Oxidation of biologically-produced elemental sulfur at neutrophilic conditions[1]

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Abstract

BACKGROUND: Previous research on a biotrickling filter for the removal of high loads of H_2S showed that accumulation of elemental sulfur (S^0) when dealing with high H_2S concentrations could lead to reactor clogging. Since S^0 can also serve as substrate for sulfur-oxidizing bacteria, this study investigates the biological oxidation of S^0 as a remediation strategy.

trickling filter occurred at a comparable rate as those reported for stirred tank reactors. When biologically-produced dried and powdered S^0 was manually added as substrate in stirred tank reactor experiments, significantly lower S^0 oxidation rates were found compared to those for freshly, *in-situ* produced biological S^0. It was speculated that either the powdered S^0 particle size or the

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surface properties hindered S^0 bioavailability even in a well stirred environment. Respirometric experiments with the same powdered S^0 and acetone-dissolved S^0 confirmed that biological oxidation of S^0 was basically limited by the solid S^0 bioavailability.

CONCLUSIONS: Therefore, results showed that S^0 oxidation basically depends on S^0 bioavailability and that S^0 volumetric oxidation rates as high as 3.48 mmols SO_4^{2-} h^{-1} L^{-1} can be achieved inside a clogged biotrickling filter probably due to the high biomass retention capacity. Overall, the results indicate that biological oxidation of S^0 can be considered a suitable strategy for S^0-clogged bioreactors unclogging.

Key words: elemental sulfur, hydrogen sulfide, biotrickling filter, sulfur oxidizing bacteria, fuel gas.

Introduction

Removal of Reduced Sulfur Compounds (RSC) such as hydrogen sulfide (H_2S) from waste or energy-rich gases has been traditionally carried out by means of physico-chemical systems, such as selective absorption or adsorption processes. Such technologies are based on the high affinity for H_2S of amine-based absorbents or ferric-based adsorbents.[1, 2]

However, both the high operating costs of the ad/absorbents' regeneration and the increasing interest in energy recovery from such gaseous effluents, due to the rising of energy prices, have speed up the development of biological systems that can improve the existing technologies for some desulfurization applications.

The oxidative side of the sulfur cycle and, more specifically, the oxidation of RSC as energy source carried out by sulfur-oxidizing bacteria (SOB) has been intensively studied by several authors[3,4] as well as its application for water or gas treatment technologies.[5,6] H_2S removal occurs by an initial absorption and dissociation into an aqueous phase (Eqs. 1 and 2) "which is pH dependent since H_2S is a weak acid (pK_{a1}: 6.9; pK_{a2} =12.92, at 25ºC)"[7] and the subsequent biologically catalyzed oxidation to elemental sulfur (S^0) or sulfate depending on oxygen availability (Eqs. 2, 3 and 4):[3]

$$H_2S_{(g)} \leftrightarrow H_2S_{(aq)} \quad \text{(absorption)} \quad (1)$$

$$H_2S_{(aq)} \leftrightarrow H^+ + HS^- \quad \text{(dissociation)} \quad (2)$$

$$HS^- + 0.5\ O_{2\,(aq)} \rightarrow S^0 + OH^- \quad \text{(biological reaction)} \quad (3)$$

$$HS^- + 2\ O_{2\,(aq)} \rightarrow SO_4^{2-} + H^+ \quad \text{(biological reaction)} \quad (4)$$

Bioreactors for the treatment of low concentrations of H_2S are nowadays commonly used at solid wastes and wastewater treatment plants[8,9], livestock farming[10]

and other industrial facilities.[5] In such applications, H_2S is biologically oxidized to sulfate by SOB according to Eq. 4 since bioreactors generally operate in presence of excess oxygen. In this situation, the most common operational difficulty comes from excess media acidification that leads to a pH drop down to toxic levels for biomass.[11, 12]

However, when high H_2S concentrations must be treated, like in the sweetening of energy-rich gases which typically contain some few thousands ppm_v of H_2S[1], only some biotechnological processes have been developed and just a few of them are already commercially available.[13]

One of the main difficulties when treating high H_2S concentrations is the capability to supply enough oxygen for the complete biological oxidation of H_2S to sulfate due to a limited O_2 mass transfer capability. Different approaches to deal with this limitation have been used. Buisman *et al.*[14] developed a process consisting of an initial absorption column and a subsequent stirred biological reactor operated under alkaline pH and S^0 producing (oxygen limited) conditions.[2] Further developments of this system have been recently published when treating H_2S loads of up to 340 g H_2S m^{-3} h^{-1}.[15]

Bailón[16] developed a biotrickling filter system able to treat 900 ppm_v of H_2S (about 24 g H_2S m^{-3} h^{-1}) where oxygen was supplied forcing the recirculation flow through an air bubble column.

A similar approach was studied by Fortuny *et al.*[17] where two lab-scale prototypes were tested and compared for the treatment of up to 10,000 ppm_v of H_2S (more than 300 g H_2S m^{-3} h^{-1}). The system viability and robustness was proved despite accumulation of S^0 onto the packing material when too low O_2/H_2S supply ratios were used was observed, which would eventually lead to pressure drop, loss of active volume, reduced EBRT, etc.

