Pig manure treatment by membrane filtration

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DISSERTATION

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- *Prantik Samanta*

Abstract

Intensive livestock farming has negatively impacted the environment by contributing to the release of ammonia and nitrous oxide, groundwater nitrate pollution and eutrophication of rivers and estuaries. In addition, the livestock husbandry and biogas digestate are considered as one of the biggest antibiotic resistance gene reservoirs which are emerging as one main threat to worldwide human health and are expected to kill 10 million people by 2050. On contrary, nutrient rich manure has always been a major focal point of resource recovery. The goal of the dissertation was to quantify the nitrogen pollution, caused by livestock farming industry, followed by nutrient recovery and antibiotic resistance genes (ARGs) removal from manure by using microfiltration (MF) -nanofiltration (NF) treatment train.

Nitrogen loss to the environment per unit meat production was found directly proportional to the virtual nitrogen factors. The relationship between total nitrogen intake and the corresponding nitrogen loss per kg meat production was found linear as well. The average nitrogen loss was calculated 150 g per kg poultry meat production. This raised to an average of 180 and 350 g per kg pork and beef production. Finally, it was found that 7000 kWh energy would require to recover the total ammonium nitrogen from beef manure per 1 Mg meat production when considering zero liquid discharge approach.

The efficiency of MF followed by the vacuum evaporation (VE) to produce ammonia water was evaluated as well. MF results showed the total suspended solids (TSS) removal above 98%. Chemical oxygen demand (COD) and total phosphorus (Tot-P) removal were found above 80%. However, nearly 80% of the ammonium nitrogen (NH_4^+-N) was recovered in the MF permeate. Thereafter, the VE of MF permeate resulted in substantial 31 gL^{-1} of NH_4^+-N condensate concentration, which was nearly 12 times higher than the initial NH_4^+-N concentration of MF permeate.

On the other hand, manure filtration by MF-NF treatment train was noticed to produce nutrient rich separate streams in reduced volumes. MF removed TSS above 98%. The COD and Tot-P retentions were found above 60 and 80% respectively, within a reduced volume which accounted for 40% of the initial MF feed volume. The NF of MF permeate by NF270 showed most promising results by concentrating overall 50 and 70% of the TN and potassium (K) within a further reduced volume.

Lastly, total 189 ARGs in raw manure and digestate samples were identified and quantified. The highest reported total ARG copy numbers in a single manure sampling site was found 1.15×10^8 copies /100 µL. The absolute concentrations of 37 ARGs were above 10^5 copies /100 µL. Highly concentrated ARGs (except aminoglycoside resistance ARGs) in feed presented high log retention value (LRV) from 3 to 5 after MF-NF treatment process. Additionally, LRV below 2 was noticed where the initial absolute ARG concentrations were ≤ 10^3 copies /100 µL.

Overall, this study demonstrates the staggering nitrogen pollution due to the meat production and urges the need for re-evaluation of market price of the meat. Initially, the VE of MF permeate compared to the raw manure and MF concentrate was found to be a viable alternative to recover nutrient and produce cleaner and concentrated ammonia water. Consequently, the MF-NF treatment train showed the ability of particle and pathogen free product water production. This could be reused in farms to wash barns, to irrigate nearby cultures or could be applied to specific fields based on the demand. However, some ARGs (tetH, strB) could still be found within the permeate of NF with up to 10^4 copies /100 µL. This calls for further investigations in future.

Zusammenfassung

Die intensive Viehhaltung hat sich negativ auf die Umwelt ausgewirkt, da sie zur Freisetzung von Ammoniak und Lachgas, zur Nitratverschmutzung des Grundwassers und zur Eutrophierung von Oberflächengewässern beiträgt. Darüber hinaus gelten die Tierhaltung und Produktion von Biogas aus Gärresten als eines der größten Genreservoirs für Antibiotikaresistenzen, die sich zu einer der größten Bedrohungen für die menschliche Gesundheit weltweit entwickeln und bis 2050 voraussichtlich 10 Millionen Menschen das Leben kosten werden. Im Gegensatz dazu war nährstoffreiche Gülle schon immer ein wichtiger Schwerpunkt der Ressourcenverwertung. Ziel der Dissertation war es, die durch die Viehwirtschaft verursachte Stickstoffverschmutzung zu quantifizieren und anschließend die Nährstoffrückgewinnung und die Entfernung von Antibiotikaresistenzgenen (ARGs) aus Gülle durch den Einsatz von Mikrofiltration- (MF) und Nanofiltration- (NF) zu untersuchen.

Es wurde festgestellt, dass der Stickstoffverlust in die Umwelt pro Einheit Fleischproduktion direkt proportional zu den virtuellen Stickstofffaktoren ist. Die Beziehung zwischen der Gesamtstickstoffaufnahme und dem entsprechenden Stickstoffverlust pro kg Fleischproduktion wurde ebenfalls als linear identifiziert. Der durchschnittliche Stickstoffverlust wurde mit 150 g je kg Geflügelfleischproduktion berechnet. Dieser Wert erhöhte sich auf durchschnittlich 180 und 350 g pro kg Schweine- und Rindfleischproduktion. Schließlich wurde festgestellt, dass 7000 kWh Energie erforderlich sind, um den gesamten Ammoniumstickstoff aus Rinderdünger pro 1 Mg Fleischproduktion zurückzugewinnen, wenn der Ansatz der Nulleinleitung berücksichtigt wird.

Die Effizienz der MF gefolgt von der Vakuumdestillation (VE) zur Herstellung von Ammoniakwasser wurde ebenfalls bewertet. Die MF-Ergebnisse zeigten, dass die Gesamtmenge der suspendierten Feststoffe (TSS) zu über 98 % entfernt werden. Der chemische Sauerstoffbedarf (CSB) und die Gesamtphosphorentfernung (Tot-P) lagen bei über 80 %. Allerdings wurden fast 80 % des Ammoniumstickstoffs (NH_4^+-N) im MF-Permeat zurückgewonnen. Danach führte die VE des MF-Permeats zu einer erheblichen NH_4^+-N Kondensatkonzentration von 31 gL^{-1}, die fast 12-mal höher war als die ursprüngliche NH_4^+-N Konzentration des MF-Permeats.

Andererseits wurde festgestellt, dass die Güllefiltration mittels MF-NF-Behandlung nährstoffreiche separate Ströme in geringeren Mengen erzeugt. MF entfernte TSS zu über 98

%. Die CSB- und Tot-P Retentionen lagen bei über 60 bzw. 80 % in einem reduzierten Volumen, das 40 % des ursprünglichen MF-Zufuhrvolumens ausmachte. Die NF von MF-Permeat durch NF270 zeigte die vielversprechendsten Ergebnisse, indem insgesamt 50 und 70 % des TN und Kaliums (K) in einem weiter reduzierten Volumen konzentriert wurden.

Schließlich wurden insgesamt 189 ARGs in Rohgülle- und Gärrestproben identifiziert und quantifiziert. Die höchste gemeldete ARG-Kopienzahl in einer einzigen Gülleprobe wurde mit $1{,}15 \times 10^8$ Kopien/100 µl festgestellt. Die absoluten Konzentrationen von 37 ARGs lagen über 105 Kopien /100 µL. Hochkonzentrierte ARG (mit Ausnahme von aminoglykosidresistenten ARG) in Futtermitteln wiesen nach dem MF-NF-Behandlungsprozess einen hohen log-Retentionswert (LRV) von 3 bis 5 auf. Darüber hinaus wurde ein LRV von unter 2 festgestellt, wenn die anfänglichen absoluten ARG-Konzentrationen ≤ 10^3 Kopien /100 µL waren. Es wurde daher festgestellt, dass die ARG-Entfernung direkt proportional zu ihrer Ausgangskonzentration ist. Folglich konnten einige ARG (tetH, strB) mit bis zu 10^4 Kopien /100 µL noch im Permeat von NF gefunden werden.

Insgesamt zeigt diese Studie die sehr hohe Stickstoffbelastung der Umwelt durch die Fleischproduktion und drängt auf eine Neubewertung des Marktpreises für Fleisch. Zunächst wurde festgestellt, dass die VE von MF-Permeat im Vergleich zu Rohgülle und MF-Konzentrat eine praktikable Alternative zur Rückgewinnung von Nährstoffen und zur Erzeugung von sauberem und konzentriertem Ammoniakwasser darstellt. Folglich zeigte die MF-NF-Behandlung die Fähigkeit, partikel- und pathogenfreies Produktwasser zu produzieren. Dieses könnte in landwirtschaftlichen Betrieben zum Waschen von Ställen oder zur ortsnahen Bewässerung wiederverwendet oder je nach Bedarf auf bestimmten Feldern eingesetzt werden.

Publications

Chapter 2, 3, 4 and 5 of this dissertation have been published in the first, second, third and fourth of the following peer reviewed articles, respectively.

Samanta, P., Horn, H., Saravia, F. (2022) Impact of Livestock Farming on Nitrogen Pollution and the Corresponding Energy Demand for Zero Liquid Discharge. Water 14 (8), 1278. https://doi.org/10.3390/w14081278

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List of abbreviations

AB	antibiotic
AMR	antimicrobial resistance
AR	ammonium nitrogen recovery
ARBs	antibiotic resistant bacterias
ARGs	antibiotic resistance genes
blaTEM	beta-lactamase gene
$C_{manure\ N}$	nitrogen concentration in manure
$C_{manure\ NH4\text{-}N}$	ammonium nitrogen concentration in manure
COD	chemical oxygen demand
DOC	dissolved organic carbon
DTN	dissolved total nitrogen
ED	energy demand
EU	European Union
K	potassium
LRV	log retention value
MF	microfiltration
MWCO	molecular weight cut-off
N	nitrogen
NC	nitrogen content
NF	nanofiltration
NH4+-N	ammonium nitrogen
NL	nitrogen loss

NM	nitrogen loss in manure
P	phosphorus
PWP	pure water permeability
QM	quantity of manure
RO	Reverse Osmosis
t	time
TC	total carbon
TN	total nitrogen
TNI	total nitrogen intake
Tot-P	total phosphorus
TSS	total suspended solids
UF	ultrafiltration
VSS	volatile suspended solids
ZLD	zero liquid discharge

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1. Overview – theoretical background and objectives

1.1. Nitrogen (N) pollution due to livestock farming

1.1.1. Wasteful N management within the European Union (EU)

Global rates of human fixation of atmospheric nitrogen (N) to reactive N have increased 20 times over the past century. This transformed the global N-cycle at a striking pace (Galloway et al., 2008). The safe boundary for anthropogenic N input is hypothesized to be exceeded by a factor of 3.5 (Rockström et al., 2009). Galloway et al. (2008) viewed the complexity of the N cycle and the close relation between the production and consumption of the reactive N via food and energy. They stated that an integrated interdisciplinary approach is required and proper strategies to be developed to optimize the need for N as a key human resource and decrease the N containing waste (Galloway et al., 2008).

The invention of the Haber-Bosch process increased the use of industrial fertilizers in agriculture and in the energy industry as well. This has raised 3 times the production of the reactive N in the EU. Approximately half of the annual reactive N input to the EU is lost as reactive N to the environment. This causes adverse impacts such as health damage due to NOx release and the eutrophication of terrestrial and aquatic ecosystem. The other half is lost to the atmosphere as unreactive N. This wasted the energy investment in the initial fixation of atmospheric inert N. The reactive N input and output increased by 4 and 2.5 times, respectively in 2000 than compared to the 1900 (Van Grinsven et al., 2013). The damage due to N pollution in EU has been calculated €70 - €320 billion. It is also expected to raise significantly by 2050 (Sutton et al., 2011a, Bodirsky et al., 2014). The key flows of reactive nitrogen in Germany is presented in Figure 1.1 (adapted from Umweltbundesamt (2014)).

The use of N fertilizer in the EU increased from 1-2 Tg around 1950 to 11 Tg around the year 2000 (Bouwman et al., 2013). The EU could use a large part of its cereal production for animal feed as the cereal yield per hectare increased rapidly at that time (Garibaldi, 2012). This in combination with large import of protein and energy rich feed stuffs allowed the strong growth of the pig and poultry sector after 1950. Per capita animal product consumption in the EU between 1960 and 2007 increased by 50% and doubled relative to 1900 (Westhoek et al., 2011, Smil, 2002). This has significantly increased the meat consumption N footprint, which provides the measure of reactive N lost to the environment per unit meat consumption (Leach et al., 2012, Klement et al., 2021).

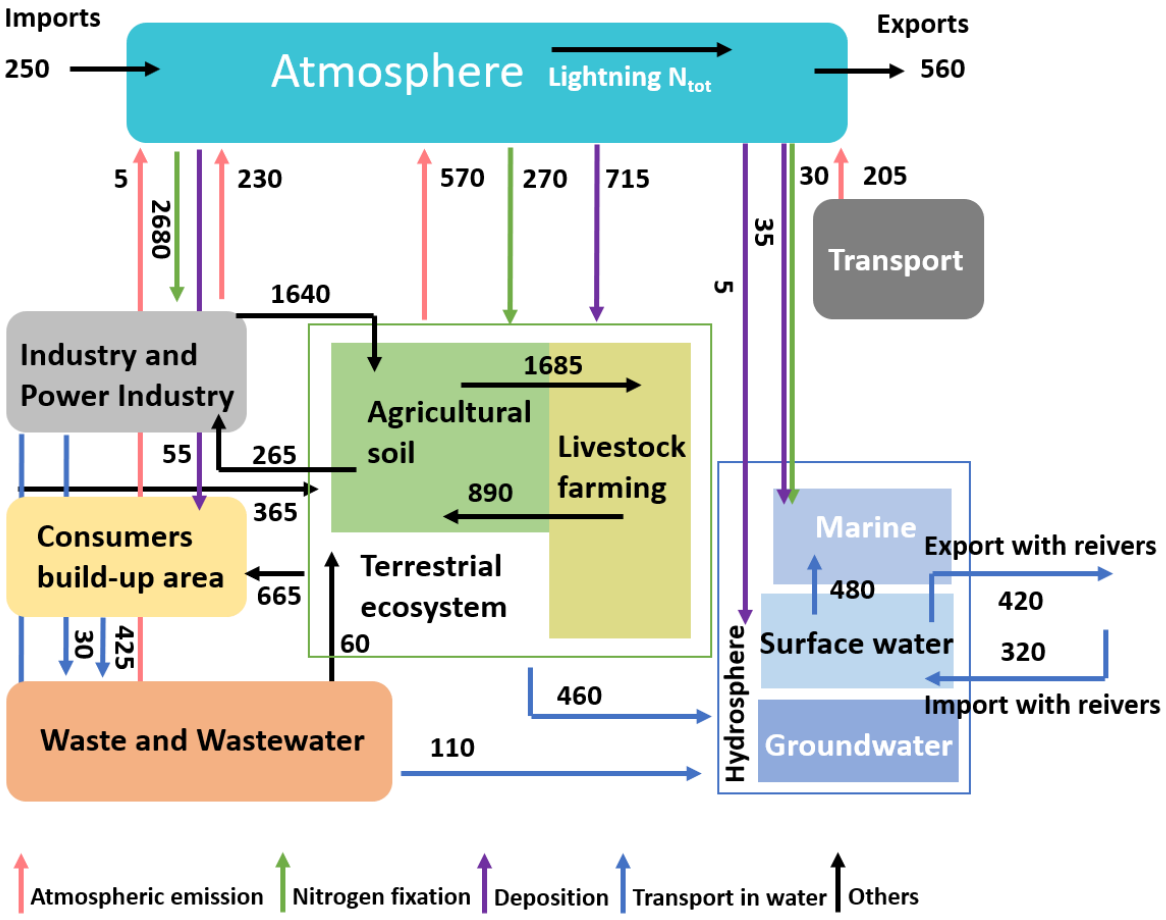

Figure 1.1. *The key flows of reactive nitrogen in Germany. All values are presented in Gg N year^{-1}. Where data are available, mean value between 2008-2010 are given. In other cases, the value for 2010 are given as the last available value. The surface water data are given for the period 2006-2011 and the data between 2005-2007 are given for the atmospheric deposition (adapted from Reactive Nitrogen in Germany, Umwelt Bundesamt 2014).*

1.1.2. Contribution of livestock manure on N pollution

Approximately, 80% of the total N footprint has been calculated as food N footprint (Godfray et al., 2010). The majority of the food N footprint was predicted as beef, pork and poultry meat nitrogen footprint (Liang et al., 2016, Shibata et al., 2017). According to literature sources, 50% - 80% of the meat N footprint is lost to soil and aquatic environment via manure (Bodirsky et al., 2014, Leach et al., 2012, Liang et al., 2016). Manure is a major source of air pollution (due to NH_3, N_2O, NOx emission) and threat to the aquatic environment (due to NO_3 leaching) in areas with high density of livestock (Steinfeld et al., 2006, Sutton et al., 2013).

Manure has a low concentration of plant nutrients. Hence, the cost of handling manure to avoid nutrients loss can therefore be higher than the cost of the mineral fertilizers. Consequently, the often mismanaged manure is the source of the air, ground and surface water pollution (Sommer and Knudsen, 2021). Animal manure N is generally in inorganic ammonium and organic forms. Subsequently, the manure N fertilizer value is lower and more variable than the commercial fertilizers. Therefore, manure organic N should be mineralized before it is available to the plants. The transformation generally happens during and outside the crop growing season. Hence, the farmers should know the availability of the manure N to crops during the seasonal growth. This ensures the right amount of the manure and the supplementary mineral fertilizer applied to the field in order to eliminate overfertilization and leaching losses (Sommer and Knudsen, 2021).

1.1.2.1. Nitrate pollution by uncontrolled manure application

Discharge of manure directly to the surface water was forbidden in 1949 but was not controlled. Until the 1980s, overflowed liquid manure from stores was discharged to the surface waters. This caused the eutrophication and depletion of oxygen in rivers and estuaries (Giola et al., 2012, Mantovi et al., 2006, Neal and Heathwaite, 2005, Clarisse et al., 2009). In addition, drinking water can also negatively be affected by nitrate concentrations, when extracted from polluted water bodies (Kastens and Newig, 2007).

Hagebro et al. (1983) showed the increase in nitrate concentration in Danish rivers and groundwaters during the 1980s (Hagebro et al., 1983). This made a continuous growth in the extent and persistence of eutrophic, hypoxic and anoxic coastal waters during that time period (Sommer and Knudsen, 2021). Another research work by Kastens and Newig (2007) in Lower Saxony of Germany during March 2005 reported that, the 86 groundwater bodies were found polluted by nitrate due to diffuse nitrate pollution by agriculture, out of the total 129 groundwater bodies, which were inspected. This problem was mentioned to be held particularly in the Hase catchment area, which was known as the most intensive livestock farming region. Consequently, the surface water bodies in Lower Saxony was reported to have high nitrate concentration as well (Kastens and Newig, 2008).

1.1.2.2. Ammonia (NH_3) emission due to manure application

The increased field application of manure N and mineral fertilizer increased the amount of NH_3 emission too. This caused severe algal blooms, anoxic conditions and killed fishes. Agriculture contributed 70% of the total NH_3 input to Danish coastal waters in 2007-2011

and leaching and runoff of contaminated groundwater (Prahastuti et al., 2019). Emission inventories showed that livestock housing, stored manure and the applied manure released 70 to 80% of the anthropogenic NH_3 emission in Europe (Kirchhoff et al., 2013, Beusen et al., 2008). NH_3 forms particles in the atmosphere, which is considered as serious health hazard (Giannadaki et al., 2018). Consequently, the depletion of the particulates and the gaseous NH_3 may cause the eutrophication of natural ecosystems as well (Duce and Tindale, 2008).

1.1.3. The EU regulations on manure application

The requirement of minimum land area for the application of manure was implemented in 1986. The intention was to limit the excessive application of plant nutrients. However, from 2009, the regulation has focused on meeting the requirements of the EU Water Framework Directive. This changed the focus from reducing N leaching from the root zone to targeting N fluxes to coastal water.

The regulations are laid down by law to fulfill the objectives in the nitrate and the Water Frame Directive and to meet good ecological conditions for ground and surface waters (N.N., 2020). At the farm, the supplied N in animal manure to fields must not exceed 170 kg N ha^{-1} $year^{-1}$. The number increased to 230 kg N ha^{-1} $year^{-1}$ for the farms, where 66% of the N is in cattle manure and the crop cover is a minimum 80% beets, grass, cereals or catch crops. The fields must be owned or rented by the farm who has a signed agreement to apply the slurry within the regulations given by the law (Sommer and Knudsen, 2021).

The EU has fixed the NH_3 emission level too. This is currently at 60,000 t NH_3-N $year^{-1}$ (De Marco et al., 2019). The emission of NH_3 may significantly affect the deposition of NH_3 at distances up to 300–500 m from livestock housing. This leads to tighter reduction in emission if there are "N-vulnerable" ecosystems nearby (Anker et al., 2019). The intention is to bring down the NH_3 deposition below the critical load capacity of the ecosystem. Increase in livestock production should not violate the requirements to keep the deposition of NH_3 to the ecosystems below the critical load capacity. The most vulnerable ecosystems possess a deposition limit span within 0.2 - 1.0 kg NH_3 ha^{-1} $year^{-1}$ (Vries et al., 2010, Fisher et al., 2007).

1.2. Livestock farming as the hub of antimicrobial resistance (AMR)

1.2.1. The occurrence, development and transmission of AMR

The world has not yet seriously taken the threat of pandemics and their environmental dimensions. The COVID-19 pandemic is a wake-up call to elevate the safety measures against

infectious diseases and their environmental dimensions (Pachauri et al., 2021). This led to the concern of another hidden pandemic. AMR is one of the main threats to global health and risks. It is adversely affecting the overall environmental sustainability which can result into catastrophic consequences (Murray et al., 2022).

1.2.1.1. What is AMR and how does it develop?

Antimicrobials are agents such as antibiotics, antiviral agents and parasiticides, which aimed to inhibit the growth of microbes. Other products such as antiseptics, pharmaceuticals and disinfectants may possess antimicrobial properties as well. The bacteria, parasites, viruses and fungi when become resistant to the antimicrobial treatments, then AMR occurs. Antimicrobials are widely used in human health care and crop and animal production as well (UN, 2022).

Overuse of antimicrobials along with the other stressors' factors (e.g. heavy metals and other pollutants) help the resistant microbes to develop in favorable conditions such as the digestive tracks of human and animals or in environmental media such as sewage, water, soil and air (Wales and Davies, 2015, Baquero et al., 2019). Furthermore, Resistance microbes, especially bacteria can cause the further development of resistance into the non-resistance bacteria. Strong evidences can be found regarding the inefficiency of the antimicrobials to cure infections, which signifies even more the AMR threat to the human, animal and plant health in the coming years (UN, 2022).

1.2.1.2. Transmit and spreading of AMR in the environment

Presence of any antimicrobial substance is not mandatory for the horizontal and vertical AMR transmission. The transmission of antimicrobial resistant microbes and antimicrobial genes (ARGs) were found to be enhanced, when the pollutant barriers are lacking severely. Such as toilets without defining barriers, usage of wastewater for irrigation and the crop fertilization with untreated manure. Thereafter, the ARGs can enrich and pioneer the spread of AMR (Bengtsson-Palme et al., 2018, Berendes et al., 2020).

Chemical pollution with antimicrobial activity (e.g. metals, pharmaceuticals and other compounds) are suspected to enhance the ARG mobilization in certain microbes or promote AMR in microbes in the environment. Consequently, air, water and soil then serve as the commuter of AMR pollution among people, animals and other environmental reservoirs (Murray et al., 2022). Figure 1.2. described the spreading of AMR in the environment.

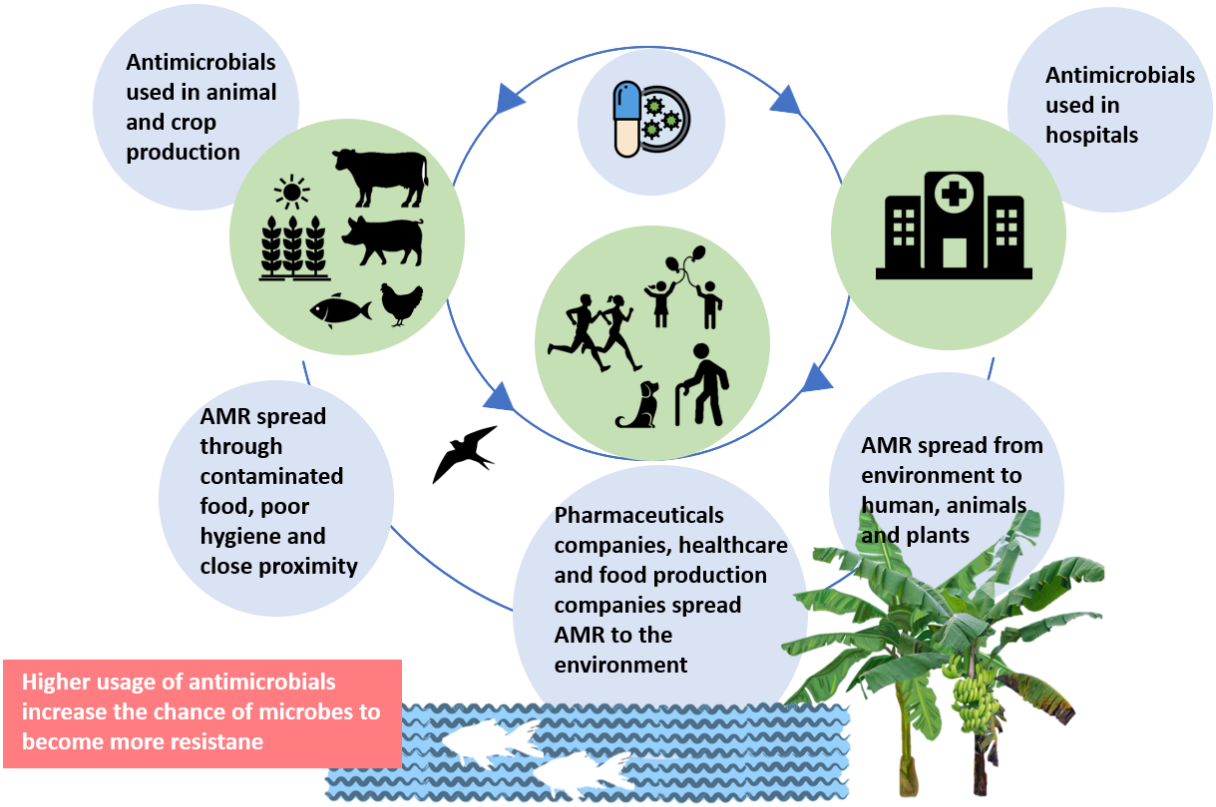

Figure 1.2. *Spreading of antimicrobial resistance in the environment (adapted from UN environment programme 2022).*

1.2.2. Animal farms as active AMR reservoirs

Antimicrobials are often used in intensive animal production to maintain livestock health, welfare and productivity. Globally, high rise in animal protein demand has led to an overuse of antimicrobials due to their effectiveness in animal growth promotion. Most veterinary antibiotics are poorly adsorbed by the animals. Therefore, a large part of it are excreted (Spielmeyer et al., 2017, Kumar et al., 2005a). Antibiotic resistance traits in manure increases by the substantial use of subtherapeutic level of antibiotics in animal feed itself (Looft et al., 2012, Binh et al., 2008). So far, the variability of the antimicrobials amount, released as an active form via animal manure and urine, is found quite large. It depends on several factors such as the administration route, drug formulation process, the health status of the animal in which it is used and many other factors (UN, 2022, Prescott, 2013).

Manure is considered as one of the major sources of antimicrobial pollution. The overuse of antibiotics in livestock husbandry turns the animal farms into ARG reservoirs (Ji et al., 2012, Whitehead and Cotta, 2013). AMR kill an estimated 700,000 people /year and it is expected to reach 10 million by 2050 (Willyard, 2017). Figure 1.3. showed the number of predicted

deaths caused by AMR compared to the other common causes of death. Unfortunately, ARGs, antibiotic resistant bacteria (ARB) and antibiotic residues are released to the soil, air and aquatic environment, when manure is used as a fertilizer (Marti et al., 2013, Li et al., 2015a, Chen et al., 2016, Beattie et al., 2018, Huang et al., 2019). Therefore, the usage of antibiotics in farms often correlates with the expansion of the related ARGs in human pathogens. This caused the spreading of animal ARBs to human ARBs as well (Forsberg et al., 2012, Smillie et al., 2011, Price et al., 2012). This mainly endangered the farm workers due to their exposure to the hazardous number of antimicrobials, if proper protective gears are not used.

