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Model Parameters for Aerobic Biological Sulfide Oxidation in Sewer Wastewater

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Abstract: Sulfide related odor and corrosion are two of the major problems associated with the operation and maintenance of sewer networks. The extent of the problems is governed by several complex and interrelated processes. Sulfide oxidation is typically the most important process for sulfide removal in wastewater from aerobic gravity sewers. Despite the significance of the process, little is known about the significance of the growth of sulfide oxidizing bacteria (SOB) during the transport of wastewater. Biological sulfide oxidation in wastewater from sewers was investigated in a series of oxygen uptake rate (OUR) experiments. The experiments showed that, for oxygen nonlimiting conditions, sulfate was produced, with elemental sulfur as an intermediate. During each experiment, the activity of the sulfide oxidizing bacteria increased significantly. This was interpreted as the result of bacterial growth related to the oxidation of intermediately stored elemental sulfur. A model concept describing biological sulfide oxidation, with intermediary storage of elemental sulfur and associated growth, was developed. The model was calibrated against the experimental results. The observed average growth rate and yield constant for the SOB were determined at 1.98 d^{-1} and 0.17 g Chemical Oxygen Demand (COD) per g sulfur, respectively. These values correspond to reported values for mixed cultures of autotrophic SOB.

Keywords: sulfide oxidation; wastewater; sewer; modeling

1. Introduction

Sewer systems have been adapted by most modern societies for the collection and conveyance of municipal wastewater. In order to ensure proper functioning of sewer systems, routine maintenance and repair is essential. In this respect, some of the most challenging problems faced by sewerage authorities are those related to the occurrence of hydrogen sulfide (H$_2$S), in terms of odor, toxicity, and corrosion of concrete and metals. In order for engineers to predict the extent of such problems, the complex nature of the sulfur cycle in sewers must be identified and investigated. Several studies, some dating back more than 50 years, have focused on the generation of sulfide in both force mains and gravity sewers (e.g., [1,2]). More recently, the processes responsible for the removal of the generated sulfide have received comprehensive scientific attention; i.e., emission of hydrogen sulfide from the wastewater to the sewer atmosphere, precipitation of metal sulfides, oxidation of dissolved sulfide, and sewer corrosion (e.g., [3–6]). The main natural processes responsible for the removal of dissolved sulfide in gravity sewers are typically chemical and biological oxidation; only under highly turbulent conditions is the rate of sulfide emission likely to exceed that of sulfide oxidation [5,7].

The knowledge of sulfide oxidation in wastewater from sewers is presently at a level where the removal of sulfide can be reasonably well predicted; for example, by incorporation into a sewer process model. An example of such a model is the Wastewater Aerobic/anaerobic Transformations in Sewers (WATS) that has been developed by the authors [8]. In the WATS model, biological sulfide oxidation is currently described by a fixed rate constant, and the model does not include the growth of sulfide oxidizing bacteria

(SOB). In addition, the main intermediate(s) and product(s) of biological sulfide oxidation under varying oxygen and sulfide levels are not fully understood and implemented in the model.

The stoichiometry of biological sulfide oxidation is complex, as sulfide can be oxidized either completely to sulfate, or incompletely by the production of intermediates, such as elemental sulfur, thiosulfate, and sulfite [9]. For the chemical reaction, thiosulfate and sulfate have been reported to be the main products [10].

It is well known that several strains of both heterotrophic and autotrophic bacteria are able to oxidize sulfide, e.g., [11,12]. Based on mass balance considerations of dissolved oxygen and sulfide, a previous study indicated that biological sulfide oxidation in wastewater produced elemental sulfur, which was not further oxidized within 4–6 h [10]. Only a few studies have reported on biological sulfide oxidation rates in wastewater. Generally, the variability of reaction kinetics is significant, making it difficult to predict appropriate parameter values.

During the transport of wastewater in force mains, sulfide levels can exceed $10\,\mathrm{g\,S\,m^{-3}}$ [1,7]. Such levels are sufficient to support significant growth of sulfide oxidizing bacteria. Under optimal growth conditions, chemostat cultures of autotrophic *Thiobacilli* have been found to produce 5–13 mg dry mass per millimole of sulfide (0.2–0.4 g dry mass per g S), when complete oxidation to sulfate takes place [13]. Thus, the concentration of SOB may increase by several g per $\mathrm{m^{-3}}$ by aerobic degradation of the sulfide generated under the anaerobic conveyance of wastewater.

The main objective of the present study was to investigate the kinetics and stoichiometry of biological sulfide oxidation in wastewater, and to quantify associated growth of the SOB. Based on the experimental investigations, a conceptual model describing sulfur transformations is proposed and calibrated. The incorporation of the proposed concept into existing sewer process models, such as WATS, will further improve its validity.

