Enhancement of methane generation by reducing nitrogen concentration during anaerobic digestion of swine manure

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Abstract

Anaerobic digestion of swine manure for the production of biogas principally CH\(_4\) may add value to manure through the production of energy. However, the large nutrient load, from total ammoniacal nitrogen (TAN – ammonium (NH\(_4^+\)) + ammonia (NH\(_3\))), in the manure and digestate is a problem during anaerobic digestion of swine manure. Low carbon:nitrogen (C:N) ratios and related NH\(_3\) inhibition are major issues affecting methane (CH\(_4\)) generation during anaerobic digestion of swine manure.

The clay mineral zeolite adsorbs NH\(_4^+\) and may be added to slurry or digestate in the anaerobic digester to reduce NH\(_4^+\) concentrations and thus mitigate inhibition by ammonia. However, the effects of the physico-chemical properties of zeolite in reducing TAN in digestate, and mechanisms for improving biogas production have not been examined. This information is vital for developing the applications of zeolite to anaerobic bioreactors to reduce TAN so that it can be used at industrial scale. In addition, the recovery of nitrogen (N) from swine manure is an option for reusing N in agriculture and reducing the amount released into the environment.

The primary objectives of this study were to develop a methodology for effective recovery of N during anaerobic digestion of swine manure, to investigate impacts of N recovery on CH\(_4\) generation, and to identify the feasibility of modifying digester by zeolite technology. To achieve the above objectives the study comprised a series of laboratory adsorption/desorption experiments and swine manure anaerobic digestion experiments with different rates of Australian zeolite. The bulk commercial Australian natural zeolite obtained from Zeolite Australia Pty Limited, Werris Creek, NSW, Australia, and zeolite that had been treated with sodium chloride (NaCl) (hereafter referred to as sodium zeolite) were used in this study.

The NH\(_4^+\) adsorption characteristics of Australian natural zeolite at aqueous solutions with high NH\(_4^+\) concentrations were investigated under a range of experimental conditions. The results showed that initial NH\(_4^+\) concentration, temperature, reaction time, and pH of the solution had significant effects on the NH\(_4^+\) adsorption capacity of Australian zeolite. Increased retention time and temperature generally had a positive impact on adsorption. The NaCl treatment increased the NH\(_4^+\) adsorption capacity of sodium zeolite by 25% at 1000 mg-NH\(_4^+\) in solution compared to the natural Australian zeolite. The maximum adsorption capacities of both natural Australian zeolite and sodium zeolite were estimated as 9.48 and 11.83 mg-N/g, respectively at 1000 mg-NH\(_4^+\)-N in solution. However, NH\(_4^+\) was adsorbed
44% and 57% less by natural and sodium zeolites respectively when zeolites were added to the solutions which consisted of other ions that resembled the ionic composition of digested manure.

Maximum anaerobic digestion of swine manure from selected hydraulic retention times (HRTs) was obtained at 16 days HRT when comparing CH$_4$ production at 8, 12 and 16 days HRT. The digestion process appeared unstable at HRTs shorter than 12 days. The C:N ratio of swine manure was 8.6:1 which was lower than optimum values for digestion and this was the reason for low CH$_4$ production at all HRTs.

Direct application of different rates of Australian natural and sodium zeolites into the digesters proved that all digesters with added zeolite produced more biogas and CH$_4$ – over 10% more than the control nil-zeolite digesters. The digesters which had natural zeolite at a dose of 40g/L resulted in the largest increase (29%) in total CH$_4$ yield from swine manure compared to the control. The lag phase of digestion was shorter with increasing zeolites doses up to 100g/L. NH$_4^+$ adsorption by zeolites increased linearly with increasing zeolite doses in the digesters. Both Australian natural and sodium zeolites adsorbed approximately similar amounts of NH$_4^+$ during the anaerobic digestion of swine manure. Natural and sodium zeolites at a dose of 100g/L reduced the TAN concentration by 50% and 52% of NH$_4^+$ (P = 0.034 compared to the control respectively. However, the increases in CH$_4$ yield of digesters that had natural and sodium zeolites at a dose of 100g/L were only 10% and 12% (P=0.013). Although NH$_4^+$ adsorption by zeolites showed a linear relationship with increasing zeolite doses, biogas, and CH$_4$ production increased linearly only up to 40g/L for natural zeolite. Variations of cations concentration inside the digesters as a result of adsorption-desorption caused by adding zeolites at different rates might be another reason for variations of CH$_4$ production from different rates of zeolites in digesters.

Both natural and sodium Australian zeolites increased CH$_4$ production significantly when cellulose was added to adjust the swine manure C:N ratio to that optimal for CH$_4$ production. The addition of zeolite, which adsorbs NH$_4^+$, affects not only the toxicity of NH$_3$ and the C:N ratio, but also the regulation of pH and this might be the reason for enhancing CH$_4$ yield. Therefore, adding Australian zeolite is the best solution for the problems associated with swine manure co-digestion with different carbon sources, resulting in enhanced CH$_4$ production rates in C:N adjusted swine manure while adsorbing substantial quantities of NH$_4^+$ from the medium.
Australian natural zeolite was shown to be a potential sorbent for the removal of NH$_4^+$, K$^+$ and P ions during anaerobic digestion of swine manure. However, higher concentrations of natural zeolite at higher pH might not be appropriate for anaerobic digestion as zeolite enhances P precipitation by releasing more Ca$^{2+}$ to the medium.

Different designs of digesters with zeolite treatment were developed and digesters with an external zeolite column (Ex-Zeo digester), with an inside zeolite bed (In-Zeo digester), and a digester without addition of zeolite (No-Zeo digester) were examined. The increases in CH$_4$ yields during the 40 day digestion period, compared to No-Zeo digester, were 5% for the EX-Zeo digester and 15% for the In-Zeo digesters. The results indicated that treatment of swine manure with 7% zeolite during anaerobic digestion has the potential to improve biodegradation of organic material in swine manure and consequently to produce more CH$_4$. Adsorption of NH$_4^+$ was lower in Ex-Zeo digesters compared to the In-Zeo digesters, and this resulted in the lower CH$_4$ production.

The research reported in this thesis contributed a number of significant findings to improve the understanding of the adsorption-desorption properties of Australian zeolite, CH$_4$ enhancement mechanisms by zeolite, effects of the addition of zeolite during the anaerobic digestion of swine manure, and their optimum rates of enhancing CH$_4$ production while mitigating N concentration inside the digesters.

The experiment with an external zeolite column connected to the digester showed the feasibility of applying zeolite as an external column to the digesters. This knowledge will enable the use of Australian zeolite during the anaerobic digestion of swine manure to enhance CH$_4$ production while mitigating NH$_4^+$ concentration inside the digesters.

**Keywords:** Anaerobic digestion, ammonium, biogas, methane, zeolite
**Declaration**

This is to certify that:

(i) the thesis comprises only my original work towards the PhD except where indicated in the Preface,

(ii) due acknowledgement has been made in the text to all other material used, and

(iii) the thesis is less than 100,000 words in length, exclusive of tables, graphs, and bibliography.

D. Thushari Nilushika Wijesinghe

August, 2017
Preface and Contribution of Authors

This thesis consists of eight chapters. The first chapter is the introductory chapter (Chapter 1) which outlines the background, objectives and significance of this research. Chapter 2 presents the literature review on the current status of swine industry and manure management, and details solutions to overcome the environmental challenges associated with waste management, anaerobic digestion, the \( \text{NH}_4^+ \) and \( \text{NH}_3 \) production and ammonia inhibition during the anaerobic digestion, nitrogen recovery methods and potential of zeolite using as \( \text{NH}_4^+ \) adsorbent and also provides a summary of the current knowledge gaps. Chapter 3 provides details of the materials and methodologies used in the study. Chapter 4 to 7 are experimental chapters. The experimental chapters 4, 5, 6 and 7 are written in manuscript format according to the specific format of the individual journals. This thesis concludes with Chapter 8, which summarises the main findings of this research and future studies.

The candidate was the primary author of all manuscripts, and co-authors included Kithsiri B. Dassanayake, Peter J Scales, Sven G Sommer and Deli Chen. The candidate was responsible for designing, conducting the experiments, collecting and analysing the data, and writing the manuscripts. The co-authors assisted in improving the experimental design, laboratory work management, analysis and the manuscripts.

The manuscripts included in this thesis are presented in the following order:

Chapter 4:


Chapter 5:

Chapter 6.1:

Chapter 6:2:

Chapter 6:3

Chapter 7:
Wijesinghe D.T.N., Dassanayake K.B., Scales P.J., Deli C., Developing an anaerobic digester with external zeolite filled column for enhancing methane production from swine manure - A feasibility study Submitted to Environmental Engineering and Management Journal.
## Contribution to Published Work

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Chapter 1

Introduction

1.1 Research background

Environmental impact of meat production, particularly from intensive livestock production systems, is a rising global concern because of rapid expansion of the industry in the recent past and the predicted increase in demand on production. It has been predicted that global meat demand may double by 2050, not only as a result of increasing global population but also partly due to increasing per capita meat consumption (FAO, 2009). Trends in global demand vary among livestock sectors, but the world per capita consumption of pork which is the most widely consumed meat product in the world will continue to rise (FAO, 2011).

In Australia, the swine industry plays an important role in sustaining the economy. Australia’s annual production is 4.8 million swines, and the industry outlook is promising due to rising demand for swine products particularly from China (APL, 2015). Australian average per capita consumption of pork is expected to rise to 27.8 Kg by 2021-2022 (ABARES, 2017).

Swine manure contains a high amount of nutrients. Nitrogen and ammonium-nitrogen (NH$_4^+$-N) content in swine manure varies between 158-1025 mg/L, and 105-726 mg/L respectively (Kruger et al., 1995). However, the main manure generated by the swine industry, slurry, may cause an environmental problem due to emissions, run off and leaching of N and phosphorous (P) to surface and ground water, and also cause eutrophication if not managed efficiently in crop production (Rao et al., 2007). Further, it may be a source of odours, spreading of diseases and fly infestation (Diez et al., 2001), and it is a source of gaseous ammonia (NH$_3$) and greenhouse gas (GHG) (Wiedemann et al., 2010). In the swine industry, about 50% of NH$_3$ emissions come from slurry storage and swine housing. The other 50% of emissions are from the land application of the slurry (Portejoie et al., 2003). The NH$_3$ emissions contribute significantly to atmospheric N load. Ammonia is a pollutant gas that increases the formation of fine particulate in the atmosphere and plays a critical role in the acidification of the ecosystems and the eutrophication (Krupa, 2003). Worldwide demand for nitrogenous fertiliser is expected to rise from a total of an 119 million tonnes in 2018, with 1.4% of annual growth rate (FAO, 2015), resulting in additional GHG emissions from the production chain of N fertilisers. Therefore, recovery of total ammonium-N (TAN = NH$_3$(l) +
NH$_4^+$ from manure and reuse in agriculture is still the best and most environmentally friendly manure management option.

Swine farming contributes to about 9% of livestock-related GHG emissions, which are about 668 million tonnes carbon-dioxide (CO$_2$)-eq (FAO, 2011). In Australia, GHG emission contribution from livestock is 16% (Garnaut, 2011), and out of these about 10% of the methane (CH$_4$) emission generated by intensive management of swine industries (DCCEE, 2011), which originate from storage of manure in the animal house and from anaerobic lagoons (Farran et al., 2000).

Therefore, the swine industry is under pressure to reduce its environmental impact while increasing its output and maintaining its profitability. Anaerobic digestion of manure may be the technology that can reduce CH$_4$ emission and produce energy, anaerobic digestion is considered the best management practice for environmental sustainable handling livestock manures (Maria et al., 2013). Capture of CH$_4$ in the anaerobic digester for energy purpose would significantly reduce the GHG emission from swine manure (Sommer et al., 2004). The CH$_4$ capturing from intensive livestock production is not very popular in Australia. However, the Australian government has implemented some programs and policies that can support mitigation of CH$_4$ emission and enhance renewable energy production (DAFF, 2008).

About 80% of the N in the digestate from feed stock of anaerobic digester is in form of TAN (Sommer et al., 2013). The low biogas production rates are another limitation for the economic feasibility of anaerobic digestion of swine manure (Bonmati and Flotats, 2003). Swine manure has a low C/N ratio, due to high nitrogen content result in lower biogas production (Mydeen et al., 2016). Very low C:N ratios are leading to an accumulation of TAN inside the digester (Resch et al., 2011). A high TAN concentration in swine manure is also contributing to a low biogas production as high TAN concentrations inhibit the anaerobic digestion process (Hansen et al., 1998). The threshold values at which CH$_4$ inhibition begin ranges from NH$_3$ concentration 80 mg/L (Koster and Lettinga, 1984) to 150 mg/L (Braun et al., 1981). TAN concentrations at higher than 1500mg-N/L at pH > 7.4 may inhibit biogas production and TAN concentration > 3000mg – N/L at all pH levels inhibits the digestion process (Van Velsen, 1979).

The technologies to recover TAN from wastewater and animal manure include ammonia stripping (Guštin and Marinšek-Logar, 2011), chemical precipitation as struvite (Yilmazel and Demirer, 2013), and ion exchange/adsorption material such as zeolite (Cooney et al.,
Zeolite addition to the digestate is also an effective method to address the ammonia inhibition process because of its high adsorptive capacity and NH$_4^+$ selectivity, resulting in enhanced biogas production during the anaerobic digestion (Montalvo et al., 2012). However, research has focused on mixing zeolite into the digestive feedstock with the aim of enhancing the CH$_4$ production rather than simultaneous maximising both NH$_4^+$ recovery and CH$_4$ production. In addition, assessing the effect of different zeolites having different characteristics (Huang et al., 2010), and impact of using Australian zeolite in anaerobic digestion process is unknown.

More knowledge of the effect of zeolite is needed about:

1. Studying impact of zeolite addition on the formation of volatile fatty acids (VFAs) and bio-physicochemical dynamics within the digester under anaerobic digestion of swine manure
2. Optimising zeolite addition to adjusting to C:N ratio
3. Improving the bioreactor configuration for optimising the digester performance with zeolite
4. Assessing potential risks associated with subsequent cation release from zeolite,
5. Develop a method to use the N-enriched zeolite is left in the bulk digestate.

1:2 Objectives of the study
The main objective of this research is to develop a procedure for effective recovery of N from swine manure during the anaerobic digestion process, to investigate its impact on the CH$_4$ generation and to identify the feasibility of modifying digester set up that facilitates handling of N-enriched zeolite separately from the bulk effluent.

The specific objectives to achieve the overall objective are as follows:

1. Determine the NH$_4^+$ adsorption properties and related kinetics of Australian zeolites (natural and NaCl treated zeolites which is known as sodium zeolites) at high NH$_4^+$ concentrations solutions (Chapter 4).
2. Determine performance of mesophilic anaerobic digestion process with a focus on NH$_4^+$ formation when digesting swine manure at different hydraulic retention times (HRTs, Chapter 5).
3. Identify the effect of Australian zeolite on anaerobic digestion of swine manure and determined the optimum addition rate of Australian zeolite that produces maximum TAN recovery at maximum CH₄ production (Chapter 6).

4. Identify the effect of adjusting C: N ratio of swine manure and determine the effect of Australian zeolite addition on C: N ratio adjusted swine manure with the aim to determine the best zeolite application rate (Chapter 6).

5. Investigate the feasibility of applying Australian zeolite to remove excess nutrients and importance of ion adsorption, desorption properties of Australian zeolite during the anaerobic digestion of swine manure (Chapter 6).

6. Improvements in the bioreactor configuration for optimising the digester performance with zeolite (Chapter 7)

1.3 Significance of the study
The results of this study will assist with improving and understanding of biogas production process from swine manure using Australian zeolite and contribute to the development of bioreactor configuration for optimising the digester performance with zeolite. This knowledge will assist to mitigate NH₃ emission while enhancing biogas production. In addition, the knowledge of ion adsorption, desorption properties of Australian zeolite during the anaerobic digestion of swine manure will help for future zeolite applications.
References


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Chapter 2

Literature Review

2.1 Background

2.1.1 Current status of swine industry
The swine industry makes an important contribution to Australian agricultural production contributing 2.3% of gross agricultural production (ABS, 2012). There are about 687 intensive pig farms in Australia which has an average annual pig population of 4.75 million pigs (ABARES, 2013). Australian pork consumption per capita has increased by 20% over the past 10 years to an average of 25 kg (ABARES, 2013) and average per capita consumption of pork is expected to rise to 27.8 kg by 2021-2022 (ABARES, 2017).

In Australia, emissions from manure of intensive livestock production are a significant source of greenhouse gasses (GHG). The combined industries of grain-fed beef cattle, piggeries, dairy and poultry account for 84% of emissions arising from manure management. Of these manure management emissions from the pig industry contributed 38% (AGO, 2007).

2.1.2 Manure production and management methods in Australian piggeries
The minimum slurry production volume for efficient intensive pig farming is about 100 L/sow/day when practising recycled slurry flushing, or 150 L/sow/day using fresh water flushing. The volume can be as high as 250 L/sow/day (Kruger et al., 1995). Swine slurry contains mainly organic matter and other suspended solids (SS) and is relatively rich in nitrogen (N), phosphorus (P), and potassium (K). The nitrogen and ammonium-nitrogen (NH$_4$-N) content of swine manure varies between 158 - 1,025 mg/L, and 105 - 726 mg/L respectively (Table 1). The concentrations depend on the type of feed, slurry management and housing system (Chastain et al., 1999). Typical nutrient compositions of swine manure are shown in Table 1.

The main manure management system in Australia is anaerobic lagoon (McGahan et al., 2000, Kruger et al., 1995) - 83% of Australian swine farmers treat swine manure in facultative anaerobic ponds (APL, 2011).
Table 1: Typical nutrient composition of swine manure

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Concentration (mg/L)</th>
<th>Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Swine manure (Australia)a</td>
<td>Swine manure (fresh weight basis, kg/g)b</td>
</tr>
<tr>
<td>Dry matter %</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Total N</td>
<td>854 (158 - 1025)</td>
<td>3.6</td>
</tr>
<tr>
<td>TAN</td>
<td>398 (105 - 726)</td>
<td>2.5</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>109 (11 – 123)</td>
<td>1.8</td>
</tr>
<tr>
<td>Potassium</td>
<td>97 – 1845</td>
<td>2.4</td>
</tr>
<tr>
<td>Magnesium</td>
<td>4 – 108</td>
<td>0.7</td>
</tr>
<tr>
<td>Sodium</td>
<td>623 (103 – 2870)</td>
<td>-</td>
</tr>
<tr>
<td>Calcium</td>
<td>8.6 – 40</td>
<td>-</td>
</tr>
<tr>
<td>Copper</td>
<td>0 – 8</td>
<td></td>
</tr>
</tbody>
</table>

a- (Kruger et al., 1995),  b- (DEFRA, 2010)

2.1.3 Environmental challenges associated with manure management

Anaerobic lagoons are widely used to treat swine manure as they remove organic matter from the waste stream. They produce low-quality effluents with high biological oxygen demand, nutrient concentrations and pathogen levels (Buchanan et al., 2013). The runoff or leaching disposal of effluents from swine farming causes surface and ground water pollution by nitrate leaching and eutrophication by overloading nutrients such as N and P. These effluents also emit putrid odours, can spread diseases and cause fly infestations (Rao et al., 2007).

Current conventional swine manure management methods, (uncovered anaerobic lagoons and direct land application) in Australia also result in the production of biogas, mostly methane (CH\(_4\)), which is a potent greenhouse gas that has more than 21 times the potential for global warming than CO\(_2\) (Farran et al., 2000). Methane emissions from slurry pits from confined pigs are 0.032 kg CH\(_4\) kg\(^{-1}\) manure, indicating that slurry pits from confined pigs are a significant source (Petersen et al., 2016). Captured CH\(_4\)-rich biogas can be used to generate electricity, and reduce GHG emissions and help to reduce odour (Maraseni and Maroulis, 2008). The effluent from anaerobic digesters could be used as a nutrient-rich liquid fertiliser when it is managed properly. However, the application of liquid effluent as fertiliser at a higher rate than plant uptake of nutrients leads to environmental problems such as eutrophication of waterways, and salinization in semi-arid areas (Burkholder et al., 2007).
About 60% to 70% of the N from the diet fed to swines is excreted in urine and faeces (Dourmad et al., 1999). Therefore, a considerable amount of NH₃ (leading to indirect N₂O emissions) is emitted during effluent treatment, while smaller volumes of N₂O emanate from land application of effluent and solids (Wiedemann et al., 2010). Nitrogen in faeces is mainly in the form of proteins, while N in urine is in the form of urea that is readily converted to NH₄⁺ by the urease enzyme (Van et al., 1999). A large proportion of inorganic N in the slurry and solid manure is readily available in the form of NH₄⁺ (Chadwick et al., 2000, DEFRA, 2010). The majority of excreted N quickly transforms into NH₃ that can readily volatilize and enter the atmosphere. NH₃ volatilization starts soon after excretion and continues through the whole manure management process (Rotz, 2004). The main pathways of N loss are therefore volatile emissions to the atmosphere, and runoff and leaching losses to surface and ground waters (Vries et al., 2001). NH₃ emissions to the atmosphere contribute to ecosystem fertilisation, eutrophication and acidification (NRC, 2003). N₂O is a GHG that contributes to global warming and its global warming potential value over a 100-year period is 310 (IPCC, 1996). The amounts of nitric oxide (NO) and N₂O released are very low but are limiting precursors in tropospheric ozone production (NRC, 2003). N accessions to surface water, either directly or from the deposition of NH₃ from the atmosphere, contributes to NO₃⁻ in surface water and promotes eutrophication (Rotz, 2004). Ground waters that exceed 50 mg NO₃⁻/L are dangerous to human health, especially for infants as it causes blue-baby syndrome (Di and Cameron, 2002). Although N is a critical nutrient needed for the survival of microorganisms, animals, humans and plants, it may be detrimental when concentrations exceed optimum levels (van et al., 2012). In addition to undesirable environmental impacts of NH₃ losses from manure, the fertiliser value of animal manure is reduced because of N losses by emissions (Philippe et al., 2011).

Despite the well documented environmental hazards of swine manure management outlined above the only innovation in swine manure management in Australia over the last 10 years has been the introduction of covers over anaerobic lagoons to minimise CH₄ emission (Buchanan et al., 2013). Currently, in Australia, environmental sustainability, climate change and natural resource management are of critical significance for the swine industry (APL, 2013).
2.1.4 Solutions to overcome environmental challenges associated with waste management

Anaerobic digestion to reduce CH$_4$ emissions and increase energy production is an environmentally sustainable method compared with other means of managing swine manure (Maria et al., 2013). However, anaerobically digested swine manure still has large concentrations of NH$_4$, phosphate (PO$_4^{2-}$), and chemical oxygen demand (COD). (Obaja et al., 2005). Up to 80% of organic N in the feed stock of anaerobic digesters is transformed to total ammonium-N (TAN = NH$_3$(l) + NH$_4^+$) (Sommer et al., 2013). Therefore, nutrients must be removed from anaerobically digested effluents before discharge to the environment to minimise adverse environmental impacts (Villegas et al., 2011). Low biogas production rates are another limitation to the economic feasibility of anaerobic digestion of swine manure (Bonmati and Flotats, 2003). Swine manure has a low C/N ratio, resulting in lower biogas production (Mydeen et al., 2016). The high TAN concentration in swine manure is another reason for low biogas production as high TAN concentrations inhibit biogas generation (Hansen et al., 1998). An integrated system of anaerobic digestion with nutrient recovery provides advantages over other manure management systems as the biogas produced is a form of renewable energy that is cheap and environmentally healthy with minimal GHG emission, nutrients can be captured for use as fertiliser, along with pathogen reduction through sanitation (Holm-Nielsen et al., 2009) and reduced nuisance from flies and odours (Birkmose, 2007). Enhancing CH$_4$ production from anaerobic digestion of swine manure would be an advantage of recovering N during the anaerobic digestion process. Therefore, it is vital to focus on recovering N during anaerobic digestion. Methane capture from intensive livestock production is well established in United States of America (USA) and the European Union (EU) (RIRDC, 2008). Its use is still limited in Australia. However, the Australian Government has implemented some programs and policies that support CH$_4$ recovery as renewable energy and in relation to reducing GHG emissions (DAFF, 2008). However, there is limited progress in recovering N in order to reduce its environmental impacts and allow its reuse as fertiliser.

2.2 Anaerobic Digestion

Anaerobic digestion is an ecosystem in which complex organic matter is converted by microorganisms into simpler products such as CH$_4$, CO$_2$, hydrogen sulphide (H$_2$S), water, NH$_3$, and residual carbon and nutrients. It has been extensively used to treat organic solid wastes (Chernicharo, 2007), and it is a proven technology for the treatment of animal manures (Gungor-Demirci and Demirer, 2004).
2.2.1 Anaerobic Digestion Processes

The breakdown of organic compounds during anaerobic digestion is typically considered as a three-phase process in which different groups of microorganism are involved in specific functions. The first phase is hydrolysis during which proteins, carbohydrates and lipids are transformed to long chain organic acids. The second step is acidogenesis in which the organic components produced by hydrolysis are converted to short-chain volatile fatty acids (VFA), amino acids, alcohols, carbon dioxide (CO$_2$) and hydrogen (H$_2$) gases by heterotrophic, fermenting bacteria. The third step is methanogenesis in which CH$_4$ is produced through two main pathways: acetoclastic methanogenesis where acetic acid is transformed to CH$_4$ and CO$_2$, and hydrogenotrophic methanogenesis which combines CO$_2$ and H$_2$ to produce CH$_4$ (Figure 1) (Sommer et al., 2013).

Acetic acid may be produced by combining CO$_2$ and H$_2$. CO$_2$ and H$_2$ are produced by two different pathways that involve two different groups of bacteria. When H$_2$ concentrations increase in the digesters, the degradation of long-chain fatty acids is inhibited (Sommer et al., 2013). Therefore, concentrations of fatty acids longer than acetic acids increase inside digesters. If biopolymers inside the anaerobic digester are completely degraded, CH$_4$, CO$_2$, H$_2$S and NH$_3$ are the final by-products produced by anaerobic digestion process (Sommer et al., 2013).

![Figure 1: Schematic representation of anaerobic digestion processes (Sommer et al., 2013)](image-url)
2.2.2 Factors affecting anaerobic digestion

Many physical and chemical parameters affect anaerobic digestion. Organic loading rate (OLR) and hydraulic retention time (HRT) are important operating parameters for anaerobic digesters (Chamy et al., 2011). The most important other factors are temperature, pH and alkalinity, and nutrient requirements (Demirel and Yenigün, 2002). These parameters have to be frequently measured to guide adjustment to ensure the performance of the reactor (Amani et al., 2010).

2.2.2.1 Organic Loading Rate (OLR)

OLR is typically expressed in terms of volatile solids (VS) - those solids in slurry that are lost on ignition of dry solids at 550°C. Anaerobic digesters treated with swine manure have been operated with OLR ranging from 0.9 to 15.5 kg VS m\(^{-3}\) day\(^{-1}\) (Hill and Bolte, 2000, Zhang et al., 1997). As the OLR increases the biogas production and VS destruction rates decrease (Vandenburgh and Ellis, 2002, Demirer and Chen, 2005). However, the recommended OLR for swine manure is 3.0-3.5 kg VS m\(^{-3}\) day\(^{-1}\) (Burton and Turner, 2003).

2.2.2.2 Hydraulic Retention Time (HRT)

The average substrate retention period inside digesters is known as HRT. The ratio of digester working volume to the mean volume of flow is defined as HRT (Equation 1) (Burton and Turner, 2003). HRT is also one of the main factors that should be considered for designing digester systems for maximum biogas production. The minimum HRT is decided by the type of substrate to be digested. A comparatively lower HRT is required to digest swine manure if it contains high fat content (Burton and Turner, 2003). The recommended HRT for mesophilic (temperature range 20 – 42 °C) digestion of swine manure is in the range of 10-20 days (Burton and Turner, 2003, Sakar et al., 2009).

\[
HRT = \frac{\text{Digester working volume}}{\text{Influent flow rate}} \quad \text{Equation -1}
\]

2.2.2.3 Temperature

Temperature plays a critical role in biogas production. Temperature variations of a few degrees affect anaerobic digestion as it affects all biological activity in the reactor. Micro-organisms in digestion reactors tolerate temperatures between 0 to 82 °C (Amani et al., 2010) and are divided into groups according to their optimal temperature. Optimum temperature ranges for psychrophilic, mesophilic, and thermophilic organisms groups are 0- 20 °C, 20 – 42 °C, and 42 – 75 °C, respectively. Most reactors operate under mesophilic conditions but
thermophilic temperatures reduce organic matter concentrations more rapidly (de Bok et al., 2004), have faster destruction rates of pathogens, and produce more biogas (Kim et al., 2006). However, the energy required for heating, the production of poor quality supernatant that contains high contents of total dissolved solids (TDS), and less process stability, are disadvantages of thermophilic reactors compared to mesophilic reactors (van Lier et al., 1997).

2.2.2.4 pH and alkalinity
The pH, alkalinity and volatile acids are intimately related to each other and are similarly important for bringing the digester reactor to optimal conditions (Lettinga et al., 1996). Each group of micro-organisms has a different optimum pH range (Appels et al., 2008). The acid-forming bacteria are less sensitive to pH compared to the methanogens and can function in a wider range of pH, from 4.0 to 8.5 (Hwang et al., 2006). Methanogenic bacteria are extremely sensitive to pH with an optimum between 6.5 and 7.2 (Turovskiy and Mathai, 2006) Therefore at the extreme low and high ranges of pH (i.e. below 6 and above 8) there are significant reductions in CH₄ production (Chernicharo, 2007). Alkalinity regulates the pH inside the reactor and should be in the range between 1000 to 3000 mg/l to avoid extreme pH fluctuations (Amani et al., 2010).

The most important ion in solution is the proton (H⁺) or oxonium (H₃O⁺) ion. The H⁺ concentrations affects inorganic and microbiological processes in animal manure. In anaerobic processes pH is controlled by the interaction of the weak and strong acid-base systems (Sommer et al., 2013). In manure, the calculated [H⁺] may be about 20% different from the real proton concentrations [H⁺] due to the high ionic strength of the slurry (Sommer et al., 2013). At very high pH levels small ions may interfere significantly with the measurements, and at Na⁺(aq) or K⁺(aq) concentrations above 0.1 mol l⁻¹ the pH may be overestimated by 0.3 pH units (Sommer et al., 1995b). At very low pH the activity of (H₃O⁺) and the ionic strength become so high that this also interferes significantly when interpreting the measurements, i.e. assessing the concentrations of the ions (Sommer et al., 2013).

Ionic strength corrections to equilibrium equations are applied by multiplying each ion concentration by an activity coefficient (Capris et al., 1975).

The equilibrium Equation 2 for HA dissociation corrected for ionic strength is given by Equation 3
\[ K_A = \frac{[H_2O^-][A^-]}{[HA]} \]  

Equation -2

\[ K_A = \frac{f_m[H_2O^-]f_m[A^-]}{[HA]} = \frac{f_m^2}{f_m^2} \]  

Equation -3

where \( f_m \) is the activity coefficient for a monovalent ion, and \( (f_m^2, K_A) \) is the corrected equilibrium constant.

Then measured pH is defined as shown by Equation 4.

\[ pH = -Log(f_m[H]) \]  

Equation -4

The main buffer components in animal manure controlling pH are liquid total inorganic carbon (TIC = CO\(_2\)(aq) + HCO\(_3^-\)(aq) + H_2CO\(_3\)(aq)), TAN and volatile fatty acids (Sommer et al., 2013). High-molecular-weight organic matter with carboxyl functional groups may also contribute to the pH buffering capacity (Sommer et al., 1995a). Buffers can be added to acidic anaerobic systems to maintain the desired pH (Sakar et al., 2009).

### 2.2.2.5 Nutrient requirement

For optimal operation of a treatment plant sufficient amounts of inorganic nutrients are required for the growth of micro-organisms. N, sulphur (S), phosphorus (P), iron (Fe), cobalt (Co), nickel (Ni), molybdenum (Mo), selenium (Se), riboflavin and Vitamin B12 are required (in decreasing order of importance) to stimulate methanogens (Chernicharo, 2007).

#### 2.2.2.5.1 C: N Ratio and Ammonia

Carbon is utilised by microbes 25–30 times faster than N during anaerobic digestion (Yadvika et al., 2004). The optimum C: N ratio for microorganisms is 30:1. Microbial growth is inhibited when the C: N ratio increases to more than 50:1 or decreases to less than 10:1 (Sommer et al., 2013). NH\(_3\) and organic N produced by degradation are the main sources of N used by microbes during anaerobic digestion (Chernicharo, 2007). However, anaerobic digestion is susceptible to inhibition by the accumulation of certain chemicals. TAN is the most significant inhibitor (Yenigün and Demirel, 2013). During the anaerobic digestion of swine manure, TAN accumulates in the digester because of the high N content of the feed (Nasir et al., 2012) and causes inhibition of microbial activity (Hansen et al., 1998). It is vital to have a good understanding of NH\(_4^+\) release and NH\(_3\) inhibition during anaerobic digestion. Therefore the focus of this study is N recovery during anaerobic digestion.
2.2.3 Ammonium release and ammonia inhibition during anaerobic digestion

2.2.3.1 Ammonium release during the anaerobic digestion

Nitrogen in the manure enters the digester mainly in two forms - NH$_4^+$ and organic N (Topper et al., 2006). The majority (95%) of N in urine is in the form of urea (Canh et al., 1998). Urea undergoes enzymatic degradation in the presence of the enzyme urease, to form NH$_4^+$ and carbonic acid (Mobley and Hausinger, 1989). Urease is commonly associated with faecal material and is considered abundant on fouled concrete floor surfaces of pig rearing houses (Braam and Swierstra, 1999). Formation of NH$_4^+$ is relatively rapid, with about 95% of the reaction complete in the first 12 hours, often before the manure enters the digester, and organic N is also converted to NH$_4^+$ by protein degradation (Topper et al., 2006). Up to 80% of organic N in the feedstock of an anaerobic digester is transformed to TAN (Sommer et al., 2013). During the anaerobic digestion of swine manure additional NH$_4^+$ was released in the hydrolysis and acidification phases (Lin et al., 2013b). Hence, the NH$_4^+$ concentration in the digester effluent is typically higher than in the raw manure (Topper et al., 2006).

