

1 Oxidation of biologically-produced elemental sulfur at 2 neutrophilic conditions¹

3
4 Marc Fortuny*; Carles Casas*; Xavier Gamisans**; Javier Lafuente*; David Gabriel*

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6 *Department of Chemical Engineering. Universitat Autònoma de Barcelona. Edifici Q.
7 Campus de Bellaterra. 08193 Bellaterra (Barcelona, Spain)

8 **Department of Mining Engineering and Natural Resources, Universitat Politècnica de
9 Catalunya, Manresa, Spain

10 Corresponding author E-mail: marc.fortuny@uab.es; +34-93-581-4795; FAX: +34-93-
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12 13 Abstract

14
15 BACKGROUND: Previous research on a biotrickling filter for the removal of high loads of H₂S
16 showed that accumulation of elemental sulfur (S⁰) when dealing with high H₂S concentrations
17 could lead to reactor clogging. Since S⁰ can also serve as substrate for sulfur-oxidizing bacteria,
18 this study investigates the biological oxidation of S⁰ as a remediation strategy.

19 RESULTS: Results indicated that S⁰ biological oxidation inside a clogged biotrickling filter
20 occurred at a comparable rate as those reported for stirred tank reactors. When biologically-
21 produced dried and powdered S⁰ was manually added as substrate in stirred tank reactor
22 experiments, significantly lower S⁰ oxidation rates were found compared to those for freshly, *in-*
23 *situ* produced biological S⁰. It was speculated that either the powdered S⁰ particle size or the

¹ Paper partially presented in III Congress on Biotechniques for Air Pollution Control, Delft (2009).

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

4 CONCLUSIONS: Therefore, results showed that S^0 oxidation basically depends on S^0
5 bioavailability and that S^0 volumetric oxidation rates as high as $3.48 \text{ mmols SO}_4^{2-} \text{ h}^{-1} \text{ L}^{-1}$ can be
6 achieved inside a clogged biotrickling filter probably due to the high biomass retention capacity.
7 Overall, the results indicate that biological oxidation of S^0 can be considered a suitable strategy
8 for S^0 -clogged bioreactors unclogging.

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

1 Introduction

2 Removal of Reduced Sulfur Compounds (RSC) such as hydrogen sulfide (H₂S)
3 from waste or energy-rich gases has been traditionally carried out by means of physico-
4 chemical systems, such as selective absorption or adsorption processes. Such
5 technologies are based on the high affinity for H₂S of amine-based absorbents or ferric-
6 based adsorbents.^{1, 2}

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

11 The oxidative side of the sulfur cycle and, more specifically, the oxidation of
12 RSC as energy source carried out by sulfur-oxidizing bacteria (SOB) has been
13 intensively studied by several authors^{3,4} as well as its application for water or gas
14 treatment technologies.^{5,6} H₂S removal occurs by an initial absorption and dissociation
15 into an aqueous phase (Eqs. 1 and 2) “which is pH dependent since H₂S is a weak acid
16 (pK_{a1}: 6.9; pK_{a2} =12.92, at 25°C)”⁷ and the subsequent biologically catalyzed oxidation
17 to elemental sulfur (S⁰) or sulfate depending on oxygen availability (Eqs. 2, 3 and 4):³



22 Bioreactors for the treatment of low concentrations of H₂S are nowadays
23 commonly used at solid wastes and wastewater treatment plants^{8,9}, livestock farming¹⁰

1 and other industrial facilities.⁵ In such applications, H₂S is biologically oxidized to
2 sulfate by SOB according to Eq. 4 since bioreactors generally operate in presence of
3 excess oxygen. In this situation, the most common operational difficulty comes from
4 excess media acidification that leads to a pH drop down to toxic levels for biomass.^{11, 12}

5 However, when high H₂S concentrations must be treated, like in the sweetening
6 of energy-rich gases which typically contain some few thousands ppm_v of H₂S¹, only
7 some biotechnological processes have been developed and just a few of them are
8 already commercially available.¹³

9 One of the main difficulties when treating high H₂S concentrations is the
10 capability to supply enough oxygen for the complete biological oxidation of H₂S to
11 sulfate due to a limited O₂ mass transfer capability. Different approaches to deal with
12 this limitation have been used. Buisman *et al.*¹⁴ developed a process consisting of an
13 initial absorption column and a subsequent stirred biological reactor operated under
14 alkaline pH and S⁰ producing (oxygen limited) conditions.² Further developments of
15 this system have been recently published when treating H₂S loads of up to 340 g H₂S m⁻³
16 h⁻¹.¹⁵

17 Bailón¹⁶ developed a biotrickling filter system able to treat 900 ppm_v of H₂S
18 (about 24 g H₂S m⁻³ h⁻¹) where oxygen was supplied forcing the recirculation flow
19 through an air bubble column.