2. Materials and Methods

2.1. Experimental Investigations

The activity and growth of SOB in wastewater from sewers were characterized in a series of oxygen uptake rate (OUR) experiments. The OUR is an activity-related quantitative measurement of the dissolved oxygen consumption for aerobic biological oxidation processes. Such experiments have been developed for investigating biological growth on both organic and inorganic substrates, as well as for characterization of wastewater and activated sludge composition (e.g., [14,15]).

OUR was measured in a completely filled reactor, specifically designed for the sulfide containing wastewater (Figure 1). The wastewater was aerated using pure O_2 (2.5 grade) until the dissolved oxygen concentration (S_O) exceeded $9\,\mathrm{g\,m^{-3}}$, approximately corresponding to 100% air saturation. Aeration was then stopped and the S_O consumption was recorded. When the S_O concentration fell below $1\,\mathrm{g\,O_2\,m^{-3}}$, the sample was aerated again, and the cycle was repeated. The OUR was calculated from the measured S_O concentration time series using a central difference approximation.

The reactor consisted of a borosilicate glass flask (Duran®, Schott AG, Germany), sealed using a butyl rubber stopper. An expansion chamber fitted to the top of the reactor allowed the wastewater volume to expand when aeration took place. During the OUR measurement, the expansion chamber was partly filled with stagnant water, efficiently inhibiting aeration of the reactor volume. In order to prevent heterogeneous catalysis of chemical sulfide oxidation, metal parts were avoided in the experimental setup. Accordingly, all tubing and connections were made of inert materials, such as Tygon© or Peek©. The wastewater contained in the reactor was mixed using a magnetic stirrer, and the reactor was immersed into a thermo-stated water bath, thereby maintaining a constant temperature of $20\,°\mathrm{C} \pm 2\,°\mathrm{C}$.
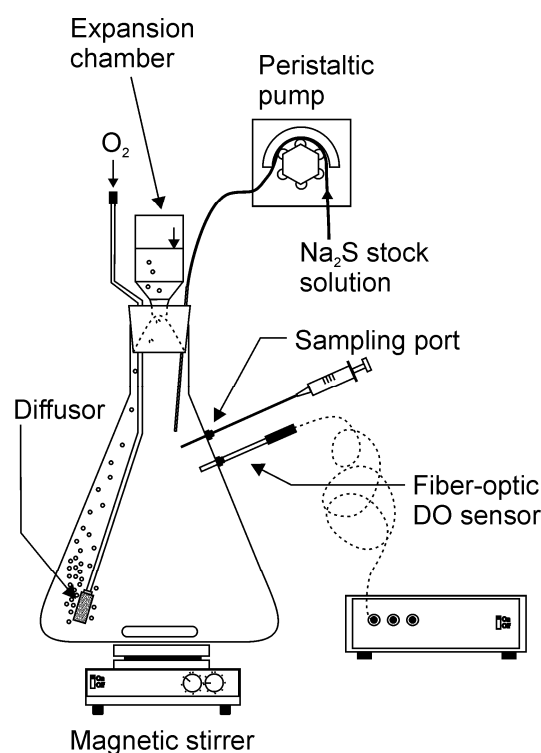
Figure 1. Illustration of the experimental setup for studying aerobic sulfide oxidation in wastewater.

Sulfide addition was initiated approximately 24 h into the experiment, at which point the readily biodegradable organic substrate was reduced and the OUR, therefore, was fairly constant in time. Sulfide was added to a final concentration of 2.5 gS m^{-3} immediately after cessation of each aeration period. This was done in 5–20 consecutive cycles by pumping 1.2 mL of stock solution (3.6 gS L^{-1}) into the reactor within 60 s, using a peristaltic pump (SCI-Q 401u, W-M ALITEA AB, Sweden). A new sulfide stock solution was prepared for each experiment by dissolving prewashed di-sodium sulfide crystals (Na$_2$S.7-9 H$_2$O, Merck, Germany) in deionized water. The concentration of the sulfide stock solution was verified by iodometric titration (APHA et al., 2005). After sulfide addition was stopped, the experiment was continued for more than 10 h in order to study further oxidation of intermediate reaction products, such as elemental sulfur or thiosulfate.