However, it is well known that SOB not only grow on sulfide oxidation but can also use S^0 according to Eq.5: [3, 18, 19]

$$S^0 + 3/2\ O_2 + H_2O \rightarrow SO_4^{2-} + 2\ H^+ \qquad \text{(biological reaction)} \qquad (5)$$

Several authors have studied the mechanism of S^0 oxidation by *Thiobacilli*.[18-20] Janssen *et al.*[21, 22] investigated the properties and stability of biological S^0 particles. Tichy *et al.*[23] studied the utilization of biological S^0 for bioleaching purposes. However, all these studies were conducted in liquid-phase, homogeneously-stirred reactors, where S^0 particles are much more biologically available than would be inside a clogged, packed bed reactor.

Since oxidation of accumulated S^0 in a biotrickling filter reactor was already reported as being the responsible for keeping the system biologically active during gas-phase shutdowns[24], the aim of this study was to investigate the biological oxidation of S^0, combined with the usually applied strategy of S^0 wash out, when trying to recover a S^0-clogged biotrickling filter. If in such a system sulfide supply can be discontinued for a period of time but oxygen supply and liquid recirculation are kept on, SOB will be forced to switch to S^0 oxidation thus leading to reactor unclogging.

Materials and methods

Biotrickling filter reactor experiments

In-situ S^0 oxidation was investigated in a lab-scale biotrickling filter reactor (BTFR) with an ancilliary unit for oxygen supply (Fig. 1). HD-QPAC® (Lantec Products Inc., CA, USA) with a 4 × 4 mm (0.16" × 0.16") grid opening cut to tightly fit inside the reactor was used as packing material. Metered amounts of H_2S, N_2 and air

using digital mass flow controllers (Bronkhorst, The Netherlands) were used to simulate a controlled biogas inflow. Mineral medium (MM) containing (g L^{-1}): NH_4Cl, 1; KH_2PO_4, 0.12; K_2HPO_4, 0.15; $CaCl_2$, 0.02; $MgSO_4 \cdot 7H_2O$, 0.2; trace elements[25], 1 ml L^{-1}, and $NaHCO_3$ as inorganic carbon source were continuously fed. Liquid phase was continuously renewed by automated timing of the MM supply, bicarbonate supply and the liquid purge, using 3 different peristaltic pumps (Fig. 1).

Continuous monitoring of the H_2S concentration in the outlet gas phase was performed using an electrochemical H_2S sensor (Sure-cell, Euro-Gas Management Services LTD, UK). On-line liquid phase monitoring included pH, oxidation-reduction potential (ORP) and dissolved oxygen (DO) measurements. A pH control by HCl or NaOH addition and a level control by liquid purge regulation were also installed. Sulfate and thiosulfate concentrations were measured using an ICS-1000 Ion Chromatography system with an IonPac AS9-HC column (Dionex Corporation).

The experiments were carried out after more than a year operating at an inlet H_2S concentration of 2,000 ppm_v (55.6 g H_2S $m^{-3}h^{-1}$), an EBRT of 180 s, a controlled pH range of 6-6.5 and excess oxygen supply. In the currently described experiment with the BTFR, an average hydraulic retention time (HRT) of 26 ± 2 h was used.

Prior to S^0 oxidation, the reactor was forced to produce and accumulate S^0 for a period of 6 weeks during which the O_2/H_2S supplied ratio was stepwise, weekly decreased from 23.6 down to 1.5 (v v^{-1}), with a maximum applied loading rate (LR) of 204.5 g H_2S m^{-3} h^{-1}. Subsequently, sulfide supply was stopped and air supply increased back to the usually applied value during normal operation (20% v v^{-1}). A 50% higher air flow supply than that normally used was also tested during the period of accumulated S^0 oxidation.

Previous research on the BTFR had shown that a high trickling liquid velocity (TLV) favored sulfate production through a better use of the supplied oxygen inside the packed bed and did not have a significant effect on accumulated S^0 wash down.[26] Therefore, two different TLV were also applied during the experiment; 3.8 m h^{-1}, the usually applied for normal operation, and 17 m h^{-1}, a value higher than those usually applied in biological reactors (< 10 m h^{-1}).[27]

Discontinuous stirred tank reactor experiments

In order to compare the different bioavailability of S^0 inside a BTFR and in an homogeneous stirred reactor, dried (60ºC, 48 h) powdered biological S^0 collected from the BTFR was fed into a 1.5 L discontinuous stirred tank reactor (DSTR) with biomass initially obtained from the same BTFR (see Table 1 for S^0 main properties; Citometry Unit, Scientific Services of the University of Barcelona, Spain).

A series of different runs were carried out either with S^0 or hydrogen sulfide ($H_2S_{(g)}$ 99%) supplied via a digital mass-flow controller (Bronkhorst, NL). After each reactor run, biomass was recovered through centrifugation (10,000 rpm, 15 min) and resuspension into fresh MM to be used in the next reactor run.

An overview of the operating conditions for the different reactor runs is given in Table 2. All reactor runs were performed under excess DO conditions (> 5 mg O_2 L^{-1}). Additionally, concentrated $NaHCO_3$ (3 g C L^{-1}) was supplied to avoid carbon limitation. A stirring rate of 400 rpm was used.

