) and free ammonia (at higher pH), it can not be ruled out that the pH effects observed were due to the change in these compounds that are known to have inhibitory effects on microbial activities. For reliable modeling of the 'pH' effects, an understanding of the underlining causes of pH inhibition is necessary.

Free ammonia concentration increases with pH as per Equation (1). It has been reported that free ammonia can cause microbial inhibition particularly in the alkaline pH range. It is therefore necessary to differentiate whether the inhibition observed in Fig. 3 is due to a higher pH or a higher free ammonia concentration resulting from a higher pH for a given total ammonium concentration. Fig. 4 shows the variation in the sulfate reduction rate when the free ammonia concentration varied between 0.1 and 25.5 mg N/L. The data shown in this figure are collected from three different tests: (1) short-term experiments performed at constant pH of 8.5 and variable total ammonium ($NH_3 + NH_4^+$) concentration (80–223 mg N/L, this study); (2) short-term experiments performed at different pH (3.9–9.0) and constant total ammonium concentration of approximately 60 mg N/L (this study); and (3) long-term experiments performed at different pH (7.6, 8.6 and 9.0) and constant total ammonium concentration of approximately 50 mg N/L (Gutierrez et al., 2009). A consistent trend is observed in Fig. 4, where the sulfate reduction rate decreases as the free ammonia concentration increases. A maximum sulfate reduction rate of 6.5 mg S/L/h was observed at free ammonia concentration of below 1 mg N/L, the lowest

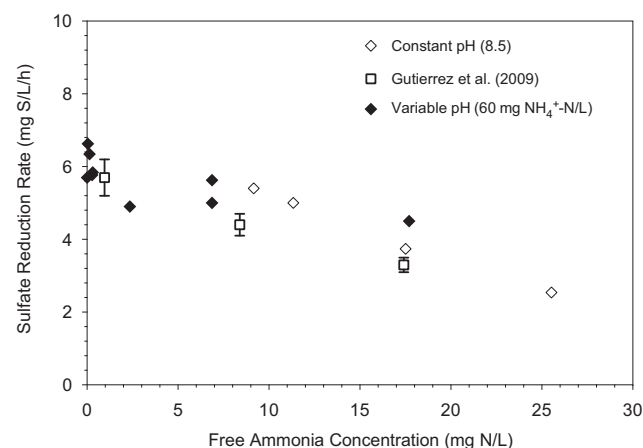


Fig. 4 – Variation of sulfate reduction rate with the concentration of free ammonia. Data obtained in this study are based on batch tests; data from Gutierrez et al. (2009) are based on long-term average rates of biofilm adapted to the specific pH.

free ammonia concentration corresponding to neutral pH and a relatively low total ammonium concentration of 50–60 mg N/L. At a free ammonia concentration of 25.5 mg N/L, the sulfate reduction rate was only 2.5 mg S/L.h. These results suggest that free ammonia likely played a significant role in the inhibitory effects reported in Fig. 3 in the basic pH range, while the direct effect of pH is not so obvious as the sulfate reduction rate decreased linearly with the increase in FA concentration irrespective of the pH. The inhibition of sulfate reduction at basic pH can therefore be modeled as free ammonia inhibition.

Batch tests were also conducted to investigate the impact of hydrogen sulfide on the sulfate reduction rate as explained in Section 2.5. The instantaneous sulfide production rates were obtained from the measured sulfide concentration data in 4 separate batch tests (Figure S2, Supplementary Information) by considering 4 data points at one time. As the overall rate of sulfide production was different in these batch studies, normalization of the rates was necessary, which was done by dividing the instantaneous rates for each of the data sets by the average sulfide production rate obtained through straight line fitting of the same data set. The normalized sulfide production rates obtained from the 4 experimental data sets are collectively presented in Fig. 5. Although some variation of sulfide production rate with H₂S concentration is observed, the data clearly suggests that the increase of H₂S concentration up to the concentration of 16 mg S/L didn't cause decrease in sulfide production rate. Maximum H₂S concentration in the experiments presented in Section 3.1 was only 6.0 mg S/L.

