Composition and metabolic significanc of microorganisms in biological airfilters

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Preface

This dissertation is submitted in partial fulfillment of the requirements for obtaining a degree of Doctor of Philosophy (Ph.D.). The dissertation consists of an extended summary of results and related literature and 5 supporting papers.

This study was carried out under supervision of Associated Professor Jeppe Lund Nielsen at the section of Biotechnology, Department of Biotechnology, Chemistry and Environmental Engineering at Aalborg University, Denmark during the period March 2007 to August 2010. A part of the study was conducted in collaboration with Assistant Professor Josh D. Neufeld at Department of Biology, University of Waterloo, Canada. Funding was provided from the Danish Ministry of Food, Agriculture and Fisheries as part of the research program 'Function of Biological Airfilters' under the Action Plan for the Aquatic Environment III.

At this point there are a lot of people to thank. A special thanks to my supervisor Jeppe Lund Nielsen for continuous enthusiasm and always having the door open. A great thank to Josh D. Neufeld and everyone in the Neufeld lab for giving me a highly memorable stay and sharing your knowledge and chemicals. Thanks to Per H. Nielsen and Andreas Schramm for constructive and valuable scientific inputs. I acknowledge Susanne Juhler, Sabine Lindholst, Anders Feilberg and Lise Bonne Guldberg who provided their assistance during field work and experimental and metrological considerations. I thank Jane Ildal, Susanne Bielidt and Marianne Stevenson for their technical support in the laboratory and Aaron M. Saunders, Aviaja A. Hansen, Caroline Kragelund and Andreas H. Kristensen for reviewing early drafts of the manuscripts and this thesis. A special thanks to everybody in the EB-group for the warm atmosphere and lots of good laughs. Last but not least, thanks to my family and all my friends for help and encouragement.

Anja Kristiansen Aalborg, August 2010

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List of supporting papers

I. **Kristiansen, A.**, Juhler, S., Nielsen, P.H., Nielsen, L.P. and Nielsen, J.L. (*in prep*). Activity microprofiling of an ammonia-loaded biofilm in a full-scale air filter.

II. **Kristiansen, A.**, Pedersen, K.H., Nielsen, P.H., Nielsen, L.P., Nielsen, J.L. and Schramm, A. (*submitted to Systematic and Applied Microbiology*). Bacterial community structure of a full-scale biofilter treating pig house exhaust air.

III. **Kristiansen, A.**, Saunders, A.M., Hansen, A.A., Nielsen, P.H. and Nielsen J.L. (*submitted to FEMS Microbiology Ecology*). Community structure of fungi and bacteria in pig confinement building bioaerosols.

IV. **Kristiansen, A.**, Lindholst, S., Feilberg, A., Nielsen, P.H., Neufeld, J.D. and Nielsen, J.L. (*in prep*). Butyric acid and dimethyl disulfide assimilating microorganisms in a biofilter treating air emissions from a pig facility.

V. Juhler, S., **Kristiansen, A.**, Nunes da Rocha, U., Feilberg, A., Lindholst, S., Plugge, C., Smidt, H., Nielsen, J.L., Nielsen, L.P. and Schramm, A (*in prep*). Identification of acetic acid- and *p*-cresol- utilizing microorganisms in biofilm from the biological airfilter of a pig house.

Supporting papers will be referred to by their roman numbers e.g. (Paper I).

Abstract

Intensified pig production brings about large emissions of ammonia (NH_3) and odorous volatile organic compounds (VOC) are emitted to the environment. Biofilters, relying on microbial oxidation, have been proven efficient in reducing these compounds.

The purpose of this Ph.D. study was to increase our understanding of microbial biofilms in biofilters. Well-functioning full-scale biofilters (exemplified by the SKOV-type) were chosen for the investigation and focus was put to obtain knowledge on the microbial community structure, identity and ecophysiology.

A clear stratification of the microbial community was observed, with ammonia oxidizing bacteria (AOB) positioned below a layer of organoheterotrophs and deeper in the biofilm. In addition, the activity profiles of different volatile organic compounds were reflected by their solubility and biodegradability, with acetic acid assimilate exclusively at the biofilm surface and phenol and *p*-cresol assimilated throughout the biofilm.

The biofilters contained highly specialized microbial communities. Few Betaproteobacterial lineages dominated the microbial community (especially *Comamonas*) together with *Gammaproteobacteria*, *Actinobacteria* and *Bacteroidetes*. The *Nitrosomonas*/*Nitrosococcus* lineage, represented by *Nitrosomonas eutropha*, was quantitative important among AOB. The bacterial population was relatively stable among different biofilters.

The biofilter community highly diverged from the microbial community in dust aerosols transported with the pig facility air emissions, revealing a high degree of microbial selection in the biofilter. This is likely caused by the high NH_3 and nitrite concentrations found in the biofilters, which often exceeds 100mM.

The assimilation of different substrate relied on few and highly specialized bacterial populations. Acetic acid was assimilated dominantly by *Comamonas*, whereas *Actinobacteria* was responsible for assimilation of butyric acid and dimethyl disulfide (DMDS). The AOB *N. eutropha* was the major participant in *p*-cresol assimilation and unidentified AOB played a role in DMDS turn-over. Fungi also participated in acetate, *p*-cresol and DMDS assimilation.

Collectively, this Ph.D. study has provided knowledge on the microbial community and their functions in biofilters. Future studies may build on these results in order to understand the mechanisms controlling the microbial community driving a well functioning biofilter and optimize the efficiency further.

Resume (abstract in Danish)

Intensiv svineproduktion medfører udledning af store mængder ammoniak (NH_3) og ildelugtende flygtige organiske forbindelser til det omgivende miljø. Biofiltre har vist sig at være særlig velegnede og effektive til at reducere disse stoffer.

Formålet med denne Ph.D. var at øge vores forståelse af den mikrobielle biomass i disse biofiltre. Velfungerende fuld skala biofiltre (eksemplifiseret af SKOV-typen) blev valgt til undersøgelsen og fokus har især været rettet mod at opnå viden om den mikrobielle samfundsstruktur, identitet og økofysiologi.

En klar stratifisering af det mikrobielle samfund blev observeret, med ammoniakoxiderende bakterier (AOB) placeret under et lag af organoheterotrofiske bakterier og dybere i biofilmen. Desuden var aktivitetsprofilter for forskellige flygtige organiske forbindelser afspejlet af stoffernes opløselighed og mikrobiel omsættelighed, med eddikesyre omsat udlukkende i biofilmens overflade og fenol og *p*-cresol omsat gennem hele biofilmen.

Biofilterne indeholdt højt specialiserede mikrobielle samfund. Få grupper indenfor *Betaproteobacteria* dominerende det mikrobielle samfund (især *Comamonas*) sammen med *Gammaproteobacteria*, *Actinobacteria* og *Bacteroidetes*. *Nitrosomonas*/*Nitrosococcus* gruppen var udbredt blandt AOB og rigt repræsenteret af *Nitrosomonas eutropha*. Det mikrobielle samfund var relativt stabil blandt forskellig biofiltre.

Samfundet i biofilterne afveg i høj grad fra det mikrobielle samfund i støv-aerosoler transporteret sammen med luftstrømmen fra svinestalden, hvilket vidner om en høj grad af selektion i biofilterne. Dette kan være forårsaget af de høje koncentrationer af NH_3 og nitrit i biofilterne, som er blevet fundet i biofilterne og som ofte overstiger 100 mM.

Optag af forskellige substrater var fordelt mellem få specialiserede fylogenetiske grupper. Eddikesyre blev primært optaget af *Comamonas*, hvor imod *Actinobacteria* var ansvarlig for smørsyre og dimethyl disulfid (DMDS) optag. *N. eutropha* var en primær aktør i *p*-cresol omsætningen og uidentifiserede AOB spillede en rolle i DMDS omsætningen. Svampe deltog også i optaget af eddikesyre, *p*-cresol og DMDS.

Samlet set har denne Ph.D. givet viden om mikrobielle samfund og deres funktioner i biofiltre. Fremtidige studier kan bygge på disse resultater med henblik på at forstå de mekanismer, der kontrollerer det mikrobielle samfund og som driver et velfungerende biofilter og dermed yderlige optimerer effektiviteten.

Introduction

1. Agriculture – food production and environmental concerns

Ever since cultivation of the land and domestication of animals first began around 10,000 years ago, agriculture has contributed substantially to meet the Worlds food demand. Agriculture has developed considerably since then and today benefits from improved crops and animals, new technologies and bigger and more condensed production units. These improvements have lead to increased productivity and lowered the production costs, although the developments mostly have been restricted to developed countries. Pork is an important part of the diet in many western and Asian countries and is an important part of the agriculture production. In this way the global production of pigs increased by approximately 34% in the period from 1990 to 2009 and the 106 million tons produced worldwide in 2009 accounted for 38% of the total meat production (Best, 2010).

In Denmark, more than 90% of the produced pork are exported which makes the country one of the World's largest exporters. In 2008, the total annual Danish pig production reached 27.4 million animals (DMA, 2009). While the number of pig producers declined from about 39,000 to about 6,300 in the period from 1988 to 2008, the average number of fattener pigs per producer increased from 381 to 2987 during the same period (DMA, 2009). Thus, the pork production has become concentrated on fewer but larger farms. The production and export of pork contribute substantially to the Danish economy by creating employment for approximately 60,000 people and accounting for nearly 6% of the total export value.