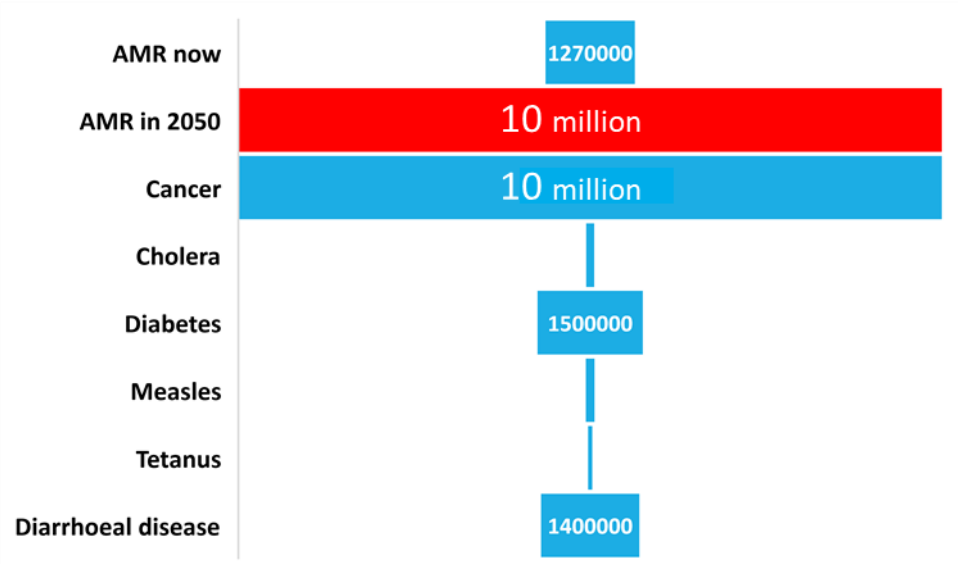

Figure 1.3. *AMR mortality prediction compared to the other common causes of death today (adapted from O'Neill 2016).*

1.3. Importance of manure

Nutrients, such as N, potassium (K) and phosphorus (P) rich manure is popular in agricultural application, in microalgae production (Almutairi et al., 2011, Cho et al., 2011) as well as in biopolymers production, due to its high volatile fatty acid concentration (Albuquerque et al., 2011). Manure is heavily used in biogas industry as well (Raven and Gregersen, 2007, Riaño et al., 2011). In general, the application of nutrient recovery techniques from manure is in high demand. Even though the high solid content, organically bound materials and its potential hazardous properties, make the recovery processes very difficult (Gerardo et al., 2013).

1.4. Nutrient recovery processes from manure

Several nutrient recovery processes from manure have already been reported, such as hydrogel application (Kioussis et al., 1999), calcium phosphate precipitation (Hosni et al., 2008, Lu and Liu, 2010), struvite precipitation (Doyle and Parsons, 2002, Uysal et al., 2010, El Diwani et al., 2007) and ammonia stripping at high temperature (Katehis et al., 1998, Saracco and Genon, 1994, Quan et al., 2009). However, these processes are very complex and require high chemical and energy inputs. An alternative to this processes is to use membranes, which can produce nutrient rich, particle and pathogen free separate streams from pig manure with relatively lower operating and maintenance cost (Masse et al., 2007, Chen et al., 2006).

1.5. Membrane filtration for manure treatment

Over the past years, membrane filtration has proven to be an attractive supplement to solid-liquid separation. Especially, when dealing with large amount of suspended solids, the pressure driven porous membrane can produce purified liquid streams (Masse et al., 2007). Membranes can retain the particles, larger than a particular size, by the application of the transmembrane pressure. Consequently, the smaller particles passes through to the permeate side (Hjorth et al., 2011). Mainly ceramic and polymeric membranes are heavily used in different application processes. Higher flux has can be achieved in the former, while the latter permits a better permeate quality (Zarebska et al., 2015). In addition, two popular filtration methods are used in membrane sphere. These are dead end filtration and cross flow filtration (Figure 1.4.). However, other filtration methods such as semi dead end filtration have been developed in recent years as well (Tuczinski et al., 2018).

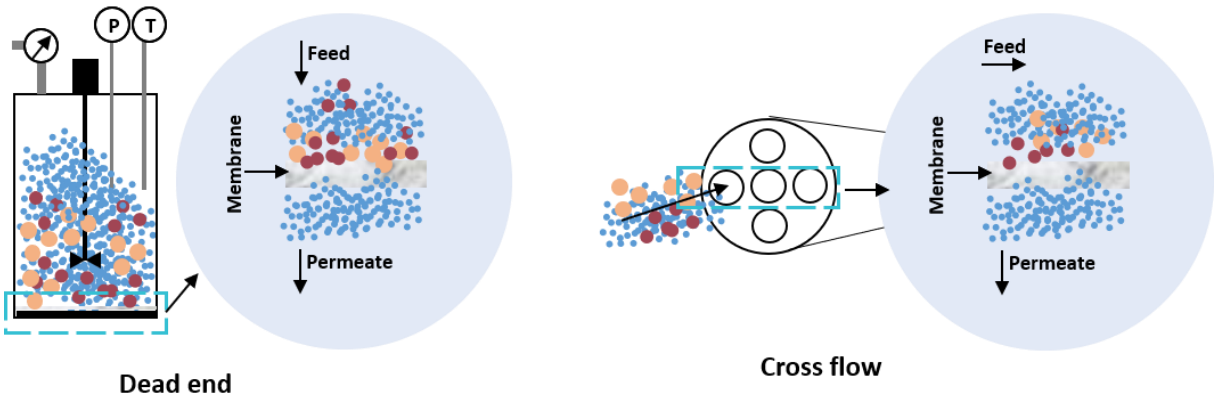

Figure 1.4. *Schematic diagram of dead end and cross flow filtration mechanism.*

1.5.1. Application of microfiltration (MF) for solid-liquid separation of manure

MF generally retains solid particles within the range of 0.1 to 10 µm. Hence, they are well suited to remove nutrients related to particles such as total phosphorus (Tot-P) (Masse et al., 2007, Hjorth et al., 2011). Additionally, dissolve nutrients such as N and K can be collected in the permeate side as well (Chen et al., 2006, Gerardo et al., 2013). However, a very dense cake layer is expected to occur on MF membranes, when dead end filtration is used. It is advisable to use MF membranes in cross flow filtration systems, where a fraction of the liquid is filtered as permeate while solids and other parts of the liquid are retained as retentate. The cross flow is able to remove most of the solids deposited on the MF surface, but some flow control reversible and irreversible fouling cannot be avoided, when dealing with larger feed volume for a longer filtration period. In addition, bacterial growth on MF surface can further reduce the flow through membranes. This is evident for manure treatment. Reversible and irreversible filter cake layer can be completely or partially removed by flushing with water and the application of chemical cleaning processes, respectively (Masse et al., 2007, Hjorth et al., 2011).

Therefore, MF process was selected for the research study. However, ARG removal efficiency by MF is poor and limited mostly to the intracellular ARGs (Slipko et al., 2019, Gros et al., 2019, Lu et al., 2020). In addition, the liquid fraction after solid-liquid separation is enriched with ammonium nitrogen (Christensen et al., 2009), which was found to be one of the major reasons alongside dissolve oxygen to exhibit strongest correlation with high ARG concentration and horizontal gene transfer (Ott et al., 2021). Hence, further treatment of MF permeate is an absolute necessity.

1.5.2. Further treatment of MF permeates by nanofiltration (NF)

The MF permeate after manure treatment generally contain significant amount of dissolved K and ammonium nitrogen (NH_4^+-N). NF is capable of further concentrating the dissolved nutrient in smaller volume and deliver purified water as NF permeate. Depending on the chosen membrane, NF can retain molecules larger than 200-400 Da, and to lesser extent the charged ions such as Ca^{2+}, Mg^{2+} and NH_4^+ (Hjorth et al., 2011). Nevertheless, one of the main advantages of using NF lies in its ARG removal efficiencies. NF are reported to eliminate ARGs above 99.99% (Lan et al., 2019, Slipko et al., 2019).

Hence, additional usage of nanofiltration would not only enhance the ARG removal efficiency, but also these would (a) reduce the volume to be transported, (b) produce

dissolved nutrients rich concentrated stream and (c) generate a purified permeate to be reused in farms for animal washing or irrigation (Al Seadi et al., 2013, Ros et al., 2020, Bonmatí-Blasi et al., 2020, Cerrillo et al., 2015, Tampio et al., 2016, Ledda et al., 2013). However, membrane fouling has always been proven to be the bottleneck when treating feed containing high organic loads and particulates (Hube et al., 2020). Chances of occurring both reversible (Kim et al., 2007) and irreversible (Kimura et al., 2017) have been mentioned previously. Therefore, proper cleaning and membrane fouling control techniques are necessary to improve the filtration performances.

1.6. Objectives of the thesis

The objectives of the thesis are as followed:

(i) To understand the real impact of livestock farming on nitrogen pollution due to substantial amount of manure generation and the corresponding energy demand for its treatment (chapter 2).

(ii) To perform long term MF of pig manure to probe the long-term stability of the filtration performance and solid-liquid separation capacity. Thereafter using VE process to produce concentrated ammonia water (chapter 3).

(iii) To further concentrate the dissolve nutrients from MF permeate using different NF membranes and compare their efficiencies and (chapter 4);

(iv) and to identify and quantify the diverse and abundant ARGs in raw pig manure and biogas digestate samples, followed by removing them using MF-NF treatment train as well (chapter 5).

2. Impact of livestock farming on nitrogen pollution and the corresponding energy demand for zero liquid discharge

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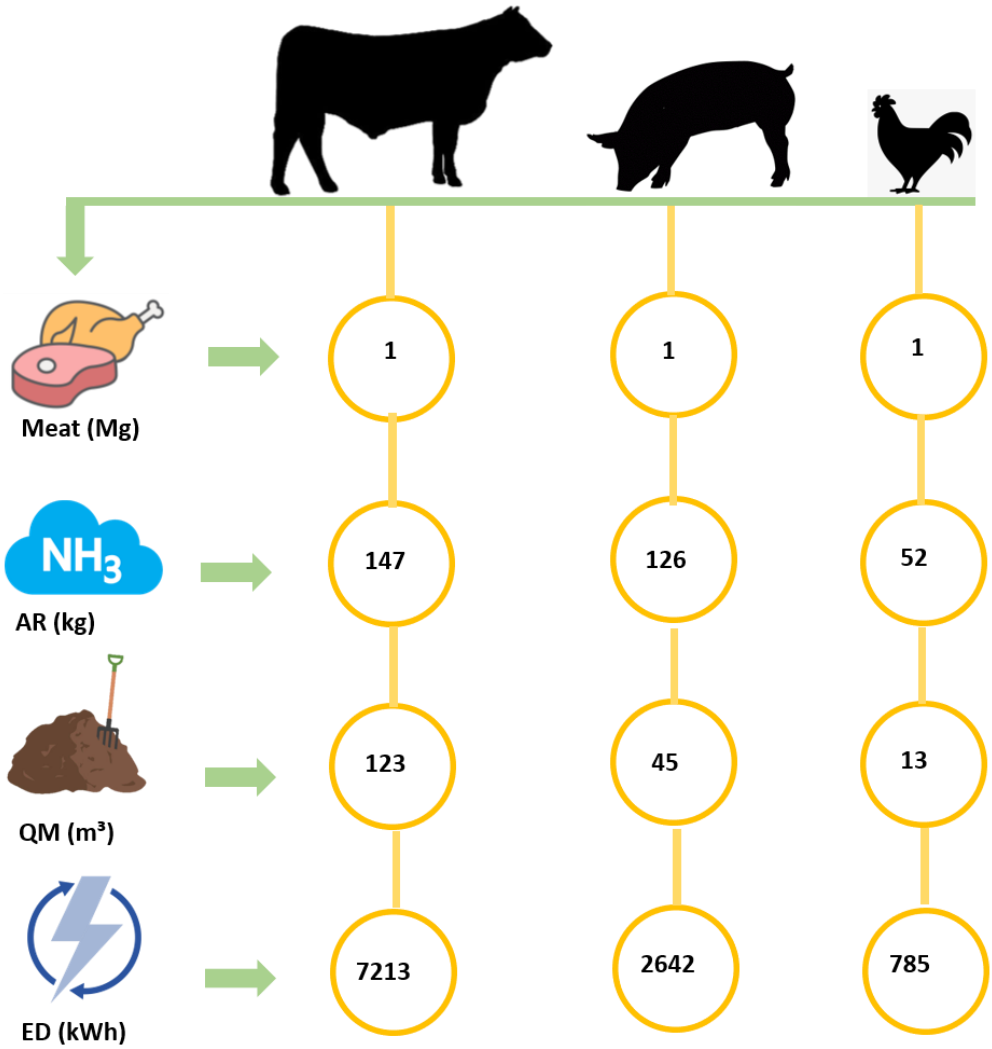

The graphical abstract of the chapter 2

2.1. Introduction

Worldwide anthropogonic release of reactive nitrogen to aquatic bodies and atmosphere have the ecosystem and human health damaging potential which has left the Haber-Bosch process as the main source of nitrogen (Sutton et al., 2011b, Gruber and Galloway, 2008, Galloway et al., 2014, Erisman et al., 2013, Conley et al., 2009). Damage related to nitrogen pollution per year in European Union (EU) has been calculated about €70 to €320 billion euros (Sutton et al., 2011a). Furthermore, nitrogen pollution by 2050 is predicted to rise significantly 102 to 156% of 2010's value and can only be controlled under strict measurement applied by individual nations (Bodirsky et al., 2014). Nitrogen foot print of a country has therefore emerged as the most useful tool to identify the reactive nitrogen emission during the production and handling of an entity, irrespective of its domestic and worldwide use (Leach et al., 2012, Oita et al., 2016, Galloway et al., 2014). Previously nitrogen footprint per capita have been calculated for Germany (Leach et al., 2012, Groenestein et al., 2019), US (Leach et al., 2012), UK (Stevens et al., 2014), Netherlands (Leach et al., 2012), Austria (Pierer et al., 2014), Australia (Liang et al., 2016), Japan (Shibata et al., 2014, Shibata et al., 2017) and Tanzania (Hutton et al., 2017).

Godfray et al. (2010) has rightly mentioned that the security and sustainability of global food consumption will largely depend on livestock source food consumption (Godfray et al., 2010). Nearly 80% of the total nitrogen foot print have been estimated as food nitrogen foot print. Consequently, 50% of the food nitrogen footprint was predicted as beef nitrogen food print followed by pork and poultry nitrogen footprint (Liang et al., 2016, Shibata et al., 2017). This has accounted for staggering one third of total nitrogen emission from the global economy (Mueller and Lassaletta, 2020) and able to reduce 0.3 – 3% of global gross domestic product (GDP) (Sutton et al., 2013). While roaming around the livestock farming and agricultural supply chain, 50 to 80% of the meat nitrogen footprint subsequently released to the atmospheric and aquatic environment via manure (Leach et al., 2012, Liang et al., 2016, Bodirsky et al., 2014). This causes severe water pollution by releasing nitrate and air pollution by releasing ammonia and greenhouse gas nitrous oxide (Bai et al., 2018, Oita et al., 2016, Bodirsky et al., 2014, Davidson, 2009, Yang et al., 2017, Lee et al., 2019, Wang et al., 2015, Aneja et al., 2008, Zhang et al., 2015).

However, the ammonium nitrogen from manure can be recovered in form of ammonia water (Samanta et al., 2022), which can further be valorized into a new end product (e.g.

fertilizers, textiles, plastics). This will promote a circular approach of resource utilization (Pikaar et al., 2017).

Several mechanical and chemical processes such as screw press, centrifugation, sedimentation, hydrogel application, ammonia stripping at high temperature, (Quan et al., 2009) were followed previously for manure treatment and nutrient recovery. However, these processes were very complex, least efficient and often required high chemical or energy demand (Hjorth et al., 2011). Later membrane filtration processes turned popular due to its higher efficiency in nutrient recovery (Masse et al., 2007). However, the demand from the fertilizer market calls for a concentrated nutrient stream production (Vaneeckhaute et al., 2017) which currently solely membrane filtration is unable to achieve (Shi et al., 2018). Therefore, zero liquid discharge approach was selected for this study. This enabled the lowering of nitrogen pollution by recovering maximum amount of ammonium nitrogen and presented highest energy consumption scenario. The average energy consumption per m^3 of manure treatment of various processes is presented in Table 2.1.

Table 2.1. *Average energy consumption per m^3 of manure treatment (N.N., 2020)*

Treatment techniques	Energy consumption (kwh) /m³ of manure treatment
Screw press	0.2 - 0.6
Decanter	1.5 - 5.0
Vacuum evaporation	10.0 - 13.0
Membrane filtration	10.0 -30.0
Zero liquid discharge	58.6 (Liang and Han, 2011)

Although many studies have already predicted the amount of nitrogen waste due to beef, pork and poultry meat production (Leach et al., 2012, Shibata et al., 2017, Stevens et al., 2014, Pierer et al., 2014), little research has been done so far on its direct correlation with manure generation and the corresponding nitrogen loss through it. Furthermore, it is the need of the hour to estimate the energy requirement to recover the potentially lost nitrogen through manure to have an outlook of the real price of meat.

Therefore, the objective of this study was to understand the impact of livestock farming on nitrogen pollution due to substantial amount of manure generation and the corresponding energy demand for its treatment.

2.2. Materials and methods

2.2.1. Manure quantification for nitrogen recovery per kg meat production

The following calculation methods allow to quantify the amount of beef, pork and poultry manure to be treated for complete nitrogen recovery corresponding to 1 kg beef, pork and poultry meat production respectively. The manure is considered to be fresh manure to avoid the nitrogen loss estimation during storage and handling (Möller et al., 2010).

2.2.1.1. Nitrogen content (NC) per kg meat

The variation of protein values among different countries (especially in EU) in beef, pork and poultry meat varies within 2 - 3% (Groenestein et al., 2019). Therefore, the average protein values per kg meat of beef, pork and poultry of 260, 210 and 270 g respectively from USDA nutrient database (Gebhardt et al., 2008) were considered for simplifying the calculation method. Protein contains 16% of nitrogen (Guo et al., 2017, Pierer et al., 2014). Hence, nitrogen content (NC) of beef (NC_{beef}), pork (NC_{pork}) and poultry ($NC_{poultry}$) per kg respective produced meat were calculated as follows:

$$NC_{beef} = (0.16 \times 260)\ g = 41.6\ g \cdot kg^{-1}$$

$$NC_{pork} = (0.16 \times 210)\ g = 33.6\ g \cdot kg^{-1}$$

$$NC_{poultry} = (0.16 \times 270)\ g = 43.2\ g \cdot kg^{-1}$$

2.2.1.2. Nitrogen loss (NL) per kg meat production

Virtual nitrogen factor (VNF), calculated from nitrogen footprint calculators, represents the amount of lost nitrogen per unit nitrogen content (NC) in respective meat (Leach et al., 2012, Guo et al., 2017) in this calculation method. The lost nitrogen (NL) amount per kg meat production was calculated as follows:

$$NL = NC \times VNF\ g \cdot kg^{-1} \quad (2.1)$$

NL of beef, pork and poultry meats are represented as NL_{beef}, NL_{pork} and $NL_{poultry}$ respectively.

2.2.1.3. Total nitrogen intake (TNI) per kg meat production

Total nitrogen intake (TNI) calculation was based on the NC and NL of per kg produced meat. TNI was calculated as follows

$$TNI = NL + NC\ g \cdot kg^{-1} \quad (2.2)$$

TNI of beef, pork and poultry meats are represented as TNI_{beef}, TNI_{pork} and $TNI_{poultry}$ respectively.

2.2.1.4. Nitrogen loss in manure (NM) per kg meat production

The average nitrogen loss (NM) in beef manure (NM_{beef}) is observed 80 % (Liang et al., 2016, Leach et al., 2012) of TNI_{beef}, followed by 54 % (Liang et al., 2016, Millet et al., 2018) of TNI_{pork} in pork manure (NM_{pork}) and 50 % (Liang et al., 2016, Malomo et al., 2018) $TNI_{poultry}$ in poultry manure ($NM_{poultry}$). Rest of the TNI are lost from plant and soil system as crop processing waste(Leach et al., 2012, Liang et al., 2016). NM_{beef}, NM_{pork} and $NM_{poultry}$ were calculated as follows:

$$NM_{beef} = 0.8 \times TNI_{beef}\ g \cdot kg^{-1} \quad (2.3)$$

$$NM_{pork} = 0.54 \times TNI_{pork}\ g \cdot kg^{-1} \quad (2.4)$$

$$NM_{poultry} = 0.5 \times TNI_{poultry}\ g \cdot kg^{-1} \quad (2.5)$$

2.2.1.5. Quantity of manure (QM) to be treated for nitrogen recovery per kg produced meat

Variation of nitrogen concentration ($C_{manure\ N}$) in manure depends on multiple reasons, e.g. animal feed quality and growth rate, manure storage and handling processes, seasonal conditions etc. (Webb et al., 2013). Therefore, the average $C_{manure\ N}$ value of 2.4, 3.4 and 6.8 gL^{-1} in beef (Sánchez-Hernández et al., 2013), pork (Xie et al., 2011) and poultry (Yangin-Gomec and Ozturk, 2013) manure were used respectively for calculating the quantity of manure (QM) to be treated for nitrogen recovery per kg produced meat.

$$QM = (NM / C_{manure\ N})\ L\ kg^{-1} \quad (2.6)$$

QM of beef, pork and poultry meats are represented as QM_{beef}, QM_{pork} and $QM_{poultry}$ respectively.

2.2.2. Ammonium nitrogen recovery (AR) from manure per kg produced meat

The ammonium nitrogen from animal manure can be recovered faster (in form of ammonia water) and valorized into new end product than compared to the other fraction of organically bound nitrogen (Samanta et al., 2022, Matassa et al., 2015). The ammonium nitrogen ($C_{manure\ NH4\text{-}N}$) concentration of 1.2, 2.8 and 4 gL^{-1} in beef (Sánchez-Hernández et al., 2013), pork (Xie et al., 2011) and poultry (Yangin-Gomec and Ozturk, 2013) manure were

considered for potential 100% $C_{manure\ NH4\text{-}N}$ recovery (AR) calculation respectively. The calculation methods were as followed:

$$AR = (C_{manure\ NH4\text{-}N} \times QM)\ g\ kg^{-1} \quad (2.7)$$

AR of beef, pork and poultry meats are represented as AR_{beef}, AR_{pork} and $AR_{poultry}$ respectively.

2.2.3. Energy demand (ED) for manure treatment

Among the available manure treatment methods, vacuum evaporation and membrane filtration are proven to be the best available alternative methods. ED of vacuum evaporation and membrane filtration for manure treatment were observed 15 and 30 kWh/ m^3 manure respectively (N.N., 2020). Nevertheless, both of these processes are only able to partial AR from manure (Samanta et al., 2022). As this calculation method was focused on the complete recovery of $C_{manure\ NH4\text{-}N}$ from manure, the usage of a different concept needed. This led to zero discharge treatment (ZLD) of manure, which presented maximum ED. Hence, an ED of 58.6 kWh /m^3 (Liang and Han, 2011), was considered for the following calculations:

$$ED = (QM \times 58.6)\ kWh\ Mg^{-1} \quad (2.8)$$

ED of beef, pork and poultry meats are represented as ED_{beef}, ED_{pork} and $ED_{poultry}$ respectively. Whereas, AR depends both on $C_{manure\ NH4\text{-}N}$ and QM, the ED is only dependent on QM.

2.3. Results and discussion

2.3.1. Nitrogen loss in meat production

VNF of beef, pork and poultry meat production of Germany (Leach et al., 2012), US (Stevens et al., 2014, Leach et al., 2012), UK (Leach et al., 2012, Stevens et al., 2014), China (Guo et al., 2017), Japan (Shibata et al., 2014), Australia (Liang et al., 2016), Tanzania (Hutton et al., 2017), Netherland (Leach et al., 2012) and Austria (Pierer et al., 2014) have been taken from previous literatures for NL and TNI calculations. The VNF, NL and TNI values of the above-mentioned countries were presented in Table SA1. NL and TNI were determined by following equation 2.1 and 2.2 respectively. NL was found directly proportional to the VNF values (Figure 2.1.A). NL and TNI were also noticed to be directly proportional to each other (Figure 2.1.B). Beef production was found to have the highest NL and TNI among all the countries, followed by pork and poultry.

Higher VNF_{beef} values reflected that the beef productions were more prone to nitrogen loss. The average nitrogen loss for poultry was calculated 150 g per kg poultry meat production. The loss raised to nearly 180 and 350 g nitrogen per kg pork and beef production

respectively (Figure 2.1.A). The substantial gap between NL and NC revealed the degree of nitrogen footprint related to the meat production. Consequently, the higher nitrogen intake led to higher nitrogen loss for meat production. Therefore, the average TNI_{beef} was noticed 10 times higher than the NC_{beef}. Although, the gap reduced to an average of 4 and 5 times for poultry and pork respectively.

Figure 2.2. presented a flow chart of the fate of nitrogen in beef, pork and poultry production, where TNI considered as 100% in each case. NM_{beef}, NM_{pork} and $NM_{poultry}$ was calculated using equations 2.3, 2.4 and 2.5 respectively. As discussed above, considerably increased NL in beef production was noticed as the relative NC value was lower than the pork and poultry. The NM_{beef} of was found 90% of the NL_{beef}. The value decreased to nearly 50 to 60 % for poultry and pork respectively. Crop processing waste for poultry and pork was found significantly high, which contributed to the other large part of the NL.

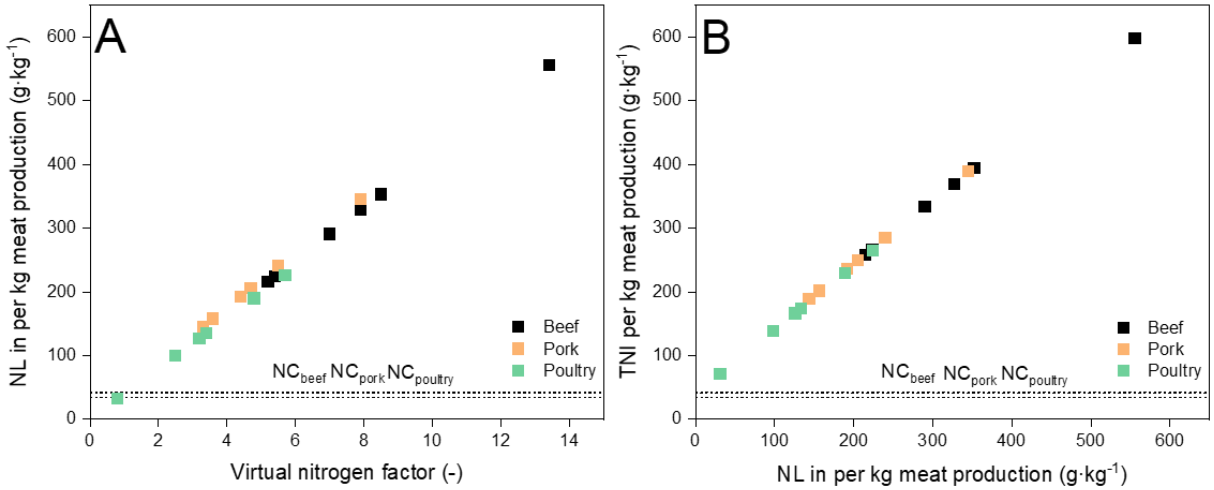

Figure 2.1. *(A) Relationship between VNF and NL and (B) the corresponding relationship between NL and TNI per kg meat production of different countries. VNF values are given in Table SA1.*

Beef production was found the most endangered for nitrogen footprint in the larger part of the world (Shibata et al., 2014, Leach et al., 2012, Pierer et al., 2014, Liang et al., 2016, Hutton et al., 2017, Stevens et al., 2014). This attributed to the substantial feed demand and steep basal metabolic rate of beef (Leach et al., 2012, Shibata et al., 2014, Eshel et al., 2014, Hulbert and Else, 2004). Therefore, NM_{beef} was observed significantly higher than NM_{pork} and $NM_{poultry}$ (Figure 2.2.). The nitrogen loss for pork and poultry meat production were

dominated by the poor manure management processes rather than feed and digestibility factors (Groenestein et al., 2019, Leip et al., 2014).

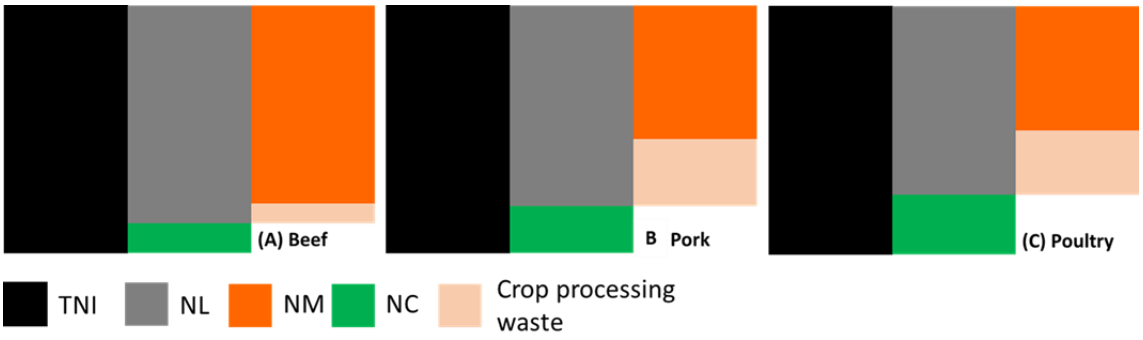

Figure 2.2. *Tree diagram of nitrogen cycle per kg (A) beef, (B) pork and (C) poultry meat production, where TNI represents 100% in each case.*

2.3.2. Comparison among countries

The NM_{beef} were found highest which corresponded to 80% of their TNI. The least VNF for beef was noticed for Austria. It was 2.5 to 3 units lesser than the other European countries. Therefore, the NM_{beef} in Austria was found to be least in Europe. NM_{beef} of the Netherlands was noticed even higher than the Germany, UK and US. Australia's NM_{beef} was calculated second highest, only second to Japan. The NM_{beef} of Japan was found nearly 5 times higher than the other Asian country China and nearly three times higher than the other European countries. Nearly 50% of both pork and poultry TNI ended up in NM. However, NM_{pork} was calculated higher than $NM_{poultry}$ due to their higher VNF values (Table SA1). A similar trend of NM among the stated countries were noticed for pork and poultry as well. However, China's NM_{pork} was found nearly doubled than the other countries apart from Japan. Tanzania had the least value of NM_{pork} and $NM_{poultry}$. The differences between NM_{pork} and $NM_{poultry}$ were not significant among the US, Australia and the other European countries.