The NH$_3$ in the anaerobic digesters exists as NH$_4^+$ and free NH$_3$; It is in equilibrium in aqueous systems as shown by Equation 7.

\[
NH_4^+ + OH^- \leftrightarrow NH_3 + H_2O \quad \text{Equation -7}
\]

TAN, the sum of NH$_3$ + NH$_4^+$, accumulates in the digester as a result of degradation of proteins, urea, and other N compounds. The ratio of NH$_3$-N to NH$_4^+$-N in an aqueous system is governed by pH and temperature (Babson et al., 2013). Free NH$_3$ has been suggested to be the actual toxic agent in anaerobic digestion processes, so increase in pH would result in increased toxicity because of the shift to a higher free NH$_3$ at higher pH values (Equation 8) (Chen et al., 2008).

The equilibrium concentrations of NH$_4^+$ are pH and temperature (T) dependent as given by Equation 7 (Sommer et al., 2013).

\[
[NH_3(aq)] = \frac{TAN(aq)}{1+[(H_3O^+(aq))/K_N]} = \frac{TAN(aq)}{1+10^{pK_N-pH}} \quad \text{Equation 8}
\]

\[
pK_N = 4 \times 10^{-8}T^3 + 9 \times 10^{-5}T^2 - 0.0356T + 10.072 \quad \text{Equation 9}
\]
Where $K_N$ is the acid ionization constant for NH$_3$ and $pK_N$ is the acid dissociation constant value for NH$_4^+$/NH$_3$ in an aqueous solution which can be estimated using Equation 9.

### 2.2.3.2 Ammonia inhibition during anaerobic digestion

The accumulation of high TAN concentrations inside anaerobic digesters inhibits methanogenesis (Chen et al., 2008). At high pH, NH$_3$ dominates, and methanogenesis is inhibited because of the accumulation of VFAs. The threshold values at which ammonia inhibition begins ranges from 80 mg/L (Koster and Lettinga, 1984) to 150 mg/L (Braun et al., 1981), and TAN concentrations of around 1700–1800 mg/L may cause reactor failure (Melbinger et al., 1971), and NH$_4^+$ concentrations more than about 1500mg-N/L are unfavourable (Zhang et al., 2000).

#### Table 2: Ammonia inhibitory effects on anaerobic digestion of swine manure

<table>
<thead>
<tr>
<th>Reactor Type</th>
<th>Reactor Temperature ($^\circ$C)</th>
<th>TAN concentration (mg/l)</th>
<th>Free ammonia concentration (mg/l)</th>
<th>Methane Reduction (relative to control) (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch</td>
<td>35</td>
<td>3000-7000</td>
<td></td>
<td>40-55</td>
<td>Zhang et al. (2014)</td>
</tr>
<tr>
<td>ASBR</td>
<td>25</td>
<td>4000</td>
<td>80</td>
<td>45.0</td>
<td>Garcia and Angenet (2009)</td>
</tr>
<tr>
<td>CSTR</td>
<td>55</td>
<td>11000</td>
<td>1450</td>
<td>50.0</td>
<td>Nakakubo et al. (2008)</td>
</tr>
<tr>
<td>Batch</td>
<td>55</td>
<td>5100</td>
<td>1400</td>
<td>36</td>
<td>Hansen et al. (1998)</td>
</tr>
<tr>
<td>CSTR</td>
<td>35</td>
<td>5020</td>
<td>257</td>
<td>71.4</td>
<td>Kroeker et al. (1979)</td>
</tr>
<tr>
<td>Batch</td>
<td>30</td>
<td>3075</td>
<td>84</td>
<td>19.8</td>
<td>Van Velsen (1979)</td>
</tr>
</tbody>
</table>

ASBR: Anaerobic Blanket Sludge Reactor, CSTR: Continuous Stir Tank Reactor

However, inhibition is mainly caused by NH$_3$, rather than NH$_4^+$ (Nielsen et al., 2013), as free NH$_3$ molecule can penetrate bacterial cell membranes due to its electrical neutrality and small size (Kell et al., 1981). The hydrophobic NH$_3$ molecule may diffuse passively in to the cell,
causing proton imbalance and/or potassium deficiency (Chen et al., 2008). From other anaerobic microorganisms, methanogenic bacteria are the least tolerant and most likely to stop their growth due to NH$_3$ inhibition (Logan et al., 2006). However, in practice swine manure anaerobic digesters perform well beyond these NH$_3$ concentrations (Sommer et al., 2013). Table 2 shows summarised results found in the literature for ammonium inhibition of swine manure.

2.2.4 Advantages of recovering ammonium from anaerobic digestion
Large amounts of nutrients in effluents from anaerobically digested swine manure are a major problem in anaerobic digestion technology (Obaja et al., 2005). Nutrients must be removed from anaerobically digested effluents before discharge to the environment to minimise adverse environmental impacts (Villegas et al., 2011). Although effluents from anaerobic digesters are used as soil amendments they must be carefully managed to avoid releases of excess N to surface waters, the atmosphere and by infiltration to groundwater (Babson et al., 2013). Digestate can be used as a soil amendment with minimal environmental effects if TAN is removed during the anaerobic process.

The C/N ratio for swine manure is around 6 to 8, depending upon pigs' growth stages (MWPS, 2000). Swine manure has a low C/N ratio, due to high nitrogen content result in lower biogas production (Mydeen et al., 2016). Accumulation of high TAN concentrations inside the anaerobic digester inhibits methanogenesis (Chen et al., 2008). Methanogenesis inhibition occurs in a TAN concentration range of 760 to 4000 mg NH$_4^+$-NL$^{-1}$ (Bousek et al., 2016). Therefore, recovering N during the anaerobic process should increase C:N ratio and mitigate NH$_3$ inhibition result in enhanced biogas production.

2.3 Nitrogen Recovery
N is essential for crop production agriculture and demand for nitrogenous fertiliser has been increasing increased from 10 Mt N in 1960 to about 90 Mt N in 1998. (Mulder, 2003). Worldwide demand for nitrogenous fertiliser is expected to rise from a total of 113 million tonnes in 2015 with an annual growth rate of 1.7% (FAO, 2011). The recovery of N from swine manure is an option for reusing N in agriculture and reducing the amount released into the environment. There are several methods for recovering N from waste streams, such as ammonia stripping, chemical precipitation as struvite, use of organic amendments, and ion exchange/adsorption.
2.3.1 Ammonia Stripping

Ammonia stripping is a physicochemical process dependent on pH and temperature. It allows for the transfer of volatile NH$_3$ (strongly dependent on pH and temperature) from a liquid to a gas phase and its subsequent recovery and concentration, by absorption (Laureni et al., 2013).

Ammonia stripping is based on the change of conditions in the effluent allowing the conversion of NH$_4^+$ in the liquid phase to NH$_3$ gas (Arogo et al., 1999). With alterations of pH and temperature, it is possible to shift the equilibrium towards the left to form a NH$_3$ gas (Lei et al., 2007), as shown in Equation 10:

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^-$$  \hspace{1cm} \text{Equation- 10}

Up to 99% of N was stripped by increasing pH above 11.5 at a temperature of 353K, with a 3 h retention time in a batch stripping column (Bonmatí and Flotats, 2003). Nielsen et al. (2013) investigated the feasibility of removing a considerable amount of NH$_3$ from the liquid phase and biogas headspace by NH$_3$ free gas circulation. However, there are some limitations to ammonia stripping, such as the requirement of controlling temperature, no opportunity to remove nitrite and nitrate, and difficulties in adjusting pH of strongly buffered wastewater (Dapena-Mora et al., 2004). Low stripping efficiency and scaling and fouling of the packing surface inside packed towers are other major problems with this process (Quan et al., 2009).

2.3.2 Chemical precipitation as struvite

Induced precipitation of struvite (magnesium ammonium phosphate - MAP (MgNH$_4$PO$_4$.6H$_2$O), is one of the techniques used for simultaneous recovery of N and P from wastewater (Doyle and Parsons, 2002). NH$_4^+$ and phosphate (PO$_4^{3-}$) removal from wastewater is achieved by the addition of magnesium (Mg$^{2+}$) and adjusting pH (Schulze-Rettmer, 1991, Webb et al., 1995). Struvite precipitation depends mainly on the pH of the solution and the Mg:N:P molar ratio (Münch and Barr, 2001).

Struvite crystals are usually stable, white, and orthorhombic (Le Corre et al., 2005). Precipitation of Mg$^{2+}$, NH$_4^+$, and PO$_4^{3-}$ in 1:1:1 molar ratio follows (the simplified) Equation 11:

$$Mg^{2+} + NH_4^+ + HPO_4^{2-} + 6H_2O \rightarrow MgNH_4PO_4.6H_2O + H^+$$  \hspace{1cm} \text{Equation 11}

However, the formation of struvite requires specific conditions and the ideal molar ratio of Mg$^{2+}$: NH$_4^+$: PO$_4^{3-}$ (from 1:1:1 to 2.5:1:1) is almost never found naturally (Fernandes et al.,
2012). Struvite can be precipitated at pH values between 7.0 and 11.5, with an optimum pH range between 7.5 - 9.0 (Hao et al., 2008). Struvite has many potential uses as a slow-release fertiliser that can be applied in a single high dose without damaging growing plants (de-Bashan and Bashan, 2004) and recovers both nitrogen and phosphorus from waste water.

2.3.3 Amendments

2.3.3.1 Biochar
Biochar is formed by pyrolysis of biomass and contains carbonaceous matter (Luque et al., 2012). Biochar is used as a soil amendment as it helps to improve soil fertility (Schulz and Glaser, 2012). Biochar used as a bulking agent decreases bulk density and increases soil aeration (Steiner et al., 2010). Improvement of microbial respiration rates and microbial growth (Jindo et al., 2012), as well as enhancing the absorption of both NH$_3$(g) and NH$_4^+$ ions are other advantages of biochar (Hua et al., 2009, Steiner et al., 2010). When poultry litter was mixed with 20% biochar during poultry litter composting that the NH$_3$ emissions were reduced up to 64% and TAN losses were decreased by up to 52% (Steiner et al., 2010). There was a potential reduction of GHG emissions from incorporating biochar into rice paddy soils in south-eastern China (Zhang et al. 2010).

However, biochar technology is still in the early stages of development and there are some concerns about its applications. The main issues of biochar are its handling, feedstock availability, and biochar system arrangement. Biochar system arrangement depends on cost, operating time, utility providers and technology reliability (Bracmort, 2009).

2.3.3 2 Lignite
Lignite (brown coal) is yellow to dark brown (rarely black) coloured material that is formed from peat at shallow depths and temperatures below 100 °C. Dry lignite consists of 60-75% carbon (EncyclopædiaBritannica., 2014).

The most important properties of lignite are its high ion exchange capacity and absorption capacity. The application of lignite to intensive cattle feedlots is an effective and economically-viable method to mitigate NH$_3$ emissions (Chen et al., 2015). Adding a mixture of nutrient-enriched sludge and lignite as a soil amendment improved soil properties and thus has the potential for application to agricultural land (Qi et al., 2011). Bulgarian lignite (after treatment of one-step steam pyrolysis-activation followed by oxidative modification) is a promising NH$_4^+$ adsorbent (Vassileva et al. 2009).
2.3.3 Fly ash
Worldwide, fly ash is generated in huge quantities from thermal power plants. Its handling and disposal are difficult, and attention has been given to its possible utilisation as a soil amendment (Masto et al., 2013). Fly ash as an agricultural soil amendment can be improved by mixing it with potentially acid-forming organic by-products such as poultry and cattle manure, or sewage sludge, which are considerably enriched in N and P (Adriano et al., 1980).

The application of a mixture of fly ash and farm manure as a soil amendment enhanced the N transformation rate and plant available-N, and therefore paddy rice productivity (Singh and Pandey, 2013). Fly ash addition to anaerobic digestion of municipal solid waste (MSW) (FA/MSW 10 and 20 g L$^{-1}$ and BA/MSW 100 g L$^{-1}$) enhanced biogas production rates (Lo et al., 2010). However, fly ash contains heavy metals that can accumulate in soil and plants. Solutions for the sustainable utilisation of fly ash should be found before their widespread application (Biedermann and Obernberger, 2005).

2.3.4 Membrane contactors
Membrane contactors are effective for removal of NH$_3$ from wastewater as they have large surface areas that allow rapid separation of NH$_3$ even with low energy inputs (Hasanoğlu et al., 2010). A membrane contactor is a device that achieves gas/liquid and liquid/liquid mass transfer without the dispersion of one phase within another (Ashrafizadeh and Khorasani, 2010). Hollow-fibre membrane contactors are attractive for removal of volatile contaminants such as NH$_3$ (Baker, 2000). Under optimum conditions, more than 99% of NH$_3$ was removed using a gas permeable hollow-fibre membrane (Ashrafizadeh and Khorasani, 2010). The volatile component in the feed readily diffuses through a gas permeable membrane and is then removed by a sweep gas driven by vacuum or reacted with a stripping solution (Nunes and Peinemann, 2001). A hollow fibre membrane could be used as a barrier between the feed and the stripping solution (Ashrafizadeh and Khorasani, 2010).

The use of hollow fibre membrane contactors has a number of advantages such as providing large surface area per unit volume which allows rapid removal of volatile compounds; independent control of liquid and gas flow rates without causing any loading, flooding or foaming; no production of secondary pollutants, and the possibility of recovery and reuse of volatile compounds (Ashrafizadeh and Khorasani, 2010). However, the economic efficiency of membrane contactors is questionable (Hasanoğlu et al., 2010).
2.3.5  Ion Exchange

Ion exchange has gained increasing attention among the methods available for treating NH$_4^+$-rich wastewater (Wang et al., 2007). Ion exchange reactions generally take place between two or more phases as liquid and solid phase. Ion transfer is governed by the ion concentrations in different phases and the ion selectivity of a given exchanger (Colella, 1996).

Ion exchangers can take the form of resins, synthetic polymeric materials and zeolite. Zeolites show comparatively high cation exchange selectivity (Colella, 1996). In addition, the zeolite can react under a wide range of temperatures and is highly compatible with the environment (Jorgensen and Weatherley, 2003, Colella, 1996). Therefore, zeolites are widely used as selective adsorbents, particularly as catalysts and molecular sieves (Colella, 1996).

2.3.5.1  Zeolite

Zeolite is aluminosilicate mineral with a highly regular micro-porous structure that has high affinity for NH$_4^+$ in water (Lin et al., 2013a). They are commonly used as adsorbents and ion-exchange media in a number of commercial applications (Inglezakis and Zorpas, 2012).

2.3.5.1.1  Potential of zeolites as an ammonium adsorbent

Interest in using zeolite for NH$_4^+$ adsorption is growing due to its relative simplicity of application and operation, and low cost (Wang et al., 2007). The use of natural zeolite for wastewater treatment has increased significantly (Widiastuti et al., 2008).

Zeolite addition to mesophilic anaerobic digestion is effective because of its ability to immobilise micro-organisms, and improve the NH$_3$/NH$_4^+$ ion equilibrium (Milan et al., 2001). It improves anaerobic digestion performance in high NH$_4^+$ concentration wastewaters such as pig, cattle and chicken manure effluents, and it also helps prevent inhibition of anaerobic digestion (Montalvo et al., 2012). Several researchers have shown the potential of a natural Australian zeolite to remove NH$_4^+$ from low strength wastewater (Cooney et al., 1999a, Booker et al., 1996, Komarowski and Yu, 1997). The saturation capacity of the zeolite mined from north of Sydney was 150 cmol/kg and its selectivity for NH$_4^+$ was higher than other cations (Ca$^{2+}$, Mg$^{2+}$, K$^+$) typically present in sewage (Cooney et al. 1999a). The NH$_4^+$ adsorption capacity of the zeolite was about 4.5 mg N/g under optimum operational conditions and NH$_4^+$ ion concentrations ranging from 25 to 50 mg NH$_4$-N/L (Booker et al. 1996). Normally livestock slurry, and effluents from anaerobic digestion of livestock manure contains NH$_4^+$ at more than 1000 mg-N/L (Ganigue et al., 2007, Terada et al., 2003). However, NH$_4^+$ adsorption onto Australian zeolite at high NH$_4^+$ levels has not yet been
investigated. Natural zeolite should always be treated before use to remove NH$_4^+$ in order to enhance its adsorption capacity (Guo et al. 2013). Alkali treatment, acid treatment, and hydrothermal treatment are commonly used to modify natural zeolite to improve its adsorption properties (Guo et al., 2013). The highest NH$_4^+$ recovery efficiency was achieved after converting an Australian natural zeolite to the sodium (Na$^+$) form (Cooney et al., 1999b, Booker et al., 1996, Komarowski and Yu, 1997). Zeolite addition is an effective way to reduce NH$_4^+$ inhibition of anaerobic digestion because of its large capacity for adsorption, and selectivity, for NH$_4^+$, resulting in enhanced biogas production (Montalvo et al., 2012). The effects of zeolite type and concentration and dosage procedures on the anaerobic digestion of N-rich wastewater have been studied by several authors. Results from studies on zeolite addition during anaerobic digestion are shown in Table 3. The main objective of previous studies of using zeolite was only to enhance CH$_4$ production rather than the recovery of maximum amounts of TAN.

Factors such as temperature, pH, reaction time, dosage, other anion and cations in water and initial NH$_4^+$ concentration all influence the NH$_4^+$ recovery performance of zeolite (Huang et al., 2010, Erdogan and Ulkü, 2011). Each different zeolite material has its own characteristics and individual research is required to identify those characteristics for particular zeolites (Huang et al., 2010). The performance of Australian zeolite during anaerobic digestion is unknown. Further research is required to investigate the optimum zeolite concentration that produces maximum NH$_4^+$ and free NH$_3$ removal while not inhibiting CH$_4$ production. In addition, the mechanism of biogas production enhancement as a result of adding zeolite to anaerobic digestion is still not very clear and not fully investigated because of the complexity of anaerobic digestion processes. The impacts of zeolite addition on the formation of volatile fatty acids (VFAs) and bio-physicochemical dynamics within the digester under anaerobic digestion of swine manure are not known. The potential risks associated with subsequent cation release from zeolite also need to be assessed, in addition, the effects of zeolite addition to feedstock liquids in relation to the C: N ratios of effluents are unknown. To date, investigations have focussed on adding zeolite to digestive feedstock and there has been no research on the bioreactor configuration by using zeolite.
Table 3: Studies regarding zeolite addition during the anaerobic digestion process

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Reactor type</th>
<th>Amount of zeolite addition (optimum)</th>
<th>Temperature °C</th>
<th>Initial NH₄⁺ concentration mg/L</th>
<th>CH₄ enhancement Percentage</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swine manure</td>
<td>Batch</td>
<td>10</td>
<td>35</td>
<td>7511</td>
<td>80</td>
<td>(Zheng et al., 2015)</td>
</tr>
<tr>
<td>Swine manure</td>
<td>CSTR</td>
<td>60</td>
<td>35</td>
<td>2182</td>
<td>20</td>
<td>(Lin et al., 2013c)</td>
</tr>
<tr>
<td>Swine manure</td>
<td>CSTR</td>
<td>20</td>
<td>55</td>
<td>1740</td>
<td>60</td>
<td>(Ho and Ho, 2012)</td>
</tr>
<tr>
<td>Swine manure</td>
<td>Batch</td>
<td>12</td>
<td>55</td>
<td>275</td>
<td>30</td>
<td>(Kotsopoulos et al., 2008)</td>
</tr>
<tr>
<td>Synthetic waste</td>
<td>Batch</td>
<td>1</td>
<td>35</td>
<td>3000</td>
<td>30.2</td>
<td>(Montalvo et al., 2006)</td>
</tr>
<tr>
<td>Swine manure</td>
<td>Batch</td>
<td>1</td>
<td>35</td>
<td>225</td>
<td>29</td>
<td>(Montalvo et al., 2006)</td>
</tr>
<tr>
<td>Swine manure</td>
<td>Batch</td>
<td>2 – 4</td>
<td>27 – 30</td>
<td>410</td>
<td>Increased</td>
<td>(Milan et al., 2001b)</td>
</tr>
<tr>
<td>NH₄⁺ rich Organic sludge</td>
<td>Mordenite</td>
<td>35</td>
<td>4500</td>
<td>1.7</td>
<td></td>
<td>(Tada et al., 2005)</td>
</tr>
<tr>
<td></td>
<td>Batch</td>
<td>50 – 100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CSTR: Continuous Stir Tank Reactor

2.4. Solid separation from swine effluent

Clogging is very common in wastewater treatment using zeolite columns and is probably caused by microbial growth and particles in the effluent wastewater (Hedstrom, 2001). Designing bioreactor configurations with external zeolite column for the purpose of convenient removal of zeolite after saturation with NH₄⁺ would be one of the promising methods of zeolite application during the anaerobic digestion process. However it is important to prevent clogging of the zeolite inside the column by recirculating leachate through the zeolite column, this section describes possible ways of avoiding clogging and fouling by filtration in combination with digesters.

Membrane filtration after removing coarse fiber materials is an appropriate technology for particle separation from wastewater. A range of technologies including membrane filtration, ultrafiltration and microfiltration membranes are efficient and effective solid-liquid separators (Masse et al., 2007). The liquid fraction obtained from ultrafiltration membrane from wastewater effluent contains large amounts of dissolved TAN and K⁺ (Sommer et al., 2013). One advantage of anaerobic digestion combined with membrane technology is an
option to retain more microorganisms inside the digester and allow operation with high biomass concentration (Wong et al., 2009, López-Fernández et al., 2011). Among the currently available filtration processes, microfiltration would be the appropriate option for our purpose (Figure 2).

![Figure 2: Spectrum of Filtration (reverse osmosis, nanofiltration, ultrafiltration, microfiltration and particulate filtration) relative to the pore size of common materials (Source: (Radcliff and Zarnadze, 2004))](image)

The different kinds of modules are used for microfiltration and ultrafiltration process according to the material and shape of the membrane (Table 4). Membranes are available in two shapes: tubular (tubes, capillaries and hollow fibres) and flat sheet forms (Futselaar and Weijenberg, 1998). The tubular membrane has been used to separate particles from swine slurry (Table 4).
Table 4: Membrane filters used in laboratory-scale anaerobic membrane reactors used to treat different types of waste water

<table>
<thead>
<tr>
<th>Wastewater type</th>
<th>Reactor Volume (L)</th>
<th>Membrane Type</th>
<th>Material Type</th>
<th>Membrane configuration</th>
<th>Pore size (μm)</th>
<th>Area (m²)</th>
<th>TMP (bar)</th>
<th>Cross-flow velocity (m/s)</th>
<th>Flux (L/m² h)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermomechanical pulping pressate</td>
<td>10</td>
<td>UF (MWCO:70 kDa) Flat sheet</td>
<td>Polyvinylidene fluoride</td>
<td>Sub-merged</td>
<td>0.03</td>
<td>–</td>
<td>–</td>
<td>5.7–6.9</td>
<td>(Gao et al., 2011)</td>
<td></td>
</tr>
<tr>
<td>Thermomechanical pulping whitewater</td>
<td>10</td>
<td>MF (MWCO:70 kDa) Flat sheet</td>
<td>Polyvinylidene fluoride (PVDF)</td>
<td>Sub-merged</td>
<td>&lt;0.4</td>
<td>–</td>
<td>–</td>
<td>4.8–9.1</td>
<td>(Lin et al., 2011)</td>
<td></td>
</tr>
<tr>
<td>Brewery with surplus yeast</td>
<td>4.5</td>
<td>MF- tubular</td>
<td>Ceramic</td>
<td>Gas-lift</td>
<td>0.2</td>
<td>–</td>
<td>–</td>
<td>6–20&lt;10</td>
<td>(Torres et al., 2011)</td>
<td></td>
</tr>
<tr>
<td>Thermomechanical pulping whitewater</td>
<td>10</td>
<td>MF (MWCO:70 kDa)- Flat sheet</td>
<td>Polyvinylidene fluoride (PVDF) coated with 1% PEBAX 1657</td>
<td>Sub-merged</td>
<td>0.03</td>
<td>&lt;0.3</td>
<td>–</td>
<td>4.3–5.2</td>
<td>(Gao et al., 2010)</td>
<td></td>
</tr>
<tr>
<td>Kraft pulp mill evaporator condensate</td>
<td>10</td>
<td>MF (MWCO:70 kDa)- flat sheet</td>
<td>Polyvinylidene fluoride (PVDF)</td>
<td>Submerged</td>
<td>0.03</td>
<td>&lt;0.3</td>
<td>–</td>
<td>5.3 ± 1</td>
<td>(Lin et al., 2010)</td>
<td></td>
</tr>
<tr>
<td>Application</td>
<td>Membrane Type</td>
<td>Area (m²)</td>
<td>Temperature (°C)</td>
<td>Flux (L/m²h)</td>
<td>Rejection (%)</td>
<td>Source</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>------------------------</td>
<td>-----------</td>
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<td>---------------</td>
<td>----------------------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kraft evaporator condensate</td>
<td>MF</td>
<td>10</td>
<td></td>
<td>0.3</td>
<td>&lt;0.3</td>
<td>5.6–12.5 (Xie et al., 2010)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simulated petrochemical</td>
<td>MF</td>
<td>58</td>
<td></td>
<td>0.45</td>
<td>0.00</td>
<td>1.5–4.5 (Van Zyl et al., 2008)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline wastewater</td>
<td>MF</td>
<td>3</td>
<td></td>
<td>0.4</td>
<td>0.2</td>
<td>200 (Akram and Stuckey, 2008)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acidified cheese whey</td>
<td>MF</td>
<td>20</td>
<td></td>
<td>0.2</td>
<td>1.25</td>
<td>137–140 (Saddoud et al., 2007)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slaughterhouse</td>
<td>UF</td>
<td>100</td>
<td></td>
<td>1</td>
<td>3</td>
<td>2–8 (Saddoud and Sayadi, 2007)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food processing</td>
<td>UF</td>
<td>500</td>
<td></td>
<td>0.32</td>
<td>1.02–1.09</td>
<td>13.1–18.9 (He et al., 2005)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slaughterhouse</td>
<td>UF</td>
<td>17</td>
<td></td>
<td>0.06–3</td>
<td>-</td>
<td>40–100 (Siegrist et al., 2005)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sauerkrout brine</td>
<td>MF</td>
<td>7</td>
<td></td>
<td>2</td>
<td>0.126</td>
<td>5–10 (Fuchs et al., 2003)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Process</td>
<td>MF Type</td>
<td>Membrane Material</td>
<td>MWCO (kDa)</td>
<td>Cross-flow</td>
<td>TMP (kPa)</td>
<td>Concentration (ppm)</td>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------</td>
<td>---------</td>
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<td>------------</td>
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<td>---------------------</td>
<td>----------------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slaughterhouse</td>
<td>MF</td>
<td>Ceremic (material: Al₂O₃)</td>
<td>–</td>
<td>2–3</td>
<td>5–10</td>
<td>(Fuchs et al., 2003)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol fermentation</td>
<td>MF</td>
<td>Skin: zirconia</td>
<td>0.14</td>
<td>0.0113</td>
<td>0.6</td>
<td>3</td>
<td>700 (Kang et al., 2002)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol fermentation</td>
<td>MF</td>
<td>Polypropylene</td>
<td>0.20 m</td>
<td>0.0129</td>
<td>0.6</td>
<td>3</td>
<td>1300 (Kang et al., 2002)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palm oil mill</td>
<td>UF</td>
<td>(MWCO: 200 kDa)</td>
<td>Cross-flow</td>
<td>1.5</td>
<td>2.3</td>
<td>26.4–30.3</td>
<td>(Noor and Fakhru'l-Razi, 1999)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palm oil mill</td>
<td>UF</td>
<td>(MWCO: 200 kDa)-</td>
<td>Cross-flow</td>
<td>0.1</td>
<td>0.024</td>
<td>1.5–2</td>
<td>(Abdurahman et al., 2011)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol-distillery</td>
<td>UF</td>
<td>(MWCO: 20 kDa)–</td>
<td>Cross-flow</td>
<td>0.0168</td>
<td>0.5–3</td>
<td>0.5–1.25</td>
<td>10–40 (Choo and Lee, 1998)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>swine manure</td>
<td>UF</td>
<td>flat sheet fluoropolymers</td>
<td>–</td>
<td>20–70</td>
<td>5–100</td>
<td>5–10 (Zhang et al., 2007)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>swine manure</td>
<td>UF</td>
<td>Polyethersulphone</td>
<td>Cross-flow</td>
<td>0.0377</td>
<td>0.3–0.7</td>
<td>5–10 (Padmasiri et al., 2007)</td>
<td></td>
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</table>

MF – Microfiltration  
UF – Ultrafiltration  
MWCO – Molecular weight cut-off  
TMP – Transmembrane Pressure
2.5 Summary of current knowledge gaps

Application of zeolite during the process of anaerobic digestion is one of the best methods to enhance CH$_4$ production and NH$_4^+$ removal compared to other methods such as air stripping, chemical precipitation as struvite, membrane contactors and using other amendments biochar, lignite and flyash. Past research has been conducted on zeolite addition to anaerobic digestion of swine manure and similar wastes and has focused on mixing zeolite into the digestive feedstock with an aim of enhancing CH$_4$ production rather than simultaneous maximising both NH$_4^+$ recovery and CH$_4$ production. This review has revealed that there are still knowledge gaps, with currently limited or no information available on:

1. NH$_4^+$ adsorption onto Australian zeolite at high NH$_4^+$ levels and related kinetics of Australian zeolites
2. Each zeolite material has its own characteristics and there is a need to study Australian zeolite as an amendment for anaerobic digestion to enhance TAN recovery and CH$_4$ production.
3. There is a need to determine the optimum zeolite concentration that produces maximum TAN removal without inhibiting CH$_4$ production on anaerobic digestion of swine manure.
4. There is a need to further understand the enhancement mechanisms associated with zeolite mixing in digestion reactors. The impacts of zeolite addition on the evolution of volatile fatty acids (VFAs) and other bio-physico-chemical dynamics within the digester under anaerobic digestion of swine manure need to be determined.
5. The effects of Australian zeolite addition on C:N ratio adjusted swine manure and the most efficacious zeolite application rate needs to be identified.
6. Bioreactor configuration for optimising the digester performance with zeolite needs to be improved.
References


Birkmose T (2007). Digested manure is a valuable fertilizer. The Future of Biogas in Europe 3, 89-94.


Chapter 3

General methodology

The research included sets of NH$_4^+$ adsorption tests and anaerobic digestion experiments conducted in the laboratory. Natural zeolite was used to conduct adsorption tests through addition to the anaerobic digestion of swine manure. The zeolite was obtained from Zeolite Australia Pty Ltd., Werris Creek, NSW, Australia. Swine manure and inoculum was collected from Berrybank Farm; Ballarat, Victoria, Australia. The piggery had a standing swine population is 20,000 pigs. After identifying the NH$_4^+$ adsorption capacity of Australian zeolites and NH$_4^+$ dynamics during the anaerobic digestion process, digestion experiments of swine manure and C/N adjusted swine manure with different rates of zeolite addition were conducted to identify the effect of zeolite addition and to determine the optimum zeolite dose. Finally, feasibility was conducted on an external zeolite column connected to an anaerobic digester. In this chapter, the general methods used in each experiment are discussed.

3.1 Zeolites for adsorption tests

3.1.1 Zeolite preparation:
Bulk commercial zeolites were obtained from Zeolite Australia Pty Ltd, Werris Creek, NSW, Australia. They were prepared by sieving (0.5mm–1mm). The sieved zeolites were then washed three times with deionised water and dried for 48 hrs at 105±1°C.

3.1.2 Preparing NaCl treated zeolite known as sodium zeolite:
A portion of the washed zeolite was treated with NaCl to modify its physio-chemical properties. The NaCl treatment was performed as a batch process that included mixing five grams of processed natural zeolite with 100 mL of 2M NaCl in a 250 mL conical flask with shaking in a thermostatic shaker at 35°C and 100 rpm for 24 h. After this treatment, the zeolite was washed three times with deionized water and dried at 105 0±1 C for 24 h.

3.1.3 Batch adsorption test
In each test, 2g of zeolite was placed in a 250 mL flask and 100 mL of appropriately diluted NH$_4$Cl solution/mixture of ions made out of pre-prepared 1M stock solutions was added to each flask. Analytical grade NH$_4$Cl, NaCl, KCl, MgCl$_2$, CaCl$_2$, K$_2$HPO$_4$ and 98% CH$_3$COOH were used to prepare stock solutions with deionized water. The desired pH level for the test was achieved by adding either 0.1 M HCl or 0.1 M NaOH. The flasks containing the NH$_4^+$ solutions/ the test medium containing the ion mix and zeolite were placed in a circular motion incubation shaker (LABWIT Scientific ZWY-2403 Series incubator shaker) (Figure 1) and agitated at 100 rpm.
3.1.4 Static leaching column study

Fifteen glass columns (55mm internal diameter and 400mm height) were used in the experiment (figure 2). Each column was filled with 150 g of zeolite (with particle size ranging from 0.5mm to 1mm). Twelve columns were fed (4 different flow rates with triplicates) with a NH₄Cl solution (1000 mg-N/L) at flow rates of 1.5 mL/min, 13 mL/min, 15 mL/min and 17 mL/min. (corresponding to residence times of 46.12 min., 5.32 min., 4.61 min. and 4.07 min. respectively). Three columns were fed with the same test medium containing the ion mix (NH₄⁺, K⁺, Ca²⁺, Mg²⁺, PO₄³⁻) and acetic acid used for the batch test at a flow rate of 1.5 mL/min. The composition of the test medium was same as for the previous tests. Solutions were fed from the top end of the column by using a peristaltic pump in the down flow mode at three different flow rates (Figure 2). The column flow experiment was conducted continuously for up to 420 minutes with different flow rates of 1.5 mL/min, 13 mL/min, 15 mL/min and 17 mL/min. Samples from the outlet were collected at different sampling time intervals to monitor the NH₄⁺ concentration.

Figure 1: Zeolite mixtures in circular motion incubation shakers

Figure 2: Schematic of experimental set-up of the column study
3.2 Design of anaerobic digesters (Chapter 5)
The lab-scale anaerobic digester was equipped with three sampling ports and a gas valve. The total volume of the digester was 5 L and the working volume 4 L (Figure 3a & b).

![Diagram of the lab-scale anaerobic digester](image1)

**Figure 3:** a. Schematic diagram of the lab-scale anaerobic digester, b. Photograph of the experimental set-up

3.3 Experimental set-up (chapter 6)
Experiments were conducted in 30, 400 mL (working volume) glass bottles (batch type digesters). Each digester was fitted with 2 stopcock valves; one for collecting gas and one for purging N at the beginning of the experiment (Figure 4). The inoculum and swine manure (substrate) were added on a VS basis at a ratio of 1:1 in all digesters. The inoculum was incubated for 14 days at 37°C. All CH$_4$ was removed before starting the experiment (Figure 6a). The digesters were filled with a 450 mL inoculum-substrate homogenised mixture (Figure 5). Zeolite was added at different rates. The dose of zeolite was 0, 10, 40, 70, 100 g/ L of swine manure.

