Soil for life

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Biologisch aanzuren van mest kansrijk voor melkveebedrijven

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Content

Samenvatting en conclusies

De Nederlandse overheid wil de uitstoot van ammoniak((NH_3) verder terugdringen. Via de Programmatische Aanpak Stikstof (PAS) dient onder andere een daling van de NH_3-emissie vanuit landbouwbedrijven in de buurt van Natura 2000 gebieden te worden gerealiseerd. Daarnaast is het de bedoeling om met ingang van 1 januari 2014 de NH_3-emissie in de melkveehouderij met 10% te verminderen via gerichte managementmaatregelen. Het aanzuren van mest in de stal is een mogelijke oplossingsrichting. Het verlaagt niet alleen de emissie uit de stal maar ook bij toedienen. Bijkomend voordeel is dat de emissie van methaan uit de mestopslag sterk afneemt.

Aanzuren van mest met sterke zuren is al langer bekend als methode om de ammoniakemissie uit de stal te reduceren. Sinds kort heeft Infarm een systeem gebaseerd op zwavelzuur operationeel in Denemarken. Voor Nederland is zo'n systeem een tijdelijke oplossing vanwege de kosten en het hoge gebruik van zwavelzuur, hetgeen leidt tot een hoge zwavelaanvoer naar percelen. Aanzuren van mest met behulp van bacteriën is eveneens een oplossingsrichting. Via het toevoegen van een gemakkelijk afbreekbare C-bron (bijvoorbeeld melasse), al dan niet in combinatie met het toevoegen van bacteriën en organische zuren, wordt de mest verzuurd. Begin jaren 90 bleek dit technisch te werken in varkensstallen, maar waren de kosten te hoog. Voor rundveemest is het in Nederland toen niet onderzocht.
In deze studie heeft het Nutriënten Management Instituut NMI in opdracht van het Productschap Zuivel nagegaan wat de mogelijkheden zijn van (microbieel) aanzuren van dunne rundermest om vast te stellen:

- in hoeverre daarmee de NH_3-emissie uit stallen en bij toediening is te verminderen;
- wat de kosteneffectiviteit is; en
- of er andere voordelen zijn te verwachten.

Naast een literatuurstudie wordt gebruik gemaakt van recente Oostenrijkse bevindingen omtrent aanzuren van mest met behulp van microben.

<u>Potentie om bij te dragen aan de reductie van de ammoniakemissie</u>
Bij aanzuren tot pH 5,5 kan een sterke reductie van de NH_3-emissie uit stal (35%), opslag (90%) en bij toedienen (85%) worden bereikt. Uitgedrukt in kg N per dierplaats per jaar, resulteert aanzuren in een NH_3-emissiereductie uit de stal van 2,4 tot 3,8 kg N $dier^{-1}$ jr^{-1}. Dit is groter of vergelijkbaar met de emissiereductie die kan worden bereikt met de meeste emissiearme stallen. Het aanzuren van mest werkt ook positief door op de NH_3-emissie na toedienen. Voor een systeem van permanent opstallen, uitgesplitst naar grondsoort, daalt de NH_3-emissie uit de gehele mestketen (vanaf stal tot en met toedienen) met 54 tot 65% wanneer de mest wordt aangezuurd tot pH 5,5 (Tabel 1).

Tabel 1. De totale reductie in NH_3-emissie (%) voor dunne rundermest aangezuurd tot pH 5,5 of 6 in vergelijking tot onbehandelde mest van koeien die het hele jaar opgestald zijn, uitgesplitst naar mesttoediening op zand, klei en veengrond.

Grondsoort	*<u>Aanzuren tot pH 5,5</u>*	*<u>Aanzuren tot pH 6</u>*
Zand	54%	35%
Klei	59%	39%
Veen	65%	44%

Door deze sterke daling van de ammoniakemissie ontstaat er ruimte op de bedrijven om meer vee te houden zonder dat het “ammoniakquotum” van een bedrijf wordt overschreden. Een verdubbeling van het veestapel
behoort dan potentieel tot de mogelijkheden. Indien tot pH 6 wordt aangezuurd dan varieert de berekende daling tussen 35 en 44%. Bijkomend voordeel is een hoger N-gehalte en hogere N-werking van de mest bij toedienen. Cumulatief betekent dit een 15 tot 30 kg hogere N-werking per ha uit toegediende mest.

Potentie om bij te dragen aan de reductie van broeikasgasemissies

De methaanvorming uit mest komt tot stilstand bij aanzuren tot beneden de pH 6. Op bedrijfsniveau kan dit ongeveer een 20% reductie van de methaanuitstoot betekenen. Op basis van beperkte en tegenstrijdige resultaten is de verwachting is dat de uitstoot van lachgas nauwelijks wordt beïnvloed.

Biologisch aanzuren

Biologisch aanzuren is sterk vergelijkbaar met het productieproces van biogas. Tijdens dit proces worden organische zuren gevormd door fermentatie van organische fracties in mest en co-producten. De organische zuren worden onder anaerobe omstandigheden omgezet in methaan, mits de pH voldoende hoog is. Door dit proces zodanig te sturen dat de pH daalt tot onder de 6 stopt de methanogenese en blijven de zuren in de mest aanwezig. Om te bewerkstelligen dat de pH sterk daalt zijn de volgende opties mogelijk: i) de microbiologische populatie wordt gewijzigd door toevoeging van zuurproducerende micro-organismen zoals Lactobacillus spp) en ii) het mestmilieu wordt gewijzigd om gunstigere omstandigheden voor zuurproducerende micro-organismen te creëren via:

- toevoegen van gemakkelijk fermenteerbaar organisch substraat;
- toevoegen van mineralen zoals zeoliet om het reactief oppervlakte te vergroten;
- het optimaliseren van de temperatuur;
- het gebruik van verse mest;
- het aanpassen van de voeding van het vee ter beïnvloeding van de mestkwaliteit; en
- het toevoegen van organisch zuur om de pH van de mest te verlagen teneinde de juiste omstandigheden voor een specifieke (groep) micro-organismen te creëren.

De verschillende studies waarin biologisch aanzuren van mest is onderzocht verschillen allemaal in de combinatie en hoeveelheden additieven. De kwaliteit en hoeveelheid van het toegevoegde organische substraat is bijvoorbeeld van invloed op de snelheid van de pH-daling, het pH-niveau dat bereikt wordt en de mate waarin een bepaalde lage pH niveau gehandhaafd wordt. Het lijkt erop dat met het toedienen van zetmeelachtige verbindingen de beste resultaten worden behaald. Door bij de opstart organische zuren te gebruiken kunnen snel gunstige condities voor zuurvormende micro-organismen worden gecreëerd. Niet duidelijk is of dit alleen nodig is bij het opstarten of dat het blijvend nodig is. Dit is belangrijk omdat organische zuren als azijnzuur en citroenzuur relatief duur zijn.
Het toevoegen van zeoliet zorgt voor een groter reactief oppervlak tussen micro-organismen en substraat. Bij toepassing in een biogasinstallatie leidde een toevoeging van zeoliet tot 50% meer gasproductie.
In Oostenrijk lopen momenteel proeven om de procescondities voor biologisch aanzuren beter in beeld te krijgen. Lab-resultaten wijzen uit dat het gebruik van azijnzuur bij het opstarten, in combinatie met enten van micro-organismen en het toevoegen van zeoliet de beste resultaten geeft. De focus ligt daar op het realiseren van een pH van 6. Voor een gerichte processturing lijkt een pH duidelijk beneden de 6 beter te zijn. Het onderzoek daar wordt voortgezet zowel in het lab als op praktijkbedrijven.

Positieve neveneffecten van aanzuren zijn dat schuimvorming in mest (hetgeen ten koste gaat van de opslagcapaciteit) niet langer voorkomt en dat mest beter mengbaar wordt.

De samenstelling, met name de pH en het gehalte fermenteerbaar organisch substraat van de mest, beïnvloedt hoe gemakkelijk (zonder veel toevoeging van substraat) de mest kan worden aangezuurd; het verzuringpotentieel. Onderzoek laat zien dat bij gebruikmaking van verse mest veel minder organisch substraat nodig is om mest aan te zuren. Verse mest heeft een lagere pH en meer organisch substraat dan mest die enkele dagen oud is. Bij aanzuren bij kamertemperatuur is soms zelfs geheel geen organisch substraat en/of toevoeging van micro-organismen nodig om mest te laten verzuren. Naast de versheid van de mest is het rantsoen ook van invloed op het verzuringpotentieel van mest. Op basis van beperkte resultaten kan het methaanproductiepotentieel van verse dunne mest (en daarmee indirect ook het verzuringpotentieel) 30 tot 50% verschillen bij verschillende rantsoenen. Rantsoenen met veel zetmeel en weinig eiwit lijken het hoogste verzuringpotentieel te hebben. Verder blijkt dat de natuurlijke pH van dunne rundermest sterk kan verschillen (van 6,8 tot boven de 8) als gevolg van het gevoerde rantsoen.

De kosten

De factoren die van belang zijn voor het kosteneffectief biologisch aanzuren lijken te zijn:

- het gebruik van dagverse mest;
- bij het opstarten organisch zuur toevoegen;
- het toevoegen van zeoliet;
- niet te lage temperaturen (bij voorkeur > 10ºC); en
- een (geringe hoeveelheid) fermenteerbaar organisch substraat (C).

Het toevoegen van organisch substraat en organisch zuur is duur en bovendien is de prijsstelling sterk variabel. Ook is nog niet precies bekend welke toevoegmiddelen en in welke hoeveelheid nodig zijn. Afhankelijk van de prijsstelling kan het accent meer op de één of de ander worden gelegd. Denkbaar is ook een mix van biologisch aanzuren en aanzuren met zwavelzuur. Verder worden de kosten voor een deel bepaald door de technische installatie.

