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Effect of oxygen on sulfur products from H_2S removal by *Thiobacillus* in a biotrickling filter column

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Abstract

This study investigated a H_2S removal process using *Thiobacillus* in a biotrickling filter column and the effect of dissolved oxygen on the sulfur products. *Thiobacillus* cultures were isolated from UASB mixed liquor of a fruit processing factory using Thiobacillus medium (for *Thiobacillus thioparus*) and Thiobacillus denitrificans medium (for *Thiobacillus denitrificans*). The two cultures were separately inoculated into two lab scale biotrickling filters with H_2S fed at the bottom of the column and culture media trickled from the top. Due to its high solubility, 30-40% of the H_2S was removed in abiotic (control) columns by dissolving into the liquid phase. More H_2S and dissolved sulfide were removed in biotic columns. With H_2S at 2000 ppmv, 89-93% removal was achieved by *T. thioparus* and 74-78% by *T. denitrificans*. The product from H_2S removal by both cultures contained more sulfur in elemental form than in sulfate form. However, element sulfur production was affected by dissolved oxygen (DO). Production of element sulfur was highest at 1- 2 mgO/L. It decreased as DO increased. To determine the species involved in the removal process, molecular identification was conducted. It revealed that four species were common in the two columns. *T. thioparus* and *T. denitrificans* were found in both columns but each predominated in the column containing their respective culture medium.

Keywords: *Thiobacillus*, Hydrogen sulfide removal, Biotrickling filter, Dissolved oxygen, Elemental sulfur, Sulfate

1. Introduction

Biogas is a product from biodegradation of organic matter in an anaerobic process such as septic tank, anaerobic filter, anaerobic digester, or Upflow Anaerobic Sludge Blanket (UASB). Biogas is generally composed of methane and other gases including H_2S. An important factor causing variation in biogas composition is the raw material, as shown in Table1 [1]. High concentrations of H_2S can result from raw material with sulfur protein and the presence of sulfur reducing bacteria [2]. H_2S limits the use of biogas as an alternative energy as it is corrosive to internal combustion engines [3]. This level is set at < 250 ppmv for a combined heat and power production plants utilizing biogas [4]. Many countries set the guidelines for the use of biogas as an alternative fuel. H_2S concentration of 23 ppm is allowed in Sweden, 5 ppm in Switzerland, 30 ppm in Germany and 100 ppm in France [5]. Due to adverse effects of H_2S on people, the Occupational Safety and Health Act (OSHA) sets maximum exposure at 20 ppm for 15 min [6].

H_2S can be removed by physical or chemical processes. These processes are normally costly, demand high energy and may produce waste with environmental implications [7]. Some microorganisms utilize H_2S to obtain energy and the gas can be converted to solid or other less toxic forms. H_2S removal thus can be achieved by biological processes with lower operational costs than those of conventional physicalchemical processes [8-9]. Oxidation of sulfide in a biological system uses oxygen as electron acceptor. Products from biological oxidation of H_2S can be elemental sulfur (S^0) or sulfate (SO_4^{2-}) depending on the amount of oxygen available (equations 1, 2). Element sulfur S^0 is the main product when oxygen is limited [10].

$$H_2S + \frac{1}{2}O_2 \rightarrow S^0 + H_2O \quad (1)$$

$$H_2S + 2O_2 \rightarrow SO_4^{2-} + 2H^+ \quad (2)$$

Sulfide Oxidizing Bacteria (SOB) encompass many genera such as *Thiobacillus, Pseudomonas, Halothiobacillus, Acidithiobacillus, Beggiatoa, Thiothrix, Thioplaca, Thiomicrospira, and Thermothrix* [11]. *Thiobacillus* is one of the genera most commonly used in removing H_2S due to its ability to grow under various environmental conditions with less stringent nutritional requirement. It can oxidize sulfide, sulfur or thiosulfate. Various thiobacilli are capable of oxidizing sulfur compounds but a facultative aerobe, *Thiobacillus denitrificans*, offers advantages over the others [12].

Biological system for gas treatment can be biofiltration, bioscrubber or biotrickling filter [13-15]. In a biotrickling filter, microorganisms immobilized on a medium are packed in a filtering column. An aqueous phase continuously trickles

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Table1 Biogas composition from different substrates [1]

Biogas composition	Organic waste	Sewage	Landfill
Methane CH_4 (% vol)	60 – 70	55 – 65	45 – 55
Carbon dioxide CO_2 (% vol)	30 – 40	35 -45	30 – 40
Nitrogen N_2 (% vol)	< 1	< 1	5 – 15
Hydrogen sulfide H_2S (ppmv)	10 – 2,000	10 – 40	50 – 300

down the column while air containing pollutants is passed through packed bed. Pollutants are absorbed and degraded by immobilized cells resulting in clean air at the outlet. Various types of bacteria have been used in previous studies. Namgung and Song [16] used *Acidithiobacillus thiooxidans* for biogas desulfurization. Toth *et al*. [17] found that 100% H_2S elimination could be achieved in a biotrickling filter with immobilized *Thiobacillus thioparus* when at least 2.5 %vol of O_2 was provided. Solcia *et al.* [18] achieved a comparable removal efficiency (98%) with the use of *Thiobacillus denitrificans* immobilized on a polyurethane foam. Successful removal was obtained from a full-scale biotrickling filter, treating 80 m^3h^{-1} of biogas with an average H_2S concentration of 3,000 ppmin a study by Tomas *et al.* [19].