DO concentration and reactor temperature were continuously monitored by an oxi340i electrode (WTW, Germany). Daily measurements of sulfate and thiosulfate were performed as described above. Also, daily measures of biomass and S^0

concentrations were carried out as described by van den Bosch *et al.*[15] and Gohering and Helbing[28], respectively.

pH acidification was controlled by automated addition of NaOH 1 M at a pH set-point of 7. The same MM as described above was used.

Respirometric experiments

Difference in bioavailability of powdered, dried biological S^0 and acetone-dissolved biological S^0 was also studied in respirometric experiments where the oxygen uptake rate (OUR) on biological oxidation of S^0 was assessed in an LFS respirometer, a continuously aerated bioreactor without continuous liquid inputs nor outputs where the oxygen concentration is measured in the liquid phase and the substrate is fed by pulses.[29]

The same MM composition as previously described was used in the respirometer that consisted of an 0.3 L well-mixed vessel continuously supplied with air (15 ml min^{-1}) through a digital mass-flow controller (Bronkhorst, NL) and with a controlled temperature of 25ºC. A detailed description of the used instrumentation can be found elsewhere.[30] pH was automatically controlled at 7.00 ± 0.01 as previously described.[29]

Both the same abovementioned dried, powdered S^0 (Table 1) and an acetone saturated S^0 solution (17 μM S^0) were used as substrates in different runs (Table 3). Run 1B consisted of a second S^0 pulse addition 3.7 h after beginning of run 1A. Prior to substrate addition in run 1B, also 20 mg C-HCO_3^- L^{-1} were added to avoid carbon limitation.

The fundamentals of the OUR calculation can be found elsewhere.[31,32] However, the experimental procedure consisted of an initial calculation of the endogenous oxygen uptake rate (OUR_{END}) and global oxygen transfer coefficient (k_La) according to

Guisasola *et al.*[29], using active and washed biomass collected from the BTFR. Upon pulse addition of either solid, powdered S^0 or acetone-dissolved S^0, the DO profiles were registered and exogenous OUR (OUR_{EX}) profiles calculated.

Possible negative effects of acetone on biological activity or experimental setup response were tested, as well as chemical oxidation of dissolved S^0.

Results and discussion

BTFR S^0 oxidation

A sulfur mass balance was carried out from the ionic sulfur species measurement (basically sulfate since thiosulfate and sulfite concentrations were under detection levels) and the sulfide LR and removal efficiency (RE) (Fig. 2). Since S^0 tends to accumulate inside the reactor, S^0 production was calculated by subtraction as previously reported.[21] According to the balance, during the S^0 accumulation period (days 148 to 192) a total amount of 56 g S-S^0 were produced from the 151 g S-H_2S removed. As it can be seen in Figure 2, there was some S^0 production from the very beginning, but it was not until high LR were applied (LR $\geq$ 87 g H_2S m^{-3} h^{-1}; $O_2/H_2S \leq 5$; day 178 onwards) that S^0 accumulation really increased. Also, RE > 99% was sustained except for the last LR increase (LR= 204.5 g H_2S m^{-3} h^{-1}, days 185 to 192) when the RE dropped down to 93 $\pm$ 1%. The drop in LR was mainly caused by mass-transfer limitation, as previously reported during the application of very similar LR[24], since no thiosulfate formation was detected, indicating very low or no sulfide accumulation that would have been chemically oxidized to thiosulfate.

As soon as sulfide supply was stopped and the air flow risen, the sulfate production rate significantly increased up to a maximum value of 223 mg S-SO_4^{2-} h^{-1}

(3.48 mmols SO_4^{2-} h^{-1} L^{-1} packed bed) between days 193 to 199, clearly showing the capacity of the system to oxidize the accumulated S^0 in a very similar rate than those reported for stirred reactors (3.5 mmols SO_4^{2-} h^{-1} L^{-1}).[21]

On day 199 the TLV was set to 17 m h^{-1} in order to increase the oxygen supply to the packed bed. Contrary to what was expected, the sulfate production rate decreased, due to an important wash out of solids that went straight to the reactor purge. Previous results[26] had shown that increasing the TLV (in the range of 0.5 to 19 m h^{-1}) could not be expected to directly improve solids wash down on the studied system. However, most probably the high amount of accumulated S^0 after day 192 and the fact that the TLV was directly increased from a low value up to almost the maximum previously tested velocity did result in a severe solids (S^0 and biomass) wash out. As a consequence, a decrease in the total amount of accumulated solids (S^0 and biomass) caused a reduction in the sulfate production rate.

Furthermore, not only S^0 oxidation is greatly dependent on the presence of the highly bio-available fine S^0 particles[23, 33], but S^0 oxidation has also been reported to be mainly a superficial process which has S^0 particles surface colonization as a prerequisite.[33] It can be speculated that the washed out solids greatly consisted of the free-S^0 particles and the superficial layers of S^0 and biomass of the biofilm, since they were the most exposed to the shear force of the trickling liquid. Therefore, it is tempting to say that both the decrease in highly bio-available fine S^0 particles and in biomass were the main cause for the sudden sulfate production rate slowdown from day 199 onwards. Further changes on the trickling liquid velocity or oxygen supply flow rate did not have any effect on sulfate production, which kept slightly decreasing until the end of the experiment (Fig. 2).