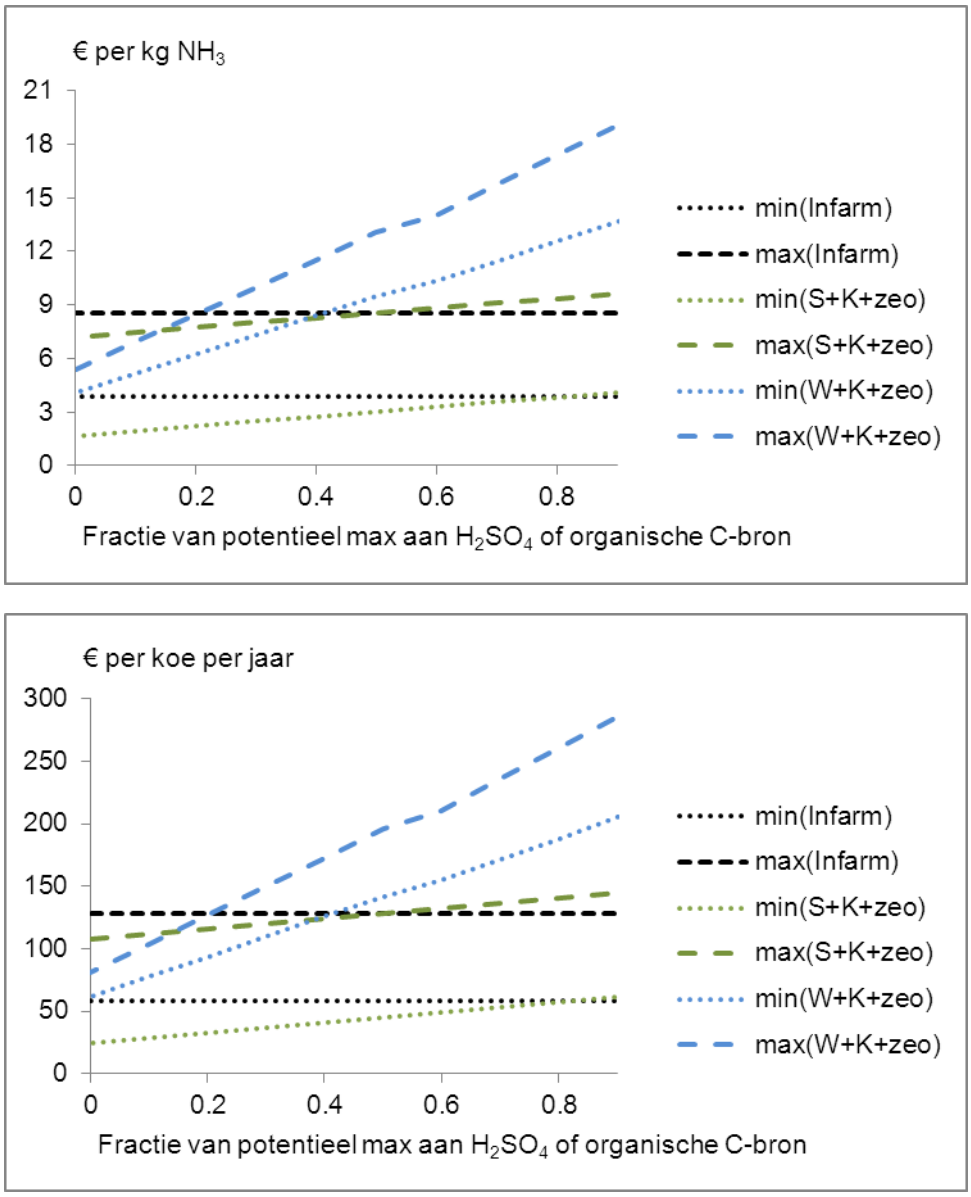
In Figuur 1 zijn de kosten per koe en per kg bespaarde NH_3 weergegeven voor zowel biologisch aanzuren als aanzuren met alleen zwavelzuur (Infarm systeem). Aanzuren met zwavelzuur kost 60 tot 130 € per koe per jaar ofwel zo'n 4 tot 8,5 € per kg bespaarde NH_3. Het wordt verondersteld dat het systeem interessant is voor ondernemers als de kosten beneden de 150 € per koe ofwel benden 10 € per kg bespaarde NH_3 blijven. Wil organisch aanzuren perspectiefvol zijn, dan moet meer dan 60% van de benodigde verzuring uit de mest zelf komen en hooguit 40% via het toevoegen van organisch substraat en of organisch zuur. Indien tijdelijk zwavelzuur gebruikt wordt dan hoeft minder dan 60% van de benodigde verzuring uit de mest zelf te komen. Indien alleen bij het opstarten toevoegmiddelen nodig zijn, dan kan biologisch aanzuren een zeer kosteneffectieve techniek zijn.

Gezien de mogelijk bandbreedte in kosten is aanvullend experimenteel lab onderzoek naar de optimale procescondities nodig voordat opschaling naar praktijkschaal plaatsvindt.

Biologisch aanzuren in relatie tot biogasproductie

Na biologisch aanzuren zou de mest gescheiden kunnen worden in een dunne en dikke fractie. Door een verlaging van de viscositeit van de biologisch aangezuurde mest gaat de mestscheiding gemakkelijker dan bij onbehandelde drijfmest. De dikke fractie kan ingezet worden voor biogasproductie zonder toevoeging van cosubstraat. Deze dikke fractie zal naar verwachting meer

methaan produceren (na verhoging van de pH in de biogasinstallatie) dan de dikke fractie van onbehandelde mest. Daarbij is het denkbaar dat de restwarmte bij de biogasproductie gebruikt wordt om biologisch aanzuren beter en sneller te laten verlopen, waardoor mogelijk minder substraat en of organisch zuur nodig is om een bepaalde mest-pH te realiseren en te behouden. Hierdoor wordt het biologisch aanzuren kostenefficiënter. Nog een stap verder is om het rantsoen niet alleen af te stemmen op de melkproductie maar ook op een bepaalde mestkwaliteit voor een betere verzuringspotentie.



Figuur 1. De kosten van het Infarm systeem (H_2SO_4 prijs varieert tussen 100 (min) en 300 (max) € per ton) en van biologisch aanzuren bij gebruikmaking van wisselende hoeveelheden H_2SO_4 (S+K+zeo) of organisch C (W+K+zeo). De bovenste figuur geeft de jaarlijkse kosten per koe weer. De onderste figuur geeft de kosten weer in € per kg bespaarde NH_3. S is H_2SO_4, K is Lactobacillus toevoeging via Kombioflor, zeo is zeoliet en W is gemalen tarwe als C-bron.

Conclusies:

- Verlaging van de pH van mest tot 5,5 door aanzuren geeft op bedrijfsniveau een reductie in ammoniakemissie van 54-66%.
- Biologisch aanzuren van mest in rundveestallen kan een kosten effectieve techniek worden om de NH_3-emissie op boerderijschaal te verlagen. De geschatte kosten voor biologische aanzuren variëren tussen de 4 en 20 € per kg bespaarde NH_3 (of 50 tot 310 € per koe). De hoeveelheid organisch substraat die nodig is bepaalt grotendeels de variatie in kostprijs. Verwacht wordt dat de kosten beneden de 10 € per kg bespaarde NH_3 kunnen blijven. Extra lab-testen zijn nodig om meer kwantitatieve informatie te verkrijgen over de optimale procescondities voor biologisch aanzuren en om een meer precieze kostprijsberekening maken.
- Positieve bijwerkingen van aanzuren zijn dat het de methaanuitstoot vermindert met 20% en dat het resulteert in meer homogene drijfmest zonder risico van schuimvorming. Dit laatste maakt een efficiënt gebruik van de mestopslagcapaciteit in ligboxenstallen mogelijk.
- Voor de korte termijn (met het oog op een snelle start) lijkt een verzuringssysteem gebaseerd op een mix van biologische en anorganische verzuring aantrekkelijk vanuit het oogpunt van risicospreiding tussen de kosten van additieven (azijnzuur, organisch substraat en H_2SO_4).
- Voor de lange termijn wordt de hoogste kostenefficiëntie verwacht voor biologische aanzuren in een fed-batch systeem. In dit systeem wordt verse mest toegevoegd aan mest die al is aangezuurd waarbij:
  - bij de start verse dunne mest direct aangezuurd wordt tot pH 5,5;
  - zeoliet en Lactobacillus spp. regelmatig worden toegevoegd;
  - een beperkte hoeveelheid fermenteerbaar organisch substraat of organisch zuur wordt toegevoegd in het geval dat de kwaliteit van het organische substraat dat aanwezig is in de vers toegevoegde mest onvoldoende is om de pH te handhaven; en
  - de temperatuur boven de 10 ^{O}C blijft.
- Indien biologisch aanzuren succesvol is dan is er een goede mogelijkheid om na scheiding de dikke fractie meer profijtelijk in te zetten als mono-substraat in een biogasinstallatie dan conventionele dikke fractie.

Summary and conclusions

Dutch policy is focused on further reducing ammonia (NH_3) emissions. The Programmatic Approach Nitrogen (Programmatische Aanpak Stikstof) aims amongst other things at realizing a reduction of the NH_3 emission from farms in the vicinity of special areas of specific natural value (Natura 2000). In addition a NH_3 emission reduction of 10% from dairy farms should be realized by management measures from January 1st 2014 onwards. Acidification of cattle slurry is a possible solution. It not only lowers the emission from the shed but emissions are also reduced when the slurry is applied to the soil. An additional advantage is that the emission of methane from slurry is also strongly reduced.

