# Characterization of microbial communities in exhaust air treatment systems of large-scale pig housing facilities

J. Haneke, N. M. Lee, T. W. Gaul and H. F. A. Van den Weghe

## ABSTRACT

Exhaust air treatment has gained importance as an essential factor in intensive livestock areas due to the rising emissions in the environment. Wet filter walls of multi-stage exhaust air treatment systems precipitate gaseous ammonia and dust particles from exhaust air in washing water. Microbial communities in the biomass developed in the washing water of five large-scale exhaust air treatment units of pig housing facilities, were investigated by fluorescence *in situ* hybridization (FISH) and 16S rDNA sequence analyses. No "standard" nitrifying bacteria were found in the washing water. Instead mainly α-*Proteobacteria*, aggregating β- and χ-*Proteobacteria*, a large number of *Actinobacteria*, as well as individual *Planctomycetales* and *Crenarchaeota* were detected after more than twelve months' operation. The main *Proteobacteria* species present were affiliated to the families *Alcaligenaceae*, *Comamonadaceae* and *Xanthomonadaceae*. Furthermore, we investigated the consumption of inorganic nitrogen compounds in the washing water of one exhaust air treatment unit during a fattening period with and without pH control. Maintaining the pH at 6.0 resulted in a *ca.* fivefold higher ammonium concentration and a *ca.* fourfold lower concentration of oxidized nitrogen compounds after the fattening period was finished.

**Key words** | ammonium, exhaust air treatment system, nitrification, pig housing

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## INTRODUCTION

Ammonia ($NH_3$) emissions from intensive livestock areas pose a severe negative impact on the surrounding environment as they may play a major contributing role in eutrophication, acidification and particulate matter emissions (Amann *et al.* 2005). In Germany, 95% (596 Gg-$NH_3$ $a^{-1}$; UBA 2009) of the $NH_3$ emissions originate from agriculture, whereas the ammonia emission rate in pig housing varies between 2.43 and 4.86 kg-$NH_3$ (animal place and year)$^{-1}$ (UMEG 2005), depending on the type of housing, the ventilation system, the inside temperature and the season.

One of the standard procedures for reducing emissions in pig and poultry housing is by the use of multi-stage exhaust air treatment systems. The cleaning stages of such systems consist of wet filter walls for the precipitation of dust and $NH_3$, as well as a subsequent biofilter for biological elimination of odorous substances. In order to facilitate chemical $NH_3$ precipitation, the pH of the washing water is often controlled by the addition of acid to the wet filter wall (Hahne 2008). The washing water also presents a potential habitat for nitrifying microorganisms, whose activities may further promote $NH_3$ solubility.

During the last decade our knowledge about the biodiversity of microorganisms involved in the nitrogen (N) cycle has expanded significantly. The process of biological (aerobic) nitrification occurs in two separate oxidation steps: (i) oxidation of ammonia to nitrous acid (nitritation) and (ii) oxidation of nitrous acid to nitrate

doi: 10.2166/wst.2010.481

(nitratation); whereby two mole protons ($H^+$) are produced from one mole $NH_3$ (Bock and Wagner 2006). With this type of conversion, ammonium ions ($NH_4^+$) are removed from the washing water, whilst the protons formed during the oxidation of ammonia push the pH-dependent dissociation equilibrium $NH_3/NH_4^+$ in the favour of $NH_4^+$. Both processes can, therefore, work advantageously on the transferral of $NH_3$ from the animal house's exhaust air into the washing water. The process of dissimilatory nitrate reduction or denitrification to gaseous nitrogen causes a proportion of the nitrogen to be removed from the washing water either under anaerobic (Zumft 1997) or aerobic (Robertson *et al.* 1995) conditions, thereby reducing the accumulation of dissolved oxidized nitrogen compounds.