20 A similar approach was studied by Fortuny *et al.*¹⁷ where two lab-scale
21 prototypes were tested and compared for the treatment of up to 10,000 ppm_v of H₂S
22 (more than 300 g H₂S m⁻³ h⁻¹). The system viability and robustness was proved despite
23 accumulation of S⁰ onto the packing material when too low O₂/H₂S supply ratios were
24 used was observed, which would eventually lead to pressure drop, loss of active
25 volume, reduced EBRT, etc.

1 However, it is well known that SOB not only grow on sulfide oxidation but can
2 also use S⁰ according to Eq.5: ^{3, 18, 19}



4 Several authors have studied the mechanism of S⁰ oxidation by *Thiobacilli*.¹⁸⁻²⁰
5 Janssen *et al.*^{21, 22} investigated the properties and stability of biological S⁰ particles.
6 Tichy *et al.*²³ studied the utilization of biological S⁰ for bioleaching purposes. However,
7 all these studies were conducted in liquid-phase, homogeneously-stirred reactors, where
8 S⁰ particles are much more biologically available than would be inside a clogged,
9 packed bed reactor.

10 Since oxidation of accumulated S⁰ in a biotrickling filter reactor was already
11 reported as being the responsible for keeping the system biologically active during gas-
12 phase shutdowns²⁴, the aim of this study was to investigate the biological oxidation of
13 S⁰, combined with the usually applied strategy of S⁰ wash out, when trying to recover a
14 S⁰-clogged biotrickling filter. If in such a system sulfide supply can be discontinued for
15 a period of time but oxygen supply and liquid recirculation are kept on, SOB will be
16 forced to switch to S⁰ oxidation thus leading to reactor unclogging.

17 Materials and methods

18 Biotrickling filter reactor experiments

19 In-situ S⁰ oxidation was investigated in a lab-scale biotrickling filter reactor
20 (BTFR) with an ancilliary unit for oxygen supply (Fig. 1). HD-QPAC® (Lantec
21 Products Inc., CA, USA) with a 4 × 4 mm (0.16” × 0.16”) grid opening cut to tightly fit
22 inside the reactor was used as packing material. Metered amounts of H₂S, N₂ and air

1 using digital mass flow controllers (Bronkhorst, The Netherlands) were used to simulate
2 a controlled biogas inflow. Mineral medium (MM) containing (g L⁻¹): NH₄Cl, 1;
3 KH₂PO₄, 0.12; K₂HPO₄, 0.15; CaCl₂, 0.02; MgSO₄·7H₂O, 0.2; trace elements²⁵, 1 ml L⁻¹,
4 and NaHCO₃ as inorganic carbon source were continuously fed. Liquid phase was
5 continuously renewed by automated timing of the MM supply, bicarbonate supply and
6 the liquid purge, using 3 different peristaltic pumps (Fig. 1).

7 Continuous monitoring of the H₂S concentration in the outlet gas phase was
8 performed using an electrochemical H₂S sensor (Sure-cell, Euro-Gas Management
9 Services LTD, UK). On-line liquid phase monitoring included pH, oxidation-reduction
10 potential (ORP) and dissolved oxygen (DO) measurements. A pH control by HCl or
11 NaOH addition and a level control by liquid purge regulation were also installed.
12 Sulfate and thiosulfate concentrations were measured using an ICS-1000 Ion
13 Chromatography system with an IonPac AS9-HC column (Dionex Corporation).

14 The experiments were carried out after more than a year operating at an inlet
15 H₂S concentration of 2,000 ppm_v (55.6 g H₂S m⁻³h⁻¹), an EBRT of 180 s, a controlled
16 pH range of 6-6.5 and excess oxygen supply. In the currently described experiment with
17 the BTFR, an average hydraulic retention time (HRT) of 26 ± 2 h was used.

18 Prior to S⁰ oxidation, the reactor was forced to produce and accumulate S⁰ for a
19 period of 6 weeks during which the O₂/H₂S supplied ratio was stepwise, weekly
20 decreased from 23.6 down to 1.5 (v v⁻¹), with a maximum applied loading rate (LR) of
21 204.5 g H₂S m⁻³ h⁻¹. Subsequently, sulfide supply was stopped and air supply increased
22 back to the usually applied value during normal operation (20% v v⁻¹). A 50% higher air
23 flow supply than that normally used was also tested during the period of accumulated S⁰
24 oxidation.