Acidifying manure with strong acids has been known for a while as a method to reduce NH_3 emission. Recently, Infarm has a system based on sulfuric in operation in Denmark. For the Netherlands, such a system is only a temporary solution due to the high costs and the use of sulfuric acid leading to a high sulfur supply to fields. Acidifying manure by means of bacteria is also a possible solution. Slurry is acidified by adding an easily degradable carbon source (for example, molasses), possibly in combination with the addition of bacteria and or organic acids. In the early 90's it was technically working in pig housing, but the costs were too high. For cattle slurry it was at that time not investigated in the Netherlands.
In this study Nutrient Management Institute NMI examines in commission of the Milk and Dairy Board the possibilities of (biological) acidification of cattle slurry to determine:

- to what extent NH_3 emissions from stables and after application can be reduced;
- the cost effectiveness; and
- if there are other benefits to be expected

In addition to the literature, use is made of recent Austrian acidification experiments using microbes.

Potential to contribute to the reduction of ammonia emissions

Acidification of slurry to a pH below 5.5 results in a strong reduction of the NH_3 emission from stables (35%), storage (90%), and application (85%). Expressed in kg N per animal per year, acidification results in an emission reduction from the stable varying between 2.4 to 3.8 kg N $animal^{-1}$ yr^{-1}. This is more or comparable to the emission reduction that can be achieved with most low-emission stables. Acidification also contributes positively to reducing emissions after application. For a system of permanent housing, depending on soil type, the NH_3 emission from the whole manure chain (from stable to application) is reduced with 54 to 65% when the slurry is acidified to pH 5.5. This strong decrease of the NH_3 emission creates room for farms to keep more cattle without exceeding the "ammonia quota" of a farm. A doubling of the herd becomes a potential possibility. If slurry is acidified to pH 6 the calculated reduction varies between 35 and 44%. An additional advantage is a higher N content and a higher N effectivity of the applied slurry. Cumulatively, this means a 15 to 30 kg N higher

Table i. Total cumulative reduction in NH_3 emission (%) after slurry is acidified to pH 5.5 or 6 compared to untreated slurry for cows that are kept permanently in the stable and when slurry is applied to either sand, clay, or peat soil.

Soil type	*Acidified to pH 5,5*	*Acidified to pH 6*
Sand	54%	35%
Clay	59%	39%
Peat	65%	44%

N effectivity per ha of the applied slurry.

Potential to contribute to the reduction of greenhouse gas emissions

The methane formation in manure comes to a standstill by acidification to a pH below 6. At farm level, it reduces methane emissions by about 20%. Based on limited and contradictory results, the expectation is that emissions of nitrous oxide are not affected.

Biological acidification

Biological acidification is very similar to the production of biogas. During this process organic acids are produced by fermentation of organic components in the manure and co-products. The organic acids will convert under anaerobic conditions into methane, provided that the pH is sufficiently high. By manipulating this process in such a way that the pH decreases below 6 the methanogenesis stops, and the acids remain present in the manure. To ensure that the pH plummets, the following options are possible: i) the microbial population is modified by addition of acid-producing microorganisms such as Lactobacillus spp.) and ii) the manure environment is changed to create more favorable conditions for acid producing microorganisms through:

- addition of easily fermentable organic substrate;
- addition of minerals such as zeolite to increase the reactive surface area;
- optimizing the temperature;
- the use of fresh manure;
- modifying the diet of cattle to influence manure quality, and
- the addition of organic acid to adjust the pH of the manure to create the right conditions for a specific (group) micro-organisms.

The various studies in which biological acidification of manure has been studied all differ in the combination and amounts of additives. The quality and quantity of the organic substrate added for example affects the rate with which the pH decreases, the pH level which is reached, and the extent to which a certain low pH level is maintained. It seems that the use of starch-like compounds gives the best results. By the start of the acidification process organic acids can be used in order to quickly create favorable conditions for acid-forming microorganisms. It is not clear whether this addition is only needed at the startup, or that it is needed permanently. This is important, as organic acids such as acetic acid and citric acid are relatively expensive. The addition of zeolite will increase the reactive surface between the micro-organisms and substrate. When used in a biogas plant it led to a 50% higher gas production.

In Austria testing is underway to get a better picture of the process conditions for biological acidification. Lab-results suggest that the use of acetic acid at the start-up, in combination with grafts of micro-organisms, and the addition of zeolite gives the best results. The focus there is to decrease the pH to a value of 6. For a targeted process control a pH clearly below 6 seems to be better. Their investigation is continuing both in the lab and on practical farms.

Positive side effects of acidification are that foaming of the slurry (which is at the expense of the storage capacity) can no longer occur, and that slurry will become more miscible.

The composition, particularly the pH and the content of fermentable organic substrate of the manure, affects how easily (with a minimum substrate addition) the manure can be acidified; the acidification potential. Research has shown that when fresh manure is used much less organic substrate is necessary in order to acidify manure. Fresh manure has a lower pH and contains more easily fermentable organic substrate than manure that is a few days old. Acidification can sometimes even occur at room temperature without the addition of organic substrate and / or micro-organisms.

In addition to the freshness of the manure, the cattle ration also affects the acidification potential of manure. Based on limited results, the methane production potential of fresh liquid manure (and hence indirectly the acidification potential) differs by 30 to 50% at different rations. Rations with much starch and little protein seem to have the highest acidification potential. Furthermore, it appears that the natural pH of cattle slurry can vary greatly (from 6.8 to over 8) as a result of the fed ration.

The costs

The factors that are important for cost-effective biological acidification appear to be:

- the use of fresh manure;
- adding organic acid at start up;
- the addition of zeolite;
- not too low temperatures (preferably >10 ° C); and
- a (small amount of) fermentable organic substrate (C).

The addition of organic substrate and organic acid is expensive and, moreover, the pricing is highly variable. Also, it is still not exactly known which additives are necessary and in what amount. Depending on the pricing a greater emphasis on one or the other can be placed. It is also conceivable to use a mixture of biological acidification and acidification with sulfuric acid. In addition, the costs are partly determined by the technical installation.

In Figure i the cost per cow and per kg saved NH_3 is shown for both biological acidification and acidification with sulfuric acid (Infarm system). Acidification with sulfuric acid costs 60 to 130 € per cow per year or about 4 to 8.5 € per kg saved NH_3. It is thought that for a system to be of interest, the costs must remain below 150 € per cow or 10 € per kg saved NH_3. For the prospects of biological acidification to be favorable, than 60% or more of the required acidification should result from the slurry itself, and at most 40% by the addition of organic substrate and or organic acid. If temporarily sulfuric acid is used, less than 60% of the required acidification should come from the manure itself. If only at the start additives are needed, biological acidification can become a very cost-effective technique.

Given the possible range in costs, additional experimental lab research into the optimal process conditions is needed before scaling up to a full-scale technique in practice.

Biological acidification in relation to biogas production

After acidification, the organic manure can be separated into a liquid and thick fraction. Due to a reduction of the viscosity of the biologically acidified manure this separation is easier than for untreated slurry. The thick fraction can be used for biogas production without adding co-substrate. The thick fraction is expected to produce more methane (after raising the pH in the biogas plant) than the thick fraction of untreated manure. In addition, it is conceivable that the residual heat in the biogas production is used to enhance biological acidification. This may result in less substrate and or organic acid needed to achieve and maintain a certain slurry pH. This makes biological acidification more cost-effective. A further step is to focus cattle feeding not only on milk production but also on a certain manure quality for a better acidification potential.