Wastewater for the experiments was sampled at the Frejlev sewer research and monitoring station, Denmark [16]. The wastewater was collected directly from a sewer pipe during dry weather periods. The Frejlev sewer catchment, upstream of the sampling site, serves approximately 2000 person equivalents, and the wastewater is entirely of domestic origin. Previous investigations have shown that the wastewater from Frejlev has a potential for both chemical and biological sulfide oxidation [10]. Compared with sulfide oxidation rates reported in the literature, the rate of chemical sulfide oxidation of wastewater from Frejlev is considered low. This makes the wastewater ideally suited for studying biological sulfide oxidation, as the chemical process will only slightly contribute to the overall process.

2.2. Analytical Procedures

The Chemical Oxygen Demand (COD) and the alkalinity of the wastewater were analyzed according to standard methods [17]. Total sulfide was measured according to the methylene blue method [17]. Samples were preserved until analysis by fixation of the dissolved sulfide in a 1% zinc acetate solution. Absorbance of the methylene blue was measured at 670 nm using a Shimadzu UV-mini 1240 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Sulfate (SO_4^{2-}), sulfite (SO_3^{2-}), and thiosulfate ($S_2O_3^{2-}$) were measured by ion-chromatography with suppressed conductivity detection (Methrom AG, Herisau, Switzerland). The sulfur anions were separated on a METROSEP A Supp

5150 column using NaHCO$_3$ 1.0 mM and Na$_2$CO$_3$ 3.2 mM as eluent, at a flow rate of 1 mL min^{-1}. The total sulfur content of the wastewater was measured by inductively coupled plasma optical emission spectroscopy (ICP-OES), following microwave assisted acid digestion in nitric acid (65%) [18]. The ICP-OES system was a Thermo iCAP 6300 duo (Thermo Fisher Scientific Inc., Waltham, MA, USA) and the microwave digestion system was an Anton Paar Multiwave™ 3000 (Anton Paar GmbH, Graz, Austria). For ICP-OES measurements, Yttrium was used as internal standard and EnviroMAT™ BE-1 certified reference material was used for quality control. All standards and acids were supplied by SCP science (Quebec, Canada) and were of Suprapur® quality.

The dissolved oxygen concentration was measured noninvasively using oxygen sensor spots glued onto the inside reactor wall and a Fibox 3 oxygen meter (PreSens GmbH, Regensburg, Germany). The light signal between the sensor spots and oxygen meter was transmitted via a 2 mm polymer optical fiber. The wastewater pH was monitored during the experiments using a Hamilton Polylite Pro pH-electrode (Hamilton Company, Bonaduz, Switzerland).

3. Results

3.1. OUR Experiments

Figure 2 shows as an example of the results of OUR experiments, with 6, 11 and 20 consecutive additions of sulfide, respectively. During the first 18–24 h of the experiments, the OUR is caused by heterotrophic transformations of organic matter. Initially, the wastewater contains significant amounts of readily biodegradable substrate, which supports substrate nonlimited growth of the heterotrophic biomass. This results in an increase of the OUR during the first 5–10 h of the experiments. After this, the bacterial growth enters a substrate-limited phase, where the remaining available substrate must first be hydrolyzed into smaller molecules that can be transported across the bacterial cell wall. Accordingly, the OUR decreases and, after 18–24 h, the OUR enters a quasi-steady-state phase, where the rate of hydrolysis is balanced by the maintenance energy requirements of the heterotrophic biomass. From this point on, the OUR related to heterotrophic breakdown of organic matter decreases slowly with time, and sulfide addition is started. A thorough description of the processes involved in aerobic degradation of organic matter in wastewater from sewers can be found in Vollertsen and Hvitved-Jacobsen [15].
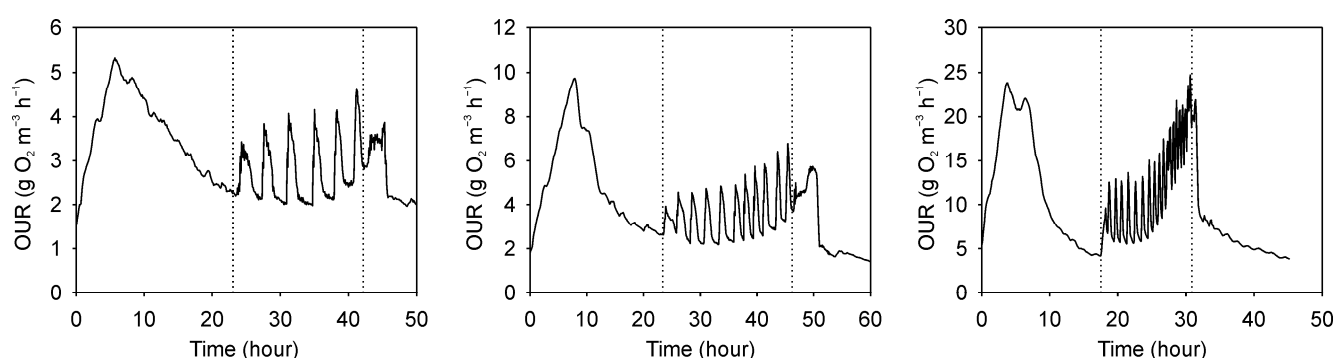
Figure 2. Results of three OUR experiments with 6, 11 and 20 consecutive additions of sulfide, respectively. The dotted vertical lines indicate the period with sulfide addition.