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

7 Discontinuous stirred tank reactor experiments

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

13 A series of different runs were carried out either with S^0 or hydrogen sulfide
14 ($\text{H}_2\text{S}_{(\text{g})}$ 99%) supplied via a digital mass-flow controller (Bronkhorst, NL). After each
15 reactor run, biomass was recovered through centrifugation (10,000 rpm, 15 min) and
16 resuspension into fresh MM to be used in the next reactor run.

17 An overview of the operating conditions for the different reactor runs is given in
18 Table 2. All reactor runs were performed under excess DO conditions ($> 5 \text{ mg O}_2 \text{ L}^{-1}$).
19 Additionally, concentrated NaHCO_3 (3 g C L^{-1}) was supplied to avoid carbon limitation.
20 A stirring rate of 400 rpm was used.

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

1 concentrations were carried out as described by van den Bosch *et al.*¹⁵ and Gohering
2 and Helbing²⁸, respectively.

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

5 Respirometric experiments

6 Difference in bioavailability of powdered, dried biological S⁰ and acetone-
7 dissolved biological S⁰ was also studied in respirometric experiments where the oxygen
8 uptake rate (OUR) on biological oxidation of S⁰ was assessed in an LFS respirometer, a
9 continuously aerated bioreactor without continuous liquid inputs nor outputs where the
10 oxygen concentration is measured in the liquid phase and the substrate is fed by
11 pulses.²⁹

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

17 Both the same abovementioned dried, powdered S⁰ (Table 1) and an acetone
18 saturated S⁰ solution (17 μM S⁰) were used as substrates in different runs (Table 3).
19 Run 1B consisted of a second S⁰ pulse addition 3.7 h after beginning of run 1A. Prior to
20 substrate addition in run 1B, also 20 mg C-HCO₃⁻ L⁻¹ were added to avoid carbon
21 limitation.

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

1 Guisasola *et al.*²⁹, using active and washed biomass collected from the BTFR. Upon
2 pulse addition of either solid, powdered S⁰ or acetone-dissolved S⁰, the DO profiles
3 were registered and exogenous OUR (OUR_{EX}) profiles calculated.

4 Possible negative effects of acetone on biological activity or experimental setup
5 response were tested, as well as chemical oxidation of dissolved S⁰.

6 Results and discussion

7 BTFR S⁰ oxidation

8 A sulfur mass balance was carried out from the ionic sulfur species measurement
9 (basically sulfate since thiosulfate and sulfite concentrations were under detection
10 levels) and the sulfide LR and removal efficiency (RE) (Fig. 2). Since S⁰ tends to
11 accumulate inside the reactor, S⁰ production was calculated by subtraction as previously
12 reported.²¹ According to the balance, during the S⁰ accumulation period (days 148 to
13 192) a total amount of 56 g S-S⁰ were produced from the 151 g S-H₂S removed. As it
14 can be seen in Figure 2, there was some S⁰ production from the very beginning, but it
15 was not until high LR were applied (LR ≥ 87 g H₂S m⁻³ h⁻¹; O₂/H₂S ≤ 5; day 178
16 onwards) that S⁰ accumulation really increased. Also, RE > 99% was sustained except
17 for the last LR increase (LR= 204.5 g H₂S m⁻³ h⁻¹, days 185 to 192) when the RE
18 dropped down to 93 ± 1%. The drop in LR was mainly caused by mass-transfer
19 limitation, as previously reported during the application of very similar LR²⁴, since no
20 thiosulfate formation was detected, indicating very low or no sulfide accumulation that
21 would have been chemically oxidized to thiosulfate.

22 As soon as sulfide supply was stopped and the air flow risen, the sulfate
23 production rate significantly increased up to a maximum value of 223 mg S-SO₄²⁻ h⁻¹

1 (3.48 mmols $\text{SO}_4^{2-} \text{ h}^{-1} \text{ L}^{-1}$ packed bed) between days 193 to 199, clearly showing the
2 capacity of the system to oxidize the accumulated S^0 in a very similar rate than those
3 reported for stirred reactors (3.5 mmols $\text{SO}_4^{2-} \text{ h}^{-1} \text{ L}^{-1}$).²¹

4 On day 199 the TLV was set to 17 m h^{-1} in order to increase the oxygen supply
5 to the packed bed. Contrary to what was expected, the sulfate production rate decreased,
6 due to an important wash out of solids that went straight to the reactor purge. Previous
7 results²⁶ had shown that increasing the TLV (in the range of 0.5 to 19 m h^{-1}) could not
8 be expected to directly improve solids wash down on the studied system. However,
9 most probably the high amount of accumulated S^0 after day 192 and the fact that the
10 TLV was directly increased from a low value up to almost the maximum previously
11 tested velocity did result in a severe solids (S^0 and biomass) wash out. As a
12 consequence, a decrease in the total amount of accumulated solids (S^0 and biomass)
13 caused a reduction in the sulfate production rate.

