MTT SCIENCE 29

Combination of biological and physico-chemical factors in the development of manure nutrient recovery and recycling-oriented technology

Doctoral Thesis

Anni Alitalo

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Abstract

Manure serves as an important source of nutrients, but is also an environmental concern. From the sustainable agriculture point of view it is of great importance to develop manure treatment technologies to recycle manure nutrients, and to reduce the odor and hygienic problems due to manure production and use. However, the development work has been greatly hampered by the complexity of manure as a raw material and requires knowledge of various physicochemical and biological factors involved in its utilization.

In this study, a sequential slurry manure processing scheme is described in which biological treatment was carried out in a series of continuously fed low-aerated tank reactors. Biological treatment was followed by ammonia stripping, conducted as a sequential stripping procedure. The biological treatment served as a means of achieving a pH increase of manure and made it possible to separate part of the nitrogen by stripping without chemical use. Limited aeration during the biological treatment was applied in order to maintain nitrogen in ammonia form, thus preventing nitrate formation and excessive CO_2 formation, and instead favoring humification processes. Efficient odor reduction and good hygienic status were also among the targeted objectives of the biological treatment.

The functionality of the biological treatment was examined by using swine and dairy slurry manure. Experiments were carried out in pilot scale using six 600-L tanks connected in series with feedback. Before the actual start of the treatment, the treatment tanks were filled with microbial seeding material. It was shown that the designed reactor system provided stability for the process and increased treatment efficiency. However, efficient pretreatment was required in order to reduce the dry matter content of the slurry manure to a level of 1–2% before the biological treatment. Part of the manure phosphorus was also removed with pretreatment. The introduced treatment system reduced the manure odors to an undetectable level or only very faint odor in four days. The pH-value at the end of the treatment increased above 8.5, and nitrogen changes occurred mainly due to ammonia volatilization during the treatments. Nitrate formation was very low with the aeration rates used. Carbon reduction during the aeration treatment depended on the initial carbon content of the manure

and varied in the different studies between 11% and 57%. The treatment also caused changes in slurry precipitation characteristics, which were observed as a color change and a decline in the concentration of divalent cations and total phosphorus. It was also shown that the six tanks in series configuration with feedback served as a good device to achieve good hygiene of the end product. At best, over 90% reductions were observed in the numbers of enteric indicator organisms. However, the result obtained varied depending on the treatment run and indicator organism.

It was shown that the buffer system in manure slurry was composed of TAN (total ammoniacal nitrogen), CO_2, HCO_3^- and CO_3^{2-} and thus can be circumvented by making use of the ammonium-carbonate reduction obtained by biological treatment. Over 30% TAN removal by air stripping was shown to be possible without use of chemicals, if the pH of the biologically treated swine manure was above 8.9.

It can be concluded that this study explains mechanisms and provides a basis for technologies used to efficiently reduce the manure odor, improve manure hygiene, and to separate nitrogen and phosphorus, enhancing the availability of manure fractions as fertilizer. The presented treatment method provides an option to increase recycling of the nutrients of manure and to reduce the odor and hygienic problems associated with manure slurry.

Keywords:

Aeration, aerobic, ammonia, buffer, continuous, humification, hygiene, nitrogen, odor, phosphorus, sequential, serial, slurry, stripping, treatment

Biologisten ja fysikokemiallisten tekijöiden yhdistäminen lietelannan ravinteiden talteenottoon ja kierrätykseen tähtäävän teknologian kehittämisessä

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Tiivistelmä

Lietelanta on tärkeä kasvinravinteiden lähde, mutta se on myös ongelmallinen ympäristön kannalta. Maatalouden kestävyyden lisääminen edellyttää, että kehitetään lannan käsittelymenetelmiä, jotka edesauttavat kierrättämään lannan ravinteita ja vähentävät sen haju- ja hygieniaongelmia. Menetelmäkehitystä on kuitenkin huomattavasti vaikeuttanut lannan kompleksisuus raaka-aineena, mikä edellyttää fysikaalis-kemiallisten ja biologisten tekijöiden ymmärrystä ja yhteensovittamista käsittelymenetelmiä kehitettäessä.

Tässä tutkimuksessa kehitettiin vaiheittainen lietteen käsittelymenetelmä, jossa biologinen käsittely toteutettiin rajoitetusti ilmastetuissa, toisiinsa sarjaan kytkettyjen reaktorien järjestelmässä. Biologista käsittelyä seurasi ammoniakin erotus ilmavirran avulla täytekappalekolonnissa strippaamalla. Erotusta tehostettiin toistamalla strippauskertoja. Lietelannan pH-arvo nousi biologisen käsittelyn aikana, mikä mahdollisti osittaisen typen erotuksen strippaamalla ilman kemikaaleja. Ilmastus pidettiin biologisen käsittelyn aikana maltillisena, jotta typpi säilyisi ammonium-muodossa ja nitraatin muodostuminen olisi mahdollisimman vähäistä. Lisäksi ilmastusta rajoittamalla pyrittiin hillitsemään hiilidioksidipäästöjä ja suosimaan humifioitumisreaktioita. Tavoitteena oli myös tehokas hajun vähentäminen ja lietelannan hygieenisyyden parantaminen biologisen käsittelyn aikana.

Biologista käsittelyä tutkittiin sian ja naudan lietelannoilla. Tutkimuksessa käytetty biologinen käsittelyjärjestelmä koostui kuudesta sarjaan kytketystä jatkuvasyötteisestä ilmastetusta prosessitankista, joista viimeisen ja ensimmäisen välillä oli takaisinkytkentä. Kokeet toteutettiin pilot-mittakaavassa 600 litran tankeissa. Ennen varsinaista käsittelyä, prosessisäiliöt oli täytetty etukäteen tuotetulla mikrobiympillä. Kehitetty reaktorijärjestelmä osoittautui toiminnaltaan vakaaksi ja tehokkaaksi. Biologisen käsittelyn edellytys oli esikäsittely, jolla lietteen kuiva-ainepitoisuus laskettiin 1-2 %:n tasolle. Neljän päivän prosessoinnin jälkeen biologinen käsittely joko poisti lietteen hajun täysin tai haju oli vain hyvin heikosti havaittavissa. Käsittelyn lopussa lietteen pH-arvo nousi 8,5 yläpuolelle ja typen muutokset tapahtuivat lähinnä ammoniakin haihtumisena käsittelyjen aikana. Nitraattia ei juuri muodostunut käytetyillä ilmastusmäärillä. Hiilen häviö ilmastuskäsittelyn aikana

riippui alkuperäisestä lietteen hiilipitoisuudesta ja vaihteli eri kokeissa 11 ja 57 prosentin välillä. Käsittely muutti myös lietteen saostusominaisuuksia. Ne havaittiin lietteen värimuutoksena ja sen kaksiarvoisten kationien ja kokonaisfosforin pitoisuuden laskuna. Tulosten mukaan biologinen käsittely paransi lietteen hygienistä laatua. Parhaimmillaan käsittely vähensi yli 90 % suolistoperäisten indikaatiomikrobien määrää. Saavutettu tulos vaihteli kuitenkin sen mukaan, miten lanta käsiteltiin ja mitä indikaatio-organismia käytettiin.

Tutkimuksessa osoitettiin, että lietteen puskurisysteemi koostui kokonaisammonium-ammoniakkitypestä, hiilidioksidista, bikarbonaatista ja karbonaatista, ja että se on kierrettävissä hyödyntämällä biologisen käsittelyn aikana saavutettua ammonium-bikarbonaattisysteemin puskurikyvyn vähenemistä. Tulosten mukaan lietteen kokonaisammoniumtypestä voitiin poistaa yli 30 % ilman kemikaalikäsittelya strippaamalla, kun biologisesti käsitellyn lietteen pH-arvo oli yli 8,9.

Yhteenvetona voidaan todeta, että tämä tutkimus selittää edellä esitettyjä mekanismeja ja luo perustan teknologioille, joilla voidaan tehokkaasti vähentää lietelannan hajua, parantaa sen hygieniaa ja lisätä lietteen jakeiden hyödynnettävyyttä lannoitteina erottamalla typpi ja fosfori. Esitetyt käsittelymenetelmät luovat mahdollisuuden parantaa lannan ravinteiden kierrätystä ja vähentää lietelantaan liittyviä haju- ja hygieniaongelmia.

Avainsanat:

Aerobinen, ammoniakki, fosfori, haju, hygienia, humifioituminen, ilmastus, jatkuva, käsittely, lietelanta, peräkkäinen, puskuri, sarja, strippaus, typpi

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The early steps towards this thesis were taken already in 2005 when I joined the Slurry manure odor removal and fractionation project led by Professor Erkki Aura at the Soils and Environment unit of MTT Agrifood Research Finland. The three-year project was funded by the Finnish Ministry of Agriculture and Forestry. During this time period early stage pilot scale experiments with swine slurry as well as the ammonia stripping experiments were conducted. Pilot scale experiments with dairy slurry were continued thereafter with the funding of Marjatta ja Eino Kollin Säätiö.

Several people were involved in the experimental and laboratory work of the experiments. I wish to thank them all for their excellent work. I am especially grateful to Senior Research Technician Risto T. Seppälä, who has been the person behind all the technical construction/equipment and has been responsible for running of continous processes. I would like to express my gratitude to Mrs. Katariina Saarela, who has been responsible for the laboratory analyses and running of the continous processes. I am grateful to my coauthors Tuomas Pelto-Huikko, Aleksis Kyrö and Johanna Nikama for carrying out most of the experiments and to Doctor Tapio Salo who helped me with statistical methods. I also wish to thank D.Ph. Minna Kahala and Laboratory Engineer Anneli Virta for their advice and assistance with biotechnological methods.

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This study was closely related to a larger R & D project, aimed at the development and market introduction of a new kind of slurry manure treatment system. I am grateful to Mr. Juha Takala for providing inspiring and broad vision into this subject area.

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List of original publications

This thesis is based on the following publications:

I Alitalo, A., Pelto-Huikko, T. & Aura, E. 2013. Application of a Series of Continuously Fed Aerated Tank Reactors System for Recycling of Swine Slurry Nutrients. Journal of Sustainable Development 6: 26-38.

II Alitalo, A., Kyro, A. & Aura, E. 2012. Ammonia stripping of biologically treated liquid manure. Journal of Environmental Quality 41: 273–280.

III Alitalo, A., Alakukku, L. & Aura, E. 2013. Process design and dynamics of a series of continuously fed aerated tank reactors treating dairy manure. Bioresource Technology 144: 350-359.

IV Alitalo, A., Nikama, J. & Aura, E. 2014. Fate of faecal indicator organisms and bacterial diversity dynamics in a series of continuously fed aerated tank reactors treating dairy manure. Ecological Engineering. Submitted.

The publications are referred to in the text by their roman numerals.

Contributions

The following table presents the contributions of the authors to the original articles of the dissertation:

	I	II	III	IV
Initial idea	EA	AA, EA	AA	AA, EA
Planning the experiment	TP, EA	AA, EA, AK	AA, EA	AA, JN
Conducting the experiment	TP, AA	AK	AA	JN
Data analysis	AA	AA	AA	AA, JN
Manuscript preparation	AA, EA	AA, EA	AA, EA, LA	AA, EA

AA = Anni Alitalo
EA = Erkki Aura
TP = Tuomas Pelto-Huikko
AK = Aleksis Kyrö
JN = Johanna Nikama
LA = Laura Alakukku

Abbreviations

DM	dry matter
CMBRs	completely mixed batch reactors
CMFRs	completely mixed flow reactors
CMR	complete mix reactor
COD	chemical oxygen demand
DO	dissolved oxygen
ED	electrodialysis
HRT	hydraulic retention time
IC	inorganic carbon
MF	microfiltration
NF	nanofiltration
N_{sol}	soluble N
N_{tot}	total N
ORP	oxidation-reduction potential
PFRs	plug flow reactors
P_i	inorganic phosphorus
P_o	organic phosphorus
P_{tot}	total phosphorus
RO	reverses osmosis
TAN	total ammoniacal nitrogen (NH_3 and NH_4^+)
TC	total carbon
TOC	total organic carbon
TS	total solids
UF	ultrafiltration
VFA	volatile fatty acids
VS	volatile solids

Contents

1 Introduction

Slurry manure is a by-product of food production, composed mainly of a mixture of feces and urine of livestock animals. During the course of history its status has changed from the originally valuable plant nutrient to disposable waste and then once again become appreciated as a raw material, the value added components and energy content of which should be recovered and recycled in the most effective way (Sommer et al., 2013). Changes in the approach to slurry revolve around global key issues: population size increase, globalization, environmental emissions and climate change, limited nutrient resources, and depleted fossil fuel reserves (Gilbert, 2009; Fedoroff et al., 2010; Godfray et al., 2010; Sutton et al., 2011; Tian et al., 2012).

Global population size has been estimated to increase from approximately seven billion today to probably over nine billion by 2050, and to level off somewhere between 9 and 12 billion people by the end of the century (UN, 2012). This will be reflected in an increase in demand for food production (Godfray et al., 2010). At the same time, the increasing standard of living is further forecasted to increase this demand, although the production of a varied, high-quality diet including animal protein is known to require additional resources (FAO, 2009). Crops may also be used for biofuels and industrial purposes (Godfray et al., 2010). It is likely that competition for land, water and energy will intensify while the effects of climate change will become increasingly evident (Tilman et al., 2001; WRI, 2005).

Limited natural nutrient resources and pressing environmental issues increase the challenges facing food production. Although phosphorus (P) resources are relatively abundant and significant globally, experts disagree on how much phosphate is left and how quickly it will be exhausted (Gilbert, 2009). In the case of nitrogen (N), industrial nitrogen fixing utilizes fossil fuels (Smil, 2001), which are a diminishing resource. Limited resources increase fertilizer prices. On the other hand, wasteful use of resources causes a global environmental threat both to water bodies and to the atmosphere (Sutton et al., 2011; Tian et al., 2012).

It is obvious that the growing need for food requires more intensive food production, which should be carried out in an ecologically, environmentaly, economically, and socially sustainable manner. In the future, manure should be managed more intelligently than at present, with a focus on high energy recovery, increasing recycling of plant nutrients, and using the most recalcitrant organic matter for soil carbon sequestration, thereby limiting negative impacts on soil, air and water quality (Jensen et al., 2013) without forgetting hygiene aspects and nuisance odors (e.g. Arnold et al., 2006; Bicudo and Goyal, 2003; Newell et al., 2010; Schiffman et al., 2004).

1.1 Characterization of animal manure

Manure is composed not only of the urine and feces of livestock, but also of the bedding, spilled feed, water used for washing, and other materials mixed with it. Livestock manure is a complex mixture from a chemical, physical and biological point of view. The content is largely dependent on the digestion of the specific feed by the individual animal (Carter and Hae-Jin, 2013). During storage, the manure characteristics change continuously due to further degradation (e.g. Zhu et al., 2001; Møller et al., 2002; Popovic and Jensen, 2012). All these factors may influence the manure properties resulting from different treatments.

Manure dry matter content is an important physical property affecting e.g. the viscosity and technical handling of manure (MWPS, 2004; Sommer et al., 2013). Manure has been classified according to its dry matter content as liquid, slurry, (semi-solid) and solid manure, but definitions have differed between countries (Kemppainen, 1989; MWPS, 2004; Sommer et al., 2013):

- Liquid manure contains a very low dry matter content and can be handled with irrigation equipment.

- Slurry manure contains 3–10% dry matter content. It flows under gravity and can be pumped, but may require special pumps for handling. Semi-solid manure contains between 10 and 20% solids. Semi-solid manure is too thick to pump.

- Solid manure contains 18–25% dry matter content or more.

Important raw material properties of manure are nutrient and other constituent concentrations. When manure handling technology is developed, the characterization of particle size distribution, nutrient fractions, odor combounds, and manure buffer capacity is often relevant.

1.1.1 Concentrations of plant nutrients

Manure has usually been characterized from the plant nutrient perspective by analyzing the basic elements of interest, mainly the physical properties of the dry matter content, pH, and the contents of the nutrients N, P and potassium (K). Additionally, calcium (Ca), magnesium (Mg) and micronutrients such as sodium (Na), chlorine (Cl), iron (Fe), copper (Cu), zinc (Zn), manganese (Mn) and boron (B) have been analyzed (Kemppainen, 1989). A comprehensive study of the manure nutrient content in Finland was presented by Erkki Kemppainen in his doctoral thesis (Kemppainen, 1989). In addition, a Finnish commercial company, Eurofins Viljavuuspalvelu Oy, collects statistics on the manure nutrient content of the samples sent by farmers for fertility analyses (Viljavuuspalvelu, 2014). Slurry manure nutrient contents in the late 1980s according to Kemppainen (1989) and some twenty years later (2006–2009) are presented I Tables 1 and 2, based on

Table 1. Average pH and contents of dry matter (DM), total N (N_{tot}), soluble N (N_{sol}), total phosphorus (P_{tot}) and potassium (K) in slurry manure according to Kemppainen (1989).

Manure type	DM	pH	N_{tot}	N_{sol}	P_{tot}	K
	(%)			(g/kg (fresh weigth))		
cow slurry	8.1	7.0	3.3	1.8	1.0	2.8
pig slurry	9.2	7.0	5.4	3.6	1.9	2.0

Table 2. Average pH and contents of dry matter (DM), total N (N_{tot}), soluble N (N_{sol}), total phosphorus (P_{tot}), potassium (K), magnesium (Mg), calcium (Ca), and sodium (Na) in slurry manure according to Viljavuuspalvelu (2014).

Manure type	DM	N_{tot}	N_{sol}	P_{tot}	K	Mg	Ca	Na
	(%)			(g/kg (fresh weigth))				
cow slurry	5.5	3.0	1.7	0.5	2.9	0.5	0.8	0.3
pig slurry	3.5	3.7	2.4	0.8	1.8	0.5	1.1	0.5

the Viljavuuspalvelu statistics (Viljavuuspalvelu, 2014).

By comparing these data, a significant difference was observed especially in pig slurry dry matter (DM) and phosphorus contents (Tables 1 and 2). In part, this was probably due to the differences in sampling techniques between these data. The samples of Viljavuuspalvelu statistics represented a larger number of samples taken by the farmers themselves, and therefore the data was possibly more heterogenous. Secondly, the differences between the two data sets were probably caused by the differences in farming practices and animal diets in the late 1980s compared to the 2000s.

1.1.2 Characteristic components of manure

Fiber and other constituent compositions

Manure processing, biogasification, and the interest in separate value-added components from manure have increased the need for detailed chemical information concerning animal manures (Chen et al., 2003). The fiber composition, including the content of cellulose, hemicellulose, and lignin in manure, can be determined by Van Soest's fiber analysis test using a reflux apparatus (Goering and Van Soest, 1970). In addition to the fiber composition, in some studies manure elemental composition, and also protein and even amino acid content have been characterized (e.g. Chen et al., 2003).

In cattle manures fiber was the main component, as the total lignocellulosic materials content in dairy manure was more than half of the dry matter (Table 3). Popovic (2012) concluded that slurry particles consist of 10–15% proteins, 15–20% humic substances/lignin, 10–30% carbohydrates/cellulose, 10–25% hemicellulose, 5% fat and 15–25% inorganic compounds such as struvite based on the data obtained from Liao et al. (2004), Rico et al. (2007); and Christensen et al. (2009). Fiber provided the substantial resources of cellulose and hemicellulose, being degradable into monosaccharides for use as feedstocks to produce value-added products such as glycols and diols (Liao et al., 2004).

Particle size distribution

Different compounds of manure fall into different particle sizes affecting, for example, the composition of fractions in separation. Particles of diameter <10 µm contained alkylaromatics, phenols and lignin monomers, carbohydrates, and N-containing compounds (e.g. peptides), whereas larger particles (10 µm – 2 mm) mostly contained fatty acids, lignin dimers and sterols in pig slurry (Aust et al., 2009). According to Masse et al. (2005), particles smaller than 10 µm represented 64% of DM in raw swine manure, and 50% of total P was associated with particles between 0.45 and 10 µm. Only 30% of the P was linked to particles larger than 10 µm and approximately 95% of organic N was associated with particles between 0.45 and

Table 3. Comparison of mean fiber and protein contents (%, DM) in cattle and swine manures according to Chen et al. (2003).

Manure type	Crude protein	Total fiber	Hemicellulose	Cellulose	Lignin
	(%, DM)				
Cattle dairy	18.1	52.6	12.2	27.4	13.0
Swine nursery	25.1	39.2	21.9	13.2	4.1
Swine grower	22.7	40.8	20.5	13.9	6.4
Swine finisher	22.0	39.1	20.4	13.3	5.4

10 µm (Masse et al., 2005). These values vary in the literature (Masse et al., 2005; Popovic, 2012) due to differences in animal feeding practices, management, and slurry storage practices.

1.1.3 Manure containing P compounds

In most studies of manure P composition, inorganic and total P have been determined. In order to improve the management of manure P, new methods have been elaborated to identify and quantify manure organic P forms (e.g. He and Honeycutt, 2001; Turner and Leytem, 2004).

Hedley fractionation

The sequential extraction schemes originally developed for soil phosphorus characterization have also been introduced to fractionate manure phosphorus (e.g. Ylivainio et al., 2008). The widely used Hedley fractionation procedure (Hedley et al., 1982) aims to categorize P into pools based on biological availability. The inorganic-P (Pi) and organic-P (Po) extracted with stronger solutions are assumed to represent less bioavailable P pools than the preceding fractions extracted with milder solutions (Table 4). The first step in Hedley fractionation extracts loosely bound water-soluble P. The second extraction is made with sodium bicarbonate ($NaHCO_3$); this extraction has been suggested to provide an estimate of plant available P. These first two fractions constitute the labile and readily plant available fraction of P, and the next two extractions, made with sodium hydroxide (NaOH) and hydrochloric acid (HCl), represent P pools which are bound more strongly. The remaining fraction is the residual fraction (Hedley et al., 1982; Sharpley and Moyer, 2000). Ylivainio et al. (2008) reported that in dairy manure, 81% of the sum of the P fractions was water-soluble (Table 4). A notable proportion (14%) of the P present was organic P and most of it was water- and $NaHCO_3$-extractable, totalling about 12% of the total P fractions.

After sequential fractionation, organic phosphorus fractions have been fractionated into even more specific organic P forms with orthophosphate-releasing enzymes. According to the study of He and Honeycutt (2001), pig and cattle manures were first sequentially fractionated into water-soluble P, $NaHCO_3$-soluble P, NaOH-soluble P, HCl-soluble P, and residual P. Part of the organic P in these fractions could be identified by the enzymatic treatments as phytate (39% for pig manure and 17% for cattle manure in water-soluble organic P), simple phosphomonoesters (43% for pig manure and 15% for cattle manure in NaOH-soluble organic P), nucleotide-like phosphodiesters (2–12%), and nucleotide pyrophosphate (0–4%).

NMR spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy can provide compound-specific information on manure phosphorus. Both solid-state and solution 31P NMR

Table 4. Concentrations of inorganic and organic P sources according to the Hedley fractionation scheme in air dried dairy manure (Ylivainio et al., 2008).

Extractant	Inorganic-P (Pi) (mg/g)	Organic-P (Po) (mg/g)
Water	3.2	0.3
$NaHCO_3$	0.2	0.2
NaOH	0.1	0.1
HCl	0.2	
Σ	3.7	0.6

spectroscopy have been used. Turner and Leytem (2004) used a two-step extraction procedure and NMR spectroscopy to quantify phosphorus compounds in extracts of swine and cattle manure. Initial extraction in $NaHCO_3$ recovered readily soluble phosphorus, whereas a second extraction in NaOH-EDTA recovered poorly soluble phosphorus. Organic phosphorus in the readily soluble fraction included DNA, phospholipids, and simple phosphate monoesters in both manures, whereas the poorly soluble fraction included poorly soluble phosphate, plus phytic acid in swine manure and a range of phosphate monoesters and diesters in cattle manure (Turner and Leytem, 2004).