Two series of digestions were carried out, each with five digesters in triplicate. After mixing swine waste and inoculum with natural and sodium zeolites at different rates according to the experimental conditions, all digesters were closed with screw cap lids. The headspaces of all digesters were flushed with N$_2$ gas to create an anaerobic environment inside the digesters. The digesters were kept in an incubator at 37 °C to maintain mesophilic conditions (Figure 6b). Each digester was mixed once a day manually.
3.4 Design of anaerobic digester (Chapter 7)

The lab-scale anaerobic digesters were equipped with three sampling ports and a gas valve. The total volume of the digester was 5 L and the working volume 4 L. A glass column (diameter 55 mm and height 400 mm, IL) with sintered borosilicate glass discs at the bottom was used as the column. The external glass column with zeolite was attached to the digester by connecting an inlet tube at the bottom through a peristaltic pump. Another tube from the top of the glass column was
connected to the gas collector. The outlet tube from the bottom of the glass column was connected to the digester after making a loop in between the peristaltic pump and column to push swine manure back into the digester. The same pump was utilised to pump swine manure from the digester to the external column. (Figure 7). This digester was known as the “Ex-Zeo” digester. Another lab-scale anaerobic digester was employed with a fixed zeolite bed digester by adding zeolite to the bottom. This is referred to as the “In-Zeo.” digester (Figure 8). The digester without zeolite was known as the “No-Zeo.” digester.

**Figure 7:** Schematic of the lab-scale anaerobic digester with external zeolite column

**Figure 8:** Schematic of the anaerobic fixed bed digester
3.4.1 Anaerobic digestion experiment
Three groups of batch experiments with 3 replicates were conducted in three different digesters, as described in section 3.4 in order to investigate the performance of a digester with a zeolite (i.e., one set of digesters with external column (Ex-Zeo digesters), one set of digesters with inside zeolite bed (In-Zeo. Digester), and one set of digesters without adding zeolite (No-Zeo. Digesters)). All digesters were continuously stirred at 350 rpm and fed with swine manure and inoculum at a 1:1 ratio. The headspace of each digester purged with N₂ to ensure anaerobic conditions inside the digesters. Each group of experiments was performed in triplicate. The experiments were conducted in batch mode at 35 °C for 40 days. Swine manure inside the digester was introduced at a flow rate of 13 mL/min into the external zeolite column using a peristaltic pump after closing valves 3 and 4 (Figure 7). The feed was introduced to the zeolite column in up flow mode while filtering through the sintered borosilicate glass disc. This was operated for 40 minutes each day to ensure circulation of 500 mL of swine manure via the zeolite column. After circulation, swine manure was returned to the digester by the same peristaltic pump, by closing valves 1 and 2. This procedure was continued up to 8 days to ensure circulation of the full volume of digestate (4 L) via the external zeolite column.

3.5 Analytical Methods
3.5.1 Biogas:
The biogas was collected using a water displacement method (Chapters 5 and 7). The volume of biogas was measured using a 1 L syringe (Hamilton Super syringe). (Chapter 6) – The syringe was connected to the digester using a Luer lock termination through the 3-way stopcock valve, connected to the digester lid. Biogas was then collected by withdrawing the syringe plunger until the headspace pressure decreased to ambient pressure (Figure 3). For all digesters, the volume of gas was measured daily during the first week of digestion, and then every 2nd day in the second week, and once a week during the following weeks.

Figure 9: Volume of biogas measuring device for the digesters
On each sampling day, gas samples (20 ml) were collected into 12 mL pre-evacuated glass vials (738W, Exetainer®, Labco Ltd., Lampeter, Ceredigion, UK). The CH₄ concentration in the biogas was analysed using gas chromatography (7890A, Agilent Technologies, USA) with three detectors (flame ionization (FID), thermal conductivity (TCD) and electron capture (ECD)) to allow simultaneous determination of methane, carbon dioxide and nitrous oxide respectively. The TCD and FID were used for quantitating carbon dioxide and methane (first channel), with the ECD being used for nitrous oxide (second channel). Columns were HayeSep Q80/100 run isothermally at 60 degrees. Pure hydrogen gas was used as the carrier gas at a flow rate of 21 mL/min in constant pressure mode at 60 degrees.

3.5.2 Total solids and volatile solids
The total solids (TS) and volatile solids (VS) content were analysed according to Standard Method 2540 B and 2540 E (APHA, 2012).

First, a clean dish was heated to 103 to 105°C for 1 h. Then cleaned dishes were stored in a desiccator until needed. The dish was weighed immediately before use. An influent or effluent sample was then added into weighed dish and again weighed. The sample was then evaporated in the dish and dried to constant weight in an oven at 103 to 105°C. The increase in weight over that of the empty dish represents the total solids. In order for volatile solids to be measured, a clean evaporating dish was ignited at 550°C for 1 h in a muffle furnace before measuring total solids. The residue from the 105°C oven drying was cooled, weighed, and dried again at 550°C to drive off volatile solids in the sample.

\[
\% \text{ TS} = \frac{C - A}{B - A} \times 100
\]

A = Weight of dish (mg)

B = Weight of fresh sample + dish (mg)

C = Weight of dried residue + dish (mg)

\[
\% \text{ VS} = \frac{C - D}{C - A} \times 100
\]

A = Weight of dish (mg)

C = Weight of dried residue + dish (mg)

D = Weight of residue and dish after ignition (mg)
3.5.3 **Total Carbon and Nitrogen:**
Swine manure after the removal of the supernatant was oven-dried at 60°C for 48 h until constant weight was obtained. Oven-dried manure was then ground using a Tissue Lyser (TissueLyser II, QIAGEN, USA) and analysed by Isotope Ratio Mass Spectrometry. Total carbon and nitrogen was quantitated using a SerCon Hydra 20-20 isotope ratio mass spectrometer (Hydra 20–20, SerCon, Crewe, UK) coupled to a SerCon ANSA-GSL elemental analyser. The principle of analysis followed the Dumas combustion method.

3.5.4 **pH, EC and TDS**
The pH, EC and TDS of the influent and effluent were measured using a pH meter (labCHEM – CP cond-TDS-sal-pH-mV-Temp. Meter).

3.5.5 **Sample preparation for measuring VFA, sCOD, NH₄⁺, cations (Ca²⁺, Mg²⁺, Na⁺, K⁺) and Total P and S**
Soluble constituents were measured from the supernatant of a sample centrifuged at 6000 rpm for 10 minutes. The supernatant was then filtered through a 0.45 µm nylon syringe filter.

3.5.6 **Volatile fatty acids:**
The VFA samples were analysed using capillary gas chromatography (GC) with an Agilent Technologies 7890B Gas Chromatograph system including an auto sampler and auto injector. This technique used a wide-bore capillary column (SGE-Analytical Science BP21 column; 12 m x 0.53 mm internal diameter (i.d.) and 0.5 µm film thickness, SGE International, Ringwood, Victoria, Australia, part number 054473). The temperature for the flame ionization detector was set at 200°C with gas flow rates of hydrogen = 40 mL/min, air = 500 mL/min and nitrogen = 30 mL/min. Helium was used as carrier gas and 0.4% of 4-methyl valeric acid was used as an internal standard.

3.5.7 **Soluble chemical oxygen demand (sCOD)**
The sCOD concentration was measured using a HACH colorimetric digestion method (Method 8000, HACH Co., Loveland, Colorado, USA) (Hach, 1992)

3.5.8 **Ammonium**
Concentrations of NH₄⁺ in pre-prepared swine manure samples were determined with a segmented-flow analyser (Skalar, SAN⁺⁺).

3.5.9 **Cations (Ca²⁺, Mg²⁺, Na⁺, K⁺) and Total P and S**
Sodium (Na⁺), potassium (K⁺), Calcium (Ca²⁺), magnesium (Mg²⁺), phosphorous(P), sulphur (S) concentrations were analysed by inductively coupled plasma atomic emission spectroscopy (ICP-AES).
References


Chapter 4

Ammonium adsorption capacity of Australian natural and sodium zeolites at high strength ammonium solutions

This chapter based on the published paper entitled:

Ammonium removal from high-strength aqueous solutions by Australian zeolite
Ammonium removal from high-strength aqueous solutions by Australian zeolite

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ABSTRACT
Removal of ammonium nitrogen (NH₄⁺-N) particularly from sources which are highly rich in nitrogen is important for addressing environmental pollution. Zeolites, aluminoisolate minerals, are commonly used as commercial adsorbents and ion-exchange medium in number of commercial applications due to its high adsorption capacity of ammonium (NH₄⁺). However, detailed investigations on NH₄⁺ adsorption and ion exchange capacities of Australian natural zeolites are rare, particularly under higher NH₄⁺ concentrations in the medium. Therefore, this study was conducted to determine NH₄⁺ adsorption characteristics of Australian natural zeolites at high NH₄⁺ concentrations with and without other chemical compounds in an aqueous solution. Results showed that initial NH₄⁺ concentration, temperature, reaction time, and pH of the solution had significant effects on NH₄⁺ adsorption capacity of zeolite. Increased retention time and temperature generally had a positive impact on adsorption. Freundlich model fitted well with adsorption process of Australian natural zeolites; however, Langmuir model had best fitted for the adsorption process of sodium (Na⁺) treated zeolites. NaCl treatment increased the NH₄⁺ adsorption capacity of Australian zeolites by 25% at 1000 mg-N/L NH₄⁺ solution. The maximum adsorption capacity of both natural Australian zeolites and Na⁺ treated zeolites were estimated as 9.48 and 11.83 mg-N/g, respectively, which is larger than many zeolites from other sources. Compared to the NH₄⁺ only medium, presence of other competitive ions and acetic acid in the medium (resembling composition in digested swine manure slurries) reduced NH₄⁺ removal of natural and Na⁺ treated zeolites by 44% and 57%, respectively. This suggests detailed investigations are required to determine practically achievable NH₄⁺-N removal potential of zeolites for applications in complex mediums such as animal manure slurries.

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KEYWORDS
Ammonium adsorption; adsorption isotherms; nitrogen recovery; waste treatment; zeolite

Introduction
Nitrogen (N) is a key plant nutrient, but it is also one of the most important environmental pollutants, which is particularly responsible for eutrophication of water bodies around the world. A major source of N pollution is animal manures, which ammonium (NH₄⁺) comprise a large fraction of the manure N with concentrations between 6 and 186 g kg⁻¹(DM) and in liquid manure more than 50% of the N is typically in the form of NH₄⁺. Anaerobic digestion of animal manure produces over 1000 mg-N/L NH₄⁺ during the process. Therefore, NH₄⁺ removal from livestock manure can be considered as an efficient method to reduce environmental pollution caused by surplus of manure-N and the related nitrogenous emissions. A wide range of chemical, physical and biological treatments have been used to remove NH₄⁺. However, NH₄⁺ removal using zeolites as adsorbent are gaining in interest due to its relative simplicity of application and the low cost operation. Zeolites are aluminosilicate minerals with a highly regular microporous structure and high affinity to ion exchange in water and are used as adsorbents and specially ion-exchange media in number of industries and for waste water treatment. However, NH₄⁺ concentrations in those aqueous waste stream materials are generally much lower than that in anaerobically digested animal manure slurries.

There are approximately 50 different types of zeolites (clinoptilolite, erionite, chabazite, mordenite, heulandite, phillipsite, etc.) available in the world with varying chemical composition and different crystalline structures. Among them, clinoptilolite is the most abundant mineral type found in large mineable deposits. Clinoptilolite has a high selectivity for NH₄⁺, ion exchange and cation adsorption capacities and is being used in water and wastewater treatments. The factors influencing the NH₄⁺ recovery potential of zeolites are temperature, pH, reaction time, zeolite dosage, the concentration of other anions and cations in the wastewater and initial NH₄⁺ concentrations of the medium. However, characteristics of zeolites, particularly adsorption properties, from different sources vary significantly. Therefore, studies of material from each source would be required to determine the efficiency of specific zeolite materials and there may also be a need for pre-treatments before use. Several studies have indicated that natural Australian zeolites have a potential to remove NH₄⁺ from waste
water that had relatively low NH₄⁺ concentrations. However, there is little or no information about NH₄⁺ removal capacity of the zeolites particularly treating liquid waste containing high concentrations of NH₄⁺ such as in digested animal manure slurries. It has also been proposed that natural zeolites should be treated to enhance its NH₄⁺ adsorption properties, and shown that a high NH₄⁺ retention efficiency could be achieved after treatment with sodium chloride (NaCl) where cations adsorbed on to zeolites were exchanged with Na⁺, producing Na⁺ treated zeolites forms. In order to explore potential for further improvements in the NH₄⁺ retention capacity, there is a need to examine the adsorption characteristics of natural and sodium treated zeolites to identify the mechanisms of the enhanced NH₄⁺ adsorption.

Therefore, a detailed characterisation of natural Australian zeolites and its sodium treated form (hereafter termed as 'sodium zeolites') was carried out. The efficiency of the adsorption process depends on number of factors such as operating conditions, adsorbent material and composition of the feed. Therefore, NH₄⁺ adsorption properties and related kinetics of natural Australian and sodium zeolites were evaluated with aqueous solutions containing high concentrations of NH₄⁺ under different operational conditions through a series of laboratory batch adsorption tests. Laboratory and industrial scale NH₄⁺ removal processes are generally performed in columns. Therefore, a column study was also included to determine residence time and breakthrough capacity. Since the primary focus of the work reported here was to establish the maximum NH₄⁺ adsorption potential of Australian Zeolites, straight animal slurries were not included in the current study. However, a test medium with a major ionic composition similar to anaerobically digested swine manure slurry was included into the evaluations. Acetic acid was added into the test medium as generally acetic acid is the dominant volatile fatty acid (VFA) in digested manures, and NH₄⁺ ions are partially exchanged during the interaction with acetic acid in the presence of acetic acid in the media.

Materials and methods

Natural zeolites used in this study were obtained from Zeolite Australia Private Limited, Werribee Creek, NSW, Australia. The bulk commercial zeolites obtained were prepared by sieving (0.5–1 mm). The sieved zeolites were then washed three times with deionised water and dried for 48 h at 105 ± 1°C. A part of the washed zeolites was treated with NaCl to modify its physicochemical properties. The NaCl treatment was carried as a batch process that included mixing 5 g of processed natural zeolites with 100 mL of 2 M NaCl in a 250 mL conical flask and shaking in a thermostatic shaker at 35°C and 100 rpm for 24 h. After this treatment, the zeolite was washed three times with deionised water and dried at 105 ± 1°C for 24 h.

Properties of natural Australian and sodium zeolites were then examined. A series of batch adsorption tests was carried out to evaluate NH₄⁺ adsorption properties of natural Australian and sodium zeolites. A static leaching column experiment was conducted to evaluate the effect of flow rate on the NH₄⁺ adsorption by natural zeolites.

Batch adsorption experiments

The batch experiments evaluated the factors affecting NH₄⁺ adsorption potential of natural Australian zeolites and whether the NaCl treatment process had any impact on the NH₄⁺ potential of natural zeolites. Five different tests were conducted to investigate the effect of different factors on NH₄⁺ adsorption (Table 1); the factors evaluated were (a) initial NH₄⁺ concentration of the solution (test no. 1), (b) temperature (test no. 2), (c) pH of the medium (test no. 3), (d) mixing (test no. 4), and (e) the contact time (test no. 5). The effect of these parameters on NH₄⁺ retention by natural zeolites and sodium zeolites were tested. A separate batch test with a medium resembling major ionic composition similar to digested swine manure was conducted to evaluate the how the presence of other ions would affect NH₄⁺ adsorption by zeolites, in order get an understanding on how digested swine manure could be treated with zeolites (test no. 6). The medium contained NH₄⁺ – 1000 mg·L⁻¹, Na⁺ – 600 mg·L⁻¹, Potassium (K⁺) – 1000 mg·L⁻¹, Calcium (Ca²⁺) – 600 mg·L⁻¹, Magnesium (Mg²⁺) – 200 mg·L⁻¹ and Phosphate (PO₄³⁻) – 150 mg·L⁻¹. Acetic acid (CH₃COOH) – 0.3 mL·L⁻¹. These concentrations were chosen based on preliminary investigation on digested swine manure generated within the research lab.

Unless otherwise specifically mentioned, temperature and contact time between the solution and zeolites were respectively maintained at 35°C and 1440 min for all tests. However, experimental conditions specific for each of the six tests are shown in Table 1. In each test, 2 g of zeolite were placed in series of 250 mL flasks and each flask was added with 100 mL of appropriately diluted NH₄Cl solutions/mixture of ions made out of pre-prepared 1 M stock solutions. Analytical grade NH₄Cl, NaCl, KCl, MgCl₂, CaCl₂, K₂HPO₄ and 98% CH₃COOH were used to prepare

<table>
<thead>
<tr>
<th>Table 1. Experimental conditions tested in batch experiment.</th>
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<td>Test</td>
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<tr>
<td>1. Effect of initial NH₄⁺ concentrations (ppm)</td>
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<td>2. Effect of pH of the solution</td>
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<td>3. Effect of contact time (min)</td>
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<td>4. Effect of temperature (°C)</td>
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<td>5. Effect of agitation (rpm)</td>
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<td>6. Effect of ion mixture (similar to digested swine manure)</td>
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stock solutions with deionised water. The desired pH levels for the test evaluating the effect of pH were maintained by adding either 0.1 M HCl or 0.1 M NaOH solutions. The flasks containing the NH₄⁺ solutions/the test medium containing the ion mix and zeolites were placed in a circular motion incubation shakers (LABWIT Scientific ZWY-2403 Series incubator shaker) and were agitated at 100 rpm except for the test no. 5 (Table 1).

At the end of each test, all the test suspensions were filtered through 0.45 μm nylon filter and the filtrates were analysed for NH₄⁺. Adsorption was determined by the change in ion concentrations from the start to the end.

**Static leaching column study**

This study used only natural zeolites. Fifteen glass columns (55 mm internal diameter and 400 mm height) were used in the experiment (Table 2). Each column was filled with 150 g of zeolites (with particle size ranging from 0.5 mm to 1 mm). Twelve columns were fed (four different flow rates with triplicates) with NH₄Cl solution (1000 mg-N/L) at flow rates of 1.5 mL/min, 13 mL/min, 15 mL/min and 17 mL/min. Three columns were fed with the same test medium containing the ion mix (NH₄⁺, K⁺, Ca²⁺, Mg²⁺, PO₄³⁻ and acetic acid) used for the batch test at a flow rate of 1.5 mL/min. The composition of the test medium was same as for the previous tests. Solutions were fed from the top end of the column by using a peristaltic pump in the down flow mode at three different flow rates. The operational conditions of the experiment are shown in Table 2. Column flow experiment was conducted continuously up to 420 min with different flow rates of 1.5 mL/min, 13 mL/min, 15 mL/min and 17 mL/min. Samples from outlets were collected at different sampling time intervals (Table 2) to monitor the NH₄⁺ concentration.

**Analytical methods**

Phase composition identification of natural Australian and sodium zeolites were analysed by X-ray diffraction of the random samples. Diffraction data was collected by using a Bruker D8 Advance X-ray diffractometer (Bruker, Billerica, MA, USA) with Cu Kα radiation (1.54 Å). Data were collected between 5 and 120° 2θ, with a step size of 0.02° and a scan rate of 2 s per step. Phase identification was completed using Materials Data, Inc. Jade 9.3 software and Quantitative Rietveld analysis using Bruker Diffrac™-EVA software.

Chemical composition of zeolites samples were determined by energy dispersive X-ray spectroscopy (EDS, Oxford, Aztec 2.3 sp 1, Oxford Instruments, Oxfordshire, UK) and inductively coupled plasma mass spectrometry (Q-ICPMS, Agilent Technologies, Santa Clara, CA, USA). The surface morphology of the natural and sodium zeolites was compared by scanning electron microscope (SEM, Quanta A200F, FEI, Hillsboro, OR, USA). The Brunauer-Emmett-Teller (BET) specific surface area and pore diameter and pore volumes of the zeolite samples were determined by nitrogen gas adsorption analyser (Micrometrics TriStar 3000 V6.04 A, Micrometrics, Norcross, GA, USA).

The concentrations of NH₄⁺ in solutions were determined by segmented flow analyser (Skalar SAN⁺++, Skalar, Breda, The Netherlands). The pH of the solution was measured by a benchtop pH meter (labCHEM-CP, TPS division, Elekon Electronics Pty Ltd, QLD, Australia).

**Data analysis**

Adsortion capacity – adsorption isotherms

The amount of NH₄⁺ adsorbed per unit weight (q) of zeolite at equilibrium (mg-N/g) and the NH₄⁺ removal efficiency (%) were determined using the Eqs. (1) and (2) respectively:

$$ q = \frac{(C_0 - C_e)V}{m} $$

Removal efficiency (%) = \( \frac{(C_0 - C_e)}{C_0} \times 100\% \) (2)

where \( C_0 \) is initial NH₄⁺ concentration (mg-N/L) and \( C_e \) is final equilibrium concentration (mg-N/L). \( V \) is the volume of NH₄⁺ solution (L) and \( m \) is the zeolite mass (g) used in the test. Ammonium adsorption capacities of zeolites were studied with adsorption isotherms using the Langmuir and Freundlich models.[25,30]

The Freundlich model assumes that energetic distribution of sites of adsorbent is heterogeneous because of the diversity of adsorption sites or adsorbed ions.[27]

The Freundlich equation is represented by following Eq. (3):

$$ q = KC_v^n $$

where \( K \) and \( n \) are the Freundlich constants; \( K \) is the capacity coefficient that helps to provide a measure of relative NH₄⁺ adsorption capacity of the adsorbent used and \( n \) is the intensity parameter.

The linear form of Eq. (3) is given by Eq. (4)

$$ \log q = \log K + \frac{1}{n} \log C_v $$

Langmuir model hypothesises a monolayer adsorption on to the surface of adsorbent assuming that the adsorption sites and

### Table 2. Operational conditions of the column study.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Operational parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column height (mm)</td>
<td>400</td>
</tr>
<tr>
<td>Internal diameter (mm)</td>
<td>55</td>
</tr>
<tr>
<td>Bed height (mm)</td>
<td>65</td>
</tr>
<tr>
<td>Column volume (L)</td>
<td>9.95</td>
</tr>
<tr>
<td>Flow rate (mL/min)</td>
<td>1.5, 13, 15 and 17</td>
</tr>
<tr>
<td>Adsortent</td>
<td>Natural zeolite</td>
</tr>
<tr>
<td>Particle size (mm)</td>
<td>0.5–1</td>
</tr>
<tr>
<td>Weight of zeolite (g)</td>
<td>150</td>
</tr>
<tr>
<td>Bulk density (g/cm³)</td>
<td>1.14</td>
</tr>
<tr>
<td>Particle density (g/cm³)</td>
<td>2.08</td>
</tr>
<tr>
<td>Mode of Flow</td>
<td>Downflow (Gravity)</td>
</tr>
<tr>
<td>Sampling time (min)</td>
<td>15, 30, 60, 120, 180, 240, 300, 360 and 420</td>
</tr>
</tbody>
</table>
adsorption energies are homogeneous, without interactions between the adsorbed molecules.\textsuperscript{[27]}

The Langmuir isotherm is represented by Eq. (5).

\[
q = \frac{q_{\text{max}}(xK_iCe)}{1 + K_iCe}
\]

(5)

\(q\) is the adsorption capacity at the equilibrium (mg-N/g), \(q_{\text{max}}\) is the maximum adsorption capacity (mg-N/L), \(K_i\) is the Langmuir constant, \(Ce\) is the \(NH_4^+\) concentration in the solution at the equilibrium (mg-N/L). The linear form of Eq. (5) is given by Eq. (6).

\[
\frac{Ce}{q} = \frac{1}{K_iq_{\text{max}}} + \frac{1}{q_{\text{max}}Ce}
\]

(6)

**Breakthrough capacity**

Breakthrough capacity is the point that the exit concentration reaches an upper limit, which is a percentage (5–10%) of the inlet concentration.\textsuperscript{[21]}

**Residence time**

The residence time (\(T_r\)) was calculated by using Eq. (7).

\[
T_r = \frac{h}{Q_{\text{in}}}
\]

(7)

\(Q_{\text{in}}\) is the flow rate (cm$^3$/h$^{-1}$) and \(r\) is the radius of the column (cm). \(h\) is the height of the zeolite column (cm) and \(\varepsilon\) is the bed porosity.\textsuperscript{[28]} Bed porosity, \(\varepsilon\) was calculated using Eq. (8).

\[
\varepsilon = 1 - \frac{\rho_b}{\rho_d}
\]

(8)

**Results and discussions**

**Mineralogical composition of Australian zeolites**

Mineral species identified through XRD phase identification primarily were clinoptilolite, quartz, microcline and mordenite (Table 3). The mineralogical composition of Australian zeolites determined by XRD phase quantification was clinoptilolite - 54.3%; quartz - 23.69%; mordenite - 13.8% and microcline - 8.2%. Clinoptilolite contents of natural zeolites vary widely depending on the source, for example, Clinoptilolite contents of US Hector, Bulgarian and Hebei China zeolites were reported to be respectively 90%, 75% and 50%.\textsuperscript{[30, 31]} Zeolites which consist of clinoptilolite with heulandite and mordenite reported show high cation adsorption capacity.\textsuperscript{[32]} Cation exchange capacity (CEC) of natural zeolites from China which consists of minerals such as heulandite, illite and montmorillonite is 0.82 meq/g.\textsuperscript{[15]}

---

**Table 3. Phase quantification of natural and sodium zeolite by XRD.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Quartz</th>
<th>Clinoptilolite</th>
<th>Microcline</th>
<th>Mordenite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural zeolite</td>
<td>23.69</td>
<td>54.3</td>
<td>8.2</td>
<td>13.8</td>
</tr>
<tr>
<td>Sodium zeolite</td>
<td>24.3</td>
<td>55</td>
<td>8.3</td>
<td>13.4</td>
</tr>
</tbody>
</table>

---

**Figure 1.** XRD patterns of natural (a) and sodium (b) zeolites.
which is lower than the CEC of Australian natural zeolites (1.5 meq/g).[17]

**Effect of sodium treatment on natural zeolite**

Comparison of mineral compositions and crystalline structures, XRD pattern between the natural and sodium zeolites through the phase identification process demonstrated that the NaCl treatment process had no impact on the mineralogy and the crystal structure of the natural material (Table 3 and Fig. 1).

The ICP-MS analysis showed that the NaCl treatment process had increased the relative Na content of the zeolite by 164%, while Ca, Mg and K contents were decreased by 36%, 6% and 6%, respectively (Table 4) compared to the levels of those in natural zeolites. Results from the EDS analysis confirmed the above trend where relatively higher Na⁺ but lower K⁺, Ca²⁺ and Mg²⁺ ions in sodium zeolites than in natural zeolites (Table 5) indicating K⁺, Ca²⁺ and Mg²⁺ ions in exchanges surfaces of natural zeolites were replaced by Na⁺ with the NaCl treatment. However, this analysis did not show any changes in trace elemental composition of the zeolite due to the NaCl treatment process (Fig. 2).

The SEM images indicated that NaCl treatment significantly altered the surface properties and microstructure of natural zeolite (Figs. 3a and b). It appeared that particles of sodium zeolite were more regular, close-grained and well-defined edges compared to the natural zeolites in which had entirely disordered crystal orientation.

Further, total pore volume and specific surface area were respectively 10% and 40% larger in sodium zeolites. However, average pore diameter in sodium zeolites was reduced by 8% compared to that of natural Australian zeolites (Table 6). Chemical or thermal treatments tend to cause migration of cations within zeolite structure, thus altering cation locations and pore openings.[17] It appears that the pore sizes of the zeolite were affected by the number and nature of the cations closer to pore openings, and thus, cation exchange may vary the sizes of pore opening in zeolites.[17] It has been suggested that when Ca²⁺ and Mg²⁺ which are closer to pore opening, are exchanged by Na⁺, the site occupied by the Ca²⁺ or Mg²⁺ are replaced by two Na⁺. Then the pore diameter is reduced. Surface area and pore volume were increased by replacing 2 Na⁺ instead of one Ca²⁺ or Mg²⁺. These results confirmed that NaCl treatment is an effective way to improve surface properties of natural Australian zeolite.

**Effects of ammonium adsorption on chemical composition**

Chemical composition of both natural and sodium zeolites before and after NH₄⁺ adsorption under initial NH₄⁺ concentration of 1000 mg-N/L, was determined by EDS (Table 5). Results showed reduced levels of all major cations in both natural and sodium zeolites after saturating them with a high-strength NH₄⁺ solution, which indicates those cations in zeolites were replaced with NH₄⁺. According to the results, changes were 100%, 83%, 68% and 39% respectively for Na⁺, K⁺, Ca²⁺, Mg²⁺ before and after adsorption of natural Australian zeolites. These results confirm that metallic cations were replaced by NH₄⁺ during the process. Zeolites consisted of aluminosilicate tetrahedral structure and cations such as Na⁺, K⁺, Ca²⁺ and Mg²⁺ are weakly bound on to that aluminosilicate structure.[14] Therefore, Na⁺, K⁺, Ca²⁺, Mg²⁺ can be exchanged with cations when zeolites are suspended in an aqueous solutions with high NH₄⁺ concentrations.[10] The SEM images indicated that morphology of zeolite structure after NH₄⁺ adsorption significantly altered the surface properties and microstructure of both natural zeolites and sodium zeolites. Their structures converted to less porous and irregular (Figs. 3c and d). Ammonium ion can enter into the pores of zeolites.[14] Therefore, less porous structures of both natural and sodium zeolite were observed after the NH₄⁺ adsorption.

**Effect of contact time and NaCl treatment on adsorption capacity**

The batch test with 1000 mg-N/L solution showed that zeolites adsorbed NH₄⁺ at a faster rate initially, however, adsorption rates slowed down after the first hour and then reached an equilibrium level after 10 h (Fig. 4). The change in adsorption rate could be due to saturation of exchange surfaces of zeolites with NH₄⁺ with time and thus reducing further NH₄⁺ adsorption potential as reflected by the Gapon equation.[14] NaCl treatment process had effectively increased NH₄⁺ adsorption capacity of natural zeolites. For example, the total NH₄⁺ adsorbed into sodium zeolites were 25% greater.

### Table 4. Chemical composition of natural and sodium zeolite (ICP-MS).

<table>
<thead>
<tr>
<th>Elements</th>
<th>Natural zeolite (wt%)</th>
<th>Sodium zeolite (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na₂O</td>
<td>0.831</td>
<td>2.063</td>
</tr>
<tr>
<td>MgO</td>
<td>0.894</td>
<td>0.050</td>
</tr>
<tr>
<td>Al₂O₃</td>
<td>11.25</td>
<td>11.38</td>
</tr>
<tr>
<td>P₂O₅</td>
<td>0.028</td>
<td>0.030</td>
</tr>
<tr>
<td>K₂O</td>
<td>1.755</td>
<td>1.633</td>
</tr>
<tr>
<td>CaO</td>
<td>2.919</td>
<td>1.047</td>
</tr>
<tr>
<td>TiO₂</td>
<td>0.191</td>
<td>0.188</td>
</tr>
<tr>
<td>MnO</td>
<td>0.104</td>
<td>0.094</td>
</tr>
<tr>
<td>Fe₂O₃</td>
<td>1.133</td>
<td>1.050</td>
</tr>
<tr>
<td>Sr (ppm)</td>
<td>568,978</td>
<td>477,273</td>
</tr>
</tbody>
</table>

### Table 5. Chemical composition of natural and sodium zeolite (EDS).

<table>
<thead>
<tr>
<th>Before NH₄⁺ adsorption</th>
<th>After NH₄⁺ adsorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Element</td>
<td>Natural zeolite (Wt%)</td>
</tr>
<tr>
<td></td>
<td>58.12</td>
</tr>
<tr>
<td>Na</td>
<td>0.37</td>
</tr>
<tr>
<td>Mg</td>
<td>0.76</td>
</tr>
<tr>
<td>Al</td>
<td>7.89</td>
</tr>
<tr>
<td>Si</td>
<td>27.68</td>
</tr>
<tr>
<td>K</td>
<td>2.3</td>
</tr>
<tr>
<td>Ca</td>
<td>1.79</td>
</tr>
<tr>
<td>Others (Wt%)</td>
<td>1.29</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>
than the natural zeolites (Fig. 4). These findings are in agreement with previous research on zeolites from other sources [14, 19, 38]. One of the reasons for increased $\text{NH}_4^+$ adsorption by sodium zeolites could be explained by differences in exchange efficiency of cations associated with their valency. Efficiency of $\text{Mg}^{2+}$ and $\text{Ca}^{2+}$ replacement by $\text{NH}_4^+$ from an exchange surface is lower than the exchange between two monovalent cations. During the NaCl treatment process, monovalent $\text{Na}^+$ replaced $\text{Mg}^{2+}$ and $\text{Ca}^{2+}$ and apparently had transformed into a unified valency.

Figure 2. Trace elements changes in natural and sodium zeolites.

Figure 3. SEM images of zeolites: (a) natural zeolites, (b) sodium zeolites, (c) natural and (d) sodium zeolites after saturation of the material with $\text{NH}_4^+$ solutions.
structure. Therefore, the NaCl treatment had facilitated exchange of NH$_4^+$ with Na$^+$ from the exchange surfaces of zeolites. In addition, the replacement of Ca$^{2+}$ and Mg$^{2+}$ with Na$^+$ results in an increased surface area and pore volume of the sodium zeolites (Figs. 3a, b and Tables 5, 6) which therefore have a greater adsorption capacity than the natural Australian zeolites.

### Table 6. Surface area and pore size of zeolites by BET.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Natural zeolite</th>
<th>Sodium zeolite</th>
</tr>
</thead>
<tbody>
<tr>
<td>BET surface area</td>
<td>m$^2$/g</td>
<td>13.69</td>
<td>19.14</td>
</tr>
<tr>
<td>Total pore volume</td>
<td>cm$^3$/g</td>
<td>0.032</td>
<td>0.035</td>
</tr>
<tr>
<td>Average pore diameter</td>
<td>nm</td>
<td>17.45</td>
<td>15.06</td>
</tr>
</tbody>
</table>

**Effects of agitation and temperature on adsorption capacity**

The agitation of the mixture of zeolites and the NH$_4^+$ solution (1000 mg-N/L) appeared to facilitate a faster adsorption compared to the non-agitated mixture. Adsorption increased rapidly up to about 60 min from the start but thereafter adsorption was steady for about 600 min until it reached saturation. Agitation increased NH$_4^+$ adsorption from 5.7 mg to 7.5 mg-N/g at equilibrium when treating a 1000 mg-N/L solution at 35°C. Agitation reduces film boundary layer, and diffusive transportation to adsorption sites is reduced. After surface adsorption sites have been “saturated” this effect is of less importance and adsorption rate become similar for agitated and untreated batches. Adsorption was increased by increasing temperature in the solution from 15 to 35°C, because rate of
NH₄⁺ diffusion across the external boundary layer and into the internal pores of zeolites could be promoted as solution viscosity decreases at a higher temperature.