The additions of sulfide revealed several important phenomena from which the likely pathway for sulfide oxidation and associated bacterial growth can be deduced. Each addition of sulfide resulted in a temporarily increase of the OUR, after which the activity returned to a baseline level. The first peak was typically lower and wider than the subsequent 3–4 peaks, which were almost identical. Hereafter, the baseline OUR increased exponentially and the peaks became higher and narrower. After the sulfide addition was

stopped, the OUR continued to increase for a period, after which it dropped to a level comparable to that before the sulfide was added.

The short-lived peaks indicate that the entire amount of sulfide added was oxidized within each aeration cycle. The apparent slow response to the first sulfide addition may be explained by a lag-phase, where the SOB adapts to the new substrate conditions, e.g., activation of the enzyme system involved in bacterial sulfide oxidation. The similar responses of the subsequent 3–4 sulfide additions suggest that only limited growth of the SOB took place during this phase. The subsequent exponential increase of the baseline OUR and the changed peaks indicate that one or more intermediates accumulated during the oxidation process, and that they served as substrates for growth of the SOB.

3.2. Reaction Stoichiometry

The observed response to the sulfide additions is the result of both chemical and biological sulfide oxidation. Previous investigations by Nielsen et al. [19] have shown that chemical sulfide oxidation produces a mixture of thiosulfate ($S_2O_3^{2-}$) and sulfate (SO_4^{2-}), and that the reaction stoichiometry is approximately 1.2 gO_2/gS. However, the chemical reaction contributes only slightly to the overall process, as the concentration of SOB increases during the experiment. Integration of the area of the peaks originating from sulfide dosing (cross-hatched area in Figure 3) resulted in an average ($\pm$standard deviation) reaction stoichiometry (R) of 0.6 ($\pm$0.1) gO_2/gS for all 14 experiments. The most likely reactions for sulfide oxidation, Equations (1)–(3), are listed below (e.g., [20,21]).

$$2\,HS^- + O_2 \rightarrow 2\,S^0 + 2\,OH^- \tag{1}$$

$$2\,HS^- + 2\,O_2 \rightarrow S_2O_3^{2-} + H_2O \tag{2}$$

$$2\,HS^- + 4\,O_2 \rightarrow 2\,SO_4^{2-} + 2\,H^+ \tag{3}$$
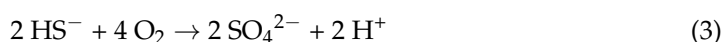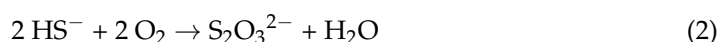
The three reactions correspond to reaction stoichiometries of 0.5, 1 and 2 gO_2/gS, respectively. It is therefore likely that the biological reaction produces elemental sulfur as an intermediate. The total oxygen uptake related to oxidation of both sulfide and intermediates (hatched and cross-hatched area in Figure 3) was 1.8 ($\pm$0.3) gO_2/gS. This is close to the expected value when sulfate is the reaction product. The small difference can be explained by thiosulfate from chemical sulfide oxidation that was not oxidized during the experiment. Thus, sulfate was most likely the product of the biological reaction—Equation (3). SOB of various groups and genera are known to oxidize sulfide to sulfate, with elemental sulfur as the main intermediate [22].
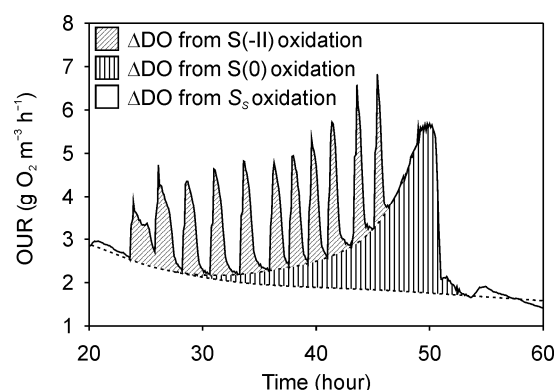
Figure 3. OUR response to 11 repeated sulfide additions. The oxygen consumed for oxidation of sulfide (cross-hatched area) and intermediates (hatched area) are indicated.