There have been a number of studies on anaerobic systems showing levels of H₂S inhibitory to sulfate production (O'Flaherty et al., 1998). However, the inhibitory sulfide levels reported in literature are much higher than those expected in sewer networks and what we applied in our tests. For example, for mixed culture with acetate as the substrate, the level of dissolved H₂S to cause 50% inhibition is reported in the range of 2.6–5.0 mmol/L (83–160 mg S/L) at the pH range of 6.5–8.0 (Koschorreck, 2008). Therefore, our results are not inconsistent with previous studies. It can be concluded that

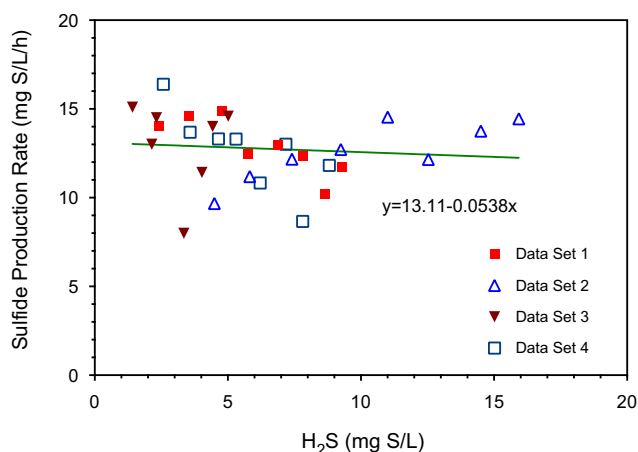


Fig. 5 – Sulfate reduction and sulfide accumulation in a batch test aimed to demonstrate that sulfate reduction is not inhibited by sulfide for sulfide levels in sewers.

the level of sulfide common to sewer systems, which normally ranges from 1 to 20 mg S/L (equivalent to 0.5–10.0 mg dissolved H₂S–S/L), should not have any inhibitory effects on sulfide production.

Acetic acid has also previously been reported to inhibit microbial activities (Ahring et al., 1995; Angelidaki et al., 1993; Fukuzaki et al., 1990; Gourdon and Vermande, 1987). These studies have been mainly conducted in high rate anaerobic systems where acetic acid and other volatile fatty acids were present at the total VFA concentration above 2–3 g/L. The levels at which total acetic acid and total propionic acid become inhibitory to microbial activity are reported to be 3 g/L and 3.7 g/L, respectively (Ahring et al., 1995). In sewers, acetic acid is the main form of VFA and is present at concentrations ranging from 10 to 60 mg/L (Sharma et al., 2008a). Other VFAs such as propionic acid and butyric acid exist at much lower concentrations than acetic acid. Due to such low concentrations, acetic acid and other VFAs are not expected to have any impacts on the biological rate.

From the discussion above, the inhibition of biofilm activity observed in Fig. 3 under acidic pH conditions was likely not caused by H₂S or organic acids. We will therefore attempt to model the inhibition under acidic pH with pH as the ‘inhibitor’.

Another possible cause for reduced sulfide production under acidic conditions could be related to the increased availability of heavy metals (Morton et al., 1991). The heavy metals bound to other inorganic compounds can be released to water phase under acidic conditions, which may result in a decrease in dissolved sulfide concentration due to the formation of metal sulfide precipitates. This phenomenon however was not investigated in this study.

3.3. Model formulation and parameter identification

Based on the results presented above, we propose to model the effect of pH on sulfate reduction by anaerobic sewer biofilms as follows:

1. For the lower range of pH, the pH effect is modeled using the lower pH inhibition expression given by Angelidaki et al. (1993). This empirical pH model is also expected to take into account the effect of free acids, if any (Batstone et al., 2002).

$$I_1 = \exp \left[-3 \left(\frac{\text{pH} - \text{pH}_{\text{UL}}^*}{\text{pH}_{\text{UL}}^* - \text{pH}_{\text{LL}}^*} \right)^2 \right] \quad (8)$$

The variables are as explained in the Nomenclature section.