As O_2 concentration is a constraint in a column and aerobic conditions yield undesirable SO_4, while anoxic condition favors elemental sulfur production, the latter is more attractive. This study investigated the effect of O_2 in H_2S removal by a biotrickling filter process using *Thiobacillus* sp. with the aim of finding conditions favoring the production of elemental sulfur. The experiment was conducted in a lab-scale biotrickling filter reactor packed with immobilized *Thiobacillus* sp. cultures. The cultures were isolated from UASB mixed liquor under conditions favorable to *T. denitrificans* and *T. thioparus*. H_2S, produced from the reaction of Na_2S and H_2SO_4, mixed with air, entering the reactor from the bottom. Removal was compared at varying levels of H_2S and air.

2. Materials and methods

UASB mixed liquor is a promising source for SOB [20] and it is also a common source of biogas. Microbial cultures used in this study were thus isolated from USAB mixed liquor of a food factory.

2.1 Characteristics of UASB mixed liquor

UASB mixed liquor was analyzed for its content of sulfide, sulfate, nitrate, dissolved oxygen, and microbial population (Table 2) according to APHA&AWWA&WEF Standard Methods for the Examination of Water and Wastewater [21]. Determination of *Thiobacillus* population was done as Total Plate Count (CFU/mL) using Thiobacillus denitrificans medium and Thiobacillus medium. The culture media are favorable to *Thiobacillus denitrificans* and *Thiobacillus thioparus*, respectively.

2.2 Isolation of Thiobacillus sp. from UASB mixed liquor

UASB mixed liquor was the source of the bacterial population used in this study. UASB mixed liquor (10 mL) was added to 100 mL of a suitable medium and incubated in an incubator shaker (Innova 4000 Incubator Shaker) at 120 rpm, 27-30 ^oC until the population was sufficient (about 10^7 CFU/mL) for immobilization. The culture media for bacterial isolation were Thiobacillus denitrificans medium and Thiobacillus medium. Thiobacillus denitrificans medium containing 5.0 g/L KNO_3, 5 g/L $Na_2S_2O_3.5H_2O$, 1.0 g/L $NaHCO_3$, 0.2 g/L K_2HPO_4 and 0.1 g/L $MgCl_2$ favors *T. denitrificans.* Thiobacillus medium containing 10 g/L $Na_2S_2O_3.5H_2O$, 1.0 g/L KH_2PO_4, 1.0 g/L NH_4Cl and 0.5 g/L $MgCl_2.7H_2O$ favors *T. thioparus* [22]. The main difference in the culture media are their nitrogen source, KNO_3 in Thiobacillus denitrificans medium and NH_4Cl in Thiobacillus medium. Both culture media are selective for the Thiobacilli group but not highly selective to specific species of Thiobacilli. The cultures obtained likely contained mixed species of Thiobacillus with one species predominating. Since Thiobacillus denitrificans medium favors *T. denitrificans* and Thiobacillus medium favors *T. thioparus*, the cultures were assumed to be *T. denitrificans* and *T. thioparus*, respectively.

2.3 Immobilization of Thiobacillus culture on filtering media

Plastic reticulated balls (20 mm diameter) were used for cells immobilization. The balls were packed in the columns (76.2 mm. diameter x 533.4 mm. high) filled with a suitable culture medium. Prior to packing, both the balls and the columns were sanitized with 70% ethanol. The bacterial cultures discussed in Section *2.2* were centrifuged (HERMLE Z383K) at 8000G (equivalent to 8181 rpm) for 10 minutes then inoculated into the columns. Each culture was immobilized in separate columns at an ambient temperature of 28 ± 2 ^oC. The culture media were changed every 2 days and the bacterial population was determined as dry weight (mgDW/ball). To remove the bacterial population from the bioballs, the balls were put in 50 mL centrifuge tubes with 20 mL of distilled water. The tube was sonicated in ultrasonic bath (Grant XUBA Analogue) for 10 minutes and then centrifuged at 8000G for 10 minutes. Loosened biofilms were then determined for volatile suspended solids (VSS) content. The columns were used for H_2S removal experiment when a sufficient population was obtained, indicated by the population on the biofilm reaching 10^7 CFU/mL (2000 – 2500 mgDW/L) (Equation (3)).