The transition period between growth on elemental sulfur, and oxygen consumption related to organic matter transformations, was very short. In Figure 3, this transition occurs after approximately 50 h, and takes less than 1 h. The short transition period indicates

that substrate limitation only occurs when the concentration of elemental sulfur is low. Similarly, the transition period from substrate nonlimited conditions to substrate depletion was also very short for the peaks related to oxidation of sulfide.

3.3. Experimental Conditions

Several factors could possibly have influenced the results. In particular, nitrification, pH effects, and sulfide emission are considered relevant. The possible implications hereof are discussed below.

Nitrifying bacteria are typically present in insignificant concentrations in domestic wastewater [3]. However, the ammonia level of domestic wastewater is sufficient to support significant growth and the corresponding oxygen consumption would complicate the interpretation of results. Experiments with activated sludge have shown that ammonia oxidizers are extremely sensitive to the presence of sulfide. Sears et al. [23] reported that sulfide concentrations as low as 0.25 g m^{-3} completely inhibit the nitrification process. In the present study, the sulfide levels were significantly higher, and nitrification was, therefore, most likely inhibited.

Fluctuations of the wastewater pH can significantly affect the rate of both chemical and biological sulfide oxidation (e.g., [10,24]). Accordingly, the wastewater pH was monitored throughout the OUR experiments. The natural pH of the wastewater from Frejlev was found to be 7.9 ($\pm$0.3, n = 14) and, as a result of its relatively high alkalinity of 8.0 eqv. m^{-3} ($\pm$0.3 eqv. m^{-3}, n = 5), the pH was fairly constant throughout the experiments. Within the first 18–24 h of the experiments, before sulfide addition was initiated, the pH decreased, typically by 0.5 pH units. This was likely due to CO_2 buildup from aerobic breakdown of organic substrates. When sulfide addition was initiated, the pH increased gradually with each successive sulfide addition, owing to the high pH of the di-sodium sulfide stock solution (pH > 12). Depending on the number of sulfide additions, the wastewater pH increased 0.2–0.7 units; i.e., to a level comparable to the initial conditions. Previous investigations have shown that, within these intervals, the kinetics of both chemical and biological sulfide oxidation is relatively unaffected [10].

A reliable interpretation of the OUR experiments depends on the ability to account for the entire mass balance. Sulfide emission during the aeration process would therefore obscure the results. However, sulfide was added in much lower concentrations than the dissolved oxygen concentration after aeration (approximately 4 g O_2 (g S)$^{-1}$). This ensured that the entire amount of sulfide added was oxidized before the following aeration took place. Routine measurements of the sulfide concentration confirmed this.

3.4. Model Development

Based on the results of the OUR experiments, a model concept for aerobic biological oxidation of reduced sulfur compounds in wastewater was developed. Details concerning the chemical oxidation processes are adapted from Nielsen et al. [19].

The OUR experiments demonstrated that biological oxidation of sulfide in wastewater does not proceed to sulfate in a single step. A likely pathway includes elemental sulfur as an intermediate. Sulfur produced by SOB can be stored in sulfur globules, located either inside or outside the cell [22]. For use in the model, dissolved sulfide and sulfate are denoted $S_{S(-II)}$ and $S_{S(VI)}$, and the elemental sulfur allotrope is denoted $X_{S(0)}$; i.e., dissolved and particulate sulfur with oxidation levels −2, 6, and 0, respectively. The concept of intermediately stored substrate is analogous to the cell internal storage of organic substrates introduced in the activated sludge model no. 3 [14].

In the OUR experiments, only limited growth could be attributed to sulfide oxidation, whereas the oxidation of elemental sulfur supported exponential growth. Similar observations have been reported in the literature. Buisman et al. [25] investigated kinetic and stoichiometric parameters of a mixed culture oxidizing sulfide and sulfur with oxygen in a chemostat. For the oxidation of sulfide to elemental sulfur, they reported a growth yield (Y_{SOB}) of 0.0015 g N (g S)$^{-1}$, while complete oxidation to sulfate resulted in a much higher

growth yield of 0.021 g N (g S)$^{-1}$. Assuming an N content of dry biomass of 12% and a COD to dry mass ratio of 1 [25,26], this corresponds to growth yields of 0.0125 and 0.175 g COD (g S)$^{-1}$, respectively.

