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Research article

Biofiltration of trimethylamine in biotrickling filter inoculated with *Aminobacter aminovorans*

Alberto Aguirre [a], Pamela Bernal [a], Daniela Maureira [a], Nicolás Ramos [a], Javier Vásquez [a], Homero Urrutia [b], Juan Carlos Gentina [a], Germán Aroca [a,*]

[a] Escuela de Ingeniería Bioquímica, Facultad de Ingeniería, Pontificia Universidad Católica de Valparaíso, Av. Brasil 2085, Valparaíso, Chile
[b] Centro de Biotecnología, Universidad de Concepción, Víctor Lamas 1290, Concepción, Chile

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ABSTRACT

Background: Trimethylamine (TMA) is the main responsible for the odor associated with rotting fish and other annoying odors generated in many industrial activities. Biofiltration has proved to be efficient for treating odorous gaseous emissions. The main objective of this work was to determine the removal capacity of TMA of a biotrickling filter inoculated with *Aminobacter aminovorans* and to evaluate the effect of H_2S on its performance.
Results: The maximum specific growth rate of *A. aminovorans* in a liquid culture was 0.15 h^{-1}, with a TMA to biomass yield of 0.10 ($g\,g^{-1}$) and a specific consumption rate of 0.062 $g \cdot g^{-1} \cdot h^{-1}$. The initial specific consumption rate of TMA was highly influenced by the presence of H_2S in liquid culture at concentrations of 20 and 69 ppm in heading space of the flasks. A BTF inoculated with *A. aminovorans* showed removal efficiencies higher than 98% in a range of loading rate of 0.2 to 8 $g \cdot m^{-3} \cdot h^{-1}$ at empty bed residence time (EBRT) of 85 and 180 s. No effect on the elimination capacity and efficiency was detected when H_2S was added at 20 and 50 ppm to the inlet gaseous emission, though the fraction of *A. aminovorans* measured by qPCR in the biofilm decreased.
Conclusions: A biotrickling filter inoculated with *A. aminovorans* can remove efficiently the TMA in a gaseous stream. The elimination capacity of TMA can be negatively affected by H_2S, but its effect is not notorious when it is forming part of a biofilm, due to its high specific consumption rate of TMA.

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1. Introduction

Volatile amines are one of the main responsible of odor nuisances in many industrial activities; usually they are generated by the decay or biological degradation of organic material. In particular, trimethylamine ($(CH_3)_3N$), is the main responsible for the odor associated with rotting fish and is one of the major sources of annoying odors generated in many industrial activities like fish-meal manufacturing processes, wastewater treatment plant, waste disposal landfills, livestock farming and hog manure, and rendering plants [1]. The source of trimethylamine (TMA) is not fully established, but there is evidence that it is produced by the action of microorganisms on choline, betaine or trimethylamine N-oxide [2]. The reported TMA odor threshold is in the range of 0.00021–0.00058 ppm while characteristic concentrations of TMA emitted in such discharges between 5 and 100 ppm [3,4,5].

In the last decade there has been an increased concern related to the presence of amines in gaseous emissions due to their toxic effects on human health because of its potentially toxic and carcinogenic effects [6]. The cost of using physical–chemical operations for depleting their presence in gaseous streams and the potential adverse effects resulting from the presence of residually persistent unknown by-products in the treated stream, have made that biological systems have been preferentially adopted [7,8,9].