QM to be treated per kg meat production was calculated using equation 2.6. High NM value and low $C_{manure\text{-}N}$, led to very high QM values for beef production in all countries compared to pork and poultry. Double $C_{manure\text{-}N}$ of poultry than pork led $QM_{poultry}$ values to be significantly lower than QM_{pork}. The trend was noticed pretty similar to the NM trends as discussed above. Japan was found with the highest QM values for all three kinds of meat. Whereas, Tanzania had the least QM_{pork} and $QM_{poultry}$ values. Interestingly, China's QM_{pork} was slightly lower than its QM_{beef}, although its NM_{pork} was much higher than the NM_{beef} per

kg meat production. This reflects the substantial differences between QM_{beef} and other QM values of a country. No significant differences of QM values were noticed among the US and the other European countries.

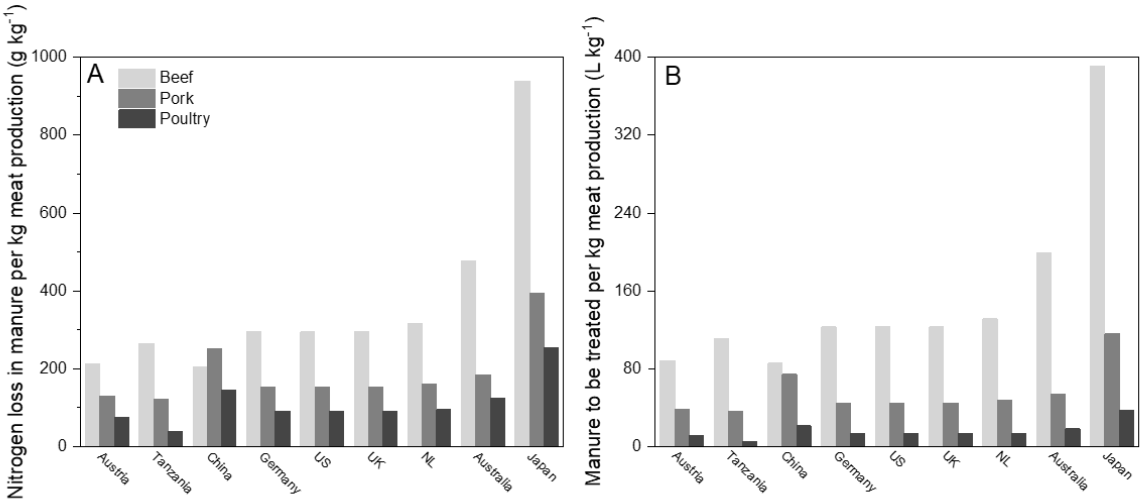

Figure 2.3. *(A) Nitrogen loss in manure (NM) and (B) the quantity of manure (QM) to be treated for nitrogen recovery per 1 kg beef, pork and poultry meat production respectively among different countries.*

Lower efficiency of nitrogen, use for feed crops and animal's stubby feed nitrogen conservation (Shibata et al., 2014) ratio led to very high quantity of NM and QM for Japan. Additionally, international food and feed trade affected Japan's overall nitrogen footprint. The country relies largely on imported food (nearly 61%). Hence, a big portion of nitrogen loss happened during production in the exporting country itself (Shibata et al., 2014). High VNF_{beef} due to very high beef consumption (Liang et al., 2016) resulted in substantial QM_{beef} amount for Australia. On contrary, pork consumption in China is the largest (Guo et al., 2017). Poor pork manure management process (Yan et al., 2014, Ma et al., 2012) intensified high VNF_{pork} for China. This decreed in China's relatively higher QM_{pork}. The US and the EU countries such as Germany, UK, Netherlands and Austria have high nitrogen nutrient recovery rate due to its advanced treatment techniques (Shibata et al., 2017, Groenestein et al., 2019). This led to their moderate to low NM and QM values. Especially, Austria's VNF_{beef} was found noticeably lower than the others (Pierer et al., 2014, Leach et al., 2012, Galloway et al., 2014, Shibata et al., 2017). Moderate meat consumption in general is considered as the main reason behind it (Pierer et al., 2014). This also reflected in relatively lower QM in Austria. Tanzania's protein consumption even lower than the WHO's calculated daily need of

75 g per adult (Schönfeldt and Hall, 2012) supported for its lower VNF values (Hutton et al., 2017). This resulted in the lowest NM and QM values for Tanzania.

2.3.3. Energy demand for ZLD and ammonium nitrogen recovery

A scaled-up version of the relation between meat production and manure generation of beef, pork and poultry is presented in Figure 2.4 (A). One Mg beef production was calculated to generate above 120 m^3 of manure. This was substantially doubled than pork and nearly 10 times of poultry manure generation per 1 Mg corresponding meat production. The manure generation was calculated by using equation 2.6.

The standard ammonium nitrogen concentrations in manure were considered (section 2.2.2) to calculate the AR by following equation 2.7. High ammonium nitrogen concentration in poultry manure led to recovery of nearly 4 kg of ammonium nitrogen from 1 m^3 manure. The value decreased to 2.8 and 1.2 kg for 1 m^3 pork and beef manure respectively (Figure 2.4 (B)). Lastly, the ED for beef, pork and poultry manure treatment for AR was calculated by following equation 2.8. The assumed ED value was corresponding to ZLD. Hence, it represented the maximum AR and the highest energy consumption scenario (Figure 2.5 (C)).

Total 147 kg ammonium nitrogen was calculated to be recovered from 123 m^3 of beef manure corresponding to 1 Mg beef meat production. The calculated AR from pork manure was 14% lesser compared to the beef manure for same quantity of meat production. Consequently, the QM was found 64% lesser for pork manure than compared to the beef manure. The AR and QM of poultry manure was calculated 64% and 89% lesser than that of beef manure per 1 Mg poultry meat production respectively. It was reduced to 58% and 4.5% respectively, when comparing with pork manure.

On the other hand, more than 7000 kWh energy was calculated to treat beef manure corresponding to 1 Mg beef meat production. The ED reduced significantly to below 3000 kWh and to nearly 800 kWh for pork and poultry manure treatment for the same amount of meat production. Therefore, the specific energy demand (SED) calculation (supporting information, Equation SA1) showed that 49 kWh energy is required to recover 1 kg of ammonium nitrogen from beef manure. The SED reduced to 21 and 15 kWh for pork and poultry manure respectively (Table SA2).

This results clearly indicates the staggering energy consumption related to manure treatment for lowering the overall nitrogen footprint in livestock farming. Recovery of ammonium nitrogen contributes to the circular approach of the economy as well. Although,

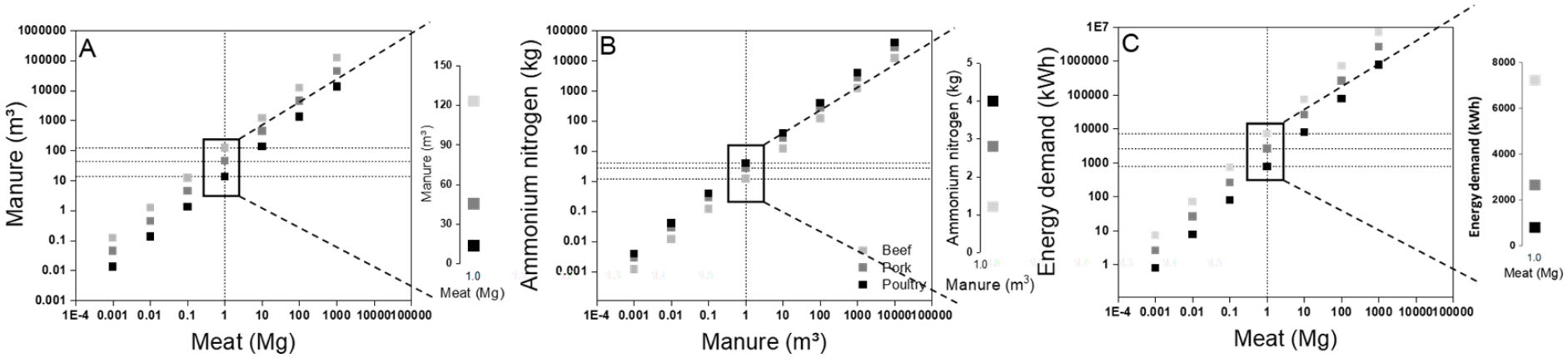

Figure 2.4. *(A) The relationship between meat production and manure generation, (B) ammonium nitrogen recovery by treating per unit m³ of manure and (C) the corresponding energy demand to treat manure per Mg of beef, pork and poultry meat production.*

considering the ED of ZLD in this approach may present the maximum ED for manure treatment. However, the substantial impact of it can't be ignored, when moving towards more sustainable livestock farming approaches.

2.4. Conclusion

The objective of this study was to understand the impact of livestock farming on nitrogen pollution by forming a direct relationship between meat production and manure generation and the corresponding energy demand for its treatment. The overall outcome of the study is given below:

(i) This is the first study that formed a direct relationship between manure generation by beef, pork and poultry per unit respective meat production. Nitrogen loss per unit meat production was found directly proportional to the virtual nitrogen factors. The relationship between total nitrogen intake and the corresponding nitrogen loss per kg meat production was found linear as well.

(ii) When comparing several countries, Japan was found to lose highest amount of nitrogen for meat production followed by Australia. Therefore, the amount of manure to be treated per unit meat production was highest for Japan. The nitrogen loss due to meat production was found relatively lesser among the US and the European countries due to their advanced nitrogen recovery systems from waste streams.

(iii) The results showed that more than 7000 kWh energy required to recover 140 kg of ammonium nitrogen from beef manure per 1 Mg meat production when considering zero liquid discharge approach. The energy demand reduced significantly to below 3000 kWh and nearly 1000 kWh for pork and poultry manure treatment for the same.

Nevertheless, this study is based on several assumptions. Standard ammonium nitrogen concentration for beef, pork and poultry manure was considered for all the countries. Whereas, it can vary depending on the animal feed, their health and manure storage conditions. In addition, the manure was considered to be fresh. Hence, any ammonium nitrogen loss due to storage was not considered.

3. Nutrient recovery and ammonia-water production by MF-vacuum evaporation treatment of pig manure

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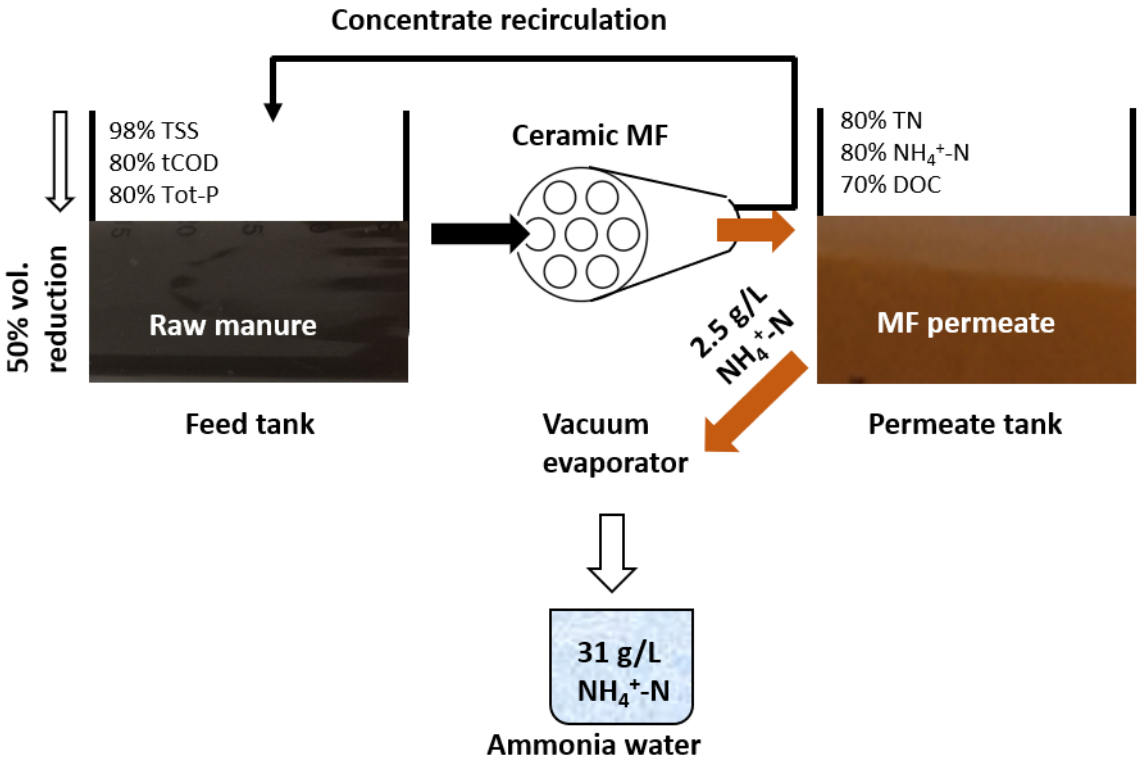

The graphical abstract of the chapter 3

3.1. Introduction

The livestock farming sector raised great concern for its environmental impact by producing substantial amount of manure and related wastewater, characterized by high organic and mineral load, mainly phosphorus (P), nitrogen (N) and potassium (K) (Petersen et al., 2007). It is well known that the inadequate disposal of manure can contribute to large environmental pollution by releasing ammonia and nitrous oxide (Kruse and Bell, 1987, Bouwman, 1990, Skiba et al., 1997) in the atmosphere and degrade the water resources by leaching nitrate into ground and surface water (Smith and Chambers, 1998, Ledda et al., 2013), as well as contributing to soil acidification (Giola et al., 2012, Mantovi et al., 2006). This may lead to further eutrophication of rivers and estuaries (Neal and Heathwaite, 2005, Clarisse et al., 2009). In addition, excess N and P concentration cause toxic algal bloom and oxygen depletion, followed by degrading the aquatic ecosystem (Carpenter et al., 1998). Therefore, manure disposal has become more tightly regulated. Especially, during the past few decades, the growing manure management problem forced European Community to draw nitrate directive guidelines to regulate groundwater nitrate pollution (Somsen, 1999).

However, nutrient (N, K, P) rich manure is popular in agricultural application to the recent production of microalgae field (Almutairi et al., 2011, Cho et al., 2011). Recent report also suggested the use of volatile fatty acids from manure for biopolymers production (Albuquerque et al., 2011). Moreover, manure is heavily used for biogas production to recover energy as well (Raven and Gregersen, 2007, Riaño et al., 2011). In general, the application of nutrient recovery techniques from manure is in high demand. Even though the high solid content, organically bound materials and its potential hazardous properties, make the recovery processes very difficult (Gerardo et al., 2013).

Previously, several nutrient recovery processes from manure have already been reported, such as hydrogel application (Kioussis et al., 1999), calcium phosphate precipitation (Hosni et al., 2008, Lu and Liu, 2010), struvite precipitation (Doyle and Parsons, 2002, Uysal et al., 2010, El Diwani et al., 2007) and ammonia stripping at high temperature (Katehis et al., 1998, Saracco and Genon, 1994, Quan et al., 2009). However, these processes are very complex and require high chemical and energy inputs. The advantages and drawbacks of using conventional mechanical processes such as, sedimentation, centrifugation and pressurized filtrations are well described in previous literature (Hjorth et al., 2011). An alternative to this processes is the use of membranes, which can produce nutrient rich,

particle and pathogen free separate streams from pig manure with relatively lower operating and maintenance cost (Masse et al., 2007, Chen et al., 2006). The comparison of mean retention values of different parameters between MF membrane filtration and these conventional mechanical processes are presented in Table 3.1. 30 to 50% higher dry matter and total phosphorus retention by MF clearly represents its advantage over the other mentioned processes.

Table 3.1. *Retention comparison between conventional mechanical manure treatment processes and MF filtration*

	Retention (%) (mean values)		
Processes (Hjorth et al., 2011, Masse et al., 2007)	Dry matter	TN	Tot-P
Sedimentation	56	33	52
Centrifugation	44	27	34
Pressurized filtration	37	15	17
MF filtration	75	15*	80*

**The mean values are taken from the current research work*

Microfiltration (MF) generally retains solid particles within the range of 0.1 to 10 µm, while ultrafiltration (UF) retains particles within 5 to 200 nm range. Hence, they are well suited to remove nutrients related to particles such as P (Masse et al., 2007, Hjorth et al., 2011). Additionally, dissolve nutrients such as N and K can be collected in the permeate side as well (Chen et al., 2006, Gerardo et al., 2013). Therefore, MF process was selected for the present research study. However, the demand from the fertilizer market calls for a concentrated nutrient stream production (Vaneeckhaute et al., 2017) which currently solely MF is unable to achieve (Shi et al., 2018).

So far, no other studies have reported on the further treatment of MF permeate by a very well-known ammonia gas stripping vacuum evaporation technique. This can be applied at low energy cost (Ukwuani and Tao, 2016) by reducing the boiling temperature by applying vacuum (Tao et al., 2019) to produce more purified and concentrated ammonia-water (Zhang et al., 2020, Tao et al., 2018, Yuan et al., 2016, Tao and Ukwuani, 2015) from the MF permeate. This can be valorized into a new end product (e.g. fertilizers, textile, plastics and cleaning products), promoting a circular approach of resource utilization (Matassa et al.,

2015, Pikaar et al., 2017). In addition, Zarebska et al. (2015) reported that concentrated ammonium sulphate solution with a nitrogen content of 6% w/w and low organic content, produced from manure could be sold at a price of 0.35 € kg^{-1} N.

Therefore, the objectives of this research study are to (i) perform long term microfiltration of pig manure to probe the long term stability of the filtration performance and solid-liquid separation capacity to produce separate nutrient rich streams, (ii) to perform short term microfiltration to check the volume reduction efficiency to reduce final transportation cost during manure management and (iii) to produce purified and concentrated ammonia water by vacuum evaporation process from MF permeate, which can be valorized into a new end product.

3.2. Materials and methods

3.2.1. Raw pig manure

Raw pig manure slurry of 200 L was collected in June 2020 from a medium size (150 to 200 pigs) pig farm, located in the state of Baden-Württemberg, Germany. Collected manure was stored at 4℃ in dark for a week prior to pilot scale membrane filtration experiments. Manure characteristics are listed in Table 3.2. Raw manure was sieved through 1 mm sieve (Supporting information, Figure SB1) to avoid particles ≥ 1 mm prior to use as the feed for the membrane filtration experiment.

3.2.2. Pilot scale membrane filtration set up

A pilot scale ceramic cross flow microfiltration (MF) system (Figure 3.1) was used for pig manure filtration. The MF system primarily consisted of controlling valves, pumps, membrane modules, manometer, flowmeters, thermometer, temperature controller and a programmable logical controller (PLC) system. An external tank of 100 L volume was used as feed tank for the experiment. A ceramic MF membrane (Al_2O_3, inopore GmbH, Germany) module of 0.2 µm pore size and 1 m Length was used for the filtration. The effective membrane area was 0.132 m^2.

Table 3.2. *Pig manure characteristics*

Characteristics	**Pig manure**
Total suspended solids (TSS, gL^{-1})	5 ± 1.5
Volatile suspended solids (VSS, % of TSS)	80 ± 5
Total Carbon (TC, gL^{-1})	5 ± 1.5
Dissolve organic carbon (DOC, gL^{-1})	2.5 ± 1.0
Total chemical oxygen demand (tCOD, gL^{-1})	12 ± 2.5
Soluble chemical oxygen demand (sCOD, gL^{-1})	7 ± 2.5
Ammonium nitrogen (NH_4^+-N, gL^{-1})	2.2 ± 1
Total nitrogen (TN, gl^{-1})	2.5 ± 1
Dissolved ammonium nitrogen (sNH_4^+-N, gL^{-1})*	1.5 ± 1
Acetic acid (mgL^{-1})	2200 ± 400
Propionic acid (mgL^{-1})	1400 ± 600
pH	7.8 ± 0.1
Cl^- (mgL^{-1})	900 ± 100
NO_2^- (mgL^{-1})	<30
Total phosphorus (mgL^{-1})	100 ± 30
Dissolved phosphorus (mgL^{-1})*	20 ± 7
Dissolved sulphate (mgL^{-1})*	43
K^+ (mgL^{-1})	1600 ± 200
Na^+ (mgL^{-1})	400 ± 100
Ca^{2+} (mgL^{-1})	150 ± 30

**Dissolved NH_4^+-N, phosphorus and sulphate were measured after filtering the raw manure by 0.45 µm membrane filters*

3.2.3. Membrane filtration procedure

At first, a long-term (7 days) filtration experiment was conducted to investigate the effectiveness of the MF system (Figure 3.1) to treat raw pig manure. Initially the feed tank was filled up with 1 mm sieved raw manure. A prefiltering sieve of 150 µm was installed after the feed tank to avoid further interference of smaller particles. Feed flow rate was controlled by the optimum opening of the bleed valve.

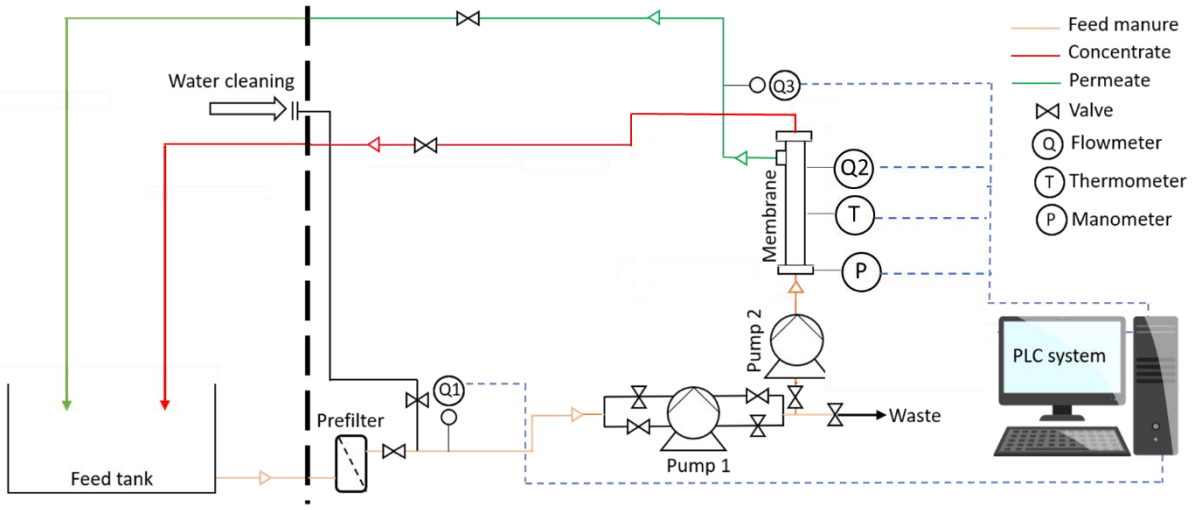

Figure 3.1. *Pilot scale cross flow membrane filtration system*

The experiment was performed at constant pressure of 2.2 bar. The feed flow rate, cross flow velocity (CF) and the permeate flow rate was monitored by Q1, Q2 and Q3 respectively. Trans membrane pressure (TMP) was controlled by adjusting the frequency of Pump 1 (a variable frequency drive pump). Pump 2 (a centrifuge pump) was used to ensure the high CF through the membrane. The experiment was carried out in 'recycle-mode'. Hence, both permeate and the concentrate, were recycled back to the feed tank to maintain the manure composition and volume in the feed tank relatively constant.

Once the long-term experiment was done, the membrane and the system were thoroughly cleaned (section 3.2.4) prior to perform a short-term filtration experiment. The short-term experiment was carried out in 'continuous -operation mode' by introducing fresh 1 mm sieved raw pig manure in the feed tank. Hence, the permeate was collected in a separate tank but the concentrate was recycled back to the feed tank as mentioned previously. The CF was maintained to remain relatively unchanged over the filtration period. The filtration was stopped once the 50% feed volume reduction was achieved.

3.2.4. Cleaning procedure

To recover the membrane flux after the completion of long- and short-term filtration experiments, the MF system was drained completely followed by performing a water cleaning process for 30 minutes to wash off the manure residues. Thereafter, the feed tank was drained, washed and filled with a diluted base cleaning agent (Atec, neu Ulm, Germany) of 100 L of pH 12. The recycle-mode filtration procedure (section 3.2.3) was performed at 40

℃ for 2 hours followed by the draining of the cleaning solution. Finally, deionized water filtration was performed by following the same method for 2 hours to wash out the residual cleaning solutions and to check the flux recovery of the membrane as well.

3.2.5. Vacuum evaporation process

The vacuum evaporation experiments were performed by using a lab scale vacuum distillation system (Heidolph, Germany) (Supporting information, Figure SB2). It was consisted with a three-neck flask, a condenser cooled by water and a vacuum pump attached with a pressure control device. The flask was submerged in a thermostatic water bath to control the temperature. 100 mL volume of each raw pig manure, permeate and the concentrate after 50% volume reduction, from the short-term experiment were used for vacuum evaporation tests. The tests were operated at 70 ℃ temperature and at a relative pressure of 100 mbar. A rotating speed of 60 rpm was maintained thoroughly. The operating parameters were remained constant during the experimental period and did not vary significantly (± 10%) from the set values. The volume of concentrated raw manure, permeate and concentrate were measured after varying operating time period (5, 8, 15 mins) by a measuring cylinder. The collected samples were then further analyzed.

3.2.6. Analytical procedures

TSS and VSS were measured according to the established methods (APHA AWWA). The tCOD, NH_4^+-N and total phosphorus were measured using rapid test kits (Hach Lange, Germany). TC, DOC and TN were measured by TOC analyzer (Shimadzu TOC-V CPN, Japan). Organic acids concentrations anions were measured by an ion chromatography (IC) system (Metrohm, Switzerland). The cations were measured by an inductively coupled plasma – optical emission spectrometry (ICP-OES, VistaPRO CCD, Fa. Varian). Electrical conductivity and pH were measured by a portable multimeter (WTW Multi 350i, Xylem, USA).

3.2.7. Calculated parameters

The MF system was controlled by a PLC system and all the corresponding data (time, pressure, temperature and flow rates) were monitored and recorded by a computer, which was connected with the data acquisition center. The operating parameters are calculated as follows:

Cross flow velocity (CF) was determined by dividing the flow rate monitored at Q2 (Figure 3.1) by the cross-sectional area of the tubular ceramic membrane. It is presented in the following equation:

$$CF = \left(\frac{Q}{A}\right) (\text{m s}^{-1}) \quad (3.1)$$

Where, Q is the in-flow rate (m^3 s^{-1}) of the membrane, which was monitored by Q2 flowmeter (Figure 3.1.) and A was the cross-sectional area (m^2) of the membrane.

The permeate flux (J) was determined by the ration of the permeate flow rate to effective membrane area.

$$J = \left(\frac{Qp}{Am}\right) (\text{L m}^{-2} \cdot \text{h}^{-1}, \text{LMH}) \quad (3.2)$$

The permeate flow rate Q_p (L h^{-1}) was monitored by flowmeter Q3 (Figure 3.1.) and A_m is referred to the effective membrane area of 0.158 m^2. Hence, the unit of flux is abbreviated as LMH.

The retention of several parameters by MF membrane was calculated by following equation:

$$R = \left(1 - \left(\frac{Cp}{Cf}\right)\right) \times 100\ (\%) \quad (3.3)$$

Where, R is the calculated retention in percent (%). C_p and C_f are the permeate and feed concentration of any parameter at a given time.

The permeate volume recovery was calculated according to equation:

$$Rec = \left(1 - \left(\frac{Vp}{Vf}\right)\right) \times 100\ (\%) \quad (3.4)$$

Where, Rec is the calculated recovery in percent (%). V_p and V_f are the permeate and feed volume at a given time.

3.3. Results and discussion

3.3.1. Long term experiment

Long term MF experiment was conducted to verify the long-term efficiency of solid-liquid separation process followed by the nutrient (N and P) recovery from pig manure. Feed and permeate samples were collected and analyzed at the same time to check the real time retention of TSS, tCOD, NH_4^+-N, TN and total phosphorus after microfiltration. Initially, 5 to 6 samples were collected on each day of the first 2 filtration days. It was then reduced to 2 to 3 samples per day for the rest of the filtration days. The obtained data is summarized in Figure 3.2.

The TSS retention was noticed above 99% throughout the filtration period. Therefore, the data was not included in Figure 3.2. All the retentions were calculated by following equation 3.3. tCOD retention was noticed nearly 50% within the first few hours of the filtration. It raised up to 80% until the beginning of the filtration day 3 and stayed nearly same throughout the rest of the filtration period. High phosphate retention (80%) was noticed within the first few hours of the filtration. The retention was noticed to be raised above 90% by the end of the filtration day 1 and stayed at the same level until the end of the filtration. The average retention was found 10 and 20% for TN and NH_4^+-N respectively until the end of filtration day 4, respectively. A slight drop of 2 to 3% retention was noticed for NH_4^+-N from filtration day 5. However, a sharp drop of TN retention was observed from filtration day 5 onwards. The final TN retention was calculated as low as 1% at the end of the filtration period.