1.1.4 Odor compounds

Manure odors are a complex mixture of volatile fatty acids (VFA), alcohols, aromatic compounds, amides (including NH_3), and sulfides (Hartung and Philips, 1994). In a study of Schiffman et al. (2001, Table 5) a total of 411 compounds were found in odorous emissions from swine facilities.

Odorous gases and volatile compounds are produced during incomplete anaerobic fermentation (Mackie et al., 1998). Anaerobic microorganisms use organic compounds as their electron donor and as sources for cell synthesis and metabolism (for energy and carbon), during which various odor-

Table 5. Heterogenity of odorous compounds. Compound group, example compound of the group, chemical formula, and odor characteristic of the compound (modified from Shiffman et al., 2001).

Compound group	Compound	Formula	Odor characteristics
Acids	Formic acid Acetic acid Propionic acid	$HCOOH$ CH_3COOH CH_3CH_2COOH	Irritant, purgent Irritant, purgent Irritant, purgent
Alcohols	Methanol Ethanol	CH_3OH	Alcoholic
Aldehydes	Formaldehyde Acetaldehyde Benzaldehyde	$HCHO$ CH_3CHO C_6H_5CHO	Pungent, rotten Pungent Almond. irritant
Amides	Acetamide N,N-dimethylformamide	CH_3CONH_2 $HCON(CH_3)_2$	Irritant, fishy, pungent
Amines	Methylamine (aminomethane)	CH_3NH_2	Initant, putrid, fishy
Aromatics	Benzene Toluene Methylstyrene	C_6H_6 $C_6H_5CH_3$ $C_6H_4(CH_3)CH{=}CH_2$	 Benzene-like Irritant
Esters	Mehyl formate	$HCOOCH_3$	Irritant
Ethers	Diethyl ether Furan	$C_2H_5OC_2H_5$ C_4H_4O	Sweet, pungent, irritant
Fixed gases	Ammonia	NH_3	Sharp, pungent
Halogenated hydrocarbons	Chloroform	$CHCl_3$	
Hydrocarbons	2-methylbutane	$CH_3CH_2CH(CH_3)_2$	Irritant
Ketones	2-propanone	CH_3COCH_3	Irritant
Nitriles	Benzen acetonitrile		Aromatic
Other N containing compounds	Pyridine	C_5H_5N	Irritant, burnt
Phenols	Phenol	C_6H_5OH	Irritant
Sulfur containing compounds	Hydrogen sulfide	H_2S	Rotten eggs

ous gases and volatile compounds are produced (e.g. Hartung and Philips, 1994). The quantity and variety of the organic matter contributes directly to odor generation and is linked to animal type, diet, manure composition and microbial fermentation (Miller and Varel, 2003). Starch and protein are primary substrates for odor compounds produced. Starch fermentation has been shown to dominate in cattle manure fermentation, whereas both protein and starch fermentation occurred in swine manure (Miller and Varel, 2003). More offensive compounds (branched chain volatile fatty acids and aromatic ring compounds) tended to be produced during protein fermentation, emphasizing the significant malodor related to swine manure (Miller and Varel, 2003).

Volatilized fatty acids and aromatic compounds have been most closely correlated to odor (Zhu et al., 1997; Powers et al., 1999; Zahn et al., 2001), and the use of VFA level to determine the odor intensity of swine manure is commonly utilized (Evans et al., 1986; Miller and Varel, 2001). Another commonly used method to measure odor is olfactometry, by which the odor concentration is measured as dilution-to-threshold in an olfactometer with human panelists (CEN, 2003). Recently, also direct on-site measurements of odor and chemical measurements by proton-transfer-reaction mass spectrometry (PTR-MS) have been used (e.g. Hansen et al., 2012).

1.1.5 Buffer capacity

Manure buffer capacity has an essential role in manure treatment processes. This is due to the buffer system resistance to pH changes, which complicate solids and P removal processes as well as N separation (Paul and Beauchamp, 1989; Husted et al., 1991; Sommer and Husted, 1995). High amounts of chemicals are required, which often make the treatment economically unprofitable.

Buffers are compounds that resist changes in pH upon the addition of acids or bases. Buffer systems are usually composed of a weak acid or base and its conjugate salt (Holman et al., 2013). The components act in such a way that addition of an acid or base results in the formulation of a salt, causing only a minor change in pH (Holman et al., 2013).

Buffer solutions achieve their resistance to pH change because of the presence of an equilibrium between the acid HA and its conjugate base A-.

$$HA \rightleftharpoons H^+ + A^- \qquad (1)$$

When strong acid is added to an equilibrium mixture of the weak acid and its conjugate base, the equilibrium is shifted to the left, in accordance with Le Chatelier's principle (e.g. Holman et al., 2013). Because of this, the hydrogen ion (H+) concentration increases by less than the amount expected for the quantity of strong acid added. Similarly, if strong alkali is added to the mixture the hydrogen ion concentration decreases by less than the amount expected for the quantity of alkali added (Holman et al., 2013).

The complex chemical buffer system in manure consists of a number of acids and bases, the most important of which are total ammoniacal nitrogen (TAN), total inorganic carbon (CO_2, HCO_3^-, CO_3^{2-}), volatile fatty acids, and other organic compounds (Paul and Beauchamp, 1989; Husted et al., 1991; Sommer and Husted, 1995).

The major buffer reactions in manure are (Husted et al., 1991; Sommer and Husted, 1995):

Enzymatic hydrolysis of urea originating from animal urine to ammonium carbonate:

$$CO(NH_2)_2 \xrightleftharpoons{\text{Urease, } H_2O} 2\,NH_4^+ + CO_3^{2-} \qquad (2)$$

Decomposition of the ammonium carbonate into NH_3 and CO_2 gases:

$$2NH_4^+ + CO_3^{2-} \rightleftarrows NH_4^+ + HCO_3^- + NH_3 \quad (3)$$

$$NH_4^+ + HCO_3^- \rightleftarrows CO_2 + H_2O + NH_3 \quad (4)$$

If the released gases are removed, equations (3) and (4) will proceed to the right. The equations show the combined effect of NH_4^+ hydrolysis generating H^+, and the release of CO_2, which in turn requires H^+; on the one hand protons are released in the hydrolysis of the ammonium, and on the other hand the liberation of carbon dioxide binds protons:

Ammonia hydrolysis:

$$NH_4^+ \rightleftarrows NH_3 + H^+ \quad (5)$$

Release of carbon dioxide:

$$CO_3^{2-} + H^+ \rightleftarrows HCO_3^- \quad (6)$$

$$HCO_3^- + H^+ \rightleftarrows CO_2 + H_2O \quad (7)$$

Because of the low equilibrium constant of equation (5) ($pKaNH_4 = 9.3$; Stumm and Morgan, 1981), volatilization of ammoniacal N only occurs under neutral to alkaline conditions. Being itself an acidifying process, NH_3 volatilization is enhanced by a high H^+ buffer capacity (Avnimelech and Laher, 1977; Vlek and Stumpe, 1978). Balancing H^+ release and uptake, the simple $(NH_4)_2CO_3$ system described in equations (2) to (7) allows complete simultaneous volatilization of NH_3 and CO_2.

VFAs may volatilize or be removed by aerobic microbial decomposition, thereby consuming H^+ (Paul and Beauchamp, 1989):

$$CH_3COO^- + H^+ \rightleftarrows CH_3COOH \quad (8)$$

1.2 Manure volumes, environmental impacts and legislation

1.2.1 Manure volumes and regional concentration

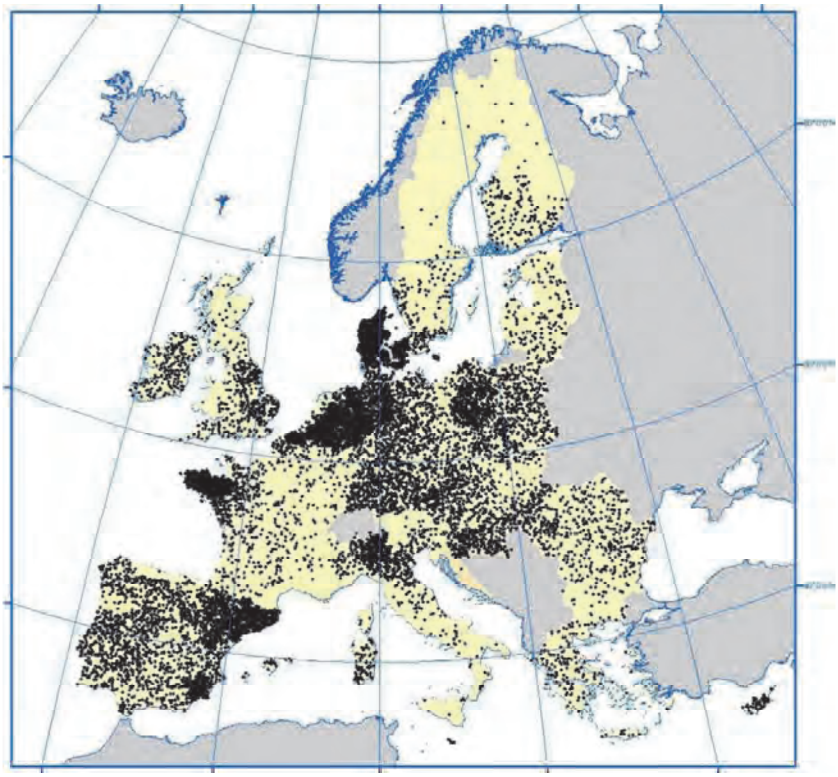
Agricultural intensification, regional specialization, concentration of production on a decreasing number of farms, and increasing farm size during recent decades have been intensive particularly in Finland (Niemi and Ahlstedt, 2010), but also in other parts of Europe too. In Finland, the regionally differentiated agricultural support has affected the specialization of agriculture and has in particular led to regional concentration of livestock production (VTV, 175/2008).

Number of livestock farms and animals

In 2012, there were 1712 pig farms in Finland with 1.3 million pigs. The number of cattle farms was 13321 with 912,800 animals, of which 31% were dairy cows (Tike, 2013). Pig farms were mainly located in south-western Finland (27%), in southern Ostrobothnia (17%) and in Ostrobothnia (16%), whereas the majority of the dairy farms were located in northern Ostrobothnia (15%) and in northern Savo (13%). The number of pig farms decreased by 72% between 1995 and 2012 but the number of pigs remained almost the same (-8%, Tike, 2013). During the same time period, the number of dairy farms decreased by 70% and the number of dairy cattle by 29%, but milk production remained on approximately the same level (Niemi and Ahlsted, 2013; Tike, 2013).

On the European level, pig production has been concentrated in a few countries, with Denmark, Germany, Spain, France, the Netherlands and Poland having more than two thirds of the breeding pigs between them (Fig. 1, European commission, 2010).

In 2011, the EU-27 had 86.2 million bovine animals. About third of them (22.8



Source: Eurostat (agr_r_animal)

Figure 1. Number of sows by region in 2008. 1 dot = 1,000 sows – NUTS 2 except DK, DE, UK (NUTS 1) (European commission, 2010).

million) were dairy cows (representing 27% of the total bovine population of the EU-27). The majority of the dairy cows were located in three different countries, i.e. in Germany (18%), France (16%) and the United Kindom (>8%) (European Commission, 2013).

Manure volumes and storage

In Finland, the annual amount of manure generated is not monitored statistically. However, based on animal numbers and official manure storage capacities, manure production was estimated to be 13 543 967 tons/year (fresh weight) in 2009 (Grönroos et al., 2009; Luostarinen and Grönroos, 2013). In terms of manure production, the most important animals were cattle and pigs, which produced more than 95% of the total amount of manure generated. Of the total amount produced in 2009, 39% was cattle slurry, 45% cattle solid, 11% pig slurry and 4% pig solid manure (Luostarinen and Grönroos, 2013).

The entire manure production in the EU that is potentially available for manure processing has been estimated to be 1.4 billion ton/year (Foged et al., 2011). The highest production has been in France, followed by Germany, and the lowest in Malta (Foged et al., 2011).

In Finland, the manure storage must be sized according to the annually generated amount of manure except for pasture manure (VNa 931/2000). With increasing production levels, manure outputs have also increased. For example, for dairy cows the increase was estimated to be 44% from

1990 to 2012 (Helstedt et al., 2013, Table 6). According to Hellstedt et al. (2013), the water volume used for e.g. washing was 9% of the total amount of slurry in the case of dairy cows and sows.

1.2.2 Environmental impacts related to animal manure

Agriculture has been identified as a significant contributor to nutrient losses to the environment, especially from livestock manure. Globally, livestock has been evaluated to excrete about 100 Tg N per year, but only 20–40% of this amount is estimated to be recovered and applied to crops (Sheldrick et al., 2003; Oenema and Tamminga, 2005). The remainder was dissipated into the environment. The estimated amounts of phosphorus (P) and potassium (K) in livestock manure were 1.5 and 3 times the amounts of P and K in mineral fertilizers, but only a fraction of manure P and K was efficiently utilized (Sheldrick et al., 2003).

Livestock production systems exert various influences on the environment, distributing emissions into the air, soils and watercourses (Fig. 2). The influences on the environment greatly depend on the livestock production system itself, the management, and the environmental conditions (Oenema et al., 2007). Additionally, the odor and hygiene influences of livestock manure have been considerable.

Nitrogen and phosphorus losses

Nitrogen leaching has caused groundwater contamination, eutrophication and also, indirectly, nitrous oxide emissions (Vitousek et al., 1997; Carpenter et al., 1998). Phosphorus losses contribute to the eutrophication of waterways. The level of nitrogen and phosphorus leaching has been influenced by soil and weather conditions and by cultivation practices (type, timing and amount of fertiliser application; crop type, timing of cultivation, and the type manure spreading technology (e.g. Shepherd et al., 2003). The overuse of fertilizer nutrients has increased the soil nutrient reserves, as found in Finland for phosphorus (Saarela, 2002). The amount of P lost to surface waters has been reported to increase with the P content of the soil (Sharpley and Rekolainen, 1996). When measures to reduce nutrient leaching are developed, an important point is that more than 90% of the loading from arable fields to surface waters in Finland has been found to enter the water bodies outside the growing season (Puustinen et al., 2007).

Gaseous nitrogen emissions

Agriculture is the main source of ammonia emissions. In Finland, 90% of ammonia emissions originate from agriculture (Grönroos et al., 2009). Ammonia emissions from agriculture arise mainly from manure (ECETOC, 1994; Grönroos et al., 1998). Animal housing, manure stores and manure spreading are the major sources of ammonia emissions. In addition to odor emission, ammonia is the main acidifying pollutant from agriculture (OECD, 2013). Manure management is one source of nitrous oxide (N_2O) emissions (Grönroos et al., 2009). N_2O is a greenhouse gas which contributes to climate change and

Table 6. Annual production of livestock manure (m^3/animal/year) in 1990 and 2012 (Hellstedt et al., 2013). Present design instruction (RMO) for manure storages in Finland (RT MMM/MTH-20919).

	1990	2012	RMO
Manure type	manure (m^3/animal/year)		
Dairy cows slurry manure	18.3	25.8	24.0
Fattening pigs slurry manure	2.0	2.4	2.0
Sows and piglets slurry manure	9.1	9.3	7.0

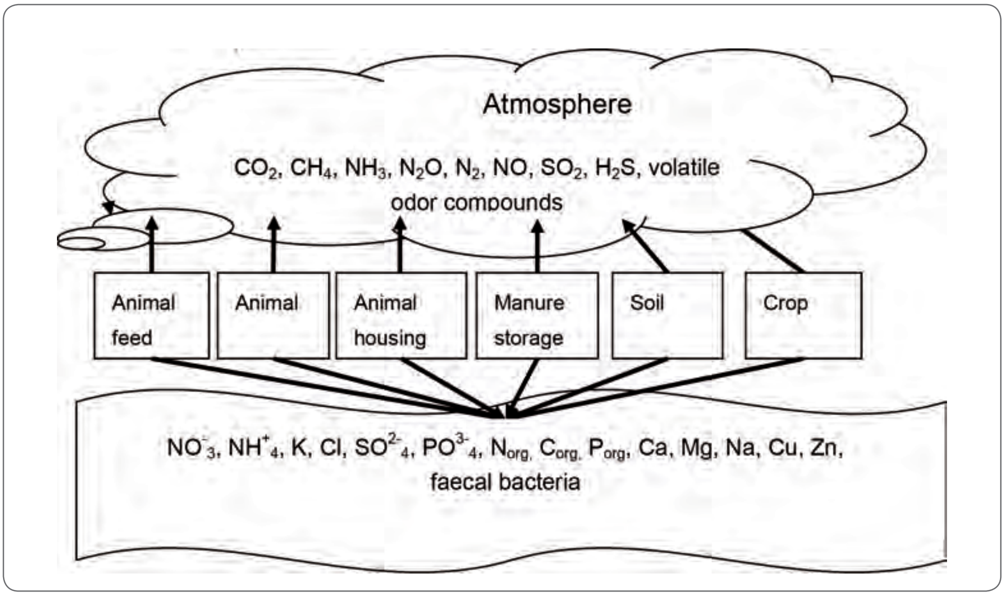


Figure 2. Possible loss pathways of nutrient elements from the feed–animal–manure–soil–crop chain. Losses via gaseous emissions to the atmosphere are shown in the upper half, losses via leaching and runoff of nutrient elements to the subsoil and to groundwater and surface waters, and accumulation of nutrients into the soil, in the lower half (modified from Oenema et al., 2007).

can catalyze the destruction of ozone (Vitousek et al., 1997). It is produced in soils aerobically during nitrification and anaerobically during denitrification. Emissions arise from the manure management and nitrogen applications (e.g. mineral fertilisers and manure) to the soil (US-EPA, 2006).

Hygiene

Livestock manure can harbour a wide range of bacterial, viral, and parasitic pathogens (McAllister and Topp, 2012). Therefore, it can pose a microbiological risk. There has been increasing concern about the effects of pathogens possibly present in animal manure on human and animal health (Bicudo and Goyal, 2003). In recent years, outbreaks of food-borne diseases caused by enteric microorganisms have received much attention, leading to increased consumer concerns about the safety of their food supply. A growing concern has been that many previously unrecognized foodborne pathogens, including Campylobacter jejuni, Escherichia coli 0157:H7, and Listeria monocytogenes, have emerged during the past twenty years (Newell et al., 2010). Adding to the problem, treatment is more difficult due to an increase in antibiotic resistance among common food-borne pathogens (e.g. Gilchrist et al., 2007). Moreover, it has been evaluated that new patterns of resistance to antimicrobial agents and new forms of virulence emerge in old pathogens and influence the risk of epidemics in the future (Velge et al., 2005; Martella et al., 2010). Some practices associated with intensified production have been estimated to have the potential to increase the risk of zoonoses (Liverani et al., 2013). The most important zoonotic pathogens and viruses in cattle and swine are listed in Tables 7 and 8.

Costs due to animal diseases have normally been associated with reductions in animal populations and production (IFAH, 2012). Even those diseases which are not classified as dangerous may result in significant costs. There are also costs related to the mitigation of disease, which include the money and resources expended to monitor, control and, in extreme cases, eliminate the disease agent (IFAH, 2012).

Table 7. Zoonotic pathogens of potential interest in cattle and swine. (Pell, 1997; Pelzer and Currin, 2007; Raizman et al., 2004; Hutchison et al., 2004, 2005; Bhaduri et al., 2005; Farzan, 2010; Esaki et al., 2004).

Pathogen	Common species	Other remarks
Campylobacter	*Campylobacter jejuni, Campylobacter coli*	Widespread in the intestinal tract of warm-blooded animals. One of the most often encountered bacteria responsible for human gastro-intestinal infections
Cryptosporidium (Protozoa)	*Cryptosporidium suis, Cryptosporidium parvum*	Some *Cryptosporidium* spp. such as *Cryptosporidium parvum* have become a public health concern
Escherichia coli	*E. coli O157:H7*	A significant number of foodborne disease outbreaks have increasingly been attributed to *Escherichia coli* O157:H7
Giardia (Protozoa)	*Giardia duodenalis (syn. G. lamblia, G. intestinalis)*	Giardia duodenalis is a commonly identified intestinal parasite of mammals, including humans. Human giardiasis, most often associated with drinking water, is frequently diagnosed in the United States
Leptospira	The severe pathogenic serovars Pomona in pigs and Hardjo in cattle have not been reported in Sweden	European Centre for Disease Control (ECDC) is pinpointing leptospirosis as an important infectious disease of particular interest in Europe. The zoonosis of leptospirosis, which is of worldwide distribution, is caused by different pathogenic serovars belonging to the genus *Leptospira* and is endemic in most tropical and temperate climates
Listeria	*Listeria monocytogenes*	
Mycobacterium	*Mycobacterium avium* subsp. *paratuberculosis (Mycobacterium paratuberculosis)*	*M. paratuberculosis* is the causal agent of paratuberculosis or Johne's disease, which is a common and chronic intestinal disease.
Salmonella	*Salmonella* can be isolated from numerous animal species and is known to be a principal zoonotic bacterium causing symptoms such as diarrhoea, fever and septicaemia.	*Salmonella* is a causative pathogen in food-borne illness. There is considerable information on antimicrobial resistance in *Salmonella* of human and food animal origin.
Yersinia	*Yersinia enterocolitica* Swine are the primary reservoir from which Y. *enterocolitica* strains pathogenic to humans are isolated	Major bacterial food-borne pathogen by the pork production and processing industry in the United States

The health risks associated with animal operations depend on various factors. The most important ones appear to be related to intensified systems with high animal densities and the concentration and virulence of pathogenic microorganisms in animal manure (Bicudo and Goyal, 2003; Létourneau et al., 2010). The nature and concentration of zoonotic pathogens excreted by animals differ according to animal species and health, nutrition, age and housing environment (Bicudo and Goyal, 2003; Cliver, 2009; Létourneau et al., 2010). In manure, bacteria and parasites persist for a time depending on geographic location of the farm, on the physicochemical composition of the manure (temperature, pH, free ammonia, and solids content), on aeration and on handling and storage management (Jones, 1982; Bicudo and Goyal, 2003; Topp et al., 2009; Létourneau et al., 2010).

The ability of the pathogens to survive for long periods and through treatment to remain infective in the environment until ingested by human or animal hosts has been an added concern (Bicudo and

Goyal, 2003). Most bacteria can survive and even multiply in environments outside the animal, such as in livestock manure (Strauch, 1991). Viruses cannot multiply outside the animal, but are capable of surviving for long periods of time (even months) in the environment, depending on environmental conditions (Spiehs and Goyal, 2007).

Odors and other air emissoins

Odors are an increasingly difficult and pressing problem for the agricultural industry. They have been a major factor causing complaints and have had a particular relevance for environmental permit issues concerning barn expansions or construction of new buildings (Arnold et al., 2006). The primary reason is the increasing unit size and concentration of large numbers of farm animals on a single farm, increasing the potential for nuisance and environmental problems including odor annoyances, greenhouse gases, ammonia and hydrogen sulfide emissions (Jacobson et al., 2001a). Dust (a combination of manure solids, dander, hair, and feed), pathogens, and flies from animal operations have also been airborne emission concerns (e.g. Jacobson et al., 2001a). A variety of potential health effects related to odorous air have been reported, including symptoms such as irritation, headache, nausea, etc. (e.g. Schiffman et al., 2004). Air emissions also pose the threat of aerosol transmissible diseases (Millner, 2009). All these problems have been forecasted to continue to increase as suburban development encroaches upon areas that are primarily used for agricultural purposes (Schiffman et al., 2004).

Livestock odors originate from three primary sources: animal buildings, manure storage units, and land application of manure (Jacobson et al., 2001a). Of these sources, land application of manure is probably the greatest source of odor emissions and complaints. The study of Arnold et al. (2006) demonstrated the importance of uncovered manure storage tanks for overall odor emissions from animal houses. Approximately half of the detected pig farm odor emissions originated from the uncovered manure storage tanks and half were from the pig farm buildings during the summer season. Temperature had a strong influence on odor emissions, as during the winter season almost no odor emissions were detected from ma-

Table 8. Some of the most important viruses affecting cattle and swine. (Martella et al., 2010; Bøtner and Balsham, 2012).