$$\text{Population in column} = \frac{(\text{mgDW/ball}) \times \text{no.of ball in column} \times 1000\text{mL}}{\text{mLcolumn}} \quad (3)$$

2.4 Biotrickling filter startup

The biotrickling filter columns used in the H_2S removal experiment included two columns. One was inoculated with the culture from Thiobacillus denitrificans medium and identified as *T. denitrificans*. The other was was inoculated with Thiobacillus medium and identified as *T.thioparus.* Two abiotic columns (without bacterial culture) were used as experimental controls for each culture. The system set up for this experiment is shown in Figure 1. The experiment was conducted at an ambient temperature of 28 ± 2 ^oC. An inorganic substrate and moisture required for bacterial growth was trickled down the column while H_2S gas was fed in at the bottom. Since H_2S was intended to be the sulfur

Table 2 Analytical methods for chemical and microbiological analyses [21].

Characterization	Method No.	Instrument
1. Sulfate (SO_4^{2-}-S)	-	IC* 761 Compact (Metrohm)
2. Nitrate (NO_3^{2-}-N)	-	IC* 761 Compact (Metrohm)
3. Dissolve Sulfide (S^{2-}-S aq)	4500-S^{2-}F	-
4. Dissolve Oxygen (DO)	4500-O B	-
5. Volatile Suspended Solids (VSS)	2540 D	-
6. pH	4500-H^+	872 pH lab (Metrohm)
7. Population of *T. denitrificans* and *T. thioparus* by Total Plate Count Method	9215 B	-

* IC :Ion Chromatography

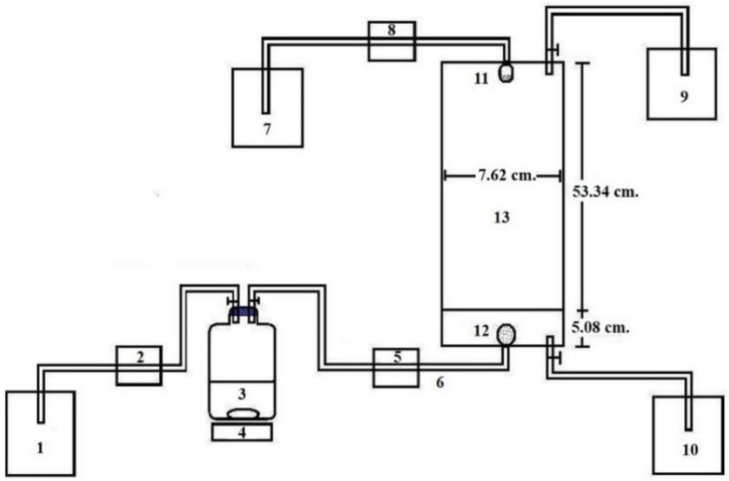

Figure 1 Experimental set up : (1)H_2SO_4, (2)peristaltic pump, (3)Na_2S, (4)magnetic stirrer, (5)air compressor, (6)inlet gas, (7)medium tank, (8)pump, (9)outlet gas tank, (10)effluent tank, (11)broth dispenser, (12)gas dispenser, (13)column, (14)H_2S sampling port, and (15)H_2S tank

source for bacteria in the columns, a suitable culture medium with no sulfur was supplied as inorganic substrate. H_2S was produced by the reaction of Na_2S and $H2_SO_4$ [23] at initial concentrations of 650 and 1300 ppmv (the amount calculated from the sulfur content of each culture media formula) for *T. denitrificans* and *T. thioparus*, respectively. The gas produced was kept in a storage bag and fed in at the bottom of the column using a peristaltic pump (Cole Parmer Masterflex LS 200-1558). Nutrients and gas were supplied at the same flow rate of 0.036 m3/h, which was the optimum rate obtained from a previous study [24]. The H_2S concentration was then increased stepwise by 270 ppmv until the concentration reached 2000 ppmv (the level of H_2S found in biogas produced from organic waste [1]).

2.5 Variation in oxygen supply into the columns

To investigate the effect of oxygen on the products of H_2S oxidation, oxygen supplied to the columns as discussed in Section *2.4* (H_2S 2000 ppmv) was varied. Oxygen was supplied via aeration of culture media. The amounts supplied (1, 2, 3, 4, 5 and 6 mgO/L) were regulated by varying the aeration times. The oxygen concentration was maintained at each level for 2 days. Samples of input and output gas as well as the effluent from the columns were collected for chemical and microbiological analysis at 24 hour intervals.

2.6 Chemical and microbiological analysis of column content

H_2S and O_2 contents in input and output gases were determined using a Piston Hand Pump Gas Detection LP-1200 (RAE system) employing a lead acetate method [25] and the Japanese Industrial Standard (JIS), respectively. Chemical analysis of the effluent was conducted to follow the biological activity in the column. Total dissolved sulfide (S^{2-}), dissolved oxygen (DO), sulfate (SO_4^{2-}), nitrate (NO_3^-), and pH were determined according to APHA&AWWA&WEF standard methods [21] as summarized in Table 2. Elemental sulfur (S^0), both in the effluent and accumulated on the bioballs, was determined using a CHNO/S elemental analyzer (TruSpec®Micro, Leco).