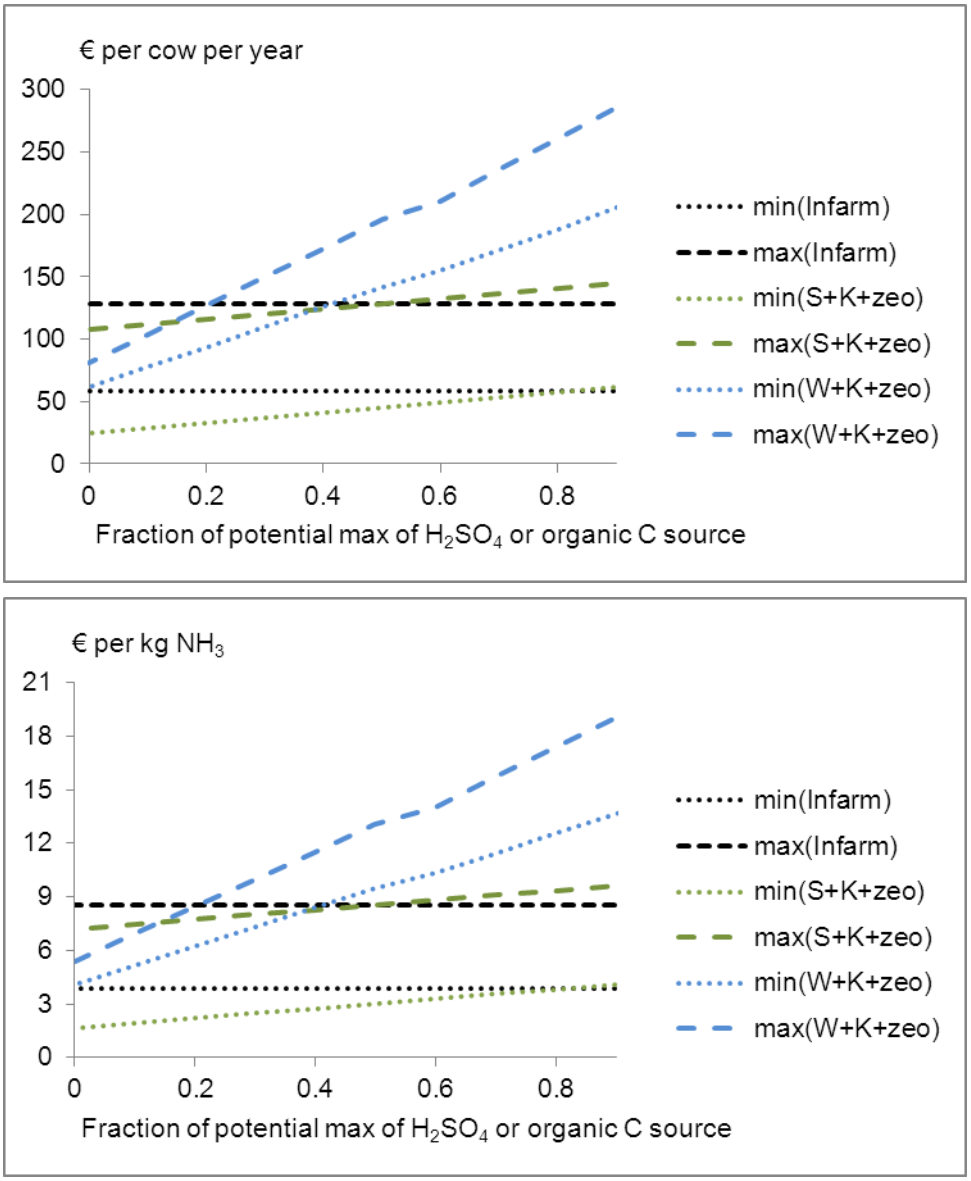


Figure i. The costs of the Infarm System (H_2SO_4 price varies between 100 (min) and 300 (max) € per ton) and of biological acidification using varying amounts of H_2SO_4 (S+K+zeo) or organic C (W+K+zeo). The upper graph expresses the annual costs per cow and the lower graph per kg saved NH_3. S is H_2SO_4, K is Lactobacillus addition via Kombioflor, zeo is zeoliet and W is the C-source ground wheat.

Conclusions:

- When the slurry pH is decreased below 5.5, NH_3 emission reductions of 54-66% are expected.
- Biological acidification of cattle slurry in cubicle houses has the potential to become a cost efficient technique to lower NH_3 emission on a farm scale. The estimated costs for biological acidification vary between 4 and 20 € per kg NH_3 saved (or 50 to 310 € per cow). This price range is to a large extent determined by the amount of C substrate needed. It is expected that the costs can be maintained below 10 € per kg NH_3. Additional lab testing is needed to get more quantitative information about optimal process conditions for biological acidification in order to make more precise cost calculations.
- Positive side effects of acidification are that it reduces methane emission and that it results in more homogenous slurry without a foam film on top. The latter results in a more efficient use of the storage capacity in cubicle houses possible.
- For the short term (in order to make a quick start) an acidification system based on a mix of biological and inorganic acidification seems to be attractive from the viewpoint of risk distribution between costs of additives (acetic acid, C substrate and H_2SO_4).
- For the long term the highest cost efficiency for biological acidification is expected to be a fed batch system. In such a system fresh manure is frequently added to manure that has already been acidified and where:
    - at the start fresh slurry is immediately acidified to pH 5.5-6;
    - zeolite and Lactobacillus spp. are regularly added;
    - a limited amount of C substrate or organic acid is added in case that the quality of the fresh manure is not sufficient as a C substrate to maintain the pH; and
    - the temperature remains above 10^oC.
- It is expected that it is more profitable to use the thick fraction of biologically acidified slurry as a mono substrate in a bio gas plant than the thick fraction of untreated slurry.

1 Introduction

1.1 General

The last decades the ammonia (NH_3) emission from dairy farms has strongly decreased as a result of the compulsory use of low emission slurry application methods and more efficient feeding strategies. Dutch policy is focused on further reducing NH_3 emissions especially in, and in the vicinity of special areas of specific natural value (Natura 2000). The Programmatic Approach Nitrogen (Programmatische Aanpak Stikstof) aims at a NH_3 emission reduction of 10% from dairy farms by January 1st 2014. Apart from the aim to further reduce NH_3 emissions, Dutch policy also aims at reducing green house gas emissions from agriculture mainly by reducing CH_4 and N_2O emissions. To comply with these aims and (future) legislation concerning NH_3 emissions it will be necessary for dairy farms to decrease NH_3 and green house gas emissions. For dairy farms situated in the vicinity of Natura 2000 areas this is particularly urgent as they are prohibited to expand when this leads to an increase in NH_3 emission. Cost effective methods to further reduce NH_3 and green house gas emissions are sought after.

A promising method to reduce both NH_3 and greenhouse gas emissions is to acidify slurry. Originally this method was developed to decrease NH_3 emissions. More recent studies have shown that slurry acidification also (strongly) reduces CH_4 emissions from stable and storage.
There are several different ways to acidify slurry. Initial research 20-25 years ago focused mainly on using nitric acid (HNO_3). This method was not accepted by Dutch authorities due to specific negative side effects related to HNO_3, like controllability and the risk of high N application rates. More recent studies focus on using sulphuric acid (H_2SO_4). The Danish company Infarm has introduced a practically applicable technique to automatically acidify slurry with H_2SO_4 in cattle and pig stables. In Denmark this is an accepted system to reduce NH_3 emissions and has been implemented on more than 80 farms. The relatively high usage of H_2SO_4 needed to acidify the slurry has some disadvantages; the S content of slurry is higher than necessary for optimal crop growth resulting in a loading of the soil and adjacent water bodies with S, and extra safety precautions must be taken to handle this strong inorganic acid.
A quite different approach, but based on the same principals, is to biologically acidify slurry by stimulating specific acid producing microorganisms in the slurry. Recent and ongoing research shows promising results which opens up new perspectives to implement biological acidification of slurry in practice.

1.2 Aim and approach

In this study an inventory is made of the potential to biologically acidify slurry in order to establish:

- to what extent NH_3 emission can be reduced from stables and during application;
- the cost effectiveness of the method; and
- other benefits of the method, e.g. reduction of greenhouse gas emissions, no slurry foaming.

The study consists of two parts. In a literature review the processes involved in the biological acidification of slurry are assessed. Special attention is given to the different additives used to stimulate the acid producing microorganisms. In the second part we have collaborated with Dr. Wenzl from the research centre Raumberg Gumpenstein in Irdning Austria. The latest experimental results are presented and discussed.
Based on these results a conclusion is drawn what the feasibility is to implement the technique of biologically acidifying slurry in dairy farms in The Netherlands. If implementation seems feasible, an inventory is made what additional information is required before implementation is possible.

2 Acidification of slurry: background and processes

2.1 Natura 2000

Dutch dairy farms in the vicinity of Natura 2000 areas are facing constraints in their ammonia (NH_3) emissions as a result of which they are not allowed to expand if this leads to increased N deposition in the sensitive areas. Natura 2000 is the Europe wide network of nature protection areas established with the aim to assure the long term survival of Europe's most valuable and threatened species and habitats. Natura 2000 is not a system of strict nature reserves where all human activities are excluded. The emphasis is on ensuring that future management is sustainable, both ecologically and economically (http://ec.europa.eu/environment/nature/natura2000).
In The Netherlands, 162 Natura 2000 areas have been assigned. In most of these areas it is important to reduce nitrogen (N) deposition to be able to achieve set nature goals. As a result no new permits were issued for activities which led to additional N emissions. This has had a negative influence on local and regional economies. Since 2009 the 'Programmatische aanpak stikstof' (further referred to as PAS) has been developed to make sure that Natura 2000 goals are met and that at the same time the economic activity in, and in the vicinity of these areas can continue to develop. Based on current legislation (https://zoek.officielebekendmakingen.nl/kst-30654-99.html), permits are issued based on an ecological test to ensure that in the area the N deposition will decrease sufficiently to ensure the conservation of the sensitive habitats. For dairy farms in the vicinity of Natura 2000 areas this means that they are allowed to expand if this does not lead to an increase in N emissions in the area. This applies to approximately half the Dutch dairy farms. Furthermore all dairy farms should have reduced their ammonia emission by 10% by January 1st 2014.

2.2 NH_3 emission from dairy farms

In The Netherlands agriculture is the main source (~90%) of NH_3 emission. Of this NH_3 emission approximately 90% is emitted from manure and 10% from artificial fertilizers (Hoogeveen et al., 2010, Table 2.1). Compared to other livestock, dairy cattle contributes most (35%) to the NH_3 emission from slurry. Of the NH_3 emission from cow manure most is emitted from the stable (~50%) and when it is applied to the field (~40%). The NH_3 emitted during grazing (9%) and from storage (1%) are relatively small.

Table 2.1. NH_3 emission from animal slurry in 2007 and expectations for 2020 (Hoogeveen et al., 2010).

	Total		dairy cows and calves			
	mln. kg NH_3		**mln. kg NH_3**		**%**	
	2007	2020	2007	2020	2007	2020
Stable	57.0	46.8	19.0	20.9	49.5	51.6
Storage	3.9	4.6	0.4	0.5	1.0	1.2
Grazing	7.8	6.5	3.5	3.1	9.1	7.7
Application	40.7	31.6	15.5	16.1	40.4	39.8
Total	109.4	89.5	38.4	40.5	100	100

For livestock in general, NH_3 emission from manure is expected to decrease with nearly 20 million kg NH_3 towards 2020. This decrease is achieved through lower emissions from stables and from applied slurry to the soil. The expected decrease mainly originates from pig and poultry farming; e.g. low

emission housing, smaller number of livestock and less manure application (Hoogeveen et al., 2010, Table 2.1). Contrary to pigs and poultry, for dairy farms neither the amount of NH_3 emitted nor the sources of emission are expected to drastically change towards 2020 compared to 2007. The overall NH_3 emission from slurry from dairy cattle is even expected to increase slightly in 2020 compared to 2007 (40.5 compared to 38.4 million kg NH_3). This increase is due to the expected increase in required feed to assure the higher milk production per cow. A decrease in the number of young stock per cow and lower N content of faeces due to diet adaptations like better balanced concentrates and more maize in roughage is expected to decrease the NH_3 emission (Silvis, 2009). Overall the contribution of dairy cattle compared to the total NH_3 emission from slurry is expected to increase to ~45% in 2020.