When using sewer process models for engineering purposes, it is, therefore, a reasonable assumption to omit the growth of SOB related to sulfide oxidation to elemental sulfur, because of the low yield coefficient. However, growth related to elemental sulfur oxidation is apparently important, and must, accordingly, be included in a model concept. Figure 4 summarizes the concept for biological sulfide oxidation with transport, transformation, and storage processes, as well as associated growth.
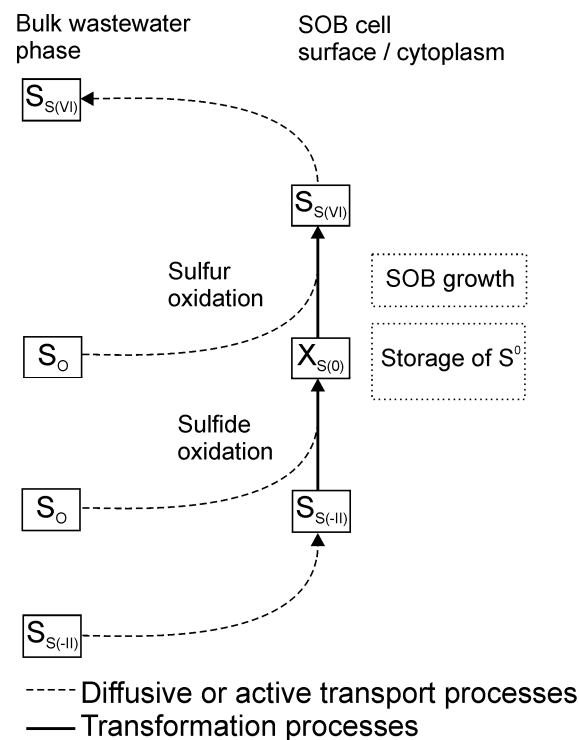
Figure 4. Model concept for biological sulfide oxidation with transport, transformation, and storage of sulfur compounds and associated growth of SOB.

Biological growth and substrate utilization are assumed first order, with respect to the biomass concentration (X_{SOB}) (Table 1). The dependence of these processes on reactant concentrations ($S_{S(-II)}$ and $X_{S(0))}$) are described by saturation (Monod) kinetics. A similar approach has successfully been applied for simulating organic matter and nitrogen transformation processes in sewers and activated sludge [3,14]. During the OUR experiments, the dissolved oxygen concentration was maintained above 1 g O$_2$ m^{-3}. This is considered sufficient to ensure nonlimiting conditions, and no dependence of the biological processes on the dissolved oxygen concentration was included in the model.

Chemical sulfide oxidation was simulated by a power function. This approach has been applied in several studies (e.g., [19,20,27]). The chemical reaction is assumed to be independent of the growth of SOB and the formation of intermediates. An autocatalytic effect of elemental sulfur has been reported for experiments conducted with buffered clean water [20]. However, the effect is insignificant compared with the reported rate of biological sulfide oxidation in wastewater [19].

Biomass decay was not included in the model concept. Hvitved-Jacobsen et al. [28] confirmed that biomass decay is only of minor importance for heterotrophic activity under sewer conditions. Thus, a similar approach was adapted in the present study.

A systematic arrangement of the kinetics and stoichiometry of the processes involved in the aerobic oxidation of sulfide in wastewater is presented in Table 1. The corresponding model parameters are listed in Table 2.

Table 1. Matrix formulation of the process model concept for aerobic transformations of sulfur components during the OUR experiments.

Process	$\frac{\partial S_{S(-II)}}{\partial t}$	$\frac{\partial X_{S(0)}}{\partial t}$	$\frac{\partial X_{SOB}}{\partial t}$	$\frac{\partial S_O}{\partial t}$	Rate Equation
Chemical S(−II) oxidation	−1			$-R_C$	$k_{S(-II)c} \cdot S_{S(-II)}^m \cdot S_O^n$
Biological S(−II) oxidation	−1	1		$-R_{B,S(-II)}$	$k_{S(-II)b} \cdot \frac{S_{S(-II)}}{K_{S(-II)}+S_{S(-II)}} \cdot X_{SOB}$
Biological S(0) oxidation		$-\frac{1}{Y_{SOB}}$	1	$-\frac{R_{B,S(0)}}{Y_{SOB}}$	$\mu_{SOB} \cdot \frac{S_{S(0)}}{K_{S(0)}+S_{S(0)}} \cdot X_{SOB}$

Table 2. Kinetic and stoichiometric parameters used in the process model outlined in Table 1.