1.1 Environmental impacts of agriculture

The intensification of agriculture has resulted in a number of environmental problems including emission of ammonia (NH_3), arising from the decomposition of manure. Agriculture accounts for around 65% of the global NH_3 emission (Erisman et al., 2007) but does locally account for a much higher percentage, like in Denmark where agriculture contributes with nearly 99% of the total NH_3 emission (Hutchings et al., 2001). Part of the air borne NH_3 is readily deposited in the environment within few kilometers of the source, while another fraction is converted to NH_4^+ salts which can travel for several days in the atmosphere before deposition (Krupa, 2003, Erisman et al., 2007). Contamination of the environment with NH_3 stimulates the primary production which eliminates vulnerable plant species and lowers the diversity. In extreme

situations, elevated primary production can cause oxygen depletion in aquatic environments, resulting in the death of fish and other animal life forms (Krupa, 2003, Smith, 2003). In combination with warm climate conditions during summer, Denmark has experienced substantial problems with oxygen depletion of coastal regions, especially during the 1980s, and cases have been reported regularly up till today. Depositions of NH_4^+ and NH_3 to the environment may also cause acidification due to the release of protons from microbial nitrification and assimilation of NH_3 and NO_3^- by plants (Krupa, 2003). Another environmental concern related to agricultural NH_3 emission is contamination of drinking water reservoirs with NO_3^-, which is produced from nitrification (Erisman et al., 2007). Denmark receives 99% of the drinking water resources from ground water reservoirs and percolation of nitrate into drinking water reservoirs poses a threat to the drinking water quality. Under anaerobic conditions, oxidized nitrous compounds are reduced by denitrification to free nitrogen (N_2) but incomplete denitrification cause accumulation of the greenhouse gas N_2O. In summary, agricultural emission of NH_3 gas leads to a range of environmental effects.

Apart from NH_3 emissions, odor nuisance from agriculture is an emerging problem. Microbial fermentation of feed in the large intestine of pigs and microbial conversion of pig manure generates a number of odorous volatile compounds. Odor nuisance is a result of both the intensive production of agriculture and increased urbanization of rural areas, minimizing the distance between production facilities and individuals living in the vicinity. In air emissions from pig facilities, more than 300 different compounds have been detected, of which the dominating species, found in concentrations exceeding ~5 parts per billion (ppb), include carboxylic acids, phenols, indoles, alcohols, aldehydes, ketones and sulfur containing compound (Schiffman et al., 2001). **Table 1** presents a selection of odorous compounds detected in air emission, their concentrations and odorous threshold. The concentration of several compounds in the emission has been detected above their odorous threshold, but adsorption to dust particles may elevate the odor nuisance experienced and the sensitivity towards odor is individual (Hammond et al., 1981, Cai et al., 2006). The exact composition and concentrations of air emissions likely vary from one facility to another, depending on the category of pigs, pig feed, stable design, ventilation system and temperature.

Table 1. Concentration, nature of smell and odorous threshold of selected VOC detected in air emissions from pig facilities. After Schiffman et al., (2001), Blunden et al., (2005) and Le et al., (2005).

Group	Compound and formula	Smell	Concentration µg m^{-3} (ppb)	Odorous threshold µg m^{-3} (ppb)
Acids	Acetic acid (CH_3COOH)	Pungent or vinegar	0.0015 – 6,700 (< 2,730)	363 (145)
	Propanoic acid (CH_3CH_2COOH)	Fecal	0.002 – 1,100 (<360)	110 (36)
	Butyric acid ($CH_3(CH_2)_2COOH$)	Fecal or stench	0.001 – 617 (<170)	15 (4)
	Pentanoic acid ($CH_3(CH_2)_3COOH$)	Fecal	0.12 – 210 (<50)	20 (5)
	Heptanoic acid ($CH_3(CH_2)_5COOH$)	Pungent	3 (0.6)	148 (28)
Alcohols	Ethanol (CH_3CH_2OH)	Alcoholic	0.8 – 150 (0.4 – 78)	55,000 (28,800)
Aldehydes	Acetaldehyde (CH_3CHO)	Pungent	0 – 36 (< 20)	339 (186)
Ketones	Acetone (CH_3COCH_3)	Irritant	0 – 21 (<9)	34,700 (14,500)
Phenols and indols	Phenol (C_6H_5OH)	Aromatic	0.0025 – 5 (<0.1)	427 (110)
	p-cresol ($CH_3C_6H_4OH$)	Fecal	41 – 80 (9 – 18)	8 (2)
	Indole ($C_6H_4(CH)_2NH$)	Fecal or stench	0.5 – 3 (0.1 – 0.7)	0.2 (0.03)
	Skatole (C_9H_9N)	Fecal or nauseating	3 (0.6)	3 (0.6)
S compounds	Methane thiol (CH_3SH)	Garlic or putrid	Detected	2 (1)
	Dimethyl sulfide (CH_3SCH_3)	Stench	0 – 18 (< 7)	6 (2)
	Dimethyl disulfide (CH_3SSCH_3)	Putrid	0 – 4 (<1)	48 (12)

1.2 Strategies and legislation related to NH_3 and odor emissions

The first Danish governmental regulation, aiming to reduce pollution from agriculture in order to improve the general water quality, was implemented in 1985 and was followed by a number of directives from the EU as well as national implementations (Grant et al., 2002, Regeringen, 2004). A goal was set to reduce the NH_3-emission of 1987 by 50% which was accomplished in 2003. The goal was reached by reducing the application of fertilizer, tilling manure in the soil after fertilization, investments in closed manure storage tanks, having crops on the fields during fall and winter and implementation of buffer zones to aquatic environments and other types of vulnerable nature etc. However, while nitrogen pollution to the environment from many agricultural sources has been decreasing, the emission from animal buildings remained almost unchanged. From 1985 to 1999, the NH_3 emission from pig facilities decreased from 18,000 to 16,000 ton N $year^{-1}$ and corresponds to 14% of the total N excreted by pigs in 1999 (Andersen et al., 2001). These values are based on the pig production methods used in 1999 and are likely lower today because less NH_3 is emitted with the new generation of housing systems (Andersen et al., 2001). However, today the emission of NH_3 from livestock building is still a major NH_3 problem in Denmark as well as in many other countries. The load of NH_3 is above acceptable levels for certain types of vulnerable nature and with the emerging of new technologies, further reduction of NH_3 and odorous compounds are required. Solutions need to be cost-effective, low in maintenance and not to influence the growth and fitness of the pigs. Initiatives and research have been conducted to reduce the nitrogen and odor emission from the source by changing the pig diet, feed additives and reduce the evaporation by decreasing the manure surface area, pH or temperature (Le et al., 2005, Ottosen et al., 2009). The application of air filters based on biological activity is another promising technique to reduce emission of NH_3 and odors from pig facilities.

2. Biofilters, a promising way to deodorize pig farming

Application of biologically based solutions for air pollution control started in the 1970s. Since then, the technology has been applied and provided an efficient tool in air pollution control on air emissions from a large range of different industries (**Box 1**) and in agriculture (Melse & Ogink, 2005, Chen & Hoff, 2009). Compared to chemical and physical remediation, such as absorption, condensation, incineration and adsorption, biological

systems have proven cost-effective in removing low concentrations (< 5 g m^{-3} air) from large air flows (500 to 100,000 m^3 h^{-1}) with a complex composition of odorous compounds (**Fig. 1**) (Devinny et al., 1999, Delhomenie & Heitz, 2005).

BOX 1: Biofilter application on other industries.

Biofilters have not only been proving useful in treating air emissions from pig facilities. Many different industries take the advantage of the resource lying in the metabolic versatile microorganism and the easy and cost beneficial application of biofilters in reducing both odors and toxic compounds to acceptable and safe levels. Examples of successful full-scale operations are odor reduction from tobacco production, animal rendering plant, odor and reduced organic sulfur compound reduction (hydrogen sulfide, dimethyl disulfide and methyl mercaptan) from a waste water treatment plant, alcohols and ketones from a fabric softener factory, elimination of a mixture of alcohols, ethers, esters, ketones, aliphatic and aromatic hydrocarbons from printing dryers, petroleum vapor biodegradation in air extracted during soil vapor extraction from polluted sites and styrene and toluene from processing of plastic. Each of the filter designs and operation is unique for their purpose and new fields are continuously being explored for the biofilter technology (Devinny et al., 1999).

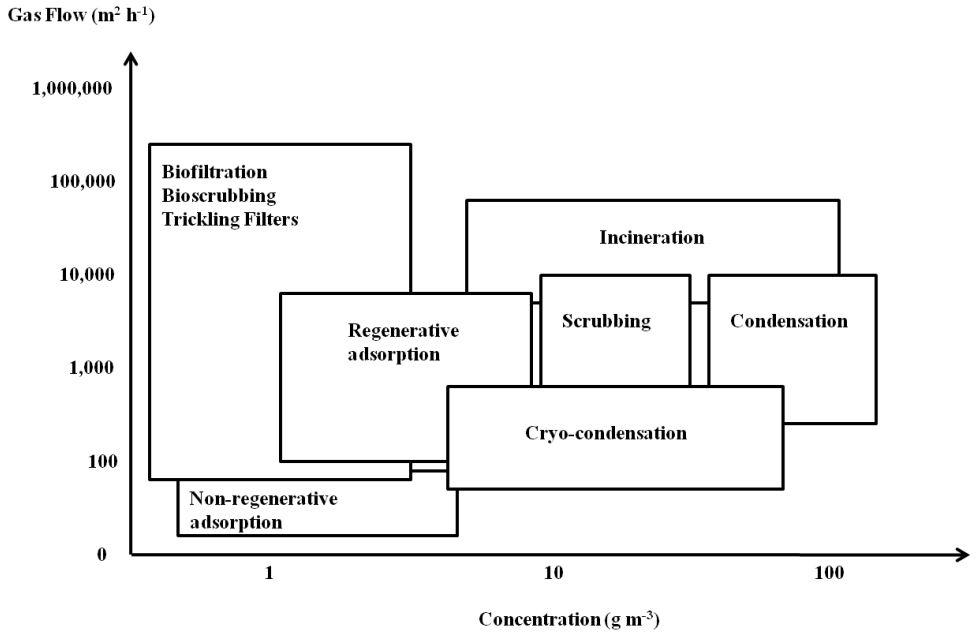

Fig. 1. Application of various air pollution control technologies based on air flow rates and concentrations to be treated. From Devinny et al., (1999).