Bacterial population density was determined as its dry weight (mgDW/L) using the technique discussed in Section *2.3*. The bacterial consortia in both columns were also of prime interest. The bacterial species were identified using a molecular technique (PCR-DGGE). Samples were aseptically collected then ultrasonically homogenized and centrifuged. The settled portion was used for DNA extraction using a Soil DNA Kit (FavorPrep™), in accordance with the protocol developed by the manufacturer. Extracted DNA was amplified using Fast Start Essential DNA Green Master (Roche) with primers U968F-GC clamp and 1401R for 16S rRNA gene of domain bacteria. Amplification was performed in a thermal Cycler® Nano (Roche). The PCR cycle consisted of an initializing step at 95ºC for 5 min followed by 25 cycles of denaturation at 95ºC for 50 s, annealing at 60ºC for 30 s and extension at 72ºC for 2 min. PCR products were checked for the number of base pairs (473 bp for domain bacteria) by electrophoresis on a 1% agarose gel. PCR products of 473 bp size were separated by DNA sequences using the DGGE technique (Bio Rad DCode™ Universal Mutation Detection System), following

the protocol developed in a previous study [26]. The selected bands of DNA fragment from DGGE were purified using a HiYield® Gel/PCR DNA Fragment Extraction kit (RBC Bioscience) and amplified with the aforementioned primer sets. A single DNA fragment was then sequenced using a DNA sequence analyzer (AIT Biotech Pte Ltd, Singapore). The DNA sequences were compared to the 16S rRNA gene sequences available in the National Center for Biotechnology Information (NCBI) database using the BLASTn search option.

3. Results

3.1 Characteristics of UASB mixed liquor

Mixed liquor from the UASB tank of a fruit processing factory was analysed to determine its chemical composition. The results were compared to the formula of Thiosulfate Mineral Salt Medium (TMSM) and are shown in Table 3. TMSM is a culture medium enriched with nutrients promoting the growth of Sulfate Oxidizing Bacteria (SOB). The amounts of sulfate (45.5 mgS/L), nitrate (141.5 mgN/L) and total dissolved sulfide (249 mgS/L) in UASB mixed liquor were lower than those in the formula. However, UASB mixed liquor could support SOB as can be seen in the populations of *T. denitrificans* and *T. thioparus* (4.3×10^4 and 7.4×10^4 CFU/mL, respectively). UASB mixed liquor should be suitable as the source of microbial consortium in the biotrickling filter experiment. This was confirmed by the results from molecular identification of bacteria in UASB granules conducted by Keyser et al. [20] in which 3 species of SOB were found among 68 species in DNA fingerprints.

3.2 Biotrickling filter startup

In the startup stage, two biotic columns (using the two Thiobacillus sp. immobilized on bioballs) and two abiotic columns were fed with H_2S gas and inorganic nutrients suitable for each culture. The concentration of the gas was increased stepwise by 270 ppmv until it reached 2000 ppmv, the level found in biogas. The changes in sulfur compounds (gaseous H_2S, total sulfide, sulfate) and bacterial population (dry weight) over a five day test period are shown in Figure 2 (abiotic columns) and Figure 3 (biotic columns).

The abiotic columns of *T. denitrificans* and *T. thioparus* were slightly different in the inorganic nutrients supplied and the initial amount of H_2S. The changes in sulfur compounds in both columns were identical. H_2S in the output gas and total sulfide increased with increasing H_2S input. In the early period (with 650 and 1300 ppmv in *T. denitrificans* and *T. thioparus* columns respectively) H_2S(g) output was approximately 25% lower than H_2S input. When H_2S reached 2000 ppmv, the decrease was found to be in the range of 30-40%. The loss in H_2S was due to the high solubility of H_2S in water. This was confirmed by the presence of total dissolved sulfide, which increased from 192 and 425 mgS/L at the start to 600 and 637 mgS/L at the end of the test in *T. denitrificans* and *T. thioparus* columns respectively. Sulfate increased slightly from the first day to the end of the test period, from 46 to 80.4 and 85.7 to 90. 9 mgS/L in *T. denitrificans* column (Figure 2(a)) and *T. thioparus* column (Figure 2(b)), respectively. Since the bacterial population was not found on the bioballs, sulfate was thus a result of chemical oxidation. Chemical oxidation of sulfide to sulfate was possible but to an extent lower than that of biological oxidation [27].