According to the sulfur balance, only 6 days after starting the S^0 oxidation period, 57% of the accumulated S^0 had been oxidized, which is a similar value to the 31-46.5% range reported by Tichý *et al.*[23] as being the percentage of highly bio-available S^0 usually found in biologically produced S^0. It took 23 days of sulfide starvation for all the accumulated S^0 to be oxidized.

Results show that even if S^0 accumulates inside a packed bed reactor, it can easily be further oxidized to sulfate if sulfide is not present. Furthermore, the time-scale for the S^0 biological oxidation is not out of consideration as a possible packed bed regeneration method, although it will most probably considerably change among different situations (packing properties, amount of accumulated S^0, oxygen supply capacity of the system and biomass content among others).

From these results it is not possible to elucidate whether S^0 oxidation only takes place once sulfide is completely consumed or whether both S^0 and sulfide oxidation occur simultaneously. However, what the results clearly show is that from day 148 to 187 the supplied sulfide was rapidly and effectively oxidized whereas S^0 oxidation, if simultaneously occurring, took place at a much lower rate since S^0 accumulated. This means that S^0 production was much faster than sulfate production (S^0 oxidation), which has already been reported in literature.[19, 34]

DSTR S^0 oxidation

An overview of the results for the different reactor runs is given in Table 4, where the specific maximum sulfate production rates were calculated using the average biomass concentration during the maximum sulfate production rate. Run 4 data belong to the oxidation of the freshly, *in-situ* produced S^0 after sulfide supply shutdown.

The results will be discussed in detail in the following sections.

Oxidation of externally fed S^0

As shown in Figure 3, when a pulse of dried, powdered S^0 was fed into the stirred reactor, an initial low S^0 oxidation rate was observed followed by a fast sulfate production phase and a final slowdown till complete stop. Both the measured and calculated S^0 concentrations are plotted, since the lack of an homogeneous S^0 sludge inside the DTSR due to S^0 sticking to the reactor wall (as previously reported)[21] did not always allow a quantitative S^0 concentration monitoring, although qualitatively providing useful information.

Run 1 (Fig. 3A) showed the slowest sulfate production (S^0 oxidation) rate and biomass growth rate, with both values representing less than a 20% of the rates for run 3 (Fig. 3B). Sulfate production rate for run 2 was very similar to that obtained for run 3 (Table 4).

The difference between results from run 1 and runs 2 and 3 probably relied in biomass adaptation to the DSTR conditions, since run 1 was performed with biomass directly taken from the BTFR where it grew as a biofilm instead of as a free cell suspension. Also, it is possible that the lower S_0 initial concentration played a role due to the previously mentioned dependence between sulfur oxidation and sulfur-surface availability. [23, 33] Therefore, runs 2 and 3 were considered as more representative of the powdered, dried S^0 oxidation capacity of the studied biomass consortium when grown as a free cell suspension. In order to compare the results from the biotrickling filter reactor and the DSTR reactor, maximum volumetric sulfate production rates were calculated (Table 4). Maximum volumetric sulfate oxidation rates from runs 2 and 3 were about three times smaller than those reported by Janssen *et al.* (3.5 mmols SO_4^{2-} h^{-1} L^{-1}) [21] or the observed in the BTFR experiment (3.48 mmols SO_4^{2-} h^{-1} L^{-1}).

Both the experiment performed by Janssen *et al.*[21] and the BTFR experiment previously described studied biological oxidation of freshly produced S^0, although in different types of reactors. However, runs 1 to 3 were performed with externally added, dried, powdered S^0 as substrate. It is tempting to suggest that the slower S^0 oxidation rates observed in runs 2 and 3 were caused by a low substrate bioavailability, which in turn could have been caused by different reasons. On the one hand, is has been previously reported that biological S^0 oxidation is basically a surface-dependent process in terms of both available surface and surface biological colonization[33], which was initially non-existent on the externally added S^0.

On the other hand, according to the particle size characterization (Table 1) 24% of the S^0 particles were smaller than 1 μm and 69% smaller than 10 μm. Janssen *et al.*[22] reported that S^0 producing bacteria form S^0 globules that are deposited inside or outside the bacterial cell, with diameters of up to 1 μm. Therefore, with 76% of the added S^0 with a particle size bigger than 1 μm, it can be speculated that S^0 particle size was, at least, partially responsible for the low S^0 oxidation rates observed.

Moreover, it has been stated that biological S^0 particles are covered by an extended proteinic polymer layer that normally confers them hydrophilic properties.[35] However, upon S^0 drying it may be very possible that those hydrophilic properties are changed or partially lost, therefore reducing S^0 bioavailability.

Oxidation of in-situ, freshly-produced S^0

In order to confirm if externally added S^0 bioavailability was negatively influencing S^0 oxidation, biological oxidation of fresh, *in-situ* produced S^0 was studied in run 4.

During the first 5.7 days of run 4, 39.2 mg S-H_2S L^{-1} h^{-1} were supplied while a DO concentration above 5 mg L^{-1} was ensured. Sulfide oxidation mainly led to S^0 formation (clearly visible by a whitish turbidity increase), although some thiosulfate and sulfate were also produced (Fig. 4A). According to the sulfur balance, an average 77.5% of the supplied H_2S was oxidized (30.4 mg S-H_2S L^{-1} h^{-1}), either as S^0 (76%), sulfate (15%) or thiosulfate (9%) and the rest would have accumulated as dissolved sulfide or stripped through the gas phase.