In biological based systems, the contaminated air stream is passed through a support material which provides contact between the air contaminants and active microorganisms growing in biofilms. The microbial community oxidizes the NH_3 and odor compounds thereby sustaining a continuous sorbing capacity of the filter. A large range of biofilters have been described in the literature and few biofilters have been patented and are commercially available (**Fig. 2**). Here some of the considerations behind the different designs, ensuring efficient removal rates, a healthy microbial community and a low price are briefly introduced.

2.1 Biofilter material

In biofilters, the support material making up the filter bed can consists of a natural materials (e.g. soil, bark, compost, peat, coconut fibers), inert or synthetic materials (e.g. lava rock, ceramics, leca nuts, perlite, polyethylene pall rings, activated carbon, extruded diatomaceous earth pellets) or a mixture (**Fig. 2**) (Shareefdeen & Singh, 2005). The organic materials are in general easier to access, dispose and cheaper compared to the inert and synthetic materials. An important feature of the support material is to provide a large surface area with suitable properties for microbial attachment. Also, the packing material should ensure a sufficient porosity to keep a low pressure drop i.e. the air permeability of the filter material across the filter bed, in order to maintain low running costs (Chen & Hoff, 2009). Organic materials generally have a higher surface area and pressure drop whereas inert and synthetic materials have low surface area (100 – 400 m^2 m^{-3}) and pressure drop (Shareefdeen & Singh, 2005).

For some organic materials, microbial degradation can cause the media to settle over time creating an increased pressure drop. The pressure drop is one of the main considerations in order to keep the operational expenses low. To reduce the extent of media settling mixing of organic material with more resistant material has been recommended (Nicolai & Janni, 2001). Due to the lower mechanical stability of organic materials the height of the filter beds is normally restricted to below 1 meter whereas biofilter beds constructed from inorganic materials has been reported up to 4 meters thus lowering the footprint needed. In addition, biofilters based on inorganic materials in general have longer lifetimes due to the mechanical stability (Shareefdeen & Singh, 2005).

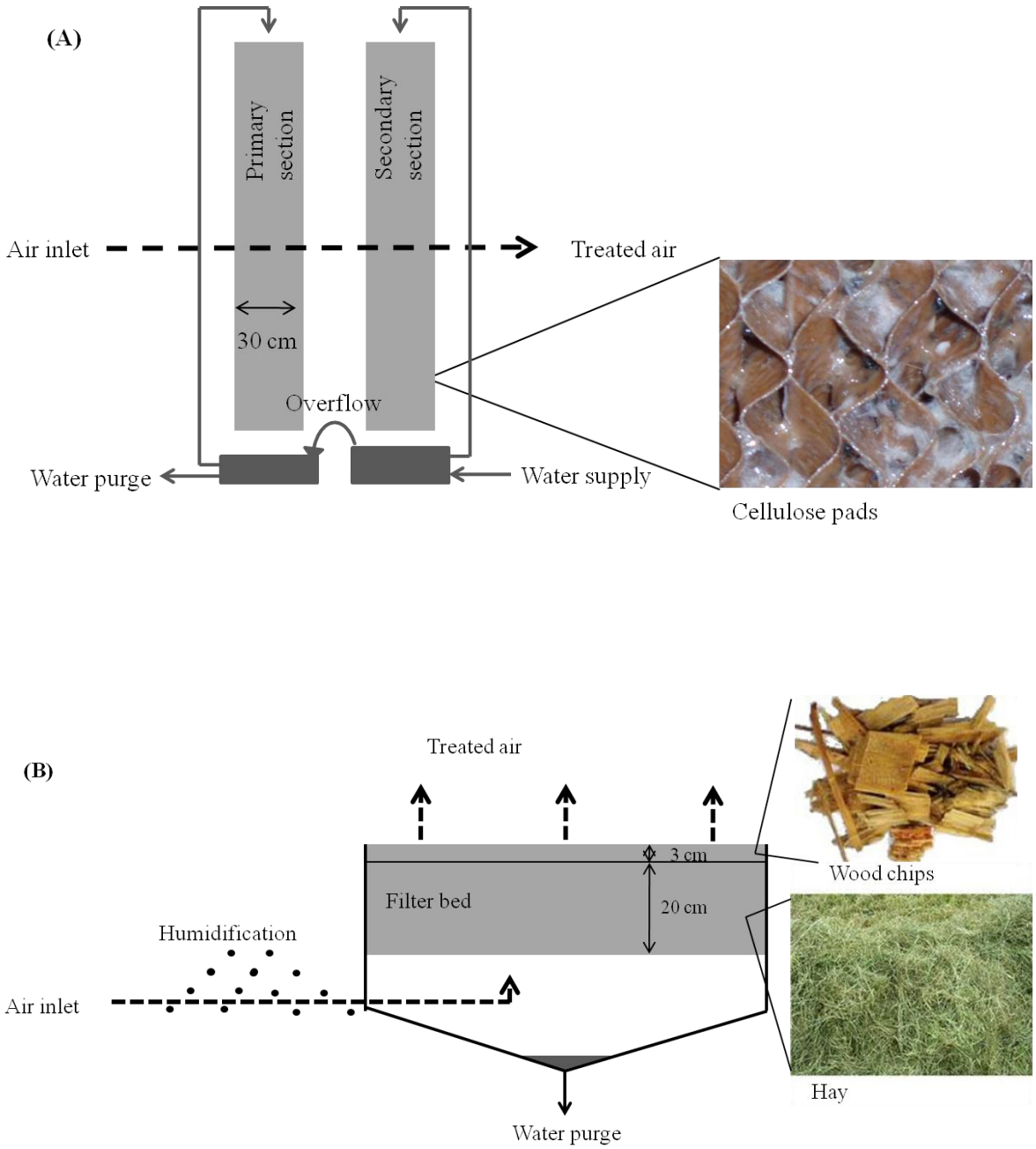

Fig. 2. Examples of biofilter systems. The airflow is shown by broken arrows and water flow by solid arrows. (A) In the SKOV-type trickling biofilter 2 cellulose pads support the active biofilm. The biofilter is continuously humidifies by recirculated water from the top; draining and fresh water supply as automatically controlled based on conductivity. (B) The Oldenburg biofilter bed consists of hay and a top layer of wood chips to hold on the moisture. The air stream is humidified by a sprinkler system before entering the filter bed. Modified with permission from SKOV A/S and after Riis et al., (2005).

2.2 Moisture content

The moisture content is a critical factor for an efficient removal of air contaminants, as the microbial activity is highly dependent on the water activity. As the airstream emitted from pig facilities normally is dry, a water spraying system applied at the filter top or to the air stream before the biofilter which keeps the water activity of the filter bed at a suitable range (between 40 to 65% by weight) (Chen & Hoff, 2009). Many organic materials have a good water holding capacity thus lowering the operational expenses. The water retention capability of the inorganic packing materials is low and a liquid phase is often trickled over the packing material to sustain the microbial community. The trickling water is applied in a circulation manner until the accumulation of waste products exceeds a selected threshold (often determined by the conductivity) upon which it is discharged. This type of filter is often termed a **trickling biofilter** (**Fig. 2A**). Due to the trickling water these filters are more adapted for the elimination of water soluble compounds but the trickling water may also contribute in removing air pollutants and wash away inhibiting products. Inadequate water supply and distribution in the biofilter beds cause the development of dry areas with low microbial activity which may develop into air channels with low air flow resistance and the elimination of air contaminants will decrease. Addition of too much water inhibits the transfer of oxygen and hydrophobic compounds and reduces of the void volume which increases the pressure drop (Swanson & Loehr, 1997).

2.3 Temperature, nutrients and pH

The microbial activity is highly dependent on the temperature and biofilters placed outside should be protected from low temperatures during winter. The temperature of air emissions from pig facilities is however in the range of 20 to 30°C which lowers the effect of the surroundings.

Microbial degradation of organic filter materials provides a slow release of nutrients, which may not be supplied in sufficient amounts from air contaminants or the moisturizing system and supports biomass growth and activity. As organic support materials provide substrates for some microorganisms part of the microbial community in these filters are not involved in the assimilation of air contaminants. For biofilters constructed of inorganic materials nutrients may have to be supplied through the trickling water which can be used to control

the biomass growth. A pH near neutral is preferred by most microorganisms and a near-neutral pH is measured in most biofilters (Chen & Hoff, 2009).

2.4 Air flow

For sufficient mass transfer of pollutants, an empty bed residence time (time period in which the air stays in an unpacked filter bed) range from less than a second to over a minute depending on the filter design and air emission (Chen & Hoff, 2009). For trickling biofilters a lower empty bed residence time can often be applied due to the lower pressure drop compared to filters constructed from organic materials. The air- and water flow can be both in the co-, cross- or counter direction and both filter types have been reported successful. However, better moisture control has been obtained for the downward co-current air- and water flow, as drying of the filter bed preferentially occur at the air inlet and in the co-current filter type a good water distribution is possible at the air inlet (Devinny et al., 1999).

3. Biofilter biofilms

Experimental and theoretical evidence suggests that surface attachment is the prevailing lifestyle of bacteria in biofiltration systems, while planktonian free swimming microbes only constitute a minor fraction (Costerton et al., 1995). Attached bacteria form biofilms, which mainly consisting of water (~90%) and a matrix of extracellular polymeric substances (EPS) excreted by the bacteria. Multiple advantages are gained by living in biofilter biofilms, including (i) close interspecies relations, which facilitates for example metabolic cooperation and genetic exchange; (ii) reduced risk of detachment due to shear stress effects and thereby being washed away from an environment with favorable living conditions; and (iii) protection from predators, bacteriophages and toxic substances as well as desiccation (Davey & O'Toole, 2000). At some point during the biofilm development, it will be inhibited in further growth due to degradation of the EPS and cell lysis causing instability, nutrient limitation and/or detachment due to the sheer force from the water flow. Thus, in time, the biofilm thickness will reach equilibrium.