2. For the higher range of pH, only the effect of free ammonia is considered using the non-competitive inhibition model of Siegrist et al. (2002). The square term in denominator is to take into account the strong inhibiting effect.

$$I_2 = \frac{1}{1 + \left(\frac{S_{\text{FA}}}{K_{\text{I,FA}}} \right)^2} \quad (9)$$

The variables are as explained in the Nomenclature section.

3. The complete 'pH' inhibition model is therefore:

For $\text{pH} < \text{pH}_{\text{UL}}$,

$$k = k_{\text{max}} \cdot I_1 \cdot I_2 \quad (10)$$

For $\text{pH} > \text{pH}_{\text{UL}}$,

$$k = k_{\text{max}} \cdot I_2 \quad (11)$$

Model parameters were determined by using the optimization algorithm described in Materials and Methods, with the confidence levels also determined. The fit between the model predictions and the experimental data reported in Figs. 3 and 4 are shown in Fig. 6(A) and (B), respectively. A good agreement between the model predictions and the experimental data validates the approach used here.

The parameters of the model (k_{max} , pH_{UL} , pH_{LL} , and $K_{\text{I,FA}}$) determined are presented in Table 4 along with the 95% confidence intervals of these estimates. The parameter values showed small variability. The Correlation Matrix (CM) computed is shown in Equation (12). The correlations between the model parameters are low in general, implying that the model has good identifiability.

	k_{max}	pH_{UL}	pH_{LL}	$K_{\text{I,FA}}$
k_{max}	1.000	0.520	-0.242	-0.418
pH_{UL}	0.520	1.000	-0.861	-0.217
pH_{LL}	-0.242	-0.861	1.000	0.101
$K_{\text{I,FA}}$	-0.418	-0.217	0.101	1.000

(12)

3.4. Model application

The SeweX model enhanced with the addition of a component for pH/free ammonia inhibition on sulfate reduction was employed to model the Tugun Elanora sewer network. Modeling was done for two cases, one without magnesium hydroxide dosing and another with its dosing.

Flow data for each of the pump stations estimated as explained in Section 2.8 was used to obtain flows in all the sewer pipes in the network. In order to calibrate the flow for the model, flow at the end of the sewer was measured on two separate occasions and corresponding total daily flows were

estimated. As the estimated and measured daily flows differed, flows from all the pump stations were modified by the ratio of average measured daily flow to the total estimated daily flow. The modified pump flow rates were then used to obtain calibrated flows for each of the sewer pipes to be used in the model. A comparison of measured and calibrated flow is presented in Figure S3 (Supplementary Information). This method of calibration produced accurate daily flows. However, there were still some discrepancies in the timing of the slugs arriving at the monitoring location, which is attributed to the difference in the actual pumping time and pumping time obtained from SCADA data collected on a different date.

Due to the dosing of magnesium hydroxide, sewage pH at the end of the network varied from 7.8 to 8.9, significantly above the pH levels of 6.9–7.6 prior to the dosing (Figure S4A, Supplementary Information). A significant decrease in dissolved sulfide levels at the end of the network was also observed (Figure S4B, Supplementary Information). Dissolved sulfide measurement showed that the total sulfide discharge was reduced from 77.9 kg S/day prior to dosing to 56.6 kg S/day after the dosing, confirming that the higher pH indeed caused a reduction (27%) in sulfide production, in agreement with laboratory results. Although the inhibitory effects of elevated pH on sulfidogenesis has been previously reported in a laboratory system (Gutierrez et al., 2009), this is the first time that such an observation has been made in a real sewer system.