Virus	Affected animals	Other remarks/aspects
Foot-and-mouth disease virus	Affects cattle, pigs and sheep	One of the world's most economically important diseases
Classical swine fever virus (and the related bovine viral diarrhea virus)	Affects cattle and pigs, is excreted in faeces by infected animals	Normally absent from Europe, but serious outbreaks of the disease can occur
Swine influenza virus	Widely distributed in pig populations, causes a relatively mild, largely respiratory, disease	Poses a threat to human health. Contains segments derived from different influenza viruses, with a potential for novel reassortment
Porcine parvovirus	Replicates within the gut and thus can be present in swine slurry	Widely distributed, being endemic in most countries; causes reproductive failure in swine
Rotavirus	Rotavirus-associated enteritis is a major problem in young calves and in weaning and post-weaning piglets	Poses a zoonotic potential, comparison of genetic sequences of human and animal rotaviruses have revealed close identity

nure storage tanks (Arnold et al., 2006). Teye (2008) and Teye and Hautala (2008) introduced a theoretical ammonia emission model and measured the ammonia emissions from dairy houses in Finland and Estonia. Ammonia emissions from dairy buildings varied between 0.04 and 0.58 g m^{-2} h^{-1}. They concluded that the critical parameters which should be considered in reducing ammonia emissions in dairy buildings were manure temperature, pH and total ammoniacal nitrogen of the manure.

1.2.3 Environmental legislation

Nitrates Directive and EU agri-environmental support

The implementation of the Nitrates Directive (91/676/EEC 1991) is one of the policy measures aimed at decreasing nitrogen discharges and losses from agricultural sources. Different measures are in place in the various EU countries to meet this directive. In Finland, the Nitrates Directive is transposed to national legislation through the Environmental Protection Act (4th February 2000/86, paragraph 11.6) and Government Decree No 931/2000 (9 November 2000), adopting the whole territory approach in the Nitrates directive instead of designating specific nitrate zones as in many other countries.

The Decree contains provisions on good agricultural practices, storage of manure, spreading and allowable quantities of fertilizers and silage liquor, analysis and recording of nitrogen in fertilizers and enforcement of the Decree (931/2000). In practice, current legislation restricts the spreading of slurry manure or urine in the autumn and prohibits spreading during the time period from November 15th to April 1st. Legislation also restricts the total amount of manure permitted to be applied per hectare according to its nitrogen content.

In parallel with the implementation of the Nitrates Directive, Finnish farmers have widely adopted the EU agri-environmental support scheme (96% coverage of the cultivated area during the second period 2000–2006). Environmental support for basic and additional measures has been paid to farmers who have met the eligibility criteria laid down in Government Decree No 644/2000 (26 June 2001) and undertaken the basic measures related to the following activity areas for five years: environmental planning and monitoring in farming, basic fertilization levels of arable crops, plant protection, filter strips, biodiversity and landscape management.

Environmental permit

The Environmental Protection Decree (169/2000) and the Environmental Protection Act (86/2000) regulate animal shelter permit requirements concerning the keeping of animals in production buildings. The environmental permit procedure estimates impacts on ground and surface waters, adverse smell and emissions into the air, manure storage, transportation and field application. Manure spreading and arable farming are not licensed activities. However, in license permissions arable land area and its adequacy in relation to the number of animals is taken into account.

1.3 Manure treatment technologies

Manure treatment has been under intensive study, and different treatment technologies have been extensively reviewed in a number of recent publications and inventory descriptions (e.g. Foged, 2010, 2011; Burton, 2007; Vanotti et al., 2009; Schoumans et al., 2010; Sommer et al., 2013). Large numbers of scientific publications have also dealt with manure treatment.

In this section, manure treatment technologies are first categorized coarsely into physical, chemical and biological methods to introduce briefly the processes involved in treatment technologies. Treatment technologies are further categorized into phosphorus recovery-oriented methods, methods to treat the liquid fraction (comprising mainly those technologies covering nitrogen separation recovery), odor treatment technologies, and methods aiming at pathogen reduction.

1.3.1 Method categorization into physical, chemical and biological processes

Physical methods

Physical methods include technologies involving the application of physical forces to treat the manure, e.g. solids-liquid separation and/or the use of heat and pressure. Solids-liquid separation has been achieved through settling or by using mechanical methods (e.g. using screens, sieves or grates), including intensified separation using drum filters, filter pressing, belt presses, screw pressing or centrifuges (e.g. Møller et al., 2000; Burton, 2007; Hjorth et al., 2010). In combination with chemicals to increase solid material flocculation, solids-liquid separation has been used more effectively to remove nutrients from manure (e.g. Hjorth et al., 2008, 2010; Walker et al., 2010). Other physical methods include drying, incineration, pyrolysis, combustion, and gasification (e.g. Schoumans et al., 2010; UNEP, 2009).

Chemical methods

Principal chemical processes include chemical coagulation, precipitation, disinfection, oxidation, neutralization, stabilization, and ion exchange methods (e.g. McCabe et al., 2005). Chemicals have also been used to control odors (e.g. Ritter, 1981; Zhu et al., 1997) and pH (Petersen et al., 2012). Electrochemical methods are incorporated in chemical methods. These methods comprise electrodialysis, electroflotation/coagulation, and electrochemical oxidation methods (e.g. Laridi et al., 2005).

Biological treatment

Biological processes make use of naturally occurring microorganisms or/and added inoculants to degrade manure in the presence of oxygen (aerobic) or in its absence (anaerobic). Manure treatment using biological methods has been designed to reduce the odor of the slurry, to reduce the slurry nutrient (e.g. nitrification-denitrification) and organic matter concentrations and for stabilization of the slurry, to reduce the content of pathogenic microbes in the slurry, and to produce energy (Ndegwa, 2003; Juteau et al., 2004; Park et al., 2005; Zhang and Zhu, 2006). The used biological methods include anaerobic treatment methods, e.g. anaerobic digestion (Nasir et al., 2012) and fermentation (Banister and Pretorius, 1998), and aerobic treatment methods, e.g. composting (Bernal et al., 2009). Sequential aerobic-anaerobic treatment methods, e.g. enhanced biological phosphorus removal (Toerien et al., 1990), have also been used.

1.3.2 Phosphorus removal and recovery technologies

Phosphorus recovery technologies have been extensively reviewed e.g by Schoumans et al. (2010). The methods relevant to the empirical work of the present study are briefly described in the following.

Solid-liquid separation

The simplest and most widely used method to treat manure phosphorus is solid-liquid separation. Foged et al. (2011) described 10 mechanical, chemical and other technologies for active separation of slurries in their inventory, and estimated the amount of livestock manure treated by separation to be 3.1% of the entire livestock manure production in the EU. The most used separation methods were separation by drum filters, screw pressing, sieves, centrifuges, and natural settling (Foged et al., 2011).

Most separation methods are based on particle size and particle density differences (Burton, 2007; Hjorth et al., 2010). More sophisticated devices such as centrifuges and chemical-enhanced settling can achieve higher separating efficiencies, but involve additional equipment and/or management requirements (Hjorth et al., 2010). The efficiency of the separation also depends on the physical (e.g. DM content, particle size) and chemical composition of the animal manure, and manure type (Zhu et al., 2001; Møller et al., 2002; Popovic and Jensen, 2012). Long storage time typically impairs the separation result by reducing the particle size of the solid material (e.g. Zhu et al., 2000).

In general, low-solids swine manure settles more easily, whereas dairy manure with its higher solids content is more easily separated by mechanical separation (Møller et al., 2002). Mechanical separation methods do not remove salts and have only had a limited capacity for separating out small (< 1 mm) particles with P, N and odorous compounds being present in high proportions in this particle class (Zhu et al., 2001; Popovic, 2012).

Chemical methods

Chemical precipitation or coagulation and flocculation with various salts of aluminum, iron and other inorganic or organic chemicals, and also lime, have been widely used to treat manure, aiming for pH change, coagulation of dry matter, and for P removal (e.g. Hjorth et al., 2008; Hjorth, 2010).

Precipitation occurs if the concentration of a compound exceeds its solubility in a solution. Thus, precipitation is achieved by a) change in ion concentration of the manure, b) change in pH, or c) by using both of these methods (e.g. Wang et al., 2005). For example, following the addition of multivalent cations (Fe^{3+}, Fe^{2+}, Ca^{2+}) to the slurry some phosphate will precipitate (Hjorth et al., 2008) due to formation of, for example, ferric phosphate ($FePO_4$), ferrous phosphate ($Fe_3(PO_4)_2$) and calcium phosphate ($Ca_3(PO_4)_2$).

In addition to precipitation, the addition of multivalent cations to the slurry manure causes coagulation of the dry matter particles of the slurry (Sherman et al., 2000; Hjorth et al., 2008). In coagulation, the multivalent cations neutralize or partially neutralize a particle's negative surface charge by adsorbing the oppositely charged ions to the particle surface, creating a double layer and thereby removing the electrostatic barrier preventing aggregation; this process is termed 'charge neutralization' (Hjorth et al., 2010).

The addition of polyelectrolyte polymers to slurry manure has been used to induce flocculation (Vanotti et al., 1996; Vanotti et al., 2002). Flocculation of small particles into larger ones increases the effective particle size by creating flocs (aggregation of small particles and smaller flocs). These flocs separate out from the liquid phase of raw slurry more rapidly than their constituent small particles alone would do (Peters et al., 2011) and they can be more easily retained by screens, or enhance separation of colloidal particles by their settling (Vanotti et al., 2002). Cationic polymers have been shown to be much more effective than anionic or neutral polyacrylamide polymers in removing organic solids from swine manure wastewater (Vanotti et al., 1996), most probably due to slurry particles carrying negative charges (Gregory, 1989). However, aluminium has been shown to be much more effective than polyacrylamides in reducing slurry total solids (TS) and P (Sherman et al., 2000). When selecting chemicals to be used for precipitation/coagulation, factors that need to be taken into account include: precipitate formed, solubility of the chemical, reaction time, and handling safety (e.g. Burns et al., 2003; Le Corre et a., 2005; Wang, 2005). Most salts of monovalent cations (e.g. Na^+, K^+) are water soluble, whereas divalent cations (Mg^{2+}, Ca^{2+}) salts are less soluble; solubility depends e.g. on crystal structure (Haynes, 2012). Trivalent ions (e.g. Al^{3+} and Fe^{3+}), although efficient precipitants, form sparingly soluble Al and Fe phosphates, which are hardly available to plants (Dao et al., 2001; Hyde and Morris, 2004).

Struvite precipitation is a phosphorus recovery technology taking advantage of chemical precipitation (Burns et al., 2002, 2003; Wang, 2005). Struvite is a crystalline precipitate, magnesium ammonia phosphate hexahydrate ($MgNH_4PO_4*6H_2O$). Struvite crystallization depends on factors such as component ion ratios, interfering ions, temperature, and solution pH (Wang et al., 2005). Struvite precipitation from manures has been typically achieved by adding Mg in a slurry (e.g. $MgCl_2$, MgO), and/or by increasing slurry pH by addition of chemicals or by using aeration (Burns and Moody, 2002; Suzuki et al., 2002). Complex organic solutions, such as manures, have required higher than stoichiometric magnesium additions to overcome complexing reactions (Burns et al., 2003). Struvite has been used as a valuable slow-release fertilizer (De-Bashan and Bashan, 2004).

Biological phosphorus removal

Enhanced biological P removal takes advantage of the ability of polyphosphate-accumulating organisms (PAOs) to accumulate large quantities of polyphosphate within their cells (Shapiro, 1967). To induce biological phosphorus removal, an alternation between anaerobic and aerobic conditions is required, together with an extracellular supply of energy in the form of easily degradable organics (Toerien et al., 1991). During the anaerobic phase, PAOs produce energy by hydrolyzing polyphosphates, take up carbon source to produce polyhydroxyalkanoates, and release soluble orthophosphate or PO_4^{3-}-P (Petersen et al., 1998). Since poly-P is broken down to PO_4^{3-}-P for energy supply, the phosphate concentration in the anaerobic phase increases. The anaerobic phase needs to be followed by an aerobic phase. In this phase, polyhydroxyalkanoates are consumed to generate energy, and PAOs accumulate large amounts of phosphorus (the phosphorus fraction of phosphorus-accumulating biomass is 5–7%) within their cells by reincorporating the PO_4^{3-}-P into new microbial cellular poly-P (De-Bashan and Bashan, 2004; Montag, et al., 2009).

The polyphosphate-accumulating organisms have a strict requirement for cyclic anaerobic, anoxic and aerobic conditions which consequently makes the bio-P-removal process rather complex (Zuthi et al., 2013). Biological P removal has been widely used for treating municipal and industrial wastewaters, but with manure slurries this technology is mainly in a developing phase.

1.3.3 Treatment of the liquid fraction after solid-liquid separation

The liquid fraction obtained after separation contains soluble components (e.g. soluble ions and N compounds) and some colloidal and suspended particles (including part of the phosphorus and organic compounds), although the largest particles have been removed (Burton, 2007; Popovic, 2012). Composition of the liquid fraction after solid-liquid separation depends on the efficiency of the preceding separation step. Almost all liquid fraction treatment methods require a pre-treatment step (e.g. prefiltration) in order to function efficiently (Hjorth et al., 2010).

Filtration

Microfiltration (MF) and ultrafiltration (UF) systems are basically highly efficient solid-liquid separators, but have not been typically used for concentrating soluble elements (Hjorth et al., 2010). Microfiltration retains particles between 0.1 and 5 μm, while UF removes macromolecules and particles in the 0.001–0.05 μm size range (Masse et al., 2007). Both processes deal with separation of suspended solids, colloids and bacteria, do not develop significant osmotic pressures and require low operating pressures (Hjorth et al., 2010). Microfiltration and ultrafiltration are usually efficient in concentrating the nutrients associated with particles, such as phosphorus, but for other constituents, e.g. ammonia and potassium, the retention requires nanofiltration (NF) and reverses osmosis (RO (Masse et al., 2007; Hjorth et al., 2010)). NF membranes retain most organic molecules with a molecular weight greater than 200–400 Da (Masse et al., 2007). NF can retain some soluble salts (charged molecules such as Ca^{2+}, Mg^{2+}, NH_4^+). Viau and Nomandin (1990) reported that a NF membrane (150 DA) operated at 2.1 MPa pressure retained 52% and 78% of TAN and potassium, respectively, in the effluent from an aerobic reactor treating pig manure. The most recent technological development is to use gas permeable membranes to remove and recover NH_3 from liquid manure taking advantage of membranes through which only NH_3 can be transported (Vanotti and Szogi, 2010).

Because of fouling, micro- and ultrafiltration membranes can only be used to separate pre-treated slurry, e.g. runoff streams from centrifuges (Hjorth et al., 2010). The fouling problems have been even more severe for nanofiltration and reverse osmosis than for micro- and ultrafiltration. Nanofiltration or reverse osmosis can therefore only be used for separation of dissolved components from the permeate produced by an ultrafiltration unit (Hjorth et al., 2010).

Reverse osmosis

Reverse osmosis is a pressure-based membrane purification technique. The principle is based on the use of pressure to force a solvent (usually pure water) to pass through a semipermeable membrane from a solution of higher concentration towards one of lower concentration, i.e. the opposite direction to that dictated by osmosis (Kucera, 2011). The osmotic pressure is related to salt concentration and external pressure. Reverse osmosis membranes theoretically retain all dissolved salts and organics with a molecular weight greater than approximately 100 Da (Masse et al., 2007). Rejection of dissolved salts ranges from 95% to greater than 99% (Masse et al., 2007). As the feed is being concentrated, osmotic pressure builds up in the system and high operating pressures must be applied (Kucera, 2011). The amount of pressure required depends on the salt concentration of the feed water. The more concentrated the feed water, the more pressure is required to overcome the osmotic pressure (Kucera, 2011). Reverse osmosis requires extensive manure pretreatments to prevent fouling, maximize membrane life, and increase flux (Masse et al., 2007).

Electrodialysis

Electrodialysis (ED) is a membrane separation method in which salt ions from one solution are transported through ion-exchange membranes to another solution under the influence of an applied electric potential difference in an electrodialysis cell (Ippersiel et al., 2012).

Electrodialysis has been used in combinations with other technologies to recover and concentrate ammonia from swine manure (Mondor et al., 2008; Ippersiel et al., 2012). Mondor et al. (2008) used electrodialysis and reverse osmosis. The results obtained by these authors suggested that, under the conditions of their experiment, a maximum total NH_3 -N concentration of about 16 g/L could be reached with the ED system. The maximum TAN concentration achievable by ED was limited by water transport from the manure to the concentrate compartment and by ammonia volatilization (17%) from the open concentrate compartment. Ippersiel et al. (2012) used electrodialysis coupled with air stripping. In this study electrodialysis was used to concentrate ammonia and then air stripping from the electrodialysis-obtained concentrate solution without pH modification was used to isolate the ammonia in an acidic solution. However, low concentrate solution pH (8.6–8.3) limited NH_3 volatilization toward the acid trap.

Ammonia stripping
Ammonia stripping comprises reducing the content of ammonia in a liquid phase by transferring it in a tower into the gas phase. The method is based on first increasing the pH of liquid manure for converting the ammonium nitrogen contained therein into ammonia (US EPA, 2000; McCabe, 2005). This is followed by driving the liquid manure through a tower filled with a packing material, promoting the volatilization of NH_3 . The liquid manure is fed into the tower at its top end while blowing air into the tower from the bottom, resulting in the desorption of ammonia (US EPA, 2000; McCabe, 2005). The ammonia gas separated into the gas phase is further passed into water or acid, in which the ammonia gas adsorbs into the liquid (Bonmati and Flotats, 2003).

In the stripping column, a large surface area between the liquid and the gas phase is needed in order to maximize the area for ammonia desorption and the rapid transfer of ammonia to the gas phase. This is achieved by filling the stripping column with carriers which promote the flow of a liquid in a thin film, while still allowing a high air flow through the column (Srinath and Loehr, 1974).

Mass transfer of ammonia into the air is also dependent on the difference in the concentration of ammonia in the liquid phase and in the air phase (US EPA, 2000). High air flow rate enables a constant low concentration of ammonia in the air, promoting the transition of ammonia into the air phase (Arogo et al., 1999).

Difficulties related to ammonia air stripping from manures have been related to high manure solids content,that has caused blockage of the tower and requires efficient pretreamtment (Rulkens et al., 1998). Moreover, air stirpping requires high pH (e.g. Bonmati and Flotats, 2003), although due to the high buffering capacity of manure the pH increase is difficult to achieve (Sommer and Husted, 1995).

Biological methods
The N contained in the liquid fraction of manure can also be removed biologically by fixing it into biomass. Of the biological N-removal systems only the algal and duckweed pond systems apply resource recovery, by using the produced algae and duckweed (Mulder, 2003; Craggs et al., 2011). Nitrogen has also been used for the production of fungi, amino acids, bacteria and yeast (Rulkens et al., 1998).

In algal ponds ammonia is assimilated into algal biomass according to the equation:

$$NH_3 + 5CO_2 + 2H_2O \rightarrow C_5H_7O_2N + 5O_2 \qquad (9)$$

The energy use in algal ponds is required for mixing and pumping. The highest value of the N-load has corresponded with the lowest efficiency (Mulder, 2003). In duckweed ponds, 41–68% of the applied N load has been assimilated in duckweed (Alaerts et al., 1996).

1.3.4 Odor treatment technologies
Odor control, targeting in buildings and facilities, in manure storage systems, and in land application, has been carried out using a variety of approaches. The odors emitted from manure storages have been controlled by covering the storage tanks with a sealed lid, floating straw, natural crust etc. (Bicudo et al., 2003). In animal buildings, odor emissions have been reduced by filtering the air coming from the production buildings and facilities using bioscrubbers or bio-filters (Sheridan et al., 2002). Odor emissions have also been controlled by treating slurry itself using aeration (Ndegwa, 2003; Juteau et al., 2004; Park et al., 2005; Zhang and Zhu, 2006), by using anaerobic digestion (Powers et al., 1997) or other biological treatment methods (Ndegwa, 2003), by using chemical or biological additives added to slurry ma-

nure (Ritter, 1989; Zhu et al., 1997; Zhu, 2000) or through diet manipulation (Sutton et al., 1999).

In order to eliminate or reduce odors associated with animal manures, several types of chemical or biological compounds have been used according to Ritter (1981): (1) masking agents that override the offensive odors, (2) counteractants chemically designed to block the sensing of odors, (3) odor absorption chemicals that react with compounds in manure to reduce odor emission, (4) feed additives affecting animal digestion, (5) oxidizing agents such as hydrogen oxide and sodium hypochlorite that chemically oxidize compounds, and (6) biological compounds such as enzymatic or bacterial products that alter the decomposition so that odorous compounds are not generated.

Several studies have demonstrated the limited success of these commercial odor treatment additives (Patni, 1992; Zhu et al., 1997; Zhu, 2000). According to Zhu (2000) this might be due to the complexity of odorous compounds in manure. Ritter (1989) postulated that some additives were only able to eliminate one or two types of odorant and that if these were not the major odorants present, the product did not work. Adding oxidizing agent can increase dissolved oxygen levels within a short time, but the effect is only temporary. In the case of microbial additives, since most indigenous bacterial genera in swine manure are strict anaerobes, they can always outcompete any added aerobes if the manure is maintained in anaerobic conditions (Zhu, 2000).

1.3.5 Treatment methods for inactivation of manure pathogens

Physical methods

Physical treatments for the inactivation of manure pathogens have included irradiation (UV, gamma and electron beam irradiation), heat treatment, drying, and photocatalytic inactivation (Martens and Böhm, 2009). Combination technologies, such as thermal drying, have also been used. The main factors influencing the inactivation of the relevant pathogens are the temperature, the exposure time and the water activity in the material (Böhm, 2008; Table 9). Low water activity increases the heat resistance of microorganisms (Martens and Böhm, 2009).

Chemical methods

Chemical methods used for inactivation of manure pathogens have included disinfection by the addition of chemicals and/or due to pH shift, and/or due to redox potential shift (Block, 2001). Chemical disinfection of liquid manure has been performed with several substances (Table 10). Depending on their chemical nature, the chemical disinfection methods are categorized as alkalis, surfactants, halogens, coal and wood tar derivatives, and miscellaneous disinfectants (Gaskin and Meyerholz). The hygienization result has been depend-

Table 9. Physical pathogen inactivation methods: exposure temperature, time, and disinfection results of treatments. Modified from Martens and Böhm (2009).

Treatment	Temperature (°C)	Exposure time	Result
Pasteurization	70	60 min	Vegetative bacteria, viruses of moderate heat resistance and all infectious parasitic stages will be inactivated
Pasteurization	90	60 min	In addition to the above, some heat sensitive spores will be inactivated
Microwave heating	80	<1s	Inactivation of vegetative bacteria, moderately heat resistant viruses, and parasites.
Thermal drying			No significant inactivation of pathogens

ent e.g. on the presence of organic and solid material in slurry, on the concentration of the used chemical, and on slurry pH (Jones, 1982; Turner and Burton, 1997; Jenkins et al., 1998).

Biological methods

Aerobic and anaerobic treatments carried out in the mesophilic or in the thermophilic temperature range have had an inactivating influence on pathogens (e.g. Martens and Böhm, 2009; Table 11).

Table 10. Chemical pathogen inactivation methods: disinfectant type, mechanisms of action, examples of chemicals used, and further remarks. (Turner and Burton, 1997; McDonnell and Russell, 1999; Block, 2001).