Symbol	Definition	Unit
$S_{S(-II)}$	Total dissolved sulfide ($H_2S + HS^- + S^{2-}$)	g COD m^{-3}
S_O	Dissolved oxygen	g O$_2$ m^{-3}
$X_{S(-II)}$	Elemental sulfur	g S m^{-3}
X_{SOB}	Sulfide oxidizing biomass	g COD m^{-3}
R_C	Stoichiometric coefficient for chemical sulfide oxidation	g O$_2$ (g S)$^{-1}$
$R_{B,S(-II)}$	Stoichiometric coefficient for biological $S_{S(-II)}$ oxidation	g O$_2$ (g S)$^{-1}$
$R_{B,S(0)}$	Stoichiometric coefficient for biological $S_{S(0)}$ oxidation	g O$_2$ (g S)$^{-1}$
$k_{S(-II)c}$	Rate constant for chemical sulfide oxidation	d^{-1}
m	Reaction order with respect to $S_{S(-II)}$	−
n	Reaction order with respect to S_O	−
$k_{S(-II)b}$	Rate constant for biological sulfide oxidation	d^{-1}
μ_{SOB}	Maximum specific growth rate of X_{SOB}	d^{-1}
Y_{SOB}	Yield constant for X_{SOB}	g COD (g S)$^{-1}$
$K_{S(-II)}$	Saturation constant for $S_{S(-II)}$	g S m^{-3}
$K_{S(0)}$	Saturation constant for $S_{S(0)}$	g S m^{-3}

3.5. Verification of the Model Concept

For verification of the concept, the model was calibrated against the measured OUR curves by varying the parameters for organic matter (see Vollertsen and Hvitved-Jacobsen [15]) and sulfur (Table 2) transformation processes, until an optimal agreement between the model and measurement was obtained. A multiparameter optimization of such complex models is difficult due to a multitude of local minima. For simplification, the saturation constants for both sulfide and elemental sulfur were, therefore, fixed at a value of 0.1 g S m^{-3}, in agreement with the fast transition from nonlimited to limited conditions. The stoichiometric coefficient for biological oxidation of $S_{S(-II)}$ to $X_{S(0)}$ was fixed at 0.5 gS (g O$_2$)$^{-1}$ —Equation (1)—and the parameter values for chemical sulfide oxidation were adapted from Nielsen et al. [19].

Figure 5 shows simulations of the three experiments from Figure 2. The measured and simulated OURs are considered to be in good agreement. This was the case, although the experimental conditions in terms of sulfide loading and wastewater activity varied significantly between the experiments. The concept (Table 1) can therefore be considered an acceptable formulation, in model terms, of the processes taking place.
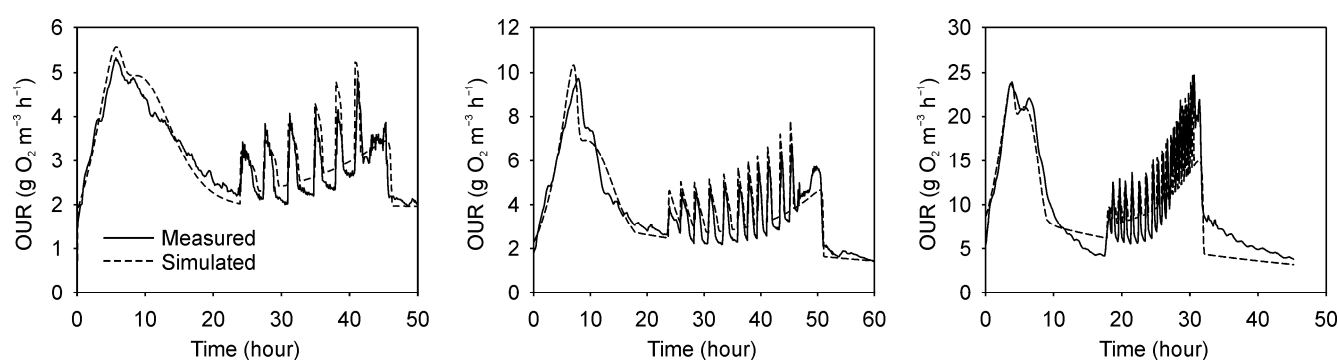
Figure 5. Model simulations of OUR measurements with the addition of sulfide.

The kinetic and stoichiometric parameter values for biological sulfide oxidation, determined by model calibration, are listed in Table 3. The initial concentration of SOB was determined at 0.59 ($\pm$0.25) g COD m^{-3}. For comparison, the initial heterotrophic biomass determined from organic matter transformations was 17.33 ($\pm$9.52) g COD m^{-3}. This value is in agreement with previous investigations on wastewater from Frejlev [26], and specifies that SOB only account for a small fraction ($\approx$3%) of the total active biomass. To the authors' knowledge, no previous studies have reported on SOB biomass concentrations in wastewater from sewers. It is, however, reasonable to expect the SOB biomass to be much smaller than the biomass responsible for the aerobic breakdown of organic matter.