The major microbial groups involved in different nitrogen metabolizing processes have been relatively extensively explored in different types of traditional biological activated sludge and biofilm processes. The N-metabolizing processes and groups observed are usually: (i) the nitritation processes due either to chemolithoautotrophic, ammonia-oxidising bacteria (AOB) affiliated to the β- and χ-*Proteobacteria* (Kowalchuk and Stephen 2001; Koops *et al.* 2006), or ammonia-oxidising *Archaea* (AOA) affiliated to the *Crenarchaeota* (Hatzenpichler *et al.* 2008); (ii) nitratation performed by nitrite-oxidising bacteria (NOB), which include α-, χ- and δ-*Proteobacteria* (Abeliovich 2006); (iii) anaerobic/anoxic oxidation of ammonia ($NH_4^+ + NO_2^- \rightarrow N_2$) performed by anammox bacteria affiliated to *Planctomycetes* (Mulder *et al.* 1995; Strous *et al.* 1999); and (iv) nitrate reduction, performed by different species of bacteria affiliated to e.g. α-, β-, χ- and ε-*Proteobacteria*, and *Archaea* (Shapleigh 2006). Unfortunately, our knowledge about the N-transforming microbiology in exhaust air cleaning units and their activity is relatively scarce.

The aim of this study was to characterize and optimize present-day exhaust air cleaning systems by a systematic exploration of operational conditions and microbiology in five industrial, multi-stage exhaust air cleaning units. For this, the concentrations of substances arising in the units' washing water during their use in pig housing were determined, and the occurrence of different bacterial groups was assessed by molecular methods.

## METHODS

### Sampling sites and experimental setup

For the investigations, five multi-stage exhaust air cleaning units used in pig fattening houses at different sites (A, B, C, D and E) in the counties of Vechta and Cloppenburg (Lower Saxony, Germany) were sampled. The fattening process was performed by the so-called "all-in, all-out" method. The relevant operational data for each of the exhaust air cleaning units and the sampling periods are shown in Table 1.

During each of the two continual sampling sessions, a three-stage exhaust air cleaning unit (A) was sampled weekly during two separate production cycles from October to December 2007 (A1), and from February to April 2008 (A2) (Figure 1a). Prior to each start of the production cycle, the cleaning unit was cleaned with ground water from a well installed on-site and the collection tanks were re-filled. In addition, during the second production cycle (A2), the pH in the second wet filter wall and collection tank was maintained at pH 6.0 by the addition of 96% sulphuric acid ($H_2SO_4$). Random sampling was done in August 2009 in five exhaust air cleaning units A3, B, C, D and E (Figure 1b). These units had different running times and all units were only roughly cleaned before the production cycle.

### Electrochemical and hydrochemical analysis

The temperature (T), electrical conductivity (EC), pH and dissolved oxygen (DO) of the washing water held in the collection tanks were measured with standard electrodes (Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany; Mettler-Toledo GmbH, Giessen, Germany). Washing water samples from the collection tanks were stored up to three hours at 4°C after sampling, subsequently filtered (Schleicher & Schuell GmbH, Dassel, Germany) and then analyzed in duplicates photometrically (XION 500, Hach-Lange GmbH, Düsseldorf, Germany) for ammonium ($NH_4$-N), nitrite ($NO_2$-N) and nitrate ($NO_3$-N) nitrogen using Spectroquant™ test kits (Merck KGaA, Darmstadt, Germany). The samples were also tested for total phosphorus ($PO_4$-P), total organic carbon (TOC) and total hydrogen carbonate/$CO_2$ using cuvette tests (Hach-Lange

**Table 1** | Pig fattening house data and sampling period for each of the exhaust air cleaning units

| | | Exhaust air cleaning unit | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | A1 | A2 | A3 | B | C | D | E |
| Sampling period | Month/Year | 10-12/07 | 02-04/08 | 08/09 | 08/09 | 08/09 | 08/09 | 08/09 |
| Type of sampling | | Continual | | | | Random | | |
| In service since | Month/Year | 10/07 | 10/07 | 10/07 | 08/03 | n. d. | 10/08 | n. d. |
| Last cleaning | Month/Year | 09/07 | 01/08 | 05/09 | 02/09 | n. d. | 06/09 | 06/09 |
| Type of animal | | Piglet | Piglet | Piglet | Piglet | Fattening pig | Fattening pig | Fattening pig |
| Number of pigs | | 1004 | 962 | 950 | 1980 | 1000 | 800 | 1500 |
| V (collection tank 1) | $m^3$ | 12.5 | 12.5 | 12.5 | 4.3 | 2.3 | 1.8 | 5.7 |
| V (collection tank 2) | $m^3$ | 5.2 | 5.2 | 5.2 | 7.2 | 3.9 | 3.0 | 9.5 |
| V (collection tank 1 + 2) | $m^3$ | – | – | – | 11.5 | 6.2 | 4.8 | 15.2 |
| V (collection tank biofilter) | $m^3$ | – | – | – | 11.5 | 6.2 | 4.8 | 15.2 |
| A (wet filter 1) | $m^2$ | 24.0 | 24.0 | 24.0 | 28.8 | 20.3 | 12.0 | 49.4 |
| A (wet filter 2) | $m^2$ | 24.0 | 24.0 | 24.0 | 28.8 | 20.3 | 12.0 | 49.4 |
| Wet filter space loading | $m^3(m^3 h)^{-1}$ | n. d. | 1015 | 792 | 1243 | n. d. | 2800 | 209 |

n. d. = no data.