2.3 Greenhouse gas emission from dairy farms

Agriculture is an important source of green house gas emissions (9% of national total) mainly due to the emission of CH_4 and N_2O. Table 2.2 shows an overview of the most important sources contributing to the green house gas emissions from agriculture. Methane and N_2O contribute almost equally. Most CH_4 is emitted from the rumen and most N_2O from soils.

Table 2.2: Contribution of the most important sources of green house gas emissions in Dutch agriculture in 2008 (Maas et al., 2010).

			Emissions	Contribution to total in 2008 (%)		
Source		Gas	Mton CO_2 eq.	Per source	Per gas	Total CO_2 eq.
Total national green house gas emissions		CH_4	17.1			
		N_2O	11.8			
		All	206.9			100
Agriculture		CH_4	9.1	49	100	4.4
		N_2O	9.4	51	100	4.5
		All	18.5	100		8.9
CH_4						
Rumen fermentation	Total	CH_4	6.5	35	71	3.1
	Cows	CH_4	5.8	31	64	2.8
	Pigs	CH_4	0.4	2.2	4.4	0.2
	Other animals	CH_4	0.3	1.6	3.3	0.1
Slurry management (emitted from stable)		CH_4	2.7	15	30	1.3
	Cows	CH_4	1.5	8	16	0.7
	Pigs	CH_4	1.1	6	12	0.5
	Other animals	CH_4	0.1	0.5	1.1	0.0
N_2O						
Slurry management (emitted from stable)		N_2O	0.9	5	10	0.4
Agricultural soils	Total	N_2O	8.5	46	90	4.1
	Direct emission from soil	N_2O	4.8	26	51	2.3
	Produced by animals on soil	N_2O	0.6	3.2	6.4	0.3
	Indirect emissions from soil	N_2O	3.0	16	32	1.4

For cows fermentation in the rumen is the main CH_4 source (~80%). Emission of CH_4 from urine and faeces excreted in the stable also contributes significantly (~20%, Table 2.2) to the total CH_4 loss . Methane is produced by CH_4 producing microorganisms that degrade volatile fatty acids which in turn have been formed by decomposition of organic matter. In slurry the CH_4 production is influenced by

temperature, acidity, residence time, and decomposability of the organic matter (Van Dooren and Smits, 2007).
For N_2O, urine and faeces excreted in the stable is a minor source (10%). Most N_2O is emitted directly or indirectly after the slurry is applied to soils (Table 2.2).
Of the total Dutch manure production most is produced by cows (75% Velthof et al., 2000). Cows thus contribute considerably to both the total CH_4 and N_2O emissions from agriculture.

To achieve Dutch climate ambitions the aim is to reduce non CO_2 greenhouse gasses by 25-27 Mton CO_2 equivalents in 2020. Of the total Dutch CH_4 and N_2O emissions respectively 54 and 80% originate from agriculture. It is thus clear that agriculture will have to contribute (considerably) to the reduction of non CO_2 greenhouse gasses. In a covenant ("Schone en zuinige Agrosectoren"), the Dutch government and the dairy sector have agreed to reduce the CH_4 emission per cow with 5% and the CH_4 emission from slurry with 15%.

To comply with these aims and (future) legislation concerning NH_3 emissions it will be necessary for all dairy farms to decrease NH_3 and greenhouse gas emissions. For dairy farms situated in the vicinity of Natura 2000 areas this is particularly urgent. A promising method to reduce both NH_3 and greenhouse gas emissions is to acidify slurry.

2.4 Acidification of slurry

Acidification of slurry was originally investigated to reduce NH_3 emissions. Numerous studies have shown the potential to effectively reduce NH_3 emissions (e.g. Pain et al., 1990, Husted et al., 1991, Bussink et al., 1994, Kai et al., 2008). All the studies in which slurry is acidified with sulphuric acid (H_2SO_4) show a strong reduction in NH_3-emission from the stable (~35%), storage (~90%), and during application in the field (~85%). The acidification of slurry has also been shown to strongly reduce methane (CH_4) emissions from slurry (Oenema en Velthof, 1993, Berg et al., 2006, Ottosen et al., 2009). Bussink (2009) calculated that this could reduce CH_4 emission with 55 kg per cow per year which equals with1150 kg CO_2 per cow per year. In addition, it was estimated that slurry acidification could reduce greenhouse gas emission with 0.6 Mton CO_2 equivalents, which matches with 2% of the Dutch climate goals concerning the reduction of non CO_2 greenhouse gasses (Bussink, 2009, Bussink and Van Rotterdam 2011).

2.4.1 Theory

Ammonia is emitted from slurry after a number of reactions have occurred in the slurry. First there is a fast conversion of mainly urea into $(NH_4)_2CO_3$. This product easily dissociates into soluble ammonium and (bi)carbonate. The ammonium (NH_4^+) is in equilibrium with ammonia (NH_3) through the reaction:

$$NH_4^+ \rightarrow NH_3 + H^+ \qquad \text{Equation 1}$$

The NH_3 in solution is in equilibrium with NH_3 in the gas phase. The proton (H^+) in equation 1 shows that the conversion of NH_4^+ into NH_3 is pH dependent. A lower pH (more H^+) results in a lower conversion and thus in a lower emission of NH_3. The pH in slurry is naturally relatively high (between 7.2 and 8.2) and reducing the pH to a value between pH 6 and 6.5 results in a 10-fold decrease in NH_3 emission (Lameijer and Vervoort, 1995). A reduction of the pH in slurry to a pH lower than 5.5 results in a reduction in NH_3 emission to practically zero.

The addition of acid does not necessarily result in an instant decrease in pH of the slurry. The amount of acid needed to decrease the pH of slurry depends on the proton buffer capacity of the slurry (Husted et al., 1991). Proton buffering is the capacity to resist a change in pH with the addition or removal of acid. Manure is a complex mixture of various substances. Adding acid will not only have an effect on the reaction between NH_3 and NH_4^+ (equation 1) but also on several other reactions in which protons are consumed.
Buffering of the pH is controlled by acid base pairs. A study with cattle slurry showed that the acid base pairs CO_2/HCO_3^- , HCO_3^-/CO_3^{2-} en NH_4^+/NH_3 in combination with $CaCO_3$ buffer the pH of manure (Husted et al., 1991). Approximately ⅓ of the buffering was controlled by the solid phase ($CaCO_3$) and the rest by the liquid phase. When $CaCO_3$ reacts with protons the formed reaction product CO_2 can evaporate from the slurry and thereby the buffer is removed from the slurry. This explains that the amount of acid that is needed to reduce the pH of slurry that has already been acidified is lower than the amount of acid needed for non-acidified slurry (Lameijer and Vervoorn, 1995).

Methane in slurry is produced by methane producing microorganisms that degrade low molecular weight organic molecules. This process is known as methanogenesis. The low molecular weight organic molecules are formed by the decomposition of organic matter. For methanogenesis to occur the conditions must be anaerobe (no oxygen). Several studies show that when the pH of slurry is decreased below pH 6, anaerobic degradation processes by microorganisms strongly decrease or even stop (Fangueira et al., 2010b, Ottosen et al., 2009, Sørensen en Eriksen, 2009). This is suggested to be caused by the presence of high concentrations of volatile fatty acids in protonated form (20-25 mM in acidified compared to <0.1 mM in untreated slurry, Ottosen et al., 2009).

2.4.2 Pros and cons of acidifying slurry

Apart from reducing NH_3 and greenhouse gas emissions, acidifying slurry has a number of advantages. The most striking characteristic of acidifying slurry is that it is a chain approach. Acidifying slurry in the stable not only reduces NH_3 emission from the stable but also reduces emissions from the slurry in the next steps in the chain: during storage and during and after application to the soil. In addition, the acidified slurry has a higher fertilizer value because the NH_3-N that is not emitted, is retained resulting in a the higher N content in the slurry. Consequently, less mineral N fertilizer is needed.

The most important disadvantage of acidifying slurry is that additives must be added to reduce the pH of the slurry. These additives may have adverse effects on certain characteristics of the slurry and on the environment after the slurry is applied to the soil. The extent and character of these effects depends on the additives used to acidify the slurry. Due to the lower pH of the slurry extra lime is also needed to maintain the pH of the soil (Bussink, 2009). From a practical point of view it is often difficult to obtain a homogeneous mixture of slurry and acid / additives. The different methods also have their own specific disadvantages.

2.4.3 Different methods to acidify slurry

At the start of the 20th century the first studies were performed to investigate the possibility to reduce NH_3 emissions by acidification of slurry (Jensen, 1928; Egnér, 1932 in Husted et al., 1991). Since then many studies have been performed on this topic and different methods have been investigated.
Slurry can be acidified in several different ways which include (based on onder andere Van Dooren and Smits, 2007):

1. an inorganic acid like hydrochloric acid (HCl), nitric acid (HNO_3) or sulphuric acid (H_2SO_4), in the order of increasing strength of the acid;
2. base precipitating salts like $CaCl_2$, (Jensen 1928 en Egnér 1932 in Husted et al., 1991);
3. inoculation with, or stimulation of, specific microorganisms that produce organic acids like lactic acid or acetic acid. This method is called biological acidification;
4. an organic acid. This is however relatively expensive and the acid can be decomposed by microorganisms in the slurry. It can be used to initiate biological acidification;
5. adaptation of the animals feed to alter the pH of the urine and faeces (Van Dongen et al., 2006). The total effect will be small but is possibly interesting in combination with option 1 and 3; and
6. feed additives (e.g. benzoic acid in pig farming). This is relatively difficult to control and to maintain. The effect of benzoic acid is about 16% reduction in NH_3 emission per pig place (Aarnink et al., 2008).