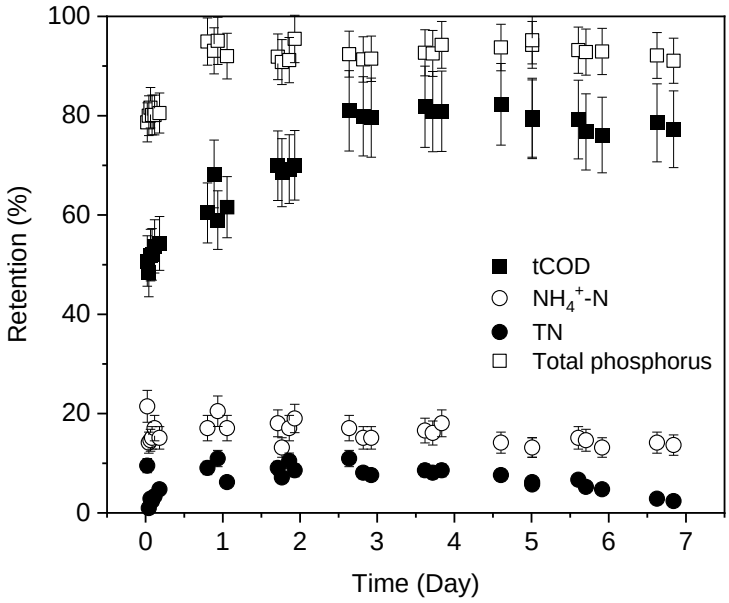

Figure 3.2. *Long term filtration of pig manure by ceramic microfiltration membrane for the retention of tCOD, NH_4^+-N, TN and Total phosphorus. Membrane pore size: 0.2 µm, applied pressure: 2.2 bars, temperature: 25°C ± 1, filtration duration: 7 days.*

The results reported in Figure 3.2 demonstrates the possibility of producing separate nutrient rich fractions with distinct composition and strength. Previous studies reported the efficiency of MF on the removal of TSS, colloids and bacteria in mixed liquor and effluents from biological reactors treating manure (Lee et al., 2001, Shin et al., 2005, Zhang et al., 2007, Zitomer et al., 2005). Viau and Normandin (1990) reported that the maximum TSS retention was achieved by a 0.2 µm membrane was 75%, which is 25% lesser than our findings. However, their feed was an anaerobic reactor fed pig manure. This kind of biologically treated manure contains large quantities of fine solids which pass through MF membranes (Masse et al., 2007). Masse et al. (2005) showed that the particle size analysis of anaerobically digested pig manure presented the particles between 0.05 and 10 µm had a biomodal distribution with peaks around 0.1 µm. Therefore, these particles easily penetrated the MF pores, influencing not only particle rejection but increasing membrane blockage. Later, Zielińska et al. (2017) reported the complete removal of TSS by MF as well. This is in accordance with the current findings.

The initial 50% tCOD retention could be attributed to the fact of 50% particulate COD presentation in the feed manure, which was well retained by the MF membrane. This is in accordance with the previous findings by González-Fernández et al. (2008). However, the

tCOD retention increased up to 80% until day 3 and stayed within similar range until the end of the filtration period. Hence, partly retention of the soluble COD could be assumed to contribute to the tCOD retention. However, Fugere et al. (2005) reported complete TSS retention by UF (0.01 µm) membranes from prefiltered raw and anaerobically digested manure but the soluble COD was not affected by the process. Similarly, Tuczinski et al. (2018) noticed nearly 50% of the tCOD removal in long term filtration experiment through 0.2 µm MF membrane, where the feed was a hydrolysis reactor effluent. On contrary, Zielińska et al. (2017) reported 80% of tCOD retention of a biologically treated wastewater using 0.45 µm pore sized MF membrane. This is in accordance with the present study. In addition, the recirculation of the concentrate and the permeate into the feed tank reduced tCOD concentration significantly to nearly 3 gL^{-1} by the end of filtration day 6, which may explain the higher and stable retention of tCOD during the latter period of the filtration. The same was noticed by Tuczinski et al. (2018). They reported a drop of tCOD concentration from 14.5 gL^{-1} to 8 gL^{-1} in the feed during MF of a hydrolysate, when the permeate recirculation to the feed tank was done for 10 days. It could also be speculated that the tCOD retention was stabilized along with the stabilization of the membrane flux during filtration (Section 3.3.3). However, further investigation is required to clarify the ambiguity.

Pig excretes 50 to 60% of their phosphorus intake in feces and urine due to the inefficiency of their digestive system to adsorb phosphorus (Hjorth et al., 2011). Detailed analysis of particle size fractions of pig manure by Masse et al. (2005) showed the predominant (70% of the undissolved phosphorus) link of phosphorus with the particles between 0.45 µm to 10 µm because small particles contain a substantial portion of the total phosphorus in pig manure. Later, Christensen et al. (2009) also noticed that more than 70% of the phosphorus in pig manure slurry are associated with particles or colloids. Moreover, the addition of phytase in pig feed, which degrades phytate to make phosphorus more easily available (Sommer et al., 2008), were found to be not affecting the total phosphorus distribution in different particle size fractions (Hjorth et al., 2011). Interestingly, only 5 % of the total phosphorus in pig manure was found in form of organic phosphorus. The remaining 95 % speculated to be bound inorganically in crystalline form or was adsorbed onto particles (Fordham and Schwertmann, 1977). Therefore, it can be stated that the high retention of TSS enhanced the phosphorus retention above 90 % throughout the filtration period. Previously, Masse et al. (2007) reported the reduction of phosphorus from 1190 mgL^{-1} in the

prefiltered raw pig manure to 150 mgL^{-1} in the permeate using MF membrane. Similarly, Waeger et al. (2010) reported 67 to 76 % phosphorus retention using 0.2 µm MF membrane while filtering an anaerobic digester effluent.

In livestock farming, 55 to 95% of the nitrogen in plant protein does not turned into animal protein and excreted via urine and dung as organically bound nitrogen (Hjorth et al., 2011). Approximately 70% of the nitrogen present in pig manure is dissolved (Christensen et al., 2009) and most of it is rapidly hydrolyzed into NH_4^+-N by the enzyme urinase (Sommer et al., 2006). Previous studies noticed the mineralization of 10% organic nitrogen of digestible compounds during in house storage (Zhang and Day, 1996, Sørensen, 1998). The mineralization of organic nitrogen dropped to 5% during outdoor storage of manure for a period of 6 to 9 months (Poulsen et al., 2001). Additionally, nearly 30% of the NH_4^+-N was found undissolved in the raw manure (Table 3.2). Therefore, the initial 10% retention of TN and a slightly higher retention of NH_4^+-N in the present study can be associated with the retention of the mineralized fraction by MF. Moreover, it can be assumed that most of the inorganic nitrogen were adsorbed on the membrane surface during the first 5 filtration days. Hence, a drop of TN retention was noticed towards the end of the filtration period.

3.3.2. Short term experiment

The short-term MF experiment was conducted mainly to probe the volume reduction efficiency of the filtration system, followed by consequent NH_4^+-N recovery from raw pig manure as well. Feed samples were collected before the start of the filtration and permeate and concentrate samples were collected once 50% feed volume reduction was achieved. The samples were then analyzed to find out the TSS, tCOD, NH_4^+-N, TN and total phosphorus retention by following equation 3.3. The chosen parameters for the study were mostly bulk parameters (e.g. TSS, tCOD, Phosphate, DOC and TN) and to a lesser extent, process specific parameters (e.g. NH_4^+-N). The manure was initially stored (section 3.2.1) for 6 weeks before the short-term experiment was executed. Thus, there was a probable chance of biological and chemical change in manure characteristics during the storage period. However, it was noticed to be too small to influence the bulk parameters of the manure. Small change in NH_4^+-N concentration was not able to alter the pH value beyond ± 0.1 unit, primarily due to the carbonate buffer. The rejection data corresponding to short term filtration is presented in Figure 3.3.

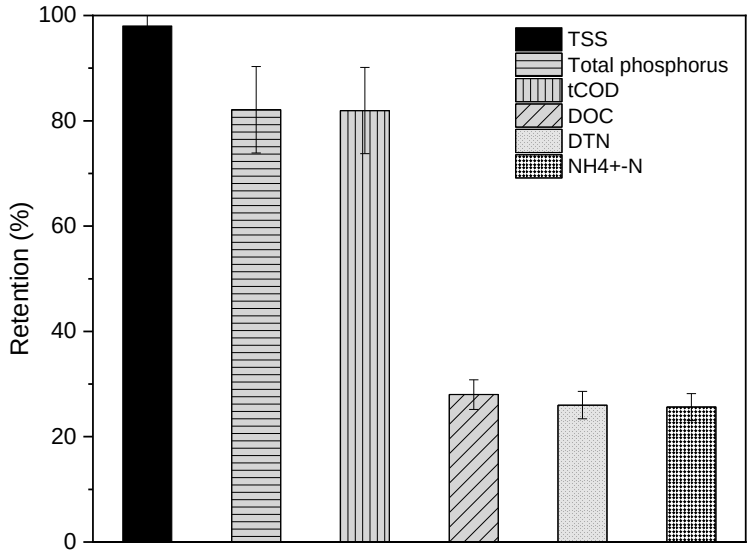

Figure 3.3. *Short term filtration of pig manure by ceramic microfiltration membrane for the retention of TSS, tCOD, NH_4^+-N, TN, DOC and Total phosphorus. Membrane pore size: 0.2 µm, applied pressure: 2.2 bars, temperature: 25°C ± 1, filtration duration: 180 minutes.*

TSS was retained completely and the total phosphorus retention was nearly 80% as it was noticed during the long-term filtration as well. The tCOD retention was observed to be above 80%, which was 20% higher than that of the tCOD retention during the first few hours of long-term filtration. In addition, considerably high DOC retention of approximately 25% was achieved. The TN retention was 10% higher than the long-term recycle mode filtration, but the NH_4^+-N retention stayed within the comparable range around 20%. Images of feed pig manure, MF concentrate and MF permeate are displayed in Figure 3.4 and their characteristics are compared in Table 3.3.

Masse et al. (2007) stated that the concentration of nutrients in reduced volume and the production of reusable water after membrane filtration are inversely correlated. According to them, the actual quantity of nutrients passing through the membranes increase along with the increasing feed concentration. They found that the passing of NH_3 through membrane caused additional NH_3 formation in the concentrate in attempt to reach equilibrium. Thus, the NH_4^+-N retention decreased slightly over the filtration. However, no drastic change in NH_4^+-N retention was noticed in the present study. Probably, the short filtration duration of approximately 3 hours was the main reason. Overall, slightly high TN retention may introduce the fact of organic nitrogen mineralization during in house storage (Sørensen, 1998, Zhang and Day, 1996). Further analysis is required to clarify the reason properly. In contrast, Thörneby et al. (1999) found the fraction of retained TSS, tCOD and

phosphorus was nearly independent of the concentration. This is in accordance with our results. Little increase in tCOD retention caused probably due to the higher adsorption on the cleaned membrane surface. Previously, Tuczinski et al. (2018) noticed > 30% volatile fatty acids (VFA) removal of from hydrolysate when filtering through 0.1 µm porous MF. They assumed that the VFAs were adsorbed on the particulate matter surface and thereby partly removed by the MF membrane. The VFA carbon content was measured to be contributing 30 to 40% of the DOC carbon content in the present study. Hence, the co-retention of VFAs along with the TSS could result in this higher DOC retention. Similarly, Viegas et al. (2020) reported 15% DOC retention using 0.1 µm ceramic MF membrane in water reclamation, whereas, Zhang et al. (2013) reported 31 to 35% DOC removal using 0.1 µm MF membrane filtering soluble algal organic matter. The retention remained consistent during the filtration period. Presence of large polymer such as proteins and polysaccharides were pointed out as the main reason of high DOC removal.

The 50% feed volume reduction was achieved within 3 hours of filtration. Higher initial feed volume would enable to reduce beyond 50% by MF in future. The objectives of the manure

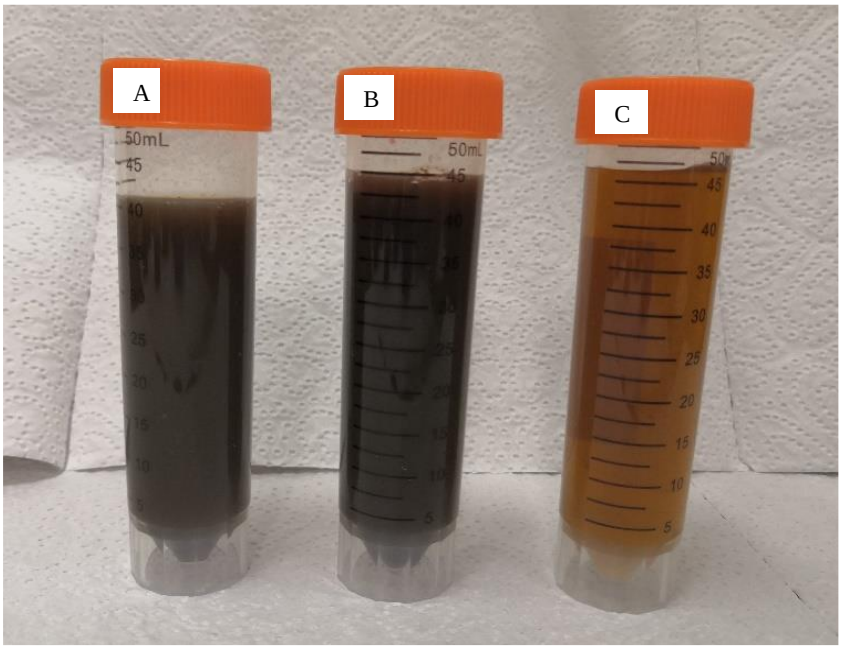

Figure 3.4. *Images of (A) MF feed pig manure slurry, (B) MF concentrate and (C) MF permeate after short term filtration.*

feed volume reduction were to mitigate problems such as (i) high transportation cost, (ii) nutrients loss to the environment and (iii) over fertilization by fractionating manure into a liquid stream enriched of nitrogen and potassium, and a phosphorus enriched solid stream (Jørgensen and Jensen, 2009). Furthermore, the MF permeate and concentrate after short term MF filtration were used in vacuum evaporation process for ammonia water production.

Table 3.3. *Characteristics comparison between MF feed manure, and MF concentrate and MF permeate after short term filtration.*

Characteristics	MF feed	MF concentrate	MF permeate
TSS (gL^{-1})	5	8	0.1
tCOD (gL^{-1})	11	18	3.5
TN (gL^{-1})*	3.2	2.5	2.5
NH_4^+-N (gL^{-1})*	3	2.5	2.4
Total phosphorus (mgL^{-1})	130	200	23

**TN and NH_4^+-N mass losses are mainly due to evaporation.*

3.3.3. Membrane performance

Feeding raw manure or digestate to NF and RO resulted in complete fouling within minutes (Hjorth et al., 2011). Therefore, MF became a popular solid-liquid separation or pretreatment process for further usage of manure and digestate in recent years (Hjorth et al., 2011, Masse et al., 2007, Shi et al., 2018, Zarebska et al., 2015).

Previous studies mentioned clear advantage of having higher production, when using ceramic membranes over polymeric ones (Zarebska et al., 2015, Shi et al., 2018). In addition, ceramic membranes possess other advantages such as, relatively narrow pore size distribution, easy cleaning process, wider range of pH tolerance and longer expected lifetime period (Bhave, 2012). Therefore, ceramic MF membrane was selected for the current research study.

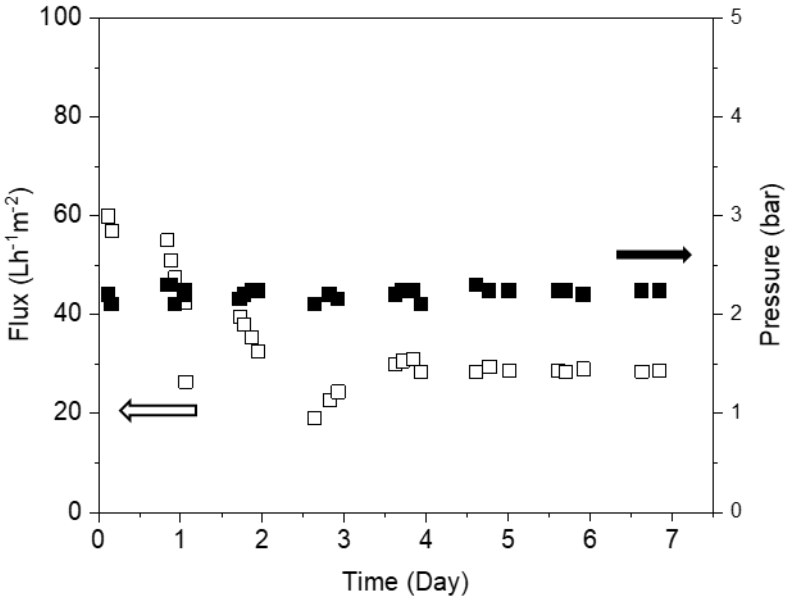

Figure 3.5. *Membrane flux and the corresponding applied pressure of pig manure filtration during long term 'recycle mode' operation period. Membrane pore size: 0.2 µm, temperature: 25° °C ± 1, filtration duration: 7 days.*

The cross-flow filtration was performed at a constant pressure of 2.2 bars. A sharp drop of permeate flux from 60 $Lh^{-1}m^{-2}$ to 20 $Lh^{-1}m^{-2}$ during the long term recycle mode filtration was noticed until the end of day 2. The flux raised up to 30 $Lh^{-1}m^{-2}$ by the start of the filtration day 4 and stayed nearly same until the end of the filtration period. The permeate flux was calculated by using equation 3.2 and the pressure and flux data during filtration are summarized in Figure 3.5. The first flux during the short-term filtration was measured after 80 mins as it was the time required for pressure stabilization. The flux was relatively unchanged from the measuring point of 80 min until 110 min at nearly 45 $Lh^{-1}m^{-2}$. Thereafter, a slight drop in flux was noticed until the end of the filtration. No backwashing with permeate during both filtrations was used as Tuczinski et al. (2018) reported that the microorganisms, which grew on the permeate due to the optimum condition, formed a dense fouling layer on the permeate side of the membrane, when permeate was used for backwashing. Fouling on the permeate side of the membrane is not possible to remove during filtration and the fouling growth is relatively easier due to the loss shear stress.

Zhang et al. (2007) reported similar sharp decline of flux from 100 $Lh^{-1}m^{-2}$ to 5 – 10 $Lh^{-1}m^{-2}$ within first 2 months of the filtration, when filtering mixed-liquor from an anaerobic reactor digesting pig manure. They noticed that the flux remained between 5 to 10 $Lh^{-1}m^{-2}$ for the course of next 4 months. Although, the membrane (UF, 20 kDa) and the filtration duration

were different from the present study, however, the nature of the flux decline followed by its stabilization was very comparable. Similarly, Tuczinski et al. (2018) observed a sharp flux decline within the first 5 filtration days before getting it stabilized around 45 $Lh^{-1}m^{-2}$ after 15 days of filtration, when filtering hydrolysate using 0.8 µm MF membrane. Later, Ravi et al. (2019) also noticed the median flux value of 46 to 49 $Lh^{-1}m^{-2}$, when filtering two different hydrolysate using 0.2 µm MF membrane. Hence, the similar initial fluxes as well as trends in flux decline are in accordance with our results.

The MF membranes are subjected to be fouled in a short period due to high TSS content in manure and digestate (Carretier et al., 2015, Karakashev et al., 2008, Bolzonella et al., 2018, Luján-Facundo et al., 2017). Lee et al. (2001) suggested that the cake build up on the membrane surface caused 95% of the total resistance. After 50 days of filtration, they noticed the permeate flux remained only 19% of its initial value. Flushing with an acidic solution at high temperature could recover only 44% of the original pure water flux. This very low flux recovery indicated the possible irreversible inorganic and organic fouling within the membrane pores (Masse et al., 2007, Zhang et al., 2007). Choi et al. (2009) mentioned the formation of cake layer and the narrowing of the pores of 0.1 µm MF membrane reduced permeate flux significantly while filtering foulant rich municipal wastewater. Additionally, biofouling may have substantial impact on MF flux reduction while filtering manure as well (Zhang et al., 2016). According to our findings, drop of membrane cross flow velocity (supporting information, Figure SB3), calculated based on equation 3.1, from 2.87 ms^{-1} to 1.38 ms^{-1} by the end of the filtration day 7, indicated the membrane channel blockage as well. However, conducting 2 hours of cleaning operation after short term experiment led to 75% of the pure water flux recovery in the present study. On contrary, Ravi et al. (2019) reported no significant membrane blockage due to cake formation or membrane fouling was observed, most probably due to high cross flow velocity and temperature.

3.3.4. Vacuum evaporation test

The nutrient recovery chain is impacted by the inputs of chemicals, energy, labor, transportation as well as the demand from the fertilizer market itself (Vaneeckhaute et al., 2017). This calls for a concentrated nutrient stream production, which currently solely membrane filtration is unable to achieve (Shi et al., 2018). Therefore, a very well-known ammonia gas stripping vacuum evaporation technique (Li et al., 2020) is used in this work

produce purified and concentrated ammonia-water (Zhang et al., 2020, Tao et al., 2018, Yuan et al., 2016, Tao and Ukwuani, 2015) from the MF permeate.

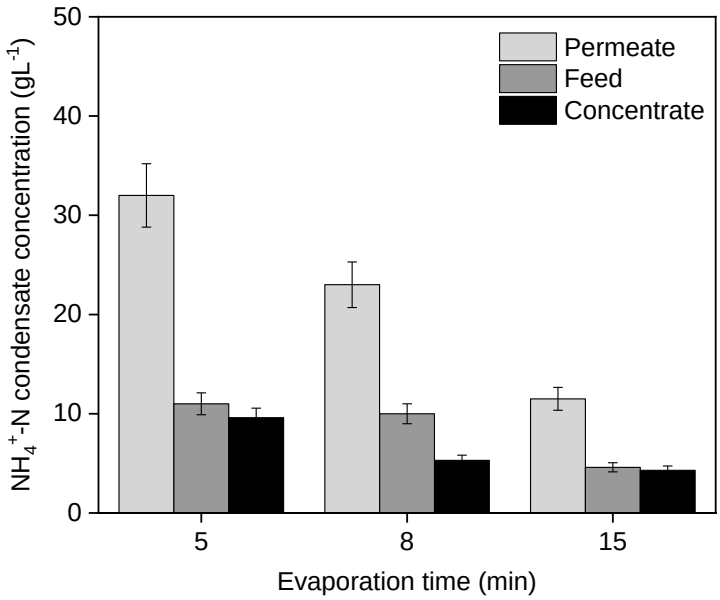

Figure 3.6. *NH_4^+-N concentration in the condensate of raw manure, MF concentrate and MF permeate after time-based vacuum evaporation process. Initial volume: 100 mL, pressure: 100 mbar, temperature: 70 °C, rotational speed: 60 rpm.*

Figure 3.6 compares the NH_4^+-N concentration after given evaporation time in the condensate of raw pig manure, MF permeate after 50% recovery and the corresponding concentrate samples. The initial raw manure feed NH_4^+-N concentration (3 gL^{-1}) was 0.5 gL^{-1} higher than MF permeate and concentrate samples. However, the condensate NH_4^+-N concentration of MF permeate after 5 mins of vacuum evaporation was found 3 to 3.5 times higher than the condensate NH_4^+-N concentration of the feed and the concentrate. The same trend was noticed after 8 and 15 minutes of evaporation as well. However, the concentration gap between the condensate samples of permeate and the feed reduced from 3 times to 1.8 times after 15 minutes of evaporation and the gap between the condensate

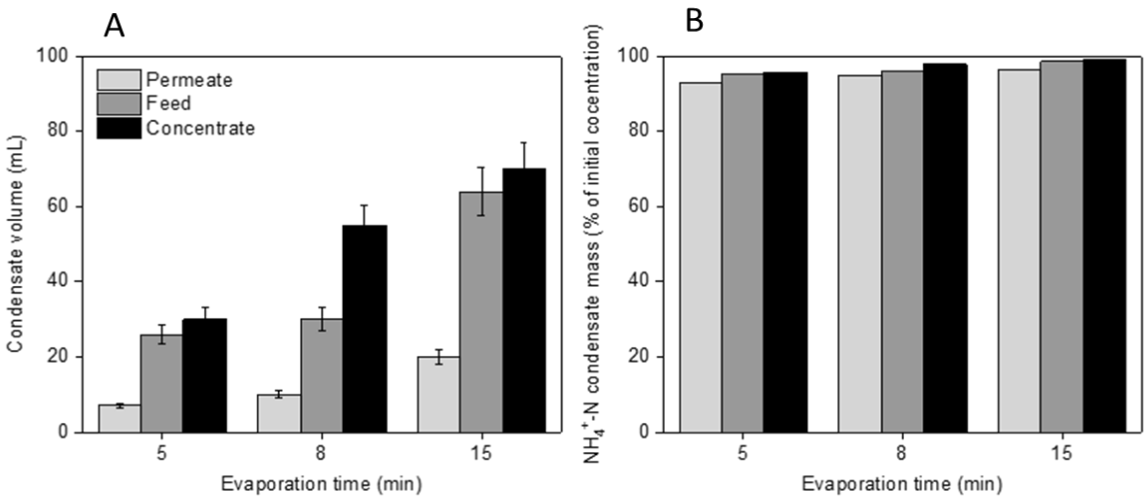

Figure 3.7. *(A) Condensate volume and (B) NH_4^+-N mass transfer to the condensate of permeate, feed and concentrate after 5, 8 and 15 minutes of vacuum evaporation period.*

concentration of permeate and concentrate after 15 minutes of evaporation reduced to 2.28 times. The overall condensate concentration dropped for all three samples with increasing evaporation time. Interestingly, the NH_4^+-N mass transfer to the condensate was above 90% for all three samples (Figure 3.7B) and a rapid water volume transfer to the condensate was noticed for feed and concentrate samples only (Figure 3.7A). The water volume transfer of the permeate sample after 15 minutes of evaporation was nearly 3.5 times lower than the other two samples.

The difference in water volume transfer between the permeate, feed and the concentrate samples could be attributed to the higher solid concentration and viscosities of the latter (Fernández et al., 2015). This is supported by the findings of Blanes-Vidal et al. (2009). They noticed that the presence of higher content of carbon in corncob maintained a longer thermophilic duration which finally lead to improve the water evaporation. Later, Fan et al. (2019) proved it by showing that the liquid pig manure with higher concentration of organic pollutants (e.g. pig effluents) was more beneficial for water evaporation. Lastly, vacuum evaporation process generally receives lower contribution of latent heat. This led to inconsistent heat distribution, resulted in abrupt onset of evaporation in a process called bumping, which led to high levels of water carryover (Guida et al., 2022). This may also partly contribute the rapid water volume transfer to the condensate, when evaporating feed and concentrate samples.

In their work, Li et al. (2016) reported the gradual vacuum evaporation of 100 mL liquid digestate at pH 8. They noticed the condensate volume after 5, 10 and 15 minutes of evaporation were 28, 60 and 70 mL respectively. These values are very comparable with our findings regarding feed and concentrate samples (Figure 3.6A). Consequently, they reported 93% of initial NH_4^+-N mass transfer to the condensate after 15 minutes of evaporation at pH 8, which is in accordance with our findings. Later, Guida et al. (2022) also noticed very high NH_4^+-N mass and water volume transfer to the condensate, while treating synthetic solution with vacuum evaporator. Similarly, they found lower ammonia concentration in the condensate when treating a complex IEX brine than compared to a simpler synthetic solution. According to them, the presence of metal cations, which are known to form metal-amine complexes (Tao et al., 2018, Ukwuani and Tao, 2016), exhibited lower volatilities and hence decreased the stripping performance (Zhang et al., 2018). This impacted negatively the ammonia distribution time to the condensate. In contrast, the NH_4^+-N mass transfer was observed to be very rapid and nearly equal for all samples in the present study. Maximum evaporation time of only 15 minutes in the current research compared to the 75 minutes of longer vacuum evaporation may explain the NH_4^+-N mass transfer hindrance faced by Guida et al. (2022).

Nevertheless, MF filtration of raw manure followed by the vacuum evaporation of the MF permeate have been proven to be a viable alternative to recover nutrient and produce a cleaner and concentrated ammonia water.

3.4. Conclusion

The main goals of the manure treatment by MF-vacuum evaporation process were to probe the long-term stability of the filtration performance and produce nutrient rich separate streams, increase the feed volume reduction efficiency and finally generate the ammonia water using vacuum evaporation. Hence, the overall advantages of using MF-vacuum evaporation process for manure treatment and management are given below:

(i) The MF cross flow system can be operated continuously for longer time period with a stable flux rate of 30 $Lh^{-1}m^{-2}$ and are able to separate more than 90% of the phosphorus in the solid fraction from the liquid fraction. The TSS free liquid fraction or the MF permeate contained more than 80% of the total initial NH_4^+-N content of the manure.

(ii) The short term recycle mode operation could reduce the initial volume up to 50% while maintaining the nutrient separation qualities at the same level. This is very important as the manure volume reduction can directly be linked with their management cost during their distribution on the agricultural land.

(iii) Lastly, the vacuum evaporation of the MF permeates compared to raw feed and concentrate stream have been proven to be a viable alternative to recover nutrient and produce cleaner and concentrated ammonia water. The evaporation duration of 5 minutes of MF permeate resulted in substantial 31 gL^{-1} of NH_4^+-N condensate concentration, which was nearly 12 times higher than the initial NH_4^+-N concentration of MF permeate. However, the vacuum evaporation system requires better mixing and temperature control tools to enable gradual thermal gradients and avoid bumping in the fluid which led to high levels of water carry over, especially for raw feed manure and concentrate.

Moreover, future field studies of this combined MF-vacuum evaporation process may yield better results. In addition, to increase the product's market value, alternative acids should be tested to investigate the possibility of different ammonium salt (such as NH_4NO_3 and $(NH_4)_2(HPO_4)$ solutions) recovery for potential use as liquid fertilizers.