Thiosulfate and S^0 formation under such oxidizing conditions clearly indicated an insufficient biological sulfide oxidation capacity that probably allowed sulfide accumulation (not measured). Sulfide accumulation was probably the cause for the slow decrease of biomass concentration observed during the first 6 days of run 4 (Fig. 4B), since sulfide becomes biologically toxic already at very low concentrations (5-30 mg L^{-1}; < 5 mg L^{-1}).[21, 34] Once sulfide accumulates in an oxidizing environment, it is chemically oxidized mainly to thiosulfate, although some chemical formation of S^0 has also been reported.[21]

However, in this case S^0 was the main product as well as sulfate, indicating that complete biomass inhibition did not actually occur. Probably both biological and chemical processes contributed to sulfide oxidation during this phase.

One day after the sulfide supply was shutdown (day 7), biomass and sulfate concentrations started increasing while S^0 and thiosulfate decreased, which indicated a fast biological activity recovery (Fig. 4). Once more the sulfur balance did not quantitatively fit the S^0 measurements but qualitatively they did show a clear S^0 concentration decrease, which was also visible by a decrease in the reactor turbidity.

Table 4 shows that the maximum volumetric sulfate production rate (calculated after day 9 of operation when thiosulfate concentration was already negligible) from run

4 was much higher than those for run 2 or 3 and actually was very similar to the value reported by Janssen *et al.*[21] or the value obtained from the BTFR experiment. Hence, it was hypothesized that the fresh, *in-situ* produced S^0 properties (particle size and surface characteristics) facilitated S^0 bioavailability and oxidation compared to dried sulfur properties.

Therefore, these results show that SOB S^0 oxidation capacity might not be so different between freshly formed S^0 accumulated into a biotrickling filter or inside a homogeneous stirred reactor. Similar volumetric S^0 oxidation rates can be obtained even if S^0 bioavailability would *a priori* seem to be much lower in a partially-clogged packed bed reactor. A possible explanation could rely on the higher biomass accumulation capacity of packed bed reactors making up for the lower substrate bioavailability compared to stirred systems. Therefore, similar volumetric oxidation rates can be achieved.

Respirometric S^0 oxidation

S^0 biological oxidation was studied in different respirometric runs with a powdered, S^0 water-suspension and acetone-dissolved (readily available) S^0. Results from a run with the S^0 water-suspension (run 1A and 1A+1B) and one with acetone-dissolved S^0 (run 2) are shown in Table 5, where the expected oxygen consumption was calculated according to the stoichiometry from Eq. 5 and only using 91% of the added substrate since it has been stated that 9% of the available electrons are estimated to be required for CO_2 fixation.[36]

Experimental runs performed with addition of pure acetone (the same volume as the acetone-dissolved S^0 pulses) showed no increase or decrease in biological activity due to acetone addition. Only a small and temporary sharp increase of DO concentration

was observed and taken into account for OUR calculations. Also, chemical oxidation of acetone-dissolved S^0 was negligible.

All experiments were carried out under exactly the same conditions of air supply, agitation, temperature and initial biomass concentration. However, the powdered S^0 runs were performed with a much higher substrate initial concentration due to S^0 handling issues and because a reduced substrate bioavailability was already expected. Still, after the first powdered S^0 addition only a very small OUR increase was observed and it rapidly reached a plateau at an average OUR of 0.011 ± 0.001 mg O_2 L^{-1} h^{-1} (Fig. 5). After about 3 hours at the same average OUR, a second powdered, water-suspended S^0 pulse was added, and another small OUR increase up to a new plateau average value of 0.023 ± 0.001 mg O_2 L^{-1} h^{-1} was observed (Fig. 5).

This indicated that the first plateau was not a real maximum OUR due to biological oxidation velocity limitation but a substrate availability-limited maximum OUR value. Since solid S^0 oxidation is a surface-dependent process, it is assumed that powdered S^0 bioavailability was limiting biological activity after the first pulse and that was the reason why upon addition of a second S^0 pulse the OUR slightly increased due to presence of more available substrate. However, it is then very plausible to think that the second maximum OUR observed was also result of a limited substrate biological availability and not a maximum biological oxidation rate.

Indeed, about 3 hours after the second pulse the OUR started increasing up to a maximum value of 0.038 ± 0.02 mg O_2 L^{-1} h^{-1} reached 24 hours after starting the experiment (Fig. 5). After 40 hours the experiment was stopped, although the initial OUR_{END} (taken as 0 mg O_2 L^{-1} h^{-1} in Fig. 5) had not been reached yet. According to Eq. 5 and to the amount of consumed oxygen and expected oxygen consumption (Table 5), the substrate had not been completely depleted yet.

Why did the OUR further increase without addition of any more substrate has not been fully elucidated yet. It has been reported that oxidation of large sulfur particles resulted in their abrasion and breaking into smaller particles,[23] which in turn are faster oxidized.[33] This might explain the observed progressive increase in the maximum OUR over S^0 oxidation. Another possibility is that a slight biomass growth occurred after more than 12h of experiment (Figure 5) and it caused the slight increase of the OUR.