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

1 According to the sulfur balance, only 6 days after starting the S^0 oxidation
2 period, 57% of the accumulated S^0 had been oxidized, which is a similar value to the
3 31-46.5% range reported by Tichý *et al.*²³ as being the percentage of highly bio-
4 available S^0 usually found in biologically produced S^0 . It took 23 days of sulfide
5 starvation for all the accumulated S^0 to be oxidized.

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

12 From these results it is not possible to elucidate whether S^0 oxidation only takes
13 place once sulfide is completely consumed or whether both S^0 and sulfide oxidation
14 occur simultaneously. However, what the results clearly show is that from day 148 to
15 187 the supplied sulfide was rapidly and effectively oxidized whereas S^0 oxidation, if
16 simultaneously occurring, took place at a much lower rate since S^0 accumulated. This
17 means that S^0 production was much faster than sulfate production (S^0 oxidation), which
18 has already been reported in literature.^{19, 34}

19 DSTR S^0 oxidation

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

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

1 Oxidation of externally fed S^0

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

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

13 The difference between results from run 1 and runs 2 and 3 probably relied in
14 biomass adaptation to the DSTR conditions, since run 1 was performed with biomass
15 directly taken from the BTFR where it grew as a biofilm instead of as a free cell
16 suspension. Also, it is possible that the lower S_0 initial concentration played a role due
17 to the previously mentioned dependence between sulfur oxidation and sulfur-surface
18 availability.^{23, 33} Therefore, runs 2 and 3 were considered as more representative of the
19 powdered, dried S^0 oxidation capacity of the studied biomass consortium when grown
20 as a free cell suspension. In order to compare the results from the biotrickling filter
21 reactor and the DSTR reactor, maximum volumetric sulfate production rates were
22 calculated (Table 4). Maximum volumetric sulfate oxidation rates from runs 2 and 3
23 were about three times smaller than those reported by Janssen *et al.* ($3.5 \text{ mmols SO}_4^{2-} \text{ h}^{-1}$
24 L^{-1})²¹ or the observed in the BTFR experiment ($3.48 \text{ mmols SO}_4^{2-} \text{ h}^{-1} \text{ L}^{-1}$).

1 Both the experiment performed by Janssen *et al.*²¹ and the BTFR experiment
2 previously described studied biological oxidation of freshly produced S⁰, although in
3 different types of reactors. However, runs 1 to 3 were performed with externally added,
4 dried, powdered S⁰ as substrate. It is tempting to suggest that the slower S⁰ oxidation
5 rates observed in runs 2 and 3 were caused by a low substrate bioavailability, which in
6 turn could have been caused by different reasons. On the one hand, it has been
7 previously reported that biological S⁰ oxidation is basically a surface-dependent process
8 in terms of both available surface and surface biological colonization³³, which was
9 initially non-existent on the externally added S⁰.

10 On the other hand, according to the particle size characterization (Table 1) 24%
11 of the S⁰ particles were smaller than 1 µm and 69% smaller than 10 µm. Janssen *et al.*²²
12 reported that S⁰ producing bacteria form S⁰ globules that are deposited inside or outside
13 the bacterial cell, with diameters of up to 1 µm. Therefore, with 76% of the added S⁰
14 with a particle size bigger than 1 µm, it can be speculated that S⁰ particle size was, at
15 least, partially responsible for the low S⁰ oxidation rates observed.

16 Moreover, it has been stated that biological S⁰ particles are covered by an
17 extended proteinic polymer layer that normally confers them hydrophilic properties.³⁵
18 However, upon S⁰ drying it may be very possible that those hydrophilic properties are
19 changed or partially lost, therefore reducing S⁰ bioavailability.

20 *Oxidation of in-situ, freshly-produced S⁰*

21 In order to confirm if externally added S⁰ bioavailability was negatively
22 influencing S⁰ oxidation, biological oxidation of fresh, *in-situ* produced S⁰ was studied
23 in run 4.

