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Comparison of the Biodegradation of Trimethylamine by *Hyphomicrobium vulgare* and *Aminobacter aminovorans*

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Trimethylamine (TMA) is the main responsible for the odor often associated with rotting fish and is one of the major sources of annoying odors generated in many industrial activities, like composting facilities, fish-meal manufacturing plants, wastewater treatment plants, landfills and livestock farms. Traditionally amines can be removed by acid scrubbers that require the continuous supply of acid and generates large amounts of wastewater. Biofiltration has been proved to be an effective and sustainable technology treating many odorous compounds but the efficient removal of TMA using biofilters has not been completely shown. There are different microorganisms with the ability to use TMA as nutrient for their growth and presents different rates of TMA bio-degradation and biomass yields. Between those *Hyphomicrobium vulgare* and *Aminobacter aminovorans* are two bacteria known for their capability to use TMA as carbon and energy source.
In order to select one of these bacteria as inoculum for biofilters, TMA bio-degradation capacity of both bacteria was determinated in batch cultures. Each bacterium was cultivated at 30°C and 200 rpm in 125 ml shake flasks with a minimum mineral medium and TMA as source of carbon. To determine the effect of H_2S on the biodegradation of TMA, H_2S was added in the headspace of the flasks, the experiments were performed using 0, 20 and 70 ppm of H_2S as initial concentration in headspace.
The maximum biomass concentration obtained during the cultures was 1.17 g L^{-1} for *Hyphomicrobium* and 0.44 g L^{-1} for *A. aminovorans*, it growth at a maximum specific growth rate of 0.15 h^{-1}, higher than the obtained for *Hyphomicrobium;* 0.09 h^{-1}. The biomass yield for TMA was 0.35 (g g^{-1}) for *Hyphomicrobium* and 0.10 (g g^{-1}) for *A. aminovorans*. The specific consumption rate was 0.47 h^{-1} in the culture of *A. aminovorans* and 0.12 h^{-1} in the culture of *Hyphomicrobium*. Although *A. aminovorans* shows higher TMA specific consumption rate than *Hyphomicrobium*, its biomass yield value (0.1 g g^{-1}) indicates that in the case of *A. aminovorans* the carbon from TMA was metabolized to other products like carbon dioxide, formaldehyde or formate in detriment of biomass production. The biodegradation of TMA by *A. aminovorans* is negatively affected by H_2S, meanwhile in the case of *Hyphomicrobium sp.* the specific rate of biodegradation of TMA is positively influenced by the presence of H_2S, making it even higher than the one obtained for *A aminovorans* without the presence of H_2S. In conclusion, due to its specific growth rate and TMA specific consume rate *Aminobacter aminovorans* is a suitable candidate to be used as inoculum for biofilters designed for removing gas emissions containing TMA when there is not H_2S in the mixture. However, if H_2S is present, *Hyphomicrobium vulgare* would be the best choice for the inoculum or being part of the inoculum of the biofilter.

1. Introduction

Volatile amines are one of the main responsible of odour nuisances in many industrial activities, usually they are generated by the decay or biological degradation of organic material. In particular, trimethylamine (TMA) $(CH_3)_3N$, is the main responsible for the odor often associated with rotting fish and is one of the major sources of annoying odors generated in many industrial activities like fish-meal manufacturing processes [Sandberg and Ahring, 1992, Hwang et al 1994, Kim et al., 2001], wastewater treatment plants (Shieh and Keenan, 1986), waste disposal landfills, livestock farming, and hog manure [Shieh and Keenan 1986, Leson and Winer,

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1991; Cao et al., 1997; Chang et al., 2004, Ho et al, 2008]. The source of TMA is not fully established, but there is evidence that it is produced by the activity of microorganisms on choline, betaine or trimethylamine N-oxide (López-Caballero et al., 2001). The reported TMA odour threshold is in the range of; 0.2 – 0.4 $\mu g \cdot m^{-3}$ (Yaws et al, 2001) while characteristic concentrations of TMA emitted in such discharges ranged in 5–100 ppm (Shieh and Keenan,1986; Hodge and Devinny, 1994). Dimethylamine (DMA) and methylamine (MA) are catabolic products of trimethylamine breakdown catalyzed by microorganisms, therefore they may be also present in the streams containing TMA (Rappert and Muller, 2005).