The changes in the columns with *T. denitrificans* (Figure 3(a)) and *T. thioparus* (Figure 3(b)) were different from those in abiotic columns. The initial bacterial population of 10^7 CFU/mL or 2,450 mgDW/L for *T. denitrificans* culture and 2,000 mgDW/L for the *T. thioparus* culture decreased slightly in the early period, then increased and reached a steady state (of 4,000 mgDW/L and 3,000 mgDW/L, respectively). The growth in the *T. denitrificans* column appeared to be greater than that of *T. thioparus*, yielding a higher population at steady state.

The H_2S outputs of the biotic columns were much lower than that of the abiotic columns. The H_2S concentration in the output gas was quite constant, in the range of 150-400 ppmv even when the H_2S input reached 2000 ppmv. H_2S removal at the start was 26% (as was observed in the abiotic columns). It then increased to 74-78% removal in the *T. denitrificans* column and 89-93 % in the *T.thioparus* column. The total dissolved sulfide was also lower in biotic columns, in the range of 110 - 300 mgS/L. Nishimura and Yoda [28] explained that H_2S was first absorbed into the liquid phase prior to biological oxidation. Along with the decrease in H_2S output and the increase in the bacterial population, sulfate, which is a possible oxidation product of H_2S, increased with the increase in H_2S input. Over the entire the test period, sulfate increased from 155 to 900 mgSO_4-S/L in the *T. denitrificans* column and from 248 to 750 in mgSO_4-S/L in the *T.thioparus* column. Elemental sulfur was noticeable on the bioballs and in the effluent. The amount of sulfur on the bioballs was 0.8 mgS/ball in the *T. denitrificans* column and 4.3 mgS/ball in the *T.thioparus* column. The increases in sulfate and bacterial population indicated biological oxidation of the dissolved sulfide in the columns.

Subtracting H_2S removal (30 and 40%) in the control (abiotic) columns from those of the biotic columns (78 and 93%) yielded net removal efficiencies of 48% for the *T. denitrificans* column and 53% for the *T. thioparus* column. It is notable that while the abiotic columns removed H_2S by converting gaseous H_2S into a dissolved form, the biotic columns removed H_2S by further oxidizing the dissolved sulfide into sulfate and elemental sulfur. H_2S was thus removed from the system more completely.

Table3 Characterization of chemical and microbiological properties of UASB mixed liquor

Characterization	UASB mixed liquor	TMSM
1. Sulfate (SO_4^{2-}; mgS/L)	45.5	78.1
2. Nitrate (NO_3^{2-}: mgN/L)	141.5	277.00
3. Dissolve Sulfide (S^{2-}aq ; mgS/L)	249	644.70
4. Dissolve Oxygen (DO ; mgO/L)	0.68	-
5. Volatile Suspended Solids (VSS ; mg/L)	5680	-
6. pH	6.8	7.0
7. Population of *T.thioparus* (CFU/mL)	4.3×10^4	-
8. Population of *T.denitrificans* (CFU/mL)	7.4×10^4	-

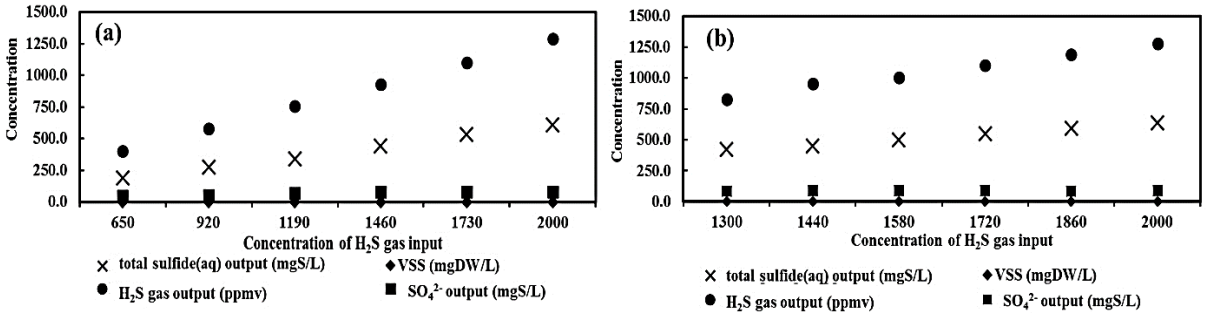

Figure 2 The changes in sulfur compounds (H_2S in the input and output gas, total dissolved sulfide and sulfate) and bacterial population on bioballs (VSS) during the start up period in the abiotic columns with (a) Thiobacillus denitrificans, and (b) Thiobacillus media