4. MF-NF treatment train for pig manure: Nutrient recovery and reuse of product water

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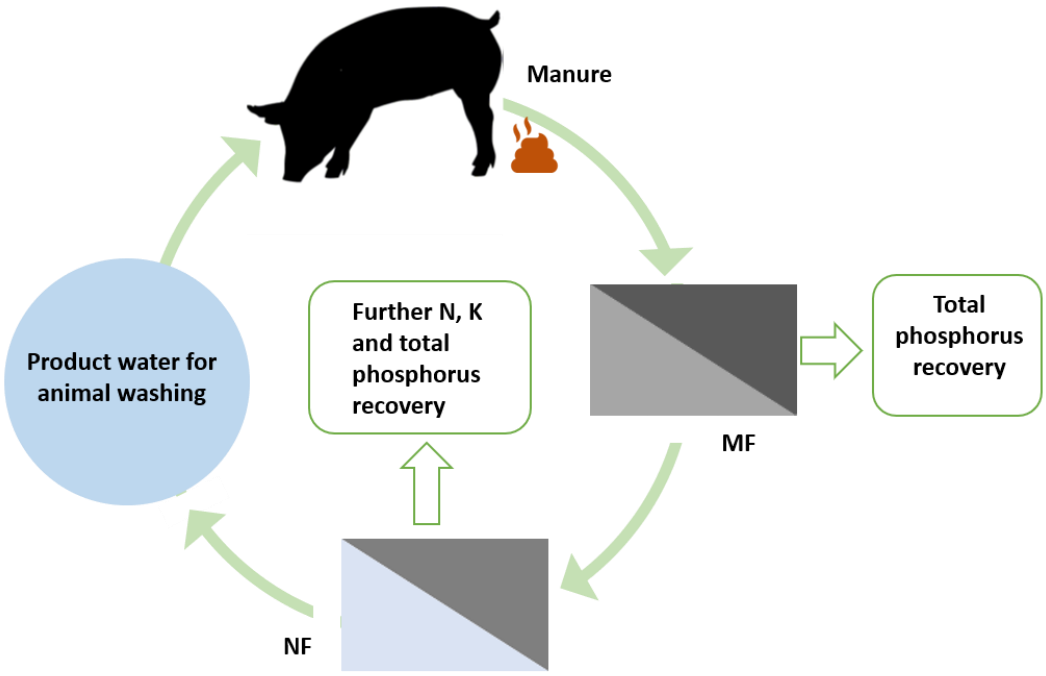

The graphical abstract of the chapter 4

4.1. Introduction

The livestock industry negatively impacts the environment by producing high organic and mineral loaded manure and wastewater (Haneklaus et al., 2016, Guo et al., 2018). Manure contributes to environmental pollution by releasing ammonia and nitrous oxide into the atmosphere (Webb et al., 2010), by leaching nitrate mainly into ground water (Ledda et al., 2013), and by increasing the soil acidification as well (Giola et al., 2012). Excess nitrogen (N) and phosphorus (P) that are released due to manure application degrades the overall aquatic ecosystem (Ngatia et al., 2019). This forced the European Community to implement nitrate directive guidelines to control the groundwater nitrate pollution (Wick et al., 2012).

On the contrary, manure is highly popular in agricultural applications for containing plant essential nutrients (Almutairi et al., 2011), in biopolymer production due to substantial volatile fatty acids concentrations (Albuquerque et al., 2011), and most importantly in biogas production for energy recovery as well (Riaño et al., 2011). Therefore, the nutrient recovery techniques from manure are in high demand, despite the presence of high solid contents, organic materials, and its potential hazardous properties (Gerardo et al., 2013).

The previously mentioned nutrient recovery processes from manure, such as hydrogel application (Kim et al., 2021), calcium phosphate precipitation (Lu and Liu, 2010), ammonia stripping (Quan et al., 2009), and struvite precipitation (Uysal et al., 2010) have proven to be very complex and required high chemical and energy inputs. Additionally, the drawbacks of using conventional mechanical processes to treat manure such as sedimentation, centrifugation, and pressurized filtrations have been well described previously (Hjorth et al., 2011). Mechanical processes such as sedimentation and centrifugation retained up to 56 and 44% of dry matter of manure. Whereas, MF could retain, on average, 75% of the dry matter content of manure. Consequently, the total phosphorus retention by MF was found 30–50% higher than the sedimentation and the centrifugation processes. However, the total nitrogen retention did not show many differences due to its significant presence in the liquid part of the manure (Hjorth et al., 2011, Masse et al., 2007, Samanta et al., 2022). Therefore, using membrane separation processes as an alternative provide an edge to the above-mentioned techniques in producing particle and pathogen-free, nutrient rich separate streams with relatively lower maintenance and operating costs (Ersahin et al., 2012).

MF can retain particles that range between 0.1 and 10 µm. Hence, it is well suited to retaining nutrients like P, which are mostly related to the solid phase in manure (Masse et

al., 2007). However, the dissolved nutrients such as K and N (mainly present as NH_4^+-N) mostly pass through the MF membranes (Gerardo et al., 2013). NF is capable of retaining major parts of the total N and K within a smaller concentrate volume (Hjorth et al., 2011, Masse et al., 2007). Therefore, the released nutrients can be concentrated using nanofiltration as a second step after MF. NF is also well known for its high micropollutants (e.g., antibiotics, antibiotics resistance genes, etc.) removal capacity (Hu et al., 2010), which enables the final product water to be reused to wash barns, irrigate nearby cultures, or apply on fields based on demand (Masse et al., 2007).

Limited studies have been reported on the application of NF as a post-treatment process after MF for manure and digestate treatment so far. However, none of the studies have compared between loose and tight NF membranes and commented on their efficiencies as a post-treatment process after MF. Therefore, the objectives of using an MF–NF treatment train in this research study to treat raw manure are to (i) perform solid–liquid separation by MF to produce nutrient rich separate streams in reduced volumes, (ii) to further concentrate the dissolved nutrients from MF permeate using different NF membranes and compare their efficiencies, and finally (iii) to produce a particle and pathogen-free product water.

4.2. Materials and methods

4.2.1. Pig manure sampling

Pig manure samples from pits of sampling sites 1 and 2, located in the state of Baden Württemberg, Germany, were collected in June 2020. Raw pig manure from the pit of sampling site 3, located in the state of lower Saxony, Germany, was collected in April 2020. The samples were collected in 10–30 L canisters and quickly stored at 4°C in the dark (Lamshöft et al., 2010) for further experiments. Site 1 contained over 500 pigs, whereas sites 2 and 3 were smaller farms. They contained overall 150–200 pigs each. The pigs of sites 2 and 3 were several months younger than the pigs of site 3. In addition, their diverse location and the growing culture resulted in different manure qualities.

4.2.2. Membrane Characteristics

The raw manure samples were initially filtered by using 0.45 µm pore sized MF membranes to eliminate the suspended solids. The MF membrane characteristics are mentioned in a previous research study by Wei et al. (2012). Three different loose NF membranes, NF270, HC50, and NTR7450 were further used for post-treatment of the MF permeate. The NF membrane characteristics are listed in Table 4.1.

Table 4.1. *NF membrane characteristics. ** (López-Muñoz et al., 2009); *'(Nitto, 2018); *** (Nyström et al., 1995, Tsuru et al., 1994)*

NF membrane	Supplier	Surface layer	pH tolerance	MWCO " [Da]	Water permeability [L h^{-1} $m^{-2}bar^{-1}$]
NF270	DuPont	Polyamide	3 to 11	300**	13.5**
HC50	Nitto	SPES*	3 to 11	1000*'	7.5*'
NTR7450	Nitto-Denko	SPES*	3 to 11	2000 - 3000***	9.2**

**Sulfonated polyethersulfone (SPES);*

"*Molecular weight cut off*

4.2.3. Membrane filtration processes

4.2.3.1. Microfiltration

Manure samples were initially sieved through a 1 mm sieve to eliminate the particles (≥1 mm). The samples were then prefiltered in a dead-end stirred cell membrane filtration system, manufactured by Merck KGaA Germany (Supporting Information Figure SC1), by using 0.45 µm pore sized MF membranes. The internal membrane diameter was 14 cm and the effective membrane area was calculated to be 154 cm^2 in the filtration cell. An initial feed volume of 500 mL was introduced in the feed tank for MF experiments. The filtrations were then performed by applying 1 bar pressure (N_2 gas, air liquid) and the rotational speed was maintained at 400 rpm. Consequently, 300 mL of permeate were collected in a sterile vial. The filtration was repeated twice for each manure sample to collect a total of 600 mL of MF permeate. The temperature was 25 °C ± 1 during the prefiltration experiments. The MF permeate samples were further analyzed for retention calculation.

4.2.3.2. Nanofiltration

An MF permeate volume of 200 mL each was used as the feed volume for the following NF experiments which were done using the same stirred cell dead-end filtration set up as mentioned in Section 4.2.3.1. Consequently, 100 mL of permeate were collected in a sterile vial after each NF experiment. The NF experiments were performed at 6.5 bar as the system could sustain maximum of 7 bar pressure. The rest of the filtration conditions were kept the same as the MF experiments. Similarly, pure water (Merck Millipore, Darmstadt, Germany)

permeability (PWP) was measured at 6.5 bar pressure before and after each NF experiment. The NF permeate samples were further analyzed for retention calculation.

4.2.4. Analytical processes

Total suspended solids (TSS) and volatile suspended solids (VSS) were measured according to the established methods (Daphne et al., 2011). The total chemical oxygen demand (tCOD), ammonium nitrogen (NH_4^+-N), and total phosphorus were measured using LCK014, LCK304, and LCK349 quick test kits (Hach Lange GMBH, Düsseldorf, Germany), respectively. The dissolved organic carbon (DOC) and dissolved total nitrogen (DTN) were measured with a TOC analyzer (Shimadzu TOC-V CPN, Kyoto, Japan). Organic acid anion concentrations were measured by an ion chromatography (IC) system (790 Personal Metrohm, Herisau, Switzerland). The cations were measured by inductively coupled plasma–optical emission spectrometry (ICP-OES, VistaPRO CCD, Fa. Varian, Mulgrave, Australia). Electrical conductivity and pH were measured by a portable multimeter (WTW Multi 350i, Xylem, Weilheim, Germany).

4.2.5. Calculated parameters

The permeate flux (J) was determined by the ratio of the permeate flow rate (Q_p) to effective membrane area (A_m).

$$J = \left(\frac{Qp}{Am}\right) (\text{L m}^{-2}\,\text{h}^{-1}) \tag{4.1}$$

The pure water permeability (PWP) was calculated by the ration of the pure water flux (J_w) to the applied pressure (TMP).

$$PWP = \left(\frac{Jw}{TMP}\right) (\text{L m}^{-2}\,\text{h}^{-1}\text{bar}^{-1}, \text{LMH}) \tag{4.2}$$

The retention calculation was done by following equation:

$$R = \left(1 - \left(\frac{Cp}{Cf}\right)\right) \times 100\ (\%) \tag{4.3}$$

Where, R is the calculated retention in percent (%). C_p and C_f are the permeate and feed concentration of any parameter at a given recovery.

The permeate volume recovery was calculated according to the following equation:

$$Rec = \left(1 - \left(\frac{Vp}{Vf}\right)\right) \times 100\ (\%) \tag{4.4}$$

Where, Rec is the calculated recovery in percent (%). V_p and V_f are the permeate and feed volume at a given time.

4.3. Results and discussion

4.3.1. Chemical characterization of manure

Pig manure characteristics vary strongly depending on various parameters such as pig feed, manure storage conditions (site location, storage duration, temperature etc.) and manure sampling methods (Tittonell et al., 2010). Chemical characteristics of pig manure slurry of three different sites are given in Table 4.2. A standard deviation of maximum 5% within the measured values was observed.

Table 4.2. *Pig manure characteristics*

Parameters	Site 1	Site 2	Site 3
TSS (g L^{-1})	3	4.9	4.7
VSS (% of TSS)	83	78	78
tCOD (g L^{-1})	11.3	11.8	10.7
NH_4^+-N (g L^{-1})	4.4	2.9	4.4
Total phosphorus (mg L^{-1})	185	103	283
pH	7.8	7.8	7.9
DOC (g L^{-1})	3.3	3	2.2
DTN (g L^{-1})	3	2	2.5
Dissolved phosphorus (mg L^{-1})*	103	19.2	105
Chloride (mg L^{-1})	1745	1083	1674
Sulphate (mg L^{-1})	39	86	108
Acetic acid (mg L^{-1})	3637	2211	1317
Calcium (mg L^{-1})	156	138	175
Potassium (mg L^{-1})	1793	1697	2737
Sodium (mg L^{-1})	895	409	850

**The dissolved phosphorus was measured after filtering the raw manure with 0.45 µm membrane filter*

In general, TSS is the combination of suspended solids that can be degraded by microorganisms and non-degradable suspended solids. The TSS ranged within 3–5 gL^{-1} in all three manure samples. Similar results were shown in previous literatures for raw manure

(Chelme-Ayala et al., 2011, Garzón-Zúñiga et al., 2007). TSS is also considered as one main contributor of tCOD. This could be noticed in the close ratios between tCOD and TSS among the manure samples (Pérez-Sangrador et al., 2012). Relatively lower NH_4^+-N concentration was found in the sample of site 2. This could be attributed to various facts such as (i) conversion of ammonium to ammonia due to longer storage may lead to further valorization or evaporation (Tao and Ukwuani, 2015) and (ii) different pig feed and growth stage as well (Dourmad and Jondreville, 2007). The total phosphorus concentration was lowest in the sample of site 2, followed by site 1 and site 3. Only 19% of the total phosphorus was dissolved in the sample of site 2. The number was raised to 55 and 44% for the samples of sites 1 and 3, respectively. This reflects the dominant presence of P in the solid fraction of manure.

The DTN in pig manure is the sum of dissolved organic N, dissolved NH_4^+-N, and dissolved nitrate N, although nitrate is not typically present in manure (Forge et al., 2016). However, parts of the organic N convert into NH_4^+-N, which then further converts into ammonia and contributes into total gradual loss of DTN (Tao and Ukwuani, 2015). This might lead to the lowest DTN value for sampling site 2 as the manure storage timing was the longest. Similar DTN values in pig manure samples were mentioned previously (Zhang et al., 2021b). DOC is generally metabolized through volatile fatty acid intermediates (Zacharof and Lovitt, 2014). Hence, the acetic acid and DOC concentrations were strongly correlated in manure samples. It was found that the organic carbon from acetic acid made up 44, 30, and 24% of the DOC in the manure samples of sites 1, 2, and 3, respectively.

4.3.2. Microfiltration of manure

MF was performed to achieve the solid–liquid separation. Feed samples were collected before the start of each MF experiment. Permeate and concentrate samples were collected after 60% recovery was achieved. The recovery was calculated by following Equation 4.4. The samples were then analyzed to probe the TSS, total phosphorus, COD, and NH_4^+-N retention by using Equation 4.3. The MF retention results are displayed in Figure 4.1.

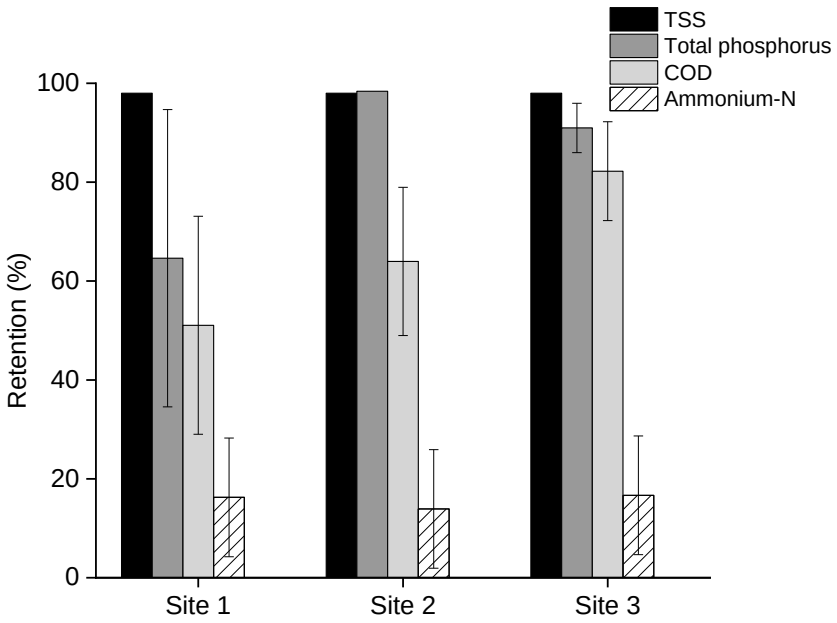

Figure 4.1. *Polymeric MF retention at 60% recovery. Pressure: 1 bar; stirring rate: 400 rpm; temperature: 25 °C.*

TSS retention of manure from all three sampling sites was above 98% (Figure 4.1). TSS-free MF permeate could visibly be observed as a transparent liquid compared to the MF feed (Figure 4.2). The total phosphorus retention remained above 80%. Short filtration duration may lead to retained dissolved P as well. The COD retention of 80% was highest in site 3. In the other sites, the retention remained within 50–60%. Low NH_4^+-N retention was found in all sampling sites. The overall NH_4^+-N retention stayed within 15–20%.

The efficiency of MF on TSS, colloids, and bacteria removal from mixed liquor and the effluents from biological reactors treating manure is well known (Gao et al., 2013, Kim et al., 2016). Different studies also showed the complete removal of TSS from manure by using polymeric MF as well (Zielińska et al., 2017).

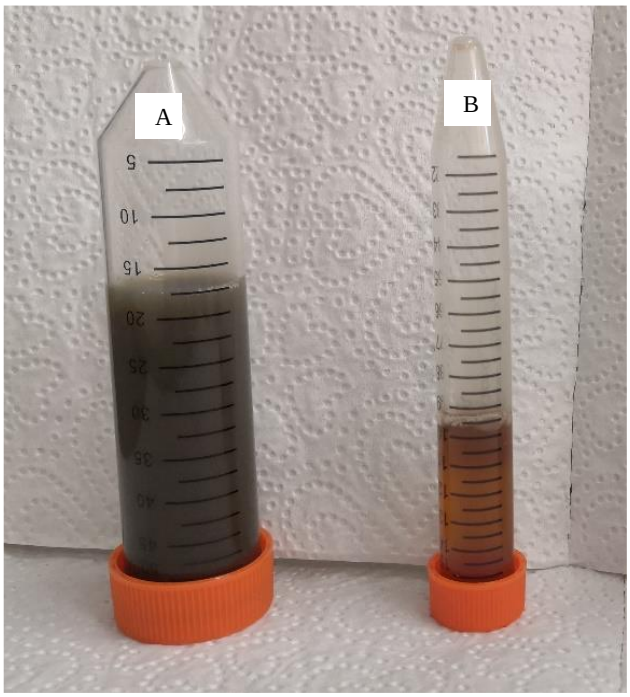

Figure 4.2. *(A) MF feed and (B) MF permeate after microfiltration performance of site 2 manure.*

Higher retention of total phosphorus could be attributed to its linkage with the particles between 0.45 and 10 µm in pig manure (Masse et al., 2005). Consequently, Christensen et al. (2009), quantified that more than 70% of the total phosphorus in pig manure slurry is associated with particles or colloids (Christensen et al., 2009). Therefore, it can be stated that the high TSS retention by MF enhanced the total phosphorus retention as well. tCOD retention of manure by MF is closely associated with the retention of the particulate organic matter content (González-Fernández et al., 2008, Zielińska et al., 2017). Hence, the manure sample of site 1 with the lowest TSS content (Table 1) presented the lowest tCOD retention by MF. Similarly, the tCOD retention of site 3 manure, which contains 56% higher TSS than site 1, resulted in 30% higher tCOD retention by MF as well. Previous studies have mentioned the dominant (>88% of TN) presence of NH_4^+-N in the liquid fraction of pig manure (Christensen et al., 2009, Mondor et al., 2008). Consequently, 5–10% mineralization of the organic N during manure storage also supports the above findings (Poulsen et al., 2001, LI and LI, 2014). Hence, the 15–20% retention of NH_4^+-N in the present study can be associated with the mineralized fraction retention by MF. In addition, MF was also expected to remove antibiotic resistance bacteria and pathogens from raw manure as well (Cheng and Hong, 2017, Kwarciak-Kozlowska and Wlodarczyk, 2020).

4.3.3. Nanofiltration of MF permeate

The objectives of performing the NF of MF permeate were to concentrate the dissolved nutrients (e.g., NH_4^+-N, K, and P) and to produce a permeate stream that can be reused. The permeate and concentrate samples were collected after 50% recovery was achieved. The recovery was calculated by following Equation 4.4. The samples were analyzed to calculate the retention by following Equation 4.3.

4.3.3.1. DTN and NH_4^+-N retention

DTN and NH_4^+-N retention by NF membranes are shown in Figure 4.3. The DTN retention of site 1 manure remained within a range of 40–50% by all NF membranes. However, the retention went above 60% for the other two sites. Interestingly, the DTN feed concentration of site 1 was 15 and 30% higher than that of sites 2 and 3, respectively. The individual membranes presented similar retention trends in all sampling sites. The retention by NF270 was the highest, followed by HC50 and NTR7450 membranes, respectively.

The NH_4^+-N contributed approximately 60–70% to the DTN. The overall NH_4^+-N retention was nearly 10–20% lower than DTN retention in all sampling sites. The retention trend was mainly unchanged. The NH_4^+-N retention differences among the membranes for site 2 remained within 5% only. However, the differences between NH_4^+-N retention by NF270 and NTR7450 membranes was found as high as 30 and 15% in sites 1 and 3, respectively. This can be attributed to their respective surface charges and pore sizes as well.

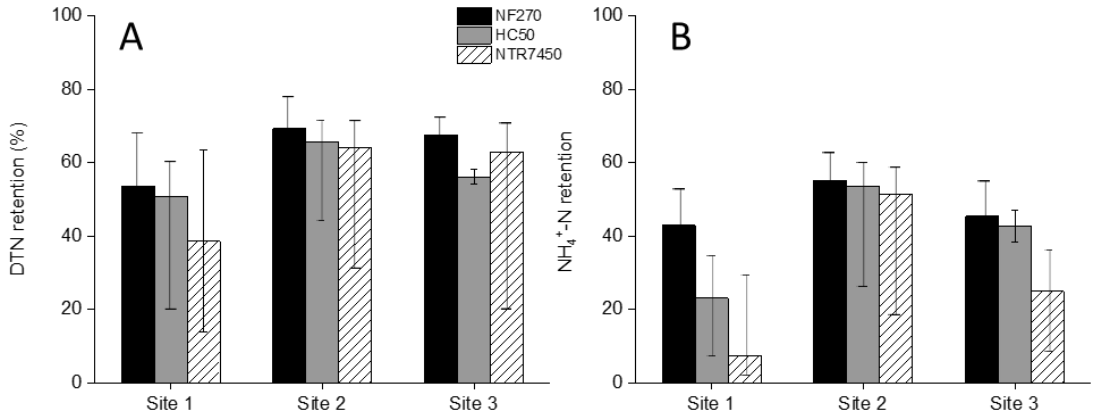

Figure 4.3. *(A) DTN and (B) NH_4^+-N retention of MF permeate at 50% recovery by NF270, HC50, and NTR7450 membranes from all sampling sites. Pressure: 6.5 bar; stirring rate: 400 rpm; temperature: 25 °C.*

Pig manure can be viewed as a mixed salt solution (Table 4.2). Therefore, the NH_4^+-N retention is assumed to be affected by the retention of the other competing ions as well (Supporting Information, Figure SC2). A previous study showed that the increase in competing ions concentration in the feed can reduce the examined ion retention (Ko and Chen, 2007). The NH_4^+-N retention by NF membranes is mainly influenced by charge interaction. It was already found that at pH7, NF270 retained 87.5% NH_4^+-N, when the filtration was performed under critical pressure. The study stressed the coupling of positively charged NH_4^+-N ions with the negatively charged active layer of the membrane (Cancino-Madariaga et al., 2011). Whereas, filtering dairy manure digested by NF270 resulted in 30–36% NH_4^+-N retention (Gerardo et al., 2013).

In addition, Hurtado and Cancino-Madariaga (2014) also observed that higher NH_4^+-N feed concentration resulted in lower retention by NF membranes. It is assumed that the increased feed concentration enhanced NH_4^+-N flux through negatively charged NF membranes (Pratofiorito et al., 2021) due to the reduction in the Donnan effect and the neutralization of the membrane surface (Bartels et al., 2005).

4.3.3.2. Phosphorus and potassium retention

The phosphorus retention by NF membranes is presented in Figure 4.4 (A). The substantial amount of phosphorus retention by MF resulted in a low phosphorus feed concentration for NF. The overall phosphorus retention was at or above 70%. In particular, NF270 retained above 90 and 80% of the remaining phosphorus from manure samples of sites 1 and 3, respectively. These were 10–20% higher than the retention by HC50 and NTR7450 membranes. Interestingly, no such retention differences between the NF membranes were noticed in the case of manure from site 2. Low feed phosphorus concentration and even lower permeate phosphorus concentration, led the analyzer to yield the limit of quantification as the permeate concentration in this case.

The potassium retention by NF membranes is displayed in Figure 4.4 (B). No real correlation between the initial feed potassium concentration and the retention was observed. The overall potassium retention was found to be within 22–54%. A similar retention trend was noticed in all manure samples. The highest retention was achieved by NF270. The retention by NTR7450 was 15–20% lower than NF270 and 5–10% lower than HC50 as well. High organic matter retention by NF (Supporting Information, Figure SC3) could visibly be noticed (Figure 4.5) as well.

Previous literature reported 96.4–97.2% phosphorus retention, when filtering dairy manure digestate using NF270. They stressed the charge repulsion effect as the main retention mechanism (Gerardo et al., 2015). It is a well-known fact that the dissolved fraction of phosphorus in manure is mostly found in the orthophosphate (PO_4^{-3}) form (Christensen et al., 2009). Ballet et al. (2007) also reported 99% retention of the divalent (HPO_4^{-2}) form of phosphate by NF NF200 membrane.

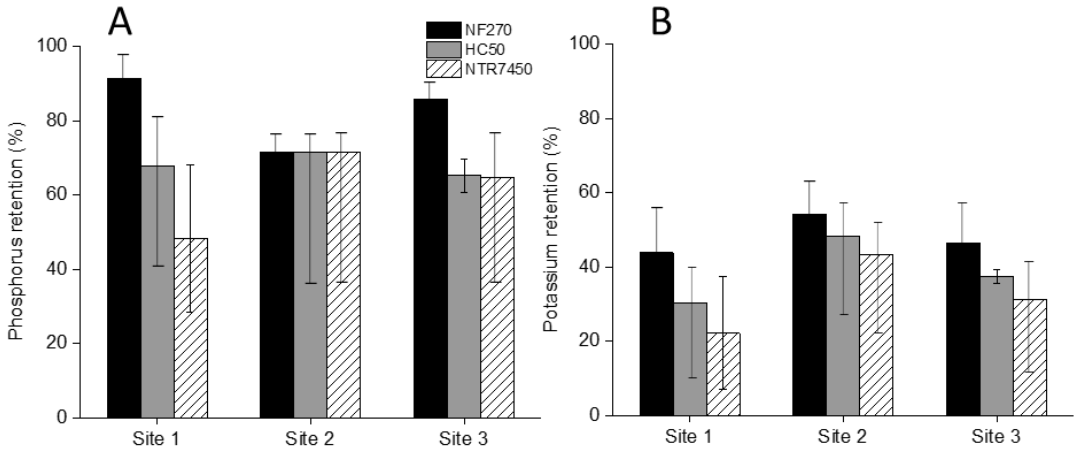

Figure 4.4. *(A) Phosphorus and (B) potassium retention of MF permeate at 50% recovery by NF270, HC50, and NTR7450 membranes from all sampling sites. Pressure: 6.5 bar; stirring rate: 400 rpm; temperature: 25°C.*

However, they carried out the experiments in a single salt solution condition, which might show very high retention (Ko and Chen, 2007). Therefore, the higher retention of the trivalent form of PO_4^{-3} by negatively charged NF membranes justifies the current findings. Previous literature has reported the proportional relation between the potassium chloride (KCl) feed concentration and the corresponding retention by NF membranes. They reported the range of KCl retention to be between 25 and 45% while filtering KCl solution of 5–15 gL-1 feed concentration, using an NF270 membrane (Al-Zoubi et al., 2007). Probably, due to the higher complexity and the presence of different ions and compounds of the feed manure, the trend was not clearly observed in the present study. Masse et al. (2007) noticed that, at higher recovery, the K retention was slightly decreased when filtering pretreated pig manure, with reverse osmosis membranes (Masse et al., 2007). Lastly, it is proven that NF retains extracellular antibiotic resistance genes from manure and digestate above 99.99% (Slipko et al., 2019, Lan et al., 2019). This facilitated the pathogen-free product water production as well.