In the last decade there has been also an increased concern related to the presence of amines in gaseous emmisions due to their toxic effects on human health due to its potentially toxic and carcinogenic effects (Xue et al. 2013). The cost of using physico-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 (Liffourrena and Lucchesi, 2014). Under the proper conditions, high removal efficiencies can be achieved by biofiltration of the gaseous contaminated emission (Kennes and Thalasso, 1989; Burgess et al, 2001).

Biological removal of amines could be accomplished by aerobic and anaerobic microorganisms (Meiberg and Harder, 1978; Rappert and Muller, 2005). In aerobic conditions, TMA is oxidized to DMA and formaldehyde by a TMA dehydrogenasa. 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 MA and formaldehyde by a DMA monooxygenase. MA is oxidized by a MA dehydrogenase or by a MA oxidase to formaldehyde and ammonia, that can be used as a source of carbon and nitrogen for biomass formation. There are other routes proposed for the conversion of MA to formaldehyde through glutamate by a *N*-methylglutamate synthase, g-glutamylmethylamide synthetase and a *N*-methyl glutatmate dehydrogenase (Lidstrom, 2006). Thus, microbial degradation would be an efficient way of eliminating TMA from contaminated environments (Kim et al 2001).

The biodegradation of TMA has been reported by several microorganisms, such as *Paracoccus* sp., *Hyphomicrobium* sp., *Pseudomonas* sp., *Methylophilus* sp., *Arthrobacter* sp., *Aminobacter* sp., *Haloanaerobacter* sp., *Nitrosomonas* sp., and *Bacillus* sp., which are known to readily utilize it as a sole carbon and energy source (Colby and Zatman, 1973; Anthony, 1975; Lobo et al., 1997; Roseiro et al, 1999, Kim et al, 2001; Chang et al., 2004; Liffourrena et al., 2010). Bioreactors packed with *Nitrosomonas* sp. have been used to remove TMA in wastewater, (Yang et al., 1994; Zita and Hermansson, 1994).

There are a few reports about biofiltration of amines as individual compounds or in complex mixtures. Chang et al. (2004) applied an aerobic biofiltration system containing entrapped mixed microbial cells to treat TMA-containing waste gas, the microbial cells were obtained from activated sludge for swan wastewater treatment, obtaining removal efficiency is higher than 90% at TMA inlet loading below 27.2 $mgN \cdot h^{-1}$, using retention time of 5.3 min.. TMA degradation in two three-stage biofilters packed with compost or sludge was investigated by Ding et al. (2007), they report the complete oxidation of TMA to NO_3^- in the compost biofilter due to the presence of nitrifying bacteria. Chung (2007) analysed the performance of a biofilter, using compost and activated carbon and inoculated with activated sludge, for treating a mixture of nitrogen containing compounds (TMA, DMA, MA, NH_3), sulphur containing compounds (H_2S, $(CH_3)_2S_2$, CH_3SH, C_2H_5SH), fatty acids and hydrocarbons generated in a compost process unit. The maximum removal for TMA was 95% (at a loading rate of 6365 $gTMA \cdot m^{-3} \cdot h^{-1}$) and 99% for MA (at a loading rate of 646 $gMA \cdot m^{-3} \cdot h^{-1}$). The removal of the other compounds was higher than 90%. These values are the highest removal of TMA, DMA and MM reported in the literature for high loading rates of these amines. Liffourrena & Lucchesi (2014) have shown that the immobilisation of *Pseudomonas putida* A in calcium alginate are capable of degrading higher concentrations of TMA than free cells. Recently, Wei et al (2015) have reported a TMA removal efficiency up to 99.9% in a Biotrickling Filter operating at thermophilic condition; 56 ºC, at a bed contact time of 25.8 s the elimination capacity was 375.2 $gTMA \cdot m^{-3} \cdot h^{-1}$.