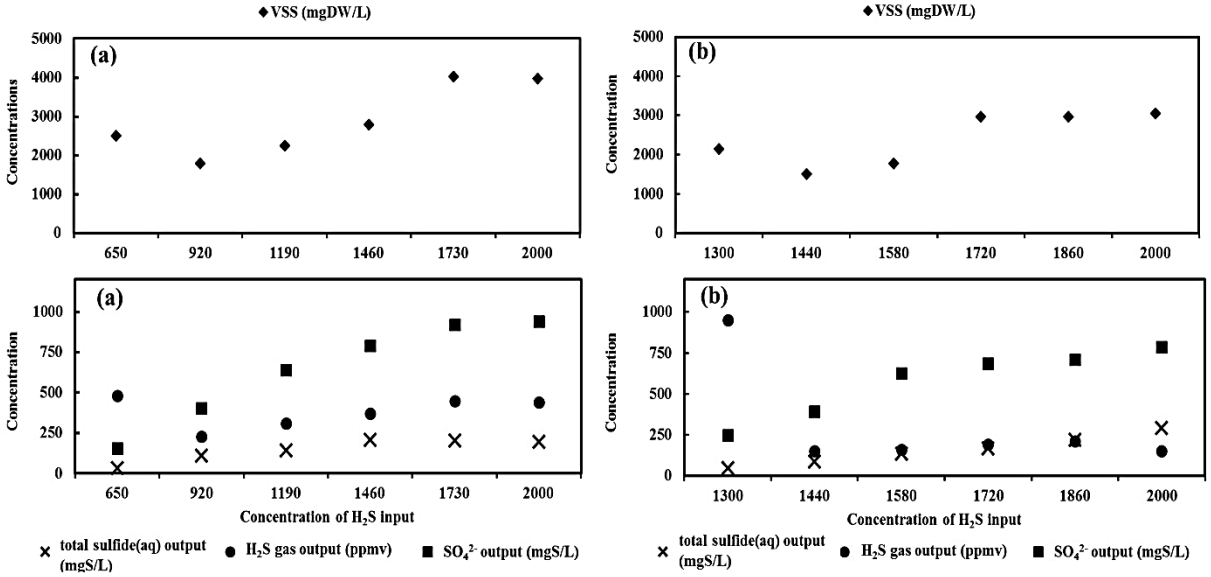

Figure 3 The changes in sulfur compounds (H_2S in the input and output gas, total dissolved sulfide, and sulfate) and bacterial population on bioball (VSS) during the start up period in (a)*T.denitrificans* and (b) *T.thioparus* columns

Both bacterial cultures isolated from UASB mixed liquor showed a potential in removing H_2S. The *T. thioparus* column gave better H_2S removal (89-93%) than *T. denitrificans* (74-78%), however the population and the oxidation product (SO_4^{2-}) of the former were lower than the latter. This indicated that the *T. denitrificans* culture gave higher yield (biomass/substrate) than *T. thioparus*. As H2S is a waste to be removed, removal performance was of more interest than yield. *T. thioparus* culture was thus considered superior.

3.3 Effect of dissolved oxygen on sulfur product of hydrogen sulfide oxidation

After the startup, the columns in steady state were tested for the effect of dissolved oxygen (DO). With H_2S input of 2,000 ppmv and DO started at lower than 1 mgO/L, oxygen supply to the columns was varied to achieve DO of 1-6 mgO/L. The results obtained from the abiotic columns were as shown in Figure 4. The H_2S in the output gas remained at 1,250-1,300 ppmv or 36% lower than that of the input gas, with the total sulfide at 600-630 mgS/L and sulfate at 93-97 mgS/L. The amounts of these three sulfur compounds were slightly different from those obtained at the end of the startup period. Sulfate increased slightly with increased DO. This was due to the oxidation of sulfide [27]. A bacterial population was not found in either abiotic column throughout the experiment.

In the biotic columns, variation in DO slightly affected the bacterial population and the amount of H_2S in the output gas (Figure 5(a) and (b)). In this phase, DO was varied in the columns at the end of the startup phase, with a H_2S level of 2000 ppmv. The populations on the bioballs in both columns were in the same range as at the end of the startup phase, 3,800-4,100 mgDW/L of *T.denitrificans* and 2890-320 mgDW/L of *T.thioparu*s. The H_2S output was also close to those obtained in the startup phase, varying over a narrow range of 370-510 ppmv in *T.denitrificans* column and 140-260 ppmv in *T.thioparus* column. H_2S removal was 78% and 91% for *T.denitrificans* and *T.thioparus*, respectively (Figure 5(a) and (b)). The H_2S gas was absorbed in the column not by biological activity but by dissolving into liquid phase. Due to its high solubility, almost all of H_2S was readily absorbed into the column. The difference in the amount removed was thus not very clear.