1 During the first 5.7 days of run 4, 39.2 mg S-H₂S L⁻¹ h⁻¹ were supplied while a DO
2 concentration above 5 mg L⁻¹ was ensured. Sulfide oxidation mainly led to S⁰ formation
3 (clearly visible by a whitish turbidity increase), although some thiosulfate and sulfate
4 were also produced (Fig. 4A). According to the sulfur balance, an average 77.5% of the
5 supplied H₂S was oxidized (30.4 mg S-H₂S L⁻¹ h⁻¹), either as S⁰ (76%), sulfate (15%) or
6 thiosulfate (9%) and the rest would have accumulated as dissolved sulfide or stripped
7 through the gas phase.

8 Thiosulfate and S⁰ formation under such oxidizing conditions clearly indicated
9 an insufficient biological sulfide oxidation capacity that probably allowed sulfide
10 accumulation (not measured). Sulfide accumulation was probably the cause for the slow
11 decrease of biomass concentration observed during the first 6 days of run 4 (Fig. 4B),
12 since sulfide becomes biologically toxic already at very low concentrations (5-30 mg L⁻¹
13 ¹; < 5 mg L⁻¹).^{21, 34} Once sulfide accumulates in an oxidizing environment, it is
14 chemically oxidized mainly to thiosulfate, although some chemical formation of S⁰ has
15 also been reported.²¹

16 However, in this case S⁰ was the main product as well as sulfate, indicating that
17 complete biomass inhibition did not actually occur. Probably both biological and
18 chemical processes contributed to sulfide oxidation during this phase.

19 One day after the sulfide supply was shutdown (day 7), biomass and sulfate
20 concentrations started increasing while S⁰ and thiosulfate decreased, which indicated a
21 fast biological activity recovery (Fig. 4). Once more the sulfur balance did not
22 quantitatively fit the S⁰ measurements but qualitatively they did show a clear S⁰
23 concentration decrease, which was also visible by a decrease in the reactor turbidity.

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

1 4 was much higher than those for run 2 or 3 and actually was very similar to the value
2 reported by Janssen *et al.*²¹ or the value obtained from the BTFR experiment. Hence, it
3 was hypothesized that the fresh, *in-situ* produced S⁰ properties (particle size and surface
4 characteristics) facilitated S⁰ bioavailability and oxidation compared to dried sulfur
5 properties.

6 Therefore, these results show that SOB S⁰ oxidation capacity might not be so
7 different between freshly formed S⁰ accumulated into a biotrickling filter or inside a
8 homogeneous stirred reactor. Similar volumetric S⁰ oxidation rates can be obtained even
9 if S⁰ bioavailability would *a priori* seem to be much lower in a partially-clogged packed
10 bed reactor. A possible explanation could rely on the higher biomass accumulation
11 capacity of packed bed reactors making up for the lower substrate bioavailability
12 compared to stirred systems. Therefore, similar volumetric oxidation rates can be
13 achieved.

14 Respirometric S⁰ oxidation

15 S⁰ biological oxidation was studied in different respirometric runs with a
16 powdered, S⁰ water-suspension and acetone-dissolved (readily available) S⁰. Results
17 from a run with the S⁰ water-suspension (run 1A and 1A+1B) and one with acetone-
18 dissolved S⁰ (run 2) are shown in Table 5, where the expected oxygen consumption was
19 calculated according to the stoichiometry from Eq. 5 and only using 91% of the added
20 substrate since it has been stated that 9% of the available electrons are estimated to be
21 required for CO₂ fixation.³⁶

22 Experimental runs performed with addition of pure acetone (the same volume as
23 the acetone-dissolved S⁰ pulses) showed no increase or decrease in biological activity
24 due to acetone addition. Only a small and temporary sharp increase of DO concentration

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

3 All experiments were carried out under exactly the same conditions of air
4 supply, agitation, temperature and initial biomass concentration. However, the
5 powdered S^0 runs were performed with a much higher substrate initial concentration
6 due to S^0 handling issues and because a reduced substrate bioavailability was already
7 expected. Still, after the first powdered S^0 addition only a very small OUR increase was
8 observed and it rapidly reached a plateau at an average OUR of $0.011 \pm 0.001 \text{ mg O}_2 \text{ L}^{-1}$
9 h^{-1} (Fig. 5). After about 3 hours at the same average OUR, a second powdered, water-
10 suspended S^0 pulse was added, and another small OUR increase up to a new plateau
11 average value of $0.023 \pm 0.001 \text{ mg O}_2 \text{ L}^{-1} \text{ h}^{-1}$ was observed (Fig. 5).