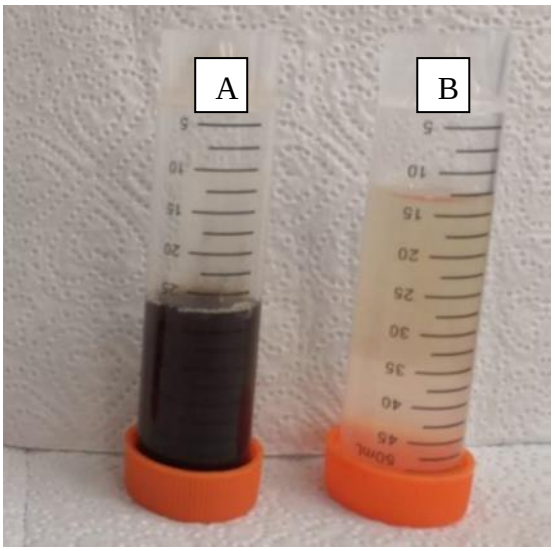

Figure 4.5. *(A) NF270 concentrate and (B) NF270 permeate of site 2 manure.*

4.3.4. Membrane performance

The permeate flux (J) and pure water permeability (PWP) were calculated by following Equations 4.1 and 4.2, respectively. The PWP results of all three NF membranes are displayed in Figure 4.6. The PWP declined by around 3 L m^{-2} $h^{-1}bar^{-1}$ for NTR7450. The permeability decline was reduced to 1–2 L m^{-2} $h^{-1}bar^{-1}$ for HC50 and was found to be lowest (0.5–1 L m^{-2} $h^{-1}bar^{-1}$) for NF270. However, the stabilized normalized flux (Supporting Information, Equation SC1) for HC50 and NTR7450 was 0.6–0.7 and 0.5–0.6 L m^{-2} h^{-1}, respectively. NF270 presented the lowest normalized flux of 0.2–0.3 L m^{-2} h^{-1} (Supporting Information, Figure SC4).

The drop in normalized flux was mainly caused due to the combined effect of reversible and irreversible fouling but the PWP decline after the filtration experiments was associated principally with the irreversible fouling (Van der Bruggen et al., 2003). Since NF270 showed the least fouling, the low normalized fluxes in the experiments with these membranes are presumably associated with the concentration polarization (CP). Winter et al. (2017) noticed that higher CP played an important role for lower MWCO membranes, while filtering natural organic matter (Winter et al., 2017). Hence, CP might largely affect the NF270 fluxes while the effect was smallest for the lower rejection NTR7450 membranes. It is also evident that the NTR7450 showed the highest fouling, followed by HC50 membranes.

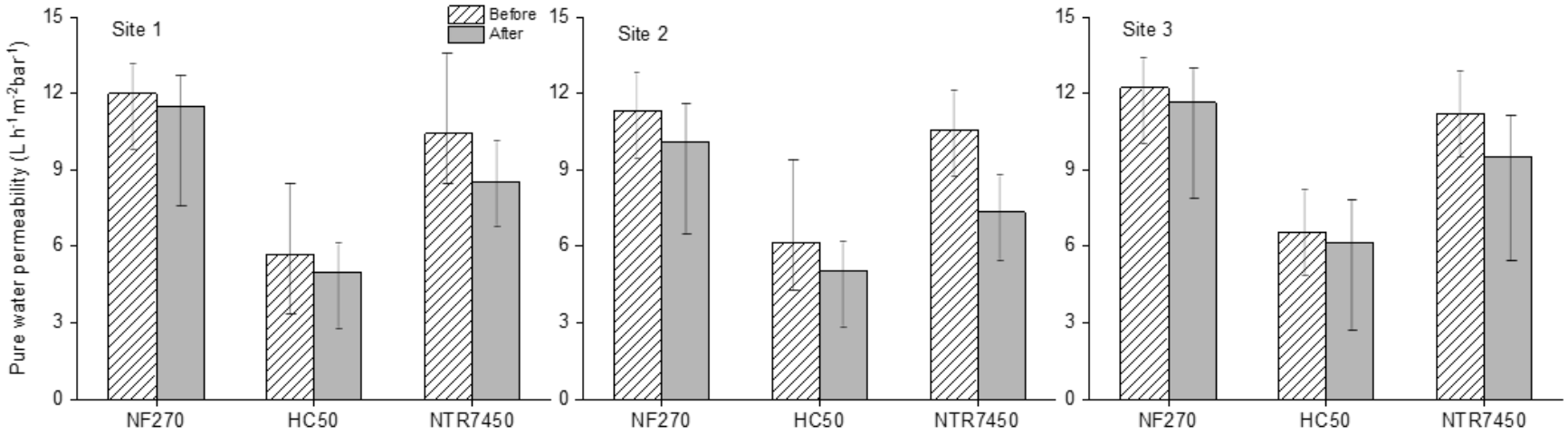

Figure 4.6. *Pure water permeability of NF270, HC50, and NTR7450 membranes, before and after filtering MF permeates from all of the sampling sites. Pressure: 6.5 bar; stirring rate: 400 rpm; temperature: 25 °C.*

4.4. Conclusions

The main benefits of the raw manure treatment by a cascade of MF and NF are to produce nutrient rich separate streams in reduced volumes. MF can separate the particulate material and NF can further concentrate the dissolve nutrients. Finally, a particle and pathogen-free product water is generated, which can be further reused in farms. Hence, the overall advantages of using a MF–NF treatment train for raw manure treatment is given below:

(i) MF retained phosphorus above 80% within a smaller MF concentrate volume, which accounted for 40% of the initial feed volume. Additionally, the MF permeate contained above 80% of the total nitrogen and most of the dissolve potassium.

(ii) NF of the MF permeate by three different NF membranes showed a maximum of 50–70% potassium and NH_4^+-N retention, respectively, within smaller NF concentrate volumes, which accounted for 30% of the initial feed volume of MF. Among all of the NF membranes, NF270 showed the most promising retention and was found to be the least prone to fouling.

(iii) Finally, the MF–NF treatment train was able to produce a particle-free final product water, which accounted for 30% of the initial feed volume of MF. This has the potential to be reused in farms to wash barns, to irrigate nearby cultures, or can be applied to specific fields based on the demand.

5. Removal of diverse and abundant ARGs by MF-NF process from pig manure and digestate

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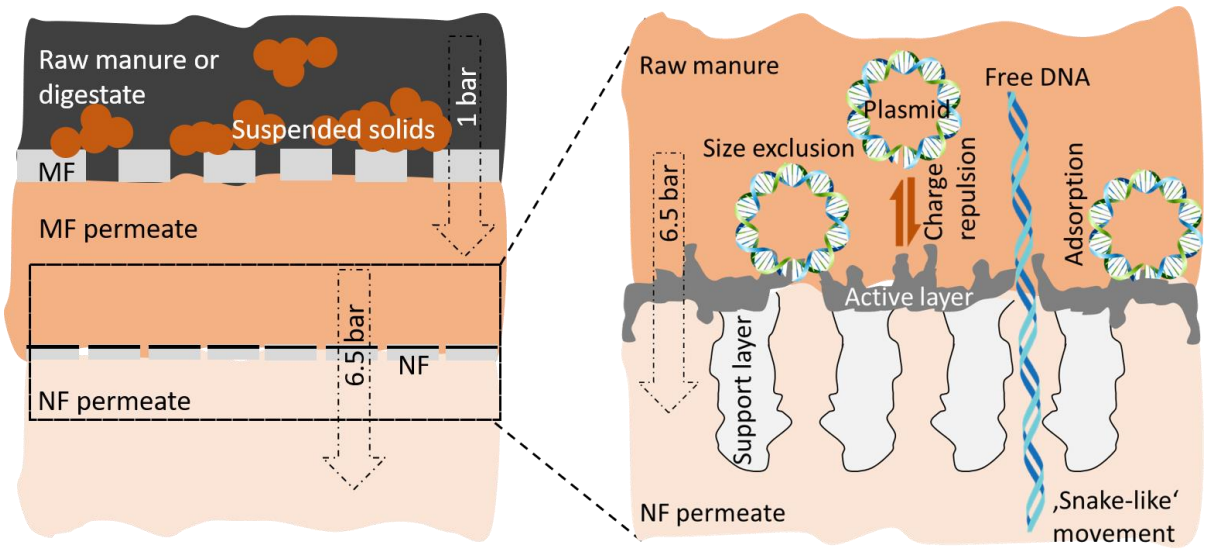


The graphical abstract of the chapter 5

5.1. Introduction

The biggest threat to the lifesaving antibiotic therapies is the spreading and aggregation of antibiotic resistance genes (ARGs) into multidrug resistance pathogens (Arias and Murray, 2009, Udwadia et al., 2012). Antibiotic resistant bacterias (ARBs) kill an estimated 700,000 people / year and it is to be expected to reach 10 million by 2050 (Willyard, 2017). Truly, the use of antibiotics in human and animal largely caused the ARG reservoir in the environment (Knapp et al., 2010). Especially, manure is considered as a major source of antimicrobial pollution which is caused by the overuse of antibiotics mostly in livestock husbandry, followed by turning farms into ARG reservoirs (Ji et al., 2012, Whitehead and Cotta, 2013).

Most veterinary antibiotics are poorly absorbed by the animals and consequently, a large part of it are excreted (Spielmeyer et al., 2017, Kumar et al., 2005b), which then unfortunately spread within soils (Chen et al., 2016), surface water (Beattie et al., 2018) and groundwater (Huang et al., 2019, Szekeres et al., 2018), when manure is applied as a fertilizer for its nutrient recycling practice. Moreover, antibiotic resistance traits in manure increases by the substantial use of subtherapeutic level of antibiotics in animal feed itself (Looft et al., 2012, Binh et al., 2008). In addition, anaerobic digestion, which is used as one of the primary treatment processes for the excrements of intensive livestock farms (Yang et al., 2010), is suspected to even increase some of the ARGs concentration (Ma et al., 2011). Therefore, the usage of antibiotics in farms often correlates with the expansion of the related ARGs in human pathogens, as well as the reason behind the spreading of animal antibiotic resistant bacteria (ARB) to human ARB (Forsberg et al., 2012, Smillie et al., 2011, Price et al., 2012). Hence, antibiotic resistance is declared as a global public health challenge which calls for immediate steps to stop its further spreading (Barancheshme and Munir, 2017, Bengtsson-Palme et al., 2018, Wernli et al., 2017).

So far, usage of multiple techniques, such as anaerobic treatment (Ma et al., 2018, Yi et al., 2017), coagulation (Li et al., 2017), advanced chemical oxidation (Michael-Kordatou et al., 2018, Fiorentino et al., 2019) and membrane bio-reactor (Le et al., 2018) have been reported as ARG removal process of various streams. However, the cost of these treatment processes was estimated pretty high due to the large usage of reagents and it can be detrimental by bringing secondary pollutants as well. Moreover, these techniques are not feasible for direct treatment of raw manure or digestate.

Recent studies reported membrane filtration processes, such as ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO), as an effective ARG removal process (Lu et al., 2020, Lan et al., 2019). Membrane filtration processes are also heavily applied as manure and digestate treatment process. Microfiltration is reported in multiple articles, to be used as an effective solid-liquid separator of manure and digestate (Tuczinski et al., 2018, Ravi et al., 2019), where both fractions have the potential to further be processed to generate bio-fertilizers (Al Seadi et al., 2013). However, ARG removal efficiency by MF is poor and limited mostly to the intracellular ARGs (Slipko et al., 2019, Gros et al., 2019, Lu et al., 2020). In addition, the liquid fraction after solid-liquid separation is enriched with ammonium nitrogen (Christensen et al., 2009), which was found to be one of the major reasons alongside dissolve oxygen to exhibit strongest correlation with high ARG concentration and horizontal gene transfer (Ott et al., 2021). Hence, further treatment of MF permeate is an absolute necessity. Therefore, additional usage of NF and RO would not only enhance the ARG removal efficiency (Lan et al., 2019, Gros et al., 2019), but also these would (a) reduce the volume, (b) produce nitrogen rich concentrate stream for using it as a direct fertilizer and (c) generate a purified stream to be further used in irrigation (Al Seadi et al., 2013, Ros et al., 2020, Bonmatí-Blasi et al., 2020, Cerrillo et al., 2015, Tampio et al., 2016, Ledda et al., 2013).

To date, a few investigations have been done on checking the efficiency of ARG removal using NF and RO processes by treating livestock waste (Gros et al., 2019), swine wastewater (Lan et al., 2019) and reclaim water (Lu et al., 2020). However, no studies have reported on the MF-NF treatment process of raw manure and digestate for the elimination of ARGs. Therefore, the objectives of this research study are (i) the identification and consequent quantification of diverse and abundant ARGs in raw pig manure and biogas digestate samples, followed by (ii) their removal using MF-NF filtration process.

5.2. Materials and methods

5.2.1. Pig manure and digestate sample collection

Pig manure samples were collected freshly in November 2020 from the pits of sampling site 1 and site 2 which are located in the state of Baden Württemberg, Germany. Digestate sample was collected in October 2020 from sampling site 3 which is located in the state of

Lower Saxony, Germany. The samples were collected in 10 L canisters and quickly stored at 4°C in dark for further experiments.

5.2.2. Characteristics analysis

Total suspended solids (TSS) and volatile suspended solids (VSS) measurements were done as per APHA AWWA (1998). Chemical oxygen demand (COD), ammonium nitrogen (NH_4^+-N) and total phosphate (PO_4^{-3}) were measured by test method of Hach Lange GmbH. pH value of pig manure and digestate were measured by using a portable WTW ProfiLine 3110 pH meter. Dissolved total carbon (DTC), dissolved organic carbon (DOC) and dissolved total nitrogen (DTN) measurement were conducted by a Shimadzu Total Carbon Analyzer TOC-5000. Acetic acid and potassium concentrations were measured by an 881 Compact IC pro (Metrohm, Switzerland) ion chromatograph and an inductively coupled plasma optical emission spectrometry (Agilent Technologies, ICP-OES 5110, Germany) respectively. Detail characteristics of the manure and digestate samples are given in supporting information (Table SD1).

5.2.3. Filtration protocols

5.2.3.1. Pre-treatment

Manure and digestate samples were initially sieved through 1 mm sieve to eliminate particles from it. The samples were then prefiltered in a dead end stirred cell membrane filtration system, manufactured by Merck KGaA Germany (supporting information Figure SC1), by using 0.45 µm pore sized microfiltration (MF) membranes to eliminate the suspended solids from it. The MF membrane characteristics are mentioned in previous research study by Wei et al. (2012). The internal membrane diameter was 14 cm and the effective membrane area was calculated 154 cm^2 in the filtration cell. Initial feed volume of 600 mL was introduced in the feed tank for MF experiments. The filtrations were then performed by applying 1 bar pressure (N_2 gas, Air liquid) and the rotational speed was maintained at 400 rpm. Consequently, 300 mL of permeate was collected in a sterile vial. The temperature was noticed 25 ℃ ± 1 during the prefiltration experiments. ARG concentrations in feed samples were measured before each MF experiment.

5.2.3.2. Nanofiltration

Permeate volume of 300 mL from MF experiments were used as the feed volume for the following nanofiltration (NF) experiments, which were done by using NF270 (DuPont, Germany) membrane in the same stirred cell dead end filtration set up as mentioned in

5.2.3.1. Consequently, 180 mL of permeate was collected in sterile vial after each NF experiment. Detailed characteristics of NF270 membranes are summarized in previous studies (Mänttäri et al., 2004, Dang et al., 2014). The NF experiments were performed at 6.5 bar as the system could sustain maximum of 7 bar pressure. The rest of the filtration conditions were kept same as the MF experiments. Similarly, pure water (MilliQ water, Millipore) flux (PWF) were measured at 6.5 bar pressure before and after each NF experiments. The NF permeate samples were additionally analyzed for ARG concentration measurements.

5.2.4. DNA extraction

Total DNA was extracted from each sample using DNeasy PowerSoil Pro Kit (Qiagen Sciences, Germany) by following manufacturer's instructions. Quality of DNA and its concentration were determined by a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, USA).

5.2.5. Smart chip qPCR analysis description

The presence and abundance of antibiotic resistance genes (ARGs) and 16S rRNA gene in each sample were analyzed using customized primer sets (Stedtfeld et al., 2018) in a high throughput method, SmartChip qPCR system. Several primer sets were designed to target sequence diversity within the gene target to more specifically assess the environmental resistome, therefore, each primer set was analysed independently. The threshold cycle (CT) of 27 was used as the detection limit (Muziasari et al., 2017, Muziasari et al., 2016, Wang et al., 2014, Zhu et al., 2013). Melting curve analysis and PCR efficiency were performed on all of the samples for each primer set. Amplicons with unspecific melting curves and multiple peaks based on the slope of melting profile were considered to be false positives and discarded from the analysis.

Briefly, the SmartChip has 5184 reaction wells with a volume of 100 nL and filled using the SmartChip Multisample Nanodispenser. qPCR cycling conditions and initial data processing was done as previously described in (Wang et al., 2014). qPCR reagents recommended by the manufacturer were used. Mean CT of three technical replicates in each qPCR reaction was used to calculate the ΔCT values, unless, the genes were detected in only one of the three technical replicates, in which case they were removed. The $2^{-\Delta CT}$ method (where ΔCT = CT detected gene – CT 16S rRNA gene) was used to calculate the relative abundances of the

detected gene in proportion to the 16S rRNA gene in each sample (Schmittgen and Livak, 2008).

5.2.6. ARG retention calculations

The log retention value of ARGs was calculated by following equation (5.1) (Slipko et al., 2019, Lan et al., 2019).

$$Log\ retention\ value\ (LRV) = Log\ (\frac{RS}{NFP}) \quad (5.1)$$

Where, RS referred to absolute ARG copy numbers per 100 µL in the raw manure and digestate samples and NFP referred to the ARG gene copy numbers per 100 µL in the nanofiltration permeate samples.

5.3. Results and discussion

5.3.1. Presence of diverse ARGs in pig manure and digestate

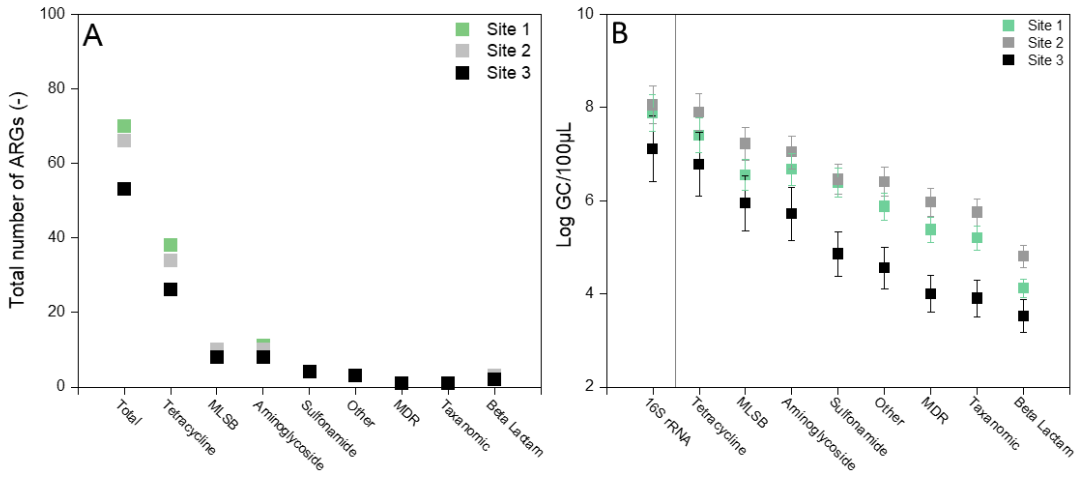


Figure 5.1. *Comparison of (A) total detected ARGs and (B) log value of ARG copy numbers among three sampling sites, conferring resistance to different antibiotics, where GC referred to genes copy numbers.*

Total 189 ARGs were detected from all the raw samples (Figure 5.1A), among which 66 ARGs were shared among manure and 53 ARGs were shared among both manure and digestate samples (supporting information, Figure SD1). Antibiotic deactivation was the main resistance mechanism confined to the detected ARGs, followed by cellular protection and efflux pumps. These samples contained ARGs conferring most dominantly resistance to tetracycline (51.9%), aminoglycoside (15.3%) and MLSB (macrolide-lincosamide-streptogramin B, 14.8%) antibiotics, followed by sulfonamide (6.3%), other groups (4.8%), ß-Lactam (3.7%), taxanomic (1.6%) and MDR (multiple drug resistance, 1.6%) (Figure 5.1A). Similarly, the log value of total ARG copy numbers per 100 µL conferring resistance to tetracycline was found highest followed by MLSB and aminoglycoside respectively (Figure 5.1B). Despite the presence of multiple ARGs resistance to ß-Lactam, the log value of total ARGs copy numbers conferring resistance to MDR and taxanomic were noticed 20% and 16% higher than ß-Lactam respectively (Figure 5.1B). Manure samples (of site 1 and site 2) contained one to two order of magnitude higher copy numbers of ARGs than digestate sample (of site 3). However, the pattern of ARG copy numbers conferring resistance to different antibiotic groups was noticed similar in both manure and digestate samples (Figure 5.1B).

Pu et al. (2018) found 83 shared ARGs in pig manure and digestate samples. Similarly, the most dominant types of ARGs were noticed conferring resistance to tetracycline (25-38%), aminoglycoside (20-29%) and MLSB (14-20%). Later, Zhang et al. (2021a) confirmed the findings, where the resistance of total 658 ARG subtypes belonged to the most frequent classes of above-mentioned group of antibiotics as well. Probable reason was directed to the high usage of these antibiotics in pig production (Krishnasamy et al., 2015). Substantial presence of ARGs resistance to tetracycline in pig manure are being mentioned in research studies since 2002 (Agersø et al., 2002, Agersø et al., 2004). Additionally, recent studies reported more variants of it (Cheng et al., 2016, Ji et al., 2012). Consequently, Zhu et al. (2013) reported frequent occurrence of aminoglycoside resistance ARGs in pig manure samples. Later, Luo et al. (2017) found 10 subtypes of aminoglycoside resistance ARGs and 4 subtypes of MLSB resistance ARGs in both pig manure and digestate samples. It is presumed that, not only the antibiotics but also the striking number of additives usage increase the prospect of coresistance in genetic element (Gillings and Stokes, 2012).

5.3.2. Absolute ARG abundances in raw manure and digestate

Pig manure and digestate samples were highly enriched with ARGs. The total ARG copy number was highest in the pig manure sample of site 2 (1.15×10^8 copies), which was one and two order of magnitude higher than site 1 and site 3 respectively (Figure 5.1B). Absolute concentrations of 37 ARGs were found above 10^5 copies per 100 µL. The absolute ARG concentrations in the raw manure and digestate samples indicated the actual ARG copy numbers per 100 µL (Figure 5.2). High enrichment of ARGs in all samples demonstrated the substantial expansion of antibiotic resistance reservoir in the sampling sites, including the enrichment of up to 38 *tet genes* in a single site, followed by 11 and 10 aminoglycoside and MLSB resistance genes respectively (supporting information Figure SD1).

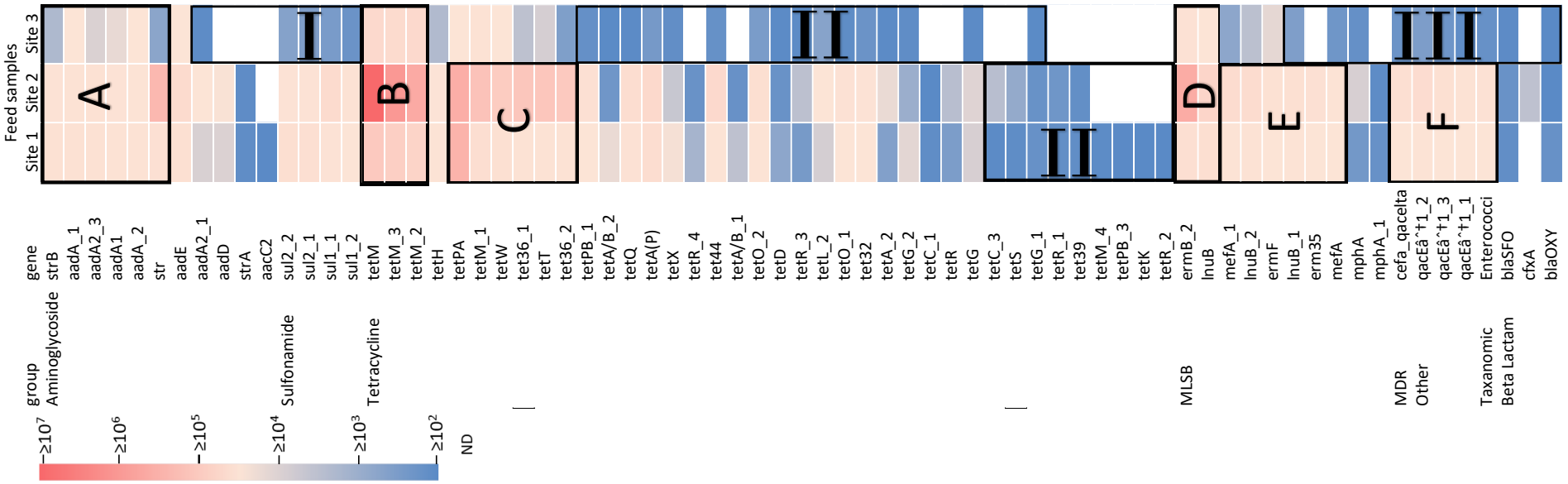


Figure 5.2. *Absolute ARG concentration (per 100 µL) profile, resistance to different antibiotic groups in each sampling site. Zone (A), (B) and (D) are enriched in all of the sampling sites; (C), (E) and (F) are enriched in site 1 and site 2 but not in site 3; Zone I, II and III denoted the absolute ARG copy numbers ≤ 10^3 per 100 µL.*

In the digestate sample of site 3, 75% of the aminoglycoside resistance genes were found above 10^4 copies per 100 µL. Although, it was reduced to 38% for *tet* genes. Consequently, 24% of the *tet* genes were found as low as 10^3 copies per 100 µL in manure sample of site 1 and 10% of the *tet* genes were not detected in manure sample of site 2. On contrary, all the ARGs resistance to sulfonamide, MDR, other and taxanomic groups were detected above 10^5 copies per 100 µL in manure samples. In addition, beta lactam resistance genes were nearly not detectable in all the samples. In general, least number of ARGs were detected in digestate sample of site 3. Their average concentration was 10^4 copies per 100 µL, which was one to two order of magnitude lower compared to the average ARG presence in manure samples.

This results not only informs the extension of the antimicrobial reservoir in large to medium size livestock husbandry and biogas plants but also shows the substantial abundances of the detected ARGs, which may lead to possible horizontal transfer in the environment (Zhu et al., 2013). The various set of detected ARGs were potentially resistance to all major classes of antibiotics, including critically important antibiotics for human medicines, such as tetracycline, macrolides and aminoglycoside (WHO, 2017). Looft et al. (2012) detected 57 ARGs from the manure of selected pigs, out of which 8 ARGs were enriched. Later Zhu et al. (2013) demonstrated the list of 62 ARGs, which were frequently detected in multiple animal farms. However, the maximum enrichment of an ARG in a single site was reported 90,000 copies per mL. This is strikingly three order of magnitude lower than the highest enrichment of the ARG (*tetM)* found in the present study. However, a few recent studies which focused on some particular *tet* and *sul* genes, have reported highest enrichment from 10^6 to even 10^{11} copies per mL (Lu et al., 2020, Lan et al., 2019), which is in accordance with the results presented here.

The average enrichment (10^4 copies per 100 µL) of aminoglycoside resistance ARGs compared to the other antibiotic groups were noticed highest in the digestate sample of site 3. It is comparable with the previous findings by Pu et al. (2018). However, depending on the anaerobic digestion conditions, the results could be turned around (Tian et al., 2016, Sun et al., 2016). In previous studies, it was observed that aminoglycoside resistance ARGs were coenriched due to their probable aggregation in the mobile genetic elements (Looft et al., 2012, Varga et al., 2009, Barlow, 2009, Heuer et al., 2009, Heuer et al., 2012). Consequently, Binh et al. (2009) mentioned that the presence of them in the integrons may cause their

enrichment as well. Presence of sulfonamide resistance ARGs in manure and digestate samples are ubiquitous and are among the most enriched ones (Zhu et al., 2013, Pu et al., 2018). The enrichment of *sul1* and *sul2* subtypes in digestate sample of site 3 were two orders of magnitude lower than the manure samples. This could be the result of the alteration of the digestate feed itself from raw pig manure to a waste mix. This is in accordance with a previous study, where Song et al. (2017) pointed, that the usage of pig manure and wheat straw mixture as digestate feed instead of raw pig manure resulted in lower *sul1* and *sul2* concentration in the digestate.

However, other studies found that the digestion operation at 35 ℃ may increase sulfonamide resistance ARGs concentration in the digestate (Sui et al., 2016, Tao et al., 2014). Hence, further studies are required to clarify the ambiguity of the enrichment of the sulfonamide resistance genes in digestate. Zhu et al. (2013) detected 22 *tet* genes, which were shared in pig manure samples of multiple animal farms. They reported *tetQ*, *tetW, tetX, tet(32), tetO, tetM, tetL and tetG* as the most abundant *tet* genes. We detected 38 *tet* genes in pig manure sample of a single sampling site (site 1) where the most abundant *tet* genes were *tetM, tetH, tetW, tetT, tetQ* and their subtypes which are in accordance with the findings by Zhu et al. (2013). The 73% increased number of *tet* genes in our study compared to the previous literature reflected our detailed sampling process and the precise ARG detection method.

Although, swine farms are considered as the hotspots of antimicrobial resistance for antibiotic free (Looft et al., 2012, Jackson et al., 2004) and antibiotic treated animals (Enne et al., 2008, Heuer et al., 2011), the enrichment of the ARGs found in this study were on par with previous literatures. In addition, Pärnänen et al. (2019) gathered large number of ARGs dataset from the different geographic regions of Europe and found that, in Germany, the abundances of gene families resistance to tetracycline and MLSB antibiotics was higher than other antibiotic groups. In particular, MLSB resistance *ermF* and aminoglycoside resistance *aadA* genes were noticed with very high prevalence. Both findings are in accordance with the present study.