**Effect of initial ammonium concentration and pH on adsorption capacity**

Adsorption of NH₄⁺ increases at increasing concentration of NH₄⁺ in the solution treated as expected and predicted by the Gapon equation (Fig. 5). Increasing NH₄⁺ provides larger difference in concentration and a greater driving force for adsorption.

Ammonium absorption by both zeolites and NH₄⁺ adsorption by natural zeolites at both low (100 mg/L) and high (1000 mg/L) NH₄⁺ concentrations increased when increasing pH from 3 to 5 and declined by increasing pH further (Figs. 6 and 7). Absorption is related to NH₄⁺ to NH₃ equilibrium which at low pH will be entirely shifted to NH₃⁺ which is the species being adsorbed. Furthermore, at low pH the H⁺ cations will compete with adsorption of NH₄⁺, therefore adsorption increases when reducing pH from 10 and optimum adsorption at pH 5 reduces when lowering pH. The optimum pH will be different for different zeolites. For example, natural Turkish zeolites optimum NH₄⁺ adsorption is at pH 4, and the optimum is pH 6 for natural Chinese zeolites. The optimum values are the most important characteristics of the zeolites to provide optimal adsorption.

**Effect of competitive ions on NH₄⁺ adsorption by zeolites**

Ammonium adsorbed by natural and sodium zeolites in the presence of other ions (Na⁺, K⁺, Mg²⁺, Ca²⁺, PO₄³⁻ and acetic acid) respectively were 5.16 mg-N/L and 4.92-N/L mg indicating the presence of other competitive ions substantially reduces NH₄⁺ adsorption potential of zeolites. These reductions were 45% and 57% respectively for natural and sodium zeolites compared to the adsorption from a pure NH₄⁺ solution. Reductions in NH₄⁺ adsorption by zeolites due to the competitive effects of other ions are caused by different mechanisms. K⁺ has strong affinity with zeolites and therefore is more preferred than NH₄⁺ in ion exchange of zeolite. The presence of Ca²⁺, Mg²⁺ ions have negative effect on the NH₄⁺ adsorption capacity, and NH₄⁺ removal is most affected by the presence of Na⁺ ion in wastewaters. These cations compete with NH₄⁺ for the adsorption sites on zeolites and therefore decrease the exchangeable sites available for NH₄⁺ adsorption. Therefore, NH₄⁺ adsorption capacities of both zeolites reduce in the presence of other ions. Even though sodium zeolite adsorbed more NH₄⁺ than the natural zeolites at a NH₄⁺ concentration of 1000 mg-N/L, a reversed trend was observed when zeolites were treated with a cocktail of ions with acetic acids (similar to found in digested swine manure). This was most probably due to formation of sodium acetate, because when sodium zeolite is exposed to the acetic acid it forms sodium acetate with high Na⁺ content zeolite (e.g. sodium zeolite). The excess of Na⁺ ions in the sodium zeolite is mainly located in the zeolite supercavities and, so, are more able and easily exchanged than the Na⁺ ions presented in other cavities of the zeolite structures.

These results confirm that NH₄⁺ adsorption by zeolites from aqueous solutions with ion mix would be much lower than the adsorption from pure NH₄⁺ solutions due to the competitive effects from other ions in the mix. These results suggest that Australian zeolites do not have preferential affinity (ion selectivity) to NH₄⁺. This is important in developing practical applications with zeolites such as in nitrogen removal from animal manure slurries and other

![Figure 6. Effect of pH on NH₄⁺ removal efficiency of natural and sodium zeolites.](image)
similar waste streams. In addition to the ionic compounds that interfere with NH$_4^+$ adsorption by zeolites, other chemical compounds and organic colloids present in waste streams might potentially alter its NH$_4^+$ adsorption ability. Such issues warrant further research.

**Leaching column study**

The average residence time of the feeding solutions in the column filled with zeolite was calculated by using Eq. 7. Average residence time of flow rates of 1.5 mL/min, 13 mL/min, 15 mL/min and 17 mL/min are 46.12 min, 5.32 min, 4.61 min and 4.07 min, respectively. Breakthrough point was assessed to be when the concentration in outflow is 10% of the concentration in the inflow. The results indicated that the breakthrough was achieved within 120 min from the start for all the flow rates tested except for the lowest (1.5 mL/min) which did not reach the breakthrough point even within 430 min for both NH$_4^+$ solution and mixture of ion solution similar to the digested swine manure (Fig. 8). The lowest flow rate of 1.5 mL/min took more time to saturate the column. The breakthrough occurred when 20% of the NH$_4^+$ adsorption capacity of zeolites was consumed. The breakthrough was seen at a low saturation of adsorption sites, because the residence time is too short for the system to reach the equilibrium. However, mixture of ion solution reached the breakthrough point earlier than the NH$_4^+$ only solution at the same flow rate of 1.5 mL/min (Fig 8). This could happen due to not only NH$_4^+$, but other competitive ions were also available for the saturation of adsorption sites in zeolites.
Ammonium adsorption mechanism onto zeolite

The Freundlich model was fitting better than Langmuir model for natural zeolites, and the Langmuir model fixed better with sodium zeolites than the Freundlich model (Table 7, Figs. 9 and 10). The results were not different from studies of removal of NH$_4^+$ by Australian natural zeolites at low NH$_4^+$ concentrations. These results indicated that natural zeolites consist of heterogeneous surface with non-uniform distribution of adsorption sites, whereas sodium zeolites have monolayer adsorption onto its surface with uniform distribution of energetic adsorption sites. Due to the NaCl treatment process, conversion of zeolite sites into homogeneous surface may be the reason for better fitting of adsorption data from sodium zeolites to the Langmuir model. The 1/n constant for both natural and sodium zeolites were less than 1 which is a measure of exchange intensity, and 1/n values below 1 represent a high NH$_4^+$
adsorption intensity. The maximum adsorption capacity of both natural Australian zeolites and sodium zeolites were estimated as 9.48 and 11.83 mg-N/g, respectively, at initial NH4\(^+\) concentrations of 10–1000 mg-N/L. To compare with natural Australian zeolites over a range of initial NH4\(^+\) concentrations, q\(_{\text{max}}\) value was 6.30 mg-N/g at initial NH4\(^+\) concentrations of 5–120 mg-N/L, and 4.5 mg/g at initial NH4\(^+\) concentrations of 25–50 mg-N/L. Under the high NH4\(^+\) concentrations, q\(_{\text{max}}\) of Turkish zeolites were 25.77 mg-N/g at initial NH4\(^+\) concentration 5–900 mg-N/L, and q\(_{\text{max}}\) of Chinese zeolites were 143 mg-N/g at initial NH4\(^+\) concentration 10–4000 mg-N/L.

Conclusion

The treatment of zeolites with NaCl had modified its surface morphology, enhanced mass transfer rate and thereby adsorption characteristics. Therefore, NaCl modification effectively increased the NH4\(^+\) adsorption capacity over natural zeolite across all levels of NH4\(^+\) concentrations tested. Ammonium adsorption by zeolites depended on the contact time, temperature, mixing, initial NH4\(^+\) concentration and the pH of the solution. Increasing initial NH4\(^+\) concentration of the solution caused increase in NH4\(^+\) adsorption. Ammonium adsorption was slightly higher at pH 5 in lower NH4\(^+\) concentrations whereas it showed remarkable difference at high NH4\(^+\) concentration. However, NH4\(^+\) adsorption capacity of Australian zeolites was comparatively lower than Chinese and Turkish zeolites at high NH4\(^+\) concentration solutions. The adsorption isotherm results indicated that Freundlich model described adsorption properties of natural Australian zeolites better than Langmuir model whereas Langmuir model provide better description of adsorption properties of sodium zeolites than Freundlich model. In the column test, NH4\(^+\) removal efficiency was increased with decreasing flow rates since average residence time increased at lower flow rates. This investigation has potential applications in reducing N content and also nutrient recovery from waste treatments systems which contain very high levels of NH4\(^+\). However, NH4\(^+\) adsorption by both Australian natural and sodium zeolites was substantially reduced (44% and 57%, respectively) when the zeolites were treated with a cocktail of ions (including NH4\(^+\) generally present in digested swine manure slurry. The purpose of present research is to develop technologies to recover NH4\(^+\)-N from aqueous wastes streams, for example animal slurries, using ion exchange materials such as zeolites. Those slurries are complex in terms of physico-chemical properties. Therefore these issues need to be further investigated for developing practical applications with Australian and other zeolites to remove NH4\(^+\)-N from aqueous waste streams.

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References

Chapter 5

Performance of mesophilic anaerobic digestion of swine manure under different hydraulic retention times (HRTs)

This chapter is based on a manuscript which was submitted entitled:

The Effect of hydraulic retention times (HRTs) on mesophilic anaerobic digestion of swine manure. Submitted to Journal Water Science and Technology.
Effect of hydraulic retention times (HRTs) on mesophilic anaerobic digestion of swine manure

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Abstract:
The performance of mesophilic anaerobic digestion of swine manure in continuous stirred tank reactors was evaluated at different hydraulic retention times (HRT). The selected HRTs were 8, 12 and 16 days. Lab scale digesters (5L) were used during the experiment. Biological oxygen demand (BOD₅), pH, volatile fatty acids and biogas production were examined every day for 30 days. The highest BOD₅ removal efficiency (57%) was observed under 16 day HRT, while the lowest (43%) was reported at 8 days HRT. Iso-butyric and iso-valeric levels declined to 0.2 mmol/L and 0.3 mmol, respectively, at steady state 16 days and 12 days HRT. Iso-butyric and iso-valeric levels at 8 days HRT was high (0.8 mmol/L) throughout the experimental period. Total CH₄ volumes produced were 294 ± 7.69 L / Kg (VS), 263 ± 2.03 L /Kg (VS) and 199 ± 4.55 L /Kg (VS) (± SD) respectively under 16, 12, and 8 days HRT. Results indicated that the performance of anaerobic digestion of swine manure was at an optimum level under 16 days of HRT and digestion process appeared to be unstable at 8 d HRTs.

Keywords: anaerobic digestion, biogas, HRT, methane, swine manure

1. Introduction
There are many physical and chemical parameters associated with the anaerobic digestion process. Organic loading rate (OLR) and hydraulic retention time (HRT) are critical operating parameters in anaerobic digesters (Chamy et al., 2011). The HRT is the time the biomass is retained in the digester. It is one of the most important variables in anaerobic digestion (Karim et al., 2005). The minimum optimal HRT is related to the type of substrate to be digested, the temperature of the
digester, design etc. HRT is relatively low for optimal digestion of swine manure as it contains high amounts of easily digestible organic fraction (Burton and Turner, 2003). The recommended HRT for mesophilic (temperature range 20 – 40 °C) digestion of swine manure is in the range of 10-20 days (Burton and Turner, 2003, Sakar et al., 2009). However, prediction of the outcome of HRT on anaerobic treatment system is impossible since it depends on the type of feed and its characteristics, reactor configuration, OLR, type of biomass and method used to evaluate the performance (Chelliapan et al., 2011). Managers of biogas plants, therefore, have to know the economic optimal retention time for running the plant with a specific biomass.

Meantime identify the process performance is important to overcome the problem such as C:N ratio imbalance, ammonia inhibition which associated with anaerobic digestion of swine manure. Therefore, in this study, optimum HRT for anaerobic digestion of swine manure in mesophilic semi-continuously fed continuous flow stirred-tank reactor (CSTR) was determined. Process performance was examined using parameters of Volatile fatty acids (VFAs), Biological oxygen demand (BOD), Ammonium (NH₄⁺) and pH at different HRTs.

2. Materials and methods

2.1 Design of anaerobic digesters

A lab-scale anaerobic digester equipped with three sampling ports and a gas valve was utilized. The total volume and the working volume of the digester were 5 L and 4L, respectively (Figure 1).

![Figure 1: Schematic diagram of the lab-scale anaerobic digester](image)
2.2 Swine manure and Inoculum

Swine manure was collected from Berrybank piggery; and inoculum was collected from anaerobic digester at Berrybank piggery Ballarat, Victoria, Australia with a standing swine population of 20,000 pigs. Table 1 shows the chemical properties of swine manure and inoculum.

| Table 1: Characteristics of swine manure and inoculum |
|---------------------------------|--------|--------|
| Parameter                       | Swine manure | Inoculum |
| Total Solid (%)                 | 2.04% | 1.03% |
| Volatile solid (%)              | 0.65% | 0.67% |
| pH                             | 6.98  | 7.40  |
| TDS (mg/L)                     | 3.81  | 4.83  |
| EC (µS/cm)                     | 6.33  | 8.45  |
| NH₄⁺ (mg/L)                    | 420   | 1175  |
| BOD (mg/L)                     | 2128.50 | 1312.50 |
| COD (mg/L)                     | 3301  | 942.50 |

2.3 Operation conditions

A mixture (4L) of substrate and inoculum, at 3:1 ratio was filled into the digesters and was continuously stirred at 350 rpm, which initially was managed in batch mode. The digesters were converted from batch mode to continuous flow mode 7 days after adding substrate and inoculum. Then all digesters were fed daily with swine manure after removing the same amount of digested manure from the digesters. Each reactor was operated at different HRT at 8 d HRT, 12 d HRT, and 16 d HRT at temperature 35°C. OLR was varied as 0.7 Kg(VS) m⁻³ d⁻¹, 0.47 Kg (VS) m⁻³ d⁻¹ and 0.35 Kg(VS) m⁻³ d⁻¹ for 8 days HRT, 12 days HRT, and 16 days HRT, respectively. The amount of biogas produced by digesters was measured daily by water displacement method.

2.4 Data collection and analysis

A sample of 20 mL was collected daily from each digester to measure total solids (TS), volatile solids (VS), pH, NH₄⁺, BOD₅ and volatile fatty acids.

The TS, VS and BOD₅ were measured according to standard methods (APHA, 2012). The concentrations of NH₄⁺ in solutions were determined by the segmented Auto analyser (Skalar SAN⁺⁺, Skalar, Breda, The Netherlands). The pH, EC and TDS of the solution were measured by a pH meter (labCHEM – CP cond-TDS-sal-pH-mV-Temp. meter TPS division, Elexon Electronics Pty Ltd., QLD, Australia).
Volatile fatty acids (VFA) were analysed by capillary gas chromatography (Agilent Technologies 7890B Agilent Technologies, Wilmington, USA) with an autosampler and autoinjector. The technique used a wide-bore capillary column (SGE-Analytical Science BP21 column; 12 m x 0.53 mm internal diameter (i.d.) and 0.5 μm film thickness, SGE International, Ringwood, Victoria, Australia, part number 054473). The temperature for the flame ionisation detector was set at 200°C with gas flow rates of hydrogen = 40 mL/min, air = 500 mL/min and nitrogen = 30 mL/min. Helium was used as carrier gas, and 0.4% of 4- methyl valeric acid was used as internal standard. The amount of gas produced by digesters were analysed by water displacement method, and the CH₄ percentage was measured by gas chromatography (GC) (7890A, Agilent Technologies, Wilmington, USA) with three detectors (flame ionisation, thermal conductivity and electron capture) to allow simultaneous determination of methane, carbon dioxide, and nitrous oxide. The TCD and FID quantitate carbon dioxide and methane (first channel), with the ECD being used for nitrous oxide (second channel). The GC columns were HayeSep Q80/100 run isothermally at 60 degrees. Pure hydrogen gas was used as the carrier gas at a flow rate of 21 mL/min in constant pressure mode at 60 degrees.

Statistical analysis of results was determined by one-way analysis of variance (ANOVA).

3. Results and Discussion

Parameters such as temperature, pH, OLR, HRT and influent (swine manure) characteristics are the main parameters related anaerobic digester operating conditions. The biogas, CH₄ production, pH, NH₄⁺ and ammonia (NH₃) concentration are important process indicators to evaluate anaerobic digestion process.

3.1 Biogas and CH₄ production:
The 16 days of HRT has maintained the highest cumulative biogas and CH₄ production throughout the 30 days of semi continuous anaerobic digestion process while the lowest was recorded from 8 HRT (Figure 2 and 3). Average CH₄ concentration at steady state was 53% at 16 days of HRT while 44% and 50% was reported in 8 days and 12 days of HRT, respectively, and the production was significantly different (p < 0.01). Digester with 8 days HRT shows a long lag phase compare to the digesters at 12 days and 16 days HRT. Lower retention times are insufficient for a stable digestion because of incomplete degradation of compounds, especially of the lipids and VFA concentrations are increasing at low HRTs due to a washout of methanogenic bacteria (Appels et al., 2008).
Figure 2: Cumulative biogas production at batch (up to 7th days) and CSTR (from 8th to 30th days) mode digesters. (Vertical bars represent standard errors, n = 3)

Figure 3: Cumulative CH₄ production at batch (up to a 7th day) and CSTR (from 7th to 30th days) mode digesters. (Vertical bars represent standard errors, n = 3)
Rodríguez Andara and Lomas Esteban (1999) found that the CH\textsubscript{4} content of anaerobic digestion process of swine manure with 60 days of HRT at 35\(^0\)C was 50%. Kim et al. (2006) reported that a high accumulated CH\textsubscript{4} yield could be obtained in a measophilic digester with a 12 days HRT over 10 days HRT in terms of food waste anaerobic digestion. The low CH\textsubscript{4} production at 8 days HRT in this study and 10 days in the study of Kim et al. (2006) was probably due a too short time for the microbial community to grow and transform the organic matter i.e. was out of microorganisms that will cause reduced biogas production (Uma Rani et al., 2012). At longer HRT, wash out is avoided and most of the organic matter is digested and biogas production is high (Ranade et al., 1989). However, the most favourable loading rates and retention time for anaerobic degradation depends on the feature of the substrate and the required effectiveness of the overall process (Gopikumar et al., 2016).

3.2 pH

The pH of digestate is affected by buffering capacity of the anaerobic digestion medium and is an indicator of the stability of the system (Mata-Alvarez et al., 2000). Anaerobic digestion process requires a consortium of microorganisms, mainly fermentative micro-organisms and methanogens involve in the process. Methanogens are the most sensitive of the microorganisms to low pH (Rittmann and McCarty, 2001). The Methanogens are most efficient at a pH range in between 6.6-7.6 for their optimum growth (Rittmann and McCarty, 2001).

Figure 4: The pH variation inside the digester at different HRT during the digestion period. (Vertical bars represent standard errors, n = 3)
The pH levels, in general, varied the desired range from 7.13 to 7.66 at all HRTs. However, pH at 16 HRT was higher than at the 8 days and 12 days HRTs digesters (Figure 4). The fluctuations observed in the present study were well within the methanogenic range, which proved that the digester could maintain the pH within a neutral range.

3.3 BOD₅ removal:
BOD₅ is an indicator organic matter transformation. The highest BOD₅ removal efficiency (57%) was observed under 16 days of HRT, while the values were 43% and 55%, respectively under HRT 8 and 12 days. The observed BOD removal, in general, was low in all treatments probably due to low hydrolysis rate of swine manure (Bonmati et al., 2001).

3.4 Volatile Fatty Acids (VFA):
The VFAs are the useful indicators of anaerobic digestion process performance and stability or imbalance (Banu et al., 2007, Ahring, 1995). The presence of high concentrations of VFAs has been reported to be inhibitory to methanogenic activity.

The level of all VFAs started to decrease after 15th days (Figure 5). After 25th day’s acetic acid, butyric acid and valeric acids concentrations were closer to zero at all 3 different HRT. The degradation of acetate was efficient after 13 days of digestion in all digesters and for acetic acid to propionic acid ratio is < 2. However, towards the end of the digestion period (after 20th day), the concentration of all fatty acids except propionic acids were low at 12 days and 16 days HRT digesters while concentrations of isoforms of butyric acid and valeric acids were comparatively higher at 8 days HRT digesters. Wagner et al. (2014) found methanogens can continue CH₄ production using propionate and acetate as the only carbon sources when the other VFAs could not be detected during the anaerobic digestion period.

According to the Hill (1982), the propionate/acetate ratio is the best process indicator, and the propionate/acetate ratio should be below 1.4 for a steady process. However, in this study, the propionate/acetate ratio increased slowly and became greater than one after approximately 16 days in 12 days HRT digesters, 18 days in 8 days HRT digesters and 21 days in 16 days HRT digesters. Wagner et al. (2014) suggested that acetate: propionate ratios ≥ 2 (i.e. 2.0, 3.0, 4.0) resulted in a faster CH₄ production and VFAs decomposition compared to acetate: propionate ratio ≤ 1 (Wagner et al., 2014). However, similarly to the results obtained from this study, Ahring et al. (1995) found the propionate: acetate ratio was > 1 was acceptable, and after the perturbation (altering operating
conditions such as HRT, OLR or the process temperature), the ratio decreased from approximately 2 to 0.3. Therefore, Ahring et al. (1995) suggested, that the propionate/acetate ratio was not important in determining process instability. Butyrate and isobutyrate fatty acids were found to be mainly good indicators (Ahring et al., 1995). However, In this study, there is no any difference of butyric concentration among 3 digesters. Hill and Holmberg (1988) also found iso-butyric and isovaleric acids levels in the digester are the best indicators for the monitoring the anaerobic digestion process. In this study, iso-butyric levels started to decline from 0.6-0.8mmols/L from 15-16 days onwards under both 12 days, and 16 days HRT while 8 days HRT continued to maintain the same at a higher level (0.7mmols/l) (Figure 5).

**Figure 5:** VFA concentrations at different HRT during the digestion period (Vertical bars represent standard errors, n = 3)
Iso-valeric levels showed the same trend as iso-butyric. At steady state on the usual process of anaerobic digestion in full-scale biogas plants, the iso-butyric level may be below detection level, and the iso-valeric level below 0.25 mM (Nielsen et al., 2007). Therefore, this study proves that iso-butyric and iso-valeric acid levels are good indicators of digester performance and the 12 days HRTs and 16 days HRTs digesters are stable than 8 HRT digesters as biogas production was also higher in 12 days and 16 days HRTs digesters than the 8 days HRT digesters.

3.5 Total ammoniacal nitrogen (TAN) concentration

Ammonium is formed in the anaerobic digestion process as a reduction product of the microbially mediated biochemical breakdown of proteins or non-protein nitrogenous compounds (Hobson and Wheatley, 1993). In the aqueous solution, total ammoniacal nitrogen (TAN) exists in two principal forms; NH$_4^+$ ion and unionised ammonia or free ammonia (NH$_3$) in a pH-dependent equilibrium state (Manyi-Loh et al., 2013).

TAN concentration increased from 489 mg/L to approximately 750mg/L in all digesters during the 7 d batch digestion (Figure 6). After 7 days digesters were converted to flow mode, and TAN levels were constant in all digesters. Comparatively, 16 HRT digesters show the highest NH$_4^+$ level and 8 HRT digesters show the lowest TAN level. TAN concentration in anaerobic digestion process increased with increasing HRT, because of more degradation at higher HRTs compared to lower HRTs and is inhibitory to anaerobic digestion (Garcia and Angenet, 2009, Nakakubo et al., 2008, Hansen et al., 1998). Ammonia toxicity is influenced by the operating pH and temperature (Garcia and Angenet, 2009). The threshold values at which CH$_4$ inhibition begin ranges from NH$_3$ concentration 80 mg/L (Koster and Lettinga, 1984) to 150 mg/L (Braun et al., 1981). The NH$_4^+$ concentrations at more than about 1500 mg NH$_4^+$-N / L at pH > 7.4 may inhibit biogas production and NH$_4^+$ concentration > 3000mg NH$_4^+$– N/L inhibits the digestion process (Van Velsen, 1979). The highest recorded TAN concentration for reactor one was 874 mg/L at pH 7.56. The TAN concentration and NH$_3$ production are lower than 45mg/L during whole digestion period in different HRT digesters, apart from being present in the slurry, also accumulate during the breakdown of proteins from the manure that has to be digested (Yenigün and Demirel, 2013).

The C: N ratio of available swine manure was 8.6, which was lower than optimum values which is between 50:1 and 10:1 (Sommer et al., 2013). The bacteria responsible for the anaerobic process required both C and N elements. However, they consume carbon roughly 30 – 35 times faster than nitrogen (Khoiyangbam et al., 2011). Therefore, the anaerobic digestion process stops when the carbon has been consumed, more NH$_4^+$ remain in the digestate. This may be the reason for lower
CH$_4$ production. Moreover, another reason is available swine manure was much diluted, and the TS % is 2%.

![TAN concentration inside the digesters at different HRT during the 30 days digestion period. (Vertical bars represent standard errors, n = 3)](image)

**Figure 6:** TAN concentration inside the digesters at different HRT during the 30 days digestion period. (Vertical bars represent standard errors, n = 3)

### 4. Conclusion

The HRT may vary depending on various factors; however, 16 days appear to be the optimum HRT for mesophilic anaerobic digestion of swine manure used in this study than 8 days and 12 days HRT. However, digestion stability reduced at 8 days HRT and isobutyric and iso-valeric acids levels were significant measured. The concentration of NH$_4^+$ - N generated in this experiment was not an inhibitory level but low C: N ratio might be the reason for comparatively lower CH$_4$ production in all digesters. It is proposed that iso-butyric and iso-valeric acid levels in the digester are the best indicators for the monitoring the anaerobic digestion process.
References


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Chapter 6

Effects of co-mixing of natural and sodium zeolites with digestive substrate on anaerobic digestion process of swine manure

This chapter is based on three submitted manuscripts entitled:

Chapter 6.1:

Chapter 6:2:

Chapter 6:3
Chapter 6:1

Effect of Australian zeolite on methane production and ammonium removal during anaerobic digestion of swine manure

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Abstract:

Anaerobic digestion is one of the most effective methods for treating swine manure by converting it into green energy, and efficiently reducing methane (CH₄) emission to the atmosphere. Low C/N ratio of swine manure and the production of high levels of total ammoniacal nitrogen (TAN) during acidogenesis due to the high N contents of swine manure considerably reduce CH₄ yield. The reduction of N during anaerobic digestion by the addition of zeolite improves CH₄ production and reduces potential environmental threats associated with ammonia (NH₃) emissions from anaerobic digestion of swine manure. The main objective of this study was to determine the optimum Australian zeolite dose that produces maximum NH₄⁺ recovery at optimum CH₄ production. In laboratory experiments, swine manure was treated with natural and sodium zeolites at 0, 10, 40, 70, 100 mg/L and digested anaerobically for 60 days. Natural zeolite at a dose of 40g/L resulted in the largest increase (29%) in total CH₄ yield from swine manure compared to the nil zeolite treatments. The lag phase of digestion was decreased with increasing zeolite doses up to 100g/L. Natural and sodium zeolites at a dose of 100g/L reduced NH₄⁺ by 50% and 52% in the media, respectively, compared to the nil zeolite treatments. However, the increases in CH₄ yield of natural and sodium zeolites at a dose of 100g/L were only 10% and 12%, respectively. Variations of cation concentrations in the digesters caused by adding zeolite at different rates might be a reason for variation of biogas and CH₄ productions from various rates of zeolites digesters.

Keywords: Ammonium, Anaerobic digestion, Methane, Swine manure, Zeolite
Highlights

- The additives of Australian zeolite in anaerobic digestion of swine manure were studied.
- Australian zeolite addition increased methane production significantly.
- Australian zeolite removed a substantial amount of ammonium from the anaerobic digestion medium.
- The anaerobic digestion process was optimized by Australian zeolite.

1. Introduction

Globally, livestock production accounts for 64% of ammonia (NH$_3$) emissions and 18% of the anthropogenic emissions of cumulated greenhouse gas (GHG) [1]. Swine farming contributes about 9% of livestock-related emissions [2]. In the swine industry, about 50% of NH$_3$ emits from slurry storage and pig housing. The other 50% emits from land application of slurry [3]. The significant NH$_3$ emissions contribute to atmospheric nitrogen (N) load, and in some parts of the world is of the same order of magnitude as emissions of nitrous oxide (N$_2$O) [4]. Ammonia is a pollutant gas that increases the formation of fine particulates in the atmosphere and plays a critical role in the acidification of ecosystems and eutrophication [5].

The swine industry around the world is under pressure to reduce its environmental impact whilst maintaining its profitability. In terms of GHG mitigation, NH$_3$ and NO emissions cannot be considered independently from CH$_4$ emissions and energy production related to manure management [6].

Anaerobic digestion is the environmentally sustainable best management practice for handling livestock manure in terms of reducing CH$_4$ emission and increasing energy production [7]. About 80% of the organic N in the feedstock of anaerobic digesters is transformed to total ammoniacal N (TAN = NH$_3$(l) + NH$_4^+$) [8]. Processes are required to remove nitrogen (N) to minimise NH$_3$ emission to the atmosphere. Elevated concentrations of TAN can inhibit anaerobic digestion processes, especially in its non-ionized free NH$_3$(l) form [9]. The C/N ratio for swine manure is around 6 to 8, depending upon their growth stages [10]. Low C/N ratio of swine manure, due to high nitrogen content results in lower biogas production [11]. Small biogas production rates are another limitation to the economic feasibility of anaerobic digestion of swine manure [12].

A number of different technologies have been tested to recover TAN, particularly from wastewater; these include ammonia stripping [13], chemical precipitation as struvite [14], and the addition of ion exchange/adsorption materials such as zeolites [15]. Zeolite addition to digestates reduced NH$_4^+$ inhibition because of its high adsorptive capacity and NH$_4^+$ selectivity, resulting in enhanced
biogas production during the anaerobic digestion [16]. Subsequently NH$_4^+$ - zeolites can be used as slow release fertiliser [17]. However, past research primarily focused on mixing zeolites into the digestive feedstock with the aim of enhancing only CH$_4$ production rather than maximising both TAN and CH$_4$ recovery. The effects of zeolite materials on CH$_4$ production and TAN vary with the types of zeolite materials, and their characteristics need to be investigated individually [18], as the most suitable zeolite material, and its application rates for enhancing biogas production, are not readily predictable [19]. The effects of Australian zeolite on CH$_4$ production is unknown.

The main objective of this study is to determine the optimum zeolite dose rate of Australian zeolite that produces maximum TAN recovery at maximum CH$_4$ production. Both Australian natural zeolite and sodium chloride modified zeolite (sodium zeolite) were used in this study.

2. Material and Methods

2.1. Inoculum, swine manure, and zeolite

The anaerobic digestion inoculum was collected from anaerobic digester at Berrybank Farm Piggery, at Windermere, Victoria, Australia, which has a standing swine population of 20,000 pigs. Swine manure was collected from the Piggery at The University of Melbourne. Table 1 shows chemical properties of swine manure and inoculum. Australian natural zeolite was obtained from Zeolite Australia Pty Ltd, Werris Creek, NSW, Australia. Sodium zeolite was prepared as described in [20].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Swine manure</th>
<th>Inoculum</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS%</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>VS%</td>
<td>69</td>
<td>67</td>
</tr>
<tr>
<td>pH</td>
<td>6.73</td>
<td>7.39</td>
</tr>
<tr>
<td>Alkalinity (mg/L)</td>
<td>2500</td>
<td>4033</td>
</tr>
<tr>
<td>Solu. COD (mg/L)</td>
<td>14725</td>
<td>1734</td>
</tr>
<tr>
<td>BOD (mg/L)</td>
<td>1991</td>
<td>204</td>
</tr>
<tr>
<td>NH$_4$ - N (mg/L)</td>
<td>664</td>
<td>1298</td>
</tr>
<tr>
<td>N%</td>
<td>2.48</td>
<td>2.97</td>
</tr>
<tr>
<td>C%</td>
<td>33.54</td>
<td>31.76</td>
</tr>
</tbody>
</table>
2.2. Anaerobic digestion experiment

Biochemical methane potential (BMP) tests were used to evaluate the enhancement in ultimate biogas/ CH\textsubscript{4} production throughout the digestion process. The BMP test used was that employed by Moller et al. (2004)[21]. This mesophilic anaerobic digestion experiment was conducted as a batch trial with different rates of Australian natural and sodium zeolites. The experiment was undertaken in thirty 500 mL (400 mL, working volume) glass bottles as batch type digesters. Each digester consisted of 2 stopcock valves, one for collecting gas and one for purging N\textsubscript{2} gas at the beginning of the digestion period. The inoculum and swine manure substrates were added on a volatile solids (VS) basis at a ratio of 1:1 in all digesters. The inoculum was incubated for 14 days at 37\textdegree{}C so that all easily digestible organic matter was removed before starting the experiment. The digesters were filled with 450 mL inoculum-substrate homogenised mixture. Zeolite was added at rates of 0, 10, 40, 70, 100 g/ L of swine manure. The experiment was conducted in triplicate. The maximum zeolite rate was designed by considering the maximum amount of TAN that could be removed from the digester without hindering the digestion process.

After mixing swine manure and inoculum with natural and sodium zeolites at different rates, all digesters were sealed tightly with screw-cap lids. The headspaces of all digesters were flushed with N\textsubscript{2} gas to create an anaerobic environment inside the digesters. The digesters were kept in an incubator at 37\textdegree{}C to maintain mesophilic conditions. Each digester was manually mixed once a day.

2.3. Analytical methods

The yield of biogas was measured daily by sampling using a 1 L syringe (Hamilton Super Syringe). Concentrations of gases in biogas were analysed by gas chromatography (7890A, Agilent Technologies, Wilmington, USA) with three detectors (flame ionisation, thermal conductivity and electron capture) to allow simultaneous determination of methane, carbon dioxide, and nitrous oxide. The TCD and FID quantitate carbon dioxide and methane (first channel), with the ECD being used for nitrous oxide (second channel). The GC columns were HayeSep Q80/100 run isothermally at 60 degrees. Pure hydrogen gas was used as the carrier gas at a flow rate of 21 mL/min in constant pressure mode at 60 degrees.

The NH\textsubscript{4}\textsuperscript{+} concentrations of digestate at the end of the digestion period were analysed with a segmented autoanalyser (Skalar SAN\textsuperscript{++}, Skalar, Breda, The Netherlands). Total solids (TS) and VS were measured according to the method described by APHA [22]. The pH of the solution was measured by a pH meter (labCHEM-CP. TPS division, Elexon Electronics Pty Ltd., QLD, Australia).
Volatile fatty acids (VFA) were analysed by capillary gas chromatography (Agilent Technologies 7890B Agilent Technologies, Wilmington, USA) with an autosampler and autoinjector. The technique used a wide-bore capillary column (SGE-Analytical Science BP21 column; 12 m x 0.53 mm internal diameter (i.d.) and 0.5 μm film thickness, SGE International, Ringwood, Victoria, Australia, part number 054473). The temperature for the flame ionisation detector was set at 200°C with gas flow rates of hydrogen = 40 mL/min, air = 500 mL/min and nitrogen = 30 mL/min. Helium was used as carrier gas, and 0.4% of 4- methyl valeric acid was used as internal standard.