Disinfectant type	**Mechanisms of action**	**Chemical**	**Remarks**
Alkalis	A pH of at least 11.5 causes the inactivation	Lime (calcium oxide or quick lime)	A pH higher than 9 will inhibit most bacteria, and some viruses, but long wet contact times are required
Surfactants	A chemical compound that lowers the surface tension of an aqueous solution, promoting wetting	Soaps, quaternary ammonium compounds	Mild disinfectants, primary value is in aiding the mechanical removal of contaminated organic material. Generally used to disinfect non-porous surfaces. Do not possess substantial viricidal, fungicidal or sporicidal properties and are generally used in final rinses after mechanical cleaning
Alcohols	Cause membrane damage and rapid denaturation of proteins and subsequent cell lysis	Ethyl alcohol (ethanol), isopropyl alcohol (isopropanol), n-propanol	Rapid antimicrobial activity against vegetative bacteria, viruses, and fungi, but are not sporidical
Aldehydes	Strong association with outer layers of bacterial cells, influence inhibitory actions	Glutaraldehyde	Broad spectrum of activity against bacteria and their spores, fungi, and viruses
Halogens/ halogen releasing agents	Highly active oxidizing agents and thereby destroy the cellular activity	Iodine, bromide and chlorine, Sodium Hypochlorite Calcium hypochlorite	Widely used for disinfectant purposes, high concentrations required where organic carbon levels are high
Silver compounds	Interaction with thiol (SH) groups, effects on enzymes, inhibition of growth, and cell division	Silver nitrate, Silver sulfadiazine	
Ozone			Viruses more resistant to ozonation than bacteria; high ozone levels are required when organic carbon levels are high
Peroxygens	Releases free oxygen rapidly/ producing hydroxyl free radicals	Hydrogen peroxide (H_2O_2) Peracetic acid (CH_3COOOH)	Broad spectrum efficacy against viruses, bacteria, yeasts, and bacterial spores
Phenols	General protoplastic toxicity and membrane active properties		Possess antifungal and antiviral properties
Bis-Phenols & Halophenols	Effects on the cytoplasmic membrane	Triclosan, Hexachlorophenole and Chloroxylenol	Bactericidal, but P. aeruginosa and many molds are highly resistant

1.4 Needs for manure treatment and the importance of nutrient recycling

Manure contains considerable amounts of nutrients useable in plant production. In Finland in 2011, the manure P content was estimated to be 17.5 million kg (8.8 kg P/ha arable land), of which 77% originated from cattle and pigs (Ylivainio et al., 2014). Salo et al. (2007) estimated that the amount of nitrogen contained by livestock manure was 42–55 kg/ha in Finland during the period 1990–2005.

At present, the manure is spread on fields situated close to the farms. However, regional concentration of production as well as increase in farm size has led to a situation in which local arable land is not sufficient to receive all the manure produced in the region (e.g. Ylivainio et al., 2014). Manure application is limited either by its nitrogen or phosphorus content. Phosphorus limitation is a result of elevated concentrations of easily soluble phosphorus on farmland (Saarela, 2002) due to the earlier overuse of P.

The use of manure as a crop fertilizer has been limited by its unfavourable N:P-ratio, relatively low nutrient concentrations, and wide variations in the amounts of nutrients and in other properties (Luostarinen et al., 2011). Moreover, part of the manure organic nitrogen in soil is mineralized at a later date, reducing the controllability of manure nitrogen in fertilization (Whalen et al., 2001). The relatively low proportion of soluble nitrogen and the high amount of phosphorus in relation to the needs of plants mean that manure fertilization cannot be optimized in the same way as inorganic fertilizers. Often additional inorganic N fertilizers are needed to supplement manure nutrient supplied for vegetation growth.

The importance of P recycling has been well recognized due to limited P reserves (Gilbert, 2009). The manufacture of N fertilizers is an energy intensive and greenhouse gas emitting process, as nearly 1 m^3 of natural gas is required per kg of anhydrous ammonia (Noble Foundation, 2001). Thus, better manure nutrient recycling involves both economic and environmental aspects. Moreover, manure application has been found to be expensive, as large volumes of slurry with relatively low nutrient content and high water content must be transported for long distances to fields (Kässi et al., 2013). Soil compaction has been found to be a serious risk when

Table 11. Biological treatment methods: exposure temperature and time, and their disinfection results. Modified from Martens and Böhm (2009).

Treatment	Temperature (°C)	Exposure time	Result
Aeration treatment of liquid manure, aerobic thermophilic stabilization (ATS)	50	23 h	Vegetative bacteria, Viruses of moderate heat resistance and infectious parasitic stages will be inactivated
	55	10 h	
	60	4 h	
Composting of solid manure	55	> 2 weeks	
	65	1 week	
Anaerobic treatment at mesophilic temperature range	30–40		Does not lead to reliable inactivation
Anaerobic treatment at thermophilic range	Above 53	Exposure time of at least 20 h	Can be effective for inactivating vegetative bacteria, viruses with moderate resistance and infectious stages of parasites

applying manure using heavy machinery on wet soils (e.g. Arvidsson et al., 2003). In Finland, limited application time during the early spring further complicates this situation as the soils are often wet, and the need to empty the manure storage tanks during late fall cause difficulties if the fall is very wet.

As a conclusion, there is a need to improve the recycling and utilization of manure as a raw material and fertilizer. In particular, the need is for concentration of nutrients, for nutrient recovery technologies, and for volume reduction. Furthermore odor and lack of hygiene are severe problems worldwide (their importance is explained in more detail in section 1.2.2 Environmental impacts related to animal manure) and efficient and cheap treatment technologies are needed. Existing waste water treatment technologies may offer approaches to reach the desired goal. The nutrient recovery and recycling in conventional waste water treatment technologies has, however, been rather weak, in particular considering N and P recycling (e.g. Ashley and Mavinic, 2009). Another aspect is that manure is a very complex material in terms of its nutrient contents, physico-chemical characteristics and its microbiological composition (e.g. Sommer et al., 2013). These specific slurry features should be taken into more detailed consideration when developing new treatment technologies. In fact, development of manure treatment technologies requires a comprehensive approach and fundamental level knowledge on the physico-chemical and biological properties of the manure.

1.5 Theoretical background of the Manure treatment system of the present study

The purpose of this section is to provide a theoretical background for the manure treatment system developed and examined in the present study. First, reactor types are reviewed. The reactor types are relevant to how the physico-chemical properties can be contolled in a system and determine the stability of the system. Physico-chemical conditions determine the operation of the entire system. The basis of the decomposition and humifiication reactions relevant to the biological processes of the presented treatment system and their dependence on the aerobic-anaerobic state of the system are also discussed.

1.5.1 Reactor types

The reactors used for waste water treatment have been commonly categorized based the operation pattern, hydraulic characteristics, unit operation occurring, and entrance/exit conditions (Reynolds and Richards, 1996; Seyrig and Shan, 2007). Common reactor configurations have included plug flow reactors (PFRs), completely mixed batch reactors (CMBRs), and completely mixed flow reactors (CMFRs). In addition, a CMFR has also been referred to as a completely mixed reactor (CMR) or continous stirred tank reactor (CSTR) (e.g. Hayes and Mmbaga, 2013).

CSTR and PFR have probably been the two most widely accepted reactor regimes used for waste water treatment (Reynolds and Richards, 1996). In water treatment, a typical reactor is a long, narrow channel, long pipe or tubular, or a series of long channels, because it typically cinvolves exposure to the disinfectant of interest for a specific duration of time (Reynolds and Richards, 1996). The main characteristic of a plug flow reactor (PFR) is that no mixing occurs in the direction of flow; however, complete mixing is assumed within the cross-sectional area of the reactor. Water and all suspended flocs of bacteria move with the same velocity along the tube reactor (Seyrig and Shan, 2007).

CSTR runs at steady state with a continuous flow of reactants and products (Seyrig and Shan, 2007). The feed assumes a uniform composition throughout the reactor; the exit stream has the same composition as in the tank. In other words, some of the elements entering the CSTR leave it immediately, because product stream is being continuously withdrawn from the reactor. On the other hand, others may remain in the reactor indefinitely, because all the material is never removed from the reactor at one time (Seyrig and Shan, 2007). In contrast to the CSTR, the PFR exhibits a continuous decrease in substrate concentration and an increase in bacterial concentration in the direction of flow (Seyrig and Shan, 2007; Hayes and Mmbaga, 2013). Hydraulic performance of a CSTR reactor has been improved by increasing the number of CSTRs in series (Reynolds and Richards, 1996). CSTRs in series have commonly been used in the biological treatment of industrial wastewater (Abu-Reesh, 2010).

1.5.2 Degradation and recondensation processes involved in biological treatment

Decompositon processes

During decomposition processes, microorganisms degrade the raw material to synthesize new cellular material and to obtain the energy for these catabolic processes. Several chemical transformations take place as complex compounds are broken down into simpler ones and then synthesized into new complex compounds (Fig. 3; Tölgyessy, 1993; Rajeshwari and Balakrishnan, 2009). Before the microorganisms can synthesize new cellular material, they require sufficient energy for these processes. The two possible modes of energy-yielding metabolism for heterotrophic microorganisms are respiration and fermentation (Jurtshuk, 1996; Madigan, et al., 2003). Respiration can be either aerobic or anaerobic (Jurtshuk, 1996; Madigan, et al., 2003; Black, 2012).

Aerobic respiration, involving oxidation-reduction reactions and with molecular oxygen as the final electron acceptor, is more efficient, generates more energy, operates at higher temperatures, and does not produce the same quantity of odorous compounds as anaerobic respiration (USDA/NRCS, 2000; Jurtshuk, 1996). Aerobes have been found to be able to utilize a greater variety of organic compounds as sources of energy, resulting in more complete degradation and stabilization of the material (Jurtshuk, 1996).

In anaerobic respiration, the microorganisms use electron acceptors other than O_2, such as inorganic nitrates (NO_3^-), sulfates (SO_4^{2-}), and carbonates (CO_3^{2-}), to obtain energy (Jurtshuk, 1996). Their use of these alternative electron acceptors in the energy-yielding metabolism has been found to produce odorous or undesirable compounds, such as hydrogen sulfide (H_2S) and methane (CH_4) (Jurtshuk, 1996; Black, 2012).

Anaerobic respiration leads to the formation of organic acid intermediates that tend to accumulate and are detrimental to aerobic microorganisms (Jurtshuk, 1996; Madigan, et al., 2003). Aerobic respiration also forms organic acid intermediates, but these intermediates are readily consumed by subsequent reactions so that they do not represent as significant a potential for odors as in anaerobic respiration (USDA/NRCS, 2000; Madigan, et al., 2003).

Fermentation is an anaerobic catabolic process, and does not require oxygen. During fermentation, energy is released from an organic compound and the final electron recipient is also an organic compound, such as lactic acid or alcohol (Madigan, et al., 2003). The fermentation metabolic pathway only uses glycolysis and produces two molecules of ATP (Madigan, et al., 2003).

Humification

Humification is a partial form of microbial decomposition, which, however, may also involve condensation and polymerization of decay products (Tölgyessy, 1993).

To a considerable extent this is an oxidative process, resulting in the formation of high-molecular-mass nitrogen compounds with a remarkable abundance of aromatic components. The humification process in soil is generally very slow, taking years to complete (Tölgyessy, 1993). In compost environments with controlled conditions, humification has been clearly faster (weeks) (e.g. Diaz et al., 2011). According to Ndewga et al. (2007a), stabilization has been reached within days during slurry aeration.

Different theories exist concerning humus formation (Stevenson, 1994). The essential part of the macromolecule is a ring structure imparted by aromatic compounds. Various side chains and functional groups are connected to the ring. The rings are interconnected by side chains and functional groups or simple bonds, so that they can polymerize to form larger molecules. The rings are formed in the humification processes from lignin components. These reactions are catalyzed mainly by microbial enzymes, and as a result very stable polymers with complex structures are obtained that can be grouped into three different types: humic acids, fulvic acids and humin (Stevenson, 1994). Humic substances containing carboxyl and phenolate groups can form complexes with ions in a solution (Hayes and Swift, 1978).

Rate of decomposition

Decomposability of different materials varies. This has an influence on e.g. required treatment time. Due to the complexity of slurry manure as a raw material, it contains materials with different levels of degradability. This complicates the definition of the required treatment time. Sugars, water-soluble nitrogenous compounds, amino acids and simple proteins, lipids, starches and some of the hemicelluloses are decomposed first at a rapid rate, whereas insoluble compounds such as cellulose, some hemicelluloses and more complex (crude) proteins are decomposed less rap-

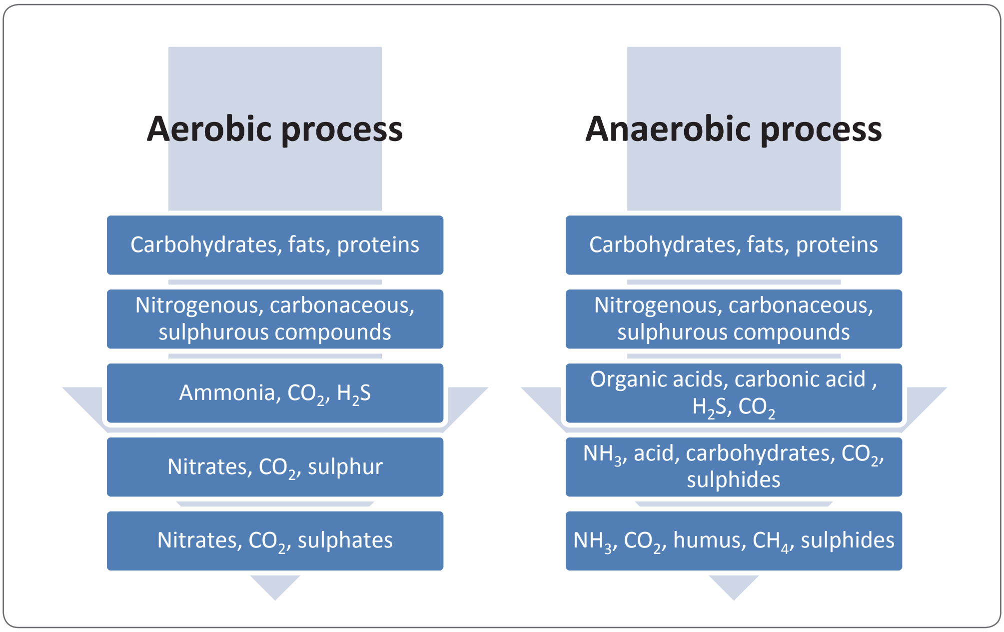


Figure 3. Decomposition of organic compounds in aerobic and anaerobic cycles (modified from Rajeshwari and Balakrishnan, 2009). This conversion is achieved through a series of reactions. These reactions serve not only to liberate significant quantities of energy, but also to form a large number of organic intermediates that serve as starting points for other synthetic reactions. Several chemical transformations take place as complex compounds are broken down into simpler ones and then synthesized into new complex compounds.

idly, and lignin, waxes and resins are decomposed only very slowly (e.g. McDonald et al., 2011).

Acceleration of biodegradation by seeding

The use of seeding is a common procedure e.g. with anaerobic digestion and activated sludge during the start up-phase. Anaerobic biodegradation of substrates requires microbial consortia with hydrolytic and methanogenic activities (Gerardi, 2003a). Populations of anaerobic microorganisms typically require a significant period of time to establish themselves to be fully effective, and so a common practice has involved seeding with an adequate population of both the acid-forming and methanogenic bacteria (Gerardi, 2003a). Long start-up times have also been reported for wastewater treatment processes (Pijuan et al., 2011). According to Pijuan et al. (2011), one of the main challenging issues for the aerobic granular sludge technology is the long start-up time of the biological process when dealing with real wastewaters. Typically, the formation of aerobic granules with nutrient removal capabilities has taken several months (Pijuan et al., 2011). Regarding composting studies, good results have been achieved by mixing the starting material with mature compost in order to supply microbes capable of degrading the lignocellulosic materials and to shorten the composting period or increase the quality of compost. For example, Wang et al. (2011) reported that lignocellulose degradation preceded 57.7% faster in compost inoculated with a lignocellolytic fungus.

1.5.3 Physico-chemical factors involved in treatment processes

In the present section the entire system controlling physico-chemical factors is discussed. These factors are relevant when the system functioning is evaluated and adjusted.

OPR and the sequence of redox reactions

Oxidation-reduction potential (ORP) and dissolved oxygen (DO) have been widely used in the control and monitoring of aeration processes (e.g. Ndegwa, 2007b; Zhu et al., 2005, 2008). An oxidation-reduction process is basically achieved by electron(s) transfer from the substance being oxidized (referred to as reductant) to the substance being reduced (referred to as oxidant). Numerous biological processes such as degradation of organic matter are essentially oxidation-reduction processes (Fig. 4, Goronszy et al., 1992).

ORP ranges required for nitrification and denitrification are typically +50 to about +225 mV and indicate the presence of dissolved oxygen (O_2) (Gerardi, 2003b; WEF, 2007). An ORP reading of +225 to +400 mV indicates the presence of oxygen and nitrate (NO_3^-) (WEF, 2007). ORP readings in the range of +50 to –50 mV indicate that no free available dissolved oxygen is present and nitrate is present as an electron acceptor (Gerardi, 2003b). There should be no free DO present in this zone so a DO meter would read zero mg/L. ORP readings less than –50 mV indicate that there is no free oxygen or nitrate present, and the microorganisms would be utilizing sulfate ($SO^{2-}{}_4$) as an electron acceptor for their energy requirements (Theodore and Gong, 2013).

Ammonia equilibria

Below are shown factors that affect ammonia volatilization from a system. Ammonia volatilization takes place at the liquid/gas interface. The volatilization tendency depends on the equilibrium constants (speed of reaction, assuming equilibrium). Ammonia nitrogen exists in aqueous solution as either ammonium ion or ammonia, according to the following equilibrium reaction:

$$NH_4^+ \leftrightarrow NH_3\ (aq) + H^+ \qquad (10)$$

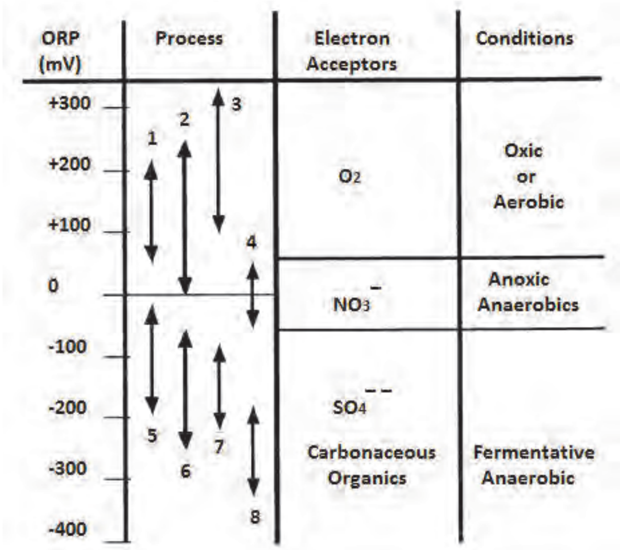


Figure 4. Oxidation-reduction potential (ORP) and metabolic process readings for organic carbon oxidation (process number 1), polyphosphate development (2), nitrification (3), denitrification (4), polyphosphate breakdown (5), sulfide formation (6), acid formation (7), and methane formation (8) (modified from Goronszy et al., 1992).

The equilibrium concentrations of NH_4^+ and NH_3 are pH and temperature (T) dependent (Fig. 5), as given by Emerson et al. (1975) (II; equation 11) and Lide (1993) (II; equation 12):

$$[NH_3] = \frac{[NH_3 + NH_4^+]}{1 + \frac{[H^+]}{Ka}} = \frac{[NH_3 + NH_4^+]}{1 + 10^{pKa-pH}} \qquad (11)$$

$$pKa = 4 \times 10^{-8}T^3 + 9 \times 10^{-5}T^2 - 0.0356T + 10.072 \qquad (12)$$

where $[NH_3]$ is the free-ammonia concentration, $[NH_3 + NH_4^+]$ is the total ammoniacal nitrogen (TAN) concentration, $[H^+]$ is hydrogen ion concentration, and Ka is the acid ionization constant for NH_3. The acid dissociation constant (pKa) value for NH_4^+/NH_3 in aqueous solution (25 °C) is 9.25 (Lide, 1993).

Carbonate equilibria

Carbon dioxide concentration at the interface determines its volatilization tendency. Carbon dioxide (CO_2) dissolves in water, producing carbonic acid (H_2CO_3). Equilibrium is established between the dissolved CO_2 and H_2CO_3 (Poling et al., 2001):

$$CO_2\,(l) + H_2O\,(l) \leftrightarrow H_2CO_3\,(l) \qquad (13)$$

This reaction is kinetically slow. At equilibrium, only a small fraction (ca. 0.2–1%) of the dissolved CO_2 is actually converted to H_2CO_3. Most of the CO_2 remains as solvated molecular CO_2.

Carbonic acid is a weak acid that dissociates in two steps (Lide, 1993):

$$H_2CO_3 + H_2O \leftrightarrow H_3O^+ + HCO_3^- \; pKa1(25\ °C) = 6.37 \qquad (14)$$

$$HCO_3^- + H_2O \leftrightarrow H_3O^+ + CO_3^{2-} \; pK_{a2}(25\ °C) = 10.25 \qquad (15)$$

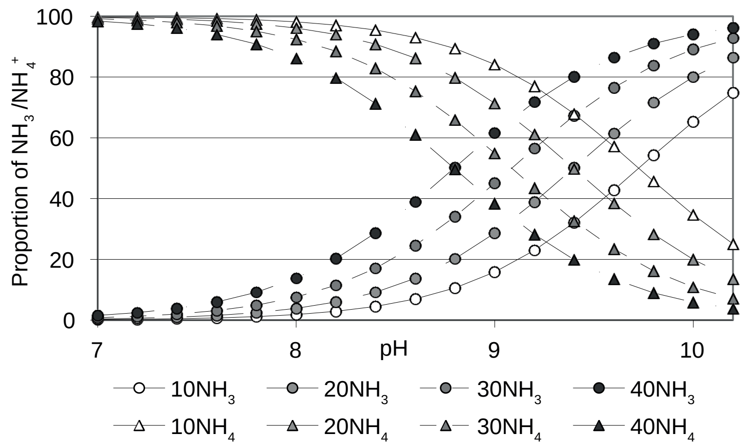


Figure 5. Proportion of NH_3/NH_4^+ ions in a solution at different pH values and temperatures (10, 20, 20 and 40 ˚C). Calculated using equatations 11 and 12.

The equilibrium between H_2CO_3 and bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions is pH- and temperature-dependent. Using carbonate mass balance and acid equilibrium expessions, the fractional amounts of all carbonate species can be found as a function of [H^+] (Poling et al., 2001; Lide, 1993):

$$[H_2CO_3] = \frac{[H^+]^2}{[H^+]^2+[H^+]Ka1+Ka1Ka2} \quad (16)$$

$$[HCO_3^-] = \frac{[H^+]Ka1}{[H^+]^2+[H^+]Ka1+Ka1Ka2} \quad (17)$$

$$[CO_3^{2-}] = \frac{Ka1Ka2}{[H^+]^2+[H^+]Ka1+Ka1Ka2} \quad (18)$$

where K_{a1} is $4.3*10^{-7}$and K_{a2} is $5.6*10^{-11}$

Figure 6 summarizes the carbonate system. The form of carbonate in the solution is very dependent on pH (Chang, 2000). At a low pH, the carbonate is present as carbonic acid, and it can evaporate. However, at high pH carbonate is present as carbonate anions and can interact with the cations present in the solution to form soluble and insoluble carbonates (Brown et al., 2011). For example, if Ca^{2+} is present, limestone ($CaCO_3$) is formed, if Mg^{2+} is present, $MgCO_3$ is formed and if NH_4^+ is present, NH_4HCO_3 is formed. The formation of the crystals depends on the relative amounts of ions in solution and on solubility product constants. Since ammonia and inorganic carbon ions are present at high concentrations in slurry manure, NH_4^+ and HCO_3^- are the predominant ions. Moreover, crystal formation and solubility is also pH dependent.