The OUR profile from run 2 is also shown in Figure 5. Substrate depletion was much faster, not only because the total amount of added substrate was smaller (Table 3) but also due to a significantly higher maximum OUR (Table 5). This is a clear indication that S^0 bioavailability was the limiting factor in runs 1A and 1B.

According to the current knowledge of S^0 biological oxidation, exogenous S^0 (assumed to be in the form of S_8 rings) must be initially converted (dissolved) to highly reactive linear S^0 to be transported through the outer or inner (depending on the reference) cell membranes for oxidation.[37, 38] Therefore, both available S^0 surface and S^0 particles surface characteristics should influence exogenous S^0 biological oxidation. It is then assumable that the difference on surface characteristics between fresh, *in-situ* produced S^0 particles and externally added dried, powdered S^0 particles would also influence S^0 oxidation.

From a reactor operation point of view, it would be interesting being able to maximize S^0 bioavailability in case of reactor clogging, where probably part of the S^0 will still keep the freshly produced S^0 properties but part of it will get dry due to channeling of the liquid and gas flows.

Conclusions

Accumulation of S^0 turns out to be the main operational problem in a biotrickling filter treating high loads of H_2S if not properly operated. Hence, biological oxidation of S^0 has been studied and compared under three different circumstances.

Oxidation of accumulated S^0 inside the biotrickling filter reactor revealed that contrary to what could be expected into a clogged, non-homogeneous packed bed reactor, volumetric S^0 oxidation rates comparable to those obtained for stirred tank reactors could be achieved. The high biomass accumulation capacity of a packed bed reactor might be responsible for it. Above all, the results showed that using the SOB capacity to oxidize accumulated S^0 under sulfide starvation can be considered as a possible mechanism for reactor recovery once accumulation of excessive S^0 has led to reactor clogging.

S^0 oxidation experiments in a stirred tank reactor showed that biological oxidation of dried, powdered S^0 particles occurs at a much slower rate than oxidation of freshly, *in-situ* produced biological S^0 particles. It is speculated that the particles size or the surface chemical properties of dried, powdered S^0 particles limited S^0 bioavailability even in a well stirred environment. This confirmed that the main rate limiting factor of S^0 biological oxidation is S^0 bioavailability.

The limited bioavailability of dried, powdered S^0 particles was finally proven in respirometric experiments which revealed much lower maximum OUR for dried, powdered S^0 oxidation than for acetone-dissolved S^0. Moreover, maximum OUR for powdered S^0 biological oxidation was substrate-limited and not kinetically limited under the tested conditions.

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Tables**:**

Table 1: Powdered biological S0 properties.

Elemental composition	S	N	C	H
% (dry weight)	88 ± 1	0.97 ± 0.01	2.9 ± 0.1	0.43 ± 0.01
Particle size distribution	< 1 μm	< 10 μm	< 40 μm	< 60 μm
% sample volume	24.1	69.0	99.1	99.999
Particle size fraction distribution	< 10 %	< 25 %	< 50 %	< 95 %
Particle diameter (μm)	0.236	1.124	4.703	29.28

Table 2: Overview of the initial conditions for the different DSTR runs.

Run Number		1	2	3	4
Substrate		S^0	S^0	S^0	H_2S
Load	(mg S-H_2S L^{-1} h^{-1})	-	-	-	39.3
S^0 pulse	(mg L^{-1})	298	1222	1128	-
Biomass	(mg N L^{-1})	20.3	18.5	16.2	21.2
Inoculum origin		BTFR	Run 1	Run 2	Run 3

1 Table 3: Overview of the respirometric experimental conditions.

2

Run		1A	1B	2
Substrate		powdered S^0	powdered S^0	dissolved S^0
Pulse	(mg S L^{-1})	24.7	26.28	2.81
	(mM S)	0.77	0.82	0.09
Biomass	(mg N L^{-1})	22.0	22.0	22.0
HCO_3^-	(mg C L^{-1})	40	+ 20	40

3

4

1 Table 4: Overview of the results for the different DSTR runs.

2

Run Number		1	2	3	4
Maximum volumetric sulfate production rate	mg $S\text{-}SO_4^{2-}$ h^{-1} L^{-1}	7.20	34.09	35.31	108.69
	mmol SO_4^{2-} h^{-1} L^{-1}	0.23	1.07	1.10	3.40
Specific maximum sulfate production rate	mg $S\text{-}SO_4^{2-}$ h^{-1} mg $N_{aver.}^{-1}$	0.48	1.69	1.77	2.87
Biomass growth rate	mg N h^{-1}	0.11	na	0.66	0.55

3

4

1 Table 5: Overview of the respirometric results with powdered, water-suspended S^0 and

2 acetone-dissolved S^0.