2.4. Data Analysis

2.4.1. Calculating the ultimate CH₄ potential at standard temperature and pressure (STP):
The ultimate CH₄ production was expressed as volume of CH₄ (L) at standard temperature (273.15 K), and pressure (101.325 kPa) per kg of volatile solids (VS) added [23, 24]. The ultimate CH₄ production at STP (Vₜₚ) was calculated using Equation 1:

\[ V_{\text{STP}} = \frac{(V_t \cdot T_s \cdot P_a)}{(T_i \cdot P_s)} \]  

Where \( V_t \) is the volume of CH₄ at time \( t \), \( T_s \) is standard temperature (273.15 K), \( P_a \) is atmospheric temperature, \( T_i \) is incubation temperature, and \( P_s \) is standard pressure (101.325 kPa)

2.4.2. Biodegradability
The percentage biodegradability (BD) during the anaerobic digestion process was calculated using the experimental biochemical CH₄ potential yield (BMPₑₓ) and the biochemical theoretical CH₄ potential yield (BMPₜ) as shown in Equation 2:

\[ BD\% = \frac{(\text{BMP}_{\text{ex}})}{(\text{BMP}_{\text{T}})} \times 100 \]  

2.4.3. Kinetics of methane production
For the kinetic characterization of each experiment, a kinetic equation for CH₄ production was applied. The lag and exponential phases of CH₄ production can be assumed to follow a first order rate and a linear regression model are shown in Equation 3[25]. Equation 3 confirms CH₄ production follows a first order kinetic model [26-28].

\[ G = G_m[1 - \exp(-k_0t)] \]  

\( G \) (L CH₄/Kg VS) is the cumulative CH₄ production at a given time \( t \) (days). \( G_m \) (L CH₄/Kg VS) is the maximum CH₄ production at an infinite digestion time (t). \( k_0 \) (days⁻¹) is an apparent kinetic constant and includes the biomass concentration, \( t \) is the time in days.
Taking natural logarithms and reordering terms results in Equation 4:

\[
\ln \left( \frac{G_m}{G_{m-G}} \right) = K_0 t
\]  

(4)

A plot of \( \ln \left( \frac{G_m}{(G_m-G)} \right) \) versus t should be a straight line in which the slope equals \( K_0 \) at the origin.

However, linear regression models cannot accurately describe and predict cumulative CH\(_4\) production through the entire digestion process, especially after the exponential phase [29]. Therefore, to assess and compare the CH\(_4\) production at different zeolite rates, nonlinear regression was utilised to achieve representative simulations and predictions. The modified Gompertz equation, as represented by Equation 5, was applied to obtain representative simulations and predictions [30].

\[
B = B_0 \exp \left\{ \exp \left[ \frac{R_m a}{B_0} (\lambda - 1) + 1 \right] \right\}
\]  

(5)

Where \( b \) is the cumulative CH\(_4\) production (L/kgVS) at digestion time \( t \) (day), \( B_0 \) is the ultimate CH\(_4\) generation or production potential (L/KgVS), \( R_m \) is the maximum CH\(_4\) production rate (L/(KgVS day)), \( \lambda \) is the lag phase time (day), and \( e \) is 2.7183.

\( R_m \) and \( \lambda \) can be determined by nonlinear regression using the SOLVER function in Microsoft Excel, which employs iterative least squares fitting routine to produce the optimal fit between data and function 5. The statistical indicator, the coefficient of determination (\( R^2 \)) was calculated by following the method of [31].

2.4.4.  
Free Ammonium nitrogen:

The equilibrium concentrations of free NH\(_3\) and NH\(_4^+\) in the aqueous solution follow the following equation 6.

\[
NH_4^+ + OH^- \leftrightarrow NH_3 + H_2O
\]  

(6)

The equilibrium concentrations of NH\(_4^+\) are pH and temperature (T) dependent as given by equation 7 [8].

\[
[NH_3(aq)] = \frac{TAN \ (aq)}{1 + [H_3O^+(aq)]/K_N} = \frac{TAN \ (aq)}{1 + 10^{pK_N - pH}}
\]  

(7)

\[
pK_N = 4 * 10^{-8}T^3 + 9 * 10^{-5}T^2 - 0.0356T + 10.072
\]  

(8)

Where \( K_N \) is the acid ionization constant for NH\(_3\) and \( pK_N \) is the acid dissociation constant value for NH\(_4^+\)/NH\(_3\) in an aqueous solution which can be estimated using equation 8.
2.4.5. Statistical analysis
Statistical analysis of the results was made by two-way analysis of variance (ANOVA) following a General Linear Model (GLM) with Minitab V17. Fisher’s LSD test was employed for comparing treatment means. Correlation analysis among the zeolite doses, physical and chemical parameters were done using Minitab V17.

3. Results and Discussion
3.1. Effect of zeolites on biogas and CH\textsubscript{4} production:
The digesters that had natural or sodium zeolites shows larger biogas and CH\textsubscript{4} production during the 60 days of anaerobic digestion than the digesters without zeolite, and the natural zeolite at 40g/L show the largest biogas and CH\textsubscript{4} production at the end of 60 days of anaerobic digestion (Fig. 1 and 2). Final biogas production with a natural zeolite dose of 40g/L was enhanced by 35%, and CH\textsubscript{4} production was improved by 29% compared to the control digesters. Both final biogas production and CH\textsubscript{4} production increased with increasing natural zeolite doses up to 40g/L and decreased with further increasing doses to 100 g/L. The application of sodium zeolite showed smaller enhancement of biogas and CH\textsubscript{4} production compared to the natural zeolites up to 40 g/L. At a dose of 70g/L sodium zeolite showed larger biogas and CH\textsubscript{4} production than the same rates of natural zeolite. Both natural and sodium zeolites at 100g/L produced more or less similar amounts of cumulative CH\textsubscript{4} production at the end of 60 days.

According to ANOVA zeolites doses had significant differences (p < 0.001) in biogas production but not between the type of zeolites (p= 0.104). Both zeolite types (p = 0.013) and zeolite doses (P < 0.001) have significant differences with CH\textsubscript{4} production. However, the significant interaction (p < 0.001) between zeolite types on biogas and CH\textsubscript{4} production indicated that the differences between zeolite types on biogas and CH\textsubscript{4} production depend on the zeolite dose. Fisher’s tests revealed significant differences between the final biogas yields and CH\textsubscript{4} yield of all natural and sodium zeolites digesters compared to the yields of nil zeolite digesters.

All digesters treated with zeolite showed larger biodegradability than those with swine manure only (Table 2). The highest biodegradability of 83% was shown by the natural zeolite digesters at the application rate of 40g/L and the least biodegradability, 64%, was shown by the digesters without zeolites. The next lowest biodegradability, 71%, was observed in the digesters with natural zeolite 100g/L which is 7% higher than digesters without zeolite. From these results, it can be estimated that optimum ultimate biogas and CH\textsubscript{4} production are achieved from digestion of swine manure with Australian natural zeolite at application rates between 10 and 40g/L.
Fig. 1: Cumulative biogas production variation over the digestion period. (Vertical bars represent standard errors, n = 3)

Fig. 2: Cumulative CH$_4$ production variation over the digestion period. (Vertical bars represent standard errors, n = 3)
Table 2: Biodegradability of swine manure at different rates of zeolites. (Standard error in parentheses, n=3)

<table>
<thead>
<tr>
<th>Zeolite Type</th>
<th>Zeolite Doses g/L</th>
<th>Biodegradability</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>0</td>
<td>64% (0.47)</td>
</tr>
<tr>
<td>Natural</td>
<td>10</td>
<td>74% (0.90)</td>
</tr>
<tr>
<td>Natural</td>
<td>40</td>
<td>83% (0.68)</td>
</tr>
<tr>
<td>Natural</td>
<td>70</td>
<td>73% (0.52)</td>
</tr>
<tr>
<td>Natural</td>
<td>100</td>
<td>71% (0.86)</td>
</tr>
<tr>
<td>Sodium</td>
<td>10</td>
<td>72% (0.78)</td>
</tr>
<tr>
<td>Sodium</td>
<td>40</td>
<td>74% (0.12)</td>
</tr>
<tr>
<td>Sodium</td>
<td>70</td>
<td>76% (0.58)</td>
</tr>
<tr>
<td>Sodium</td>
<td>100</td>
<td>72% (0.10)</td>
</tr>
</tbody>
</table>

Milan et al. (2001) [32] tested natural zeolite application rates in the range of 0.2-10g/L of digestate and found CH₄ yield increased up to zeolite doses of 2-4 g/L and then CH₄ production was increasingly inhibited above zeolites dose 6 g/L. Tada, Yang, Hanaoka, Sonoda, Ooi and Sawayama [19] found that only natural mordenite from a range of zeolites (i.e., mordenite, clinoptilolite, zeolite 3A, Zeolite 4A) contributed to CH₄ enhancement during anaerobic digestion of ammonium-rich organic sludge. However, in this study, biogas and CH₄ production increased over 10% compared to the control at all doses of natural and sodium zeolites (Table 3).

Table 3: Summary of percentage of biogas and CH₄ enhancement at different rates of zeolites application. (Standard error in parentheses, n=3)

<table>
<thead>
<tr>
<th>Zeolite Type</th>
<th>10 g/L</th>
<th>40 g/L</th>
<th>70 g/L</th>
<th>100 g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enhanced % of biogas</td>
<td>Enhanced % of CH₄</td>
<td>Enhanced % of biogas</td>
<td>Enhanced % of CH₄</td>
</tr>
<tr>
<td>Natural</td>
<td>18(1.99)</td>
<td>35(1.70)</td>
<td>29(1.05)</td>
<td>13(1.36)</td>
</tr>
<tr>
<td>Sodium</td>
<td>14(1.06)</td>
<td>17(0.72)</td>
<td>15(0.18)</td>
<td>21(2.04)</td>
</tr>
</tbody>
</table>

The CH₄ yield from anaerobic digestion of different substrates was enhanced by adding zeolites because they reduce the free ammonium-N (FAN)/NH₄⁺-N ratio by reducing free NH₃ and NH₄⁺
ions in the digestate, and they also have the capacity to immobilise microorganisms [16]. However, reductions of CH₄ yield at zeolites doses higher than 40g/L was observed in this study. This could be explained by several factors. The zeolite additions to the digesters increased the total solids. Therefore, free available water inside the digesters was reduced, and this affects the transport of nutrients and metabolites in the vicinity of the zeolite particles, and associated microorganisms [16, 33].

The increased CH₄ yield per g of zeolites decreased with increasing zeolites doses. The data from the increased CH₄ yield per 1 g of zeolites in each digester versus zeolites dose are well described by an exponential decay curve (Fig. 3). The rate constants of natural zeolites and sodium zeolite are 31.27 g and 41.67 g respectively.

![Fig. 3: Exponential decay functional fitting of increased CH₄ production per g of zeolite versus Zeolite doses.](image)

3.2. Kinetics Models

3.2.1. First order kinetics

Representation of the experimental data as indicated by Equation 2 (Plot of Ln(Gₘ/Gₘ-G) values as a function of digestion time for different types of digesters) gave straight lines with the intercept very close to zero. The parameter k₀ was calculated by standard linear regression and showed a significance level of 95%. Regression coefficients are higher than 0.96 in all cases (Table 4). Apparent kinetic constant, K₀, for the natural zeolite dose 40g/L was 0.065, which was the highest k₀ among all treatments, indicating fastest kinetic behaviour.
Table 4: Values of the kinetic constant, $K_0$, determined according to the first order model used.

<table>
<thead>
<tr>
<th>Zeolite Type</th>
<th>Zeolite Doses g/L</th>
<th>$K_0$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Zeolite</td>
<td>0</td>
<td>0.057</td>
<td>0.98</td>
</tr>
<tr>
<td>Natural</td>
<td>10</td>
<td>0.057</td>
<td>0.98</td>
</tr>
<tr>
<td>Natural</td>
<td>40</td>
<td>0.065</td>
<td>0.96</td>
</tr>
<tr>
<td>Natural</td>
<td>70</td>
<td>0.061</td>
<td>0.97</td>
</tr>
<tr>
<td>Natural</td>
<td>100</td>
<td>0.060</td>
<td>0.98</td>
</tr>
<tr>
<td>Sodium</td>
<td>10</td>
<td>0.056</td>
<td>0.98</td>
</tr>
<tr>
<td>Sodium</td>
<td>40</td>
<td>0.061</td>
<td>0.98</td>
</tr>
<tr>
<td>Sodium</td>
<td>70</td>
<td>0.063</td>
<td>0.98</td>
</tr>
<tr>
<td>Sodium</td>
<td>100</td>
<td>0.062</td>
<td>0.98</td>
</tr>
</tbody>
</table>

According to the two-way ANOVA, only zeolites doses had a statistically significant difference with $k_0$ ($p < 0.001$). Fisher’s tests revealed significant differences between the $k_0$ of the natural and sodium digesters at the zeolite application rates of 40g/L, 70g/L and 100g/L compared to the $k_0$ of the nil zeolite digesters. The $K_0$ kinetic constant includes the biomass concentration [34]. Zeolites have a large capacity to immobilise microorganisms [16, 34]. Anaerobic digesters with 40g/L natural zeolites showed higher microbial activity than other digesters used in the experiment. The apparent viscosity of digestion media would increase with increasing zeolites doses, thus reducing the mass transfer between microbes and the substrate, and thereby decelerating the process [34]. Zeolites released $Ca^{2+}$, $Mg^{2+}$, $Na^+$, $K^+$ ions into solution whilst they adsorb other cations. Therefore, the types of zeolites and their different dosage rates alter the amount of these cations in the digestate. The light metal ions including $Na^+$, $K^+$, $Ca^{2+}$, and $Mg^{2+}$ are required for microbial growth and, consequently, affect specific growth rates [35]. While moderate concentrations of these ions stimulate microbial growth, excessive amounts slow down growth and even higher concentrations can cause severe inhibition or toxicity [36].

3.2.2. The modified Gompertz model

According to the modified Gompertz plots of biogas accumulation in the all digesters used in this experiment, the results for all nil-zeolite and both natural and sodium zeolites at different rates had data that followed the modified Gompertz growth curve with $R^2 > 0.997$ (Fig. 4). The maximum CH$_4$ production rate, 16 L/Kg VS/day was shown by digesters that contain natural zeolites at 40g/L. The lag phase of digestion was shorter with increasing zeolites dose (Table 5).
Fig. 4: Modified Gompertz plots of CH$_4$ accumulation.

Table 5: Values of fitting modified Gompertz equation at different rates of zeolite application.

<table>
<thead>
<tr>
<th>Zeolite Type</th>
<th>Zeolite Doses</th>
<th>B$_0$</th>
<th>R$_m$</th>
<th>$\lambda$</th>
<th>R$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Zeolite</td>
<td>0</td>
<td>370</td>
<td>11.91</td>
<td>1.28</td>
<td>0.9972</td>
</tr>
<tr>
<td>Natural</td>
<td>10</td>
<td>428</td>
<td>14.24</td>
<td>1.08</td>
<td>0.9998</td>
</tr>
<tr>
<td>Natural</td>
<td>40</td>
<td>478</td>
<td>16.01</td>
<td>1.20</td>
<td>0.9998</td>
</tr>
<tr>
<td>Natural</td>
<td>70</td>
<td>419</td>
<td>13.36</td>
<td>0.53</td>
<td>0.9959</td>
</tr>
<tr>
<td>Natural</td>
<td>100</td>
<td>408</td>
<td>13.56</td>
<td>0.43</td>
<td>0.9954</td>
</tr>
<tr>
<td>Sodium</td>
<td>10</td>
<td>417</td>
<td>13.26</td>
<td>0.49</td>
<td>0.9974</td>
</tr>
<tr>
<td>Sodium</td>
<td>40</td>
<td>427</td>
<td>14.00</td>
<td>0.29</td>
<td>0.9990</td>
</tr>
<tr>
<td>Sodium</td>
<td>70</td>
<td>440</td>
<td>14.99</td>
<td>0.77</td>
<td>0.9978</td>
</tr>
<tr>
<td>Sodium</td>
<td>100</td>
<td>418</td>
<td>14.26</td>
<td>0.61</td>
<td>0.9977</td>
</tr>
</tbody>
</table>

In all digesters with added zeolite, the lag phase was shorter, and maximum CH$_4$ production was larger than digesters without zeolite. This may be due to the special surface structure of the aluminium silicate minerals of clinoptilolite which was more favourable for carrying
microorganisms on surfaces [37]. Therefore the addition of Australian zeolite could not only enhance the CH$_4$ production rate but could also shorten the lag phase of anaerobic digestion and increase the ultimate biogas and CH$_4$ production.

3.3. **pH and alkalinity:**
The optimum pH range for CH$_4$ formation is between 6.0 and 8.0 and pH values outside that range cause a significant reduction of CH$_4$ production [38]. Buffering capacity (alkalinity in anaerobic digestion processes) provides resistance to large and rapid changes in pH, and determining alkalinity is a more reliable method than direct measurements of pH, as an accumulation of short chain fatty acids will reduce the buffering capacity significantly before the pH decreases [39]. Sufficient alkalinity, between 1000–3000 mg/L as CaCO$_3$, avoids extreme pH decreases in the anaerobic digestion process [40]. Both pH and alkalinity were reduced with increases of zeolites doses. Sodium zeolites showed lower pH and alkalinity than natural zeolites. However, both pH and alkalinity were in the desired ranges in all digesters (Fig. 5). The pH and alkalinity at the end of 60 days in nil-zeolite digesters were 7.79 and 6300 mg/L, and the values were not decreased to below 7.54 and 4566 mg/L, respectively, with the introduction of different natural or sodium doses of zeolites.

![Fig. 5: pH and alkalinity variation with the zeolite doses in the digesters. (Vertical bars represent standard errors, n = 3)](image-url)
The alkalinity and pH increased during the anaerobic digestion process because of NH$_4^+$ production as a result of protein hydrolysis followed by consumption of volatile fatty acids and the dissolution of CO$_2$ produced by the methanogenic bacteria [41]. The decreases in NH$_4^+$ concentration in the digestate may be because of NH$_4^+$ adsorption onto the zeolites and the removal of CO$_3$ as a result of precipitation of Ca$^{2+}$ and Mg$^{2+}$ released by zeolite and could be the major reasons for the lower values of alkalinity and pH in the digesters treated with zeolites.

3.4. Effect of zeolites on ammonia and ammonium concentrations

Removal of NH$_4^+$ increased with increasing doses of both natural and sodium zeolites (Fig. 6 and Table 6). According to the two-way ANOVA, only zeolite doses had a statistically significant effect on NH$_4^+$ concentration ($p < 0.001$). Fisher’s tests also revealed significant differences between the NH$_4^+$ concentrations, except between natural and sodium zeolites digesters at the rate of 10g/L compared to the NH$_4^+$ concentration of the nil zeolite digesters. There were no significant differences between NH$_4^+$ concentrations at the same rates of natural and sodium zeolite addition.

Table 6: Summary of percentage NH$_4^+$ reduction at different rates of zeolites application, (Standard error in parentheses, n=3)

<table>
<thead>
<tr>
<th>Type of zeolites</th>
<th>Reduced % of NH$_4^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10g/L</td>
</tr>
<tr>
<td>Natural Zeolite</td>
<td>5(4.7)</td>
</tr>
<tr>
<td>sodium Zeolite</td>
<td>4(0.75)</td>
</tr>
</tbody>
</table>

The free NH$_3$(l) concentration in nil-zeolite digesters was 104 mg/L. The threshold values at which CH$_4$ inhibition begins ranges from the NH$_3$ concentration of 80 mg/L [42] to 150 mg/L [43]. The addition of 10 g zeolites / 1L digestate, resulted in an NH$_4^+$ concentration reduction of 5%. The ammonia concentration inside the digester with a zeolite application rate of 10g/L was 85 mg/L, and increasing the zeolite application rates to over 10g/L, NH$_3$ concentrations inside the digesters were lowered to less than the 80 mg/L, and NH$_3$ concentrations inside the digesters were below the inhibitory levels.

Sodium zeolite adsorbed 25% more NH$_4^+$ than natural zeolites from 1000 mg NH$_4^+$-N /L pure NH$_4$Cl solution [20]. The reason for no significant differences between NH$_4^+$ adsorption by sodium zeolites or natural zeolites in this study can be explained by the following reasons. Natural zeolite contain exchangeable Ca$^{2+}$ which precipitates at pH >7 as carbonate, or with other anions, resulting in excess NH$_4^+$ adsorption [44]. Another reason is that in the presence of Na$^+$ in the digestate the
first ionic exchange process between the zeolites and aqueous Na\(^+\) result in the production of sodium zeolite, as expressed by Equation 9. In addition, ionic exchange processes might take place between zeolites and the mixture of the NH\(_4^+\) solution as expressed by Equation 9. Thereafter, natural zeolites can also adsorb NH\(_4^+\) in a similar way to the sodium zeolites as the efficiency of Na\(^+\) replacement by NH\(_4^+\) from exchange surface is easier than the exchange between divalent and monovalent cations (Equation 10).

\[
\text{Zeolite} - \text{C}^{n+} + n\text{Na}^+ \rightarrow \text{Zeolite} - (\text{Na}^+)_n + \text{C}^{n+} \quad (9)
\]

\[
\text{Zeolite} - (\text{Na}^+)_n + n\text{NH}_4^+ \rightarrow \text{Zeolite} - (\text{NH}_4^+)_n + n\text{Na}^+ \quad (10)
\]

With increasing zeolites doses NH\(_4^+\) removal was increased and free NH\(_3\)(l) production was reduced, but it did not enhance CH\(_4\) production progressively up to the 100g /L zeolite application rate. Even though sodium zeolite removed the same amount of NH\(_4^+\), it did not increase CH\(_4\) production as much as natural zeolite. Tada et al. (2005) [19] showed that zeolite 3A had a similar capacity for NH\(_4^+\) removal as mordenite. However, zeolite 3A did not enhance the CH\(_4\) yield. Results from this study also showed that natural zeolite provided an additional effect on enhancing CH\(_4\) yield compared to digesters without zeolite. The addition of natural zeolite reduced NH\(_4^+\) in solution by ion exchange and the release of Mg\(^{2+}\), Ca\(^{2+}\), and Na\(^+\), whereas sodium zeolite adsorbed NH\(_4^+\) and mainly released Na\(^+\) [45]. The optimum level of these cations stimulate microbial growth and are antagonistic to NH\(_4^+\) inhibition [43]. Variation of the amount of these cations might be another reason for biogas and CH\(_4\) yield variation within the treatments.

**Fig. 6:** Ammonium, and ammonia concentrations inside the digesters with natural and sodium zeolites. (Vertical bars represent standard errors, n = 3).
3.5. Effects of cation adsorption, desorption by zeolites on anaerobic digestion

Although the cations Na\(^+\), K\(^+\), Ca\(^{2+}\), and Mg\(^{2+}\) stimulate microbial growth and have antagonistic effects on NH\(_4\)\(^+\) inhibition [43], higher concentrations are toxic to anaerobic digestion processes [35, 36]. Zeolites are microporous aluminosilicate minerals with loosely bound cations, with Na\(^+\), K\(^+\), Ca\(^{2+}\), and Mg\(^{2+}\), which can be readily replaced by other cations in the same solutions [46].

Fig. 7a and 7b show final Na\(^+\), K\(^+\), Ca\(^{2+}\), and Mg\(^{2+}\) concentrations of nil-zeolite, and natural and sodium zeolites at application rates of 10g/L, 40g/L, 70g/L and 100g/L. The concentrations of Ca\(^{2+}\), Mg\(^{2+}\), and Na\(^+\) should be below 3,000, 720, and 1,000 mg/L, respectively, for optimum methanogenic activity [47-49]. The concentration of K\(^+\) exceeded the 400mg/L threshold that causes digestion failure because of the inhibition of acidogenic microorganisms [35].

![Graph showing cation concentrations](image1)

**Fig. 7a:** Cations concentration changes with natural zeolite doses, **7b:** cations concentrations changes with the sodium zeolite doses inside the digesters. (Vertical bars represent standard errors, n = 3)
Except for the nil-zeolite and natural and sodium zeolites application rates of 10g/L, the other treatments resulted in desirable concentrations of K\(^+\). This might be because both natural and sodium zeolites have high affinities for K\(^+\) adsorption. The Na\(^+\) concentrations in nil-zeolite digesters, all natural zeolite digesters, and 10g/L sodium zeolite digesters, were less than 1000 mg/L, which is favourable for methanogenesis. In all other sodium treatments, the Na\(^+\) concentration exceeded optimum levels, and this might be the reason for the comparatively smaller CH\(_4\) production of sodium digesters compared to the natural zeolite digesters, even though those digesters had smaller NH\(_4^+\) concentrations. Mg\(^{2+}\) concentrations in the digesters increased with increasing zeolite doses, and they were higher than desirable levels (i.e. 720 mg/L) above the 40g/L zeolite dose. The Ca\(^{2+}\) concentrations in all digesters were within the optimum range, which is less than 3000 mg/L. Except for Ca\(^{2+}\), the other cations, K\(^+\), Na\(^+\) and Mg\(^{2+}\), showed linear relationships with the zeolite dose rate. However, excess Ca\(^{2+}\) in the digestate leads to carbonate (CO\(_3^{2-}\)) and phosphate (PO\(_4^{3-}\)) precipitation [50]. The pH can be a key factor in controlling the quantity and crystalline structure of calcium phosphates obtained by precipitation and at constant pH. The Ca/P ratio strongly affects the precipitation efficiency of calcium phosphate [51]. This might be the reason for the observed Ca\(^{2+}\) variation among the digesters.

3.6. Effects of zeolites addition on volatile fatty acids (VFAs) production

VFAs are important intermediate by-products in the process of CH\(_4\) production. A range of VFAs occur in anaerobic digestion media, and they have diverse and cooperative effects on bacteria and other archaea [52]. In strongly buffered systems only VFAs can be considered as reliable for monitoring anaerobic digestion processes [53]. It is the best indicator of impending reactor failure, with several authors suggesting iso-butyric, iso-valeric [54], propionic acid [53], or the ratio of propionic/acetic acid [55] as the most appropriate indicator. However, according to our previous studies, iso-butyric and iso-valeric fatty acids are the most suitable indicators of anaerobic digestion of swine manure. In the current study, VFAs analyses of all digester effluents showed less than 0.2 mMol/L iso-butyric acid, less than 0.01 mMol/L iso-valeric acid concentration and there were no iso-valeric acids in any digesters (Fig. 8), The lowest concentration of these VFAs indicated completed digestion over the 60 day period.

The propionic acid concentration of 12 mMol/L resulted in significant inhibition of methanogens [56]. Propionic acid concentrations in all digesters solutions were less than 0.001mMol/L. Acetic acid concentrations in all digesters were less than 0.1mMol/L. However, digesters that contained sodium zeolite showed smaller concentrations of acetic acid than digesters that contained natural zeolite at the same rates of application. The adsorption of carboxyl acids (acetic acid and propionic acids) takes place only in pores on =Si-O-Si= sites and not on cation exchange sites [57]. Sodium

94
zeolite has larger pore volume than natural zeolite [20]. This might be a reason for smaller acetic acid concentrations in solutions of digesters with sodium zeolite. However, reduction patterns similar to acetic acids were not observed for other VFAs. Their molecular size might be too large in comparison with the pore sizes of zeolites. However, butyric, iso-butyric, valeric and iso-valeric, were either completely non-detectable or present in low concentrations, in properly functioning digesters [54]. Therefore, non-detectable levels of butyric acid and very low levels of iso-butyric, valeric and iso-valeric acids in the digesters in this study indicated proper functioning of all digesters.

![Fig. 8: Volatile fatty acids variation with the zeolite doses in the digesters. (Vertical bars represent standard errors, n = 3)](image)

3.7 Correlation analyses

The correlations between physical and chemical parameters of zeolite suspensions during the anaerobic digestion process were determined. Results are presented in Table 7. The significant positive correlations ($p < 0.01$) are shaded in green and negative correlations ($p < 0.01$) are shaded in yellow. Zeolite doses did not show any correlation with biogas and CH$_4$ but showed strong significant negative correlation with NH$_4^+$, pH, TDS, EC and acetic acids. However, zeolite doses did not show any correlation with VFAs other than acetic acid thereby showing that Australian zeolite has the ability to adsorb acetic acids. As expected, VS and COD have negative relationships with biogas and CH$_4$ production, and NH$_4^+$ has a positive correlation with pH, TDS, EC, alkalinity and VS. Although the correlation between acetic acids and pH should be negative, it showed positive correlation mainly because acetic acids concentration was affected by zeolite doses.
Table 7: Correlation analysis of physical and chemical parameters

<table>
<thead>
<tr>
<th>Zeolite Dose</th>
<th>Biogas</th>
<th>Methane</th>
<th>Ammonium</th>
<th>pH</th>
<th>TDS</th>
<th>EC</th>
<th>Alkalinity</th>
<th>COD</th>
<th>VS</th>
<th>Acetic</th>
<th>Propionic</th>
<th>Valeric</th>
<th>Hexanoic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biogas</td>
<td>0.117</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methane</td>
<td>0.142</td>
<td>0.982</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammonium</td>
<td>-0.973</td>
<td>0.098</td>
<td>-0.125</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>-0.775</td>
<td>0.335</td>
<td>-0.351</td>
<td>0.72</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDS</td>
<td>-0.957</td>
<td>0.3</td>
<td>-0.327</td>
<td>0.926</td>
<td>0.73</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC</td>
<td>-0.956</td>
<td>0.315</td>
<td>-0.347</td>
<td>0.928</td>
<td>0.737</td>
<td>0.998</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkalinity</td>
<td>-0.538</td>
<td>0.317</td>
<td>-0.346</td>
<td>0.567</td>
<td>0.519</td>
<td>0.585</td>
<td>0.605</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COD</td>
<td>-0.14</td>
<td>0.859</td>
<td>-0.876</td>
<td>0.125</td>
<td>0.283</td>
<td>0.304</td>
<td>0.328</td>
<td>0.274</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VS</td>
<td>-0.572</td>
<td>0.615</td>
<td>-0.643</td>
<td>0.573</td>
<td>0.523</td>
<td>0.703</td>
<td>0.707</td>
<td>0.539</td>
<td>0.658</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Acetic</td>
<td>-0.716</td>
<td>-0.042</td>
<td>-0.033</td>
<td>0.64</td>
<td>0.715</td>
<td>0.625</td>
<td>0.623</td>
<td>0.328</td>
<td>0.077</td>
<td>0.285</td>
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</tr>
<tr>
<td>Propionic</td>
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<td>0.26</td>
<td>0.214</td>
<td>0.017</td>
<td>0.166</td>
<td>0.036</td>
<td>0.023</td>
<td>-0.185</td>
<td></td>
<td></td>
<td></td>
<td>0.166</td>
<td>0.057</td>
</tr>
<tr>
<td>Valeric</td>
<td>0.318</td>
<td>0.035</td>
<td>-0.004</td>
<td>-0.235</td>
<td>-0.624</td>
<td>-0.232</td>
<td>-0.236</td>
<td>-0.246</td>
<td></td>
<td></td>
<td>0.052</td>
<td>0.032</td>
<td>-0.408</td>
</tr>
<tr>
<td>Hexanoic</td>
<td>-0.211</td>
<td>-0.021</td>
<td>-0.042</td>
<td>0.217</td>
<td>-0.019</td>
<td>0.26</td>
<td>0.249</td>
<td>-0.046</td>
<td></td>
<td>0.139</td>
<td>0.174</td>
<td>0.114</td>
<td>0.357</td>
</tr>
<tr>
<td>Heptanoic</td>
<td>-0.279</td>
<td>0.012</td>
<td>-0.031</td>
<td>0.283</td>
<td>-0.024</td>
<td>0.331</td>
<td>0.317</td>
<td>0.007</td>
<td>0.081</td>
<td>0.251</td>
<td>0.152</td>
<td>0.403</td>
<td>0.378</td>
</tr>
</tbody>
</table>

Note: Positive correlation with a p < 0.01 are highlighted in green and negative correlations with a p < 0.01 are highlighted in yellow.
However, valeric acids showed a negative correlation with pH, and other VFAs did not show any correlation. The majority of VFAs were positively correlated with each other, [52]. The VFAs, except acetic acid and valeric acid, were not present in great amounts, and this might be the reason for not to observing a relationship among VFAs and other parameters.

4. Conclusion
All digesters with added zeolite showed more biogas and CH₄ production than the nil-zeolite digesters. The digesters with natural zeolite at 40g/L showed the largest biogas and CH₄ production at the end of 60 days of anaerobic digestion. Final biogas production was enhanced by 35%, and CH₄ production was improved by 29% compared to the control of the digesters with natural zeolite of dose 40g/L. Similar to biogas and CH₄ production, all digesters treated with zeolite showed larger biodegradability than those with swine manure only. The greatest biodegradability of 83% was shown by the natural zeolite digesters at the application rate of 40g/L, and the least biodegradability, 64%, was shown by the digesters without zeolite. The modified Gompertz equation gave a good approximation of the maximum CH₄ production per day (Rₘₖ), the lag phase (λ) and CH₄ production potential B₀ with coefficients of determination (R²) over 0.997. In all digesters with added zeolite the lag phase was shorter and maximum CH₄ production was larger than digesters without zeolite. Australian zeolite was proven to be effective in adsorbing NH₄⁺ from aqueous media. This study confirms that Australian zeolite, either in natural or sodium chloride treated form, significantly enhanced biogas generation during anaerobic digestion of swine manure, while adsorbing substantial quantities of NH₄⁺ from the media during the process. Although NH₄⁺ adsorption by zeolites showed a linear relationship with increasing zeolite doses, biogas, and CH₄ production increased linearly only up to 40g/L for natural zeolite. The effects of NH₄⁺ adsorption by natural and sodium zeolites were more or less similar in the current study. However, digesters with sodium zeolites had smaller CH₄ production compared to the digesters with natural zeolite. Sodium zeolite released more Na⁺ into the digesters, and Na⁺ levels in those digesters exceeded the desired limit for microorganisms. In general, total NH₄⁺ adsorption by zeolites appeared positively correlated with biogas/CH₄ production supporting the preposition that reduction in soluble NH₄⁺ levels in anaerobic digestion media favours methanogenesis process. Variations of cations levels inside the digesters as a result of adsorption-desorption caused by adding zeolites at different rates might be another reason for variations of biogas and CH₄ production from different rates of zeolites digesters.
Acknowledgement:
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References


Chapter 6:2

Biogas improvement by adding Australian zeolite during the anaerobic digestion of C: N ratio adjusted swine manure

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Abstract:

Maintenance of the ideal carbon: nitrogen (C:N) ratio with a minimum level of TAN is a key challenge for achieving maximum potential CH\textsubscript{4} production through the anaerobic digestion process of swine manure. Biogas production can be enhanced by adding zeolite into the anaerobic digestion medium. The effects of zeolite addition to the feed with an optimal C:N ratio is unknown. The main objectives of this study were to identify the effect of adding Australian zeolite to swine manure with a C:N ratio adjusted to 30 and to determine the best zeolite application rate. The addition of Australian zeolite, enhance the CH\textsubscript{4} production rate and decreased the lag phase of anaerobic digestion in batch production. It was shown that the optimal addition rate of zeolite was 40 g/L. The effect was due to reduction of dissolved ammonium from the digestate. Therefore, adding Australian zeolite could be an opportunity when using a feed high in nitrogen in biogas production.