Henry's law

Volatilization is dependent on NH_3 or CO_2 concentration at the interface according to Henry's law. Henry`s law states that at constant temperature the partial pressure of a given gas in air is directly proportional to the concentration of that gas in liquid, when the dissolved gas is in equilibrium with gas in the air (Chang, 2000).

$$p_{gas}=hC \quad (19)$$

where

- p_{gas} is the partial pressure of the gas (often in units of atm)

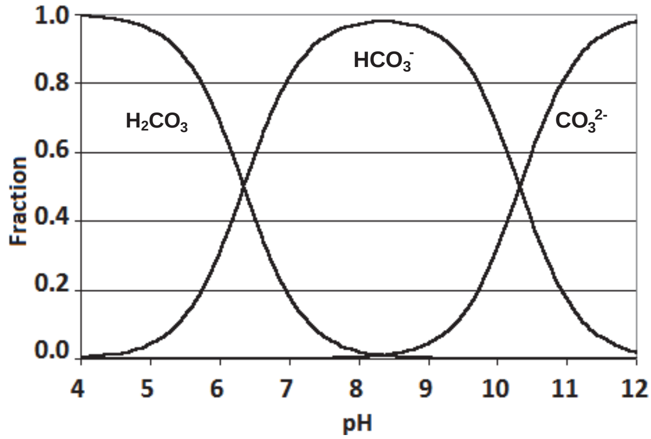


Figure 6. Proportion of carbonic acid/bicarbonate ion/carbonate ion (H_2CO_3 / HCO_3^- / CO_3^{2-}) in a solution at different pH values at 25°C. Modified from Chang (2000).

- C is the concentration of a gas at a fixed temperature in a particular solvent (in units of M or mL gas/L)
- h is Henry's law constant (often in units of atm/M)

The Henry's law constant (h) depends on temperature (Chang, 2000). It increases with increasing temperature. Therefore, the higher the temperature, the higher the numerical value of the Henry's Law constant and the lower the solubility of the compound in water, and the easier the compound is volatilized (stripped).

The solubility constant value is equal to the inverse Henry's constant value (Chang, 2000). Accordingly, the solubility of a gas in a liquid depends on the partial pressure of the gas in the air at the interface. The numerical value of Henry's Law constant increases with increasing temperature, and thus the solubility of the gas in a liquid decreases with increasing temperature (Fig. 7).

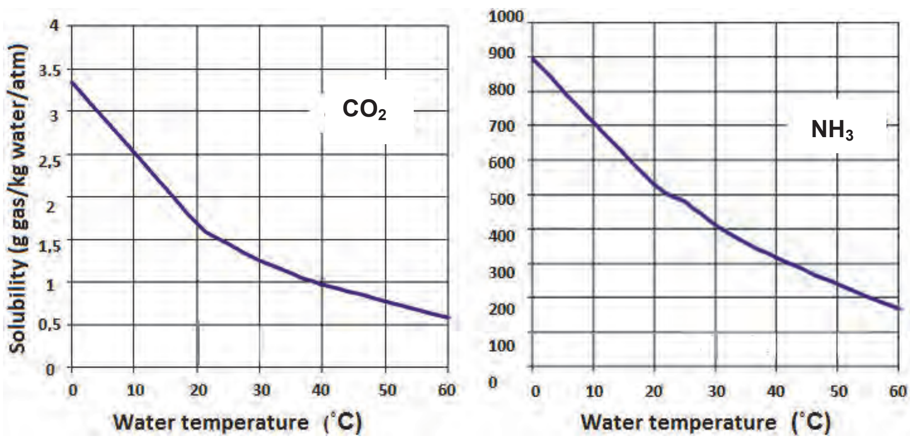


Figure 7. Solubility of carbon dioxide (CO_2) and ammonia (NH_3) in water at one atmosphere (101.3 kPa) in different temparatures. Modified from the engineering toolbox (www.EngineeringToolBox.com).

2 Objectives of the study

In the present study, a new slurry manure treatment scheme was proposed and examined. The treatment scheme comprised biological treatment of manure with decreased contents of solids and P in a specially designed reactor regime followed by ammonia separation by ammonia stripping.

The general aim of the study was to investigate the feasibility of slurry manure treatment conducted in the designed reactor system. One research question was: how does moderate or only slight aeration affect slurry manure properties when conducted in a series of continuously fed aerated tank reactors? Limited aeration was applied in order to maintain N in ammonia form, thus preventing nitrate formation, avoiding abundant CO_2 formation and favoring humification processes. Both swine and dairy slurry manure were studied. Further, swine slurry manure was treated by ammonia stripping after biological treatment in order to separate N. It was studied whether biological treatment alone was sufficient to increase slurry manure effluent pH to a level enabling N separation by ammonia stripping without the use of chemicals. The theoretical basis of each process step was also discussed.

Focusing on the biological treatment process, the more specific objectives and hypotheses of the study were to:

1. Examine the operational conditions, dynamics and stability of the serial treatment system, and to study process efficiency of the system (III).
2. Investigate whether it is possible to efficiently increase slurry manure pH without turning ammonium to nitrate with aeration treatment or losing it in gaseous form, or losing a substantial fraction of the carbon during biological treatment (I, III, Supplemental data).
3. Investigate whether the slurry-precipitating characteristics can be strengthened and how the system influences odor (I).

The hypotheses were: the hydraulic retention time of the serial system is short (days) and the system is stable; the limited aeration increases the pH of slurry manure but nitrate formation is still limited; and the biological treatment reduces the odor of slurry manure.

Focusing on the hygienic state of the process and changes in microbial community composition during the manure slurry processing, the more specific objectives and hypotheses of the study were to:

1. Evaluate the used seeding preparation and seeding during the start-up phase.
2. Study the hygienic state of the process by measuring viable counts of bacteria (IV, Supplemantal data).
3. Study the changes in microbial community composition and to compare the community composition of treated slurry with that of untreated slurry manure and of the soil used as the origin of the inoculant (IV).

The hypotheses were: the use of seeding and feedback of treated effluent enhances the functioning of the system; the biological treatment improves the hygienic state of the slurry manure; and the microbial community of treated slurry manure is different from that of untreated manure.

Focusing on ammonia separation by ammonia stripping, the more specific objectives and hypotheses of the study were to:

1. Investigate feasibility of the sequential stripping procedure: (i) increasing the swine slurry manure pH without use of chemicals and with part of the slurry buffer capacity removed, and (ii) increasing the slurry pH with chemical treatments in between the stripping cycles (II).

2. Investigate changes in swine slurry manure buffer properties during stripping (II).

3. Evaluate ammonia (NH_3) stripping of the biologically treated dairy slurry manure and the effect of magnesium oxide (MgO) treatment on stripping performance (III).

The hypotheses were: the amount of chemicals can be reduced by combining the biological and chemical methods to increase the pH; ammonia removal by stipping lowers the buffer capacity of slurry manure; and the ammonia stripping is one option to produce mineral-form N fertilizer from slurry manure.

Focusing on overall applicability of the designed manure treating technology and on the achieved environmental benefits, the more specific objective of the study was to:

1. Evaluate different process stages from the physicochemical and agricultural end-use viewpoints (I, II, III, IV).

The hypothesis was that the developed slurry manure treatment system can separate P and N from the manure and that the nutrients are in plant avaibale form, and the system clearly reduces the harmful odor of slurry manure.

3 Materials and methods

The slurry manure characteristics and treatment systems used in this work are described in detail in articles I-IV. Moreover, supplementary material is presented on swine manure treatment (Supplemental data). The supplementary data presents the results of one study which was conducted with swine slurry manure using biological aeration treatment equipment including eight tank reactors in seriers. The performance and hygiene aspects of the biological process are examined.

3.1 Properties of raw slurries

Swine slurry manure was taken from the collecting pit of a growing-finishing farm (I, II). Raw dairy slurry manure was obtained from the MTT (MTT Agrifood Research Finland) dairy farm (III, IV). The characteristics of raw swine and dairy slurry manures are presented in Table 12.

3.2 Technical description of the treatment system and treatment processes

The designed slurry manure treatment scheme consisted of a biological aeration treatment process conducted in a specially designed reactor regime followed by ammonia separation employing ammonia stripping (Fig. 8, I and II). Before the aeration treatment, manure dry matter and P content were decreased by separation (A in Fig 8; I and III). The initial stage of the treatment was a biological process, in which the system was filled with a microbial inoculum produced from soil-water suspension (B in Fig. 8, I:3.1). During biological treatment, slurry manure was continuously fed to the serial treatment system and maintained under limited aeration to keep N in ammonia form, thus preventing nitrate formation, avoiding abundant CO_2 formation, and instead favoring humification processes. Feedback effluent from the last tank was used to inoculate the first tank (Fig. 9). Feedback also functioned as a buffer mechanism against process interferences (III). Increased pH facilitated the subsequent N separation with ammonia stripping when the treatment was continued with N separation (C in Fig. 8; III).

3.2.1 Pre-treatment processes

Prior to aeration treatment, part of the slurry manure solid content was removed by sedimentation or by mechanical means using separation (step 1 in Fig. 9). Swine slurry manure was separated by natural unforced sedimentation in an intermediate tank before transferring the liquid phase to a serial treatment system (I, II, Supplemental data). Dairy slurry manure was mechanically separated (Bauer separator s650) into liquid and solid phases (III, IV).

Table 12. Mean dry matter (DM) and chemical composition of raw slurry manures before sedimentation or mechanical separation (I:Table 1, III:Table 2). TOC, total organic carbon; N_{tot}, total nitrogen; NH_4^+-N, ammonium nitrogen; P_{tot}, total phosphorus.

Manure type	DM	TOC	N_{tot}	NH_4^+-N	P_{tot}	K	Mg	Ca	Na
	(%)			(mg/kg (fresh weight))					
Swine slurry(I)	1.85	3930	2255	1649	451	1188	203	635	668
Dairy slurry(III)	4.40		2190	1240	400				

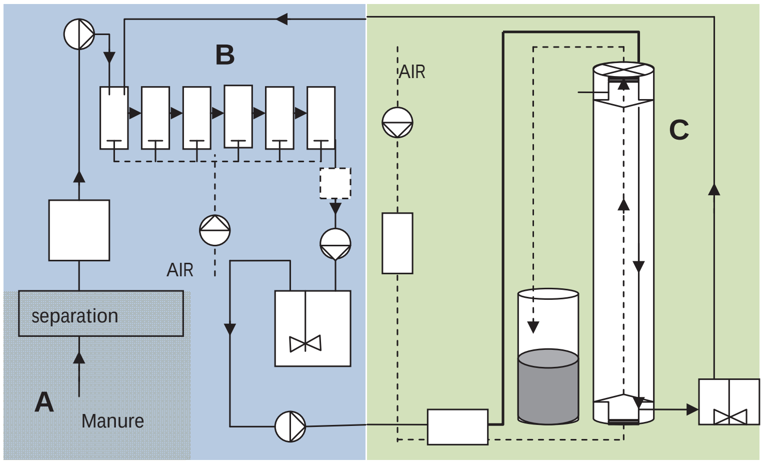


Figure 8. Schematic representation of the designed manure treatment sheme including solid separation step (A), biological aeration treatment equipment (B) and ammonia stripping equipment (C). For a more detailed technical description of A and B see Figure 9 and for C see publication II.

3.2.2 Serial treatment system

Biological aeration treatment

Both swine and dairy slurry manure aeration was conducted in pilot scale in a series of continuously fed aerated tank reactors consisting of six 600 l treatment tanks (hydraulic volume 2.96 m^3 in I and II; 2.40 m^3 in III and IV) connected in series by polyvinyl tubing (65 mm in inner diameter) (I; step 2 in Fig. 9). Before start-up the treatment reactors were filled with seeding material (an inoculant) and the slurry manure treatment was initiated with small amounts of slurry fed into the system (20–50 l/day). The feed-in flow in each tank was to the bottom of the tank and the outflow was through the crosswise upper corner in relation to the input flow. The surface effluent level remained constant when slurry manure ran gravitationally from one tank to another (I; Fig. 9). Recirculation (feedback) of effluent slurry from the last tank to the first tank was also introduced (step 3 in Fig. 9).

One study was conducted with swine slurry manure using biological aeration treat-

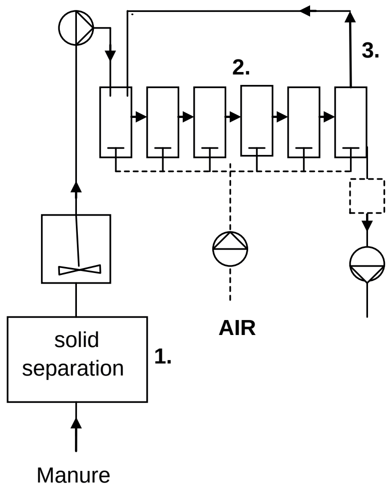


Figure 9. Components of the biological aeration treatment equipment. Prior to feeding, solids separation is performed (1.). Aeration equipment consists of six aerated tank reactors (á 600 l) connected in series (2.). Part of the effluent slurry is feedback from the last tank to the first tank of the serial system (3.) (I).

ment equipment including eight tank reactors in series. The results of this study are present as supplementary data.

In the first study (I), the aeration equipment was the most primitive. Perforated tubes were used for aeration and mixing, air pressure was produced by a compressor and a pressure meter controlled the feed-in air pressure to each tank, which was adjusted by a pressure regulator. In the other studies (II, III, IV and supplemental data), aeration was performed using membrane diffusers and air flow was produced by a high pressure blower and adjusted by manual rotameters. The treatment tanks were equipped with mechanical foam breakers, and gases released during processing were collected separately from each tank via an outlet duct.

Dairy manure treatment differed from swine slurry manure treatment systems in that feedback was NH_3 -stripped solution after biological treatment (Fig. 8, III). Thus, in the dairy manure treatment system, slurry was transferred from the sixth process tank by gravitation to an additional overflow container, from which it was transferred with a submersible pump to a 1 m^3 plastic container (Fig. 8). From this container the slurry was then transferred with a hose pump into a stripping tower. After stripping, effluent was transferred to a container from which a part was continuously fed into the first treatment tank (feedback) (Fig 8; III: Fig. 1).

Ammonia stripping

The stripping tower was constructed using polyethylene plastic tubing (3.2 m height × 0.4 m internal diameter) (Fig. 8; II: Fig.1). The tower was randomly packed with 1.5 m^3 of plastic rings, giving a specific area of 126 m^2 of the filling material. Stripping was conducted with continuous forced air flow from the bottom of the tower. Slurry manure was continuously pumped into the top of the tower and distributed there evenly (II). A circulating warm water system was used to warm up the incoming liquid and air. The tower was insulated to maintain the desired temperature. The continuous effluent exiting the tower was collected in a closed container. Air containing NH_3 was collected in water in a wet washer (II). Air stripping experiments were performed with a liquid flow rate of 20.83 L h^{-1} and an air flow rate of 20.83 x 10^3 L h^{-1}. Thus, the liquid/air ratio was adjusted to 1:1000 (II, III).

3.3 Experimental designs

The functioning and feasibility of the proposed slurry manure treatment system was examined by carrying out controlled experiments (I, II, III, supplementary data) and by analyzing the microbial community composition (IV, supplementary data). Experimental designs of each study are presented in Table 13 and the reseach focus in each study in Table 14. Biological treatment experiments consisted of study periods of variable lengths during which the treatment system was loaded with variable feeding rates (I, III). Air stripping experiments included pretests using a pure ammonium reference solution with different concentrations, with or without warming the feed-in liquid and air, in order to test the general function of the stripping tower and optimal treatment parameters (II). Thereafter stripping experiments were continued with slurry manure (II, III).

3.4 Analytics

Methods used in the thesis are summarized in Table 15. Detailed descriptions of methods can be found in the original publications I-IV.

3.4.1 Chemical methods and buffer capacity

The measurements of process conditions and control (Table 15) were carried out daily at a depth of approximately 10 cm. Of the nutrients the concentrations of K,

Table 13. Experimental designs and parameters for the biological treatment in each study (I–IV, supplementary material).

Manure	Pre-treatment	Study period	HRT (days)	Loading (L/d)	Feedback ratio	Aeration intensity	Studied parameters	Article
Swine	sedimentation	1*7 days 1*21 days	4. 3	734 & 978	0.8±0.2:1	0.7–7.2 mg/L DO	DO, pH, TS, VS, COD,VFA, odor, color	I
Swine	sedimentation	72 days	6.3	506	1.0	1.1–7.2 mg/L DO -216–(+)279 mV	DO, ORP, pH, TS, TOC, hygiene	Suppl. material
Swine	sedimentation		9	330	1.0		ORP, pH, TS, TOC,VFAs	II
Dairy	mechanical separation	4*30 days	6.5–96	50 to 350	1.5–1.6	-172–(+)308 mV	ORP, pH, TS, TOC	III
Dairy	mechanical separation	4*30 days	43, 44, 48 & 6.7	50 to 350	1.5–1.6	-172–(+)308 mV	ORP, pH, TS, VS hygiene,16SrRNA	IV

HRT, hydraulic retention time; DO, dissolved oxygen; TS, total solids; VS, volatile solids; COD, chemical oxygen demand; VFA, volatile fatty acids; ORP, oxidation reduction potential; TOC, total organic carbon

Table 14. Research focus in each study (I–IV).

Manure	Study focus	Studied parameters	Article
Swine	Biological treatment performance	Fate of pH, carbon (TC, IC, TOC) and nutrients (N_{tot}, NH_4^+, NO_3^-, K^+, Mg^{2+}, Ca^{2+}, Na^+) during biological treatment	I
Swine	Humification	Influence of biological aeration treatment on slurry color and precipitating characteristics	I
	Odor	Fate of VFAs and odor intensity during biological treatment	I
Swine	Hygiene	Changes in enteric indicator organisms in slurry during biological treatment	Supplem. material
Swine	Ammonia stripping and biological pH increase	Evaluate the ability to remove ammonia from biologically treated slurry manure without chemical pH increase	II
	Sequence stripping	Evaluate the repeated cycles of stripping on NH_3 removal	II
Swine	Buffer capacity	Evaluate changes in buffer properties during stripping	II
Dairy	Process dynamics and treatment stability, loading increase from 50 to 350 L d_{-1}, fixed aeration, varying TS load	Effect of loading on oxidation–reduction potential (ORP), total solids content (TS) and pH	III
Dairy	Treatment efficiency	Fate of carbon (TC, IC, TOC) and nutrients (N_{tot}, NH_4^+, NO_3^-, K^+, Mg^{2+}, Ca^{2+}, Na^+) during biological treatment	III
Dairy	Stripping performance	Stripping performance and the effect of MgO treatment on stripping effectiveness	III
Dairy	Hygiene	Changes in enteric indicator organisms in slurry during biological treatment	IV
Dairy	Microbial community composition	Changes studied by 16S rRNA gene sequence analysis in different treatment tanks of the system and comparison to the community composition of untreated slurry and soil (the origin of the used inoculant)	IV

TC, total carbon; IC, inorganic carbon; TOC, total organic carbon; N_{tot}, total nitrogen; VFA, volatile fatty acids; MgO, magnesium oxide

Mg, Ca, and Na ions were analyzed with an atomic absorption spectrophotometer. P_{tot} and N_{tot}, after oxidation with peroxodisulfate and NH_4^+ -N, NO_3^- -N, were analysed colorimetrically using a QuikChem Auto Analyzer (I–IV, supplementary data).

Buffering curves (Table 15) were obtained by titrating the treated swine slurries against 1.0 mol L^{-1} HCl and 1.0 mol L^{-1} NaOH. To evaluate pH responses to increasing rates of base added, curves of the amount of base added vs. pH were plotted (II). Another buffer experiment was performed with corresponding congruent samples after carbonate exclusion. Carbonates were removed by acidifying the samples to pH 4 (II).

3.4.2 Microbiological methods

Selection and enrichment of initial microbial populations

At the installation stage, the tanks were filled with effluent (sub-cultured inoculant). The procedure of sub-culturing is described in article I.

Enumeration of enteric indicator organisms in slurry samples

The hygienic state of the process was monitored by measuring viable counts of bacteria, including total counts and counts of enteric indicator microorganisms including fecal coliforms, *E. coli*/coliforms, fecal streptococci and sulfite-reducing clostridia (IV, supplementary data).

Nucleic acid methods

Nucleic acid methods included DNA extraction, PCR amplification of bacterial 16S rRNA, plasmid isolation and sequencing and analyzing of the sequence data (IV).

3.4.3 Data analysis and statistics

Statistical evaluation of the influence of different treatment parameters on slurry chemical composition (e.g. solids content, pH, nutrient contents, enteric counts) during biological treatment and the sequential stripping procedure were determined using the MIXED procedure of SAS (Littell et al., 1996) (I,II,III, IV, supplementary data). Pairwise means comparisons were performed using the mixed default statement (t-test). Linear regression analysis of SAS was used to evaluate the influence of tank (of a serial system consisting of six treatment tanks in series) on odor, TS, VS, TOC, COD, VFA nutrient contents and enteric counts (I, IV, Supplemental data).

Table 15. The methods used in this study. Symbols as in Tables 13 and 14.

Method	Described and used in
Process monitoring, process control	
pH	I, II, III, IV
Dissolved oxygen (DO)	I
Redox potential (ORP)	II, III, IV
Chemical methods	
Solids (TS, VS)	I, II, III, IV (VS I)
Carbon (TC, IC, TOC)	I, II, III
Chemical oxygen demand (COD)	I
Volatile fatty acids (VFA total)	I, II
Individual VFAs	II
Odor intensity	I
Color change	I
Buffer capacity	
Titration curve	II
Nutrients	
N_{tot}, NH_4^+, P_{tot}, PO_4^{3-},	I, II, III, IV
Ca^{2+}, Mg^{2+}, K^+ ,Na^+	
Microbiological methods	
Hygienic indicator microbes	IV
Total bacterial counts	IV
DNA extraction	IV

4 Results

4.1 Effects of pre-treatment on manure composition

Swine and dairy slurry manure had inherently different dry matter contents, and further differences in physicochemical composition led to differences in process feed contents between these manure types after pre-treatment by partial solids separation (Table 16; I and III). Swine slurry manure solids were removed using natural settling. TS removal efficiency reached 70% after settling (I). At the best, more than 80% of the swine manure total P and nearly 90% of the phosphate phosphorus was removed before biological treatment (I).

Dairy slurry manure was separated with a screw press. TS removal efficiency was 33% after separation (III). However, less than 5% of N_{tot} and only 12.5% of total phosphorus was removed with mechanical separation (Tables 12 and 16). In the case of dairy slurry slight sedimentation was observed after mechanical separation (III).

4.2 Biological treatment

4.2.1 Operational conditions, dynamics and stability of the serial treatment system

All the experimental runs were conducted at room temperature. The temperature in each treatment reactor was 15–24°C and no significant temperature increase due to aeration was observed between the sequential tanks (I, III).

To measure the oxidative state of the aerated tanks, DO and/or Redox-values were measured in each treatment tank. The DO content of swine slurry manure varied from 0.6 to 7.2 mg/L (six tanks; I: Table 2) and from 1.1 to 7.2 mg/L (eight tanks; Fig. 10, supplementary data). ORP levels ranged between -200 and +300 mV in the treatment tanks (Fig. 10, supplementary data; III: Fig. 2).

Pattern of DO and ORP in a serial system

The changes in ORP and DO with time in different treatment reactors (swine slurry manure Fig. 10, supplementary data; dairy slurry manure III: Fig. 2) were used to control and monitor the aeration processes. It was found, as in other studies (e.g. Ndegwa et al., 2007b), that ORP was a better indicator than DO to monitor the process. With respect to ORP values, a certain pattern was easily detected: in general the lowest value was detected in raw slurry, which then increased from one tank to the next, reaching its highest level in the last tank (III: Fig. 2). The observed DO values were erratic (Fig. 10; I: Table 2). Raw slurry was clearly distinguishable from the aerated tanks, but differences between the tanks or the relative order of the tanks were difficult to determine solely on the basis of the obtained DO-values.

Defining optimal operation parameters based on ORP-values

The ORP-values provided a useful tool to monitor and evaluate the process state. ORP changes were observed with a delay in subsequent tanks of the treatment system in the case of both increase and decrease in ORP (III: Fig. 2). A systematic drop in redox value from one tank to the next was an indicator of process failure. The feed TS content appeared to contribute to a rapid decrease in ORP values in the first and thereafter in the subsequent tanks as a result of increasing supply (III: Figs. 2 and 3).