GmbH, Düsseldorf, Germany). The acid capacity ($K_{S4,3}$) and total suspended solids (TSS) were determined according to standard methods (Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung (German Standard Methods for the Examination of Water, Wastewater and Sludge) 2010).

## Fluorescence *in situ* hybridisation (FISH)

Samples of washing water and biomass from the bottom of the collection tanks of the exhaust air cleaning units were collected for FISH at the end of production cycles A1 (December 2007) and A2 (April 2008), as well as during the

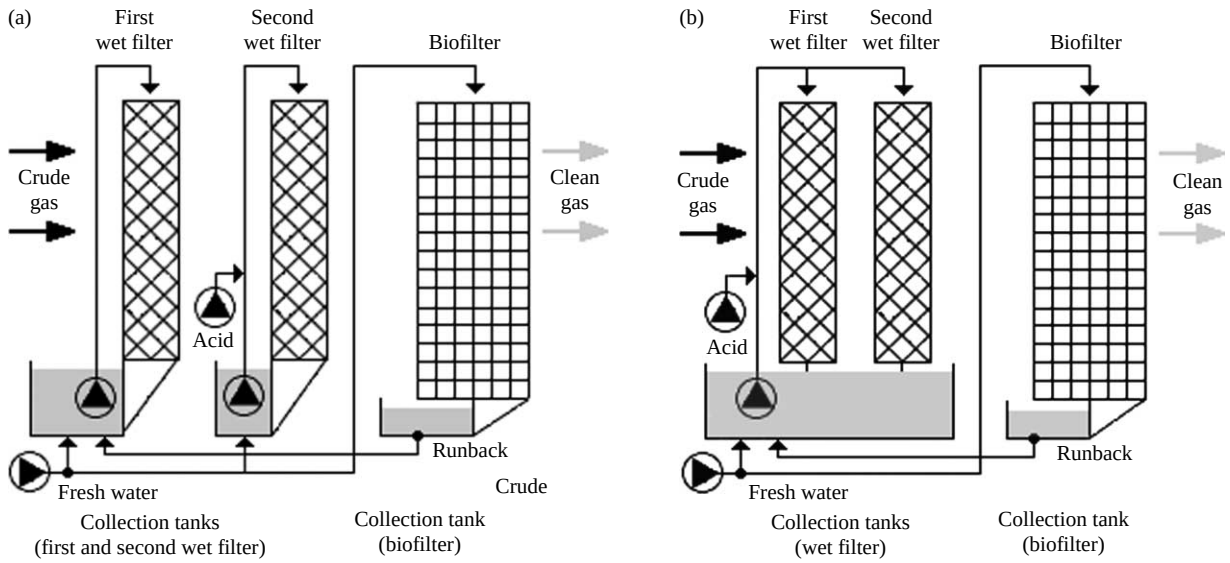


**Figure 1** | Schematic diagrams of two types of three-stage exhaust air cleaning units: (a) Unit with two wet filters, which are sprayed separately with washing water from two different tanks (unit A); (b) Unit with two wet filters, which are sprayed with washing water from a communal collection tank (units B, C, D and E).

production cycles of A3, B, C, D and E (August 2009). The biomass was fixed with (i) 4% paraformaldehyde (PFA), and (ii) 96% ethanol. FISH was performed as described by Amann (1995), using 32 different group-specific oligonucleotides (Thermo Electron GmbH, Ulm, Germany), labelled with either 5(6)-carboxyfluorescein-N-hydroxysuccinimide ester (FLUOS) or hydrophilic sulphoindocyanine dyes (Cy3, Cy5) at the 5′ end. In addition, samples of biomass were air dried on a microscope slide for Gram's stain (Jenkins *et al.* 1993). All the probes used with their corresponding formamide (FA) concentration are listed in Table 2.