In biologically based filters, biofilm thicknesses vary both between and within filters. In a trickling biofilter, a patchy distribution of biofilms has been observed, being up to few

millimeters thick but with an overall decrease in thickness following the air flow (Juhler et al., 2009) (**Paper IV**) (**Fig. 3**). Due to excess substrate and nutrient loads, biomass formation can cause an increased pressure drop and clogging of filters. Under such conditions, the biofilm have to be removed by physical methods such as flushing at high flow (Shareefdeen & Singh, 2005).

4. Substrate availability

Gaseous contaminant removal in biofiltration systems, depend on effective mass transfer from the air phase to the aqueous phase and diffusion in a thin boundary layer of water into the biofilm (**Fig. 4**). The boundary layer varies in thickness depending on the biofilter design and management and represents a potential barrier impeding especially the transfer of hydrophobic compounds.

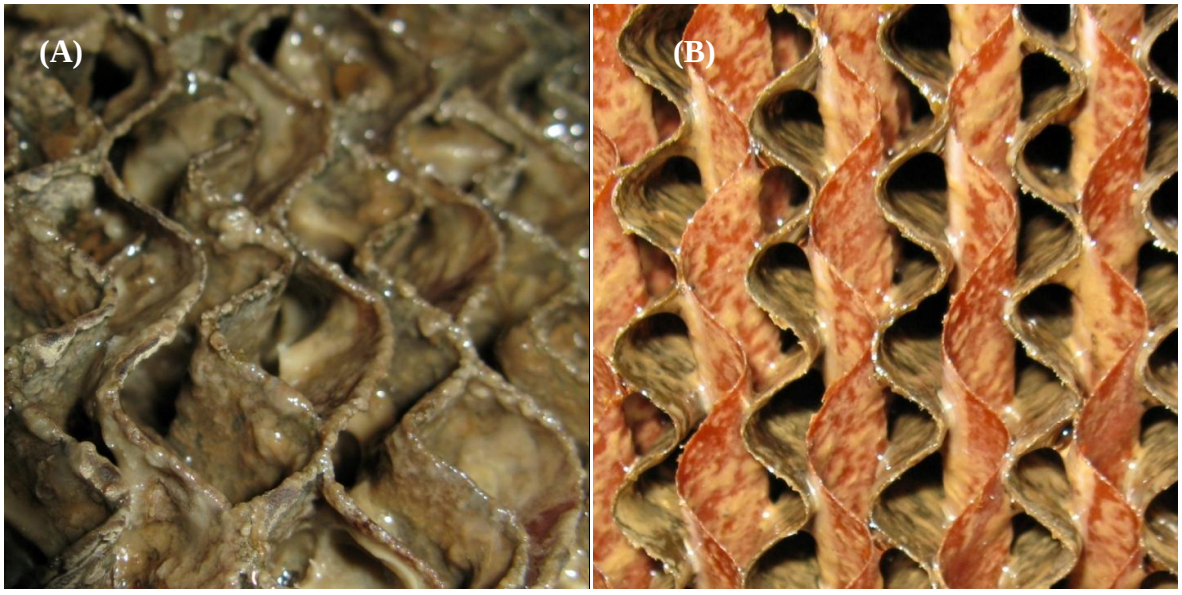

Fig. 3: Trickling biofilter with a biofilm covering the support material at the air inlet (A) and outlet (B).

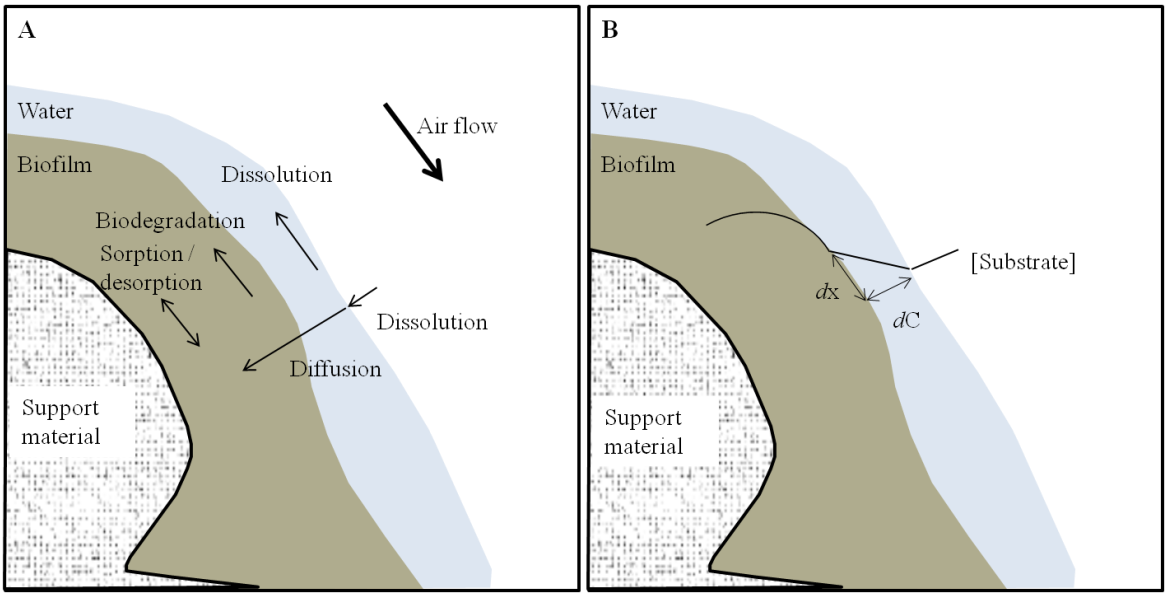

Fig. 4. Transfer mechanisms of air contaminants in biofilter biofilms (A) and the concentration gradient arising from these mechanisms (B). Modified from Devinny & Ramesh (2005) and Swanson & Loehr (1997).

4.1 Mass transfer to the aqueous phase

The mass transfer rate ($J_{air\text{-}l}$) of contaminants from the air to the liquid phase is often described as:

$$J_{air\text{-}l} = K_l \times a \times (C^*_l - C_l) = K_l \times a \times (C_g/H - C_l)$$ (Nielsen et al., 2009)

where K_l is the overall transfer rate constant per unit time, a is the transfer area, C_g is the concentration in the gas phase, C_l is the concentration in the liquid phase, C^*_l is the concentration at equilibrium and H is the Henry's Law Constant. Thus, the mass transfer of a given compound depends on the surface area, the chemical properties of the specific compound and the concentration gradient given as the difference between the equilibrium and the actual concentration of the contaminant in the liquid phase.

The Henry's Law Constant relates to the proportion of a compound being dissolved under equilibrium. For example, short chain fatty acids such as formic acid, acetic acid, butanoic acid and propanoic acid, which are commonly detected in pig facility air emissions, have high Henry's Law Constants[1] (~10^3 – 10^4 M atm^{-1}) revealing high dissolution abilities, as is also seen for aromatic compounds like phenol and *p*-cresol (~10^3 M atm^{-1}) (Sander, 1999).

On the other hand, the Henry's Law constants of organic sulfur containing compounds, like methane thiol, dimethyl sulfide (DMS) and dimethyl disulfide (DMDS), are very low (~0.1 – 1 M atm^{-1}) and compounds like H_2S (~0.1 M atm^{-1}), NH_3 (~10 M atm^{-1}) and CH_4 (~10^{-3} M atm^{-1}) also show low water solubility (Sander, 1999).

4.2 Transport mechanistic in the aqueous phase

Once dissolved, some air contaminants establish ionic equilibrium. The ionic equilibrium is dependent on the character of the compound and pH. For example, at pH 7 more than 99% of the dissolve NH_3 is found at its ionized form (NH_4^+). Thus, despite NH_3 only shows poor solubility (H ~ 60 M atm^{-1}) (Sander, 1999) protonation increase the soluble fraction 100 times. Keeping the moisture content of a biofilter at 28%, 47% and 55% (w/w), average NH_3 removal efficiencies were 6%, 49%, 81%, respectively, reflecting the dependency of water activity of a filter on the removal efficiency (Nicolai & Janni, 2001). Likewise, short chain fatty acids are highly soluble (H ~ 10^3 - 10^4 M atm^{-1}) (Sander, 1999) and have pK_a values around 4.8 but as they are weak acids, they show a high degree of deprotonation at neutral pH which increases the dissolved fraction further. On the other hand, phenolic compounds, such as phenol and *p*-cresol, which are also weak acids, have a pK_a value around 10 and therefore only show weak deprotonation at neutral pH. The substrate solubility affects the penetration depth of the compound into the biofilm and thus the substrate availability.

In trickling biofilters the bulk flow of the trickling water cause movement of dissolved air contaminants towards the biofilm surface (**Fig. 4**). However, in boundary water layers, close to the biofilm, and in the biofilm diffusion is the dominant transport mechanism. The mass transfer (J_{diff}) of a compound by diffusion is proportional to the concentration gradient ($\delta C/\delta x$) and the substrate-specific diffusion coefficient in water (D_w) as described by Fick's First Law of diffusion:

$$J_{diff} = -D_w \frac{\delta C}{\delta x}$$

Once contaminants have been transferred to the liquid phase and transported to the vicinity of the microorganisms, they may be utilized as an energy and/or carbon source by the microorganisms. The microbial consumption of dissolved contaminants drives the overall

concentration gradients in the liquid phase (**Fig. 4**). However, contaminants may also undergo abiotic reactions (**Fig. 4**). Dissolved contaminants may sorb to the biofilm, trapped particles, support material or to microbial cells. In this way, it has been found that microorganisms in the aqueous phase, support material and biofilms increase the solubility or partitioning of hydrophobic compounds like α-pinene compared to water (Miller & Allen, 2004). Sorbed contaminants may not be available for microbial degradation but may gradually desorp and enter the water or air phase due to equilibrium and thus can act as a buffer capacity to changing air emission concentrations. Thus, the physicochemical characteristics of the air contaminants and the filter design determine their biological availability.

5. The microbial activity in biofilters

The microbial community is the catalyst behind the removal of volatile organic carbon (VOC) and NH_3 by biofilters. As VOC and NH_3 are dominating contaminants in air emissions from pig facilities organoheterotrophic microorganisms and ammonia oxidizing bacteria are expected to dominate the microbial community.