On the other hand, oxygen showed stronger effects on sulfur compounds related to H_2S oxidation (Figure 6). As DO increased, total dissolved sulfide slightly decreased from 180.8 to 145.0 mgS/L in the *T.denitrificans* column and 162.7 to 139.9 mgS/L in the *T.thioparus* column. Dissolved sulfide is the form of sulfur that bacteria absorb for utilization [28]. The decrease in the remaining amount

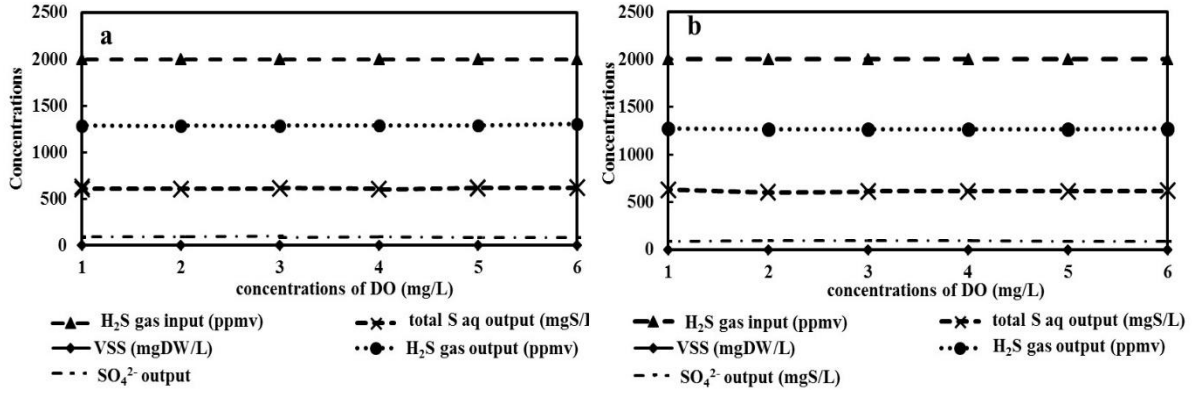

Figure 4 The changes in sulfur compounds (H_2S in the input and output gas, dissolved H_2S and sulfate) and bacterial population on bioballs (VSS) in abiotic columns of (a)*T.denitrificans* and (b) *T.thioparus* at varying DO levels (1-6 mgO/L)

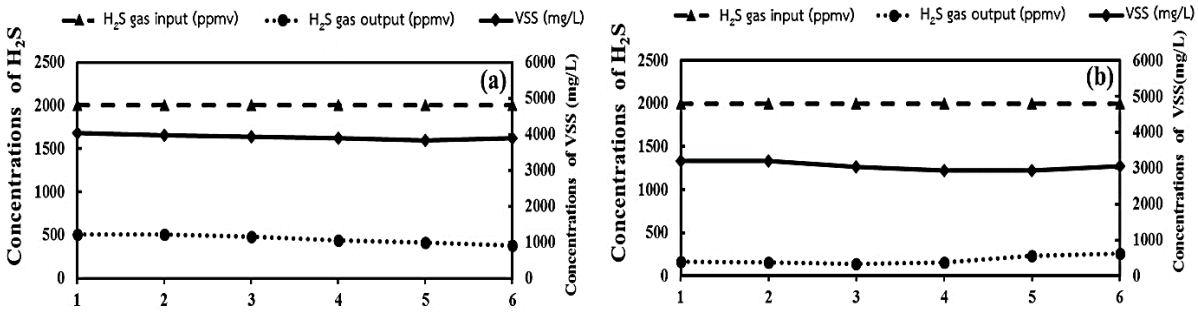

Figure 5 The changes in H_2S (H_2S in the input and output gas) and bacterial population (mgDW/L) in biotic column of (a)*T.denitrificans* and (b) *T.thioparus* columns at DO levels of 1-6 mgO/L

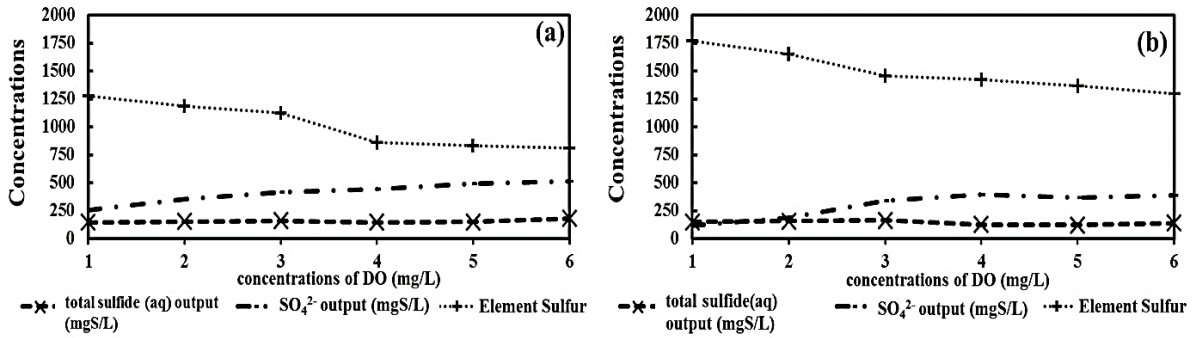

Figure 6 The changes in sulfur compounds (dissolved sulfide, sulfate and element sulfur) in biotic columns of (a) *T.denitrificans* and (b) *T. thioparus* columns at DO levels of 1-6 mgO/L