Key words: Ammonium, biogas, C:N ratio, methane, zeolite
**Highlights**

- Australian zeolites increased CH$_4$ production significantly for the anaerobic digestion of swine manure with added cellulose to give a C:N ratio optimal for biogas production.
- Increasing zeolite dose from 10g/L to 100g/L decreased the lag phase of a batch anaerobic digestion process.
- The removal of NH$_4^+$ increased linearly with an increase of zeolite dose.
- The highest CH$_4$ production was measured at a natural zeolite application rate of 40g/L.

**1. Introduction**

Animal manure is a biomass used world wide for biogas production (Sommer et al., 2013). Most often, animal manure is co-digested with organic biomass containing a high concentration of easily degradable organic matter (Abouelenien et al., 2014). Using biomass high in nitrogen (N) for biogas production will result in a high concentration of total ammonia nitrogen (TAN = ammonia (NH$_3$(aq)) + ammonium (NH$_4^+$)), and the NH$_3$ which is related to high pH and temperature may inhibit methane (CH$_4$) production (Hansen et al., 1998). On the other hand, a high carbon to N (C:N) ratio may cause poor growth of microorganisms and also a low CH$_4$ production. It is, therefore, generally accepted that the C:N ratio of a biomass should be in the range 20–30 to give a high and stable anaerobic digestion (Borowski et al., 2014). To avoid low C:N ratios, which may cause accumulation of TAN in the digesters and inhibition of the microorganisms (Resch et al., 2011), manure can be co-digested with organic wastes with a high C:N ratio. Swine manure contains a high N concentration and this may inhibit CH$_4$ production, (Rajagopal et al., 2013), therefore, swine manure is often co-digested with organic waste with a high concentration of digestible organic matter and a low concentration of N (Rajagopal et al., 2013). Zeolite addition during the anaerobic digestion process is favourable because zeolite has a high capacity for retention of microorganisms, can improve the NH$_3$/NH$_4^+$ ion equilibrium and reduce the TAN concentration (Montalvo et al., 2012).

To our knowledge, there are no studies of zeolite addition to a co-digestion process with a C:N ratio at 30 (considered to be optimal for the anaerobic digestion process). Therefore, this study aims at examining if biogas production can be improved by adding zeolite to a swine manure adjusted with cellulose to give a C:N ratio at 30 and identify the most optimal zeolite addition rate for CH$_4$ production from this biomass.
2. Methods
Mesophilic anaerobic digestion experiments were conducted as a batch trial with different rates of Australian natural and sodium zeolites added to swine manure with a C:N ration adjusted to 30 by adding cellulose.

2.1 Inoculum, swine manure, cellulose (microcrystalline powder) and zeolite
The inoculum was collected from the anaerobic digester at Berrybank Farm Piggery, at Windermere Victoria, Australia. The farm has a standing swine population is 20,000 pigs. The inoculum was incubated 14 days at 37°C to remove easily digestible organic matter before starting the experiment. Swine manure was collected from a pig pen at the animal house in the University of Melbourne. The main characteristics of swine manure were: pH 6.73, total solids (TS) 6.62%, and volatile solids (VS) 69% from %TS and C:N ratio 10 and initial NH$_4^+$ Concentration 664 mg/L. The main characteristics of inoculum were: pH 7.39, TS 2.63; and VS 67% from %TS and the NH$_4^+$ concentration 1298 mg/L.

Cellulose (microcrystalline powder) obtained from Sigma-Aldrich was added as a carbon source in all digesters. 25g of cellulose (microcrystalline powder) was added (amount of added cellulose based on C, N and total solids (TS) content of digestion medium) to each swine manure digester (400 mL, containers) to adjust the C:N ratio of the manure to 30. Australian Natural zeolite was obtained from Zeolite Australia Pty Ltd, Werris Creek, NSW, Australia. Sodium zeolite was prepared as described in an earlier publication (Wijesinghe et al., 2016). Australian zeolite contains 54% clinoptilolite as the main mineral species and the porous structure of zeolite accommodates a wide variety of cations, such as Na$^+$, K$^+$, Ca$^{2+}$, Mg$^{2+}$ and others (Wijesinghe et al., 2016). Both Australian natural and sodium zeolites were dried overnight at 105°C before being used in an experiment.

2.2 Anaerobic digestion experiment
The anaerobic digestion experiment was conducted in 36, 500 mL (working volume) glass bottles (batch type digesters). The inoculum and swine manure were added on a VS basis at a ratio of 1:1 in all digesters. Then 25g of cellulose (microcrystalline powder) was added to each digester (400 mL) to adjust the C:N ratio of the swine manure to 30. After preparing the C:N adjusted swine manure digesters, Australian natural zeolite, and sodium zeolite were added at different rates. The dose of zeolite was 0, 10, 40, 70, 100 g/ L. All these treatments were in triplicate. After mixing the substrate and inoculum with natural and sodium zeolites
at different rates, all digesters were closed. The headspaces of all digesters were flushed with N₂ gas to create an anaerobic environment inside the digesters. The digesters were placed in an incubator at 37°C to maintain mesophilic condition for 60 days. Each digester was manually shaken once a day to mix the digestate.

2.3 Analytical methods:
Biogas production was measured daily using a 1L syringe (Hamilton Super Syringe). The CH₄ concentrations in the biogas were analysed using gas chromatography (7890A, Agilent Technologies, Wilmington, USA). The NH₄⁺ concentration of digestate at the end of the digestion period was analysed using a segmented Auto analyser (Skalar SAN⁺⁺, Skalar, Breda, The Netherlands). The TS and VS were measured according to the method described in APHA (APHA, 2012). The pH of the solution was measured with a pH meter (labCHEM-CP. TPS division, Elektron Electronics Pty Ltd., QLD, Australia). The VFA of samples were analysed with the use of capillary gas chromatography (GC) using a Gas Chromatograph system (Agilent Technologies 7890B Wilmington, USA) with an autosampler and auto-injector.

2.4. Data Analysis
2.4.1. Free Ammonium nitrogen:
The equilibrium concentration relationship between free NH₃ and NH₄⁺ in the aqueous solution is depicted in equation 1. The NH₃ concentration in the digestate was calculated using equation 2 (Sommer et al., 2013).

\[
\text{NH}_4^+ + \text{OH}^{-} \leftrightarrow \text{NH}_3 + \text{H}_2\text{O} \tag{1}
\]

\[
\left[\text{NH}_3(\text{aq})\right] = \frac{T\text{AN}(\text{aq})}{1+[\text{H}_3\text{O}^+(\text{aq})]/K_N} = \frac{T\text{AN}(\text{aq})}{1+10^{pK_N-pH}} \tag{2}
\]

\[
pK_N = 4 \times 10^{-8}T^3 + 9 \times 10^{-5}T^2 - 0.0356T + 10.072 \tag{3}
\]

Where K_N is the acid ionization constant for NH₃ and pK_N is the acid dissociation constant value for NH₄⁺/NH₃ in aqueous solution, TAN total ammoniacal nitrogen (mol/L), T temperature (Kelvin), pH is –log(H⁺) in H⁺ in mol/L.

2.4.2 Kinetics of CH₄ production:
Mathematical modelling of microbial growth is used to estimate important parameters such as the specific CH₄ production rate, CH₄ production potential and lag period for the study of
microbial growth under different physical and chemical status. The modified Gompertz equation (Equation 4) was applied to obtain representative simulations and predictions.

\[
B = B_0 \exp \left[ \exp \left( \frac{R_m \lambda}{B_0} (\lambda - 1) + 1 \right) \right]
\]

Where \( B \) is the cumulative CH\(_4\) production (L/kg VS) at a given digestion time \( t \) (day), \( B_0 \) is the accumulated CH\(_4\) generation (L/Kg VS), \( R_m \) is the maximum CH\(_4\) production rate (L/Kg VS day), \( \lambda \) is the lag phase time (day), and \( e \) is 2.7183.

### 2.5. Statistical analysis
Statistical analysis of results was made with Two-way analysis of variance (ANOVA) following a General Linear Model (GLM) with the statistical software Minitab V17, Fisher’s LSD test was employed for comparing treatment means. Correlation analysis relating CH\(_4\) production to the zeolite addition rate, physical and chemical parameters were performed using the statistical software Minitab V17.

### 3. Results and Discussion:
#### 3.1. Effect of zeolites on biogas and methane production
The addition of natural and sodium zeolites to the digesters increased biogas and CH\(_4\) production significantly \((p < 0.01)\) in relation to the control (without zeolite) (Fig. 1a and 1b). The highest biogas and CH\(_4\) production were from the digesters with an added natural zeolite at 40g/L. In this case, the biogas production was 69% higher than the control and CH\(_4\) production 87% higher. The addition of sodium zeolite into the digesters didn’t increase biogas and CH\(_4\) production as much as natural zeolite. The sodium zeolites desorbed Na\(^+\) upon cation exchange, and high amounts of desorbed Na\(^+\) might be the reason for lowering CH\(_4\) production at zeolite application rates higher than 10g/L. The optimum accumulated of biogas and CH\(_4\) production was achieved from swine manure digestion with Australian natural added zeolite at a rate of 40g/L. The CH\(_4\) yield from anaerobic digestion was enhanced by adding zeolite because zeolite reduces TAN concentration and free NH\(_3\) from the digestate, and it has a capacity of immobilisation of microorganisms (Montalvo et al., 2012). Microorganisms have a tendency to adhere to solid surfaces enhancing their activity (Weiβ et al., 2011). The maximum improved biogas yield was attributed to the reduction in TAN at the zeolite application rate 10g/L digestate (i.e 0.5 L/VS biogas/1 mg/L NH\(_4^+\) reduction). When zeolite doses increased up to 100g/L digestate, improved biogas yield per reduction in TAN reduced up to 0.3 - 0.06 L/VS biogas/1 mg/L NH\(_4^+\) reduction. The addition
of zeolite, which adsorbs $\text{NH}_4^+$, thus having an effect not only on the toxicity of $\text{NH}_3$ and the C:N proportion but also on the regulation of acidity (pH) of the waste studied (Milan et al., 2010).

C:N adj. SM = C:N adjusted swine manure, NZ = Natural Zeolite, SZ = Sodium Zeolite
10, 40, 70, 100 – Zeolite Doses g/L of swine manure

**Fig. 1**: a. Cumulative biogas production, b. Cumulative $\text{CH}_4$ production at the different zeolite doses in C:N adjusted swine manure digesters. (Vertical bars represent standard errors, n = 3)
Table 1: Values of the kinetic constants of Modified Gompertz equation

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$R_m$</th>
<th>$\lambda$</th>
<th>$B_0$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C:N adj. SM</td>
<td>45.68</td>
<td>2.08</td>
<td>190</td>
<td>0.998</td>
</tr>
<tr>
<td>C:N adj. SM +NZ10g/L</td>
<td>30</td>
<td>1.6</td>
<td>191</td>
<td>0.995</td>
</tr>
<tr>
<td>C:N adj. SM +NZ40g/L</td>
<td>27.59</td>
<td>1.32</td>
<td>257</td>
<td>0.997</td>
</tr>
<tr>
<td>C:N adj. SM +NZ70g/L</td>
<td>27.99</td>
<td>1.46</td>
<td>265</td>
<td>0.998</td>
</tr>
<tr>
<td>C:N adj. SM +NZ100g/L</td>
<td>29.99</td>
<td>1.54</td>
<td>297</td>
<td>0.999</td>
</tr>
<tr>
<td>C:N adj. SM +SZ10g/L</td>
<td>25.18</td>
<td>1.59</td>
<td>223</td>
<td>0.999</td>
</tr>
<tr>
<td>C:N adj. SM +SZ40g/L</td>
<td>34.3</td>
<td>1.48</td>
<td>255</td>
<td>0.996</td>
</tr>
<tr>
<td>C:N adj. SM +SZ70g/L</td>
<td>33.1</td>
<td>1.33</td>
<td>246</td>
<td>0.994</td>
</tr>
<tr>
<td>C:N adj. SM +SZ100g/L</td>
<td>25.83</td>
<td>0.71</td>
<td>421</td>
<td>0.999</td>
</tr>
</tbody>
</table>

C:N adj. SM – C:N adjusted swine manure, NZ- Natural Zeolite, SZ – Sodium Zeolite

$R^2$ values obtained from the modified Gompertz model are higher than 0.97 in all treatments, indicating the modified Gompertz model is a good fit for the first stage of the two-phase digestion period. The lag phase became shorter when adding zeolite into the digesters (Table 1).

3.2. Effect of zeolites on pH, alkalinity, and VFA

Both pH and alkalinity were reduced by adding zeolite. Sodium zeolite digesters showed lower pH than natural zeolite.

![Fig. 2: Volatile fatty acids variation with the zeolite doses in the digesters. (Vertical bars represent standard errors, n = 3)](image-url)
However, both pH (with variation within digesters from 5.95 to 7.67) and alkalinity (variation within digesters from 4223 to 5000 mg/L) were at the desired level for all zeolite treated digesters. Iso- butyric, iso-valeric fatty acids and propionic acid concentrations in all C:N adjusted swine manure digesters were higher than the desired level indicating incomplete digestion (Fig. 2). However, pH and alkalinity were at desirable levels in all digesters. Thus, a buffer system formed in co-digestion, allowing a high concentration of VFAs without decreasing pH.

3.3 Effect of zeolite on ammonia and ammonium concentration inside the digester

The two-way ANOVA indicated that zeolite dose rate has a statistically significant correlation with NH$_4^+$ concentration ($p < 0.001$). NH$_4^+$ concentration in the digestion medium reduced linearly with increasing zeolite dose. According to Fisher’s tests, there is a significant difference between the NH$_4^+$ concentration at all doses compared to the NH$_4^+$ levels in the nil zeolite digesters. Significant differences were not observed between NH$_4^+$ concentrations and the same rate of natural and sodium zeolites digesters. However, removal of NH$_4^+$ increased linearly with an increase of zeolite dose of both natural and sodium zeolites (Fig. 3). When zeolite was added, the NH$_3$ concentration was lower than the inhibitory level (i.e. 80 mg/L) in all zeolite treated digesters.

Although the NH$_4^+$ concentration decreased in the digesters with increasing zeolite dose, biogas/CH$_4$ production increased continuously up to 40g/L and declined at concentrations above this level. Although the sodium zeolite removed similar amounts of NH$_4^+$ as the natural zeolite, biogas/CH$_4$ production was not enhanced as was seen with the addition of natural zeolite.

The addition of natural zeolites reduced the NH$_4^+$ concentration in the solution by ion exchange releasing Mg$^{2+}$, Ca$^{2+}$ and Na$^+$ whereas the sodium zeolite adsorbed NH$_4^+$ by releasing mainly Na$^+$ (Wijesinghe et al., 2016). It is proposed that the optimum level of these cations stimulate microbial growth and are antagonistic to NH$_4^+$ inhibition, (Braun et al., 1981). The high cation concentration (Salt) may reduce CH$_4$ production and a high Na$^+$ concentration may be more problematic than a high concentration of divalent cations.
Fig. 3: Ammonium, NH$_3$ concentrations inside the digesters with natural and sodium zeolites at different rates. (Vertical bars represent standard errors, n = 3)

4. Conclusion
Adding natural Australian zeolite to swine manure in anaerobic digestion to a level of 40g/L significantly increased the biogas and CH$_4$ production rate while adsorbing a substantial quantity of NH$_4^+$ from the medium during the process. Although the effects of NH$_4^+$ adsorption by natural and sodium zeolites were more or less similar, digesters with sodium zeolite produced less CH$_4$ compared to the natural zeolite digesters.

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References


Chapter 6:3

Removal of excess nutrients by Australian zeolite during anaerobic digestion of swine manure

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Abstract
Significant amounts of nitrogen (N), phosphorus (P), potassium (K) and other nutrients are available in digested effluents after anaerobic digestion. Improper management of digested effluents can lead to several environmental impacts such as eutrophication of surface waters, fish toxicity and ammonia emissions. Zeolite has a high affinity for NH4+ in water. However, only a few studies have reported simultaneous removal of the other major nutrients by zeolites. There is no information available on the simultaneous removal of major nutrients by Australian zeolite during anaerobic digestion. The objective of this study was to investigate the feasibility of using natural and NaCl-treated Australian zeolites to simultaneously remove excess nutrients from anaerobically digested swine manure. Ion adsorption and desorption properties of Australian zeolite during the anaerobic digestion of swine manure were investigated. Two experiments were conducted: the first was an adsorption experiment with multi-component solutions that corresponded with the ionic composition of swine manure digestates. The second experiment determined the effects of zeolite dose rates during anaerobic digestion of swine manure on the removal of N, P and K from solution. Adsorption isotherms confirmed selectivity for K+ over NH4+ by Australian natural and sodium zeolites. Therefore NH4+ removal was considerably reduced when there was simultaneous K+ uptake. Natural zeolite desorbed more Ca2+ during K+ and NH4+ adsorption than sodium zeolite. The ion exchange reaction was independent of the presence of P. P removal was very dependent on the pH of the medium. Natural Australian zeolite was shown
to be a potential sorbent for the removal of \(\text{NH}_4^+\), \(\text{K}^+\) and \(\text{P}\) during the anaerobic digestion of swine manure.

**Keywords:** adsorption, anaerobic digestion, ammonium, phosphorous, potassium, zeolite

1. **Introduction**

   Anaerobic digestion is a well-established technology for converting animal manure into renewable energy (Cantrell et al., 2008), and a promising method of mitigating greenhouse gas (GHG) emissions (Kaparaju and Rintala, 2011). At the end of anaerobic digestion significant amounts of nitrogen (N), phosphorus (P), potassium (K), and other nutrients, are available in digested effluents, which are essential for plant growth, so effluents have potential as fertilisers. Improper management of digested effluents leads to several environmental issues such as accumulation of nutrients in water bodies and eutrophication and fish toxicity (Painting et al., 2007). Improper effluent management also increases the potential for ammonia (\(\text{NH}_3\)) volatilization as a consequence of an increase in total ammoniacal nitrogen (TAN - ammonium (\(\text{NH}_4^+\) + \(\text{NH}_3\))) and elevated pH values in the effluent during anaerobic digestion (Pain et al., 1990, King et al., 2012, Sommer and Husted, 1995). Therefore, effective removal or recovery of nutrients from effluents from anaerobic digestion is essential for environmental protection (Lei et al., 2007).

Zeolite is a crystalline aluminosilicate mineral with well-defined three-dimensional microporous structure (Santen and Neurock, 2006). Zeolite has a high affinity for \(\text{NH}_4^+\) in water (Lin et al., 2013, Wijesinghe et al., 2016). Different types of natural zeolites from various origins, including Australia, have been broadly investigated for removal of \(\text{NH}_4^+\) from aqueous solutions (Cooney et al., 1999, Komarowski and Yu, 1997, Widiastuti et al., 2011, Wijesinghe et al., 2016). However, only a few studies reported the simultaneous removal of other nutrients by zeolites. One study focused on the simultaneous removal of nitrogen (N) and potassium (K) by zeolite (Guo et al., 2008), two studies on the simultaneous removal of N and P by zeolite (Wu et al., 2006, Lin et al., 2014), and two studies showed the application of natural zeolite and calcium ion removal, including both N and P (Karapınar, 2009, Montalvo et al., 2011). This study is the first to explore the potential of Australian zeolite for removal of excess nutrients during anaerobic digestion. There has been no detailed investigation of the ion composition in digesters as a result of adding zeolite. The objective of this study was to examine the feasibility of applying Australian natural and NaCl-treated zeolites to remove excess nutrients. As such, the importance of ion adsorption
and desorption properties of Australian zeolite during anaerobic digestion of swine manure were investigated.

2. Methods

2.1 Adsorption Experiment with multicomponent solutions
Batch experiments using seven solutions with different compositions (Table 1) were conducted to evaluate the effects of competing ions on the adsorption capacity of Australian natural and sodium zeolites. Four grams of zeolite (0.5–1 mm) and 100 mL solution, prepared according to Table 1, were added to 200 mL conical flasks and shaken at 37°C at 100 rpm for 24 h. The pH of each solution was adjusted to 7 by adding either 0.1 M HCl or 0.1 M NaOH solutions. The ions used in this experiment were NH$_4^+$ - 1000 mg N/L, Na$^+$ - 600 mg/L, K$^+$ - 1000 mg/L, Ca$^{2+}$ - 600 mg/L, Mg$^{2+}$ - 200 mg/L and PO$_4^{3-}$ - 150 mg P/L, and acetate (CH$_3$COO$^-$) - 300 mg/L. These concentrations were selected according to a preliminary investigation of digested swine manure which showed that these concentrations are typical of those found. Analytical grade NH$_4$Cl, NaCl, KCl, MgCl$_2$, CaCl$_2$, K$_2$HPO$_4$, H$_3$PO$_4$ and CH$_3$COOH were used to prepare stock solutions with deionized water. The final concentrations remaining in the solution were analysed after filtering through a 0.45µm nylon filter.

2.2 Zeolite doses in anaerobic digestion with swine manure
Mesophilic anaerobic digestion experiments were conducted as a batch trial using glass mini-digesters (500 mL working volume). The inoculum (collected from Berrybank Farm, Windermere, Victoria, Australia) and swine manure (collected from the University of Melbourne, Australia) were introduced into the digesters at a ratio of 1:1 on a volatile solid (VS) basis. One set of digesters were operated with swine manure and inoculum only. In order to determine the effects of adjusting the C:N ratio another set of digesters were operated with swine manure and microcrystalline cellulose with an initial inoculum to swine manure at a ratio of 1:1 VS. 7% microcrystalline cellulose (Sigma-Aldrich) was added to adjust the C:N ratio of swine manure to 30. Those digesters were designated as C:N ratio adjusted swine manure digesters. Australian natural zeolite and sodium zeolite doses were selected based on previous studies which enhanced methane generation by adding during the anaerobic digestion process of swine manure. The mixtures of swine manure, inoculum and
cellulose were treated with four doses (10, 40, 70, and 100 g/L) of Australian natural zeolite (obtained from Zeolite Australia Pty Limited, Werris Creek, NSW, Australia) and sodium zeolite (prepared as described in Wijesinghe et al. (2016)) separately. Controls for each material without added zeolites were also maintained. All treatments were replicated three times. Nitrogen gas was flushed through the headspace of each digester to promote anaerobic conditions inside the digesters. The digesters were placed in an incubator at a constant temperature of 37°C and manually shaken daily to mix the contents. The total digestion period was 60 days.

Table 1: Composition of ion mixtures

<table>
<thead>
<tr>
<th>Test No</th>
<th>Zeolite Type</th>
<th>CH$_3$COO$^-$ (mg/L)</th>
<th>PO$_4^{3-}$-P (mg/L)</th>
<th>NH$_4^+$-N (mg/L)</th>
<th>K$^+$ (mg/L)</th>
<th>Ca$^{2+}$ (mg/L)</th>
<th>Na$^+$ (mg/L)</th>
<th>Mg$^{2+}$ (mg/L)</th>
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2.3 Chemical analyses

At the end of the digestion period, samples of digestate were centrifuged at 6000 rpm for 8 min and the supernatant gently decanted for chemical analysis. The NH$_4^+$ concentrations of digestates at the end of anaerobic digestion were analysed with a segmented auto-analyser (Skalar SAN$^{++}$). Cations Na$^+$, K$^+$, Ca$^{2+}$, Mg$^{2+}$ and P and S were measured using inductively coupled plasma spectrometry (ICP-OES, Optima, Model 8000, Perkin Elmer), and acetate was measured using a gas chromatograph (Agilent Technologies, Model 7890A, USA). The chemical composition of zeolite samples was measured by energy dispersive X-ray
spectroscopy [EDS, (Oxford, Aztec 2.1 sp 1, Oxford Instruments, Oxfordshire, UK)]. The surface morphologies of the zeolites were compared by scanning electron microscope (SEM, QuantaA200F, FEI, Hillsboro, OR, USA).

2.4 Data Analysis

2.4.1 Ion Adsorption
Ion adsorption and desorption were calculated using the following equation:

$$Q = \frac{(C_i - C_e) V}{m}$$  \hspace{1cm} (1)

Where Q is the amount of ion adsorption in mg/g of zeolite, Ci is the initial ion concentration (mg/L), Ce is the concentration of ion at equilibrium, (mg/L), V is the volume of solution and M is the mass of zeolite (g).

2.4.2 Adsorption isotherms:
The Langmuir isotherm model is widely used for describing adsorption equilibria (Guo et al., 2008). The Langmuir model was used to determine the maximum adsorption capacities of NH$_4^+$ and K$^+$ of zeolites during the anaerobic digestion of swine manure.

The Langmuir isotherm is represented by Eq. (2).

$$q = \frac{q_{\text{max}} k_L C_e}{1 + k_L C_e}$$  \hspace{1cm} (2)

where q is the adsorption capacity at equilibrium (mg - cation /g), $q_{\text{max}}$ is the maximum adsorption capacity (mg-cation/L), $k_L$ is the Langmuir constant and Ce is the NH$_4^+$ or K$^+$ concentration in the solution at equilibrium (mg-cation/L).

The linear form of Eq. (2) is given by Eq. (3).

$$\frac{C_e}{q} = \frac{1}{k_L q_{\text{max}}} + \frac{C_e}{q_{\text{max}}}$$  \hspace{1cm} (3)

2.4.3 Statistical analysis
The significance of differences in NH$_4^+$, K$^+$, Na$^+$, Mg$^{2+}$, Ca$^{2+}$, P and S concentrations in the digester solutions with the natural and sodium zeolite types, manure types (swine manure and C:N adjusted swine manure) and zeolite doses (0g/L, 10g/L, 40g/L, 70g/L and 100g/L) were
tested by two-way ANOVA. Differences between the values were considered statistically significant at P < 0.001. Where significant treatment effects were determined, Fisher's LSD test was applied to separate differences between treatment means at a confidence level of 95%.

3. Results and Discussion:

3.1 Adsorption:
The adsorption of NH$_4^+$, K$^+$, PO$_4^{3-}$ and CH$_3$COO$^-$ at equilibrium by Australian natural and sodium zeolites from 7 multi-component solutions are presented in Figure 1. Test solutions no. 1 and no. 2 showed very little CH$_3$COO$^-$ and PO$_4^{3-}$ removal. In the presence of NH$_4^+$ (test solutions 3 to 7), sodium zeolite adsorbed 25% more NH$_4^+$ than natural zeolite. Introducing CH$_3$COO$^-$ to the NH$_4^+$ solution resulted in a small reduction in NH$_4^+$ adsorption, of 2.3% by natural zeolites and of 4.8% by sodium zeolites. Introducing CH$_3$COO$^-$, PO$_4^{3-}$ and K$^+$ into the solution reduced NH$_4^+$ adsorption by 39% and 44% with the Australian natural and sodium zeolites, respectively. Australian natural and sodium zeolites adsorbed 12.3 mg/L and 13.5 mg/L NH$_4^+$, respectively. Adding Ca$^{2+}$ and Mg$^{2+}$ into the solutions (Test solutions 6 and 7) reduced NH$_4^+$ adsorption further, to 5.16 mg/L and 4.92 mg/L in natural and sodium zeolite systems, respectively. K$^+$ adsorption by natural and sodium zeolites were decreased by 18% and 19% in the presence of all ions. However, PO$_4^{3-}$ removal was enhanced by the presence of Ca$^{2+}$ or Mg$^{2+}$ ions. The latter is implied as a precipitation reaction.

These results indicate the effects of K$^+$ on reducing NH$_4^+$ adsorption by zeolite are significant. The presence of CH$_3$COO$^-$, Mg$^{2+}$, Ca$^{2+}$ ions at the concentrations studied also reduced NH$_4^+$ adsorption. The effects of Na$^+$, Ca$^{2+}$, Mg$^{2+}$ ions on NH$_4^+$ adsorption by both natural and sodium zeolites were similar. The selectivity of adsorption of competing cations on zeolite agreed with the original investigation of Ames (1960): K$^+$ > NH$_4^+$ > Na$^+$ > Ca$^{2+}$ > Mg$^{2+}$.

Sodium ion was the main cation desorbed by NH$_4^+$ adsorption from sodium zeolite, and Ca$^{2+}$ was the dominant cation exchanged upon NH$_4^+$ adsorption by natural zeolite at high concentrations (Figure 2). At higher NH$_4^+$ concentrations, Ca$^{2+}$ started to dominate the ion exchange process after most of the Na$^+$ was desorbed (Lin et al., 2013). K$^+$ showed the least desorption reflecting that K$^+$ has a higher affinity for zeolite than NH$_4^+$ (Ames, 1960). The K$^+$
desorbed by NH$_4^+$ adsorption might be reabsorbed onto zeolite by exchanging with other cations (Lin et al., 2013).

**Figure 1:** Ion adsorption by natural and sodium zeolites from test solutions. (Vertical bars represent standard errors, n = 3)

**Figure 2:** Ion desorption of natural and sodium zeolites by test solutions. (Vertical bars represent standard errors, n = 3)
The large hydrated radii of Mg\(^{2+}\) likely hinders its exchange for NH\(_{4}^{+}\) and might be responsible for the smaller amounts of desorption (Kantiranis et al., 2011). According to the concentration of desorbed cations the order of the degree of total cation desorption from Australian natural and sodium zeolites at steady state was Ca\(^{2+}\)>Na\(^{+}\)>Mg\(^{2+}\)>K\(^{+}\) and Na\(^{+}\)>Ca\(^{2+}\)>Mg\(^{2+}\)>K\(^{+}\), respectively. The order of the degree of total cations desorption from a Greek zeolite-rich rock was Ca\(^{2+}\)>Na\(^{+}\)>Mg\(^{2+}\)>K\(^{+}\) (Kantiranis et al., 2011).

3.1.1 Adsorption Isotherms

The adsorption of NH\(_{4}^{+}\) and K\(^{+}\) from anaerobically digested swine manure by Australian natural and sodium zeolites were well described by the Langmuir adsorption isotherms (\(R^2>0.95\)). The results are consistent with the zeolites having monolayer adsorption with uniform distributions of adsorption sites. The parameters of the Langmuir equations are presented in Table 2.

According to the Langmuir adsorption isotherms the maximum NH\(_{4}^{+}\) adsorption capacity of both Australian natural and sodium zeolite were estimated as 6.75, and 6.47 mg-N/g, respectively. The maximum K\(^{+}\) adsorption capacity of both Australian natural and sodium zeolites were estimated as 19.7 and 19.5 mg-K/g, respectively, in anaerobically digested swine manure at pH 7.7 (±0.09). The maximum NH\(_{4}^{+}\) adsorption capacity of natural and sodium zeolites in a solution containing only NH\(_{4}^{+}\) were 9.48 mg-N/g and 11.83 mg-N/g respectively (Wijesinghe et al., 2016). Similarly, Liu and Lo (2001) found NH\(_{4}^{+}\) and K\(^{+}\) adsorption by Canadian zeolite (pebbles) from composting leachates were 3.06 mg-N/g and 32.7 mg – N/g. Guo et al. (2008) found that with a pseudo-second order model at equilibrium that uptakes of NH\(_{4}^{+}\) and K\(^{+}\) by zeolite with 85% clinoptilolite were 6.3 mg-N/g and 20.9 mg-K/g, respectively, in the presence of individual ions.

Table 2: Langmuir adsorption parameters for NH\(_{4}^{+}\) and K\(^{+}\) removal by natural and sodium zeolites

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameter</th>
<th>NH(_{4}^{+})</th>
<th>K(^{+})</th>
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</thead>
<tbody>
<tr>
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<td>Natural Zeolite</td>
<td>Sodium Zeolite</td>
<td>Natural Zeolite</td>
</tr>
<tr>
<td>R(^2)</td>
<td>0.993</td>
<td>0.956</td>
<td>0.998</td>
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<tr>
<td>Langmuir</td>
<td>q(_{max}) (mg/L)</td>
<td>6.75</td>
<td>6.47</td>
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<tr>
<td>K(_L)</td>
<td>0.40</td>
<td>0.11</td>
<td>0.0042</td>
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</table>
The uptakes of $\text{NH}_4^+$ and $\text{K}^+$ were 3.01 mg-N/g and 10.6 mg -K/g when both ions were present in the solution. These results confirmed that $\text{NH}_4^+$ adsorption from swine manure by natural and sodium zeolites were significantly affected by the presence of $\text{K}^+$. In this multicomponent system, there was competition between $\text{NH}_4^+$ and other cations such as $\text{Ca}^{2+}$, $\text{Mg}^{2+}$, and $\text{K}^+$. The zeolites showed selectivity for $\text{K}^+$ ions over other cations, and there was also secondary selectivity for $\text{NH}_4^+$

3.2 Scanning electron microscopy imaging (SEM) and energy dispersive X-ray spectroscopy (EDS) results

The SEM images and EDS results were obtained for both natural and sodium zeolites from a mixture of all ions in experiment 1, and for natural and sodium zeolites at the application rate of 40g/L.

**Figure 3**: SEM images of natural and sodium zeolites after ion adsorption
The SEM images revealed differences after treatment. The more regular structure of natural zeolite and sharp edges of sodium zeolite were converted to less porous and irregular structures after treatment with test solutions. When zeolite samples were treated with swine manure, they showed less porosity compared to the zeolites in experiment 1 (Figure 3).

The reason for this is colonisation of clinoptilolite particles by microorganisms by spontaneous adhesion to preferentially sheltered areas such as pits (Weiß et al., 2011). Among the zeolite samples in anaerobic digestion treatments, the natural zeolite showed the least porosity, and precipitates were observed on zeolite surfaces compare to the sodium zeolites (Figure 3).