A comparison was made between the measured and predicted dissolved sulfide levels for the two cases, firstly comparing the dissolved sulfide profiles (Figure S5, Supplementary Information). Although hydraulic retention times and hence the sulfide peaks will be largely dependent on actual pumping patterns, assumptions had to be made since SCADA data for all the stations on the day of dissolved sulfide measurement was not available. It was therefore not possible to precisely predict the timing and magnitude of individual sulfide peaks. The comparison was therefore made in terms of mean, maximum and minimum dissolved sulfide concentrations, which is shown in Fig. 7. The enhanced sewer model was able to predict the sulfide levels at the end of the sewer network without pH elevation very well. However, the model without considering the pH effect on sulfate reduction did not adequately predict sulfide production when pH was elevated by magnesium hydroxide addition. By considering the pH

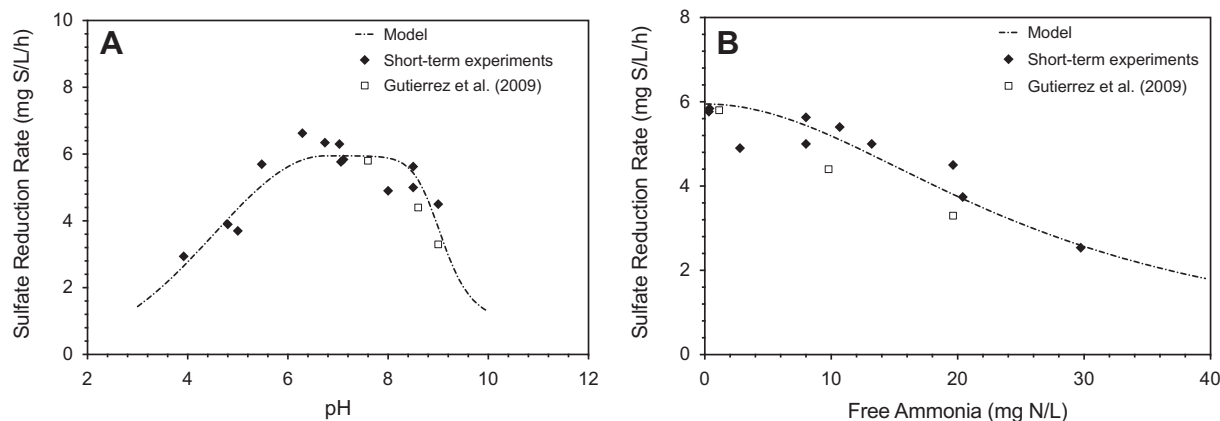


Fig. 6 – Comparison of modeling results with measured data: (A) at different pH levels (B) at different free ammonia levels.

Table 4 – Parameter values.

Parameter	Value	Unit
k_{\max}	5.95 ± 0.02	mg S/L-h
pH_{UL}	6.75 ± 0.06	Units
pH_{LL}	1.30 ± 0.09	Units
$k_{\text{I,FA}}$	26.15 ± 0.3	mg N/L

effect on sulfate reduction using the model discussed in the previous section, a close match between the measured and model predicted data could be obtained as illustrated in Fig. 7.

The good agreement between the model-predicted and measured sulfide levels in the case of magnesium hydroxide addition indicate that the ‘pH’ inhibition model(s) adopted and calibrated using laboratory data is a valid and useful tool for predicting sulfide formation under varying pH conditions. This model can be used to facilitate optimization of chemical dosing for effective sulfide control in sewer systems.

4. Conclusions

The impact of pH on sulfidogenesis by anaerobic sewer biofilm was studied and a model to predict this impact was developed, calibrated and validated. The key conclusions are:

- Sulfate reduction rate by sewer biofilms vary with pH, reaching its maximum in the neutral pH range (6.5–7.5). The rate decreases with the shift of pH away from this range, reaching approximately 50% of the maximum rate at pH 4.0 and 9.0. The inhibiting effects of pH on sulfate