Biological removal of amines could be accomplished by aerobic and anaerobic microorganisms. In aerobic conditions, TMA is oxidized to diethylamine (DMA) and formaldehyde by a TMA dehydrogenase. A second pathway for utilization of TMA is due to a TMA monooxygenase that oxidize TMA to TMA N-oxide that is subsequently demethylated by a TMA demethylase to DMA and formaldehyde. DMA is oxidized to methylamine (MA) and formaldehyde by a DMA monooxygenase. MA

Abbreviations: BTF, biotrickling filter; c_{in}, inlet TMA concentration; c_{out}, outlet TMA concentration; DGGE, denaturing gradient gel electrophoresis; DMA, diethylamine; EBRT, empty bed residence time; EC, elimination capacity ($gTMA \cdot m^{-3} \cdot h^{-1}$); F, flow ($m^3 \cdot h^{-1}$); H, height (m); ID, inside diameter (m); L, loading rate ($gTMA \cdot m^{-3} \cdot h^{-1}$); MA, methylamine; OD, outside diameter (m); PVC, polyvinyl chloride; qPCR, quantitative polymerase chain reaction; RE, removal efficiency (%); TMA, trimethylamine; V, volume (m^3).

* Corresponding author.
E-mail address: german.aroca@pucv.cl. (G. Aroca).
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is oxidized by a MA dehydrogenase or by a MA oxidase to formaldehyde and ammonia that can be used as a carbon and nitrogen source for some microorganisms present in a biological treatment system. There are other routes proposed for the conversion of MA to formaldehyde through glutamate by a *N*-methylglutamate synthase, g-glutamylmethylamide synthase and a *N*-methyl glutamate dehydrogenase [10]. Thus, microbial degradation would be an efficient way of eliminating TMA in industrial gaseous emissions. There are a few reports about biofiltration of amines as individual compounds or in complex mixtures. Chang et al. [11] used a biofiltration system containing a mix of microorganisms obtained from an activated sludge of a wastewater treatment plant to treat a TMA-containing waste gas, obtaining a removal efficiency higher than 90% at TMA inlet loads below 27.2 $mgN \cdot h^{-1}$, using a long retention time of 318 s. Ding et al. [12] showed the complete oxidation of TMA to NO_3^- in the compost biofilter due to the presence of nitrifying bacteria. Ho et al. [13] also showed that a biofilter inoculated with a nitrifying microorganism *Arthrobacter* sp. removes efficiently TMA and NH_3 from the exhaust air of a swine waste storage pit. The inoculation of *Paracoccus* sp. CP2 and *Arthrobacter* sp. CP1 as inoculum into a biofilter allowed the removal of TMA in a mixture with DMA and MA at EBRT of 60 s treating emissions containing TMA in a range of 10–100 ppm [14]. Wan et al. [15] reported the biofiltration of waste gas containing high concentration of TMA using a Biotrickling filter (BTF) packed with ceramic particles and inoculated with B350 a mixture of microorganisms that contains 28 species and several enzymes (Biosystems Co., USA) showing a maximum EC of 13.13 $g \cdot m^{-3} \cdot h^{-1}$ with a RE of 64.7% at 55 s EBRT. Liffourrena and Lucchesi [9] have shown that *Pseudomonas putida* A immobilized in calcium alginate is capable of degrading higher concentrations of TMA than free cells. Understanding of microbial community compositions in biofilters plays an important role in seeking biological limiting factors related to the removal efficiencies of TMA and other compounds from waste gas and further enhancing the performance of biofilters [16,17]. Molecular fingerprints methods such as Denaturing gradient gel electrophoresis (DGGE) has been successfully used for showing the presence of specific microorganisms [13], and other techniques like qPCR allows to quantify the presence of an specific microorganism in a biofilter.

Aminobacter aminovorans is a microorganism known for its ability to use TMA as carbon and energy source. Rappert and Muller [18] reported that the degradation of TMA is strongly inhibited by reduced volatile sulfur compounds that are usually present in industrial emissions causing odor nuisance where TMA is also present. The main objective of this work was to determine the removal capacity of TMA of a biotrickling filter inoculated with *A. aminovorans* and to evaluate the effect of H_2S on its performance.