The EDS results from experiment 1 showed the concentration of C was higher in sodium zeolite compared to the natural zeolites, and when zeolites were added to the digesters the C concentration was greater or similar in both types of zeolites (Table 3).

| Table 3: EDS results from ion adsorbed zeolites (Standard error in parentheses, n=3) |
|-----------------|-------|-------|--------|--------|
| Element | NZ @Exp.1 Wt% | SZ@Exp.1 Wt% | NZ @Exp.2 Wt% | SZ@Exp.2 Wt% |
| C      | 2.86 (0.03) | 4.54(0.13) | 8.28(0.79) | 8.71(0.14) |
| O      | 49.84 (0.57) | 47.26(0.13) | 47.78(0.74) | 49.13(0.52) |
| Na     | 0.89 (0.02) | 1.17 (0.02) | 0.40(0.01) | 0.83(0.28) |
| Mg     | 0.57 (0.01) | 0.41(0.01) | 0.50(0.05) | 0.35 (0.08) |
| Al     | 6.89 (0.04) | 6.62(0.03) | 5.69(0.11) | 6.42(0.70) |
| Si     | 31.16 (0.59) | 34.03(0.27) | 22.63(1.97) | 30.72 (0.67) |
| P      | 0.28 (0.01) | 0.18(0.01) | 3.35(0.40) | 0.04 (0.03) |
| K      | 4.04 (0.05) | 3.14(0.11) | 2.54(0.12) | 2.03 (0.21) |
| Ca     | 1.19 (0.02) | 0.75(0.08) | 5.67(0.52) | 0.52(0.08) |
| Fe     | 2.28 (0.05) | 1.90(0.02) | 2.71(0.12) | 1.25(0.08) |

NZ – Natural Zeolite, SZ – Sodium Zeolite

Adsorption of volatile fatty acids (VFAs) such as acetic acid might be a reason for this. Compared to the Na\(^{+}\) content of untreated natural zeolite, Na\(^{+}\) content of natural zeolite in experiment 1 (Test solution no.7), and Na\(^{+}\) content in the natural zeolite in the digesters (experiment 2) increased by 141% and 29% respectively. Na\(^{+}\) adsorption by natural zeolite
might be the reason for this. However, during digestion the Na$^+$ content of sodium zeolite decreased by 8% compared to the results from EDS of sodium zeolite in a previous study (Wijesinghe et al., 2016). The K$^+$ concentrations increased in zeolite after the treatments because of its adsorption. However, Ca$^{2+}$ and total P contents in natural zeolite markedly increased during digestion (by 1096% for Ca and 376% for P) compared to the natural zeolite in experiment 1. Lin et al. (2014) also observed Ca$^{2+}$ and P increased in zeolite after it was treated with wastewater. Precipitation of Ca$_3$(PO$_4$)$_2$ onto NH$_4^+$ loaded zeolite may be the reason for increases of Ca$^{2+}$ and P in zeolite after treatment with wastewater (Karapinar, 2009).

### 3.3 Effects of zeolites on NH$_4^+$ removal from digesters

The removal of NH$_4^+$ increased linearly with increasing zeolite doses for both natural and sodium zeolites (Figure 4).

![Figure 4](image)

**Figure 4:** The NH$_4^+$ removal percentages of digesters of swine manure and C:N adjusted digesters with zeolite doses. (Vertical bars represent standard errors, n = 3)

Optimising the substrate C:N ratio enhanced the efficiency of anaerobic digestion greatly (Wang et al., 2014). The C:N ratio adjusted swine manure produced more NH$_4^+$ as a result of enhanced digestion. However, zeolite removed a considerable amount of NH$_4^+$ from the high concentrations of NH$_4^+$ in solutions (Wijesinghe et al., 2016, Lin et al., 2013). Therefore digesters with adjusted C:N ratios showed larger removal rates in the presence of zeolite than
digesters with unadjusted swine manure. Two-way ANOVA was applied to the NH₄⁺ concentration results of the digesters that used natural and sodium zeolites with swine manure and C:N ratio adjusted swine manure. The only significant interaction was between manure types (swine manure and C:N ratio adjusted swine manure) and zeolites doses (0, 10g/L, 40g/L, 70g/L and 100g/L) (p < 0.001). The interaction between zeolites types (natural and sodium) and zeolites doses (p=0.533) and the interaction between zeolites types and the manure types (p=0.549) and the interaction of zeolite types, zeolite dose, and manure types, were not significant (p = 0.943). Fisher’s tests revealed significant differences between the NH₄⁺ concentrations of the natural and sodium digesters at the zeolite application rates of 40g/L, 70g/L and 100g/L compared to the NH₄⁺ concentration of the nil zeolite swine manure digesters.

3.4 Effects on zeolite on cation concentrations in digesters

The cations Na⁺, K⁺, Ca²⁺, and Mg²⁺ are present in the influent of anaerobic digesters and are released by the breakdown of organic matter (Grady Jr et al., 2011). They are required for microbial growth and, consequently, affect specific growth rates like any other nutrient (Chen et al., 2008). While moderate concentrations stimulate microbial growth, excessive amounts slow down growth, and even higher concentrations can cause severe inhibition or toxicity (Soto et al., 1993).

3.4.1 Potassium

The average K⁺ concentration of nil-zeolite digestions was 910 mg/L and 1004 mg/L in C:N ratio adjusted swine manure digestions. Those levels decreased linearly with increasing doses of both natural and sodium zeolites (Figure 5).

A two-way ANOVA model was fitted to the K⁺ concentration data for the digestions with natural or sodium zeolites with swine manure or C:N ratio adjusted swine manure. The interaction of zeolite types (natural and sodium), manure type (swine manure and C:N ratio adjusted swine manure), and zeolite doses (10g/L, 40g/L, 70g/L, 100g/L) with K⁺ concentration inside the digesters were not significant. The interaction between zeolite type and zeolite dose (p = 0.316) and the interaction between zeolite type and manure type (p=0.791) are not significant. Significant differences were only shown by the interaction of zeolite doses and manure types.
Figure 5: K$^+$ concentration changes in the digesters with the zeolite doses. (Vertical bars represent standard errors, n = 3)

Both natural and sodium zeolites showed larger adsorption capacities of K$^+$ than for NH$_4^+$. Compared to the nil-zeolite digesters, 84% and 82% reductions of K$^+$ concentrations were observed in the digesters that had natural and sodium zeolites at 100g/L of digestate, respectively. Low concentrations of K$^+$ are favourable for anaerobic digestion, and inhibition by K$^+$ is induced at 400 mg/L (Chen et al., 2008). Relieving K$^+$ inhibition is beneficial for acidogenic microorganisms (Fernandez and Forster, 1994) as at higher K$^+$ levels there is a passive influx of K$^+$ that neutralises the membrane potential (Jarrell et al., 1984). Therefore, application of zeolites to anaerobic digestion is beneficial as it keeps K$^+$ concentrations at low concentrations.

3.4.2 Sodium

The average Na$^+$ concentrations of both nil-zeolite swine manure and swine manure zeolite adjusted digesters were similar at the end of 60 days (i.e. 694mg/L and 695 mg/L) The Na$^+$ concentrations increased linearly with increasing zeolite doses, either natural or sodium, in both swine manure and C:N ratio adjusted swine manure digesters (Figure 8). It reached a maximum of 965 mg/L with 100g/L natural zeolite. Sodium zeolites released more Na$^+$ while adsorbing other ions and it reached 952 mg/L in the digesters at 10g/L zeolite and 2250mg/L
at 100g/L of sodium zeolites. The optimum concentration of Na\(^+\) for mesophilic anaerobic digestion is 100-350 mg/L (McCarty, 1964). The Na\(^+\) concentration favourable for methanogens is < 1000 mg/L (Lee et al., 2012). The greater concentrations in sodium zeolite might be one reason for smaller CH\(_4\) production in sodium zeolites digesters compared to the natural zeolite digesters (Chapter 6.1 & 6.2). According to the two-way ANOVA, the interaction among zeolites types (natural and sodium), manure types (swine manure and C:N ratio adjusted swine manure), and zeolite doses (10g/L, 40g/L, 70g/L, 100g/L), and the interaction between manure types and zeolite doses (p =0.018), and the interaction between zeolite types and manure types (p = 0.202) were not significant. The only significant interaction was the interaction between zeolite types and zeolite doses (p < 0.001).

**Figure 6:** Na\(^+\) concentration changes in the digesters with zeolite doses. (Vertical bars represent standard errors, n = 3)

### 3.4.3 Magnesium

Average Mg\(^{2+}\) concentrations in nil-zeolite swine manure digesters and C:N ratio adjusted swine manure digesters were 230 mg/L and 1458 mg/L, respectively. With larger zeolites doses the Mg\(^{2+}\) concentration increased linearly (Figure 9) as a result of the release of Mg\(^{2+}\) by zeolites in response to the adsorption of other ions. According to experiment 1 Australian natural zeolite desorbed more Ca\(^{2+}\) than Mg\(^{2+}\) but in swine manure digesters more Mg\(^{2+}\) was present in the digestate than Ca\(^{2+}\). Excess Ca\(^{2+}\) in the digestate leads to carbonate and phosphate precipitation (Klass, 1998). Ca\(^{2+}\) precipitation might be the reason for lower Ca\(^{2+}\)
concentrations than Mg$^{2+}$ ions in the digesters. Mg$^{2+}$ ion decreases may be expected in the digestion media as a result of struvite (magnesium ammonium phosphate hexahydrate) formation. However, decreases in pH due to the addition of zeolite may inhibit struvite formation. Struvite precipitation occurs at pH 8.0 and above (Wang et al., 2005). In this study, the pH values of the digestion media were less than 8 as adding zeolite caused reductions in the pH of the digestion media. Therefore, struvite formation was diminished in zeolite treated digesters. Correspondingly, Mg$^{2+}$ concentrations linearly increased with increasing zeolite doses from 10g/L to 100g/L. Australian natural zeolite released more Mg$^{2+}$ than sodium zeolite with the adsorption of K$^+$ or NH$_4^+$ ions, thus more Mg$^{2+}$ was observed in natural zeolite digesters than sodium zeolite digesters. However, Mg$^{2+}$ contents were much higher in digesters with C:N adjusted swine manure compared to swine manure digesters, as a result of faster digestion rates at the optimum C:N ratio.

Figure 7: Mg$^{2+}$ concentration changes in the digesters with the zeolite doses. (Vertical bars represent standard errors, n = 3)

According to the two-way ANOVA, the interaction among zeolites types (natural and sodium), manure types (swine manure and C:N ratio adjusted swine manure), and zeolites doses (10g/L, 40g/L, 70g/L, 100g/L) and interaction between manure types and zeolites doses and the interaction between zeolites types and manure types were significant (p < 0.001). According to Fisher’s multiple comparisons, except swine manure digesters with natural and sodium zeolite application rate 10g/L, other digesters significantly varied from the control (nil-zeolite digesters).
3.4.4 Calcium

K\(^+\) and NH\(_4\)\(^+\) adsorption and Na\(^+\) and Mg\(^{2+}\) desorption showed linear relationships with increasing zeolite doses in the digesters, but Ca\(^{2+}\) concentrations did not show a linear relationship with zeolite doses (Figure 7). Above the 10g/L zeolite application rate, digesters with sodium zeolites showed greater Ca\(^{2+}\) concentrations than natural zeolite digesters, and natural zeolite released more Ca\(^{2+}\) than sodium zeolite during adsorption (Figure 7). According to the two-way ANOVA analysis of Ca\(^{2+}\) concentrations inside the digesters, with the exception of the interaction between zeolite types and manure types, (p = 0.607) other interactions (zeolite type * zeolite doses, manure types * zeolite doses, manure types*zeolite types* zeolite doses) were significant (p < 0.001).

Calcium mitigates NH\(_4\)\(^+\) toxicity as an antagonistic ion (McCarty and McKinney, 1961). The desorbed Ca\(^{2+}\) from zeolites could have a similar effect in the present study. Ca\(^{2+}\) is known to be essential for the growth of certain strains of methanogens (Murray and Zinder, 1985). The addition of Ca\(^{2+}\) can have a positive impact on reactors in which retention of biomass is desired (Chen et al., 2008). The performance of swine manure anaerobic digestion improved with increasing Ca\(^{2+}\) levels in the digestions and reached its maximum at 3 g/L (Ahn et al., 2006). However, excess Ca\(^{2+}\) in the digestate leads to carbonate and phosphate precipitation (Klass, 1998). Calcium carbonate (CaCO\(_3\)) precipitation is dependent on the Ca\(^{2+}\) concentration and the COD removal efficiency (Van Langerak et al., 1998). The Ca\(^{2+}\) in the wastewater reacts with PO\(_4\)^{3-} generating substances such as hydroxyapatite, dicalcium phosphate, and octa calcium phosphate (Lee et al., 2015). The desorbed Ca\(^{2+}\) ions from zeolite may have precipitated with dissolved PO\(_4\)^{3-}. Figure 8 shows that the Ca\(^{2+}\) concentration inside the digesters decreased with increasing pH. The pH is a key factor in controlling the quantity and crystalline structure of calcium phosphates obtained by precipitation, and at constant pH, the Ca/P ratio strongly affects the precipitation efficiency of Ca\(_3\)(PO\(_4\))\(_2\) (Mekmene et al., 2009). These might be the reasons for no increase of Ca\(^{2+}\) levels inside the digesters with increasing zeolites doses, and the reason for higher Ca\(^{2+}\) concentrations in sodium zeolites digesters than natural zeolite.

Swine manure contains large concentrations of lipids, 30–40%, based on chemical oxygen demand (COD) (Chynoweth et al., 1999). Lipids are readily hydrolyzed to glycerol and long-chain fatty acids (LCFAs), which are the main inhibitory factor of anaerobic microorganisms (Ma et al., 2015).
Therefore, adding zeolite may reduce the inhibitory effect of LCFAs on anaerobic digestion in an indirect way since the LCFAs from swine manure digestate precipitate with Ca\(^{2+}\) as a calcium salt.

### 3.5 Effect of zeolite on phosphorus and sulphur in the digesters

#### 3.5.1 Total Phosphorus

P is one of the key nutrients that control bacterial growth. The total P concentration was reduced with the addition of zeolite, and digesters with natural zeolite showed smaller P concentrations than those with sodium zeolite. There was no dissolved P observed above the zeolite application rates of 40g/L in any of the digesters with swine manure. However, the total dissolved P concentrations were higher in digesters with C:N adjusted swine manure, and zeolite application rates of 70 and 100g/L in both natural and sodium zeolites, and at 40g/L in sodium zeolite (Figure 9). The pH values of these digesters were comparatively lower than the digesters that had low total P concentration (Figure 9). Therefore, P removal from digested effluent increased with increasing zeolite doses and pH.
Figure 9: Total P concentration changes in the digesters with the zeolite doses. (Vertical bars represent standard errors, n = 3)

Figure 10: Relationship of $\text{Ca}^{2+}$ and P with pH. (Vertical bars represent standard errors, n = 3)

A larger concentration of zeolite at higher pH might not be suitable for anaerobic digestion as zeolites enhance $\text{PO}_4^{3-}$ precipitation by releasing more $\text{Ca}^{2+}$ into the medium. However, there is no $\text{Ca}^{2+}$ limitation caused by adding zeolites during the digestion process as zeolites release...
more Ca\textsuperscript{2+} by ion adsorptions. According to the two-way ANOVA analysis of total P concentration inside the digesters, except for the interaction between zeolite types and manure types, other interactions (zeolite type * zeolite doses, manure types * zeolite doses, manure types*zeolite types* zeolite doses) are significant (p < 0.001).

3.5.1.1 The mechanism of P removal by Australian natural zeolite

Zeolites remove P by anion exchange and electrostatic attraction (Dionisiou et al., 2013, Jiang et al., 2013) or chemical interactions (Wu et al., 2006). Dissolved PO\textsubscript{4}\textsuperscript{3-} precipitates with Ca\textsuperscript{2+} derived from the zeolite lattice during ion exchange processes (Lin et al., 2014). Experiment 1 showed P adsorption by zeolite increased with the presence of Ca\textsuperscript{2+} in solution. Total dissolved P can be present in a range of chemical forms depending on the pH, i.e. H\textsubscript{3}PO\textsubscript{4} at pH 2; H\textsubscript{2}PO\textsubscript{4}\textsuperscript{-} at pH 3-6.5; HPO\textsubscript{4}\textsuperscript{2-} at pH 7.5-12; PO\textsubscript{4}\textsuperscript{3-} at pH > 12 (Tisdale, 1993).

At relatively high pH values the surfaces of adsorbents carry more negative charges and repulses negatively charged species in solution (Chen et al., 2013). As the pH increases OH\textsuperscript{-} competes strongly with phosphate for active sites, which affects adsorption capacity (Chen et al., 2013). Total P concentrations decreased in the solution with increasing pH suggesting that neither the mechanisms of anion exchange or electrostatic attraction caused the removal of P by Australian natural zeolite (Figure 5). The SEM images of Australian natural zeolite recovered from the digester at pH 7.7 showed precipitate which cannot be seen in zeolite at pH 7. According to the EDS results, concentrations of Ca\textsuperscript{2+} and total dissolved P increased (i.e., 1096% and 376%) in natural zeolites at pH 7.7 compared to the zeolites used in experiment 1, test solution 7 (pH 7). This indicates the amount of Ca\textsuperscript{2+} and P increased on zeolite in digestion media because of phosphate precipitation on zeolite particle surfaces. Karapınar (2009) found Ca\textsubscript{3}(PO\textsubscript{4})\textsubscript{2} precipitate-coated zeolites surface increased with an increase in particle size of zeolite. As described above, and as shown in Figure 10, pH is a key factor controlling the quantity and crystalline structure of Ca\textsubscript{3}(PO\textsubscript{4})\textsubscript{2} precipitate. The average pH of experiment 1 was 7, and the average pH in zeolite 40g/L digesters was 7.7. This pH difference might be a possible reason for a larger percentage of Ca\textsuperscript{2+} and P on zeolites in these digesters. At more or less similar pH, with increasing zeolite doses, total dissolved P concentrations inside the digesters decreased (Figure 10). According to experiment 1, Ca\textsuperscript{2+} is the major ion desorbed by Australian natural zeolite. With increasing zeolite doses, more Ca\textsuperscript{2+} ions accumulated in the solution because of displacement by other ions (Equation 4 and 5). At pH around 7.7 there would be HPO\textsubscript{4}\textsuperscript{2-} ions in the solutions. Therefore desorbed Ca\textsuperscript{2+} could react to form hydroxyapatite (HAP) (Ca\textsubscript{5}OH (PO\textsubscript{4})\textsubscript{3})
(Equation 6). $\text{Ca}_5\text{OH(PO}_4\text{)}_3$ has the highest thermodynamic stability among the different kinds of calcium phosphate precipitates (Koutsoukos, 2000). The reaction (Equation 6) is favourable at pH > 9 but it takes place at pH > 7 (Vanotti et al., 2003). Therefore Australian natural zeolite can be used to remove P during the anaerobic digestion process of swine manure. The adsorption of $\text{K}^+$ and $\text{NH}_4^+$ by zeolites may assist in removal of P as natural zeolite desorbed more $\text{Ca}^{2+}$ by displacement by $\text{K}^+$ and $\text{NH}_4^+$

$$\text{Zeolite} - \text{Ca}^{2+} + 2\text{K}^- \rightarrow \text{Zeolite} - 2\text{K}^- + \text{Ca}^{2+} \quad (4)$$

$$\text{Zeolite} - \text{Ca}^{2+} + 2\text{NH}_4^+ \rightarrow \text{Zeolite} - 2\text{NH}_4^+ + \text{Ca}^{2+} \quad (5)$$

$$5\text{Ca}^{2+} + 3\text{HPO}_4^{2-} + 4\text{OH}^- \rightarrow \text{Ca}_5\text{OH(PO}_4\text{)}_3 \downarrow + 3\text{H}_2\text{O} \quad (6)$$

3.5.2 Sulphur

Sulphate derived from a protein-rich diets (Hansen et al., 1999), feed additives for improving feed utilisation (antibiotics and chemotherapeutics) (Varel and Hashimoto, 1981), and disinfectants, which are used for preventing infectious diseases, result in S compounds in swine manure (Hilpert et al., 1984, Poels et al., 1984). The S concentration inside the digesters decreased with increasing zeolite doses, and Australian natural zeolites removed more S than sodium zeolites (Figure 11). Sulphur is an essential nutrient for methanogens (O'Flaherty et al., 1998). The optimal level of sulphur reported in the literature varies from 1 to 25 mg S/L (Scherer and Sahm, 1981). However, total sulphide concentrations above pH 7.2 were identified as inhibitory to all microorganisms (O'Flaherty et al., 1998). Zeolites are promising materials for adsorbing S compounds (Velu et al., 2002). A larger amount of S was observed in C:N ratio adjusted swine manure digesters compared to the swine manure digesters (Figure 11). Enhancing digestion at the optimum C:N ratio may be the reason for higher concentration of S in C:N ratio adjusted swine manure digesters than the swine manure only digesters. However, zeolite addition showed a reduction in the S content inside the digesters and Australian natural zeolites showed larger removal of S than sodium zeolite. Therefore adding zeolite to anaerobic digestion would be another benefit of zeolite application as it removes S.

According to the two-way ANOVA analysis of S concentrations inside the digesters, the only significant interaction was that between manure types and zeolite doses. (p < 0.01).
Figure 11: S concentration changes in the digesters with zeolite doses. (Vertical bars represent standard errors, n = 3)

3.6 Correlation analysis

The correlations between physical and chemical parameters and zeolite doses during the anaerobic digestion were determined. The results are presented in Table 4. Significant strong positive correlations (p < 0.01) are shaded in green, and strong negative correlations (p < 0.01) are shaded in yellow. As expected, zeolite doses had strong positive relationships with Na\(^+\), Ca\(^{2+}\), Mg\(^{2+}\) and NH\(_4^+\) removal % and strong negative correlations with K\(^+\), NH\(_4^+\) and pH of the digested effluent. Total dissolved P was strongly positively correlated with Ca\(^{2+}\) and Mg\(^{2+}\). Both P and Ca\(^{2+}\) were strongly negatively correlated with pH. These results reflect that Ca\(^{2+}\), Mg\(^{2+}\) and P were present in the digested effluent at lower concentrations at higher pH values. Other than P adsorption by zeolites, P removal can be enhanced by Ca\(^{2+}\) released by zeolites at high pH. This relationship was reported by several authors (Montalvo et al., 2011, Lin et al., 2014). The NH\(_4^+\) concentrations did not show any correlation with Ca or P. Similarly, Nuray (2009) and (Karapınar, 2009) found there was no significant effect of NH\(_4^+\) on the precipitation of PO\(_4^{3-}\).
Table 4: Correlation analysis of physical–chemical parameters

<table>
<thead>
<tr>
<th>Zeolite Dose</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>P</th>
<th>S</th>
<th>Ca²⁺</th>
<th>Mg²⁺</th>
<th>NH₄⁺</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>0.646</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K⁺</td>
<td>-0.95</td>
<td>-0.617</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.392</td>
<td>0.392</td>
<td>-0.32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>-0.325</td>
<td>-0.088</td>
<td>0.457</td>
<td>0.453</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>0.533</td>
<td>0.371</td>
<td>-0.446</td>
<td>0.943</td>
<td>0.367</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>0.805</td>
<td>0.506</td>
<td>0.606</td>
<td>0.789</td>
<td>0.096</td>
<td>0.856</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>-0.908</td>
<td>-0.581</td>
<td>0.938</td>
<td>-0.284</td>
<td>0.586</td>
<td>-0.388</td>
<td>-0.683</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>-0.554</td>
<td>-0.428</td>
<td>0.497</td>
<td>-0.952</td>
<td>-0.343</td>
<td>-0.975</td>
<td>-0.882</td>
<td>0.439</td>
</tr>
<tr>
<td>NH₄⁺ removal %</td>
<td>0.87</td>
<td>0.446</td>
<td>-0.829</td>
<td>0.567</td>
<td>0.19</td>
<td>0.653</td>
<td>0.872</td>
<td>-0.837</td>
</tr>
</tbody>
</table>

Note: Positive correlation with a p < 0.01 is highlighted in green and negative correlations with a p < 0.01 are highlighted in yellow.

4. Conclusions

This study investigated Australian natural and sodium zeolites for simultaneous removal of N, K and P during the anaerobic digestion of swine manure. Adsorption isotherms confirmed a higher selectivity for K⁺ than for NH₄⁺ by Australian natural and sodium zeolites. Therefore NH₄⁺ removal was considerably reduced by K⁺ uptake. The orders of the degree of total cation desorption from Australian natural and sodium zeolites at steady state were: Ca²⁺ > Na⁺ > Mg²⁺ > K⁺ and Na⁺ > Ca²⁺ > Mg²⁺ > K⁺ respectively. Australian natural zeolite was shown to be a potential sorbent for the removal of NH₄⁺, K⁺ and P when added to the anaerobic digestion of swine manure. However, larger concentrations of zeolite at higher pH might not be appropriate for anaerobic digestion as zeolite removed P resulting in inadequate available P for microbial growth. The Australian natural zeolite produced during the anaerobic digestion process has potential as a nutrient-rich slow release fertiliser.
References


Chapter 7

Developing an anaerobic digester with external zeolite filled column

This chapter is based on a manuscript which is submitted entitled:

Developing an anaerobic digester with external zeolite filled column for enhancing methane production from swine manure - A feasibility study. Submitted to Environmental Engineering and Management Journal.
Chapter 7

Developing an anaerobic digester with external zeolite filled column for enhancing methane production from swine manure - A feasibility study

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Abstract:

Development of digesters with an external zeolite column facilitates the convenient removal of the zeolite with TAN, without disturbing the continuous anaerobic digestion process. A digester with an inside zeolite bed (In-Zeo) and digester without adding zeolite (No-Zeo) were employed to compare the process performance with digester with external zeolite column (EX-Zeo). The cumulative, CH\textsubscript{4} yields were 5\% and 15\% greater in the EX-Zeo, and the In-Zeo digesters respectively compared to the No-Zeo digesters. Also, the % VS reduction was 49\%, 55\%, and 41\% respectively in the Ex-Zeo, In-Zeo and No-Zeo digesters. The results indicated that treatment with 7\% zeolite during anaerobic digestion has the potential to improve biodegradation of swine manure. The addition of zeolite appeared to reduce TAN from the digestate, thereby enhancing the CH\textsubscript{4} yield. Zeolite could be used either internally or externally to enhance CH\textsubscript{4} production through anaerobic digestion of swine manure.

Key words: Ammonium, External column, Methane, Swine manure, Zeolite

Highlights:

- A novel Ex-Zeo digester was developed to identify the feasibility of the addition of a zeolite column as an external column to the anaerobic digestion process of swine manure
- The highest biogas and CH\textsubscript{4} production were obtained from internal zeolite digester and digester with external zeolite column
- Australian zeolite is identified as an effective way of enhancing CH\textsubscript{4} production from anaerobic digestion process by removing ammonia inhibition and Potassium inhibition
1. Introduction
Anaerobic digestion of animal manure with biogas capture provides both greenhouse gas savings and revenues from generating electricity by converting biogas to electricity (Chia-Wei Chang, 2015). Despite the advantages of anaerobic digestion, poor operational stability caused by factors such as feed compositions and total ammoniacal nitrogen (TAN) (TAN is a summation of ionised ammonium nitrogen (NH$_4^+$) and free (unionised) ammonia nitrogen (FAN) concentrations) limits anaerobic digesters being widely adopted and commercialised (Amani et al., 2010). Accumulation of high TAN concentrations inside the anaerobic digester inhibits methanogenesis (Chen et al., 2008). Methanogenesis inhibition occurs in a TAN concentration range of 760 to 4000 mg NH$_4^+$- NL$^{-1}$ (Bousek et al., 2016) and the threshold values at which ammonia inhibition begin ranges from a FAN concentration of 80 mg/L (Koster and Lettinga, 1984) to 150 mg/L (Braun et al., 1981). In addition, Swine manure has a low C/N ratio, due to high nitrogen content result in lower biogas production (Mydeen et al., 2016). The C/N ratio for swine manure is around 6 to 8, depending upon their growth stages (MWPS, 2000), which is too low for efficient anaerobic digestion process to maximize the methane production (Wu et al., 2010). Therefore, low CH$_4$ production and VFA accumulation inside the reactor are very common in the anaerobic digestion process of nitrogen-rich animal slurries such as swine manure (Hansen et al., 1998).

Zeolite adsorb NH$_4^+$ from aqueous solutions (Wijesinghe et al., 2016) hence can alter the C:N ratio of swine manure. Additionally, zeolite addition was identified as an efficient manner to address the NH$_4^+$ inhibition process, resulting in enhanced biogas production during anaerobic digestion (Montalvo et al., 2012). Importantly, improvement in bioreactor configuration for optimising digester performance with zeolites is not well studied. A fixed zeolite bioreactor was studied by Wang et al. (2011) and Zheng et al. (2015) at the zeolite application rate of 4 g/L. In these studies, zeolite was added into the digester. It is a major drawback of this configuration that digesters have to be opened to remove zeolite after NH$_4^+$ saturation. As such controlling ammonia inhibition was the focus, not recovery of maximum TAN from the digested animal manure without hindering biogas production. Development of digesters with external zeolite columns facilitates the convenient removal and filling of zeolite after saturation with TAN, without disturbing the continuous anaerobic digestion process. Utilisation of the higher application rates of zeolite mitigates TAN losses from the digested effluent. Therefore, in this study, a digester with an external zeolite column (Ex-Zeo digester) was developed to treat swine manure under mesophilic anaerobic conditions. A
higher zeolite application rate than studied in the literature was selected. Anaerobic digestion process performance was evaluated throughout the experiment. An anaerobic digester with an internal zeolite bed (In-Zeo digester) and digester without added zeolite (No-Zeo digester) were employed to compare anaerobic digestion process performance to an external zeolite column. Before the anaerobic digestion trial, a column test was used to identify the adsorption behaviour of zeolite in the column.

2. Materials and Methods

2.1. Column adsorption studies
Nine glass columns with 150 g Australian natural zeolite (particle size 0.5mm to 1 mm) were arranged to determine the adsorption kinetics under dynamic conditions. The volume of the column and bed height was 0.15 L and 65 mm respectively. All columns were fed with an NH\textsubscript{4}Cl solution (1000 mg-N/L) continuously for up to 420 minutes using a peristaltic pump in down flow mode with flow rates of 13 mL/min, 15 mL/min and 17 mL/min.

2.2. Design of the anaerobic digester
A lab-scale anaerobic digester equipped with three sampling ports and a gas valve was used. The total volume of the digester was 5 L and the working volume 4 L. The digester was a glass column (diameter 55 mm and height 400 mm, IL) with sintered borosilicate glass discs at the bottom. The external glass column with zeolite was attached to the digester by connecting an inlet tube to the base of the digester via peristaltic pump. Another tube from the top of the glass column connected the gas collector. An outlet tube from the bottom of the glass column was attached to the peristaltic pump to move swine manure into the digester using the same peristaltic pump as that used to move swine manure from the digester to the external column (Fig.1a). Another lab-scale anaerobic digester was employed as the internal zeolite bed digester (Fig. 1b).
Fig. 1: a Schematic diagram of the lab-scale Ex-Zeo digester b. Schematic diagram of the In-Zeo digester
2.3. Inoculum and swine manure

The anaerobic digestion inoculum was collected from the anaerobic digester at Berrybank Farm Piggery and swine manure were collected from Berrybank Farm Piggery, at Windermere Victoria, Australia. The farm has a swine population of 20,000. Table 1 shows the chemical properties of the swine manure and inoculum.

2.4. Zeolite:

Australian natural zeolite was obtained from Zeolite Australia Private Limited, Werris Creek, NSW, Australia. This Australian zeolite contained 55% clinoptilolite. The physical and chemical characteristics of the Australian zeolite were reported in Wijesinghe et al. (2016). The zeolite was dried overnight at 105°C before use in experiments. 70g/L of zeolite was added to the zeolite column based on the results from preliminary studies. This ensured optimum CH₄ production corresponding to optimum NH₄⁺ removal.

Table 1: Characteristics of inoculum and swine manure (Standard error in parentheses, n=3)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Inoculum</th>
<th>Swine manure</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS %</td>
<td>3.68 (0.08)</td>
<td>2.28 (0.06)</td>
</tr>
<tr>
<td>VS % (%TS)</td>
<td>69 (0.74)</td>
<td>74 (0.44)</td>
</tr>
<tr>
<td>Total N % (%TS)</td>
<td>6.51 (0.11)</td>
<td>5.5 (0.12)</td>
</tr>
<tr>
<td>Total C % (%TS)</td>
<td>32.87 (0.71)</td>
<td>33.41 (0.91)</td>
</tr>
<tr>
<td>pH</td>
<td>7.46 (0.02)</td>
<td>7.12 (0.01)</td>
</tr>
<tr>
<td>EC (mS)</td>
<td>11.47 (0.04)</td>
<td>8.09 (0.06)</td>
</tr>
<tr>
<td>TDS (ppk)</td>
<td>5.54 (0.04)</td>
<td>8.77 (0.06)</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>2192 (26)</td>
<td>7268 (291)</td>
</tr>
<tr>
<td>BOD (mg/L)</td>
<td>582 (21)</td>
<td>1494 (40)</td>
</tr>
<tr>
<td>Alkalinity (mg/L)</td>
<td>3800 (58)</td>
<td>3033 (88)</td>
</tr>
<tr>
<td>N-NH₄⁺ in the slurry (mg/L)</td>
<td>1247 (16)</td>
<td>656 (23)</td>
</tr>
<tr>
<td>K⁺ in the slurry (mg/L)</td>
<td>764 (28)</td>
<td>471 (6)</td>
</tr>
<tr>
<td>Ca²⁺ in the slurry (mg/L)</td>
<td>65 (7)</td>
<td>11 (2)</td>
</tr>
<tr>
<td>Mg²⁺ in the slurry (mg/L)</td>
<td>10 (1)</td>
<td>2 (0.6)</td>
</tr>
<tr>
<td>Na⁺ in the slurry (mg/L)</td>
<td>242 (10)</td>
<td>251 (4)</td>
</tr>
<tr>
<td>Total S in the slurry (mg/L)</td>
<td>30 (1)</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>Total p in the slurry (mg/L)</td>
<td>76 (2)</td>
<td>58 (6)</td>
</tr>
</tbody>
</table>
2.5. Anaerobic digestion experiments

Three groups of experiments were conducted in three different digesters configurations to investigate the performance of the zeolite columns (i.e. Ex-Zeo digester, In-Zeo digester and No-Zeo digester). All digesters were continuously stirred at 350 rpm and fed with swine manure and inoculum in a 1:1 ratio. The headspace of each digester was purged with N₂ to ensure anaerobic conditions inside the digesters. Each group of experiments was performed in triplicate. The experiments were conducted in batch mode at 37 °C for 40 days.