5.1 Ammonia oxidation

Concentrations of NH_3 emitted from pig facilities normally range from 5 to 60 ppm (Radon et al., 2002). In biologically based filters, NH_3 is oxidized by bacteria in the two-step nitrification process. In the first step, NH_3 is oxidized by ammonia oxidizing bacteria (AOB) to NO_2^- and in the second step, NO_2^- is oxidized by nitrite oxidizing bacteria (NOB) to NO_3^-.

In biologically based airfilters, the running expenses are often kept low by for example reducing the supply of fresh water. This cause accumulation of dissolved compounds from the air emissions or biological waste products. Nitrogen compounds (NH_4^+, NO_2^- and NO_3^-) are the dominating ions measured and have been detected at concentrations close to or above 100 mM (Melse & Moi, 2004, Juhler et al., 2009) which is 100 to 1000 times above the concentrations in natural environments or even waste water treatment plants (Daims et al., 2001). Oxidation of NH_3 by AOB causes elevated concentrations of NO_2^-, H^+ and HNO_2 (the protonated form of NO_2^-). HNO_2 is a widely known inhibitor which restrain the

AOB activity until the H^+-balance is decreased again by the continuous dissolution of NH_3 (Anthonisen et al., 1976). Thus, the nitrification activity of trickling biofilters is thought to be self-regulated but also to balance the pH around neutral values in filters with limited acid production. In some trickling biofilters, no NO_3^- production is detected and NO_2^- appears as the end product (Melse & Moi, 2004). This phenomenon has often been hypothesized to take place due to inhibition of NOB by HNO_2 and NH_3. Both NO_2^- and NH_3 are also inhibiting heterotrophic activity though different species have different tolerances (Rowe et al., 1979, Lee et al., 2000).

In anaerobic zones, NO_3^- and NO_2^- may be further processed to N_2 by denitrifying bacteria, if biodegradable organic compounds are available. Intermediates of the denitrification process include the greenhouse gas N_2O, which may be emitted from the filter if not all steps of the denitrification process are completed (Yasuda et al., 2009). Therefore, minimal emission of this compound is preferred. Some microorganisms are known only to contain part of the denitrification pathway, thus causing larger emissions of N_2O. A high degree of control of the denitrification process is therefore preferred.

5.2 Heterotrophic activity

In biofilter biofilms, oxygen has been observed to penetrate several hundred micrometers into the biofilm depending on the loading of organic compounds (Juhler et al., 2009). Under oxic conditions, VOC are oxidized to carbon dioxide, biomass or EPS by heterotrophic microorganisms. Low molecular weight compounds (e.g. alcohols, aldehydes, carboxylic acids and ketones) are readily transferred across the microbial cell membrane and require a less complex enzymatic mechanism compared to more complex compounds. These compounds are therefore considered readily biodegradable compared to more complex compounds (Shareefdeen & Singh, 2005). In the deeper anaerobic parts of the biofilm, VOC may be fermented or oxidized through denitrification (Devinny et al., 1999).

Highly odorous organic compounds containing sulfur like dimethyl sulfide (DMS), dimethyl disulfide (DMDS) and methane thiol are also emitted from pig facilities. Methane thiol is auto-oxidized to DMDS, which can be further oxidized by bacteria to sulfate or

sulfide (Sipma et al., 2004). Thus, population of sulfide-oxidizing bacteria may be present in airfilters.

Most research on biologically based filter systems have so far been conducted under constant laboratory conditions addressing the removal of NH_3, H_2S or one or few a selected VOC. In this way, promising results on the removal efficiencies of filters have been obtained, revealing high removal efficiencies (> 80%) for a large range of different VOCs and NH_3 as well as new knowledge on the role of physicochemical parameters (Sheridan et al., 2003, Babbitt et al., 2009, Hort et al., 2009). However, full-scale biofilters are challenged by irregular loadings with variable concentrations and compound compositions, temperature fluctuations, gas channeling and dust particles. Even so, high odor and NH_3 removal efficiencies have been obtained (> 90%) for filters treating air emissions from pig facilities, though rates are usually fluctuating (Hartung et al., 2001, Sheridan et al., 2002, Melse & Ogink, 2005). Detecting the removal efficiency of individual chemical groups by trickling biofilters have revealed high and stable removal efficiencies for carboxylic acids and phenols (80 – 100%), reflecting their high solubility and biodegradability, whereas only 0 to 50% of the organic sulfur compounds were removed (Feilberg et al., 2010). To archive high odor reduction, high removal efficiencies of sulfur compounds are also needed which has been proven possible in an activated carbon biofilter (Ho et al., 2008).

6. Aim and objectives

Previous studies on biofilters installed into the exhaust system of pig facilities have almost exclusively focused on the physiochemical parameters and their influence on the removal efficiency. These studies have provided valuable knowledge on the biofilter design and operation. Despite the key function of the microbial community in the NH_3 and odor reduction process from pig facility air emissions, only limited information is available on their identity and function as well as the community structure. From mostly lab-scale biofilters treating one or few VOC and/or NH_3, initial information on the microbial aspects has been gained, despite the microbial selective pressure differ compared to full-scale biofilters due to different operational parameters and conditions (e.g. substrate concentrations, substrate complexity and temperature). In addition, most studies looking into the microbial aspects use cultivation dependent methods (Lipski & Altendorf, 1997, Khammar et al., 2005), though it is well-known that techniques dependent on cultivation do not reflect the *in situ* community structure, nor is the *in situ* function reflected by the substrate uptake patterns of isolated organisms (Wagner et al., 1993, Amann et al., 1995). Only a small number of studies have applied molecular methods to identify the microbial communities, investigate their substrate utilization and community stability in full-scale biofilters (Alexandrino et al., 2001, Friedrich et al., 2002, Friedrich et al., 2003, Friedrich & Lipski, 2010). While the microbial community removing NH_3 in biofilters, treating air emissions from pig facilities, has recently been characterized (Juhler et al., 2009), no study has been performed to investigate the microbial community structure as well as identity and function using molecular techniques.

The aim of this project was therefore to gain knowledge on the microbial community in biofilters treating air emissions from pig facilities. The SKOV-type trickling biofilter (**Fig. 2A**) is one of the few commercially available biofilters, which today is installed in full-scale at a small number of Danish pig facilities, and has proven stable and efficient in reducing NH_3 and odorous compounds. Therefore, the focus in the present study was on this type of filter. A broad range of molecular techniques were applied in order to gain knowledge on:

- the spatial distribution of the microbial communities and their activity, vertically in the biofilm from the substratum to the biofilm surface and along the air flow axis (**Paper I**)

- the microbial identity and community composition (**Paper II**)

- the microbial community in the pig stable air emissions for comparison with the biofilter microbial consortium, in order to evaluate the potential of air emissions from pig facilities as inoculums (**Paper III**)

- the ecophysiology of the detected microbial species in assimilating selected key odorous compounds representing different chemical odorous groups (acetic acids, butyric acid, *p*-cresol and DMDS) (**Paper IV, V**)

7. Community structure within the biofilm

Visual observations of biofilter biofilms have revealed structures having a patchy distribution and varying in thickness from barely being visible to few millimeters thick (**Paper I, IV**). Heterogeneity is, however, not only seen at the surface, but also in terms of chemical and microbial gradients vertically from the surface to the substratum. In the EPS, diffusion is the only mode of substrate transportation which, due to oxidation and formation of waste products in biofilms, causes gradients to arise.

Oxygen is the primary electron acceptor for most microbial processes in the biofilter biofilm. Aerobic respiration forms a decreasing O_2 gradient through the biofilm, creating microoxic conditions, as revealed by studies using O_2 profiling (**Fig. 5**), and the development of an anoxic zone in deeper parts of the biofilm (Juhler et al., 2009). The O_2 penetration is determined by microbial activity, which again depends on the substrate load and the biodegradability of the air contaminants. As a result, in the biofilm of the SKOV-type trickling filter, a low O_2 penetration and a high O_2 consumption rate has been measured close to the air inlet where both the VOC concentration is high and readily biodegradable compounds are available (Juhler et al., 2009). Organoheterotrophic organisms are generally stronger competitors for O_2, due to their relatively low K_m value for O_2 and a fast growth rate compared to nitrifying bacteria or anaerobic organisms (Satoh et al., 2000). For this reason, aerobic organoheterotrophs are able to outnumber nitrifying bacteria in environments with high VOC availability and they are found in a dense layer at the surface of biofilter biofilms (1 – 20 μm) (**Paper I**). If readily available VOCs are depleted before O_2, a niche is available for AOB and they are repeatedly observed in layers just below organoheterotrophic bacteria or deeper in biofilter biofilms (**Fig. 6**) (**Paper I**).

Gradients for each VOC also arise vertically through the biofilm due to the microbial activity. The load and biodegradability of the different VOC determine the penetration depth. Through the application of microautoradiography (**Box 2**) on intact biofilm under defined conditions, the site of assimilation of representative groups of VOC was observed (**Paper I**). Assimilation of acetate, which is considered a readily biodegradable substrate, was exclusively detected at the biofilm surface. Contrary, *p*-cresol, which is considered less biodegradable than acetate, was assimilated at the biofilm surface but also by micro-

colonies in deeper parts of the biofilm where also phenol was assimilated (**Fig. 7**). These gradients reflect the hydrophobicity and biodegradability of the VOC as well as gradients of the microbial species oxidizing the specific compounds

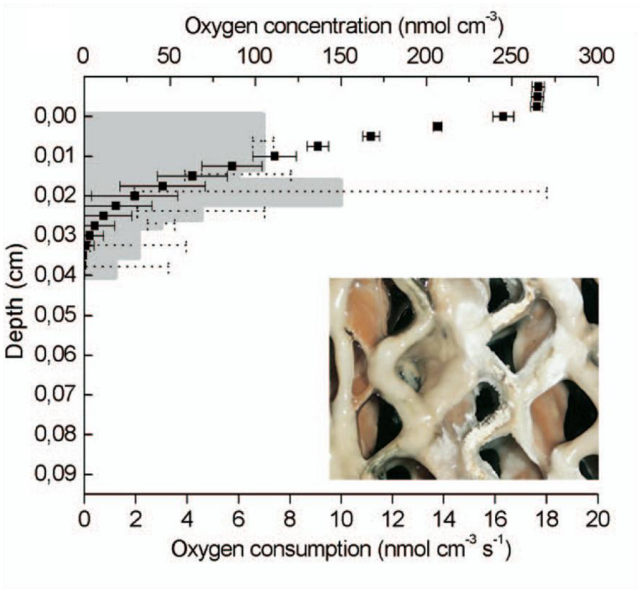

Fig. 5. Oxygen profile (squares) and O_2 consumption (bars) obtained by microsensor measurements of a biofilter biofilm at the biofilter air inlet. From Juhler et al. (2009).