3

Run		1A	1A+1B	2
Maximum OUR	mg O_2 L^{-1} h^{-1}	0.673	1.395	3.548
Measured O_2 consumption	mg O_2	-	15.06	1.02
Expected O_2 consumption	mg O_2	-	20.87	1.15

4

5

Figures

Figure 1:

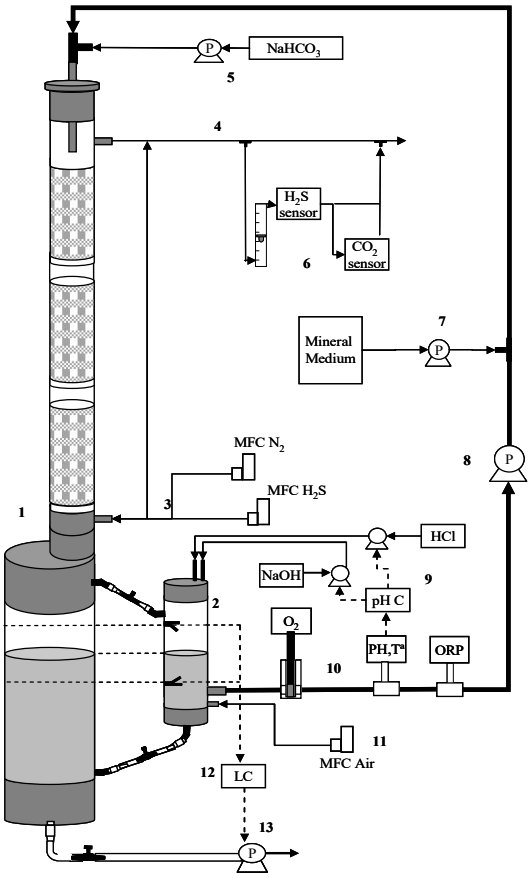


Figure 1: Schematic of the biotrickling filter reactor. 1: Main reactor; 2: Air supply compartment; 3: Gas inlet; 4: Gas outlet; 5: HCO_3^- supply; 6: Gas monitoring; 7: MM supply; 8: Recirculation pump; 9: pH control; 10: Liquid monitoring; 11: Air supply; 12: Level control; 13: Liquid purge.

Figure 2:

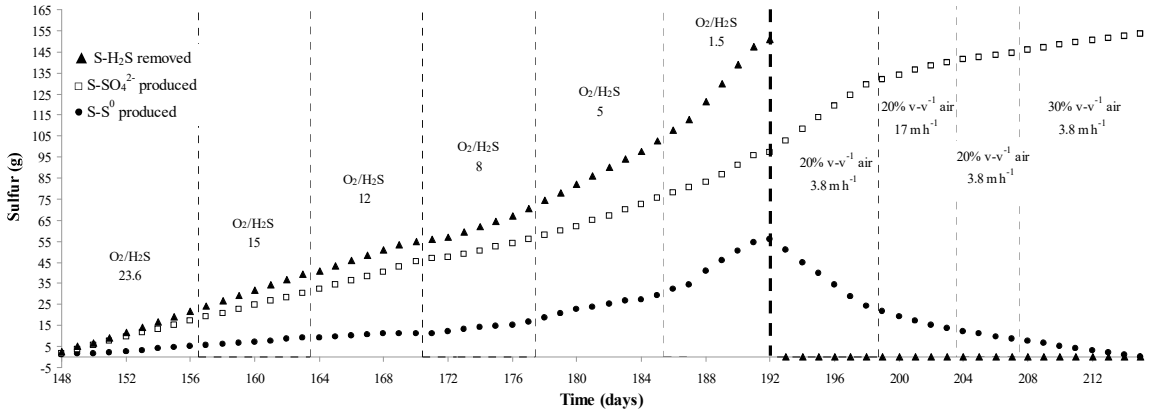
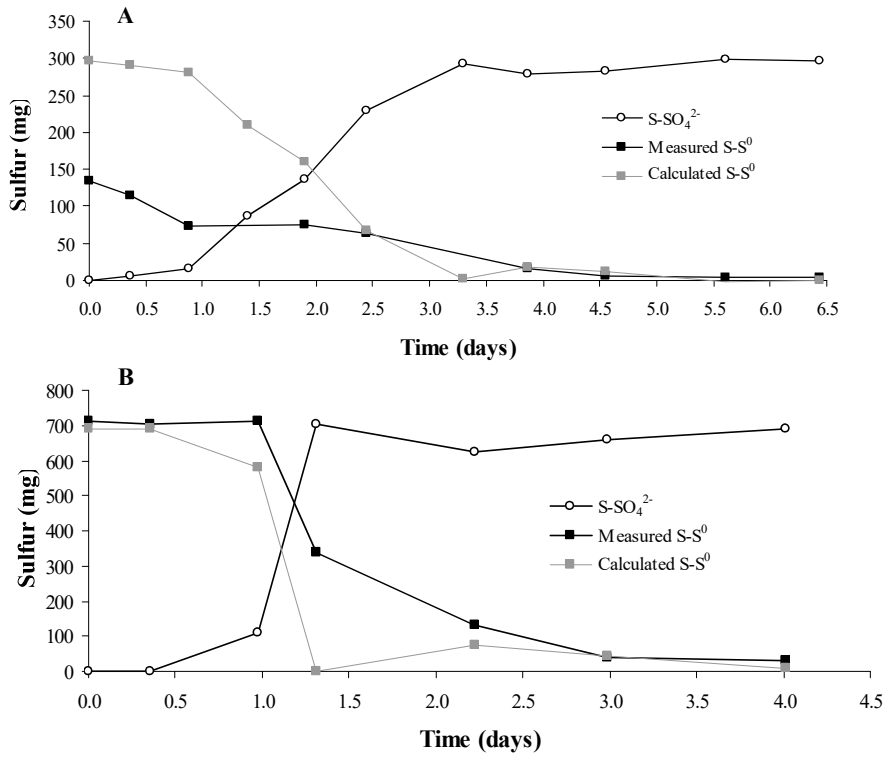


Figure 2: Sulfur mass balance in the biotrickling filter reactor. The thick dashed line separates the S^0 accumulation period (left side) from the S^0 oxidation period (right side). The thin dashed lines separate periods with different O_2/H_2S supplied ratios during S^0 accumulation (days 148 to 192) and different O_2 supply or trickling liquid velocity periods during S^0 oxidation (days 192 to 215).