Swine manure inside the digester was introduced at a flow rate of 13 mL/min into the external zeolite column by a peristaltic pump after closing valves 3 and 4 (Fig.1a). The feed was introduced to the zeolite column in up flow mode while filtering it through a sintered borosilicate glass disc. This was operated for 40 minutes for each day to ensure the circulation of 500 mL of swine manure via the zeolite column. After sending 500 mL through the zeolite column, swine manure in the external column was collected again into the digester using the same peristaltic pump by closing valves 1 and 2. This procedure was continued up to 8 days to ensure circulation of the full volume of digestate (4 L) via the external zeolite column.

2.6. Analytical methods

Biogas was collected using a water displacement method, and the volume of biogas was monitored daily analysed by gas chromatography (7890A, Agilent Technologies, Wilmington, USA). Samples from all digesters were collected daily until 8 days and then collected once in 4 or 5 days. Samples of leachate prior to the column and in the column were collected. The total solids (TS) and volatile solids (VS) were measured according to standard methods (APHA, 2012). The pH of the effluent was determined using a pH meter (labCHEM-CP, TPS division, Elexon Electronics Pty Ltd., QLD, Australia). Bicarbonate alkalinity was determined using a colorimetric method. Soluble chemical oxygen demand (COD) was measured using a SCOD analyser Hanna reactor with a high range of reagent (Hanna Instruments Australia, Victoria, Australia). Sodium (Na⁺), potassium (K⁺), Calcium (Ca²⁺), magnesium (Mg²⁺), total phosphorous (P) and sulphur (S) concentrations were analysed by inductively coupled plasma (ICP- OES, Optima, Model 8000, Perkin Elmer). The amount of NH₄⁺ adsorbed by zeolite was determined after 2M KCl extraction (Chapman, 1965). The concentrations of NH₄⁺ in effluent and in the KCl extraction solution were measured using a segmented flow analyser (Skalar SAN⁺⁺, Skalar, Breda, The Netherlands).
2.7. Data Analysis

2.7.1. Calculations

2.7.1.1. Ammonium adsorption

The amount of NH$_4^+$ adsorbed per unit weight of zeolite over a certain period for which solution went through zeolite in the column was determined by the following formula.

\[
q = \frac{(C_i - C_t)Qt}{m} \quad (1)
\]

Where q is the amount of NH$_4^+$ adsorbed in a period of time, mg NH$_4^+$ - N / g of zeolite. C$_i$ is the initial NH$_4^+$ concentration, mg NH$_4^+$ - N /L. C$_t$ is the NH$_4^+$ concentration after solution sending through the zeolites column after a certain period mg NH$_4^+$ -N /L. Q is the flow rate of the solution through the column (L/min). t is the period that the solution went through the column, min.

2.7.1.2. Adsorption Efficiency:

The NH$_4^+$ adsorption efficiency was determined using Equation 2

\[
E = \left(\frac{m_e}{m_o}\right) * 100 = \left(\frac{qm}{C_iQt}\right) * 100 \quad (2)
\]

Where, E is the NH$_4^+$ adsorption efficiency %, and m$_e$ is the total NH$_4^+$ adsorption by zeolite in the column at a given time t. m$_o$ is the amount of NH$_4^+$ - N (mg) when solution runs through the zeolite in the column at a given time.

2.7.1.3. Free Ammonium nitrogen

The equilibrium concentrations among FAN and NH$_4^+$ in the aqueous solution are depicted in the following equation.

\[
\text{NH}_4^+ + \text{OH} \leftrightarrow \text{NH}_3 + \text{H}_2\text{O} \quad (3)
\]

The equilibrium concentrations of NH$_4^+$ are pH and temperature (T) dependent as given by equation 4 (Sommer et al., 2013).

\[
[N\text{H}_3(aq)] = \frac{T\text{AN (aq)}}{1+([H_3O^+ (aq)]/K_N)} = \frac{T\text{AN (aq)}}{1+10^pK_N-p\text{H}} \quad (4)
\]

\[
pK_N = 4 \times 10^{-8}T^3 + 9 \times 10^{-5}T^2 - 0.0356T + 10.072 \quad (5)
\]

Where K$_N$ is the acid ionization constant for NH$_3$ and pK$_N$ is the acid dissociation constant value for NH$_4^+$/NH$_3$ in an aqueous solution which can be estimated using equation 5.
2.7.1.4. **Kinetics of CH$_4$ production – First order model:**

For the kinetic characterization of each experiment, the kinetic equation for CH$_4$ production was applied. This equation confirms CH$_4$ production follows a first order kinetic model (Bilgili et al., 2009; Dubey et al., 2013).

\[
G = G_m [1 - \exp(-k_0 t)]
\]  

(6)

$G$ (L CH$_4$/Kg VS) is the cumulative CH$_4$ production at a given time $t$ (days). $G_m$ is the maximum CH$_4$ production (L CH$_4$/Kg VS) at an infinite digestion time ($t$). $K_0$ is an apparent kinetic constant (days$^{-1}$) and includes the biomass concentration. $t$ is the time (days).

On taking natural logarithms and reordering terms, Equation 6 can be re-expressed as

\[
\ln\left[\frac{G_m}{G_m - G}\right] = K_0 t
\]  

(7)

A plot of $\ln\left[\frac{G_m}{G_m - G}\right]$ versus $t$ should be a straight line with slope equals to $K_0$ at zero intercept.

2.8. **Statistical analysis**

The biogas, CH$_4$, NH$_4^+$, cation and anion data were analysed using Minitab software package version Minitab 17. A one-way ANOVA tested the significance of differences between digesters. Differences between the values were considered statistically significant at $P < 0.001$. Where significant treatment effects were determined, Turkey’s pairwise comparison test was applied to separate differences between digesters at a probability level of $P < 0.05$.

3. **Results and Discussion:**

3.1. **Column Test – analysis of NH$_4^+$ adsorption:**

Australian natural zeolite removes NH$_4^+$ ions from aqueous solution (Wijesinghe et al., 2016). The amount of NH$_4^+$ adsorption was improved at a flow rate of 13 mL/min and reached its maximum 9.48 mg NH$_4^+$-N /g of zeolite when it was passing a 5500 mL bed Volume. The advantage of a higher contact duration among zeolite and NH$_4^+$ solution at lower flow rates enhanced the NH$_4^+$ adsorption properties of zeolite inside the column. The NH$_4^+$ adsorption properties of zeolite in the column was improved by interruption or slowing down the infiltration while the solution was going through the zeolite column (Sprynskyy et al., 2005). The efficiency of NH$_4^+$ adsorption by the zeolite in the column decreased with an increase of flow rates and with time.
3.2. Anaerobic digestion process performance:

3.2.1. Biogas and CH$_4$ production:

All three digesters exhibited a significant difference ($p < 0.001$) with respect to cumulative biogas and CH$_4$ production at the end of 40$^{th}$ day. Although they did not show a clear difference in biogas and CH$_4$ production during the first 10 days, In-Zeo digesters always exhibited a higher biogas and CH$_4$ yield than the other two digesters (Fig. 2a and 2b). Biogas production of Ex-Zeo, In-Zeo, and No-Zeo digesters were 308 (±0.86) (L/VS Kg), 319 (±1.75) (L/VS Kg) and 287 (±3.86) (L/VS Kg) respectively. CH$_4$ production was increased by 5% in the Ex-Zeo digesters, and 15% in In-Zeo digesters compared to the No-Zeo digesters. Also, the % volatile solids reduction was 49%, 55%, and 41% in the Ex-Zeo, In-Zeo and No-Zeo digesters respectively. These results indicate that anaerobic digestion of swine manure with 7% zeolite is an effective way of degradation of swine manure and consequently improving CH$_4$ yield. Even the Ex-Zeo digesters exhibited higher CH$_4$ production compared to the No-Zeo digesters, and it was 8% lower than the In-Zeo digesters. The CH$_4$ yield from anaerobic digestion of different substrates was enhanced by adding zeolite because it improves the FAN/NH$_4^+$–N equilibrium by reducing free ammonia and NH$_4^+$ ion from the digestate and it has a capacity to immobilise microorganisms (Montalvo et al., 2012). Therefore, In-Zeo digesters more effectively remove TAN than Ex-Zeo digesters and have a higher capacity of immobilisation of microorganisms compared to Ex-Zeo digesters (for the configuration tested).

3.2.2. Kinetic Model

According to the plot of Ln (Gm/Gm-G) values as a function of digestion time (days) for 3 different types of digesters, representation of the experimental data as indicated in equation 6 gave straight lines with the intercept virtually at zero. The parameter $k_0$ was calculated by a standard linear regression model (implemented in Minitab 17). Regression coefficients are higher than 0.95 in all cases. The apparent kinetic constant, $K_0$ for CH$_4$ gas production, was 0.060, 0.063 and 0.062 respectively for the Ex-Zeo, In-Zeo and No-Zeo digesters. The best kinetic behaviour was shown by the In-Zeo digesters whereas the worst was shown in the Ex-Zeo digesters. The $K_0$ kinetic constant includes the biomass concentration (Milán et al., 2003). Colonisation of clinoptilolite particles by microorganisms was demonstrated, revealing spontaneous adhesion to preferentially sheltered areas as pits (Weiß et al., 2011). In the Ex-Zeo digester, some biomass is retained with the zeolite in the column. However, digestate was not running continuously via the zeolite column, resulting in the colonised
microorganisms no longer being able to participate in the digestion process continuously and this might be the reason for the poorer behaviour of the Ex-Zeo digesters.

Fig. 2: a. Cumulative biogas production b. Cumulative methane production during 40-day mesophilic anaerobic digestion of swine manure in 3 different reactors. (Vertical bars represent standard errors, n = 3)
3.2.3. pH and alkalinity:
The optimum pH range for CH₄ formation is pH between 6.0 and 8.0. pH’s below 6 and above 8 causes a significant reduction in CH₄ production (Chernicharo, 2007). Buffering capacity provides resistance to major and rapid changes in pH. Determining the digester imbalance using alkalinity is a more reliable method than direct measurements of pH, as an accumulation of short chain fatty acids will reduce the buffering capacity significantly before the pH decreases (Ward et al., 2008). Sufficient alkalinity between 1000–3000 mg/L as CaCO₃ avoids extreme pH changes in the anaerobic digestion process (Amani et al., 2010). Results indicate that the Ex-Zeo digester, In-Zeo digester and No-Zeo digester experienced pH fluctuations from an initial pH of 7.36 to 7.7, 7.64 and 8.00 respectively. Total alkalinity decreased from the second day in all digesters, and then increased from 1867 mg to a maximum of 3100 CaCO₃/L in the Ex-Zeo digesters, from 3433 to a maximum of 3500 mg CaCO₃/L in the In-Zeo digesters and from 3200 to a maximum of 4433 mg CaCO₃/L in the No-Zeo digesters with a slight variation with the time. Both alkalinity and pH parameters were lower in the In-Zeo digesters and Ex-Zeo digesters than the No-Zeo digesters with the In-Zeo digesters showing the lowest pH and alkalinity compared to other two. The alkalinity and pH of swine manure decreased after digestate was sent through the zeolite column. These pH and alkalinity reductions after adding zeolite into the digesters were also reported by (Lin et al., 2013b) and Montalvo et al. (2014). The alkalinity and pH in the digestate increased because of NH₄⁺ production as a result of protein hydrolysis. This was then followed by consumption of volatile fatty acids and the dissolution of CO₂ produced by methanogenic bacteria (Lin et al., 2013b). The decrease of NH₄⁺ concentration in the digestate because of NH₄⁺ adsorption onto the zeolite and the removal of CO₂ as a result of precipitation of Ca²⁺ and Mg²⁺ released by the zeolite could be the major reasons for the lower value of alkalinity and pH in the digesters associated with zeolite. However, zeolite in the columns of the Ex-Zeo digesters might not have sufficient time to contact with digestate with the 13 mL/min flow rate. This might be the reason for the higher pH and alkalinity values of the Ex-Zeo digesters than the In-Zeo digesters.

3.2.4. Ammonium and free ammonia
Total ammoniacal nitrogen, apart from being present in the manure to be digested, also accumulate during the breakdown of proteins and urea (Sung and Liu, 2003). The NH₄⁺-N concentration increased in the No-Zeo digesters with the time (Fig. 3a). This is evidence for ammonification of protein and urea in the digestate. In-Zeo digesters, NH₄⁺-N concentration
decreased within the first 10 days, and it maintained a lower level than the No-Zeo and Ex-Zeo digesters (Fig. 3a).

In the Ex-Zeo digesters, the \( \text{NH}_4^+ \)- N concentration of digestate gradually decreased at a decreasing rate while digestate was circulating through the zeolite column. The \( \text{NH}_4^+ \) concentration before and after the zeolite column shows a reduction of \( \text{NH}_4^+ \)- N concentration at outlet samples at a decreasing rate with time (Fig. 3b). At the end of the 40\textsuperscript{th} day, the \( \text{NH}_4^+ \)-N concentrations of In-Zeo digesters and Ex-Zeo digesters depict 45%, and 23% reduction respectively compare to the No-Zeo digesters mainly as a result of \( \text{NH}_4^+ \) adsorption capacity of the zeolite. KCl extraction of zeolite proved zeolite in the In-Zeo digesters adsorbed 8.57(±0.046) mg \( \text{NH}_4^+ \)-N/g of zeolites and 3.71 (±0.048) mg \( \text{NH}_4^+ \)-N /g of zeolite. Since the total contact time of digestate with the zeolite in the column was approximately 300 min within 8 days, less \( \text{NH}_4^+ \) adsorption was observed compared to the In-Zeo digesters. Nguyen and Tanner (1998) have shown the \( \text{NH}_4^+ \) breakthrough capacity of zeolite increased by 29- 57% with decreasing flow rate from 15.9 mm/min to 0.47 mm/min and by 2 to 4 times with a decline in particle size from 2-2.83 mm to 0.25- 0.5 mm under a 15.9 mm/min loading flow rate and suggested this was probably the result of the sufficient contact time for \( \text{NH}_4^+ \) adsorption and diffusion into zeolite.

The ammonium adsorption capacity of natural zeolite in the presence of other ions (\( \text{Na}^+ \), \( \text{K}^+ \), \( \text{Mg}^{2+} \), \( \text{Ca}^{2+} \), \( \text{PO}_4^{3-} \) and acetic acid) at pH 7, reduced 47% compared to the adsorption from solution with only \( \text{NH}_4\text{Cl} \) (Wijesinghe et al., 2016). However, \( \text{NH}_4^+ \) adsorption by zeolite in the In-Zeo digesters (pH around 7.5-7.6) reduced only 10% compared to the adsorption with only \( \text{NH}_4^+ \) ions in solution. The increase in \( \text{NH}_4^+ /\text{NH}_3 \) removal at pH around 7.5 to 7.6 suggests the interplay of molecular adsorption mechanism (Lin et al., 2013a). The other reason is \( \text{Ca}^{2+} \) ions precipitation is encouraged at pH > 7, and it can be precipitated with carbonate and other anions, resulting in excess \( \text{NH}_4^+ \) adsorption (Lin et al., 2014).

The highest FAN concentration i.e. 90 \( \text{NH}_3\)-N mg/L was observed in the No-Zeo digesters towards the end of digestion period with an increase of pH up to 8. In-Zeo digester shows the lowest \( \text{NH}_3\)-N (L) concentration, i.e. lower than 30 mg/L in digestate, and Ex-Zeo digester shows less than 50 mg \( \text{NH}_3\)-N/L of FAN concentration.
The ammonia inhibitory concentration depends on the microorganisms adaptation potential (Hansen et al., 1998). Therefore, a broad range of inhibitory values are found in the literature, i.e. 80 mg NH$_3$-N/L at pH 7.6-7.8 under ambient temperature (Koster, 1986) and TAN 1000 and FAN 60 mg/L at pH 7.5 and 55 °C (Kayhanian, 1994). Reducing NH$_4^+$ concentration, lowering pH, and reducing FAN in the digestate are accomplished by adding zeolite during the anaerobic digestion process thus mitigating ammonia inhibition.

**Fig. 3a:** NH$_4^+$-N inside the digesters  
**Fig. 3b:** NH$_4^+$-N concentration before sending via the zeolite column and after sending via the column in Ex-Zeo digesters. (Vertical bars represent standard errors, n = 3)
3.2.5. Total C and N
The C:N ratio of the swine manure tested here was 7.4 (±0.12). The optimum C:N ratio for microorganisms is 30:1. Microbial growth is inhibited when C: N ratio increases more than 50:1 and C: N ratio less than 10:1 (Sommer et al., 2013). The process which was aided by adding natural zeolite during swine manure anaerobic digestion, enhanced CH₄ production by preventing ammonia inhibition and regulating the C:N ratio via the NH₄⁺ adsorption. Carbon % reductions were 46% , 53% 41% respectively for Ex-Zeo, In-Zeo and No-Zeo digesters. These results also confirm the higher degradation and enhancing CH₄ production because of adding zeolite during the anaerobic digestion process.

The lowest total N amount, 4.64 g was observed in the In-zeo digesters and N reduction was 17%, 36%, and 3.6% respectively in the Ex-Zeo, In-Zeo and No-Zeo digesters. However, according to the N mass balance, losses were observed. The comparatively higher amount, 3.6% of losses was observed from the No-Zeo digester whereas 2.3% and 1.7% from Ex-Zeo and In –Zeo digesters. Ammonia can be released during anaerobic digestion depending on temperature and pH (Strik et al., 2006). This might be a possible reason for N losses from the digesters.

3.2.6. Major Cations (sodium (Na⁺), potassium (K⁺), calcium (Ca²⁺), and magnesium (Mg²⁺))
While moderate concentrations of cations (Na⁺, K⁺, Ca²⁺, and Mg²⁺) stimulate microbial growth, excessive amounts slow down the growth, and even higher concentrations can cause severe inhibition or toxicity (Soto et al., 1993). The concentration of K⁺ is higher in the No-Zeo digesters at 700 mg/L. The In-Zeo digesters and Ex-Zeo digesters show a lower concentration of K⁺. At the end of the digestion period, the In-Zeo digesters reduced its K⁺ concentration up to 100 mg/L (Fig. 4a). The K⁺ concentration gradually decreased in the Ex-Zeo digesters while the digestate was circulating via the zeolite column (Fig. 4a). Digestate from the zeolite column shows K⁺ concentration decreased at decreasing rate when digestate flowed through the column (Fig. 5a). Zeolite (Clinoptilolite) species have a high adsorption affinity of NH₄⁺ and K⁺(Ames, 1960). Low concentrations of K⁺ are favourable to anaerobic digestion and potassium inhibition is induced at 400 mg/L (Chen et al., 2008). At the higher K⁺ level, it leads to a passive influx of K⁺ ion that neutralise the membrane potential (Jarrell et al., 1984). Therefore, application of zeolite in anaerobic digestion is beneficial as it keeps K⁺ concentration at a lower level.
Sodium concentrations inside the No-Zeo digesters were the lowest compared to the other two digesters. By the second day, Na\(^+\) content in the In-Zeo digesters increased from 250 mg/L to 425 mg/L and then gradually decreased up to 296 mg/L at the 10\(^{th}\) day and the Na\(^+\) concentrations were relatively stable with minor fluctuations (Fig. 4b) thereafter. However, Na\(^+\) content in Ex-Zeo digesters increased while digestate was circulating via the zeolite column from 250 mg/L to 350 mg/L and then remained stable around 325 mg/L throughout the digestion period. The Na\(^+\) content from the Ex-Zeo digesters decreased rapidly compared to the digestate before circulation (Fig. 5b) and after the 5\(^{th}\) day, the Na\(^+\) content is more or less similar indicating the instant exchangeable behaviour of Na\(^+\) in the zeolite. Many kinds of natural zeolite are rich in Ca\(^{2+}\) and K\(^+\) ions, partially replacing equivalents of Na\(^+\) ions (Rao, 2006). Na\(^+\) is essential for growth of anaerobic microorganisms because of its role in the formation of adenosine triphosphate or the oxidation of nicotinamide adenine dinucleotide, NADH (Dimroth and Thomer, 1989). This might be the reason for the reduction in Na\(^+\) content within first 10 days. The Na\(^+\) concentration for methanogens is favourable at < 1000

Fig.4: Cations concentration changes with time inside the Ex-Zeo, In-Zeo and No-Zeo digesters. (Vertical bars represent standard errors, n = 3)
mg/L (Lee et al., 2012). In all digesters, the Na⁺ concentration is within the desirable range for microorganisms.

The initial concentration of Mg²⁺ of the swine manure is very low. It increased up to 200 mg/L within 10 days at the In-Zeo digesters and then show relative stability with some fluctuation. The Mg²⁺ content in the Ex-Zeo digesters gradually increased until digestate was circulating via the zeolite column and then it showed minor fluctuations around 50 mg/L. However, no Mg²⁺ ion accumulation was observed in the No-Zeo digesters (Fig.4c). The Mg²⁺ level at digestate after circulating through the zeolite column shows higher concentration than in the inlet, and its concentration increased at an increasing rate at the outlet with time (Fig.5c). This is indicative that additional Mg²⁺ comes into the zeolite digesters as a result of release of Mg²⁺ while adsorbing other cations in the swine manure. The optimum Mg²⁺ concentration for anaerobic microorganisms is 300 – 720 mg/L (Schmidt and Ahring, 1993). Therefore, there is no inhibitory effect of Mg²⁺ because of adding zeolite. However, there is no Mg²⁺ level increased in the No-Zeo digesters. The Mg²⁺ ions which are released during the substrate degradation possibly were utilised by microorganisms, and there is a potential for struvite formation at pH 7.8 (Wang et al., 2005). These might be the reasons for the lowest level of Mg²⁺ in the No-Zeo digesters.

The Ca²⁺ concentration increased in all reactors by the second day (Fig. 4d). For the In-Zeo digesters, Ca²⁺ went up to 300 mg/L and then gradually decreased and maintained a very low level, below 50 mg/L. The Ca²⁺ level for the Ex-Zeo digesters and No-Zeo digesters was 25 mg/L after the 2nd day, and after the 16th day, no or an insignificant amount of Ca²⁺ can be observed (Fig.4d). However Ca²⁺ levels at the outlet of the external zeolite columns show zeolite released Ca²⁺ at a decreasing rate (Fig.5d). The addition of Ca²⁺ has a positive impact on reactors in which retention of biomass is desired (Chen et al., 2008). However, excess Ca²⁺ in the digestate leads to carbonate and phosphate precipitation (Klass, 1998). The Ca²⁺ ions released by zeolites during the ion exchange process precipitate dissolved phosphate, depending on the concentration of NH₄⁺ (Lin et al., 2014). The Long-chain fatty acids (LCFA) in the digestate are precipitated with Ca²⁺ as the calcium salt (Ahn et al., 2006). This is the possible reasons for the reduction of calcium along the anaerobic digestion process even as zeolite releases Ca²⁺ while adsorbing other ions. The LCFAs, which are the main inhibitory factor on anaerobic microorganisms (Ma et al., 2015). Therefore, adding zeolite reduces the inhibitory effect of LCFAs from anaerobic digestion in an indirect way since the
LCFAs from swine manure digestate precipitate as a calcium salt. Each ion desorbed by the zeolite (i.e., Na\(^+\), Ca\(^{2+}\), and Mg\(^{2+}\)) has their individual benefits for the anaerobic digestion process as all these ions are antagonistic to ammonia inhibition.

**Fig.5:** Cations concentration before sending via the column and after sending via the column in Ex-Zeo digesters. (Vertical bars represent standard errors, n = 3)

### 3.2.7. NH\(_4^+\) and K\(^+\) removal time constants for the Ex-Zeo digesters:

The NH\(_4^+\) and K\(^+\) removal time constants related to NH\(_4^+\) removal from digestate for the Ex-Zeo digesters were calculated using NH\(_4^+\) and K\(^+\) removal/adsorption after flow of the digestate through the zeolite column and fitting an exponential decay curve (i.e. first order kinetics). The first-order kinetics time coefficient is 0.1 for NH\(_4^+\) and 0.115 for K\(^+\) and the reciprocal of this gives the NH\(_4^+\) removal time constant of 10 days and 8.7 days. The correlation coefficient \((r^2)\) is 0.97 and 0.96. This indicates that a first order kinetic constant gave an adequate representation of the physical behaviour of NH\(_4^+\) removal and K\(^+\) removal.
from the digestate because of their adsorptions by the zeolite in the column. Comparatively higher first order kinetics of K\textsuperscript{+} indicates zeolite’s preference to adsorb K\textsuperscript{+} over NH\textsubscript{4}\textsuperscript{+}.

3.2.8. Total phosphorous (P) and Sulphur (S)
In the No-Zeo digesters, total P concentration slightly increased on the second day, decreased rapidly within 10 days and then slightly decreased with minor fluctuation around 50 mg/L. Total P concentration in the In-Zeo digesters, rapidly decreased within the first 4 days and was then relatively stable and maintained a concentration of approximately 13 mg/L. In the Ex-Zeo digesters, total P concentration reduced for up to 4 days and then it showed a minor fluctuations around 17- 20 mg/L (Fig. 6a). Total P level at the outlet from the zeolite column was always higher for the first 4 days, and then, no clear difference between the inlet and outlet. Zeolite removes total P based on the mechanisms of anion exchange and electrostatic attraction (Dionisiou et al., 2013; Jiang et al., 2013). Apart from this process, dissolved phosphate precipitate as Ca\textsuperscript{2+} ions are released from the zeolite lattice during other ion exchange processes (Lin et al., 2014). Struvite precipitation is enhanced between pH 7 - 11 (Burns et al., 2001). This might be another reason for the reduction of P along the digestion process, especially in the No-Zeo digesters. The pH of the No-Zeo digesters increased up to 7.8, and it is more favourable to struvite formation than the other reactors.

In the In-Zeo digesters, S concentration rapidly reduced within first 3 days and then S was not observed (Fig 6c). The Ex-Zeo digesters, S reduction, was observed while digestate was circulating via the zeolite column and as a result of that S level inside the Ex-Zeo digesters decreased within 8 days, and after 10 days, S was not observed in Ex-Zeo digesters. The S concentration was decreased from 14mg/L to 3 mg/L in the No-Zeo digesters, and towards the 40\textsuperscript{th} day of digestion, it was stable.

The optimal level of sulphur reported in the literature varies from 1 to 25 mg S/L (Scherer and Sahm, 1981). However, total sulphide concentrations above pH 7.2 are identified as inhibition of all microorganisms (O’Flaherty et al., 1998). Zeolite is promising material for the adsorption of S compounds (Velu et al., 2002). Therefore adding zeolite with anaerobic digestion process would be another additional benefit of zeolite application.
4. Conclusion:
The developed novel Ex-Zeo digester ensures the feasibility of the addition of a zeolite column to the anaerobic digestion process of swine manure. Zeolite is identified as an effective way of enhancing CH$_4$ production by removing ammonia inhibition, Potassium inhibition, and reducing the total P and S levels in the digestate as well. The Zeolite external column with continuous lower flow rate needs to be carried out to provide more comprehensive information on the optimisation of external zeolite column use to enhance CH$_4$ production.

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References


Chapter 8

General Discussion and Conclusion

In this study the NH$_4^+$ adsorption and ion exchange capacities of an Australian zeolite at high NH$_4^+$ concentrations were investigated in detail. Different rates of natural and NaCl-treated zeolites were added to swine manure and the C:N ratio adjusted in the mixture. The aim was to understand the effects of zeolite addition and determine the optimum zeolite rate for maximum CH$_4$ production and the mitigation of the effects of NH$_4^+$ concentration inside the anaerobic digester. The adsorption and desorption properties of natural and NaCl-treated Australian zeolites during anaerobic digestion of swine manure were comprehensively analysed. An anaerobic digester with an external column was developed for convenient replacement of NH$_4^+$ saturated zeolite.

8.1: Major findings

The major results from each chapter were as follows:

1. Ammonium removal from high-strength aqueous solutions using an Australian zeolite (Chapter 4)

Zeolite has high affinity for NH$_4^+$. However, the characteristics of each zeolite are different and therefore investigations of the detailed mechanisms of NH$_4$ adsorption on the Australian zeolite and its NaCl-treated form (sodium zeolite) at high NH$_4$ concentrations are necessary. The major results were as follows:

I. Initial NH$_4^+$ concentration, reaction time, temperature, and pH of the solution had significant effects on the NH$_4^+$ adsorption capacity of zeolite.

II. NaCl treatment increased NH$_4^+$ adsorption capacity of Australian zeolite by 25\% at 1000 mg-N, NH$_4^+$ solution.

III. Compared to the NH$_4^+$ only medium, the presence of other competing cations and acetic acid in the medium (resembling the composition in digested swine manure slurries) reduced NH$_4^+$ removal by natural and NaCl-treated zeolites by 44\% and 57\% respectively.

2. Performance of mesophilic anaerobic digestion process with swine manure under different HRTs (Chapter 5)

Identifying anaerobic digestion performance, especially NH$_4^+$ production during the anaerobic digestion of swine manure, is important for determination of the ammonium
inhibition method. The prediction of the process performance and optimum HRT for the anaerobic treatment system is difficult since it depends on the type of feed and its characteristics, reactor configuration, OLR, and type of biomass and method. Therefore, process performance was investigated at different HRTs. Based on the results the following conclusions were made:

I. 16 days HRT digester produced the maximum CH$_4$ production from the selected HRTs. Therefore, 16 days was found to be the optimum HRT for mesophilic anaerobic digestion of swine manure used in this study when studies were conducted at 8, 12 and 16 days HRTs.

II. Iso-butyric and iso-valeric acid levels in the digester were the best indicators of the progress of anaerobic digestion of swine manure.

3. Effects of co-mixing of zeolites with digestive substrate on anaerobic digestion of swine manure (Chapter 6)

The effects of zeolite on CH$_4$ production and TAN vary with the types of zeolite material. Their characteristics need to be investigated individually as the most suitable zeolite material and its application rates for enhancing biogas production are not readily predictable. Therefore, the effects of Australian zeolite on CH$_4$ production, and the optimum rate of Australian zeolite that produces the maximum TAN recovery at maximum CH$_4$ production, were identified. In addition, the effects of zeolite addition on C:N ratio adjusted swine manure were determined.

The major results were as follows:

a. Effects of zeolite on anaerobic digestion of swine manure

I. Australian zeolite, either in a natural or NaCl-treated form, significantly enhanced biogas generation while adsorbing a substantial quantity of NH$_4^+$ from the medium during the process.

II. NH$_4^+$ adsorption by zeolite showed a linear relationship with increasing zeolite dose, and biogas and CH$_4$ production increased linearly up to natural zeolite addition rates of 40g/L, above which it started to decrease

III. Digesters with sodium zeolites showed smaller CH$_4$ production than digesters with natural zeolites.

IV. The lag phase of anaerobic digestion was shorter with increasing zeolite doses, up to 100g/L.
V. The effects of NH$_4^+$ adsorption by natural and sodium zeolites were more or less similar in swine manure anaerobic digestion.

VI. Variations of cation levels inside the digesters as a result of adsorption-desorption caused by adding zeolite at different rates might be another reason for variations of biogas and CH$_4$ production.

VII. Total NH$_4^+$ adsorption by zeolite was positively correlated with biogas/CH$_4$ production supporting the proposition that reduction in soluble NH$_4^+$ levels in anaerobic digestion favours the methanogenesis process.

b. Removal of other ions from zeolite during the anaerobic digestion of swine manure.

I. Adsorption isotherms confirmed higher selectivity for K$^+$ than for NH$_4^+$ on Australian zeolites. Therefore NH$_4^+$ removal was significantly reduced under conditions of simultaneous K$^+$ uptake.

II. The order of cation desorption from an Australian natural zeolite and sodium zeolite at steady state was Ca$^{2+} >$Na$^+ >$Mg$^{2+} >$K$^+$ and Na$^+ >$Ca$^{2+} >$Mg$^{2+} >$K$^+$, respectively.

III. More Ca$^{2+}$ was desorbed by natural zeolite when there was significant K$^+$ and NH$_4^+$ adsorption. P removal by precipitation with Ca$^{2+}$ is dominant relative to P adsorption onto zeolite.

IV. The presence of high concentrations of zeolite at high pH might not be appropriate for anaerobic digestion since zeolites enhance P precipitation by releasing Ca$^{2+}$ into the medium, resulting in reduced P availability for microbial growth.

4. Developing an anaerobic digester with an external zeolite column to enhance methane production from swine manure - A feasibility study.

It is important to determine the method for the most convenient removal and re-filling of zeolite after saturation with TAN, without disturbing the continuous anaerobic digestion process. Therefore, a digester with an external zeolite column was developed. An anaerobic digester with an internal zeolite bed and a digester without added zeolite were employed to compare anaerobic digestion process performance to an external zeolite column. Results show the treatment of swine manure to enhance CH$_4$ production through anaerobic digestion is aided by the presence of zeolite deployed either in the medium or in an external column.
8.2 Future studies

This research has shown that CH$_4$ production can be enhanced through the reduction of nitrogen concentration during anaerobic digestion of swine manure. It was observed that larger doses of Australian zeolite reduced NH$_4^+$– N linearly but increases in CH$_4$ yield were marginal at doses of zeolite above 40g/L. A model that can be used to determine the optimum dose of Australian zeolite so as to maximise CH$_4$ production is an important next step.

A bioreactor configuration with an external zeolite column was developed for optimising the digester performance with zeolite and to allow easy replacement of NH$_4^+$ saturated zeolite. However, the external column worked with low efficiency and the approach needs to be further developed. It is preferable that future work includes a continuous feed test since zeolite treatment with the external column was restricted to eight days in the present study. Further, a study of the nitrogen dynamics during the anaerobic digestion is needed to confirm claims of NH$_4^+$ removal from the digester configuration. Solutions are also needed to avoid clogging of the zeolite matrix/medium inter- and intra-particle pore spaces due to organic particulate matter and microbial colonisation. The addition of membrane filtration into the system, where only the liquid portion separated from the bulk solids is sent through the zeolite column, is a potential solution. Amongst the available spectrum of filtration processes (Radcliff and Zarnadze, 2004), microfiltration would appear to be the appropriate option. Membranes are available in two shapes; as tubular (tubes, capillaries and hollow fibres) and flat sheet forms (Futselaar and Weijenberg, 1998). Using a tubular membrane to filter the swine manure before sending it through the zeolite external column would be a desired option, as indicated in Figure 1.

In addition to the above, all the digestions with zeolite addition were investigated in batch mode. Continuous experiments with zeolite in either an internal or external configuration are recommended, including but not limited to:

I. Developing a continuous mode anaerobic digester with an external zeolite column would allow the digester to operate in different OLR and HRT regimes and allow better optimisation of operating conditions.

II. A continuous study would allow an appropriate replacement cycle of the Australian zeolite to be determined through a study of NH$_4^+$ concentration at the inlet and outlet of the external column.
III. The experiments would produce fully loaded zeolite where the feasibility of its use as an effective fertiliser in agriculture.

IV. The work would produce samples for regeneration trials.

**Figure 1:** Experimental setup with tubular membrane and zeolite column

**Reference**


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