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

20 Indeed, about 3 hours after the second pulse the OUR started increasing up to a
21 maximum value of $0.038 \pm 0.02 \text{ mg O}_2 \text{ L}^{-1} \text{ h}^{-1}$ reached 24 hours after starting the
22 experiment (Fig. 5). After 40 hours the experiment was stopped, although the initial
23 OUR_{END} (taken as $0 \text{ mg O}_2 \text{ L}^{-1} \text{ h}^{-1}$ in Fig. 5) had not been reached yet. According to Eq.
24 5 and to the amount of consumed oxygen and expected oxygen consumption (Table 5),
25 the substrate had not been completely depleted yet.

1 Why did the OUR further increase without addition of any more substrate has
2 not been fully elucidated yet. It has been reported that oxidation of large sulfur particles
3 resulted in their abrasion and breaking into smaller particles,²³ which in turn are faster
4 oxidized.³³ This might explain the observed progressive increase in the maximum OUR
5 over S⁰ oxidation. Another possibility is that a slight biomass growth occurred after
6 more than 12h of experiment (Figure 5) and it caused the slight increase of the OUR.

7 The OUR profile from run 2 is also shown in Figure 5. Substrate depletion was
8 much faster, not only because the total amount of added substrate was smaller (Table 3)
9 but also due to a significantly higher maximum OUR (Table 5). This is a clear
10 indication that S⁰ bioavailability was the limiting factor in runs 1A and 1B.

11 According to the current knowledge of S⁰ biological oxidation, exogenous S⁰
12 (assumed to be in the form of S₈ rings) must be initially converted (dissolved) to highly
13 reactive linear S⁰ to be transported through the outer or inner (depending on the
14 reference) cell membranes for oxidation.^{37, 38} Therefore, both available S⁰ surface and S⁰
15 particles surface characteristics should influence exogenous S⁰ biological oxidation. It is
16 then assumable that the difference on surface characteristics between fresh, *in-situ*
17 produced S⁰ particles and externally added dried, powdered S⁰ particles would also
18 influence S⁰ oxidation.

19 From a reactor operation point of view, it would be interesting being able to
20 maximize S⁰ bioavailability in case of reactor clogging, where probably part of the S⁰
21 will still keep the freshly produced S⁰ properties but part of it will get dry due to
22 channeling of the liquid and gas flows.

1 Conclusions

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

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

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

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

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7

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4 prokaryotes. *Eng Life Sci* **7** (4): 301-309 (2007).
- 5

1 Tables:

2 Table 1: Powdered biological S0 properties.

3

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

4

5

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

2

Run Number	1	2	3	4
Substrate	S ⁰	S ⁰	S ⁰	H ₂ S
Load (mg S-H ₂ S 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

3

4

5

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	mg S-SO ₄ ²⁻ h ⁻¹ L ⁻¹	7.20	34.09	35.31	108.69
sulfate production rate	mmol SO ₄ ²⁻ h ⁻¹ L ⁻¹	0.23	1.07	1.10	3.40
Specific maximum sulfate					
production rate	mg S-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 S⁰ and
2 acetone-dissolved S⁰.

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

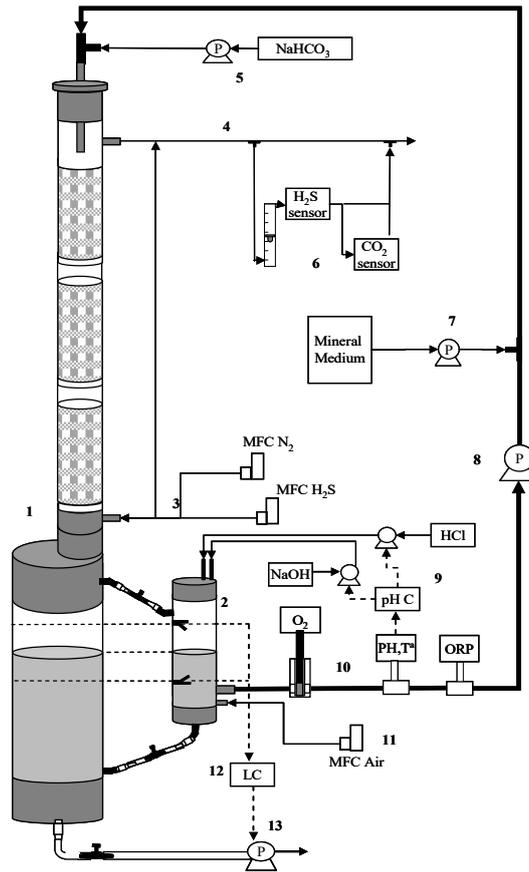
5

1 Figures

2 Figure 1:

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7 **Figure 1:** Schematic of the biotrickling filter reactor. 1: Main reactor; 2: Air supply