The specificity of the diverse ARGs and their fate in raw and treated samples reflected the influence of antibiotics, particularly the residues (Gao et al., 2012, Rodriguez-Mozaz et al., 2015, Li et al., 2015b, Bengtsson-Palme et al., 2016). The alarming enrichment of ARGs at farm level might exhibit the threat to human population by getting transferred from livestock animal to human related bacteria (Marshall and Levy, 2011, Smillie et al., 2011).

5.3.3. Removal of ARGs from raw manure and digestate by nanofiltration

The World Health Organization (WHO) referred antimicrobial resistance as the emerging threat to the treatment against infections caused by parasites, viruses and bacteria in their Global Report on Surveillance (WHO, 2014). The substantial use of antibiotics made the presence of resistant genes and the mobile genetic elements ubiquitous in all possible environment. In particular, livestock husbandry identified as the antimicrobial reservoir which has the potential to spread ARGs and the mobile genetic elements into the environment (Cheng et al., 2016, Sui et al., 2016, Chen et al., 2010). The concerns may hinder the further reuse of manure and digestate in agriculture. Hence, a suitable antimicrobial resistance removal technology is the need of the hour.

Membrane filtration processes, including MF to RO has been applied as an effective process to remove ARGs from pig wastewater and digestate in recent years (Gros et al., 2019, Lan et al., 2019, Wang and Chen, 2020). MF is widely used as a solid-liquid separation process for manure and digestate treatment (Tuczinski et al., 2018, Ravi et al., 2019). Therefore, in this study, MF of raw pig manure and digestate was initially used to remove suspended solids from it. Although, a previous study stated, that MF could separate ARGs only to some extent (Le et al., 2018), especially the intracellular ARGs by removing almost all bacteria (typically 0.5 – 5.0 μm) (Lan et al., 2019), however, the absolute concentration difference between the feed and the permeate after MF remained within one to two order of magnitude (Lu et al., 2020). In addition, Gros et al. (2019) noticed no difference of *tetW* concentration between solid and liquid fraction of livestock waste and no retention of *ermT*, *qnrA* and *qnrB* by MF was observed by Lu et al. (2020) using MF membrane. Most important, MF could not retain extracellular or free DNAs, which could result in the dissemination of the ARGs, that are encoded within these DNAs, into the soil and aquatic environment (Slipko et al., 2019). In this work, the MF permeates were directly filtered by NF membrane to minimize the further transmission probability, followed by detecting ARGs in the NF permeate to evaluate the final retention using the MF-NF process.

The highly concentrated ARGs in raw samples are presented in zone A to F and the low concentrated ARGs in raw samples are presented in zone I to III of Figure 5.2. Similarly, the LRV of these highly concentrated ARGs after MF-NF process are presented in zone A to F and the LRV of the low concentrated ARGs after MF-NF process are presented in zone I to III of Figure 5.3. Apart from the highly concentrated aminoglycoside resistance genes (Figure 5.2,

Zone A), all the other ARG enriched zones (Figure 5.2, Zone B to F) showed LRV from 3 (99.9 %) to as high as 5 (99.999 %) after the MF-NF process (Figure 5.3, Zone B to F). Consequently, LRV ≤ 2 (≤ 99 %) was noticed in Zone I, II and III of Figure 5.3, where the initial absolute ARG concentrations were ≤ 10^3 copies per 100 µL (Figure 5.2, Zone I, II and III). In addition, ARG removal was found to be directly proportional to its initial concentration in the feed apart from mostly aminoglycoside resistance and a few tetracycline resistance genes (Supporting information, Figure SD2 A, B and C).

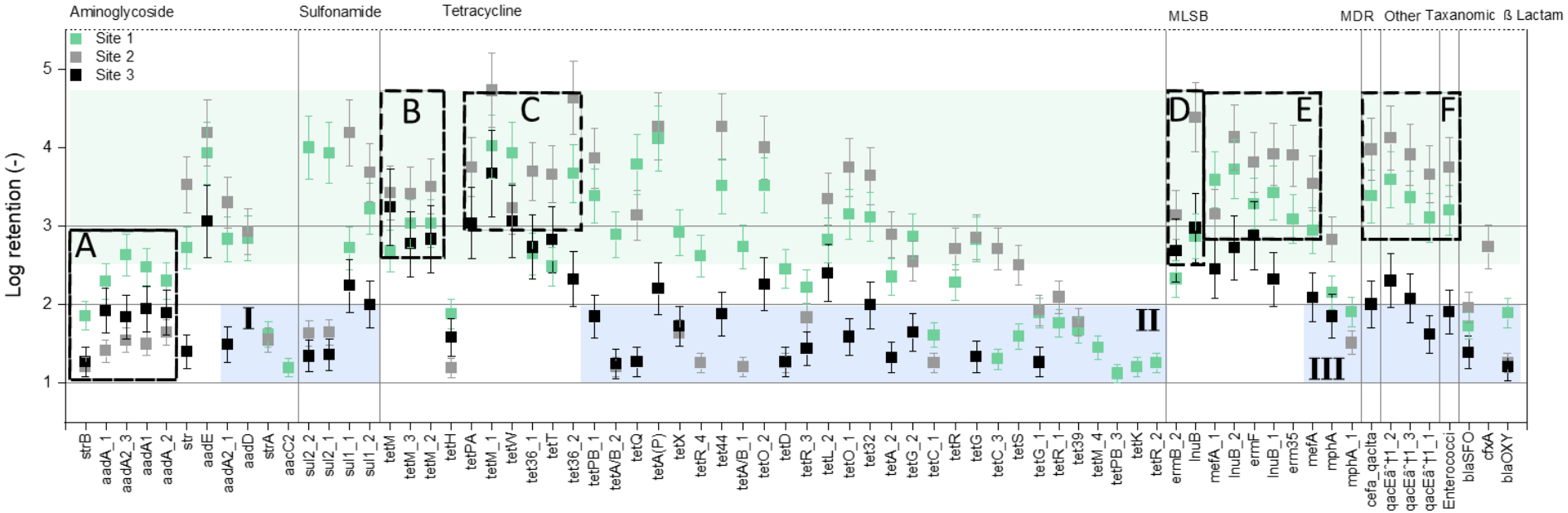


Figure 5.3. *Log retention value (LRV) of individual ARGs of pig manure and digestate samples of sampling site 1, 2 and 3. Zone (A), (B) and (D) were enriched in raw samples of all the sampling sites; (C), (E) and (F) were enriched in the raw samples of site 1 and site 2 but not in the raw digestate sample of site 3; Zone I, II and III denoted the absolute ARG copy numbers ≤ 10^3/100 µL in the raw samples of all sampling sites.*

Despite being enriched with ARGs in all samples, the average LRV remained only 1.5 of aminoglycoside resistance genes, represented in Zone A of Figure 5.3. The lowest LRV was found 1.2 for *strB* in the manure sample of site 2 and in the digestate sample. Pärnänen et al. (2019) gathered large number of ARG dataset from various European countries and noticed the persistence of aminoglycoside resistance ARGs (*aadA* and *strB*) after treatment in more than 90% of the samples. In addition, Gros et al. (2019) mentioned, that the ARGs with low retention after RO, were directly linked with class I integrons (Cheng et al., 2013, Chen and Zhang, 2013, Subirats et al., 2018). Hence, the similarities in low retention of aminoglycoside resistance genes in this study might be attributed with their linkage in class I integrons (Binh et al., 2009) as well. The LRV of sulfonamide resistance genes (subtypes of *sul1* and *sul2*) was 4 in manure samples. Lan et al. (2019) reported the LRVs of *sul1* and *sul2* genes were 5.29 and 6.13 respectively after NF process. High initial concentration of ARGs was found as the key reason for this very high retention. Similarly, Lu et al. (2020) found the LRVs of *sul1* and *sul2* were 2.8 and 3.3 respectively after RO filtration. High efficiency of eDNA removal by RO was mentioned as a major reason for higher removal. These are in accordance with the present study. However, LRV of *sul_1* and *sul_2* in the digestate sample of site 3 were 1.32 and 1.36 respectively. Interestingly, the lower retention could directly be linked to the lower initial concentration of the genes in (Figure 5.3, Zone I). In addition, Gros et al. (2019) linked the low retention of *su1* after RO with their linkage to class I integrons. A similar trend was noticed for *tet* genes as well. Highly enriched *tet* genes such as *tetM*, *tetW*, *tetT* and their subtypes in zone B and C were also retained efficiently after NF process. The maximum LRV of *tetM*, *tetW* and *tetT* genes were noticed 3.46, 4.72 and 3.93 respectively. These results are in accordance with previous studies, where max LRV of *tet* genes after NF and RO was reported between 2.5 to 7.84 (Gros et al., 2019, Lu et al., 2020, Lan et al., 2019). However, the LRV of *tet* genes with low initial concentration (≤ 10^3 copies per 100 µL) were below 2 (Figure 5.3, Zone II). In manure of site 1, lowest retention was noticed for *tetPB_3*, *tetK* and *tetR_2* genes. Their LRVs were between 1.11 to 1.25. The similar range of LRVs was noticed for the lowest retained *tetR_4* and *tetA/B_1* genes in manure sample of site 2 and *tetA/B_2* and *tetQ* genes in digestate sample of site 3. Lu et al. (2020) noticed low LRV of 1.6 of *tetB* gene after RO filtration. Interestingly, they mentioned that the initial concentration of *tetB* gene before RO filtration was below 10^3 copies. This is in accordance with the present study. Furthermore, the LRV between 3 to 4.41 was noticed for MLSB, MDR, other

and taxanomic resistance genes in zone D, E and F of Figure 5.3 which were enriched in raw manure and digestate samples (Figure 5.2, Zone D, E and F). The LRV above 2.3 for MLSB resistance *erm* genes after RO filtration were reported in previous studies (Lu et al., 2020, Gros et al., 2019), which is in accordance. Subsequently, The LRV of ß Lactam resistance genes in manure and digestate samples were below 2. The lowest LRV of 1.18 was noticed for *bla*OXY in manure of site 2 and in the digestate sample. Cristóvão et al. (2021) noticed only 90.59% removal of *bla*NDM gene after NF with Desal 5 DK membrane. However, the removal rate of other *bla* (*bla*KPC*, bla*OXA-48 *and bla*VIM) genes were reported above 99.6%. Dissemination of aerosol near the sampling point was mentioned as the reason for low ARG presence in NF permeate. In summary, the LRV of enriched genes after NF process was higher than 3 to as high as 5. However, the retention of genes with low initial concentration remained below 99 % (LRV 2).

Size exclusion mechanism was previously mentioned as one of the prime reasons for ARG removal by membrane filtration process (Arkhangelsky et al., 2011, Breazeal et al., 2013, Latulippe et al., 2007, Latulippe and Zydney, 2009). A recent study by Cheng and Hong (2017) assessed the sizes of the plasmids of *bla*NDM-1, *bla*CTX-M-15 and *bla*OXA-48 ARGs by dynamic light scattering technique and were noticed to be within 460 to 560 nm in diameter. On the other hand, the average pore diameter of NF270 was reported 0.84 nm (Nghiem and Hawkes, 2007), which was 560 to 660 times smaller than the previously mentioned plasmid diameter. Therefore, in our study, the size exclusion of ARGs by NF270 is also considered as one of the main ARG retention mechanisms. Electrostatic charge repulsion was considered as the next major ARG retention mechanism by NF. The hydrophilicity of the extracellular plasmids are evident due the exposed sugar-phosphate bond of DNA (Westhof, 1988). In support, Cheng and Hong (2017) also found, that the zeta potential value of the above-mentioned three plasmids were greater than -22 mV. Consequently, the zeta potential of NF270 membrane was reported -24.7 mV at pH 8 (Nghiem and Hawkes, 2007). Therefore, electrostatic charge repulsion mechanism might play major role for ARG retention in the present study as well. This is in accordance with Ager et al. (2009), who reported the retention of negatively charged plasmid molecules enhanced when filtering with negatively charged membranes. However, Slipko et al. (2019) observed higher adsorption of ARGs on less charged membranes. Moreover, in our study, ARG retention was found largely proportional to its initial enrichment in the feed. It is in accordance with findings of Slipko et

al. (2019). They hypothesized that the free DNA molecules, which adsorb on the membrane surface, subsequently blocked the passage through membrane, followed by reduction in ARG permeation. The similar findings were noticed by Lan et al. (2019), where extremely high level presence of *sul* and *tet* genes in raw swine wastewater lead to LRV of 4.98 to 9.52 after NF and RO treatment of the sewage. Lastly, the interaction of free DNA molecules with manure and digestate matrix might serve as an additional ARG removal mechanism by NF270 (Breazeal et al., 2013, Slipko et al., 2019).

Although multiple studies reported the complete removal of ARGs by RO, NF and UF application, especially in case of wastewater post treatment (Schwermer et al., 2018, Lamba and Ahammad, 2017), however, we observed some ARGs (e.g. *tetH, strB* etc.) were present at a concentration of 10^3 to 10^4 copies per 100 µL in NF permeate. This in accordance with the findings where nearly same ARG concentration was observed in NF and RO permeate (Gros et al., 2019, Lu et al., 2020), when filtering livestock waste and reclaim water. According to Gros et al. (2019), fouled membrane permeates more ARGs when compared with clean membranes. Tang et al. (2007) observed fouled NF270 membrane turned considerably less negative in presence of DOC and calcium divalent ions. Therefore, carefully considering the manure and digestate composition where DOC concentration was 10 times higher (supporting information, Table SD1), it might be hypothesized that the severe membrane fouling followed by reduced electrostatic repulsion effect may lead to the permeation of some ARGs. However, previous studies proposed that the permeation of these DNAs, which were 500 to 600 times bigger in size compared to the membrane pores, could only possible when the DNAs could be stretched and elongated through the pores while possessing a 'snake-like' movement (Arkhangelsky et al., 2011, Latulippe and Zydney, 2009, Latulippe et al., 2007). Arkhangelsky et al. (2011) found that the DNA penetration was linearly correlated to the applied pressure and was completely unaffected by its length. They observed a critical pressure threshold of 2 to 3 bars must be reached to stretch out the DNAs. This is in accordance with the present study where all the NF experiments were performed at 6.5 bars. Interestingly, the latest findings of pores or voids in so called non-porous membranes (Li et al., 2018, Song et al., 2019) may influence the permeation of certain ARGs as well. However, this needs further investigations.

5.4. Conclusions

Pig manure and digestate containing abundant and diverse ARGs along with its sheer volume is considered as a major antibiotic resistance reservoir and a public health hazard. In this present study, total 189 ARGs were detected from all the raw samples, among which 66 ARGs were shared among manures and 53 ARGs were shared among both manure and digestate samples. The highest reported total ARG copy numbers in a single manure sampling site was 1.15×10^8 copies. This highly alarming ARG numbers indicated the uncontrolled use of antibiotic in pig farm expanded antimicrobial reservoir in the farm environment.

The combination of prefiltration by MF, followed by nanofiltration by NF270 membrane investigated herein represented their suitability for raw pig manure and digestate treatment. Results indicated that various ARGs and 16S rRNA genes could effectively be removed to LRV above 3 to as high as 5 by this advance membrane filtration process. Size exclusion and electrostatic repulsion were considered as the main ARG removal mechanisms by NF270. Interestingly, ARG removal was found directly proportional to its initial concentration in the raw manure and digestate samples. Nevertheless, some points which needs further investigation are given below:

(i) Further removal of some ARGs (e.g. *tetH, strB*) which were present at a concentration of 10^3 to 10^4 copies per 100 µL in NF permeate.

(ii) Better pretreatment of the raw manure and digestate samples for further fouling reduction of NF270.

Furthermore, the established guideline values of raw manure application as a fertilizer should be monitored rather strictly to prevent soil and groundwater antimicrobial pollution as well as their uptake by crops.

Lastly, with the rise of the antibiotic's consumption in livestock production, human health is facing a bigger issue of antimicrobial resistance. Several studies have already claimed the correlation of animal farming with the rise of ARG concentration in the nearby groundwater and surface water which might result in diseases outbreak, virulence and enhance the transmission. The present study could only raise the awareness to an elevated level by presenting the strikingly high concentration of ARGs that were found in the manure and digestate.

6. Summary and conclusion

Intensive livestock farming has negatively impacted the environment by contributing to the release of ammonia and nitrous oxide, groundwater nitrate pollution and eutrophication of rivers and estuaries. On contrary, nutrient rich manure has always been a major focal point of resource recovery. Hence, the overall objective of the thesis was to evaluate the efficiency of nutrient recovery from manure and the consequent pollution reduction by combined membrane filtration processes. The conclusions of the individual chapters are summarized:

6.1. Impact of livestock farming on nitrogen pollution

The study formed a direct relationship between manure generation by beef, pork and poultry per unit respective meat production. Nitrogen loss and virtual nitrogen factor were found proportional to each other. Japan was found to lose highest amount of nitrogen for meat production followed by Australia. This is due to the amount of manure to be treated per unit meat production was highest for Japan.

Finally, it was found that more than 7000 kWh energy required to recover 140 kg of ammonium nitrogen from beef manure per 1 Mg meat production when considering zero liquid discharge approach. The energy demand reduced significantly to below 3000 kWh and nearly 1000 kWh for pork and poultry manure treatment for the same.

This study clearly indicated the staggering energy consumption related to manure treatment for lowering the overall nitrogen footprint in livestock farming. Recovery of ammonium nitrogen contributed to the circular approach of the economy as well.

6.2. Performance of MF-VE process for ammonia water production

The MF cross flow system operated continuously for longer time period with a stable flux rate of 30 $Lh^{-1}m^{-2}$ and it was able to separate total suspended solids above 98 % and retained total phosphorus above 90% (Figure 6.1.A). The short term recycle mode operation could reduce the initial volume up to 50% while maintaining the nutrient separation qualities at the same level.

Evaporation duration of 5 minutes of microfiltration permeate resulted in substantial 31 gL^{-1} of NH_4^+-N condensate concentration, which was nearly 12 times higher than the initial NH_4^+-N concentration of microfiltration permeate (Figure 6.1.B).

MF filtration of raw manure followed by the vacuum evaporation of the MF permeate have been proven to be a viable alternative to recover nutrient and produce a cleaner and concentrated ammonia water.

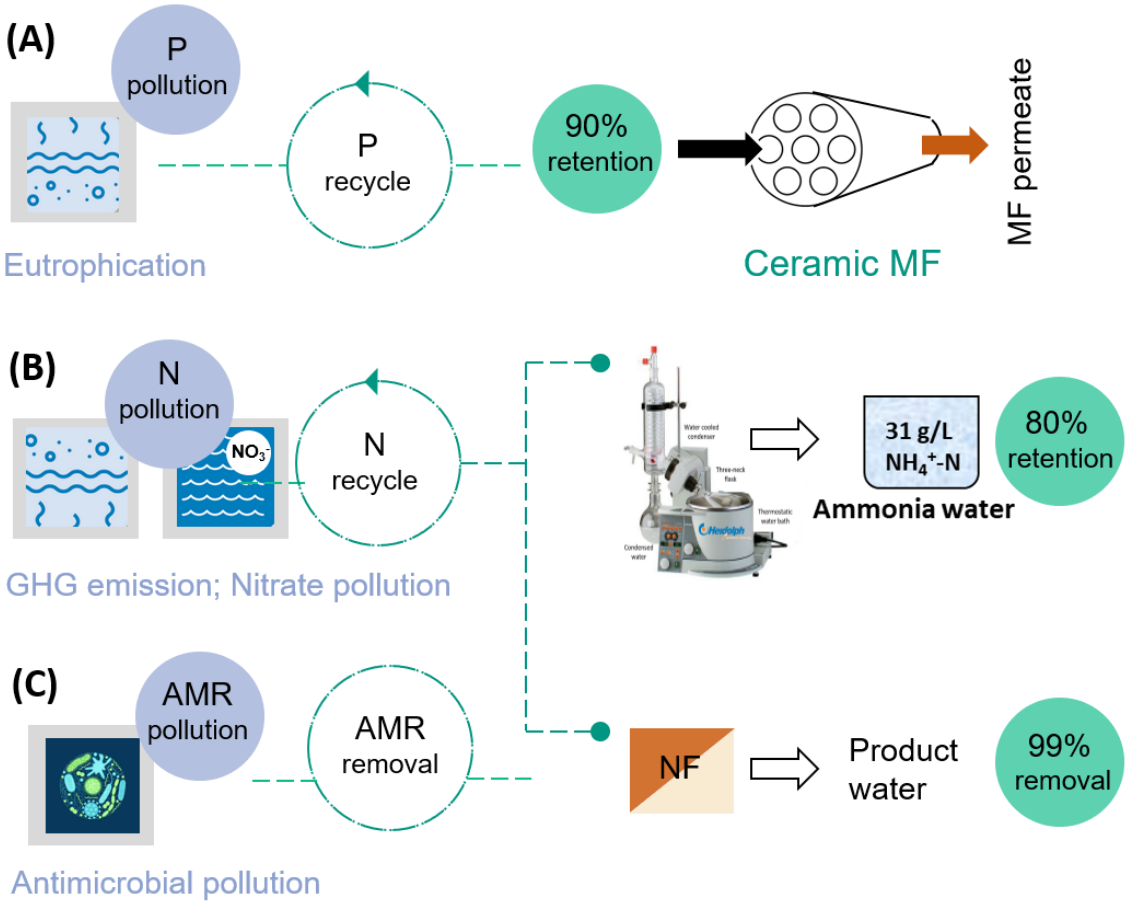


Figure 6.1. *Summary of (A) phosphorus retention by ceramic MF, (B) nitrogen retention by MF-VE and MF-NF treatment train and (C) the removal of antimicrobial pollution by MF-NF treatment process.*

6.3. Application of MF-NF treatment train for nutrient recovery

MF retained phosphorus above 80% within smaller concentrate volume, which accounted for 40% of the initial microfiltration feed volume. NF of the MF permeate by three different nanofiltration membranes showed maximum 50 to 70% potassium and nitrogen retention respectively within smaller NF concentrate volume (Figure 6.1.B). NF270 showed the most promising retention and was found to be least prone to fouling.

Hence, the MF–NF treatment train was able to produce a particle-free final product water, which accounted for 30% of the initial feed volume of MF. This has the potential to be reused in farms to wash barns, to irrigate nearby cultures, or can be applied to specific fields based on the demand.

6.4. Removal of ARGs by MF-NF treatment train

Total 189 ARGs were detected from all the raw samples, among which 66 ARGs were shared among manures and 53 ARGs were shared among both manure and digestate samples. The highest reported total ARG copy numbers in a single manure sampling site was 1.15×10^8 copies. Various ARGs and 16S rRNA genes could effectively be removed to log retention value above 3 to as high as 5 by microfiltration-nanofiltration process (Figure 6.1.C). Size exclusion and electrostatic repulsion were considered as the main ARG removal mechanisms by NF270.

Interestingly, ARG removal was found directly proportional to its initial concentration in the raw manure and digestate samples.

Lastly, with the rise of the antibiotic's consumption in livestock production, human health is facing a bigger issue of antimicrobial resistance. The present study could not only raise the awareness to an elevated level by presenting the strikingly high concentration of ARGs that were found in the manure and digestate but also showed their proper elimination process as well.

7. Supporting information

7.1. Supporting Information A

Table SA1. *Comparison of VNF, TNI and NL of per kg meat production among different countries.*

Country	Germany	US	UK	China	Japan	Australia	Tanzania	Netherlands	Austria
				VNF					
VNF_{beef}	7.9	7.9	7.9	5.2	27.3	13.4	7	8.5	5.4
VNF_{pork}	4.4	4.4	4.4	7.9	12.9	5.5	3.3	4.7	3.6
$VNF_{poultry}$	3.2	3.2	3.2	5.7	10.7	4.8	0.8	3.4	2.5
				TNI (g/kg)					
TNI_{beef}	369.2	369.2	369.2	257.2	1174.1	597.4	332.9	394.1	265.5
TNI_{pork}	236.0	236.0	236.0	389.0	607.6	284.1	188.0	249.2	201.1
$TNI_{poultry}$	165.9	165.9	165.9	264.6	462.0	229.0	71.1	173.8	138.2
				NL (g/kg)					
NL_{beef}	327.8	327.8	327.8	215.70	1132.62	555.94	290.42	352.65	224.04
NL_{pork}	192.33	192.33	192.33	345.30	563.88	240.42	144.25	205.45	157.36
$NL_{poultry}$	126.36	126.36	126.36	225.08	422.52	189.54	31.59	134.26	98.72

Specific energy demand (SED) refers to the energy demand (ED) per kg ammonium nitrogen recovery (AR) from manure:

$$\text{Specific energy demand (SED)} = \text{ED/AR (kWh kg}^{-1}) \qquad \text{Equation (SA1)}$$

Table SA2. *Specific energy demand per kg ammonium nitrogen recovery from beef, pork and poultry manure*

	Beef	Pork	Poultry
Specific energy demand (kWh kg^{-1})	49	21	15

7.2. Supporting Information B

Figure SB1. *Raw pig manure sieving through 1mm sieve*

Figure SB2. *Lab scale vacuum evaporation system.*

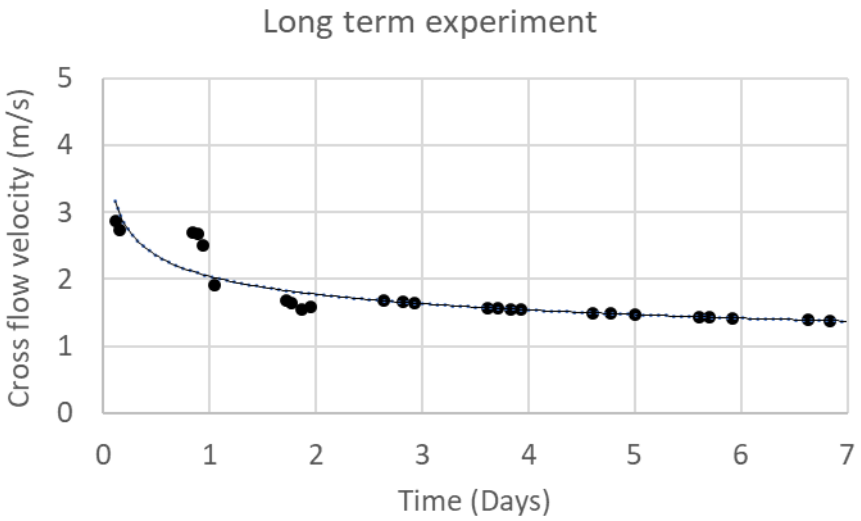


Figure SB3. *Membrane cross flow velocity during the long-term filtration period.*

7.3. Supporting information C

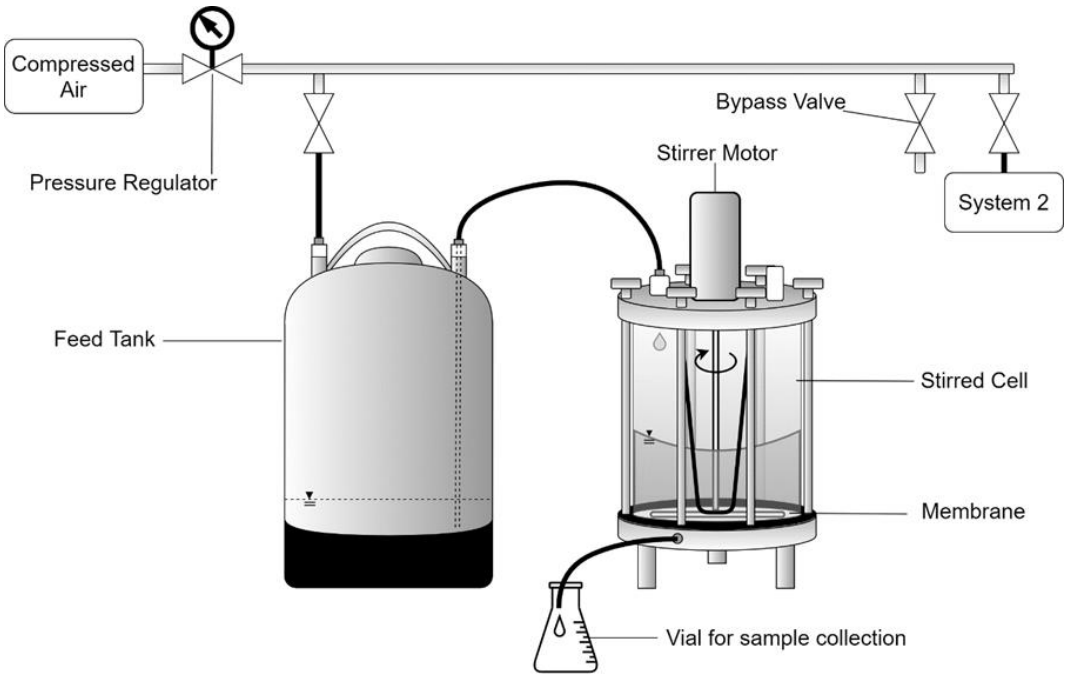


Figure SC1. *Stirred cell dead end membrane filtration system*

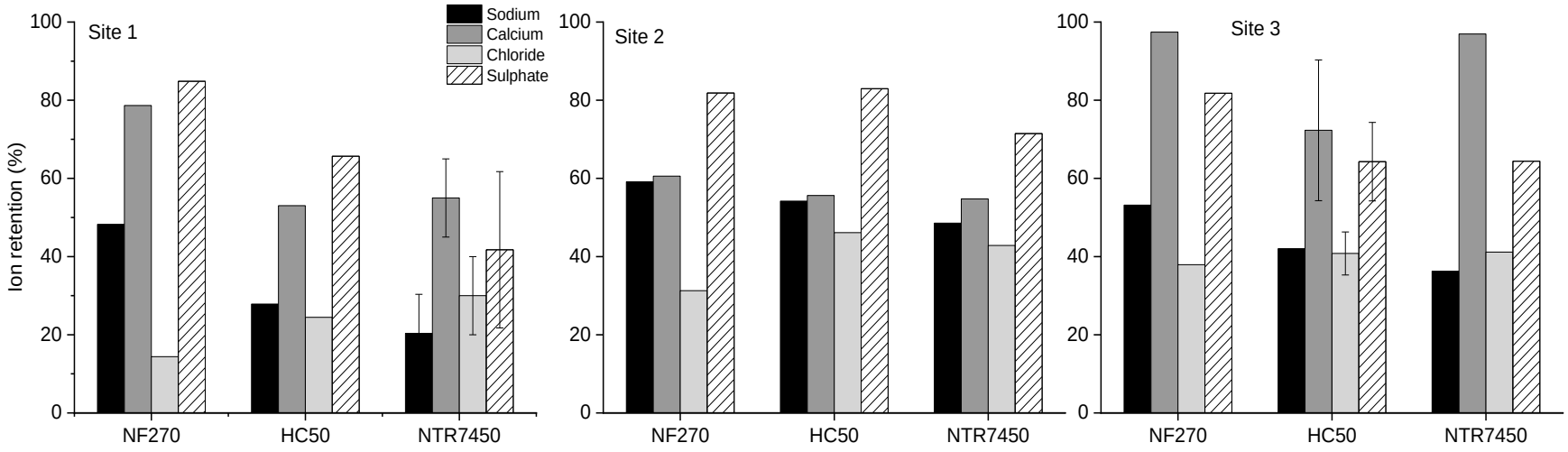


Figure SC2. *Ion retention of MF permeate at 50% recovery by NF270, HC50 and NTR7450 membranes from all sampling sites. Pressure: 6.5 bar, stirring rate: 400 rpm, temperature: 25°C.*