2. Materials and methods

2.1. Microorganism and preparation of the inoculum

Aminobacter aminovorans (DSM 7048) was used in all the experiments. The liquid culture medium used was the Colby and

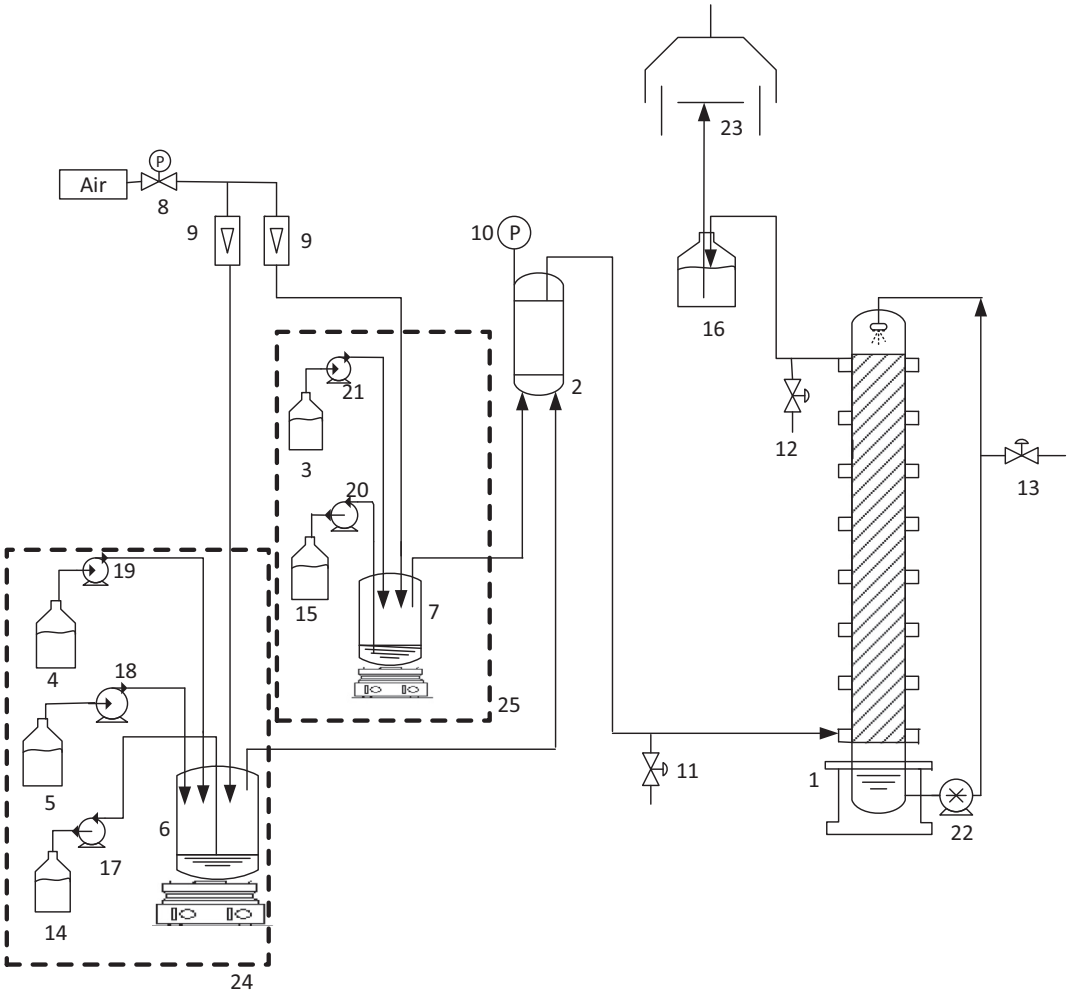

Fig. 1. Experimental set up. 1: Biotrickling filter; 2: Gas mixer; 3: Solution of trimethylamine; 4: Solution of HCL; 5: Solution of Na_2S; 6–7: Heating plate with magnetic stirring; 8: Manifold; 9: Rotameter; 10: Manometer; 11–12–13: Sampling ports; 14: Container for disposal of H_2S generation solution; 15: Container for disposal of Trimethylamine generation solution; 16: Acid solution for TMA absorption; 17–18–19–20–21–22: Peristaltic pumps; 23: Gas extraction system; 24: Generation of gaseous H_2S; 25: Generation of gaseous TMA.