Table 16. Chemical composition of the slurry manure (± standard deviation (SD)) after solids separation pre-treatment. Raw manure properties are presented in Table 12 and symbols in Tables 13 and 14.

	DM	pH	NH_4^+-N	N_{tot}	PO_4^{3-}	P_{tot}	TC	IC	TOC	K^+	Ca^{2+}	Mg^{2+}	Na^+
Paper	(%)							(mg/L)					
Swine slurry													
I	0.55±0.25		1367±4	1509±22	21±6	82±4	2626±82	1543±94	1083±165	964±20	160±11	49±4	361±6
II	1.01±0.62	7.9±0.25	1494±180	1816±372	50±16	252±403	2774±171	1302±10^2	1471±78	952±196	204±79	82±182	284±53
Dairy slurry													
III	2.94±0.01		1220±10	2190±20		350±10							
III (III*)	2.42	7.6	1296±142	2169±200	155	310±53	$110^2$3	1701	9322±640	2801	819	353	516
III (IV*)	2.16±0.51	7.9	859±71	1590±344	142	230±111	6496	1151	5335±716	442	218	2108	197

*Study periods III and IV in article III.

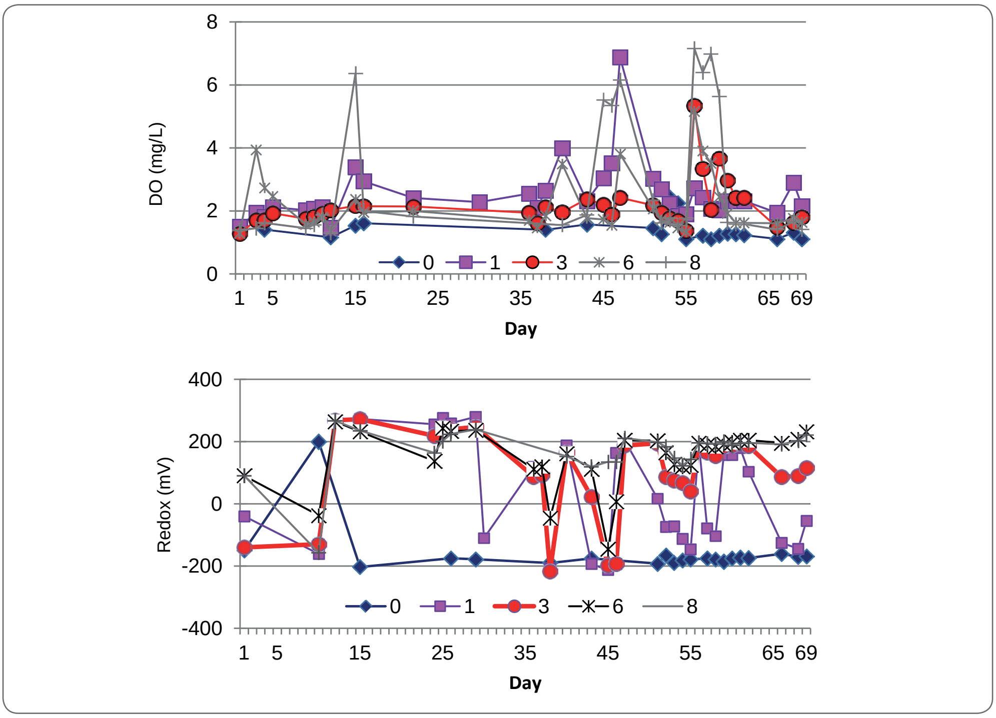


Figure 10. Comparison of dissolved oxygen (DO) and Redox values during biological aeration treatment of swine slurry manure in a series of continuously fed aerated tank reactors. Measurements were made in untreated manure (0) and in individual tanks (1, 3, 6 and 8) of the serial treatment system (supplementary data).

The obtained results suggest that a feed containing less than 1% TS content of swine slurry manure allowed a serial system loading with a maximum of 978 L/day, corresponding to 3 days HRT-value of the treatment system with the used aeration rates (I). Higher solids content of the feed decreased the maximum loading volume. Dairy slurry manure with less than 2% TS content allowed the system to be loaded with up to 350 L/d, corresponding to 6.8 days HRT-value (III).

The obtained results suggest maintaining the redox-values of the treatment tanks ideally as follows: 1st tank at zero or slightly above, 3rd tank at around 100–180, and the 6th tank at 180–200 mV. Corrective measures should be initiated no later than when the ORP has fallen below zero in the third tank of the serial system (I and III).

Effect of aeration on pH

The average pH of pre-treated slurry manure before aeration was between 7 and 8 irrespective of slurry type (Table 17). However, after biological treatment the pH increased above 8.5 and even an average value of 8.8 was reached in studies II, III. In some of the studies individual measured pH-values reached almost 9.0 (e.g. II: Table 2). The pH-increase due to aeration treatment appeared to depend on process loading. As shown in Table 17, lower average pH-values were reached with shorter hydraulic retention time values (I: Table 3) or with higher carbon loading (III: Table 3). On the other hand, long hydraulic retention time increased the ammonia volatilization from the treatment tanks, which resulted in reduction of pH-values in the last tanks of the serial tanks system (III: Fig.4).

4.2.2 Physico-chemical changes due to treatment

Effect of treatment on solids and carbon content

There was a clear decrease in the TS levels of slurry manure due to the treatment (Table 17). Effluent from the last tank contained on average 10, 31 and 16% less TS compared to slurry before aeration in the three experiments with swine manure (Table 17). Therefore, the higher the initial TS concentration was, the more TS content of swine manure was decreased due to aeration treatment.

In the case of dairy slurry manure the effect was less marked. The average TS reduction percentage was 21% during period III and 20% during period IV (Table 17; III).

Carbon reduction during aeration treatment depended on initial carbon content and varied between studies from 11 to 57% (Table 17). Swine slurry manure treatment led to an average of 13% TC and 11% TOC content reduction (effluent from the last tank compared to feeding) in the first study (I: Fig. 2C) when the TS content of the feed was very low. When the feed TS concentration was higher than in paper I, 29% TC and 29% TOC reduction in the second study (II: Table 1) and similarly 25% TC and 26% TOC reduction in supplementary data (Table 17) were recorded.

During dairy slurry manure treatment, effluent from the last tank contained on average 44–49% less TC and 47–57% less TOC compared to slurry before aeration in the two periods studied (Table 17; III: Tables 2 and 3). Greater carbon reduction was obtained with slurry having a lower carbon content (III: Period IV), when the greatest change in the amount of carbon occurred in the first treatment tank. Lower carbon content led to a 50% higher TOC reduction between tanks 1 and 6 (III).

Odor

The odor generation potential of the swine slurry was evaluated based on the VFA levels in the slurry. In addition, odor intensity was evaluated by a human panel (I).

The biological treatment of slurry manure significantly reduced the odor intensity compared to that of untreated manure (I: Fig. 4B) when measured by sensing. The untreated slurry manure was evaluated as having a very strong odor, whereas in the last treatment tank no odor or only a very faint odor was reported (I: Fig.4B and Table 4). The best result was obtained with a longer hydraulic retention time (HRT 4 days). The odor intensity reduction due to biological treatment was only evaluated in the first study (I) with swine slurry, but similar observations (very effective odor reduction) were also made during the other studies (II, III and IV).

The VFA concentration was a less sensitive measure of the odor than the evaluated odor intensity. The VFA concentration in swine slurry before aeration varied between 404 and 484 mg/L (I: Fig. 4A) and 799±235 mg/L (II: Table 1). After treatment the VFA concentrations varied between 330 and 484 mg/L (I: Fig. 4A) and 525±27 mg/L (II: Table 1) and were much higher than the level (below 230 mg/L) regarded as representing manure without an offensive odor, although odor intensity was reduced to a level of no odor or only a very faint odor.

Color

Swine slurry color change and darkness intensification from one tank to the next was observed visually and also confirmed by absorbance value comparison as an indicator of humification (I). The absorbance values were increased from 2.244 for untreated slurry to values ranging from 3.079 to 4.000 in the last tanks of the treatment system (I). The peaks were observed at wavelength 382–383 nm (I: Fig. 6).

Table 17. Average feed concentration (Feed), effluent concentration (Effl.) and change of concentration during processing (Red.%) (I–III, supplementary material (Suppl.)) Per.III and IV: the study periods III and IV of paper III. Other symbols as in Tables 13 and 14.

	Manure	pH	TS	TOC	TC	IC	N_{tot}	NH_4^+-N	P_{tot}	PO_4-P	Ca^{2+}	K^+	Mg^{2+}	Na^+
	Paper		(%)					(mg/L)						
Feed	Swine	7.1-7.4	0.55	1083	2626	1543	1509	1367	82	21	160	964	49	361
Effl.	I	8.3-8.6	0.49	959	2288	1329	1487	1339	51	18	136	970	24	360
Red.%			10.5	11.4	12.9	13.8	1.5	2.1	37.8	13.1	15.2	-0.6	50.4	0.2
Feed	Swine	7.9	1.01	1471	2774	1302	1816	1494	252	50	204	952	82	284
Effl.	II	8.8	0.70	10^{3}8	1995	957	1548	1292	166	40	148	942	53	272
Red.%	HRT=9 d		30.7	29.4	28.1	26.5	14.8	13.5	34.1	20.0	27.5	1.1	35.4	4.2
Feed	Swine	8.1	0.60	1471	2774	1302	1545	1320	96	42	161	1034	35	316
Effl.	Suppl.	8.8	0.51	1089	2070	981	1306	1150	645	34	103	1050	145	321
Red.%	HRT=2.3d		16.1	26.0	25.4	24.6	15.5	12.9	32.9	18.2	36.1	-1.5	58.2	-1.8
Feed	Dairy	7.6	2.32	9322	110^{2}3	1701	2169	1296	310	155	819	2801	353	516
Effl.	III/Per.III	8.5	1.84	4972	6169	1197	1047	438	166	57	464	2919	392	561
Red.%			20.7	46.7	44.0	29.6	51.7	66.2	46.6	63.0	43.4	-4.2	-11.2	-8.7
Feed	Dairy	7.9	1.90	5335	6496	1161	1590	859	230	142	442	2108	218	197
Effl.	III/Per.IV	8.8	1.52	2293	3306	1013	1167	510	89	35	141	2650	268	313
Red.%			20.0	57.0	49.1	12.8	26.6	40.7	61.4	75.5	68.0	-25.7	-22.6	-59.0

4.2.3 Effect of treatment on manure nutrient content

The nitrogen content changes of slurry manure occurred mainly due to ammonia volatilization during treatments. However, in the first study with swine manure there were almost no changes in nitrogen concentrations during treatment (Table 17; I: Fig. 3A). In other studies with swine slurry manure, an average of 15% total N reduction and 13% ammonium N reduction was observed due to treatment (Table 17, II, and supplemental data). The used ammonia stripping influenced N concentrations with dairy slurry manure treatment (III). Slurry NH_4^+-N content decreased below half its original concentration in the first treatment tank during the third study period, and the corresponding decline during the fourth period was 40% (Table 17; III: Table 3). This was due to the feedback dilution effect. During the course of the treatment (between tanks 1 and 6) neither the N_{tot} nor NH_4^+-N content of slurry manure decreased significantly (III). Overall, effluent from the last tank contained on average 27 and 52% less N_{tot} and 41 and 66% less NH_4^+-N compared to the slurry manure before aeration in the two periods studied (Table 17). Higher N reductions were obtained with originally higher N content slurry (III, period III). Nitrogen mineralization was observed in the case of slurry with a higher solids content (III, period III).

Irrespective of the study, the amount of nitrate was low, with concentrations varying between 9.9 and 31.2 mg/L in the first study with swine slurry manure (I) and less than 9 mg/L during dairy slurry manure treatment (III). Accordingly, nitrate formation was low with the aeration rates used. However, the very low solid content of the slurry manure appeared to expose the material to easier nitrate formation (I).

Of the P_{tot} 33–61% and of the phosphate phosphorus 13–76% was removed as a result of biological treatment (Table 17). With swine slurry manure feeding, phosphorus contents were low due to the pre-treatment. More than 80% of the total P of swine manure and nearly 90% of the phosphate phosphorus had been removed before biological treatment (study I). Despite the low phosphorus content of the feed, up to 38% of the swine manure total P and 20% of the phosphate phosphorus was removed during biological treatment (Table 17). With dairy slurry manure, 47–61% of the total phosphorus and 63–76% of the phosphate phosphorus were removed, respectively, during the process (Table 17, III). The greatest P_{tot} concentration changes occurred in the first tank irrespectively of the study. As was observed previously with respect to TS and carbon reductions, it appeared that phosphorus reduction also depended on the initial content: the higher the concentration in the feed, the higher was the reduction.

Magnesium and calcium ion concentration changes were similar to those of P_{tot} (Table 17). Predictably, monovalent Na^+ and K^+ ion concentrations during processing remained almost unchanged irrespectively of the study (Table 17).

4.2.4 Total bacterial counts and enteric indicator organisms in slurry

Total bacterial counts

Total bacterial numbers were enumerated in dairy slurry manure. Total aerobic bacterial counts in raw dairy slurry varied from 2.95 105 to 3.24 106 cfu/g. Mean aerobic counts in aerated tanks varied from 1.88 105 to 6.17 106 (Fig. 11A; IV: Fig. 2). Total counts were increased (on Day 33 and 61 samples) or only slightly decreased (on Day 133 sample) compared to raw slurry and there were only small differences between the aerated tanks. On Day 172 total counts of samples with a short HRT value (6.7 d) were significantly decreased in the last treatment tank (Fig. 11A; IV).

Enteric indicator organisms

Swine slurry manure contained initially higher viable counts of enteric bacteria compared to dairy slurry manure irrespectively of time points of the study periods, with the exception of Enterobacteria (Fig. 11; IV: Fig. 2). Furthermore the reductions obtained (%) in the numbers of hygienic indicators due to treatment were higher with swine slurry manure compared to dairy slurry manure, with the exception of fecal coliforms (Table 18; IV: Table 2). This could be exclusively due to higher original counts in swine slurry, since after the treatment the numbers of indicator bacteria were higher in swine slurry manure than in dairy slurry manure (Fig. 11). Of the individual indicators, the numbers of fecal streptococci remained higher throughout the treatment compared to *E. coli* and other coliform bacteria in dairy slurry manure, although the opposite was observed in swine manure.

Aeration treatment was conducted with fixed HRT values with swine slurry manure, whereas with dairy slurry manure the HRT differed with one sampling time from the other samplings. The numbers of E.coli (attained reduction-% varied from 93.9 to 98.4) and total coliforms with swine slurry manure were significantly reduced irrespectively of sampling time (Table 18), whereas with dairy slurry manure different HRT values influenced the result obtained: with higher HRT values (days 33, 61 and 130, HRT=43–48 days) the numbers of *E. coli* and total coliforms were significantly reduced, whereas with shorter HRT value (day 172, HRT=6.7 days) wide variation was observed in the numbers of these indicator microbes from one tank to the next and there was no decrease in the last tank compared to the original count (Fig. 11, IV: Fig. 2 and Table 2).

Fecal coliform reduction in the last tank was 93.9% of the original count even with

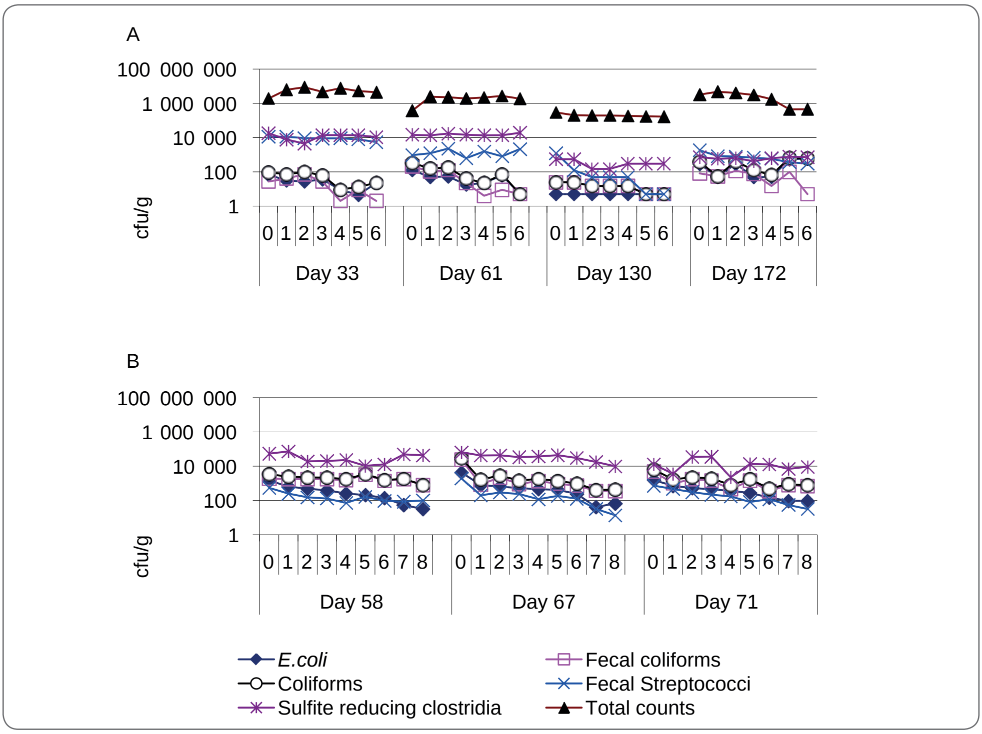


Figure 11. The geometric means of fecal indicator bacteria and total counts (cfu/g) during aerobic treatment at different sampling times in untreated manure (0) and A. tanks (1–6) of the treatment system of dairy manure (HTR 43–48 days for sampling days 33, 61 and 130, 6.7 days for day 172=6.7, **IV**), and B. tanks (1–8) of the serial treatment system of swine manure (HTR 6.3 days, supplementary data).

the shorter HRT (IV: Table 2) with dairy slurry manure, whereas with swine slurry manure more variation was observed in the reduction percentages (59.9–98.5%) (Table 18).

Short retention time did not appear to influence the reduction of fecal streptococci with dairy slurry manure either. Their count was markedly reduced during treatment, except on day 61, when there was a marked variation in the numbers of fecal streptococci between the treatment tanks (Fig. 11). With swine slurry manure good reduction percentages were attained in the numbers of fecal streptococci (81.7–95.7%) without variation between the sampling dates (Table 18).

Of all the indicator microbes, treatment had no or only little influence on sulfite-reducing clostridia, irrespectively of sampling time or manure type (Fig. 11 and Table 18, III: Fig. 2, III: Table 2).

4.2.5 Bacterial diversity dynamics

Microbial diversity dynamics in separated dairy slurry manure and in aerated dairy slurry manure in different treatment tanks (Tanks 2 and 6) of the serial system were studied. The aim was to study changes in microbial community composition due to aeration and between the treatment tanks. In addition, the soil sample (the origin of an inoculant) microbial composition was studied and was compared to the microbial composition of the treatment tanks.

Table 18. Statistical differences in numbers of enteric organisms between the different time points of the study period (Fig. 11B). Numbers of hygienic indicators (*E. coli*, Faecal coliforms, Coliforms, Enterococci, sulfite-reducing clostridia and total counts) were analyzed with the MIXED procedure of SAS. Linear regression analyses (intercept, factor, R^2 and p-values shown). Swine manure (supplementary data).

Analysis of variance			Linear regression				Reduction
Microbe	Exp Day	Level average (cfu/g)	Intercept	Factor	R^2	p	(%) tanks 0–8
E. coli	58	$4.09*10^{2\ a}$	$9.69*10^2$	$-1.40*10^2$	0.740	<0.003	97.9
	66	$8.36*10^{2\ a}$	$2.18*10^3$	$-3.37*10^2$	0.481	<0.038	98.4
	71	$4.71*10^{2\ a}$	$1.05*10^3$	$-1.45*10^2$	0.703	<0.003	93.9
Faecal	58	$1.77*10^{3\ a}$	$2.03*10^3$	-64.7	0.072	<0.485	59.9
coliforms	66	$3.59*10^{3\ a}$	$1.03*10^4$	$-1.68*10^3$	0.344	<0.097	98.5
	71	$1.41*10^{3\ a}$	$2.74*10^3$	$-3.32*10^2$	0.446	<0.049	85.9
Total	58	$2.18*10^{3\ a}$	$3.00*10^3$	$-2.05*10^2$	0.430	<0.055	76.3
coliforms	66	$4.42*10^{3\ a}$	$1.25*10^4$	$-2.02*10^3$	0.363	<0.086	98.5
	71	$1.89*10^{3\ a}$	$3.79*10^3$	$-4.77*10^2$	0.530	<0.026	87.9
Entero	58	$1.77*10^{2\ a}$	$3.34*10^2$	-39.2	0.530	<0.026	81.7
bacteria	66	$3.46*10^{2\ a}$	$9.11*10^2$	$-1.41*10^2$	0.424	<0.057	99.3
	71	$2.44*10^{2\ a}$	$5.50*10^2$	-76.6	0.819	<0.001	95.7
Sulfite	58	$3.43*10^{4\ a}$	$4.31*10^4$	$-2.27*10^3$	0.082	<0.454	19.3
reducing	66	$3.61*10^{4\ a}$	$5.73*10^4$	$-5.28*10^3$	0.781	<0.016	85.0
clostridia	71	$1.48*10^{4\ b}$	$1.96*10^4$	$-1.19*10^2$	0.067	<0.501	26.0

Level averages between experiment days within a column having different letters indicate a significant difference at $p< 0.05$.

† nd, not defined.

Changes in microbial community composition in the 2nd and 6th treatment tanks

The major influence of aeration was the decrease in the number of Firmictus in aerated slurry tanks compared to raw manure before aeration and the increase in the numbers of Deinococcus-Thermus and Proteobacteria in aerated tanks (IV).

Due to the aeration treatment, the number of clones belonging to the phylum Firmicutes decreased from 44% in untreated slurry to 6% in the 2nd tank of the serial system and continued to fall to 3% in the 6th treatment tank (IV: Fig. 3; IV: Table 3). The proportion of Deinococcus-Thermus increased from 10% in untreated slurry to 39% in the 2nd treatment tank, but fell to 29% in the 6th treatment tank. Correspondingly, the proportion of Proteobacteria increased from 10% to 45% in the 2nd treatment tank and continued to increase up to 63% in the 6th treatment tank (IV).

The species diversity of the Proteobacteria was also comprehensive. Clone libraries from tanks 2 and 6 included classes of Alpha-, Beta-, Delta-, Epsilon- and Gammaproteobacteria (IV: Table 3; IV: Fig. 4). Deinococcus-Thermus Predominant OTUs detected in the 2nd and 6th tanks made up a single cluster in the phylogenetic tree, suggesting that the various Deinococcus-Thermus representatives were closely related, irrespectively of their origin (untreated, tank 2, tank 6) (IV: Fig. 4). The proportion of the class Bacilli, member of the phylum Firmicutes, increased in the 2nd tank, but no Bacilli were present in the last treatment tank. Clones belonging to the phyla Bacteroidetes and Chloroflexi appeared in the 2nd treatment tank and Planctomycetes in the 6th treatment tank. Untreated slurry did not contain measurable levels of these phyla, whereas the soil from which the inoculum originated contained Planctomycetes. However, the Planctomycetes in the 6th tank and in the soil belonged to different classes and families.

Soil - the origin of the inocolumn

The original inoculant was prepared from a soil-water suspension (III). Acidobacteria were the most abundant phylum of soil bacteria (38%), dominated by subgroup Gp6 (IV: Fig. 2; IV: Table 3). Acidobacteria were followed by the phyla of Proteobacteria (29%), Gemmatimonadetes (15%), Verrumicrobia (6%), Planctomycetes (3%), Firmicutes (3%) and Chloroplast (3%). The clone libraries of soil and treatment tanks did not share any sequence at the genus level, but some at the family level and several at the order level (IV).