### Microscopy and image analysis

The microscope slides for microscopic image analysis were mounted with an anti-bleaching agent (Citifluor, UK) and evaluated with a Zeiss Axiovert 100 M laser scanning confocal microscope (Carl Zeiss AG, Göttingen, Germany), equipped with a UV laser (351–364 nm), an argon ion laser (450–514 nm) and two helium/neon lasers (543 nm and 633 nm) and the standard Zeiss software package.

### DNA isolation, 16S rRNA gene amplification, cloning and sequencing

The biomass (TSS see Table 3) collected during the production cycles of A3, B, C, D and E was extracted with the FastDNA® Spin Kit for Soil (MP-Biomedicals GmbH, Eschwege, Germany) according to the manufacturer's instructions. The polymerase chain reaction (PCR) was used to amplify the bacterial 16S rRNA genes using two different universal primer pairs: 27F and 1492R, as well as 8F and 1542R as described in Galkiewicz and Kellogg (2008). Each 25-$\mu$l reaction contained 25–222 ng DNA template, 0.1 mM of each of the four deoxynucleoside triphosphates (dATP, dCTP, dGTP and dTTP), 0.1 $\mu$M of each forward and reverse primer, 5x Green GoTaq buffer including $MgCl_2$ and 0.5 U of Taq DNA polymerase (Promega GmbH, Mannheim, Germany). The thermocycling conditions started with a 3-min denaturation step at +94°C, followed by 25–35 cycles of denaturation for 40-sec at +94°C, 40 sec of primer annealing at +55°C and 130 sec of chain extension at +72°C. This cycle was repeated 30 times and followed by a final elongation for 10 min at +72°C. The PCR products of analogue samples were pooled. Purified (QIAquick PCR Purification Kit, Qiagen, Hilden, Germany) 16S rRNA gene fragments were cloned into the pCR®2.1 vector using the TOPO-TA cloning® kit (Invitrogen GmbH, Karlsruhe, Germany). According to the manufacturer's protocol (EasyPrep® Pro, Biozym, Germany), 148 white clones from all units were obtained for plasmid-DNA isolation. Sequencing of the plasmid-DNA was performed by using the oligonucleotide primers T7 and M13rev(−29) provided by the company Eurofins MWG Operon (Ebersberg, Germany).

### Phylogenetic interference

Fourty-nine sequences obtained from 114 clones of all units were chosen for phylogenetic investigations. Gene fragments were sequenced bidirectionally and some of the fragments could be aligned by manual overlapping to obtain a 16S rRNA gene with more than 1450 bp. To determine the probable phylogenetic affiliation, a comparison of the sequences was undertaken with the aid of the Basic Local Alignment Search Tool (BLAST; Altschul *et al.* 1990). The BLAST sequences chosen were those with the highest degree of similarity to the sequences found in this study. These were then aligned with additional 16S rDNA reference sequences available in molecular databases (GenBank: http://www.ncbi.nlm.nih.gov/Genbank/) belonging to various bacteria belonging to similar taxa using the program ClustalX (Larkin *et al.* 2007). The phylogenetic tree (Figure 3) was calculated using the neighbour-joining algorithm and visualised using the program TreeView X.

## RESULTS AND DISCUSSION

### Electrochemical and hydrochemical parameters

During the continual sampling periods A1 and A2 from autumn 2007 to spring 2008, the mean washing water temperatures varied between 13.3 and 18.9°C (A1) and between 12.4 and 16.6°C (A2), respectively. The temperatures in the two separate collection tanks (Figure 1b) did