Zatman medium [19], in g/L: K_2HPO_4 1.2, KH_2PO_4 0.62, $(NH_4)_2SO_4$ 0.5, $MgSO_4 \cdot 7H_2O$ 0.2, NaCl 0.1, $CaCl_2 \cdot 6H_2O$ 0.05, $FeCl_3 \cdot 6H_2O$ 1, $CuSO_4 \cdot 5H_2O$ 5, in $\mu g \cdot L^{-1}$ $ZnSO_4 \cdot 7H_2O$ 70, H_3BO_3 10, $MnSO_4 \cdot 5H_2O$ 10, $Na_2MoO_4 \cdot 2H_2O$ 10, $CoCl_2 \cdot 6H_2O$ 5. Trimethylamine was added in the range of 1.5–2.5 $g \cdot L^{-1}$ depending on the experiments. TMA was used as sole carbon and energy source. Biomass concentration was determined by turbidimetry at 600 nm dry weight method using a standard curve made by the dry weight method. The measured pH of the liquid culture medium was 7.0. The flasks used in the experiments and generation of inoculum were incubated in an orbital shaker at 30°C and 200 rpm.

2.2. Biotrickling filter

A biotrickling filter (BTF) was set up by using a transparent tube of PVC of 0.077 m inside diameter (ID) and 1.7 m of height with gas sampling ports located every 0.15 m from inlet to outlet. Polyethylene rings (OD = 15 mm, ID = 13 mm, H = 10 mm), with an external specific surface area of 316 m^{-1} and 77% bulk void fraction were used as a support for the biofilm. The total packing volume (V) was 5.6 L.

Fig. 1 shows a diagram of the experimental set-up. The system includes devices to generate gaseous TMA and H_2S to feed into the BTF. Gaseous TMA was generated by passing air in a container where a solution of TMA is dropped at 30°C. Gaseous H_2S is generated by mixing solutions of Na_2S and HCl in a container where humidified air is passing through it.

The BTF was inoculated with 0.4 L of an active culture of the microorganism growing in exponential phase, and re-circulating it throughout the column to promote adsorption of the cells to the support. The biomass concentration in the recirculating liquid was measured for observing the adsorption of the cell to the support. After 30 d a biofilm was clearly observed over the support. During the biofiltration experiments, 50 mL/min of culture medium without TMA was continuously circulated throughout the column to keep the viability of the biofilm.

The operation of the BTFs was characterized by measuring the TMA removal efficiency (RE) in %, and elimination capacity (EC) in $gTMA \cdot m^{-3} \cdot h^{-1}$ at different TMA loading rates (L) in $gTMA \cdot m^{-3} \cdot h^{-1}$ after reaching steady state and calculated according to Eqs. (1), (2) and (3) respectively. The gas flow was kept constant at 2 or 4 $L \cdot min^{-1}$, i.e.: 170 s and 85 s EBRT, the loading rate was adjusted by varying the concentration of TMA or H_2S at the inlet of the BTF. A steady state was considered to be reached when the variation in *RE* was less than 5% in consecutive days. These parameters were determined according to the following equations, where c_{in} is the Inlet TMA concentration, c_{out} is the Outlet TMA conce-ntration, F: flow (m^3/h):