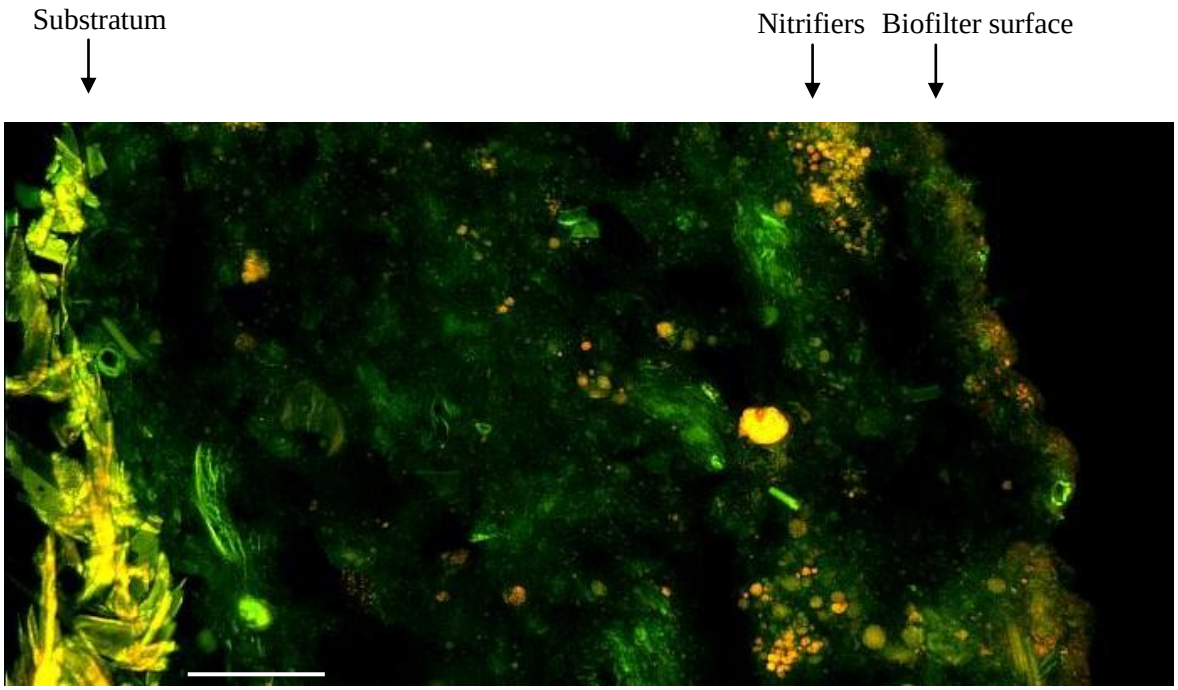

Fig. 6. Vertical distribution of bacteria in a trickling biofilter biofilm. *Betaproteobacteria* are hybridized with the BET42a probe (red) and the general EUBmix probe is shown in green. Nitrifies (orange microcolonies) are observed in the middle and upper layer below organoheterotrophs. The scale represents 100 µm.

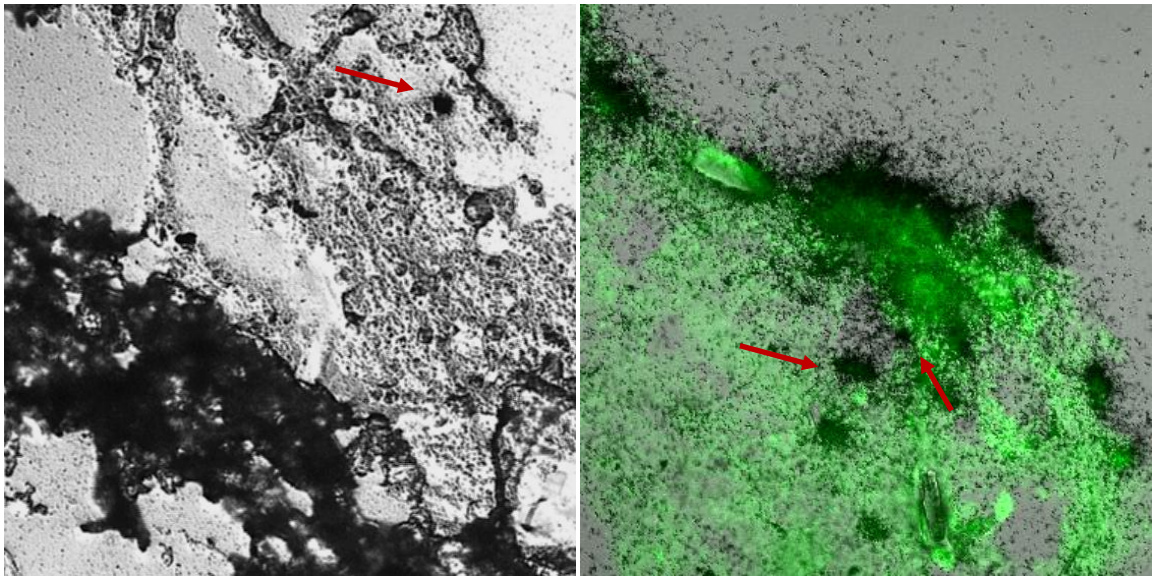

Fig. 7. MAR images of intact biofilm which had been incubated with (A) phenol and (B) *p*-cresol as a substrate (**Paper I**). The biofilm has been stained with FISH in image B using the EUBmix probes. The support material can be seen at the lower part of image (A). Active microcolonies covered by silver grains are indicated by arrows.

Dense and active biomass is mainly present in close contact with the air (**Paper I**, Devinny et al., 1999). In deeper parts of the biofilm, and especially near the substratum, both the fluorescent intensity and the cell density decrease and cell wall remnants are detected, suggesting substrate limitation (**Paper I**, Devinny et al., 1999). The starvation and cell lysis in deeper parts of the biofilm make the structure unstable and parts of the biofilm may break off due to the sheer force from the water flow.

8. The biofilter microbial community

A range of physiochemical factors affects the microbial community establishing in biofilters. The microbial community in these filters is influenced by not only different VOC compositions but also different biofilter design and operation.

8.1 Nitrifying bacteria

In biological air filters treating air emissions from pig facilities, the nitrifying community is a functionally important part of the microbial community. The ammonia-oxidizing community has been detected constituting up to 15% of the FISH detectable community (**Fig. 8**) (**Paper II, IV**) and the *Nitrosomonas*/*Nitrosococcus*-lineage, including

Nitrosomonas eutropha, is a dominant group (**Paper II**, **V**, Friedrich et al., 2002, Juhler et al., 2009). The finding of *N. eutropha* is in line with its relatively high growth rate at high NH_3 concentrations and a high tolerance for NO_2^- (Koops et al., 2003). The high concentrations of NO_3^- detected in some filters suggest the presence of NOB although they have not yet been identified (**Paper IV**, Juhler et al., 2009).

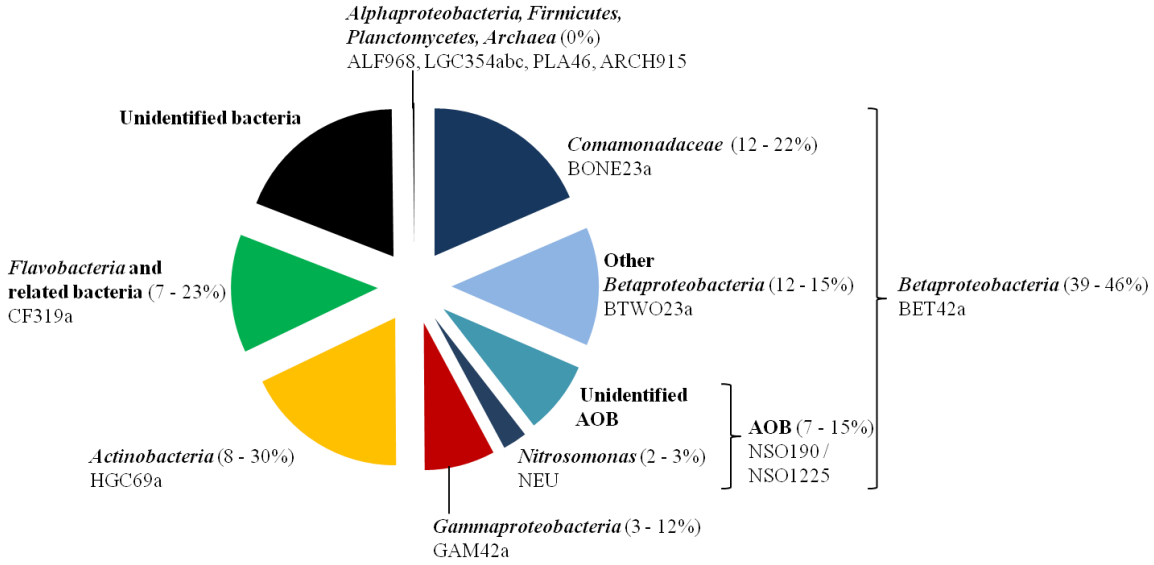

Fig. 8. Microbial community distribution in SKOV-type trickling biofilters as determined by fluorescence *in situ* hybridization. The FISH probe and its phylogenetic target group are indicated. The numbers are summaries from **Paper II**, **IV** and **V**.