1 Figure 3:

2

3



4

5 **Figure 3** Sulfur balance for runs 1 (A) and 3 (B) in the DSTR with measured sulfate and S^0

6 concentrations as well as calculated S^0 concentrations from sulfate production.

7

Figure 4:

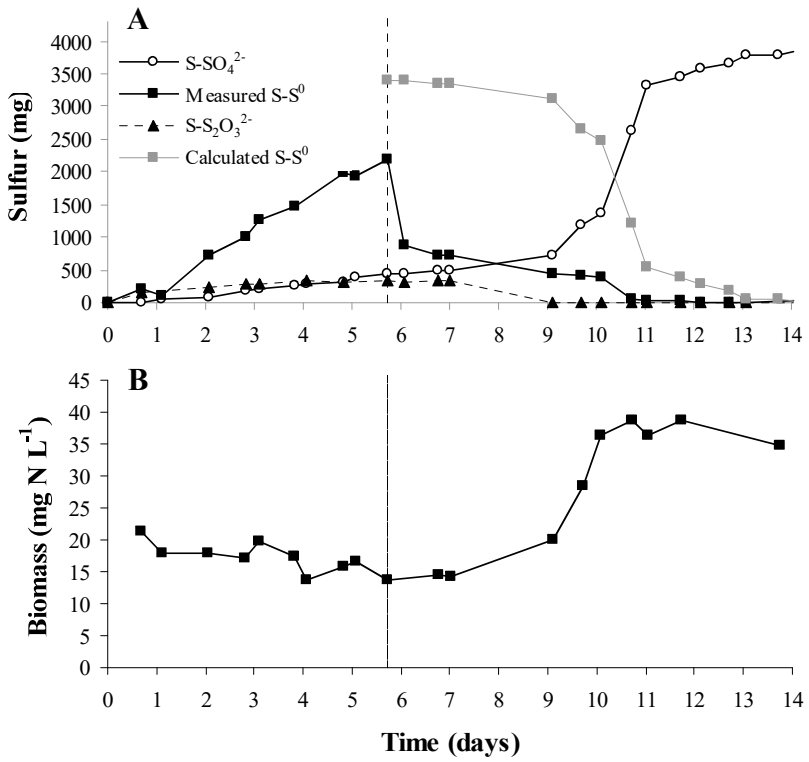
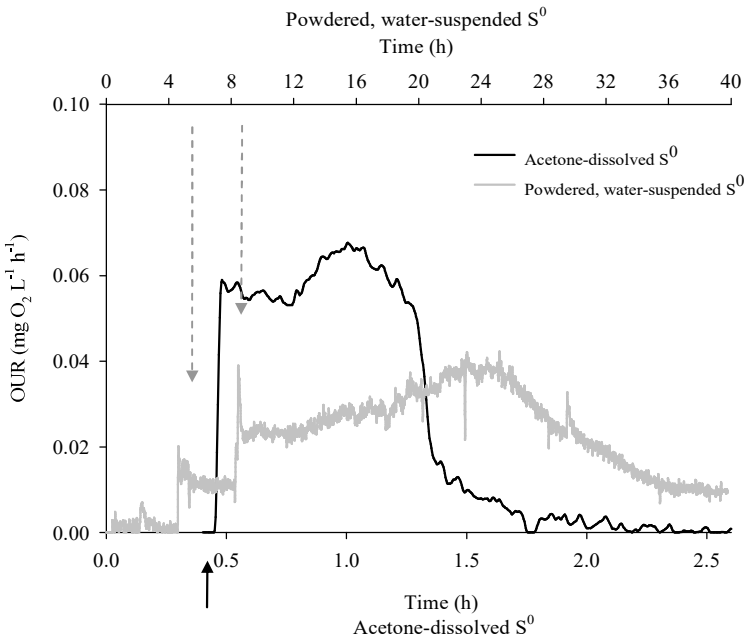


Figure 4 Sulfur balance (A) and biomass concentration (B) during run 4 in the DSTR. The dashed vertical line indicates sulfide supply shutdown. Calculated S^0 concentration based on sulfate production and sulfur mass balance is only represented from sulfide supply shutdown onwards.

1 Figure 5:

2

3



4 **Figure 5** OUR profiles from the two consecutive powdered, water-suspended S^0 pulses are
5 represented on the upper horizontal axis (additions indicated by the gray dashed arrows). The
6 acetone-dissolved S^0 pulse is represented on the lower horizontal axis (addition indicated by the
7 solid black arrow).

8