To decrease the accumulation of NH_3 during amine biodegradation, some nitrifying microorganisms have been tested inside biofilters with good results. Ho et al. (2008) worked with a biofilter of granulated activated carbon inoculated with *Paraccocus* and *Arthrobacter*, a denitryfing bacteria, and at high loading rate (93 g $TMA \cdot m^{-3} \cdot h^{-1}$) achieved a TMA removal higher than 85%. The same authors also achieved good DMA and MA removal, higher than 90%, at high loading rates; 89,4 $gDMA \cdot m^{-3} \cdot h^{-1}$ and 70 $gMA \cdot m^{-3} \cdot h^{-1}$).

Aminobacter aminovorans is a microorganism known for its ability to use TMA as carbon and energy source but Rappert and Muller (2005) reported that the degradation of TMA is strongly inhibited by dimethyl disulfide, therefore the efficiency of the oxidation of amines would be reduced when sulphur compounds are present, it is a common situation in industrial emissions causing odor nuisance. On the other hand, *Hyphomicrobium*

vulgare is a bacterium with a wide range of metabolic abilities that allow to degrade TMA in the presence of other compounds including reduced sulphur compounds. The main objective of this work was to determine the rates of TMA biodegradation of those two microorganisms, *Hyphomicrobium vulgarae* and *Aminobacter aminovorans* and the effect of H_2S, in order to select one of them for inoculating biofilters for the removal of TMA from odorous gas emissions.

2. Materials and Methods

Hyphomicrobium vulgarae (ATCC 27499) and *Aminobacter aminovorans* (DSM 7048) were used in all the experiments. They were cultivated in specific ATCC minimum mineral salts mediums (Table 1) using an initial concentration of 2.4 $g \cdot L^{-1}$ TMA as sole carbon and energy source. To determine the effect of H_2S on the biodegradation of TMA, H_2S was added in the headspace of the flasks, the experiments were performed using 0, 20 and 70 ppm of H_2S as initial concentration in headspace.

The liquid cultures were incubated in an orbital shaker at 30 °C and 200 rpm. Experiments were performed in stoppered flasks of 125 mL provided with mininert valves (VICI, USA) with 23 mL of medium at pH 7.0 and inoculated with 2 mL of an active culture with a cell concentration of 0.01 g L^{-1}.

Gas samples of 0.5 mL were taken from headspace of the flasks to determine the concentration of TMA by gas chromatography (Clarus 500, Perkin Elmer), using a packed column (Carbopack B/4% Carbowax 20M/0.8% KOH, Supelco) and a flame ionization detector (FID). The carrier gas used was helium at a gas flow rate of 20 mL min^{-1}. The temperatures at the injector and detector were 100 and 200 °C, respectively. The oven was heated from 90°C to 150°C at rate of 4°C min^{-1}. The concentration of H_2S was determined by GC using a Supelpack S column and a flame photometric detector (FPD), the temperatures at the injector and detector were 60 and 400 °C respectively, using helium as carrier flow gas at 30 ml/min.

The concentration of biomass was determined by measuring optical densities at 600 nm and converted to dry weight of biomass using calibration curves previously made for each microorganism.

Table 1. Culture media used in the cultivation of Aminobacter and Hyphomicrobium.