Table 3. Kinetic and stoichiometric parameter values for biological sulfide oxidation, determined by calibration of the model presented in Table 1.

Symbol	Definition	Value 1	Unit
X_{SOB}	Initial concentration of SOB	0.59 ($\pm$0.25)	g COD m^{-3}
$R_{B,S(-II)}$	Stoichiometric coefficient for biological $S_{S(-II)}$ oxidation	0.5 [2]	g O$_2$ (g S)$^{-1}$
$R_{B,S(0)}$	Stoichiometric coefficient for biological $S_{S(0)}$ oxidation	1.3 ($\pm$0.3)	g O$_2$ (g S)$^{-1}$
$k_{S(-II)b}$	Rate constant for biological sulfide oxidation	63.8 ($\pm$20.0)	d^{-1}
μ_{SOB}	Maximum specific growth rate of X_{SOB}	1.98 ($\pm$0.59)	d^{-1}
Y_{SOB}	Yield constant for X_{SOB}	0.17 ($\pm$0.10)	g COD (g S)$^{-1}$
$K_{S(-II)}$	Saturation constant for $S_{S(-II)}$	0.1 [2]	g S m^{-3}
$K_{S(0)}$	Saturation constant for $S_{S(0)}$	0.1 [2]	g S m^{-3}

1. Average value (standard deviation), n = 14. [2] Constant parameter value, c.f. text.

A stoichiometric coefficient of 1.5 g O$_2$ (g S)$^{-1}$ is expected for the biological oxidation of $X_{S(0)}$ ($R_{B,S(0)}$) when sulfate is the product. Considering the variability of the determined parameter value, there is no reason to reject the assumption that sulfate was the reaction product. The slightly lower value could be explained by residual elemental sulfur when the experiments were terminated. For the interpretation of the data, it was assumed that the entire amount of sulfide was completely oxidized within each experiment. Thus, any residual elemental sulfur would have resulted in a lower value of $R_{B,S(0)}$. It is well known that some bacteria, e.g., *Beggiatoa*, lack the ability to oxidize sulfide completely to sulfate, but accumulate intracellular elemental sulfur [29].

The rate constant for biological sulfide oxidation was determined at 63.8 ($\pm$20.0) d^{-1}. According to the model concept, the initial biological sulfide oxidation rate can, therefore, be estimated at 37.6 g S m^{-3} d^{-1} when the SOB concentration is 0.59 g COD m^{-3} and reactant concentrations are nonlimiting. This agrees with previously reported values for biological sulfide oxidation in wastewater (e.g., [10,30]).

The growth rates of the SOB determined from model calibration are consistent with the literature values on autotrophic SOB determined in batch experiments. For comparison, Chen et al. [31] reported a growth rate of 2.87 d^{-1} for *Thiobacillus thiooxidans* oxidizing elemental sulfur at 30 °C. The yield constant was in good agreement with reported values

for mixed cultures; e.g., the value of 0.175 g COD (g S)$^{-1}$ reported by Buisman et al. [25]. In addition, the value was lower than reported maximum yields for *Thiobacilli* grown in chemostats [13]. Considering the growth rate and yield constant, it is realistic for the SOB concentration to double during transport in extended sewer systems.

Overall, the parameter values agree well with the literature values. Correlation analysis showed that all kinetic and stoichiometric parameters could be considered statistically independent. However, there were some correlations between the concentrations of heterotrophic biomass involved in organic matter transformations and SOB (R = 0.73). This is not surprising, as variations in wastewater strength are expected to affect all wastewater constituents.

4. Conclusions

This study investigated biological sulfide oxidation in a series of batch experiments with wastewater from a sewer. The experiments showed that the reaction produced sulfate, with elemental sulfur as an intermediate. During each experiment, the activity of the sulfide oxidizing bacteria increased significantly as the result of bacterial growth related to the oxidation of intermediately stored elemental sulfur. The initially present sulfide oxidizing biomass accounted for only a small fraction ($\approx$3%) of the total active aerobic biomass.

Based on the experiments, a model concept describing biological sulfide oxidation, with intermediary storage of elemental sulfur and associated growth, was developed. The model was successfully calibrated against the experimental results using realistic parameter values.

The results of this study show that sulfide levels typically found in sewer systems are sufficient to support significant growth of sulfide oxidizing bacteria, thereby increasing the potential for sulfide oxidation. The developed model concept can be integrated with existing sewer process models, such as the WATS model, for predicting sulfide build-up in sewer systems, thereby improving the model validity.

Author Contributions: A.H.N. and J.V. conceived and designed the experiments; A.H.N. performed the experiments, analyzed the data and wrote the paper; J.V. revised the paper. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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