8 compartment; 3: Gas inlet; 4: Gas outlet; 5: HCO_3^- supply; 6: Gas monitoring; 7: MM supply; 8:

9 Recirculation pump; 9: pH control; 10: Liquid monitoring; 11: Air supply; 12: Level control;

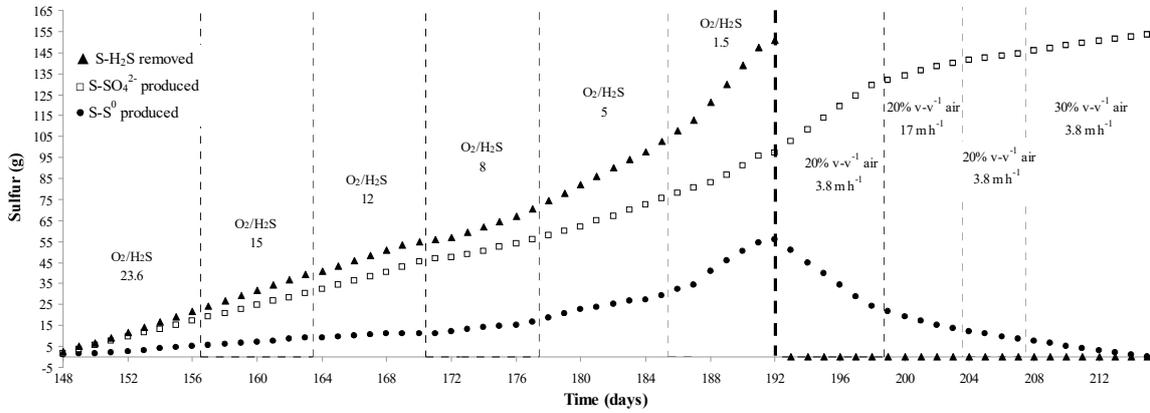
10 13: Liquid purge.

11

1 Figure 2:

2

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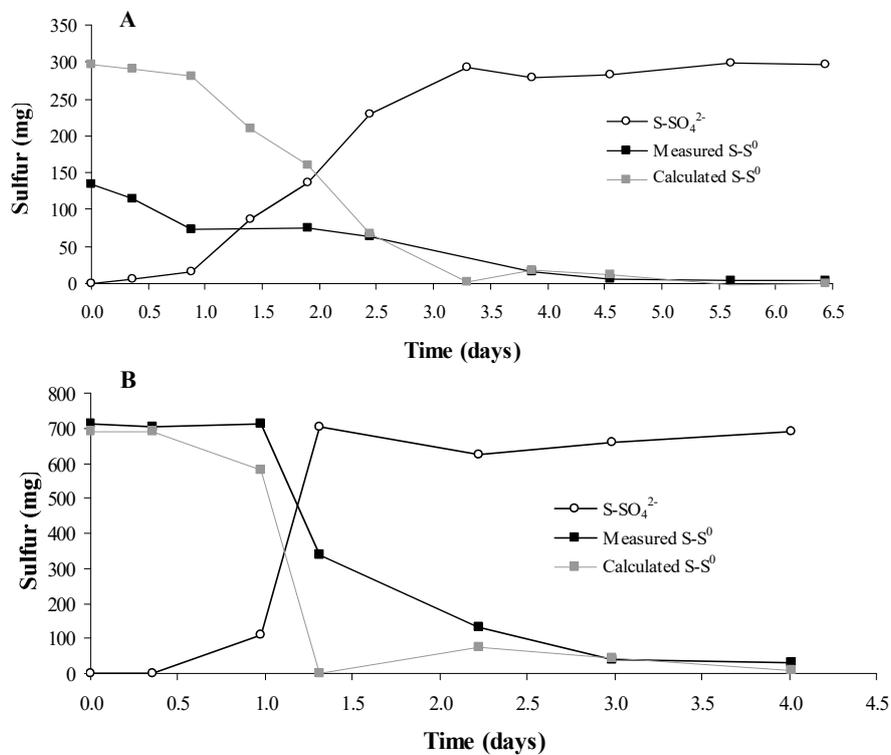
5 **Figure 2:** Sulfur mass balance in the biotrickling filter reactor. The thick dashed line separates
6 the S^0 accumulation period (left side) from the S^0 oxidation period (right side). The thin dashed
7 lines separate periods with different O_2/H_2S supplied ratios during S^0 accumulation (days 148 to
8 192) and different O_2 supply or trickling liquid velocity periods during S^0 oxidation (days 192 to
9 215).