**Table 2** | 16S and 23S rRNA-targeted oligonucleotide probes used in this study

| Probe | Target group | FA [%] | Reference* |
|---|---|---|---|
| AMX368 | all ANAMMOX bacteria | 15 | pB-00953 |
| ALF1b | α-P*roteobacteria*, some δ-*Proteobacteria*, *Spirochaetes* | 20 | pB-00017 |
| ALF968 | α-P*roteobacteria*, except for *Rickettsiales* | 20 | pB-00021 |
| Amx1900 | ANAMMOX bacteria, Candidatus *Brocadia anammoxidans*, Candidatus *Kuenenia stuttgartiensis* | 30 | pB-00406 |
| ARCH915 | *Archaea* | 0 | pB-00027 |
| ARC344 | *Archaea* | 0 | pB-00026 |
| Ban162 | Candidatus Brocadia anammoxidans | 40 | pB-00408 |
| Beta1 | β-*Proteobacteria* | 35 | pB-01372 |
| Beta2 | β-*Proteobacteria* | 35 | pB-01373 |
| BET42a | β-*Proteobacteria* | 35 | pB-00034 |
| CFX1223 | *Chloroflexi* (green nonsulphur bacteria) | 35 | pB-00719 |
| CF319a | most *Flavobacteria*, some *Bacteroidetes*, *Sphingobacteria* | 35 | pB-00042 |
| CREN499 | most *Crenarchaeota* | 0 | pB-00054 |
| DELTA495a† | most δ-*Proteobacteria* and most *Gemmatimonadetes* | 35 | pB-00432 |
| DELTA495b† | some δ-*Proteobacteria* | 35 | pB-00433 |
| DELTA495c† | some δ-*Proteobacteria* | 35 | pB-00434 |
| EPSY549 | ε-*Proteobacteria* | 35 | pB-01321 |
| EUB338‡ | most *Bacteria* | 0-50 | pB-00159 |
| EUB338 II‡ | *Planctomycetales* | 0-50 | pB-00160 |
| EUB338 III‡ | *Verrucomicrobiales* | 0-50 | pB-00161 |
| GAM42a | χ-*Proteobacteria* | 35 | pB-00174 |
| HGC69a | *Actinobacteria* (high G + C Gram-positives) | 25 | pB-00182 |
| Kst157 | Candidatus *Kuenenia stuttgartiensis* | 25 | pB-00407 |
| LGC | *Firmicutes* (low G + C Gram-positives) | 25 | pB-01040 |
| NIT3 | *Nitrobacter* spp. | 40 | pB-00241 |
| NON338 | negative control probe complementary to EUB338 | 20 | pB-00243 |
| NSO190 | β-*proteobacterial* ammonia-oxidizing bacteria | 55 | pB-00249 |
| NSO1225 | β-*proteobacterial* ammonia-oxidizing bacteria | 35 | pB-00248 |
| Nsv443 | *Nitrosospira* spp. | 30 | pB-00250 |
| Ntspa712 | most members of the phylum *Nitrospirae* | 50 | pB-00254 |
| Sca1309 | Candidatus *Scalindula* | 5 | pB-00951 |
| PLA46 | *Planctomycetales* | 30 | pB-00283 |

*Escherichia coli* 16S rRNA numbering (Brosius *et al.* 1981).
*probeBase accession number as a reference according to Loy *et al.* (2007).
†DELTAmix consist of equimolar mix DELTA495a, DELTA495b and DELTA495c including corresponding competitors.
‡3EUBmix consist of equimolar mix of EUB338, EUB338 II and EUB338 III.

not differ from each other. Higher temperatures between 21.4 and 23.1°C were measured during the random sampling during August (A3, B, C, D and E). The dissolved oxygen concentration of the washing water during the sampling periods A1 and A2 was always above 5 $mgO_2\ l^1$ in both the first and second stages, while during the sampling periods A3, B, C, D and E, the values varied between 6.0 and 8.2 $mgO_2\ l^1$. These high values were caused by the filter

**Table 3** | Results of the continual (towards the end of fattening periods of A1 and A2) and random sampling (during fattening periods of A3, B, C, D, E) of the washing water and biomass from the five multi-stage exhaust air cleaning units