Compound	Aminobacter	Hyphomicrobium
K_2HPO_4	1.20 g L^{-1}	-
Na_2HPO_4	-	2.13 g L^{-1}
KH_2PO_4	0.62 g L^{-1}	1.36 g L^{-1}
$(NH_4)_2SO_4$	0.50 g L^{-1}	0.50 g L^{-1}
$MgSO_4x7H_2O$	0.20 g L^{-1}	0.20 g L^{-1}
NaCl	0.10 g L^{-1}	-
$CaCl_2x6H_2O$	0.05 g L^{-1}	-
$CaCl_2x2H_2O$	-	0.006 g L^{-1}
$ZnSO_4x7H_2O$	0.07 mg L^{-1}	-
H_3BO_3	0.01 mg L^{-1}	-
$MnSO_4x5H_2O$	0.01 mg L^{-1}	-
$MnSO_4xH_2O$	-	1.00 mg L^{-1}
$Na_2MoO_4x2H_2O$	0.01 mg L^{-1}	1.50 mg L^{-1}
$CoCl_2x6H_2O$	0.005 mg L^{-1}	-
$CuSO_4x5H_2O$	0.005 mg L^{-1}	-
$FeSO_4x7H_2O$	1 mg L^{-1}	3.0 mg L^{-1}
$FeCl_3x6H_2O$	1 mg L^{-1}	-

3. Results and discussion

The maximum biomass concentration obtained during the cultures was 1.17 g L^{-1} for *Hyphomicrobium* and 0.44 g L^{-1} for *A. aminovorans* (Figure 1). The biomass yield for TMA was 0.35 (g g^{-1}) for *Hyphomicrobium* and 0.10 (g g^{-1}) for *A aminovorans*. The maximum specific growth rate of *A. aminovorans* was 0.15 h^{-1}, higher than the obtained with *Hyphomicrobium;* 0.09 h^{-1}.

The consumption of TMA in both cultures is shown in Figure 2. The specific consumption rates of TMA were 0.47 h^{-1} and 0.12 h^{-1} for *A. aminovorans* and *H. vulgare* respectively (Table 2). Although *A. aminovorans* shows higher TMA specific consumption rate than *Hyphomicrobium*, its yield in biomass value (0.1 g g^{-1}) indicates that in the case of *A. aminovorans* the carbon from TMA can be metabolized to carbon dioxide, in detriment of biomass production, according to the reports found in literature. The metabolic versatility of *Hyphomicrobium*, could be a an advantage over *A. aminovorans* for its selection as inoculum for systems dealing with the removal of TMA in mixtures with other compounds, i.e.: reduced sulfur compounds, usually present in gaseous emissions from industrial operations where is present. Successful TMA removal in

biofilters has been reported (Wan et al. 2011) using mixed cultures; therefore both microorganisms could be used as inoculum of biological system designed to remove amines from a complex gas mixture.

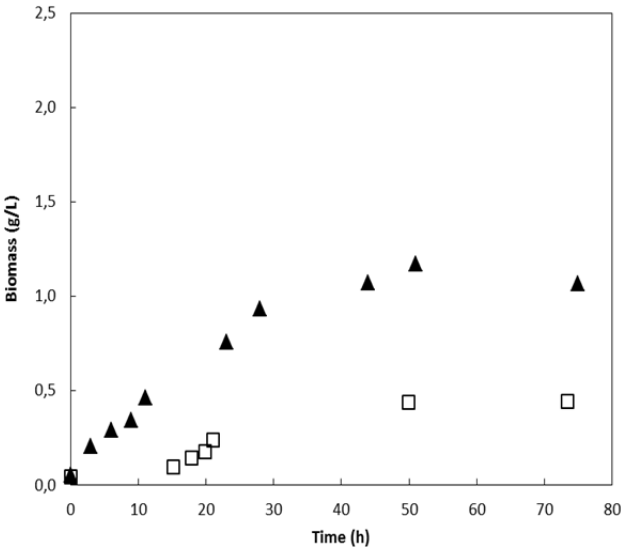

Figure 1. Biomass growth for Hyphomicrobium (▲) and Aminobacter (□) in batch cultures using TMA as sole carbon and energy source.

Table 2. Maximum specific growth rate (μ_{max}), biomass yield ($Y_{x/s}$) and specific rate of TMA bio-degradation (r_{TMA}) for Hyphomicrobium and Aminobacter.