4.3 Buffer system in the slurry and ammonia stripping

4.3.1 Ammonia stripping

Ammonia stripping with the ammonia reference solution

The constructed ammonia stripping device was tested by using different reference solutions (NH_4^+-N concentration from 300 to 1700 mg/L, II). The stripping performance with the used operation parameters was good (II). Over 90% ammonia removal percentages were reached irrespectively of the NH_3 content of the reference solution (II: Fig. 2). However, the NH_3 removal efficiency was shown to be directly dependent on the air and liquid temperatures (II).

Ammonia stripping of the biologically treated manure without chemicals

Over 30% total ammoniacal nitrogen (TAN) removal by air stripping was shown to be possible if the pH of the biologically treated swine manure was above 8.9 (II: Fig. 3A). The NH_3 removal with biologically treated swine slurry varied between 65 and 67% and TAN removal between 20 and 32% when the pH of the biologically treated manure was above 8.9 (II: Fig. 3A). With lower pH values (pH-value variation from 8.6 to 8.9), 6 to 26% TAN removal was reached (II: Fig. 3A).

However, with biologically treated dairy slurry (pH value 8.8 before stripping), only 6.2% of TAN was removed by air stripping (III: Table 3, III: Fig. 6B). The low removal percentage with dairy slurry was probably due to low ammonium concentration in slurry before stripping and also due to the high TS content of the slurry (III).

Ammonia stripping of the biologically treated manure with the chemicals used

With biologically treated dairy slurry, MgO was added before stripping. This increased the slurry pH from 8.5 to 8.9 and facilitated 15.6% TAN removal (III: Table 3, III: Fig. 5B). Compared to the dairy slurry stripping result without MgO addition, the achieved result was better, but was still very low compared to the results achieved with biologically treated swine slurry without chemical additions (II).

Repeated stripping procedure after first stripping

Due to a decrease of pH after the first stripping, stripped swine manure was treated with 0.5 kg m^{-3} magnesium oxide (MgO) and stripped again. The use of MgO resulted in pH increase even to 9.2 (II: Table 2) before the second stripping. However, the pH reached varied widely between experiments. The second stripping decreased the slurry TAN by 15 to 26% after MgO treatment (II: Fig. 4A).

The second stripping was followed by calcium hydroxide ($Ca(OH)_2$) treatment to increase the pH of the twice stripped manure and then continued with a third stripping. In the third stripping, 29 to 58% of the TAN was removed compared with the TAN content before $Ca(OH)_2$ addition (II, Table 2). Overall, after three consecutive stripping occasions, 59 to 86% of the original TAN of swine slurry had been removed (II: Table 2, Fig. 4A).

4.3.2 Buffer system composition in the slurry

Buffer capacity curves were drawn in order to identify the acid and base species making a major contribution to the buffer capacity in slurry (II). All the samples showed differences in buffer capacity when different amounts of TAN were present in a sample (Figure 12). Taking into account the acid dissociation constant (pKa) values (6.38, 10.32 and 9.25) for CO_2/HCO_3^-, HCO_3^-/ CO_3^{2-} and NH_4^+/NH_3 , respectively, it appears likely that the peak at pH 6 to 7 was attributed to HCO_3^-, the peak at 9 to 10 included the buffer capacity contribution from NH_4^+/NH_3 and at pH 10 to 10.5 the peak was due to CO_3^{2-} (Fig. 12; II: Fig. 5B). It was shown that the reduction in buffer capacity of the slurry was due to ammonia and carbonate removal during stripping (Fig. 12; II: Fig. 5A and B).

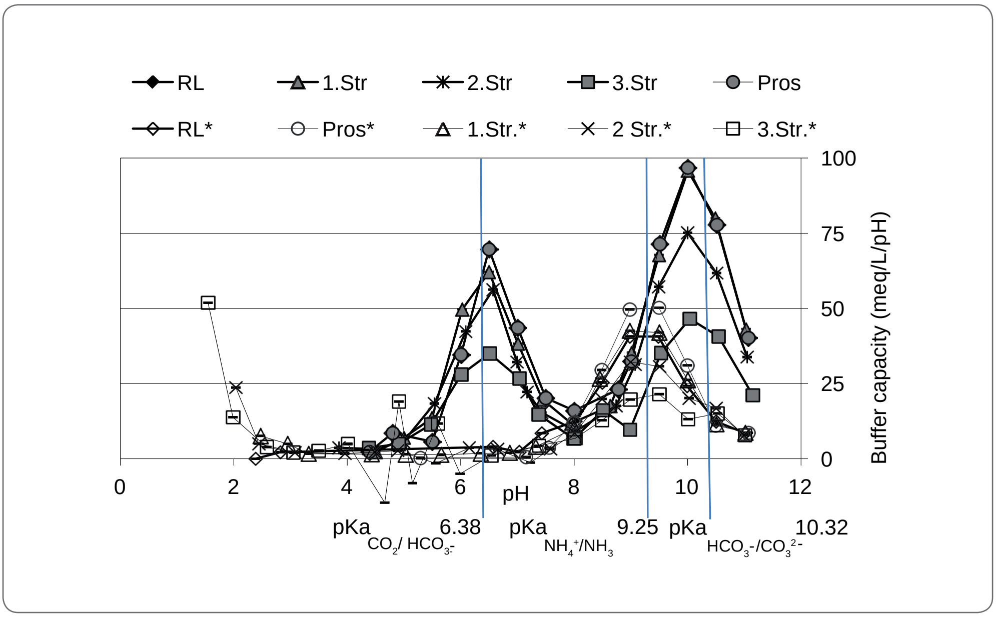


Figure 12. Buffer capacity curves for untreated swine slurry (RL), biologically treated swine slurry (Pros), and the treated swine slurry after the first stripping (1.Str), second stripping (2.Str), and third stripping (3.Str) (II). * Slurry samples with carbonates previously removed. Pka values marked with vertical lines for CO_2/HCO_3^-, HCO_3^-/ CO_3^{2-} and NH_4^+/NH_3 .

5 Discussion

5.1 Solids separation before aeration treatment

As a pre-treatment, solids separation of slurry manure was carried out in order to achieve suitable solids content prior to biological processing (I and III). The goal was to reduce manure TS content to 1% or lower in order to achieve efficient biological treatment. According to Zhu et al. (2008), in order to reduce aeration time and rates the manure solids content should not exceed 1%. Oxygen transfer rate and efficiency are affected by factors such as the surface area in contact with air, mixing, temperature, and the amount of solids or other constituents in the manure (Martin and Loehr, 1976; Westerman and Zhang, 1997; Ndegwa, 2004). According to Zhu et al. (2005), the oxygen transfer coefficient in slurry at 4.0% total solids level was reduced to about a quarter of the value in slurry at 0.5% total solids level. According to those authors, the energy consumption for slurry with 4% total solids is nearly four times that for slurry with 0.5% solids content in order to achive the same DO level in the treated slurry. Finer solid particles also decompose faster and to a greater extent than coarse particles (Westerman and Zhang, 1997). According to Ndegwa (2004), with solids separation improved aeration efficiency and better contact between the microbial biomass and the dissolved substrate was achieved, thus boosting the aeration treatment and biostabilization.

Sedimentation alone decreased the swine slurry manure dry matter content to ca. 1% or below (I). The achieved good sedimentation result was probably due to raw slurry initially containing a low solids content (1.85%) before the sedimentation step (I). This agrees with the results of Ndegwa et al. (2001), who found that the best results in manure separation were achieved with 1.0 and 2.0% solids levels. Natural settling reduced swine manure TS content by 70%, total P content by 80% and almost 90% of the phosphate P (I). The results are in agreement with the previous results of Ndegwa et al. (2001) and Zhu et al. (2004).

Dairy manure mechanical separation with a screw press resulted in an average of 2.9% TS content in the liquid phase (III). Separation removed 33% of the manure TS and 12.5% of the P_{tot}. The obtained results were in agreement with previous research conducted by Møller et al. (2002) with freshly collected dairy manure. Screw press separation efficiency is dependent on its ability to retain particles of certain size. According to Møller et al. (2002), the screw press separator only retained particles > 1 mm, which were low in phosphate compared to smaller particles. This was also probably the explanation for the achieved low nitrogen separation result (less than 5% of N_{tot} separated) with a screw press (III).

It was likely that after the solids separation step, finer compounds remained in the liquid fraction. Those compounds probably included odor compounds and compounds that were precursors for odor generation, as was reported e.g. by Zhang and Westman (1997) and Jacobson et al. (2001b). Supporting this, mechanical separation methods have been considered to have only a limited capacity for separating out odorous compounds (Zhu et al., 2001; Ndegwa et al., 2002).

5.2 Theoretical basis for the designed reactor type

The reactor type used in the present study was a system of six continuously fed aerated tank bioreactors grouped by serial connection conducted with feedback (I, III). This reactor type was designed on the basis of data in the literature on reactor performance. The goal with the reactor design was to achieve stable and efficient treatment in treatment facilities of comparatively small size.

5.2.1 Several bioreactors grouped by serial connection

Chemical reaction theory states that any monotonic reaction proceeds most rapidly in a plug flow reactor (PFR) due to the substrate gradient developed with that configuration. Therefore, an ideal PFR configuration would achieve specific performance goals in a smaller reactor volume than any other configuration (Levenspiel, 1972). However, Erickson and Fan (1968) recommended the use of a tanks-in-series configuration for activated sludge systems to reduce back mixing and approximate the plug flow-type substrate gradient. Hydraulic performance of a CSTR reactor can be improved by increasing the number of CSTRs in series (Reynolds and Richards, 1996). For the same total reactor volume (Vt) it is possible to approach the performance of a PFR (in terms of retention time) by increasing the number of CSTRs (n) in series (in this case each CSTR in the series has a volume of Vt/n) (Reynolds and Richards, 1996). CSTRs in series have commonly been used in biological treatment of industrial wastewater, such as activated sludge basins which are cascade connected (Abu-Reesh, 2010). This arrangement of reactors offers a number of advantages such as increased stability of the treatment plant when subjected to pulse load and also enhanced degree of degradation by adoption of activated sludge recycling (Abu-Reesh, 2010).

Based on the above literature, a configuration of six tanks in series was designed. This was assumed to provide small reactor volume of the plug flow-type reactor design, but more stability compared to plug flow design.

5.2.2 Feedback

In the present study feedback was introduced in the system in order to increase the stability of the treatment process. In biological systems it is well known that most parameters must stay under control within a narrow range around a certain optimal level under certain environmental conditions (Thomas and D'Ari, 1990). Deviation from the optimal value of the controlled parameter can result from the changes in internal and external environments. Homeostasis in biological systems maintains thermal, chemical, and biological conditions through feedback (Thomas and D'Ari, 1990).

In practice, feedback was carried out by recycling a part of the treated material back to the process. In this respect, the designed reactor system rather resembled a recycle reactors design (Hill and Root, 2014) than an active sludge system (Reynolds and Richards, 1996).

5.2.3 Use of limited aeration

Limited aeration was applied in the designed series reactor system. Manure aeration using limited aeration is used for manure stabilization (Ndegwa et al., 2007), odor reduction (Ndegwa, 2003), and hygienization purposes (Heinonen-Tanski et al., 2006), most frequently carried out, however, in single tank systems with continual or intermittent aeration. More efficient biological treatment technologies are based on nitrification-denitrification processes, in which nitrogen is lost, not captured (Reynolds and Richards, 1996). This study aimed at determining the level of aeration that would accelerate humification reactions, but would not lead to nitrate formation. Reduced odor and improved hygiene were also targeted goals.

5.3 Benefits of the serial treatment system

The functional goals of the designed serial manure treatment system were to achieve A) treatment stability towards system failures, B) treatment efficiency (short HRT), and C) improved process control. Besides the functional goals, the aims of the biological treatment were to achieve: 1. effective odor reduction; 2. good hygienic quality; 3. stable treatment product concurrently, and 4. preservation of nitrogen in NH_3 /NH_4^+ form in a liquid phase in order to continue the treatment with ammonia stripping.

5.3.1 Treatment stability towards system failures

It was clearly shown on the basis of the obtained ORP-values that the six tanks system in series provided stability for the treatment of dairy manure (III). Compared to the single tank system, in a serial system changes in the subsequent tanks occurred with a delay, thus indicating the dynamic character of the treatment system (III). Partly this was achieved with serial configuration, which effectively prevented material from being mixed between tanks and allowed only part of the material to flow from one tank to the next in a given time (III). Feedback also functioned evidently as a buffer mechanism against process interferences and was able to provide a system very resilient towards external influences, as described by Åström and Murray (2009).

In activated sludge treatment systems, a part of the settled material, the sludge (often called return activated sludge), is returned to the head of the aeration system to re-seed the new wastewater entering the tank (Reynolds and Richards, 1996). In the present system, feedback was not only the sedimented microorgnisms (activated sludge), but part of the effluent (partial recycle stream) exiting from the last tank of the serial system (I, III). In chemical reactor design, however, partial recycle stream is used when a substrate cannot be completely processed in a single pass, such as with an insoluble substrate (Hill and Root, 2014). These reactors continue to move the same substrate through the reactor so that the effective contact time is high enough to allow the substrate to be processed. Recycle reactors also allow the reactor to operate at high fluid velocities (Hill and Root, 2014). This is important because it minimizes the bulk mass transfer resistance to the transport of the substrate (Conroy, 1997).

5.3.2 Treatment efficiency

The HRT time of the designed system was measured in days (I, III), indicating efficient manure treatment. Factors that might influence the treatment efficiency of a serial tank system with recycle were feedback dilution effect (III) and the indication of different decomposability of organic matter in different treatment tanks based on the obtained redox profile (III), and the community compositional changes observed in different treatment tanks (IV).

The feedback significantly reduced the concentrations of carbon and cationic ions in the first treatment tank (III, I). Thus, it was probable that feedback enhanced carbon reduction due to a dilution effect in a system with high organic load (as explained in III). If ammonia is a process inhibiting factor, its removal from the recycle stream leads to a more efficient process. The feedback TAN concentration was deliberately lowered by NH_3 stripping in order to improve the treatment process and to avoid NH_3 inhibition (III). Ammonia inhibition has been commonly acknowledged as an inhibition factor in anaerobic digestion, as methanogens have been reported to be sensitive to ammonia (Chen et al., 2008). However, studies concerning ammonia inhibition in aerated livestock slurry are scarce, although ammonia in aerated slurry with higher pH is evidently present to a higher degree in ammonia form compared to anaerobic digestion. Free ammonia has been suggested to be the main cause of process inhibition, since it is freely membrane-permeable and may diffuse passively into the cell, causing proton imbalance and/or potassium deficiency (Sprott and Patel, 1986; Gallert et al., 1998). According to Kokkonen et al. (2006), accumulation of ammonium in solution affected the mobilization rate and final reaction equilibrium. They showed that accumulation of the gaseous reaction products, e.g. NH_3-N and CO_2, in the system, instead of volatilization to surrounding air, slowed the reaction rate down.

Indication of the different decomposition in different treatment tanks suggested that easily degradable material diminished already in the first treatment tanks, whereas more refractory material accumulated in the last tanks (III). Composting studies have revealed organic matter decomposition and microbial succession (e.g. Nakasaki et al., 2005). The microorganisms contributing to organic matter decomposition have changed as the composting progress proceeds (Nakasaki et al., 2005). The observed community compositional changes between different treatment tanks (IV) support the idea that microbial succession was spread within the different treatment tanks of the serial system. Whether or not this would lead to decreased microbial competition between successive tanks, and thereby increase treatment efficiency, remained however uncertain.

5.3.3 Odor reduction

It was shown that biological treatment in a series of continuously fed aerated tank reactors efficiently reduced the odor of the swine slurry manure (I). Concerning the odor reduction, serial configuration with feedback was one probable reason for increased treatment efficiency. Although the obtained results were preliminary and did not extend to individual compounds, it was noticeable that odor was reduced from one tank to the next, clearly waning towards the end of the process (I). On a theoretical basis the following was thought to provide an explanation: Based on odor characteristics even tiny amounts of odorants have been found to produce a strong smell because the additive and/or synergistic effects of hundreds of compounds increase the strong odor intensity (Schiffman et al., 2001). Based on the flow characteristics of serial tank reactors, only part of the solution flows from one tank to the next at a time, and consequently the mixing of the entire volume is prevented (I, III). Therefore, an example can be presented in which odor compounds concentrations are reduced according to a decreasing geometric series. In the first tank odorants are diluted by half due to the feedback dilution effect (assuming feedback only contains stabilized organic matter and its volume equals that of the feed volume). Only part (for example one tenth) of the odorants existing in the 1st tank flows to the 2nd tank. From the second tank again only 1 tenth of the odorants flow to the next container, and so on. Thus, in the last (6th) tank, the residual concentration of odorants is only $0.5*10^{-6}$. Thus, it is reduced to one part in a million of the original concentration.

5.3.4 Advanced process control

With regard to process control the serial system provides several benefits over a single tank system (III). The obtained results showed how process control became easier with a serial system compared to a single tank system. It was concluded that process failure was easily predictable (III) and the dynamic system gave time to start corrective measures in time. Moreover, the serial treatment system allows advanced process control (over aeration) according to demand. Aeration demand in the first tank was higher than in the following tanks, and thus aeration could be adjusted to a higher level in the first tank and to lower levels in the last tanks according to demand, thereby saving energy (III). However, drawbacks were reported (Yoon, 2011) relating to the high aeration demand in the first tank: 1) due to bubble coalescence, the high upstream oxygen demand makes it hard to dissolve enough oxygen to support biological COD degradation, 2) due to the bubble coalescence and high surfactant-like molecules in wastewater, oxygen transfer efficiency is low upstream, 3) the high ratio of nutrients to microorganisms causes higher diffuser fouling in upstream, which again makes it harder to dissolve enough oxygen in the local space.

5.4 Process conditions – Biological and physico-chemical factors in a serial system

5.4.1 Treatment start-up and inoculation

The slurry manure was treated biologically in the serial tank system. Before starting the treatment process, the tanks were filled with microbial seeding material (I). Manure aeration is commonly conducted without using any external microbial additives or seeding (e.g. Zhu et al., 2005). Microbiological additives have been used for odor reduction purposes (Ritter, 1981; Zhu et al., 1997; Zhu, 2000). However, it is acknowledged that commercial digestive deodorants which contain bacteria and enzymes added in manure have had limited success in controlling odor (e.g. Ritter, 1981). It has been reported, neverthless, that a commercial microbiological additive has functioned effectively during the manure composting process (e.g. Nakai et al., 2004) and even very small amounts of additives have resulted in a good result (Wang et al., 2011).

The use of seeding is a common procedure e.g. with anaerobic digestion and activated sludge during the start-up phase to enhance degradation activities and to shorten the start-up times and treatment periods (Gerardi, 2003a; Pijuan et al., 2011). In the present study, the aim was to carry out seeding in order to increase the degradation potential in treatment reactors (IV). The idea was in particular to improve the odor reduction potential of the biological treatment. Moreover, slurry manure itself (anaerobic source) was not seen as the best source for enriching the most suitable microbial population for aerobic treatment.

Seeding material was produced from an agricultural soil with a long history of manure applications and an abundant earthworm population (I, IV). The aim was to take advantage of the large reservoirs of genetic information in soil (Torsvik and Øvreås, 2002). It is probable that soil (especially a soil that has been under intensive agricultural use and received abundant manure applications) contains capacity for utilization of diverse odor compounds.

Preparing the seeding material

A seeding material was produced by using soil as a starting material (soil:water ratio 1% v/v). The procedure is described in more detail in publication I. Naidu at al. (2010) described a methodology to produce aerated tea compost, a water-based compost extract containing a high population of beneficial microbes. The compost:water ratio was 1:5 (w/v). Compared to the tea compost production (Naidu et al., 2010), the used soil:water relation in the present study was much lower. The number of total bacteria in tea compost was105 mL^{-1} (Naidu et al., 2010), but was not measured in the present study.

Seeding and the treatment start-up

In the present study, the start up-phase was to fill the treatment tanks with the produced effluent. Thereafter, slurry manure feed was started with a continuous feeding rate (with small amounts of slurry, 20–50 L/day), monitoring the ORP and odor formation simultaneously and adjusting the feeding accordingly (I). Regarding anaerobic digestion, a general guideline is that the seed material should be twice the volume of the fresh manure slurry during the start-up phase, with a gradual decrease in amount added over a three-week period

(DaSilva, 1979). Thus, the used seeding volumes in the present study were much higher related to feed raw manure volumes (the feeding rate of 50 L/day represents one tenth of the first tank volume and one sixtieth of the entire system volume). Simultaneously recycling stream (feedback) equivalent to the feeding volume was recycled from the last tank to the first tank (I).

In the composting process, the desired activities have been achieved with even very small amounts of inoculant use with preselected microorganisms. According Wang et al. (2011), 57.5% faster lignocellulose degradation was reached by inoculating compost with a lignocellulolytic fungus. Fungal suspensions with a concentration of 1×10^9 colony-forming units (cfu) mL^{-1} were used at a concentration of 5 mL kg^{-1} of compost during the building of heaps. In another composting study, each composting heap was inoculated with a volume of microbial suspension to reach a level of 10^6 to 10^7 colony-forming units g^{-1} (cfu g^{-1}) of waste (Vargas-García et al., 2006). In a study of Sasaki et al. (2006), a mixture comprising 400 kg of beef cattle manure and chaff, and 100 kg of mature beef manure compost was well mixed and then mixed with 10 kg of microbial addition.

The reason for successful inoculant use in composting (e.g. Wang et al., 2011) but unsuccessful use with manure (microbiological additives added in manure for odor reduction, e.g. Zhu, 2000) is probably due to material differences between these two materials and differences in treatment conditions. Not only does aerobic composting occur under aerobic conditions (and added inoculants are predominantly aerobes), but respiration intensity is also probably relatively low in dry compost material compared to stirred slurry manure. In fact, the treatment conditions are comparable to submerged (liquid) fermentation (liquid manure) and solid substrate fermentation (composting) (fermentation types reviewed e.g. Raimbault, 1998; Subramaniyam and Vimala, 2012).

For this reason the seeding procedure in the present study was carried out as implemented (I) by adding only a small volume of slurry manure to a large volume of seeding material and conducting feedback in order to increase the survival of aerobes in a liquid state with high organic load.

In the present study with the start-up phase and with the seeding material preparation, the question was probably also one of adaptation (I). Several studies have revealed the importance of bacterial adaptation to a wide range of ammonia concentrations (Angelidaki and Ahring, 1993; Angenent et al., 2002), but have not determined whether the adaptation was a consequence of metabolic transition of the already existing microbial population or of the growth of new cultures adapted to the different ammonia concentrations (Rajagopal et al., 2013).

Viable bacterial counts

Total counts were enumerated in order to provide basic information on the numbers of bacteria in slurry during treatment, as well as on overall microbial function. In general there were higher total counts in aerated tanks than in raw slurry (IV). This was also supported by the 16S rRNA results (IV). With short HRT value (HRT=6.7), however, total counts significantly decreased in the last treatment tanks of the serial system. Perhaps treatment time was sufficient to inactivate anaerobic/ facultative anaerobic species in aerobic conditions in the last tanks of the serial system, but too short for aerobes to evolve.

Indicator microorganisms were used as a marker of the hygenic state of the treatment and in order to provide evidence for the possible occurrence of ecologically similar pathogens in a system. Differences in enteric counts were observed between the slurry manure type, between the indicator organisms and between the different HRT values (IV). Higher numbers of enteric bacteria in swine manure compared to dairy manure in the present study were probably related to the low solids content of the dairy slurry manure (found e.g. Jones, 1982). The levels of *E. coli*, fecal (thermotolerant) coliforms and total coliforms in raw dairy slurry were considerably lower in the present study than in the earlier study of Heinonen-Tanski (1999).

Comparing individual indicators, fecal streptococci have been more resistant to stress than *E. coli* and the other coliform bacteria (OECD/WHO, 2003), and this was also seen in the results with dairy manure (Fig. 11A). With swine manure, however, the opposite result was obtained (Fig. 11B).