In The Netherlands most research on slurry acidification was performed in the 80's and 90's of the previous century. The research mainly focused on using nitric acid (HNO_3). The Dutch government however did not acknowledge this to be an acceptable method. The main reasons were controllability, risk of high N application rates, and an increase in N_2O emissions as a result of increased denitrification rates (Berg et al., 2006, Oenema and Velthof, 1993). In later years, research shifted to using sulphuric acid (H_2SO_4).
The use of H_2SO_4 has a number of advantages over HNO_3. The most important advantages are that H_2SO_4 does not result in an increased N_2O emission, that the slurry can be acidified to a less low pH with the same effect, and that this pH is more stable compared to using HNO_3.

2.5 Potential NH_3 emission reduction for large farms

Recently, the Danish company Infarm has introduced a practically applicable technique to automatically acidify slurry from cattle and pig housing using sulphuric acid (H_2SO_4). In Denmark this is an accepted system to reduce NH_3 emissions and has been implemented on more than 80 farms. A thorough investigation of the Infarm system and the applicability for the Dutch situation is described in Bussink (2009) and Bussink and Van Rotterdam (2011). Here we will shortly summarize to what extent NH_3 emission can be reduced from stables and during application for large farms in The Netherlands.

Due to the investment and maintenance costs of the Infarm system, it is mainly suitable for large farms. In the study by Bussink and Van Rotterdam (2011) the potential NH_3-emission reduction was calculated based on 6 scenario's; limited grazing (Lg) and permanent in the stable (Ps) for 3 types of ration (N-poor, average, and N-rich). Results are presented in Table 2.3.

Table 2.3. NH_3-emission reduction from slurry after emission from stable and after application of the slurry to either sand, clay, or peat soil for cows permanent in the stable (Ps) and limited grazing (Lg).

		Untreated			*Acidified to pH 5.5*				*Acidified to pH 6*			
	Milk urea content	*20*	*24*	*29*	*20*	*24*	*29*	*decrease*	*20*	*24*	*29*	*decrease*
NH_3-emission (kg N cow^{-1} yr^{-1})												
Stable	Ps	8.0	9.2	11	5.2	6.0	7.0		6.4	7.4	8.8	
	Lg	7.0	7.8	8.9	4.5	5.1	5.8		5.6	6.2	7.1	
Application	Sand	5.7	5.9	6.1	0.9	0.9	1.0		2.3	2.4	2.5	
	Clay	8.3	8.6	8.9	1.3	1.4	1.4		3.4	3.5	3.6	
	Peat	14	15	15	2.3	2.4	2.5		5.7	6.2	6.2	
TAN- content slurry (kg N cow^{-1} yr^{-1})												
Initial		58	61	65	58	61	65		58	61	65	
After stable	Ps	50	52	54	53	55	57	5.8%	52	54	56	3.5%
	Lg	51	53	56	53	56	59	5.0%	52	55	58	3.5%
After application	Ps: Sand	44	46	48	52	54	56	17%	49	51	54	12%
	Ps: Clay	41	43	45	51	53	56	23%	48	50	53	17%
	Ps: Peat	35	37	38	50	52	55	43%	46	47	50	30%

Acidification with the Infarm system on large farms results in an expected NH_3 emission reduction from the stable of 2.4 – 3.8 kg N $animal^{-1}$ yr^{-1}. This is comparable to an emission reduction that can be obtained with most emission- poor stables. After the acidified slurry is applied to the field the lower pH of the slurry also positively affects the NH_3-emissions from the field; NH_3 emission decreased from 26% (trailing foot) to 4% when acidified tot pH 5.5

A summary of the overall reduction in NH_3 emission when the whole chain is considered (stable to application) is shown in table 2.4. Acidification with the Infarm system is expected to result in an overall reduction in NH_3 emission of 53-66% when the slurry is acidified to pH 5.5 (Table 2.4). When the slurry is acidified to pH 6 this reduction is slightly lower but still the NH_3 emission can be reduced with 34 – 45%. The decrease in NH_3 emission results in a higher N content of the slurry and a higher N effectivity of applied slurry. As a result N effectivity of applied slurry can increas with 15 – 30 kg N per hectare or otherwise N fertilizer use can decrease with 15 – 30 kg N per hectare.

Table 2.4. Total overall reduction in NH_3 emission (%) after slurry is acidified to pH 5.5 or 6 compared to untreated slurry for cows that are kept permanently in the stable and when slurry is applied to either sand, clay, or peat soil.

	Acidified to pH 5.5			*Acidified to pH 6*		
Milk urea content	*20*	*24*	*29*	*20*	*24*	*29*
Sand	55	54	53	36	35	34
Clay	60	58	58	40	39	37
Peat	66	65	63	45	44	43

By adding H_2SO_4 to slurry the sulfur (S) content of the slurry strongly increases. The amount of sulphuric acid that is needed to decrease the pH of the slurry to 5.5 depends on the N content of the slurry (Sørensen et al., 2009). For Dutch dairy cows the range in amount of sulphuric acid needed to decrease the pH to 5.5 varies between 7.5 and 12 kg H_2SO_4 ton^{-1} slurry depending on N content of the slurry. For slurry with an average composition of 4.1 kg N ton^{-1} approximately 10 kg H_2SO_4 ton^{-1} is needed (Bussink 2009). For limited grazing and permanent in the stable this would result in an amount

of S added to the soil of respectively 117 and 165 kg S ha^{-1}. This is much more than is taken up by the crop (20 – 50 kg S ha^{-1}). This results in leaching of sulfate from the soil to surface and / or groundwater where the target value for sulfate (100 mg l^{-1}) may be approached. An additional disadvantage of using H_2SO_4 is that it is a strong inorganic acid and thus a safety hazard (Borst, 2001). Consequently, strict safety precautions must be taken to prevent a calamity (Clemens et al., 2002).

Studies using sulphuric acid show that decreasing the pH of slurry has a large potential to decrease NH_3-emissions and also green house gas emissions. As stated above, the use of sulphuric acid also has major disadvantages and will thus only be a temporary solution. It is thus desirable to find other methods to decrease the pH of slurry that don't have undesirable side-effects.
It is possible to biologically acidify slurry by stimulating specific acid producing microorganisms (Lameijer and Vervoort, 1995, Hendriks and Vrielink, 1997, Clemens et al., 2002, Wenzl et al., 2009, Somitsch et al., 2008). The theory, recent developments, and applicability in Dutch dairy farms is investigated in this study.

3 Biological acidification of slurry

3.1 History and recent developments

Biological acidification of slurry has not been studied as extensively as acidification using an inorganic acid. In the 90's of the previous century a Dutch patent was published concerning biological acidification of slurry (Lameijer and Vervoort, 1995). Subsequent studies, in which the pH was reduced to below 6 show a strong reduction in NH_3 emission from biologically acidified slurry. Both from the stable (Hendriks and Vrielink, 1997) and when applied to the soil (Clemens et al., 2002). Hendriks and Vrielink (1997) however concluded that due to the quantity and type of additives needed to acidify the slurry and to maintain the low pH, the method was too expensive to be practically implemented. More recently, biological acidification has been investigated by Clemens en Wulf (2005) and in Austria by Wenzl et al. (2009) and Somitsch et al. (2008). The prospects from these studies are positive and in Austria research was continued on a larger scale in 2011. Recent results from Austria will be discussed in Chapter 4.

Because biological acidification is still in an experimental phase, no studies are known to have investigated the direct effect on greenhouse gas emissions. In this study we will explore the potential to reduce greenhouse gas emissions based on theory and the results found when acidifying slurry with inorganic acids.

3.2 Theory

Biological acidification is very similar to the biogas production process (Figure 3.1). Simplified three steps can be distinguished. In the first step hydrolysis take place by fermenting bacteria. Fermenting bacteria
decompose long chains of complex carbohydrates, proteins and lipids in shorter parts.

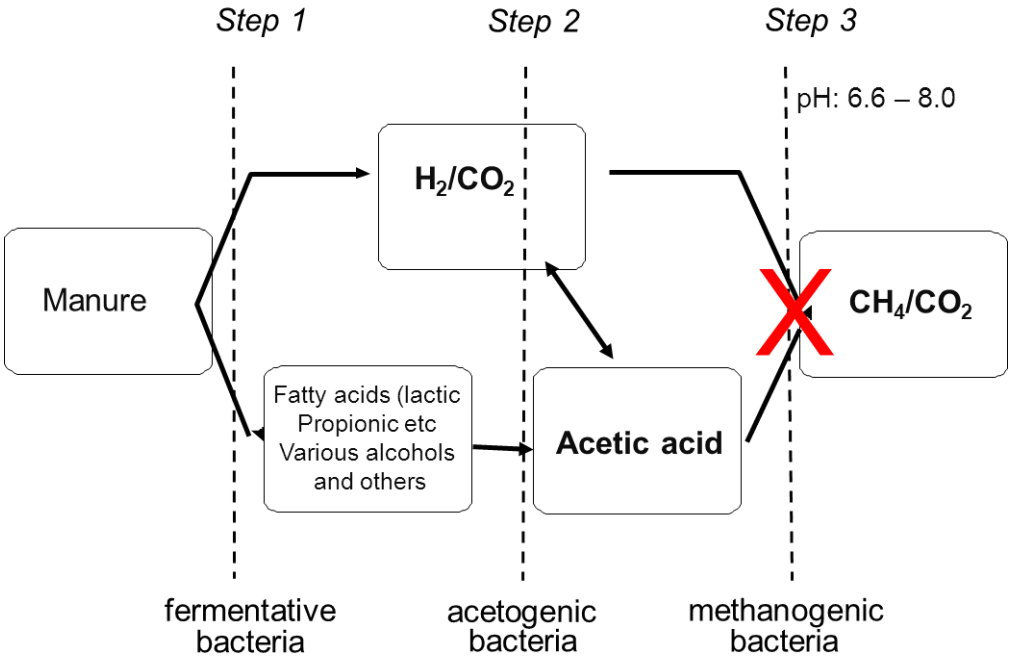


Figure 3.1. A simplified three step stage of fermentation of organic manure.