8.2 Heterotrophic bacteria

The microbial community of biofilters normally constitutes major portions of *Proteobacteria*, *Actinobacteria* and *Firmicutes* but also the *Cytophaga-Flavobacteria* cluster, *Plancomycetes* and *Archaea* have been detected (Friedrich et al., 1999). However, the composition and abundance of these groups differentiates between biofilters. *Alpha-*, *Beta-* and *Gammaproteobacteria* accounted for a major portion of the community in a biofilter composed of crushed tree roots, having a pH ranging from 1.5 to 5 and treating emissions from an animal rendering plant (von Keitz et al., 1999) whereas *Alphaproteobacteria*, *Actinobacteria* and *Firmicutes* dominated the detectable community in a lab-scale biofilter constructed of crushed wood chips and bark compost, a pH around 4 and treating styrene contaminated air (Friedrich et al., 1999). Factors like composition of the waste gasses, biofilter support material, pH and moisture content influence the

microbial community composition establishing. Biofilters constructed, operated and treating emission of comparable composition reveal a high degree of similarity within the microbial community. Thus, in trickling biofilters of the SKOV-type treating emissions from pig facilities, dominating *Betaproteobacteria* have been detected with major contributions from *Burkholderiales* and the beta-2 subgroup. In addition, *Gammaproteobacteria*, *Actinobacteria* and the *Cytophaga-Flavobacteria* cluster have been detected at different abundances (**Fig. 8**) (**Paper II, IV, V**).

At the species level, moderate richness (66 to 118 operational taxonomic units) have detected in the SKOV-type filter treating emissions from pig facilities, which was estimated to cover at least 75% of the bacterial community (**Paper II**). In contrast, a prediction of minimum 855 unique types was made (determined by restriction enzyme digestion) in a biofilter constructed of crushed tree roots and treating VOC emitted from an animal rendering plant (Friedrich et al., 2002). A high selective pressure due to e.g. the high NH_4^+ and NO_2^- concentrations in the SKOV-type filter likely reduce the number of species able to survive and grow. Furthermore, in biofilters using organic support materials, additional microbial niches are available and therefore support a more diverse community.

In the SKOV-type filter, *Gammaproteobacteria* was represented by the genera *Thermomonas*, *Luteimonas*, *Dokdonella* and *Rhodanobacter* whereas *Arthrobacter*, *Microbacterium*, *Brevibacterium*, *Mycobacterium* and *Rhodococcus* represent *Actinobacteria*. The *Cytophaga-Flavobacteria* group was highly divers but almost exclusively affiliates with sequences of unknown identity (**Paper II**). Several genera (e.g. *Comamonas*, *Pusillimonas*, *Nitrosomonas Microbacterium* and *Leadbetterella*) have been rediscovered among different SKOV-type filters (**Paper II, IV, V**). Thus, at the phylum, sub-phylum and genus level similarities between SKOV-filters operated under similar conditions are apparent. Some of the detected genera in the SKOV-type biofilter have also been detected in other biofilters (e.g. *Arthrobacter*, *Brevibacterium*, *Rhodococcus* and *Rhodanobacter*) (Friedrich et al., 2002, Khammar et al., 2005, Friedrich & Lipski, 2010) and 3 of the OTUs detected in the SKOV-type filter had their closest related organism among sequences obtained from a biofilter treating VOCs though from an animal rendering plant (Friedrich et al., 2002).

From the obtained results it can be speculated, that a number of core species exists in biologically based airfilters able to survive and grow under these conditions and making up a substantial part of the microbial community. The quantitative distribution of these core species shared between filters likely depends on differences in design, the physicochemical conditions in the filter and the types and concentration of VOC. Such core of microbial species is known to be shared between waste water treatment plants (WWTPs) operated after the principle of enhanced biological phosphate removal. In these WWTPs a number of core species, constituting 7 functional groups, each including 3 – 7 species/genera, have been identified. This core makes up 60 - 90% of all bacterial detectable by the EUBmix probe, though the abundance of each probe defined group change in time and space (Nielsen et al., 2010).

8.3 Eukaryotes

Higher eukaryotes have also been observed in biological based airfilters among others fungi (**Fig. 8**). Fungi grow well under dry conditions and their possible role in air emission reduction has been explored. Fungi are able to degrade a large array of compounds and it has been speculated that the hydrophobic filaments positioned directly in the air phase may facilitate a high mass transfer of hydrophobic pollutants (Kennes & Veiga, 2003). Fungi which have been detected in biofilters include *Aspergillus*, *Penicillium*, *Fusarium*, *Scopulariopsis*, *Bullera*, *Mucorales* and *Trichosporon* (unpublished data, **Paper V**). However, a major concern is that fungi may release large quantities of spores, which may cause allergenic problems in the nearby environment. Other eukaryotes in air filters include protozoa, rotifers, larvae, mites, flies and nematodes which are feeding on the bacterial and fungal community (**Fig. 9**) (Shareefdeen & Singh, 2005, Juhler et al., 2009). The activity of these eukaryotes, feeding on the biofilm, may provide partial biomass control and returns elements bound in biomass to the microbial community. Thus, biofilters support a complex ecosystem.

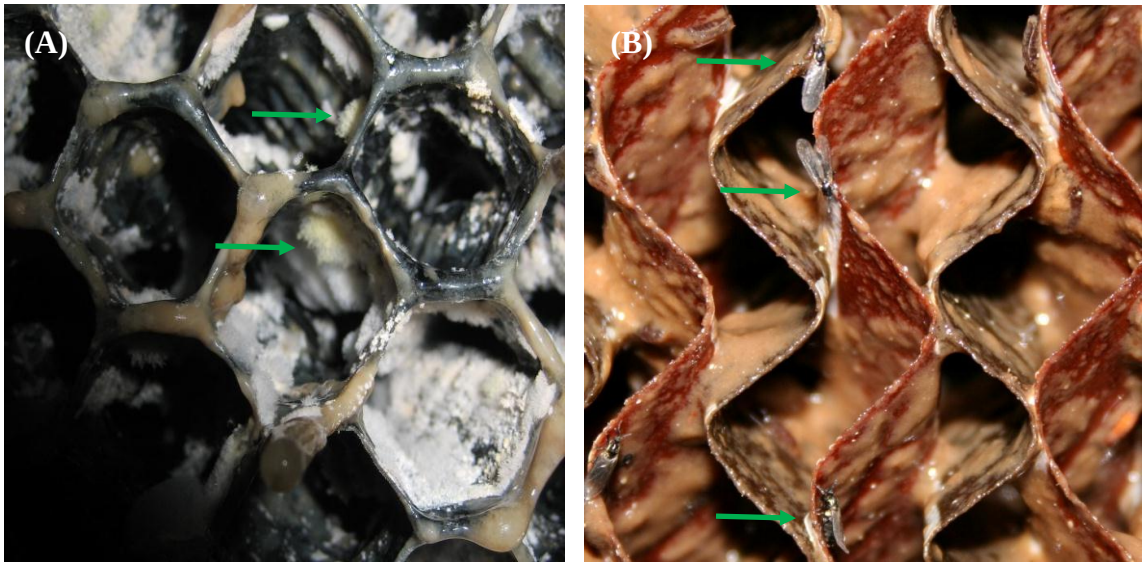

Fig. 9. Occurrence of fungi (A) and flies (B) in biofilters indicated by arrows.

9. Microbial spatial structure following the air flow

Substrate consumption in the biofilm of the filter inlet section affects substrate availability downstream. This is often observed by denser biofilm formations at the air inlet of biotrickling filters where higher substrate concentrations are available compared to deeper parts. Likewise, measurements on the aerobic activity have shown a significant decrease along the air flow (Juhler et al., 2009). When treating a mixture of different VOC the dissolution ability and biodegradability is reflected by the longitudinal position for their removal in the biofilter. Oxygenated compounds are mainly reduced close to the air inlet whereas the removal of aromatic compounds mainly is conducted in the lower parts of biofilters (Friedrich et al., 2003, Khammar et al., 2005). In the SKOV-type filter, the primary section receives the air directly from the stable and the water from the secondary section, whereas the secondary section receives partially treated air from the front section and was partially supplied with tap water. Therefore, gradients of NH_3 and (toxic) oxidation products develop along the air flow in such biofilters and easy biodegradable compounds like carboxylic acids are mainly removed by the primary section whereas the reduction of aromatic compounds is mainly conducted by the secondary section (**Paper IV**) (Juhler et al., 2009).

Stratified removal of different VOC has been correlated with a shift in the community structure (Khammar et al., 2005, Babbitt et al., 2009) whereas no clear longitude microbial

gradient has been detected by others (Friedrich et al., 2003). In the SKOV-type filter, additional diversity has been detected in the primary section by a higher number of OTUs. Additional, higher numbers of *Actinobacteria* was detected in the front section compared to the secondary section where an increased numbers of the *Cytophaga-Flavobacteria* group was detected (**Paper II**).

10. Microbial inoculation of biofilters

Stable removal efficiencies and operational conditions of biofilters are generally reflected by fairly stable microbial communities (Sercu et al., 2006, Babbitt et al., 2009, Jiang & Tay, 2010). Only upon physiochemical changes (e.g. concentration or composition of air pollutants) shifts are revealed depending on the magnitude of disturbance and during start-up phases shifts away from the initial consortium are normally seen (Cai et al., 2006, Jiang & Tay, 2010).

Biofilters constructed from organic material harbor an indigenous microbial population which over time develops into an active filter community whereas trickling biofilters, constructed of inorganic support material, need an external microbial source in order for a microbial population to establish. The selective pressure in biofilters is, however, high and only few of the indigenous microorganisms and the microorganisms brought to the biofilter from external sources by air and water flow or from inoculation thrive in the new environmental conditions.