$$RE = ((c_{in} - c_{out})/c_{in}) \cdot 100 \quad \text{(Equation 1)}$$

$$EC = (c_{in} - c_{out}) \cdot F/V \quad \text{(Equation 2)}$$

$$L = c_{in} \cdot F/V \quad \text{(Equation 3)}$$

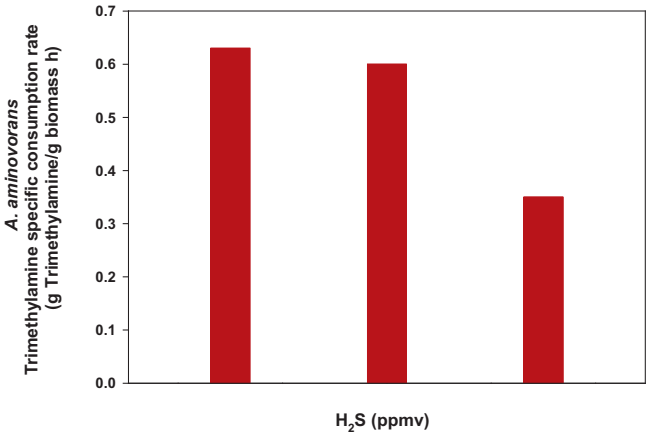

Fig. 3. Effect of H_2S on the specific consumption rate of Trimethylamine by *A. aminovorans*.

2.3. Determination of concentration of TMA and H_2S in gaseous phase

TMA was measured by gas chromatography (Clarus 500, Perkin Elmer, USA), using a capillary column Equity-1, 30 m long and a flame ionization detector (FID). As carrier gas was used a mixture of air, hydrogen and helium at a flow rate of 20 $mL \cdot min^{-1}$. The temperatures of the injector and detector were 120 and 200°C, respectively. The oven was heated from 75°C to 150°C at a rate of 20°C min^{-1}. The concentration of H_2S was determined by using an infrared detector (Drager, X-am 5000, Germany).

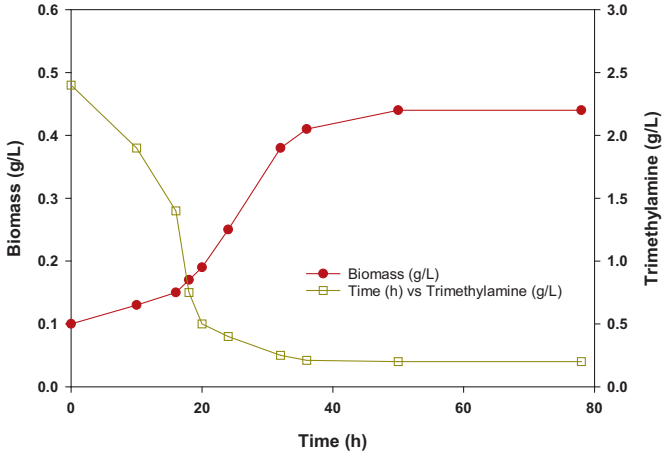

Fig. 2. Kinetic of biomass growth (•) and TMA consumption by *A. aminovorans* (□) in batch cultures using TMA as sole carbon and energy source.

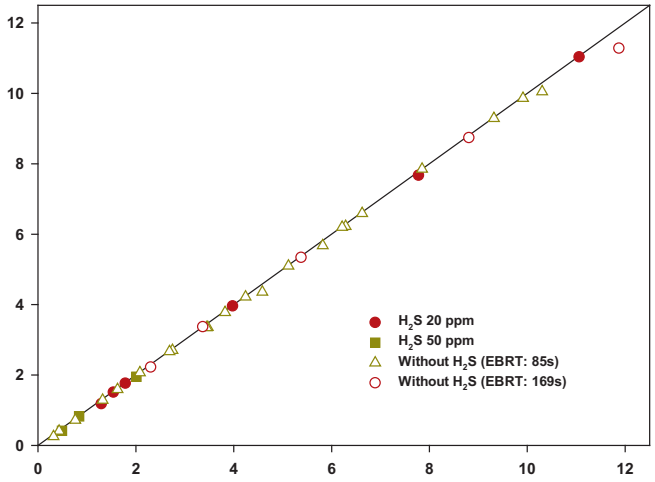

Fig. 4. Elimination capacity vs loading rate of TMA.