| | | Exhaust air cleaning unit—collection tank | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A1-1 | A1-2 | A2-1 | A2-2* | A3-1 | A3-2 | B-1 | C-1 | D-1 | E-1 |
| pH | – | 7.0 | 6.9 | 7.2 | 5.9 | 6.5 | 4.1 | 5.7 | 6.9 | 6.5 | 6.5 |
| $K_{S4,3}$ | mmol $l^{-1}$ | 16.7 | 19.7 | 14.9 | 3.8 | 6.7 | n. d. | 0.4 | 7.0 | 7.4 | 8.1 |
| $HCO_3/CO_2$ | g $l^{-1}$ | 3.92 | 5.40 | 4.17 | 1.26 | 3.24 | 0.82 | 0.73 | 2.26 | 2.94 | 2.96 |
| TOC | g $l^{-1}$ | 1.15 | 0.37 | 0.95 | 0.17 | 0.59 | 0.22 | 0.23 | 0.37 | 0.71 | 0.57 |
| $NH_4$-N | g $l^{-1}$ | 4.14 | 4.54 | 5.45 | 24.28 | 7.00 | 5.48 | 5.30 | 1.85 | 6.73 | 6.04 |
| $NO_2$-N | g $l^{-1}$ | 2.93 | 4.15 | 3.76 | 0.79 | 2.15 | 0.30 | 0.30 | 1.19 | 2.09 | 2.40 |
| $NO_3$-N | g $l^{-1}$ | 0.81 | 0.11 | 0.63 | 0.20 | 2.60 | 4.18 | 3.11 | 0.68 | 1.64 | 2.60 |
| $PO_4$-P | mg $l^{-1}$ | 32.4 | 14.6 | 34.6 | 7.8 | 17.7 | 22.6 | 22.0 | 14.3 | 37.2 | 13.5 |
| TSS | g $l^{-1}$ | n. d. | n. d. | n. d. | n. d. | 14.8 | 9.8 | 9.1 | 1.3 | 6.8 | 4.3 |

n. d. = no data.
*pH maintenance at 6.0.

walls being sprayed with water, which facilitated the oxygen dissolving in the washing water. These results show that neither the temperature nor the oxygen concentration could impose a limiting effect on the nitrification process in the washing water. The consumption of acid in the second collection tank (A2-2) to maintain a pH of *ca.* 6.0 resulted in roughly 1.8 kg-$H_2SO_4$ (animal place and year)$^{-1}$. Without the acid, the mean pH of the washing water in the first and second collection tank during A1 and in the first collection tank during A2 decreased from 8.5 to 7.0 during the 6th and the 7th week of fattening and remained at this level to the end of the production cycle. Parallel to this pH decrease, the mean acid capacity reduced three-fold till day 55, although the acid capacity increased again during the rest of the production cycle the acid capacity. The pH decrese and the reduction in acid capacity after a month of the production cycle are obvious indications of biological nitrification processes starting to occur in the washing water.

These indications were further confirmed by the formation of oxidized nitrogen compounds in the washing water in all of the collection tanks as the ammonium concentration increased after the 7th week of the production cycle (Figure 2). The measurements listed in Table 3 show that a mean concentration of 4.71 g-$NH_4$-N $l^{-1}$ and 3.61 g-$NO_2$-N $l^{-1}$ was reached in the washing water without the application of acid after three months. The application of acid resulted in a *ca.* fivefold higher concentration of ammonium (Figure 2a) and a more than fourfold lower concentration of nitrite (Figure 2b) in the collection tanks. However, the mean nitrate concentration in the first collection tanks was more than four times higher than in the second tanks independent of the acid dosage (Figure 2c). The delay (*ca.* one month) in increase in nitrite and nitrate concentrations could be explained by the fact that at the beginning of the production cycle the exhaust air cleaning unit was thoroughly cleaned and fresh water was placed in the tanks. It is possible that the direct input of dust particles or organic carbon compounds from the pig house influenced the development of those microorganisms.

Whether the lower oxidation activity in the tank A2-2 was due to the lower pH and/or the higher ammonium concentration has to be elucidated in future investigations. Both factors are considered to be inhibitory according to conventional explanatory models (Anthonisen *et al.* 1976).

If the measurements recorded during A1 and A2 are compared with A3, i.e. roughly after one year of operation (and thus also after a three-month production cycle with a previously cleaned unit), significantly higher nitrate concentrations could be observed in a later operational phase (Table 3). This may indicate changes in the microbial community in the washing water, which were confirmed by microscopic analyses as discussed in the next section.