In the second step acid producing bacteria convert the intermediates that were formed in the first step into acetic acid but also into alcohols, other organic acids, carbon dioxide, amino acids etc.. The acid producing bacteria are facultative anaerobic and they can grow under acid conditions. To produce acetic acid they need oxygen and carbon for which they use dissolved or bound oxygen. They thereby create an anaerobic environment which is essential for methane producing bacteria. In step three methane producing bacteria decompose compounds with a low molecular weight to methane and carbon dioxide. Anaerobic conditions and a pH above about 7 are essential for a high methane production.

In biological acidification steps are taken to prevent the production of methane (step 3 in Figure 3.1) by maintaining a low pH (below 6) and less strict anaerobic conditions. Specific acid producing microorganisms are stimulated to convert organic substrate (e.g. degradable carbohydrates) into acids (e.g. lactic acid or acetic acid, Lameijer en Vervoort, 1995, Hendriks en Vrielink, 1997, Clemens en Wulf, 2005). In more detail this stimulation is achieved in two different ways; i) the microbiological population is altered by adding a specific acid producing microorganism (i.e. Lactobacillus spp) and ii) the environment in the slurry is altered to create conditions that activate and stimulate specific acid producing microorganisms by:

- adding easy decomposable organic substrate;
- adding minerals like zeolite to increase the reactive surface;
- optimizing the temperature;
- using fresh manure; and
- adjusting the diet of the cattle.

For example addition of glucose stimulates the production of lactic acid by lactic acid producing bacteria:

$$C_6H_{12}O_6 \rightarrow 2\ CH_3CHOHCOOH \qquad \text{Equation 2}$$

To reduce NH_3 emissions to sufficiently low levels the pH of the slurry must be decreased to a value below 6.5, preferably between 4.5 and 6.2 and must be maintained at this level (Lameijer and Vervoort, 1995). To achieve this, often a combination is used of both adding specific microorganism and influencing the environment. Once activated, the microorganisms must continue to produce acid to maintain the low pH. If the manure itself is insufficient as a C source, fresh substrate is continuously needed. One of the questions is how to minimize the addition of substrate because this can be quite costly (Hendriks and Vrielink (1997).

3.3 The manure itself

The composition of the manure that is used directly affects biological acidification. The slurry contains acid producing microorganisms and substrate for these organisms. The slurry composition also directly affects the NH_3 emission; a lower N content and lower pH of the slurry both result in lower NH_3 emissions.

3.3.1 The substrate

Cattle slurry itself is an important C source for microorganisms. However, the quality of cattle slurry differs, depending on the cattle diet and the milk production level. Relatively little is known about manure quality in relation to acidification, but some information about the methane production potential is available. When suppressing the methane production (step 3, Figure 3.1) the methane production potential is equal to the acidifying potential. Kryvoruchko et al. (2006) measured under standardized

conditions the methane production of the excreta of six dairy cows which were fed different amounts of hay, maize and grass silage and concentrates. The lowest and highest methane production showed a 33% difference. This means in fact that there is a substantial difference in acidifying power between dairy manures. A recent investigation in Germany (Bugdahl, 2011) of thirty fresh dairy cattle slurries confirm these findings. The lowest and highest level of methane production showed a 50% difference. First observations in practice suggest that methane production from dairy cattle slurry is highest on intensive dairy farms (relatively high milk yield and concentrate usage). Most publications (Lameijer and Vervoort, 1995; Hendriks and Vrielink,1996; Clemens and Wulf, 2005; Somitsch et al,. 2008) mention that under conditions found in practice the self acidifying power of manure is too low to obtain a pH below 6. This may partly be the result of the relatively low temperature of normal manure, quality of the manure, the age of the manure, and the degree to which conditions are anaerobic.

The acidifying potential of day fresh slurry is higher compared to older slurry. Day fresh slurry has a lower pH due to a higher volatile fatty acid (VFA) content and more (endogenous) substrate in the form of low molecular weight molecules for the acid producing microorganisms (Lameijer and Vervoort 1995). As a result respectively less acid is needed to decrease the initial pH and less substrate is needed to feed the acid producing microorganisms in fresh manure compared to old manure. Lameijer and Vervoort (1995) showed that the amount of acid needed to acidify pig slurry nearly doubled when the slurry was first stored for 16 weeks instead of when day fresh slurry was acidified.

Miller and Varel (2001) did not need an additional C substrate to realize a sharp pH drop. In their experiments fresh and aged beef cattle manure was incubated during 36 days at room temperature (20-23 ^{o}C) under anaerobic conditions (after gassing with N2). Their results showed a fast drop of pH (Figure 3.2). After 10 days the pH was 4 for the fresh slurry and 5.5 for the aged slurry. Methane

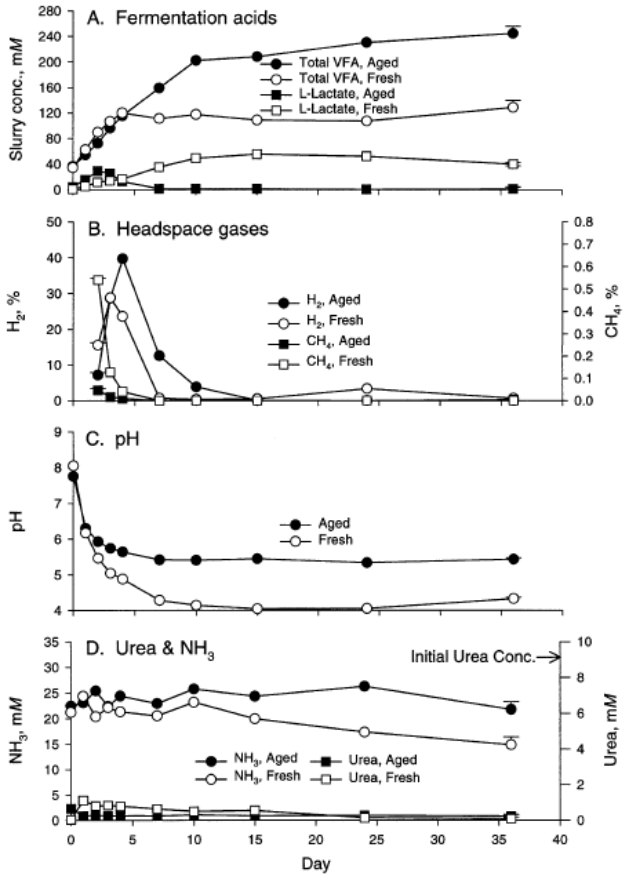


Figure 3.2. Production of total volatile fatty acid (VFA) and fermentation gases (H_2 and CH_4) in fresh and aged manure slurries in relation to pH, L-lactate, NH_3, and urea contents (obtained from Miller and Varel, 2001).

production stopped when the pH dropped below pH 6. Furthermore the results showed a much higher lactate and lower volatile fatty acid concentration in fresh slurry than in aged slurry. Starch fermentation was the dominant process for the pH drop and the lactate production (Miller & Varel, 2002). When starch is no longer available, protein fermentation (old slurry) may take place. In contrast Clemens and Wulf (2005) did not find a pH decrease during anaerobic incubation of (aged) slurry. An additional C source, sugar or starch, was needed to obtain a pH drop. In line with Miller and Varel (2001) the pH drop at 20 ^{o}C was faster and to a lower level than at 8 ^{o}C.

The results suggest that little or no additional C substrate is necessary when at least fresh cattle slurry of good quality (easy fermentable substrate which most likely will result from cattle diets with high starch content) is used and temperature is relatively high. In all other cases additives (C substrate, zeolite, organic acids) may be necessary to obtain and maintain a low pH.

3.3.2 pH

The natural pH of pig and cattle slurry can vary considerably. This was for instance found in Austria where almost 140 cattle slurry samples were analyzed for composition (Figure 3.2). In the Netherlands, the pH of manure is rarely measured. The limited data for cattle slurry (Monteny et al., 2002) also show a similarly large variation as found in Austria.

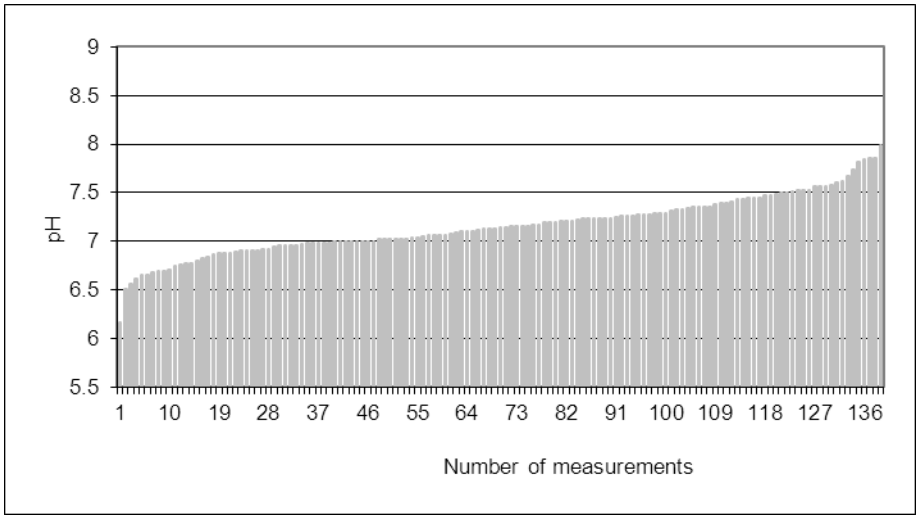


Figure 3.2. De pH of cow slurry in Austria (source: Wenzl personal communication).