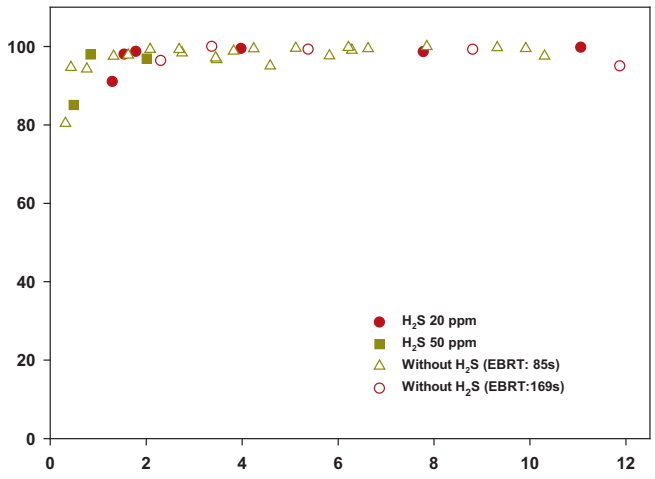

Fig. 5. Removal efficiency vs loading rate of TMA.

2.4. Effect of the hydrogen sulfide on the consumption and biofiltration of TMA

To determine the effect of H_2S on the biodegradation of TMA, H_2S was added to the headspace of stoppered flasks of 125 mL, provided with mininert valves (VICI, USA) with 23 mL of medium at pH 7.0, inoculated with 2 mL of an active culture of a cell concentration of 1 g L^{-1} of *A. aminovorans*. The experiments were performed using 0, 20 and 69 ppm of H_2S as initial concentration in the headspace. The liquid cultures were incubated in an orbital shaker at 30°C and 200 rpm. The effect of H_2S was measured by calculating the initial specific rate of TMA consumption of the culture. To determine the effect of H_2S on the biofiltration of TMA, a continuous amount of H_2S was added to the inlet gas stream at a loading rate of 1 and 2,5 $mg \cdot m^{-3} \cdot h^{-1}$.

2.5. Determination of A. aminovorans and total bacteria by qPCR in biofilm

The fraction of *A. aminovorans* in the established biofilm of the BTF was done by measuring the amounts of the microorganism and the total bacteria by qPCR.

2.5.1. Sampling and DNA extraction

4 g of support media was taken from the BTF and the biofilm was detached in 50 mL of still sterile water by sonication during 10 min and agitation using a vortex for 1 min. The biomass suspension was centrifuged at 6000 rpm for 10 min. The pellet was re-suspended in sterile water and the DNA was extracted using DNeasy Ultraclean Microbial kit (QIAGEN, USA), obtaining 50 μL of DNA which was used for qPCR assays.

2.5.2. Quantification of A. aminovorans and total bacteria by qPCR

The quantification of *A. aminovorans* and total bacteria was done using an AriaMx Real Time PCR system (Agilent Technologies, USA). The total volume of qPCR reaction was 25 μL (2 μL of extracted DNA, 12.5 μL of Brilliant II SYBR Green qPCR mastermix, 0.15 μL of forward primer (100 μM), 0.15 μL of reverse primer (100 μM) and 10.2 μL of water). The primers used for quantifying the *A. aminovorans* 16S rRNA gene were: Forward primer (5′-3′) GGCAATCTCGAGTCCGAGAGAG; Reverse primer (5′-3′) CTTCCTCGCGGCTTATCACC [20]. The primers used for quantifying the total bacteria 16S rRNA gene were: forward primer (5′-3′) ACTCCTACGGGAGGCAG, reverse primer (5′-3′) GACTAC CAGGGTATCTAATCC [21]. qPCR conditions for *A. aminovorans* were 1 cycle of 10 min at 95°C followed by 40 cycles (30 s at 95°C, 1 min at 55.8°C, 30 s at 72°C). For total bacteria the conditions were 1 cycle of 10 min at 95°C followed by 40 cycles (30 s at 95°C, 1 min at 50°C, 30 s at 72°C). The number of copies of 16S rRNA gene for *A. aminovorans* and total bacteria were determined from standard curves made by using known amounts of 16S rRNA gene copy numbers of *A. aminovorans* and total bacteria obtained previously from PCR using genomic DNA extracted from *A. aminovorans DSM 7048*.