A good linear correlation ($R^2 = 0.97$) of the washing water's EC with its mineral nitrogen compound concentration ($NH_4$-N, $NO_2$-N and $NO_3$-N) could be observed in all the collection tanks of A1 and A2 during the continual

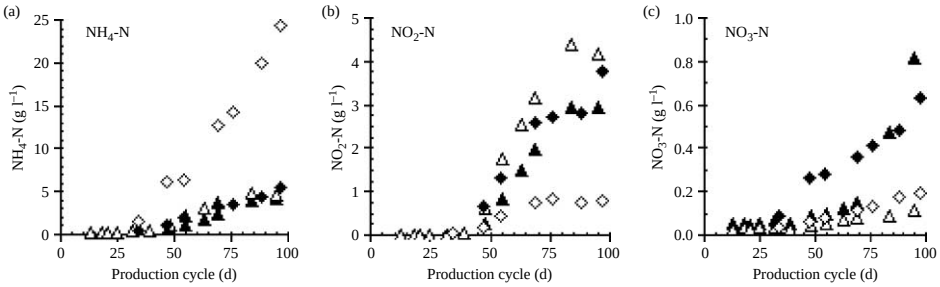


**Figure 2** | Concentrations of (a) ammonium nitrogen ($NH_4$-N), (b) nitrite nitrogen ($NO_2$-N) and (c) nitrate nitrogen ($NO_3$-N) in [g $l^{-1}$] in the washing water tanks of the first and second wet filter walls during production cycles in [d] A1 and A2 (filled triangle = A1 tank 1, empty triangle = A1 tank 2, filled diamond = A2 tank 1, empty diamond = A2 tank 2).

data collection (data not shown), as the nitrogen compounds formed the major proportion of the ions present. The EC, as a reflection of the ammonium concentration, already served in some of the exhaust air cleaning units as a control parameter for the removal of sludge and washing water when a certain value is reached. Using this parameter assumes that there is a high ratio of ammonium to nitrite/nitrate, which, however, was rarely observed during these investigations. The $NH_4^+$:$NO_2^-$:$NO_3^-$ ratio observed, may lead to a severe degree of inaccuracy in the maintenance of adequate sludge removal and washing water replacement.

To reach a predefined sludge age in the collection tanks as well as to ensure a better substrate supply for the microorganisms in the sedimenting biomass, agitation of the washing water in the collection tanks through the use of pumps in combination with optimized fluid mechanics is essential. Furthermore in addition to the washing water, it is certain that the biofilm which developed on the surface of the wet filter walls was also involved in the nitrogen turnover.

## Microscopy and FISH

FISH analyses of biomass developed during the first (A1) and second (A2) production cycles in the collection tanks did not show any significant amounts of "typical" aggregates of AOB/β-*Proteobacteria* and χ-*Proteobacteria* that are regularly observed in activated sludge from aerobic waste water treatment (e.g. Wagner *et al.* 1993). Instead, numerous, individual rod-like β-*Proteobacteria* (Beta1) were detected. When the unit was sampled roughly one year later (A3), a large number of aggregating β- and χ-*Proteobacteria* were found. Bacteria from these taxa were also detected in the four other units (B, C, D and E). "Classical" AOB (*Nitrosomonas*, *Nitrosospira* spp.) and NOB (*Nitrobacter* spp.) were never recorded by FISH from any of the five units. The lack of these bacteria has often been reported in other nitrogen-containing habitats (e.g. Schramm *et al.* 1998). However, a plausible explanation for the absence of nitrifying bacteria could be the high detection level with standard FISH (about $10^{5-6}$ cells $ml^{-1}$). In future we plan to explore this further with other molecular-based methods, such as quantitative-PCR (qPCR).

In units A3, B, C, D and E (i.e. after at least one year of operation), bacteria of the class *Planctomycetes* could also be detected, including individual coccoid anaerobic ammonia-oxidising bacteria. α-*Proteobacteria* and a conspicuously large number of high GC-containing gram-positive bacteria (*Actinobacteria*) could be detected in all five units. The latter formed flake-like structures made of antler- and rod-like bacteria. These structures could also be observed with Gram's stain. Aggregates of large rod-shaped δ-*Proteobacteria* (mainly DELTA495a) could be detected in A1 and A2, which were no longer detectable after one year of operation. Individual *Archaea* and *Crenarchaeota* – and thus possibly some potential AOA–could also be detected in all five units.