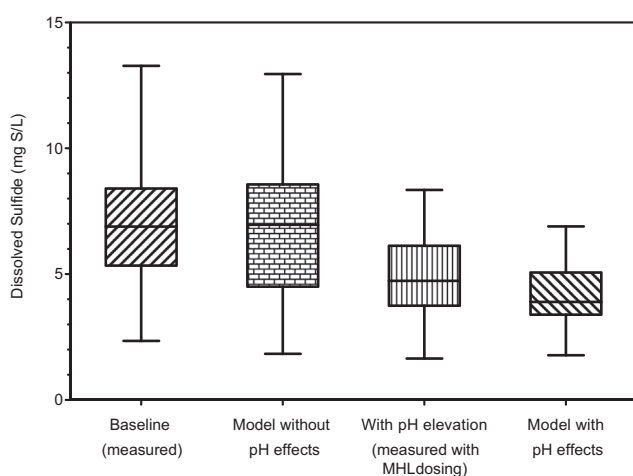


Fig. 7 – Multiple box and whisker plots showing a comparison of modeling results with measured field data with and without magnesium hydroxide dosing at the end of the sewer network (top line: the maximum level, the bottom line: the minimum level, top line of the box, 75%ile, the bottom line of the box, 25%ile; middle line of the box, the average level). Model results without pH effects are to be compared with the baseline results, while the model results with pH effects are to be compared with the measured results with pH elevation.

reduction in the basic range have been verified through a field study.

- Free ammonia likely plays an important role in the inhibitory effects in the alkaline pH range, where the direct effect of pH is not obvious. The inhibition of sulfate reduction at a pH higher than the neutral range could be adequately modeled as free ammonia inhibition.
- Inhibition of sulfate reduction by sewer biofilm under acidic pH conditions is likely caused by the low pH itself rather than by the inhibitory effects of H_2S or organic acids. The inhibition of sulfate reduction at a lower pH could therefore be modeled as pH inhibition.
- To correctly predict sulfide production in sewers, it is important to take the impact of pH variation on sewer biofilm activities into consideration. The inhibition models developed in this study can be used to characterize such effects.

Acknowledgments

The authors acknowledge the financial support provided by the Australian Research Council (d) and many members of the Australian water industry through LP0882016 the Sewer Corrosion and Odour Research (SCORE) Project (www.score.org.au). The authors would like to acknowledge the support provided by Gold Coast City Council and Shaun Corrie in field measurements and data collection. The authors wish to thank Jing Sun for assistance with running some of the laboratory experiments.

Nomenclature

k	Reaction rate (mg S/L-h)
k_{\max}	Uninhibited reaction rate (mg S/L-h)
I_1, I_2, I_3, I_4	Inhibition terms
pH	Wastewater pH
pH_{LL}	Lower pH drop-off value where the growth rate is approximately 50% of the uninhibited rate
pH_{UL}	Upper pH drop-off value where the growth rate is approximately 50% of the uninhibited rate
pH_{LL}^*	pH at which there is complete inhibition
pH_{UL}^*	pH at which no inhibition occurs
S_{H}	Hydrogen ion concentration (mol/L)
S_{AC}	Acetic acid concentration (mg/L)
$S_{\text{H}_2\text{S}}$	Dissolved molecular hydrogen sulfide concentration (mg S/L)
S_{FA}	Free ammonia concentration (mg N/L)
$K_{\text{I,H}}$	Hydrogen ion concentration at which 50% inhibition occurs (mol/L)
$K_{\text{I,Ac}}$	Acetic acid concentration at which 50% inhibition occurs (mg/L)
$K_{\text{I,FA}}$	Free ammonia concentration at which 50% inhibition occurs (mg N/L)
$K_{\text{I,H}_2\text{S}}$	Un-dissociated H_2S concentration at which 50% inhibition occurs (mg S/L)
$[\text{NH}_3]$	Free ammonia concentration (mg N/L)
$[\text{NH}_{\text{total}}]$	Total ammonium + ammonia concentration (mg N/L)
K_{a}	Equilibrium constant for ammonia
T	Wastewater temperature ($^{\circ}\text{C}$)

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.watres.2013.11.019>

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