3. Results and discussion

3.1. Growth kinetic of A. aminovorans using TMA

Fig. 2 shows the kinetic of biomass growth and TMA consumption by *A. aminovorans* in liquid culture. The maximum biomass concentration obtained was 0.44 g L^{-1}, the maximum specific growth rate 0.15 h^{-1} and the specific consumption rates of TMA was 0.062 h^{-1}. The calculated biomass yield for TMA was 0.10 (g g^{-1}). It shows that *A. aminovorans* has a great ability for TMA consumption.

3.2. Effect of H_2S on Oxidation of TMA by A. aminovorans

Fig. 3 shows the effect of H_2S on the biodegradation of TMA, measured as initial specific rate of TMA consumption ($g_{TMAconsumed} \cdot g^{-1}_{biomass} \cdot h^{-1}$). As can be seen, the presence of H_2S have a strong influence on the specific rate of TMA consumption decreasing in 50% when 69 ppm of H_2S is present in the gas in contact with the liquid culture.

3.3. Biofiltration of TMA in the BTF inoculated with A. aminovorans

After the starting up period of 60 d, the BTF inoculated with *A. aminovorans* showed removal efficiencies higher than 98% in a range of loading rate of 0.2 to 8 $g \cdot m^{-3} \cdot h^{-1}$ at EBRT of 85 and 180 s. These results are better than those reported by other authors [12,13,15] showing that a BTF inoculated with *A. aminovorans* can be efficiently used to remove TMA present in a gaseous stream.

After 8 months of operation of the BTF, H_2S was added into the inlet of the biofilter containing TMA, at loading rates of 1 and 2.5 $mg \cdot m^{-3} \cdot h^{-1}$. The results of TMA EC and RE are also showed in Fig. 4 and Fig. 5. Table 1 shows the amount of *A. aminovorans* contained in the biofilm and the total bacteria quantified by qPCR. The amount of *A. aminovorans* decreased in the biofilm by 80% and 90% in the lower and upper zone of the column respectively after 50 d of continuous adding of H_2S. As can be seen in Fig. 4 and Fig. 5, no effect on the EC and RE was detected when H_2S was added to the inlet gaseous emission, though the fraction of *A. aminovorans* measured by qPCR in the biofilm decreased.

Table 1
Fraction of *A aminovorans* in the biofilm of the BTF before and after the addition of H_2S into the inlet emission.

Time and conditions	BTF level	*Aminobacter aminovorans* 16S rRNA gene copy number/g of support media	Total bacteria 16S rRNA gene copy number/g of support media	*Aminobacter aminovorans* fraction in relation to total bacteria
Day 0 of BTF operation	Lower zone	$6.52 \cdot 10^6$	$5.60 \cdot 10^8$	1.2%
(addition of H_2S to the inlet started)	Upper zone	$9.37 \cdot 10^6$	$2.00 \cdot 10^8$	4.7%
Day 50 of BTF operation	Lower zone	$2.15 \cdot 10^6$	$8.67 \cdot 10^8$	0.25%
(addition of H_2S to the inlet ended)	Upper zone	$3.79 \cdot 10^6$	$7.43 \cdot 10^8$	0.51%

4. Conclusions

A biotrickling filter inoculated with *Aminobacter aminovorans* can remove efficiently the TMA from a gaseous stream. Even though the removal capacity of TMA can be negatively affected by H_2S, this effect is not notorious when cells are forming part of a biofilm, keeping the efficiency and removal capacity of TMA of the biofilter.

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