showed the extent of biological utilization. Together with the decrease in dissolved sulfide, sulfate amunt showed an increasing trend as DO increased. Varying DO from 1 to 6 mgO/L, sulfate concentrations increased from 230 to 520 mgS/L in *T.denitrificans* column and from 115 to 384 mgS/L in *T.thioparus* column. Oxygen showed clear effect on the production of elemental sulfur. Elemental sulfur was found accumulated on the bioballs and in the liquid effluent of the column. In both columns, the amount of element sulfur was highest at DO of 1-2 mgO/L. It was then decreased as DO was increased to 6 mgO/L. In the *T. denitrificans* column, element sulfur decreased from 1250 to750 mgS/L. In the *T. thioparus* column, it decreased from 1750 to 1250 mgS/L. This, together with the sulfate result, showed that the products of H_2S oxidation were strongly influenced by the DO level. It was notable that the *Thiobacillus* consortium yielded more sulfur in elemental form than in sulfate form. Considering the impact on the environment, *Thiobacillus* consortium are attractive for H_2S oxidation.

3.4 Microbial diversity on biofilm

Molecular identification of the bacterial populations attached to the bioballs in both biotic columns revealed existence of various bacterial species. From the DNA fingerprints (Figure 7) six distinctive bands was obtained from *T.thioparus* sample and four bands from *T.denitrificans*. The common bands found in both columns were not unexpected since both cultures were isolated from the same source (UASB mixed liquor) and the culture media used were not highly selective for a specific group. Determination of DNA sequences of the distinctive bands and analysis of the sequences revealed that the predominant species in the *T. thioparus* column were related

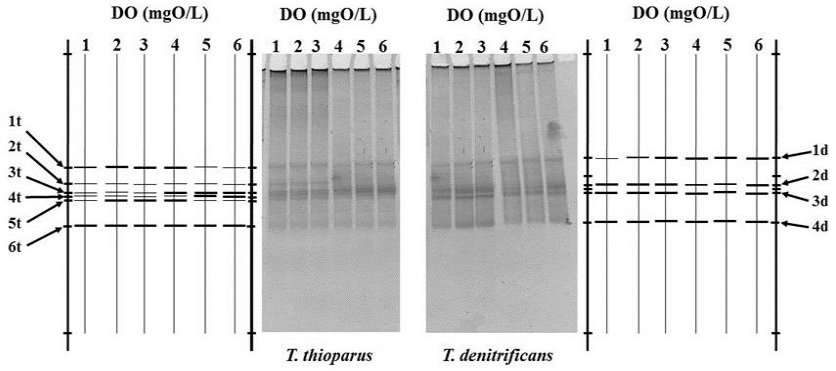

Figure 7 DGGE fingerprints of PCR-amplified DNA of Thiobacillus populations immobilized on bioballs in *T.denitrificans* and *T.thioparus* columns at DO levels of 1-6 mgO/L

to *Thiobacillus thioparus* DSM 505 (97% similarity), *Pseudomonas fluorescens* Pf1 (97% similarity), *Microbacterium* sp. CHQ-1 (98% similarity), *Microbacterium* sp. D14B (99% similarity), *Thiobacillus denitrificans*DXM 807 (98% similarity) and *Halothiobacillus* sp. HL1 (97% similarity). The predominant species in the *T. denitrificans* column was related to *Thiobacillus thioparus* DSM 505 (97% similarity), Microbacterium sp. CHQ-1 (98% similarity), *Thiobacillus denitrificans* DXM 807 (98% similarity) and *Halothiobacillus* sp. HL1 (97% similarity). Even though *T. thioparus* and *T. denitrificans* were found in both columns, the respective intensities of DNA bands were different.

4. Conclusions

The UASB mixed liquor of fruit processing factory was a good source of a mixed bacterial population for H_2S oxidation. *Thiobacillus* cultures could be isolated using a Thiobacillus medium (as *T.thioparus*) and Thiobacillus denitrificans medium (as *T.denitrificans*). They were successfully used in a biotrickling filter as immobilized cells to remove H_2S. At 2000 ppmv, 91% and 78% H_2S removal could be achieved for *T.thioparus*, *T.denitrificans*, respectively. Due to its high solubility, H_2S was first absorbed into the biotrickling filter column as a dissolved sulfide which was then used by bacteria. *Thiobacillus* populations yielded sulfur products both in elemental and sulfate forms. In both columns, the production of elemental sulfur was highest at a DO of 1-2 mgO/L. It then decreased as DO was increased. Since sulfate is a pollutant, H_2S removal by Thiobacillus populations is attractive, especially when DO is low.

Molecular identification showed that the two populations consisted of four common bacterial species. *T.denitrificans* and *T.thioparus* were found in both populations, but they predominated in their respective culture medium.

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