10

1 Figure 3:

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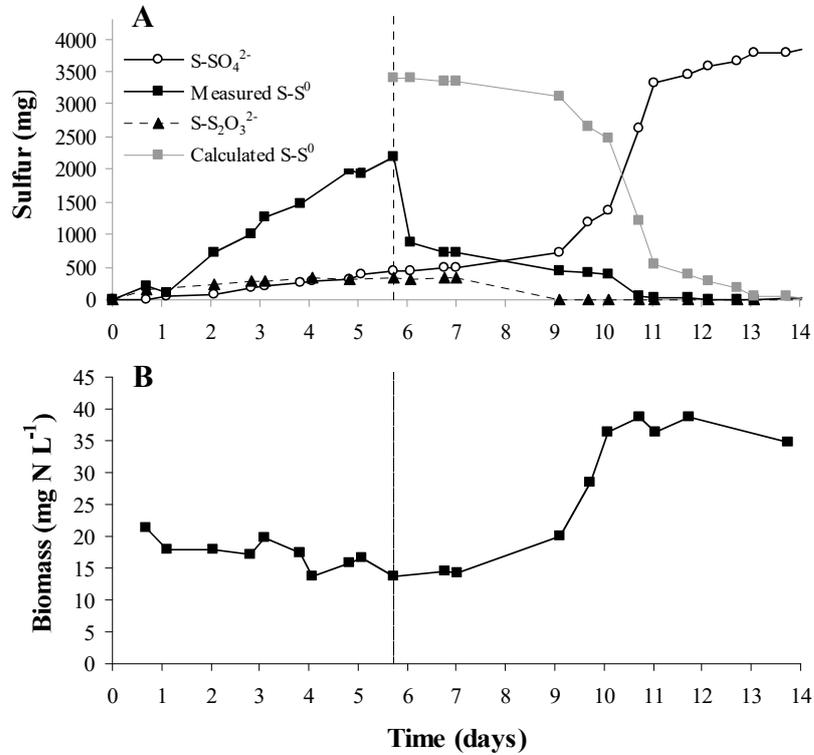
5 **Figure 3** Sulfur balance for runs 1 (A) and 3 (B) in the DSTR with measured sulfate and S^0
6 concentrations as well as calculated S^0 concentrations from sulfate production.

7

1 Figure 4:

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3



4

5 **Figure 4** Sulfur balance (A) and biomass concentration (B) during run 4 in the DSTR. The

6 dashed vertical line indicates sulfide supply shutdown. Calculated S⁰ concentration based on

7 sulfate production and sulfur mass balance is only represented from sulfide supply shutdown

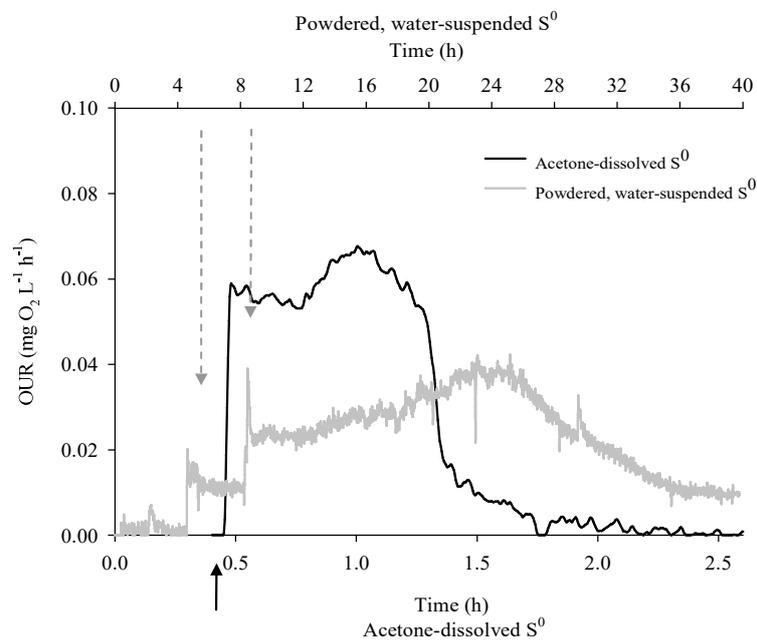
8 onwards.

9

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