Air emissions from agricultural buildings harbor a microbial community emitted along with dust particles. The microbial community of air emissions from pig stables have however been found mainly to be composed of anaerobic and facultative aerobic bacteria like *Clostridium* and *Streptococcus* (**Paper III**) which are not found to be quantitative significant in the oxic biofilter environment (**Paper II**). Though aerobic species also have been found in low amounts in pig facility air emissions there is inevitably a period of time before achieving full efficiency of a newly established filter ranging from several days to months. With a suitable inoculum this time period can be reduced.

Biofilter treating air emissions containing complex hydrocarbons have successfully been inoculated with soil contaminated with hydrocarbons (Devinny et al., 1999). Also, cultures

enriched for the ability to degrade selected compounds or activated sludge from WWTP has been used to provide an active microbial community, thriving in a nutrient rich environment and able to oxidize waste compounds. Filter inoculated with a complex community have been found to perform better during the start-up period and have additionally showed higher performance stability at extreme loading rates and pH changes compared to filters inoculated with pure cultures (Sercu et al., 2007). The higher functional diversity of complex inoculums compared to pure or enriched cultures give a better guarantee that one or a few species are able to survive the selective pressure and remove pollutants of interest. Complex cultures may additionally contain species which yet are not known to possess the ability to oxidize key odorous compounds. Yet, using enriched inoculums from WWTP a high degree of selection has been detected (Babbitt et al., 2009, Jiang & Tay, 2010). If a core of microbial species exists in biofilters treating air emissions with similar compositions, using biofilm from well-functioning biofilters will be efficient for biofilter inoculation, in order to reduce the time period for the biofilter to reach stability.

11. Ecophysiology of the biofilter bacteria

With the use of molecular tools it has proven possible to identify microorganisms responsible for the elimination of key odorous compounds from the pig facility air emissions (**Box 2**). In this way, different bacteria have been identified being involved in the assimilation of a range of different air contaminants under conditions reflecting the competitive *in situ* environment. Two studies have focused on the microbial community assimilating fatty acids (**Paper IV, V**). Assimilation of acetic acid was dominated by *Comamonas* (*Betaproteobacteria*) but few numbers of *Stenotrophomonas* and *Rhodanobacter* (*Gammaproteobacteria*) and the fungi *Trichosporon* also participated. The actionbacterial genera *Microbacterium*, *Rhodococcus*, *Propionibacterium*, *Dietzia*, *Gordonia,* and *Janibacter* were responsible for butyric acid assimilation. *Gordonia* has also been identified as part on the active community assimilating hexane in a biofilter treating emissions from an oil mill (Friedrich & Lipski, 2010). Among odorous aromatic compounds emitted from pig facilities only *p*-cresol assimilating bacteria has been identified and revealing the AOB *Nitrosomonas eutropha* as the major contributor together with fungi (**Paper V**). Traditionally, AOB are thought to be autolithotrophic but several studies have revealed the uptake of small amount of organic substrates such as *p*-cresol,

pyruvate and even triclosan by nitrifiers under both cultivation and environmental conditions (Keener & Arp, 1994, Daims et al., 2001, Roh et al., 2009). One study has been conducted on the assimilation of sulfur containing compound using DMDS as a substrate (**Paper IV**). *Actinobacteria* were identified as the major contributor but fungi also participated. These results suggest that the removal of odorous substances in pig facility biofilters relies on few, highly specialized bacterial populations.

Whether the detected species detected are specialized in oxidizing a single type of VOC or assimilate several odorous compounds remain unknown. Species specialized in utilizing one substrate are more vulnerable compared to generalists in a changing environment as biological based filter can be with fluctuating composition and concentration of VOC. However, the SKOV-type biofilter is showing stable reduction efficiencies for fatty acids and phenols revealing functional stability of the microbial community and resistance towards such fluctuations. In general, such stability is correlated with the presence of several species with different response of environmental factors within each functional group (Botton et al., 2006, Nielsen et al., 2010) as was detected for the fatty acids but not *p*-cresol assimilating community.

Box 2: Methods for linking the *in situ* physiology with microbial identity.

Stable isotope probing (SIP): In SIP the sample of interest is incubated with stable isotopically labeled substrate (**Fig. 10A**) to tag the active community. After sufficient labeling, fatty acids, DNA or RNA are extracted, and labeled molecules are separated from unlabeled by density centrifugation. The composition of membrane fatty acids are unique to various taxonomic groups and can be used for identification of the active community. Fatty acids are identified by gas chromatography-mass spectrometry. Another molecule used for identification of the active community is the 16S rRNA gene. The 16S rRNA gene can be retrieved directly from the extracted DNA through PCR or from RNA through cDNA construction and PCR.

Microautoradiography-fluorescence *in situ* hybridization (MAR-FISH): In MAR-FISH radioactive labeled substrate is used to tag the active community (**Fig. 10B**). After ended incubation the sample is fixated and immobilized, and FISH is performed with fluorescently labeled probes targeting taxonomic groups of interest. Following, the sample is covered by a radiation sensitive film which detects the labeled cells, and through light microscopy MAR-positive cells appear with a cover of silver grains and the fluorescent signal from FISH-positive cells is visualized by fluorescence microscopy.

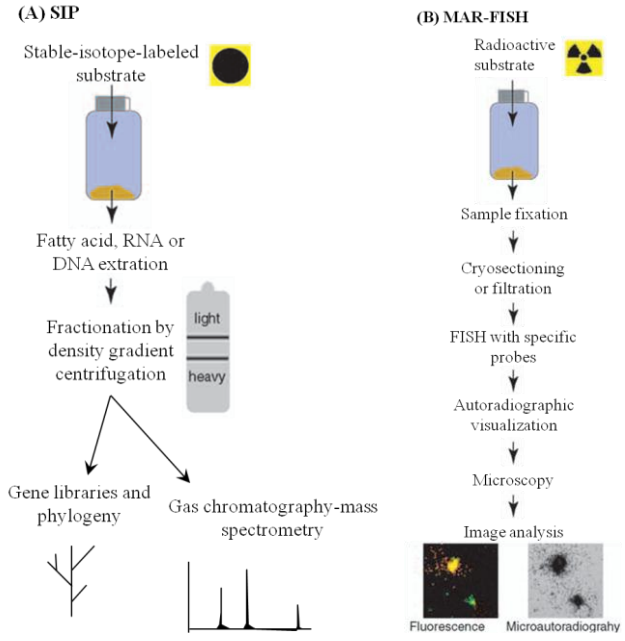

Fig. 10. Overview of the methods: stable isotope probing (A) and microautoradiography combined with fluorescence *in situ* hybridization (MAR-FISH). After Wagner et al. (2006).

Project conclusions

Developments in the biofilter technology have so far largely been conducted by treating the microbial community as a 'black box' despite their central role for the biofilter function. In this project, the microbial community structure and the *in situ* function of the active community in well-functioning and full-scale trickling biofilters (exemplified by the SKOV-type) were investigated. The main conclusions of this Ph.D. are:

- Most bacteria were present throughout the biofilter biofilm but through closer examination vertically in the biofilm a clear stratification of heterotrophic and nitrifying bacteria was observed. Nitrifying bacteria were positioned deeper in the biofilm reflecting the competitiveness of this functional group. Additionally, stratification of the substrate degradation was observed, with acetate exclusively assimilated at the biofilm surface, whereas *p*-cresol and phenol also were degraded deeper in the biofilm, reflecting the stratification of the microbial community.
- Moderate microbial diversity was detected in the trickling biofilter, but the results suggested, that a stable and specific core the bacterial populations existed among filters designed and operated similarly and treating comparable air emissions.
- The microbial populations in the trickling biofilters were dominated by *Betaproteobacteria*, with major contribution from the genus *Comamonas*, but *Gammaproteobacetria*, the *Cytophaga-Flavobacteria* group of *Bacteroidetes* and members of the *Actinobacteria* were also abundant.
- Microorganisms carried by air emissions from pig facilities were mainly anaerobes or facultative anaerobs (*Clostridium* and *Streptococcus*), which did not establish in the biofilter reflecting a high selective pressure. An external inoculation source (e.g. from another biofilter) could likely decrease the start-up period required for a filter to become efficient and stable.
- Selected odorous compounds were assimilated predominately by few dominating but defined groups of microorganisms. Acetic acid was assimilated by *Comamonas*, butyric acid by a few species of *Actinomycetales*, *p*-cresol by *Nitrosomonas eutropha* and DMDS by *Actinobacteria* and betaproteobacterial AOB. Fungi also participated in the assimilation of acetic acid, *p*-cresol and DMDS.

Future perspectives

The continuous expansion and the concentration of the pork production on big farm facilities increase the demands for solutions in order to eliminate NH_3 and odor from air emissions. Biofilters, relying on an active microbial consortium reducing the air contaminants, provide a promising tool for cheap and reliable air pollution control not only on pig farm facilities but also on many other industries.

Despite that the operation of biofilters to a large extent relies on the ability to control the microbial activity and that microbial systems are known to be complex, research on biofilters is often conducted with only limited attention to the microbial community. To optimize and stabilize the removal efficiency of NH_3 and key odorous species by biofilters, a better knowledge on the microbiology is needed. Knowledge on the microbial composition, the role of individual species in the transformation of the compounds and factors (e.g. NO_2^- and NH_3 concentrations) controlling their abundance and activity can be used to create valid models. These models can be used to optimize the factors controlling the stability and removal efficiency of the microbial community and hence the biofilter.

Within the last years, a range of molecular tools have emerged which are able to provide high throughput sequence data directly from environmental samples at a reasonable prize. With such tools available, the generation of metagenomic data from different biofilter designs, which are operated under different conditions, is possibly. Such data provides information on the species present, how they are influenced by the physiochemical factors and the possible metabolic processes in the community and by the different species. In addition, the construction of metatranscriptomic data can provide further insight into the microorganisms involved in the oxidation of key odorous compounds and how the transcription of genes is influenced under different environmental conditions. From such data FISH probes can be designed targeting core species quantitatively abundant and involved in the removal of key odorous compounds. Such probes can be used in trouble shooting by providing a fast method for screening biofilter microbial communities for the presence or abundance of key species.

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