## 16S rDNA analysis

Fourty-nine clones of the established clone library from A3-1 (13 clones) A3-2 (12 clones), B (11 clones), C (5 clones) and E (7 clones) were affiliated to the *Proteobacteria*. Unit D provided no prokaryotic sequences so far. Approximately 43% (21 clones) belonged to the β-*Proteobacteria* and 41% (20 clones) to the χ-*Proteobacteria*. The remaining sequences could either be classified as *Firmicutes* (1 clone), *Actinobacteria* (1 clone), *Bacteriodetes* (2 clones) or

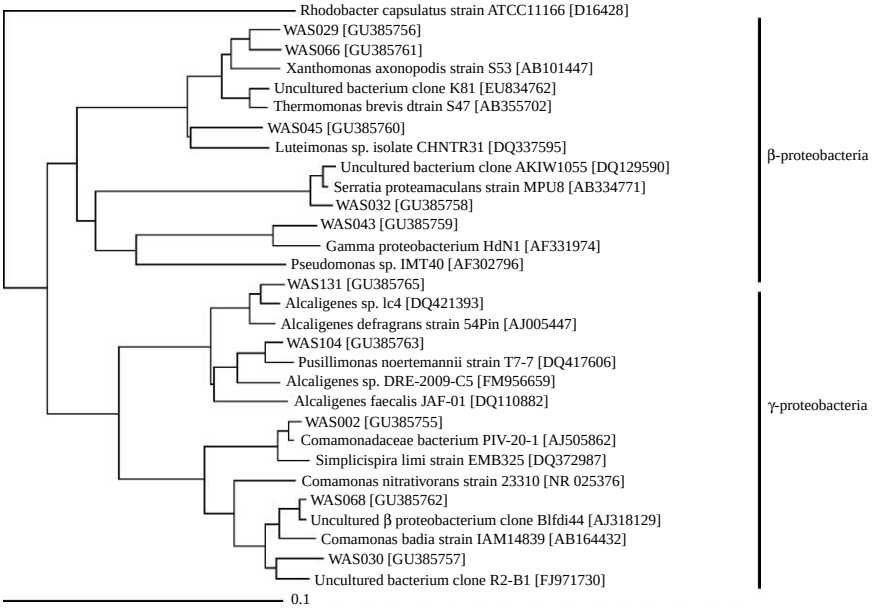


**Figure 3** | Phylogenetic tree based on the comparative analysis of 16S rRNA gene sequences from clones retrieved in this study and reference sequences obtained through BLAST analysis. The tree is based on the results of neighbour joining analysis of nearly complete sequences (>1450 nucleotides). Sequences obtained from the present study are indicated with "WAS" and their corresponding clone number. The scale bar represents the expected changes per nucleotide position. *Rhodobacter capsulatus* was used as an outgroup sequence.

*Tenericutes* (1 clone). The high number of *Proteobacteria* supports the observations made with FISH. The β-*Proteobacteria* were mainly affiliated to *Comamonadaceae* (found in all units) and *Alcaligenaceae* (only found in A3). The χ-*Proteobacteria*, found in all units, consisted predominately of bacteria from the *Xanthomonadaceae* (Figure 3).

The nearly complete 16S rDNA gene sequences of the clones retrieved in this study had a high degree of similarity (>0.95 identity) to bacteria that are found in nitrifying and denitrifying activated sludge, sewerage polluted with organic material or waste air systems. Contrary to the expectations from the microscopic evaluation, only one sequence was found that could be classified as gram-positive bacteria with a high GC content (*Actinomycetales*).

## CONCLUSIONS

The present study on the microbial communities and their activities in five industrial exhaust air cleaning units used in pig housing facilities show definite biological oxidation processes (nitritation) occurring in the collection tanks without pH control. Instead of "standard" nitrifying bacteria (e.g. *Nitrosomonas*, *Nitrobacter*), other potential AOB and NOB, especially β-*Proteobacteria* (*Comamonadaceae*, *Alcaligenaceae*) and χ-*Proteobacteria* (*Xanthomonadaceae*) were involved in the N-turnover. An inhibition of nitrification observed during the chemical cleaning stage when the pH was maintained at 6.0, was either due to the high ammonium concentration, sulphate concentration, or the low pH. Technically, the incipient nitrification processes in the washing water could specifically be used to increase its absorbency of ammonia as these processes can be relatively easily controlled and monitored by pH sensors. In addition the EC is suitable for the controlled removal of $NH_4^+$ in the washing water–if its ratio to $NO_2^-$ (and $NO_3^-$) is taken into consideration. The results of this study show that on-site technically controlled biological cleaning processes help to prevent high nitrogen loads in the washing water. Further treatment of the washing water should be considered by the use of additional, separate denitrification reactors.

## ACKNOWLEDGEMENTS

We gratefully acknowledge the financial support by the German Research Foundation (DFG-Project Va 346/2-1).

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