Of all the indicator microbes, treatment had no or only little effect on sulfite-reducing clostridia, irrespectively of sampling time or slurry type (Fig. 11, Table 18). Sulfite-reducing clostridia are spore-forming and therefore have been more resistant to treatment and disinfection processes (Gould and Hurst, 1969; NRC, 2004).

Bacterial community composition

A shift in the bacterial community composition from obligate or facultative anaerobes to aerobically growing bacteria was observed due to aeration (IV). Differences in community composition between the aerated tanks were also observed (IV), although the result is preliminary and needs confirmation. It has previously been reported that microbial communities have been able to change very rapidly, and that variability in community composition has changed even more rapidly (e.g. Redford and Fierer, 2009). Research areas under intensive study at the moment (such as the colonization process of the infant gut microbiome) (e.g. Koenig et al., 2011) could provide new interesting insights on this subject area in the future. How microbiomes are established and maintained, is one of the leading questions in biology (Scheuring and Yu, 2012).

5.4.2 Aerobic decomposition under limited aeration and humification

The process of decomposition is initially rapid, but slows down considerably as the supply of readily decomposable organic matter becomes exhausted (USDA/NRCS, 2000). Consequently, the last tank in the series contained further degraded, more stable material compared to the material in the first tank, and the aeration demand in the last tank in the series was obviously less than in the first tanks (III). Therefore, it was also very likely that there were differences in compound composition between the tanks. The observed community compositional changes between different treatment tanks supported this (IV). However, further research is required to identify the compound composition changes in the systems.

Humification

During the treatment process slurry manure color changed from gray to dark brown, indicating humus formation (I). Humus formation is often considered to occur solely under anaerobic conditions (e.g. Rajeshwari and Balakrishnan, 2009). However, if aeration is kept within a limited range, humus-like condensation products may also form under aerobic conditions (Tölgyessy, 1993).

In the present study, humification was studied using spectroscopic methods only (I). The observed dark color was linked to a recondensation process forming humic substances such as fulvic and humic acids (I). The typical dark color of humic and fulvic acids is due to the presence of aromatic nuclei (Stevenson, 1984). Humic substances behave like weak acid polyelectrolytes and the occurrence of anionic charged sites accounts for their ability to retain cations (Hayes and Swift, 1978).

Thus, this behavior probably enhanced coagulating behavior (complexation with divalent cations in a system), as observed during biological treatment (I).

Problematic compounds in manure treatment are those low molecular weight compounds that are electrically neutral, highly water-soluble and difficult to remove by precipitation or filtration (Zhu et al., 2001; Ndegwa et al., 2002). These compounds include volatile fatty acids, low molecular weight carbohydrates (simple sugars) and other dissolved compounds, e.g. organic acids, phenols, nitrogen and sulfur compounds, low molecular weight proteins etc. that have been considered to be responsible for most offensive odor emissions (Zhang and Westerman, 1997; Jacobson et al., 2001b). Aeration is used for odor control (I, III), because these compounds are easily consumed by microorganisms (Jacobson et al., 2001b). Microbial metabolites of these processes are probably involved in humification processes. Humification is enhanced by high amounts of soluble organic compounds and limited aeration, and probably serves as a natural coagulant facilitating the precipitation of these weakly precipitating compounds (I).

In soil conditions the humification process has been found to be generally very slow, taking years to reach completion (Tölgyessy, 1993). Under controlled conditions, as in a compost environment, humification proceeds much faster, in weeks (e.g. Diaz et al., 2011). However, during slurry aeration, stabilization proceeds even faster, in days (Ndegwa et al., 2007a). The obtained results showed color intensification occurring in a very short time, suggesting a very rapid humification process (I). The reasons for accelerated humification processes during slurry aeration were probably the low C/N of the raw material (C/N = 2), small particle size of the organic material, sufficient O_2 concentration due to aeration (but not too intensive), and high water content. Moreover, aeration mixed the suspension thoroughly (I), which was probably the most important factor.

ORP values under limited aeration

The measured ORP values ranged between -200 and +300 mV in treatment tanks when the dairy manure was treated (III). ORP in separated slurry before aeration treatment was circa -200 mV and ranged from 0 to 200 mV in treatment tanks when the process functioned in the desired way (III). It was devised that ideally the redox-values of the treatment tanks should be kept as follows: 1st tank at zero or slightly above, 3rd tank at around 100–180, and the 6th tank at 180–200 mV (III). These ORP values were within the range reported by Ndegwa et al. (2007a), using limited aeration, and on a level high enough to reduce the odor potential in the manure (at +35 mV or higher) according to Zhu et al. (2002).

The measured ORP values remained below +225 mV and no significant amount of nitrate was formed (I, III), although ORP values were on a level favoring nitrification (III, Fig. 4). Therefore nitrate formation was probably prevented by other factors. According to Strauss (2000), nitrification rates were influenced by many factors: the template factors including pH, temperature, and dissolved oxygen and within these constraints C:N ratio, organic carbon availability, and nitrogen availability in the system. Many studies have revealed the negative effect of organic carbon (in particular labile organic carbon) on nitrification (Strauss and Dodds, 1997; Strauss, 2000). The mechanism responsible for the inhibition of nitrification when organic carbon is abundant is probably increased competition between nitrifying and heterotrophic processes (Strauss, 2000). This was probably also the reason in the present study, although many mechanisms were evidently involved in a complex system. The obtained characterization results revealed Nitrosomonas in the second treatment tank but no nitrifying organisms were detected from the sixth treatment tank (IV).

The low nitrate formation was an aim because nitrate cannot be removed by ammonia stripping. Likewise, the nitrate which returns with recycle back to the first treatment tank is vulnerable to denitrification due to the low ORP value in the tank.

5.4.3 pH and its relationship with the release of NH_3 and CO_2 from the system

In the present study, significant pH increase due to aeration was observed (I, II, III). The pH change in aerated manure slurry is controlled by a complex chemical buffering system (Husted et al., 1991; Sommer and Husted, 1995; Paul and Beauchamp, 1989). Probably the most important role in slurry is played by the ammonium bicarbonate system (II, Husted et al., 1991). Ammonia in solution is neutralized by dissolved CO_2 to form ammonium bicarbonate, keeping the pH at around neutral. An increased CO_2 release from the system in relation to NH_3 leads to a pH increase, whereas the opposite leads to a pH decrease. This is explained according to the maximum solubility of these two components (II). The solubility difference between these two components is 1.7 g CO_2 L^{-1} compared with 535 g NH_3 L^{-1} in pure water at 20°C at 1 atm pressure (Chang, 2000).

In fact, the system is an interrelationship between the equilibrium constants, Henry's law, and the weak acid-base relationship. These factors apply both in biological aeration treatment as well as in ammonia stripping processes (explained in more detail in section 1.5.3, Physico-chemical factors involved in treatment reactors).

In aerated tanks, the solution pH affects the proportions of carbonate and ammoniacal nitrogen which are present in bicarbonate and ammonia form (Figs. 5 and 6). Above a pH-value of 8.5, the proportion of bicarbonate decreases with increasing pH (Fig. 6; Chang, 2000); at the same time the proportion of NH_3 increases (Fig. 5; Chang, 2000). Thus, both CO_2 and NH_3 are volitilizated from the system. However, due to the solubility difference between these two compounds, more CO_2 is volitilizated in relation to NH_3 (Chang, 2000).

5.5 The role of manure buffer system in manure treatment

In the present study, it was shown that biological aeration treatment facilitated a slurry manure pH increase that in turn facilitated ammonia removal, reducing the needed chemical consumption (II). Although the manure buffering system plays a central role controlling manure pH change and coagulation processes, and causes substantial chemical consumption (Husted et al., 1991; Sommer and Husted, 1995; Paul and Beauchamp, 1989), the number of studies in which manure buffering capacity has intentionally been reduced is limited.

Buffer composition

In the present study, it was shown that manure buffer capacity was composed of TAN and the CO_2, HCO_3^-, CO_3^{2-} system (II). The obtained result was in agreement with the study of Husted et al. (1991). In their study, the major buffer components in manure were found to be ammonium, bicarbonate and a solid phase of carbonate. It was also shown that the reduction in buffer capacity of the slurry was due to ammonia and carbonate removal during ammonia stripping (II).

Selection of a precipitating agent

In the present study, MgO was used to increase manure slurry pH and to precipitate the residual P in the effluent (II and III). MgO addition was performed either after biological treatment (III) or after the first stripping cycle and subsequent nitrogen removal (II) using a dose of 0.5 kg m^{-3}. The most important reason for the selection of a divalent rather than a trivalent precipitant was the plant availability of the formed precipitate (Dao et al., 2001; Hyde and Morris, 2004). Although divalent ions precipitate less organic matter than the trivalent ions, they are reasonably effective P precipitants. The other reason is that aluminium and iron salts, although commonly used for solids removal in waste water treatment, are rather ineffective in manure treatment because of the high buffer capacity in slurry manure. Due to high pH and a highly buffered system, too rapid polymerization occurs, creating insoluble precipitated aluminium and iron polymers, resulting in Al/Fe(III) being surrounded and thereby neutralized by negatively charged oxygen atoms/hydroxides, with consequent loss of flocculation/coagulation function (Jiang and Graham, 1998). Therefore manure treatment with Al/Fe salts is in general inefficient and requires rather high doses of Al/Fe.

Influence of the buffer system on chemical use in ammonium separation

The results showed that a better precipitation result was obtained with MgO dosing after first stripping (II) compared to MgO dosing after biological treatment (III) when using the same amount of MgO. This supported the ammonia reduction effect on the need for chemicals. It appeared that ammonia stripping also affected the pH increase. After ammonia stripping, higher pH values were reached with MgO dosing compared to MgO dosing after biological treatment (II and III).

5.6 Sequential nitrogen separation

Air stripping requires a high pH for the NH_4^+ to separate in the tower as NH_3 gas. It is difficult to change the pH of the manure because of the high buffering capacity of slurry manure (e.g. Sommer and Husted, 1995). Large amounts of chemicals are required, which makes the process economically unprofitable. As NH_3 is removed, the pH of the solution decreases (also observed in II and III), and the effectiveness of the tower to separate N is diminished.

In order to reduce the needed chemical consumption a new sequential stripping procedure was proposed. The sequential treatment scheme included (i) increasing the slurry pH without chemical use and with part of the slurry buffer capacity removed and (ii) increasing the slurry pH with chemical treatments in between the stripping cycles (II).

It was shown that over 30% total ammoniacal nitrogen removal by air stripping was possible without chemical use, if the pH of the biologically treated swine manure was above 8.9 (II). The slurry was further subjected to repeated cycles of stripping with MgO and $Ca(OH)_2$ additions after the first and second strippings, respectively, to increase slurry pH in between the stripping cycles. After three consecutive stripping cycles, 59 to 86% of the original ammonium had been removed (II).

It was important to observe that the buffer system in slurry manure was shown to consist of NH_3 and carbonate systems and it was also shown that these compounds were removed during biological treatment and stripping cycles (II). Therefore, less chemicals are needed when slurry manure pH is increased sequentially between the stripping cycles than when changing the slurry ammonium completely to NH_3 before stripping. A patent was applied for this observation (Kokkonen et al., 2013).

In addition to ammonia removed by air stripping, part of the ammonia (on average 15% total N and 13% ammonium N; Table 17, II and supplementary data) was released during aeration treatment. This ammonia can be preserved for further use, if the NH_3 released during the biological treatment is collected and led to an air scrubber.

5.7 Hygiene

One of the main goals related to biological aeration treatment was to improve the hygienic quality of the slurry (IV). Hygienic quality measurements consisted of commonly applied methods based on enumeration of enteric indicator organisms by cultivating samples on a specific agar and defined temperature for a certain time period. Pathogen reduction can be reached by various means, although the method was not directly addressed towards pathogen reduction. The successive procedures have had a hygiene increasing effect (e.g Böhm, 2008). The hygiene influences of treatment steps and their ability to inactivate pathogens are evaluated below.

Solids separation

In the present study, hygiene indicator organisms were analyzed in dairy slurry after the solids separation step (IV). Solids separation probably improved hygiene in the liquid fraction as indicated by the lower levels of indicator organisms compared to the reported average counts in raw slurry (IV). However, in some studies pathogens have been found both in the solids and in the liquid fractions of the source separation systems (e.g. Letourneau et al., 2010), indicating that solids separation does not necessarily lower the indicator organism counts in the liquid fraction. The other mechanisms that probably resulted in lower counts were related to storage time before slurry feeding in a slurry treatment system (IV). However, high solids content and low temperatures have been found to promote survival of pathogens (Strauch, 1991). According to Jones (1982), survival was greatest at temperatures below 10°C and in slurries containing more than 5% solids.

Aeration treatment in a series of continously fed aerated tank reactors

The obtained results after biological aeration treatment revealed good hygienic state although the reduction percentages reached were not high, particularly in the case of dairy manure due to its relatively low initial enteric counts (IV, Fig. 11 and Table 18). This was in agreement with the results of Heinonen-Tanski (1999). Aeration treatment has been commonly been applied to achieve reduction in the counts of intestinal bacteria or viruses (e.g. Heinonen-Tanski et al., 2006; McGarvey et al., 2007). Pathogen reduction during aeration was probably due to a variety of factors such as O_2 sensitivity, temperature, high concentration of free ammonia, and high pH (e.g. Jenkins et al., 1998).

Martens and Böhm (2009) concluded on the basis of data presented by Meyer (2001) that aeration treatment should be operated in at least two vessels connected in series in order to achieve a sufficient exposure time and to avoid hydraulic short circuits during the addition and removal of slurry during operation. Based on this conclusion, one could expect a six treatment tank system connected in series to provide a means for achieving a good hygiene result. The present aerated treatment system also contributed to aspects such as pH shift, high redox potential and antagomism, which were listed by Martens and Böhm (2009) as factors expected to lead to a more or less rapid inactivation of pathogens.

Ammonia stripping

The effluent solution after ammonia stripping was not evaluated based on hygiene indicator measures. However, increased temperature has been found to be the most effective factor in pathogen inactivation (Martens Böhm, 2009). Although the residence time in ammonia stripping was relatively short, the repeated chemical additions (pH shift) were agood hygienization verification. However, further research is needed on the effect of ammonia stripping on hygienization.

5.8 Applicability of the developed manure treatment technology

The aim of this section is to evaluate the novel treatment system (I–IV) in a broader sense and to analyze its practical advantages. Benefits and weaknesses of the process steps are considered.

Solids separation – effect on manure use as a fertilizer

The obtained results demonstrated the difficulty to get all the material treated. It was evident that part of the solid material of both raw swine and dairy slurry was sedimented on the bottom of the manure storage tank and into a container before the manure was pumped forward to the first treatment tank (I–III). Treating all the slurry requires efficient mixing in the storage and reservoir tanks from where it is pumped forward in the process.

Mechanical separation of dairy manure did not affect the manure N content although it lowered the manure P content slightly (III). Therefore, as N was the limiting factor when applying dairy manure on ley, separation had no influence on dairy manure application rates (Table 19). Sedimentation was a more efficient means to lower the swine manure solids and P content (I), contributing significantly to the permitted fertilizer application rates. Nitrogen being the limiting factor, slurry manure application rate was increased from 75 tn/ha to over 112 tn/ha (Table 19). However, as stated in previous studies (e.g. Ndegwa et al., 2001), slurry dry matter content has a strong influence on the obtained sedimentation result, and as manure solid content is increased over 2% the sedimentation result is degraded. In the present study, the used swine manure differed considerably in its average slurry manure TS composition (3.5%, Table 2). Thus, in practice the requisite TS reduction might be difficult to reach by sedimentation.

Biological aeration treatment – odor emissions and hygienic quality of manure

Biological aeration treatment influenced in particular manure odor (I) and hygienic quality (IV). Odor has been a major factor causing complaints and it has a particular relevance to environmental permit issues concerning barn expansions or construction of new buildings. Odor emissions can be significantly reduced by reducing the odor potential of slurry manure. Preliminary results of swine manure showed that biological treatment reduced the odor intensity to a level of no odor or only a very faint odor (I). The aeration treatment was probably able to reduce the odor emissions originating from the manure storage. The odor problem is particularly intense at the time of application and this approach would greatly facilitate odor reduction. This study did not reveal whether the achieved odor reduction continues after storing (aerated slurry). This issue requires further research.

The other major advantage was that the slurry after aeration treatment had a clearly reduced content of pathogenic organisms (IV). Although manure storage has a hygiene improving influence, pathogens may survive for long periods. Pathogens are likely to survive viable even longer, for several months or even years, in soils where they are protected from exposure to UV radiation and desiccation (e.g. Nicholson et al., 2005).

Biological treatment was observed to reduce the viscosity, improving the pumping properties of slurry. This can probably also affect the spreading of manure on fields by improving the infiltration of effluent into the soil and providing for irrigation of effluent. On the other hand, the high pH of aerated slurry manure may promote ammonia emissions during manure spreading. After biological treatment, nitrogen remained the limiting factor and application rates were only increased slightly to 130 t ha^{-1} at the best (Table 19).

The major drawback related to aeration treatment is the required energy consumption (Westerman and Zhang, 1997). Therefore, it is essential to relate the aeration to the actual oxygen need based on real time ORP measures in treatment tanks (III). This enables the achievement of good treatment results by using low aeration rates, as was shown in the present study (I, III). By insulating the treatment reactors, the heat energy released during the process has been used for hygienization (Juteau, 2006). The captured heat energy can alternatively be exploited by using heat pump technology for e.g. heating the farm buildings.

In the present study nitrous oxide (N_2O) emissions were not measured. Normally nitrous oxide (N_2O) is formed by biological denitrification under anaerobic conditions, but N_2O as well as nitric oxide (NO) can also be formed as a by-product of the microbial nitrification. Béline et al. (1999) reported that relatively high N_2O emissions were observed during aerobic biological treatment of livestock effluents (up to 20% of the total N). However, these emissions could be reduced to almost zero if good treatment conditions were applied (Béline and Martinez, 2002; Loyon et al., 2007). Based on the observed carbon reductions (Table 17), it was evident that carbon dioxide emissions occurred during aeration treatment. However, with limited aeration, the effort was to curb substantial carbon losses (I). Further research is needed to optimize the aeration level with regard to avoiding nitrogen oxide emissions while evading substantial carbon dioxide emissions and maximizing the heat energy released.

Air stripping – N removing

The obtained results showed that, regarding manure application rates as fertilizer, the greatest benefits were achieved already during pre-treatment of solids separation, which clearly reduced the manure P content (Table 19). Continuing the treatment with biological aeration and thereafter by sequential stripping allowed efficient nitrogen separation, reducing the manure N content by 70% (II) and enabling the manure N fixation into mineral form. The N- and P-poor reject can, for example, be sprinkled, on the fields nearby a farm using a high application rate (260 t/ha, Table 19).

Table 19 Nutrient contents of separated fractions (I–III, Supplementary data) and corresponding manure application rates when manure is utilized as a fertilizer for silage ley in the establishment year.

Manure	Fraction	TS	N_{tot}	NH_4^+	P_{tot}	Application rate, N limit[a)] (t/ha)	Application rate, P limit[a)] (t/ha)	Criterion application rates[b)] (kg/ha)	
		(kg/ton manure)							
Dairy	Raw manure	44.0	2.2		0.40	78	90	N 170	P 36
III	Separated liquid		2.2		0.35	78	103		
	Separated solid								
	After aeration&stripping no MgO	1.0		0.23	162	157			
	After aeration&stripping+MgO	1.2		0.09	146	405			
Swine	Raw manure	18.5	2.3	1.6	0.45	75	80	N 170	P 36
I	Slurry after sedimentation	5.5	1.5	1.4	0.08	113	444		
	After aeration	4.9	1.5	1.3	0.05	114	706		
II	Slurry after sedimentation	10.1	1.8	1.5	0.25	94	143		
	After aeration	7.0	1.5	1.3	0.17	110	217		
	Slurry after sedimentation*	9.9	1.9	1.6	0.49	87	73		
	After stripping	7.5	0.7	0.5	0.04	260	850		
Suppl.	Slurry after sedimentation	6.0	1.5	1.3	0.10	110	374		
data	After aeration	5.1	1.3	1.2	0.07	130	557		

a)Application rate according to the N and P contents of the slurry manure at different treatment stages
b)Criterion application rates of N and P for ley according to the Agri-Environmental protection Act. P rates on ley on establishment year when soil P-value is fair and ley is sown with cereal
*calculated mean from the sequence stripping procedure results II: Table 2.

6 Conclusions

A new biological manure treatment scheme was proposed and evaluated on a pilot scale. The treatment consisted of a biological treatment operated in a specially designed reactor regime using 600-L tanks followed by ammonia separation conducted by ammonia stripping.

Biological treatment in a series of continuously fed tanks applying limited aeration accelerated humification of the organic matter of slurry manure and increased the manure pH. Reduced odor and improved hygienic quality of slurry manure were also among the achieved results of the treatment. The designed system was operated effectively with HRT (hydraulic retention time) being 3 to 7 days when the DM (dry matter) content of manure was reduced by pretreatment to below 2%. The optimum redox profile of a stable system followed an increasing trend.

It was shown experimentally that the designed treatment system can be loaded with a 3 to 4 days HRT value, if the DM content of the slurry manure is low enough (in swine manure less than 1%). The treatment system was loaded with mechanically separated dairy slurry manure (DM 1.5 to 2%). In this case, the system could be loaded with a maximum of 350 l per day, corresponding to 6.8 days HRT-value. The results obtained suggest keeping the redox values of the treatment tanks ideally at zero or slightly above in the 1st tank, at around 100–180 in the 3rd tank and at 180–200 mV in the 6th tank.

It was shown that several benefits could be achieved when using a series of continuously fed aerated tank reactors and feedback. The serial system proved to be stable and resistant to process disturbances, and process failure was easily predictable and thus easy to control. The efficiency of the treatment increased by feedback via a number of tentative mechanisms: feedback dilution probably enhanced carbon reduction and feedback probably stabilized and enhanced microbial function in the first tank.

Due to limited aeration and feedback, some changes in the carbon and nutrient contents of the slurry manure were observed during biological treatment. Carbon reduction varied in different studies between 11% and 57% and depended on the initial carbon content. Nitrogen changes occurred mainly due to ammonia volatilization and varied from 0 to 15 % in swine manure. In dairy manure, an average of 52% maximum decrease was observed when ammonia-reduced feedback was conducted after air stripping. Nitrate formation was low with the aeration rates used. The concentrations of the total phosphorus and divalent ions were also decreased. This was suggested to be due to the humic substances formed during biological treatment. Humic substances behave like weak acid polyelectrolytes and the occurrence of anionic charged sites accounts for the ability to retain cations.

The most marked advantage of the biological treatment was its effect on odor and hygiene. Odors were reduced efficiently to an insignificant level or only very faint odor was observed after four days of treatment. It was also shown that the six tanks in series configuration with feedback served as a good means to achieve a good hygiene result of the treated manure. At best, over

90% reduction was observed in the numbers of enteric indicator organisms.

It was shown that the buffer system resisting pH change in slurry manure was composed of TAN (total ammoniacal nitrogen), CO_2, HCO_3^- and $CO_3^{2-}$and can be circumvented by making use of the pH increase obtained by biological treatment, thus reducing the chemical consumption. Over 30% TAN removal by air stripping was shown to be possible without chemical use, if the pH of the biologically treated swine manure was above 8.9. It was shown that biological aeration treatment and thereafter N separation conducted by sequential stripping allowed efficient nitrogen separation by reducing the manure N content by 70%.

In the future, it would be interesting to study the preparation of seeding material, the effect of seeding and changes in microbial community composition in a serial system in more detail using the latest analyzing tools available (e.g. using 454 pyrosequencing technology). It would also be interesting to study the changes between the different treatment tanks on a compounds level (e.g. using metabolomic techniques) and focusing on humification to examine changes in molecular sizes and relationships with odor reduction.

As a final conclusion of this study, the importance of deep understanding of the various biological and physicochemical factors involved in complex raw material cannot be overestimated when developing manure treatment technologies. Combination of these factors may provide new insights in development work and provide advantages (such as reducing chemical consumption and energy demand) in manure treatment technologies.

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