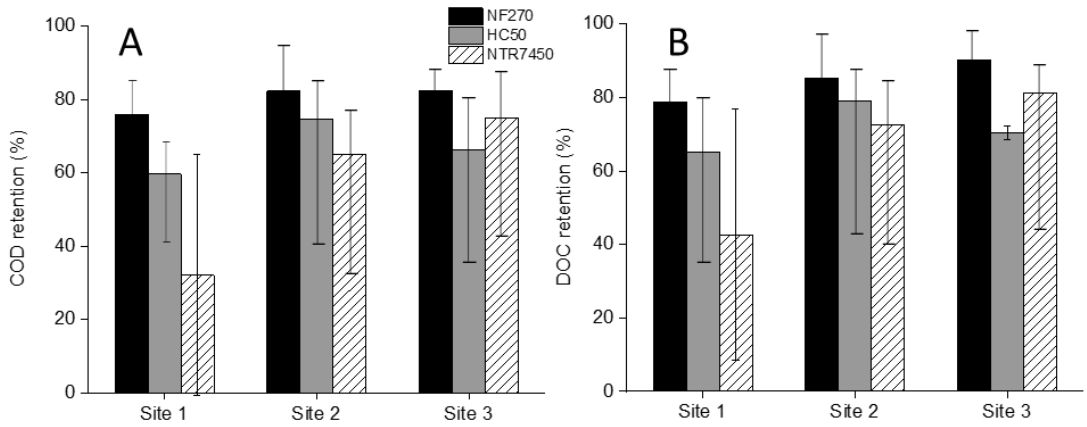


Figure SC3. *(A) COD and (B) DOC retention of MF permeate at 50% recovery by NF270, HC50 and NTR7450 membranes from all sampling sites. Pressure: 6.5 bar, stirring rate: 400 rpm, temperature: 25°C.*

Normalized flux was calculated by following equation SC1, where Jw is pure water flux before MF permeate filtration and Jp is the flux during MF permeate filtration.

$$Normalized\ flux = (Jp/Jw) \quad \text{(SC1)}$$

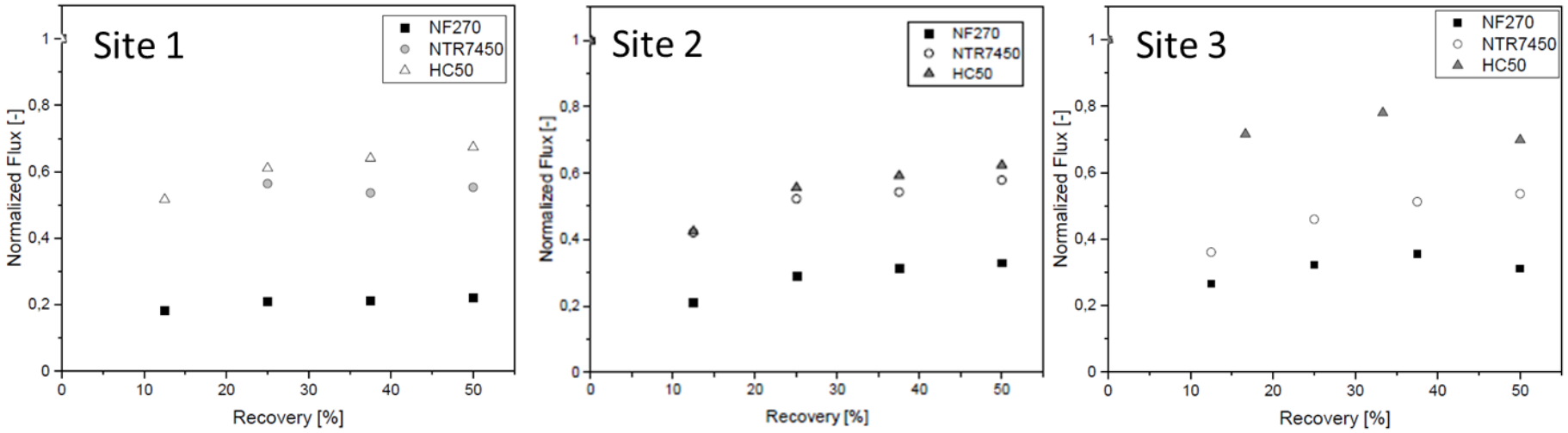


Figure SC4. *Flux during filtrations of NF270, HC50 and NTR7450 membranes while filtering pig manure from all sampling sites. Pressure: 6.5 bar, stirring rate: 400 rpm, temperature: 25°C.*

7.4. Supporting information D

Table SD1. *Characteristics of the pig manure and digestate samples*

Parameters	Site 1 Pig manure	Site 2 Pig manure	Site 3 Digestate
TSS [gL^{-1}]	3	4.9	1.8
VSS [% of TSS]	83.3	78	67.8
COD [gL^{-1}]	11.3	11.8	19.5
NH_4^+ - N [gL^{-1}]	4.4	2.9	1.8
PO_4^{-3} [mgL^{-1}]	394	323	245
pH	7.8	7.8	7.8
TOC [gL^{-1}]	4.9	4.3	3.9
DOC [gL^{-1}]	3.3	3	1.8
DTN [gL^{-1}]	3	2	1.5
Acetic acid [mgL^{-1}]	3637	2211	<100
K^+ [mgL^{-1}]	1794	1698	2663

Table SD2. *ARG characteristics*

AB groups	SL No.	ARGs	Forward Primer	Reverse Primer
Aminoglycoside	1	strB	GCTCGGTCGTGAGAACAATCT	CAATTTCGGTCGCCTGGTAGT
	2	aadA_1	GTTGTGCACGACGACATCATT	GGCTCGAAGATACCTGCAAGAA
	3	aadA2_3	CAATGACATTCTTGCGGGTATC	GACCTACCAAGGCAACGCTATG
	4	aadA1	TGTACGGCTCCGCAGTG	CACGGAATGATGTCGTCGTG
	5	aadA_2	CGAGATTCTCCGCGCTGTA	GCTGCCATTCTCCAAATTGC
	6	str	AATGAGTTTTGGAGTGTCTCAACGTA	AATCAAAACCCCTATTAAAGCCAAT
	7	aadE	TACCTTATTGCCCTTGGAAGAGTTA	GGAACTATGTCCCTTTTAATTCTACAATCT
	8	aadA2_1	ACGGCTCCGCAGTGGAT	GGCCACAGTAACCAACAAATCA
	9	aadD	CCGACAACATTTCTACCATCCTT	ACCGAAGCGCTCGTCGTATA
	10	strA	CCGGTGGCATTTGAGAAAAA	GTGGCTCAACCTGCGAAAAG
	11	aacC2	ACGGCATTCTCGATTGCTTT	CCGAGCTTCACGTAAGCATTT
Sulfonamide	12	sul2_2	TCATCTGCCAAACTCGTCGTTA	GTCAAAGAACGCCGCAATGT
	13	sul2_1	TCCGGTGGAGGCCGGTATCTGG	CGGGAATGCCATCTGCCTTGAG
	14	sul1_1	CGCACCGGAAACATCGCTGCAC	TGAAGTTCCGCCGCAAGGCTCG
	15	sul1_2	GCCGATGAGATCAGACGTATTG	CGCATAGCGCTGGGTTTC
Tetracycline	16	tetM	GGAGCGATTACAGAATTAGGAAGC	TCCATATGTCCTGGCGTGTC
	17	tetM_3	CATCATAGACACGCCAGGACATAT	CGCCATCTTTTGCAGAAATCA
	18	tetM_2	GCAATTCTACTGATTTCTGC	CTGTTTGATTACAATTTCCGC
	19	tetH	TTTGGGTCATCTTACCAGCATTAA	TTGCGCATTATCATCGACAGA
	20	tetPA	GGAAACCTTAGTTCAGTGACTTGG	CCCATTTAACCACGCACTGAA
	21	tetM_1	TAATATTGGAGTTTTAGCTCATGTTGATG	CCTCTCTGACGTTCTAAAAGCGTATTAT
	22	tetW	ATGAACATTCCCACCGTTATCTTT	ATATCGGCGGAGAGCTTATCC
	23	tet36_1	AGAATACTCAGCAGAGGTCAGTTCCT	TGGTAGGTCGATAACCCGAAAAT
	24	tetT	CCATATAGAGGTTCCACCAAATCC	TGACCCTATTGGTAGTGGTTCTATTG
	25	tet36_2	TGCAGGAAAGACCTCCATTACAG	CTTTGTCCACACTTCCACGTACTATG
	26	tetPB_1	TGGGCGACAGTAGGCTTAGAA	TGACCCTACTGAAACATTAGAAATATACCT
	27	tetA/B_2	GCCCAGTGCTGTTGTTGTCAT	TGAAAGCAAACGGCCTAAATACA
	28	tetQ	CGCCTCAGAAGTAAGTTCATACACTAAG	TCGTTCATGCGGATATTATCAGAAT
	29	tetA(P)	AGTTGCAGATGTGTATAGTCGTAAACTATCTATT	TGCTACAAGTACGAAAACAAAACTAGAA
	30	tetX	AAATTTGTTACCGACACGGAAGTT	CATAGCTGAAAAAATCCAGGACAGTT
	31	tetR_4	CGCGATGGAGCAAAAGTACAT	AGTGAAAAACCTTGTTGGCATAAAA
	32	tet44	CTCATGTAGATGCAGGAAAGACG	GTAACTGCTGCCTGAATTGTGA
	33	tetA/B_1	AGTGCGCTTTGGATGCTGTA	AGCCCCAGTAGCTCCTGTGA
	34	tetO_2	CAACATTAACGGAAAGTTTATTGTATACCA	TTGACGCTCCAAATTCATTGTATC
	35	tetD	AATTGCACTGCCTGCATTGC	GACAGATTGCCAGCAGCAGA
	36	tetR_3	CGCGATAGACGCCTTCGA	TCCTGACAACGAGCCTCCTT
	37	tetL_2	ATGGTTGTAGTTGCGCGCTATAT	ATCGCTGGACCGACTCCTT
	38	tetO_1	ATGTGGATACTACAACGCATGAGATT	TGCCTCCACATGATATTTTTCCT
	39	tet32	CCATTACTTCGGACAACGGTAGA	CAATCTCTGTGAGGGCATTTAACA
	40	tetA_2	CTCACCAGCCTGACCTCGAT	CACGTTGTTATAGAAGCCGCATAG
	41	tetG_2	CATCAGCGCCGGTCTTATG	CCCCATGTAGCCGAACCA
	42	tetC_1	CATATCGCAATACATGCGAAAAA	AAAGCCGCGGTAAATAGCAA
	43	tetR	CCGTCAATGCGCTGATGAC	GCCAATCCATCGACAATCACC
	44	tetG	TCGCGTTCCTGCTTGCC	CCGCGAGCGACAAACCA
	45	tetC_3	TGCGTTGATGCAATTTCTATGC	GGAATGGTGCATGCAAGGAG
	46	tetS	TTAAGGACAAACTTTCTGACGACATC	TGTCTCCCATTGTTCTGGTTCA
	47	tetG_1	TCAACCATTGCCGATTCGA	TGGCCCGGCAATCATG
	48	tetR_1	CAATCCATCGACAATCAC	GACAATCAGCTACTTCAC
	49	tet39	TATAGCGGGTCCGGTAATAGGTG	CCATAACGATCCTGCCCATAGATAAC
	50	tetM_4	TGGCAAGACGAGTTTGACTGA	GATCGCTCCACTTCAGCGATAA
	51	tetPB_3	ACACCTGGACACGCTGATTTT	ACCGTCTAGAACGCGGAATG
	52	tetK	CAGCAGTCATTGGAAAATTATCTGATTATA	CCTTGTACTAACCTACCAAAAATCAAAATA
	53	tetR_2	ATGAGTTCGGCCAGAATTTCC	GGTTGTGCGCGAAATGATT
MLSB	54	ermB_2	GAACACTAGGGTTGTTCTTGCA	CTGGAACATCTGTGGTATGGC
	55	lnuB	GGATCGTTTACCAAAGGAGAAGG	AGCATAGCCTTCGTATCAGGAA
	56	mefA_1	CCGTAGCATTGGAACAGCTTTT	AAACGGAGTATAAGAGTGCTGCAA
	57	lnuB_2	AAAGGAGAAGGTGACCAATACTCTGA	GGAGCTACGTCAAACAACCAGTT
	58	ermF	TCTGATGCCCGAAATGTTCAAG	TGAAGGACAATTGAACCTCCCA
	59	lnuB_1	TGAACATAATCCCCTCGTTTAAAGAT	TAATTGCCCTGTTTCATCGTAAATAA
	60	erm35	TTGAAAACGATGTTGCATTAAGTCA	TCTATAATCACAACTAACCACTTGAACGT
	61	mefA	CCGTAGCATTGGAACAGCTTTT	AAACGGAGTATAAGAGTGCTGCAA
	62	mphA	TCAGCGGGATGATCGACTG	GAGGGCGTAGAGGGCGTA
	63	mphA_1	CTGACGCGCTCCGTGTT	GGTGGTGCATGGCGATCT
MDR	64	cefa_qacelta	TAGTTGGCGAAGTAATCGCAAC	TGCGATGCCATAACCGATTATG
Other	65	qacEΔ1_2	CCCCTTCCGCCGTTGT	CGACCAGACTGCATAAGCAACA
	66	qacEΔ1_3	GTCGGTGTTGCTTATGCAGTCT	CAACCAGGCAATGGCTGTAA
	67	qacEΔ1_1	TCGCAACATCCGCATTAAAA	ATGGATTTCAGAACCAGAGAAAGAAA
Taxanomic	68	Enterococci	AGAAATTCCAAACGAACTTG	CAGTGCTCTACCTCCATCATT
Beta Lactam	69	blaSFO	CCGCCGCCATCCAGTA	GGGCCGCCAAGATGCT
	70	cfxA	TCATTCCTCGTTCAAGTTTTCAGA	TGCAGCACCAAGAGGAGATGT
	71	blaOXY	CGTTCAGGCGGCAGGTT	GCCGCGATATAAGATTTGAGAATT

Description	Total genes No.	Tetracycline	Aminoglycoside	MLSB	Sulfonamide	Other	Beta Lactam	Taxanomic	MDR
Site 1 manure	70	38	11	10	4	3	2	1	1
Site 2 manure	66	34	10	10	4	3	3	1	1
Site 3 digestate	53	26	8	8	4	3	2	1	1
Site 1 N270 perm	16	7	6	2	1	0	0	0	0
Site 2 NF270 perm	25	15	6	2	2	0	0	0	0
Site 3 NF270 perm	13	4	6	1	2	0	0	0	0

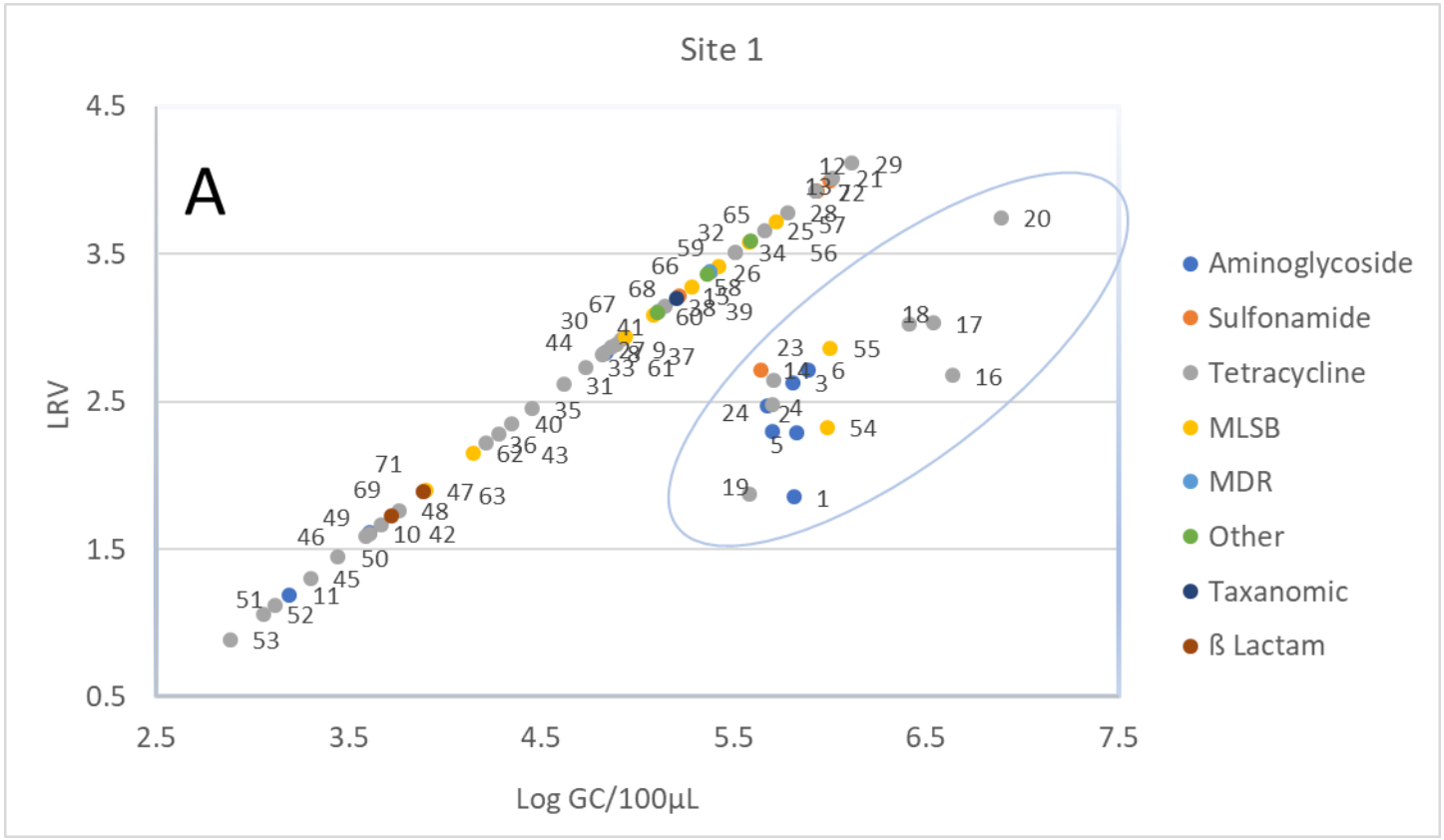
Figure SD1. *Number of detected ARGs.*

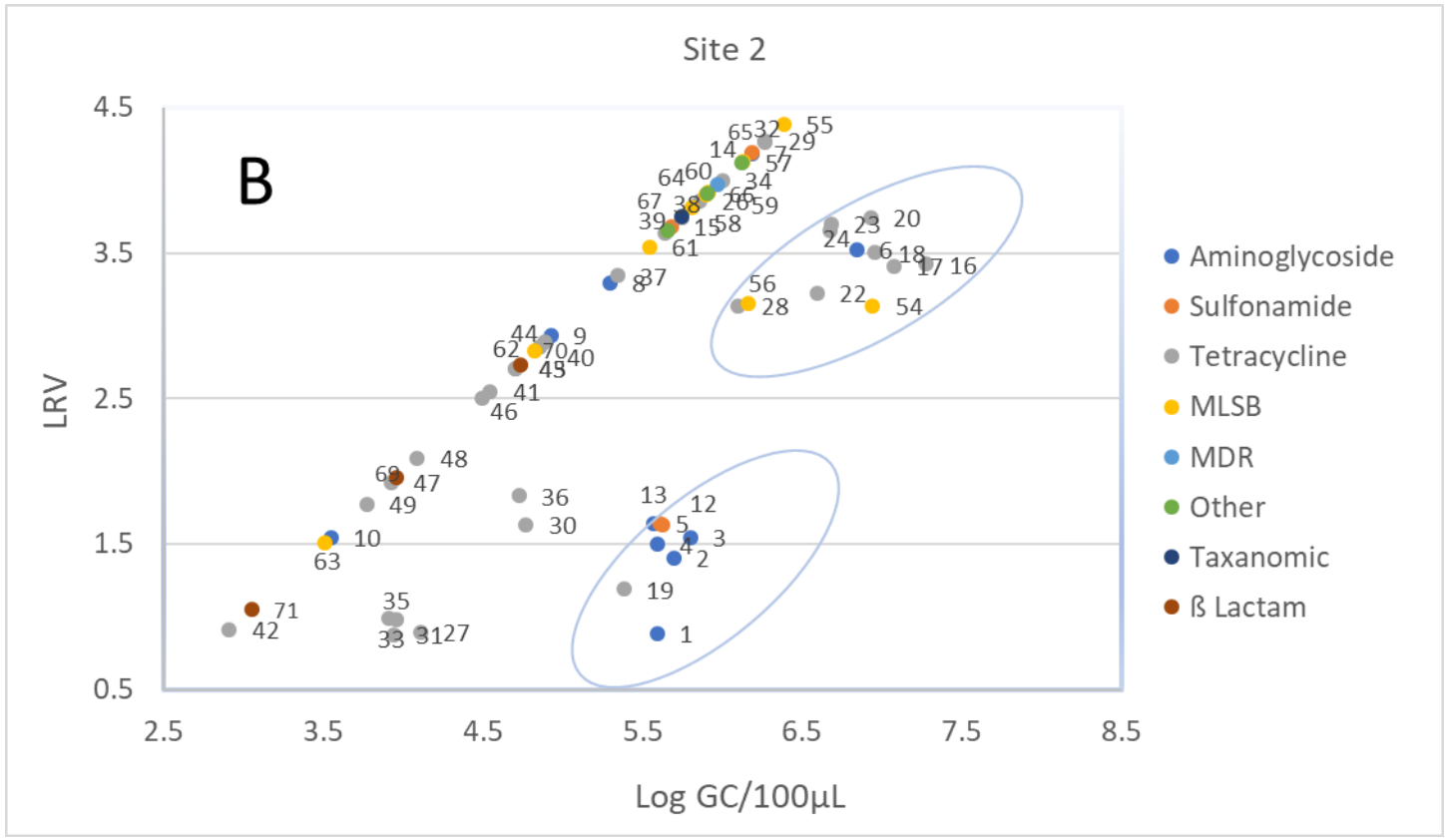
ARG retention was calculated by following equation (SD1).

$$R = \left[\frac{Cf - Cp}{Cf}\right] \times 100\ \% \qquad \text{(SD1)}$$

Where, C_f = Absolute ARG copy numbers per 100 µL volume in raw manure or digestate

C_p = Absolute ARG copy numbers per 100 µL volume in permeate after nanofiltration by NF270


Site 1
A
LRV
Log GC/100µL
4.5
3.5
2.5
1.5
0.5
2.5
3.5
4.5
5.5
6.5
7.5
Aminoglycoside
Sulfonamide
Tetracycline
MLSB
MDR
Other
Taxanomic
ß Lactam


Site 2
B
LRV
Log GC/100µL
4.5
3.5
2.5
1.5
0.5
2.5
3.5
4.5
5.5
6.5
7.5
8.5
Aminoglycoside
Sulfonamide
Tetracycline
MLSB
MDR
Other
Taxanomic
ß Lactam

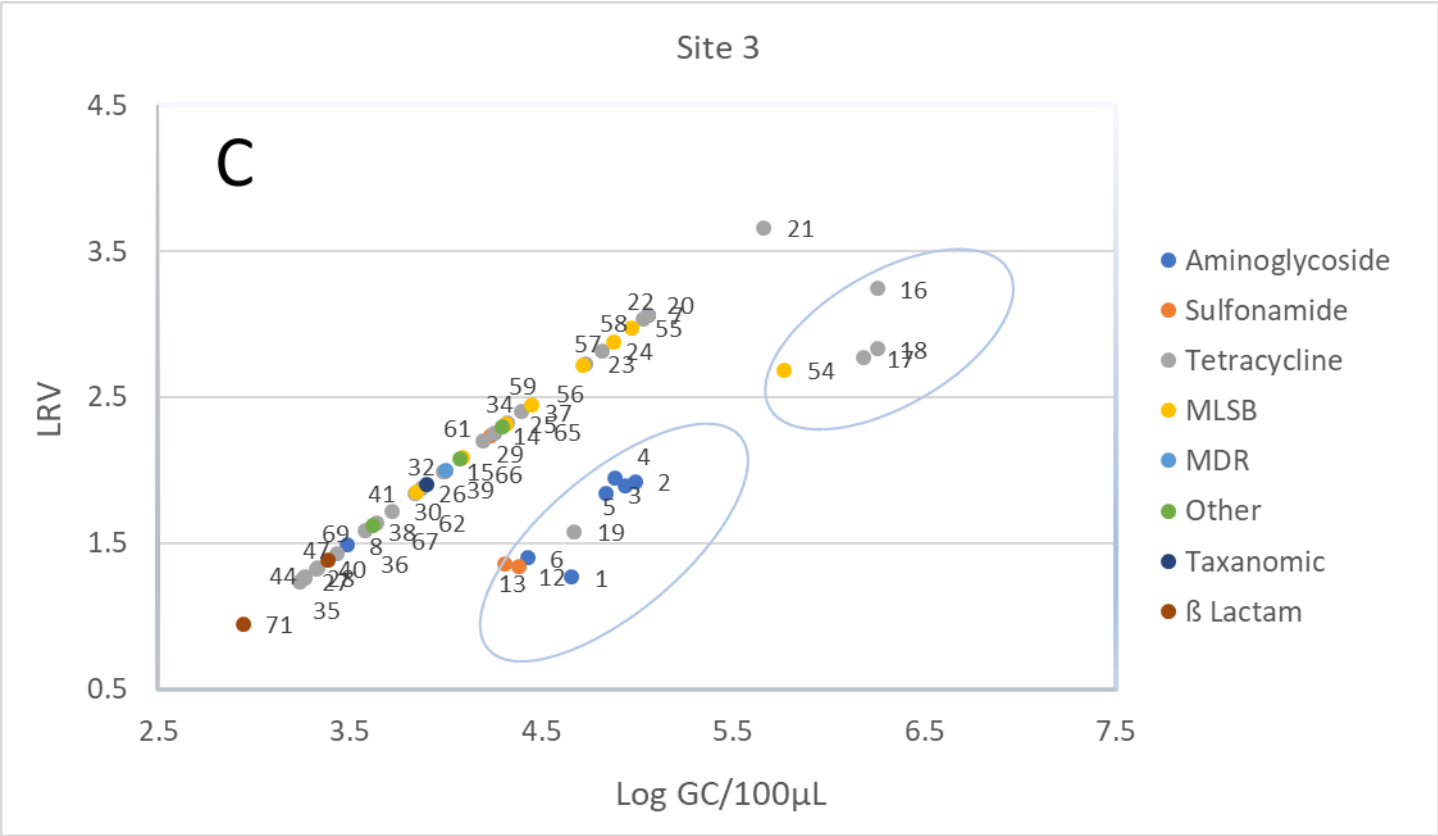


Figure SD2. *Relation between ARG concentration in the feed and their consequent removal by MF-NF process of manure from (A) site 1, (B) site 2 and (C) site 3. Every ARG is represented with a unique serial number which is mentioned in Table SD2.*

8. Verification of the contribution from co-authors

Title: Impact of livestock farming on nitrogen pollution and the corresponding energy demand for zero liquid discharge

Journal: Water MDPI

Authors: Prantik Samanta, Harald Horn, Florencia Saravia

Position in the dissertation

The content of this manuscript has been included in chapter 2

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Journal: Membranes MDPI

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