Microorganism	μ_{max} (h^{-1})	$Y_{x/s}$ ($g_{biomass}$ g-1_{TMA})	r_{TMA} (h^{-1})
Hyphomicrobium v.	0.09	0.35	0.12
Aminobacter a.	0.15	0.10	0.47

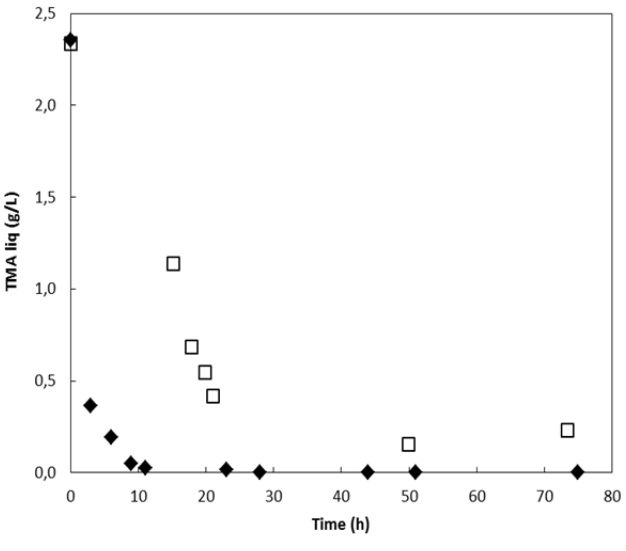

Figure 2. TMA consumption by Hyphomicrobium (▲) and Aminobacter (□)

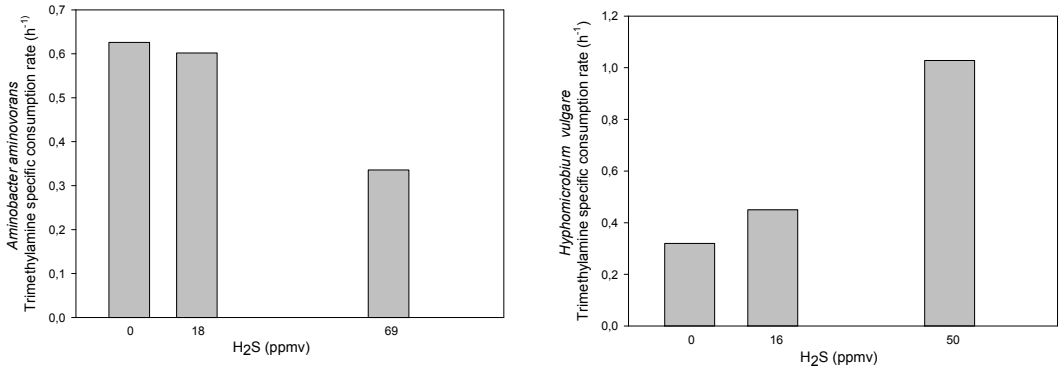

Figure 3. Specific consumption rate of Trimethylamine by *a. Aminobacter aminovorans* and *b. Hyphomicrobium vulgare*.

Figure 3 shows the effect of H_2S on the biodegradation of TMA, measured as initial specific rate of TMA consumption ($g_{TMAconsumed} \cdot g^{-1}_{biomass} \cdot h^{-1}$) for both microorganisms. As can be seen in the figures, even though the specific rate of consumption of TMA is higher for *A. aminovorans*, the presence of H_2S have a strong influence the specific rate of consumption of TMA. The biodegradadtion of TMA by *A. aminovorans* is negatively affected by H_2S, meanwhile in the case of *Hyphomicrobium sp.* the specific rate of biodegradation of TMA is positively influenced by the presence of H_2S, making it even higher than the one obtained for *A aminovorans* without the presence of H_2S.

4. Conclusions

In conclusion, due to its specific growth rate and TMA specific consume rate *Aminobacter aminovorans* is a suitable candidate to be used as inoculum for biofilters designed for removing gas emissions containing TMA when there is not H_2S in its composition. However, if H_2S is in the gaseous mixture, *Hyphomicrobium vulgare* would be the best choice for the inoculum or being part of the inoculum.

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