The variation in slurry pH can be (partly) attributed to the composition of the cattle feed. A reduction in pH of manure can for instance be achieved by additives in pig feed (e.g. benzoic acid, Aarnink et al., 2008). The pH of slurry can also be decreased by manipulation of the Dietary Cation Anion Difference by feeding more anions compared to cations (Van Dongen et al., 2006).
In Austria Wenzl and Somitsch developed a fully automatic system in which the slurry pH is followed continuously. On two cattle farms, the pH was measured over longer periods of time. On one of these farms considerable variations in the pH of the slurry were measured (sometimes more than one pH unit). These pH variations could be related to a change in the feed composition. Wenzl (personal communication) also found a positive relationship between the pH and N content of slurry and a negative relationship between pH and the ratio C content over organic N content (Figure 3.3). A lower pH can thus be expected for slurries with a lower N content and when the slurry contains more C per

organic N molecule. The N and C content of manure are a direct result of how the animals are fed. Other factors that determine the pH of manure in addition to food, are ration design, food hygiene, animal health and stall hygiene, and also the microbiological composition.

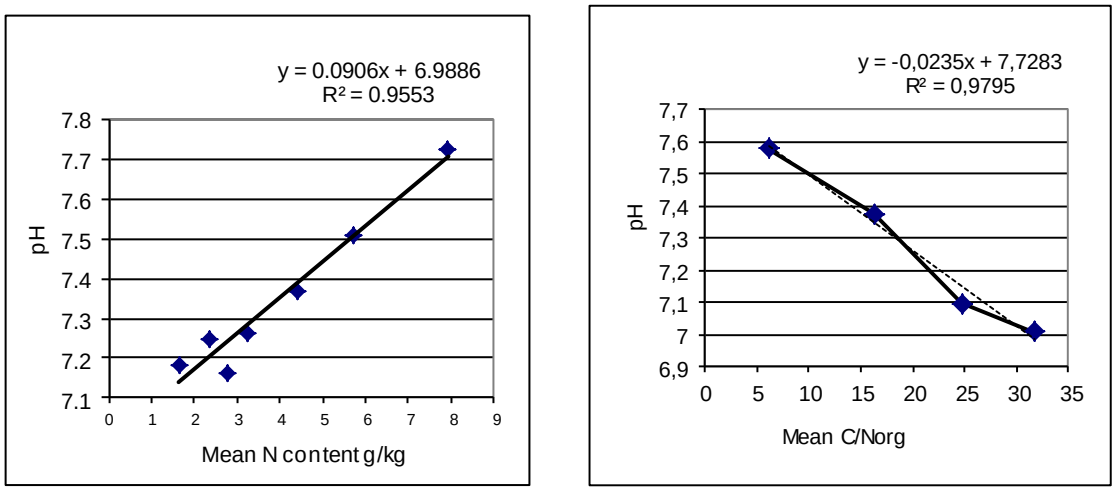


Figure 3.3: Relationship between pH and the average N content in slurry (A) and between pH and the ratio C content over organic N content (B). (Source: Wenzl personal communication)

3.3.3 N content

A reduction of N content in the ration, and thus a direct reduction in NH_3 emissions from the faeces, can also be achieved by diet adaptations (Duinkerken et al., 2003). The emission can be reduced by reducing the rumen degradable protein balance of the ration (referred to in Dutch as OEB). Part of the OEB reduction can be achieved through the roughage and another part through a reduction in the use of protein rich concentrates. A reduction in the OEB in roughage can be achieved by using low protein forages such as maize, the reduction of N fertilization on grassland and harvesting the grass at a later point in time.

It is also possible to manipulate the ileal digestible protein content (referred to in Dutch as DVE). An excess of DVE in the ration is not utilized in the cow and is excreted as urinary N and urea in the milk. Limited grazing or summer feeding contributes to achieve a balanced diet. With unlimited grazing this is difficult to achieve because grass supply, grass composition and grass intake are rarely well balanced concerning protein requirements.

Clearly there are opportunities to change the composition of slurry through dietary changes to make it more favorable in terms of biological acidification and direct NH_3 emissions. The most simple to implement precaution to effectively biologically acidify slurry is to use day fresh manure and not slurry that has been stored for some time (Miller & Varel, 2001).

3.4 Additives

Different methods have been developed to biologically acidify slurry. These vary in adding, whether or not combined, the following additives (Lameijer and Vervoort, 1995; Somitsch et al., 2008):

1. an (in)organic acid to reduce the pH of the starting slurry to create the right conditions for a specific (group) of microorganisms;
2. inoculation with a specific acid producing microorganism.;
3. a substrate for the acid producing microorganisms; and
4. colloidal material upon which microorganisms can fix, for example zeolite.

In the following paragraphs the different additives will be discussed one by one.
In literature the various studies investigating biological acidification of slurry all vary in their usage of (a combination of) additives. The effect of different combinations of additives on the pH of the slurry as described in the various studies is discussed in a subsequent paragraph (§ 3.4.5).

3.4.1 Addition of acid

Acid producing microorganisms do not per definition thrive at the normal pH range of the starting slurry. Lameijer and Vervoort (1995) found that it is necessary to decrease the initial pH once to about 6.5, in order to activate the specific microorganisms. Ideally this activates the acid producing microorganisms and they start to produce acid *in situ* which further decreases the pH of the slurry.
Acidification of the initial slurry can be achieved by adding an organic acid, an inorganic acid, or a batch of acidified slurry. Adding a batch of biologically acidified slurry has the advantage that at the same time the slurry is also inoculated with acid producing microorganisms. However the incubation experiments of Miller & Varel (2001) suggest that is not strictly necessary to lower the initial pH

3.4.2 Inoculation with acid producing microorganisms

Inoculation with acid producing microorganisms is done to alter the composition of the microbial community in the slurry. The slurry is preferably treated with Lactic acid producing bacteria because lactic acid is little volatile (Lameijer and Vervoort, 1995). For an optimal functioning of these bacteria different studies find that it is necessary to decrease the pH of the starting slurry (Hendriks and Vrielink, 1996; Lameijer and Vervoort, 1995), whereas in others this is less obvious (Miller& Varal, 2001; Clemens & Wulf, 2005) .

3.4.3 Addition of substrate

To stimulate or maintain the activated acid producing microorganisms, additional substrate can be added to the slurry. There are three possibilities; i) directly adding substrate in the form of an easily degradable C source, e.g. glucose, ii) adding microorganisms or enzymes that are capable of degrading macromolecules to substrate for the acid producing microorganisms, and iii) adding macromolecular compounds in combination with microorganisms or enzymes that are capable of degrading these macromolecules (Lameijer and Vervoort, 1995). The advantage of the last option (option iii) is that such macromolecular compounds are waste products and are thus much cheaper than the easily degradable C substrate. An advantage of stimulating the degradation of endogenous macromolecules (present in the slurry, option ii) is that the viscosity of the slurry is reduced. This has advantages when separating the slurry into a liquid and solid fraction (Lameijer and Vervoort, 1995).
A different option is using a fed batch system in which fresh slurry is added to slurry that is biologically acidified. In this case all fermentable substrate of the fresh slurry can be used, nothing is lost due to undesired decomposition processes. From biogas production it is known that not using day fresh manure can reduce biogas output by 30% (www.praktijkbioenergie.nl presentation Teeselink). Apart from the loss of fermentable substrate, decomposition processes may also increase slurry pH.
At the start of the acidification process in the fed batch system the bacteria have been activated by decreasing the pH of the starting slurry and the slurry has been inoculated with lactic acid producing bacteria. In this system a C source is added in the form of the endogenous substrate present in the added day fresh manure (Lameijer and Vervoort, 1995). Thereby the cattle diet itself has an influence on the quality of the C source in the fresh manure, which can be an important factor as shown earlier. To determine the potential of such a fed batch system the process conditions must be further investigated.

Several studies show that if, and to what extent the pH decreases after a C source is added, depends on the amount and type of C source added. Additionally, the rate with which the pH decreases depends on the C source. For some C sources, the pH rises again after the pH of the acidified slurry reaches a minimum. This can be explained by several processes (Clemens et al., 2002 Clemens and Wulf, 2005): I) the produced organic acids are volatile and evaporate from the slurry, ii) organic acids are degraded aerobically, or iii) the organic acids are anaerobically degraded to CH_4 and CO_2. One example is the aerobic and anaerobic degradation of lactic acid:

$$CH_3CHOHCOOH + 3O_2 \rightarrow 3CO_2 + 3H_2O \qquad \text{Equation 3}$$

$$2CH_3CHOHCOOH \rightarrow 3CH_4 + 3CO_2 \qquad \text{Equation 4}$$

At low pH, the anaerobic decomposition is unlikely because methanogenesis is inhibited at pH < 6 (Oenema and Velthof, 1993). However, as the pH increases, this process can again become significant. If the acidification has not converted all of the carbonate buffer then the pH may also rise again in the course of time as a result of the conversion of HCO_3 into CO_2 gas and water (Lameijer and Vervoort, 1995).

3.4.4 Addition of colloidal material

Colloidal material is added to increase the reactive surface area of microorganisms with substrate and thus enhancing the conversion rate of substrate into acid. According to Lameijer and Vervoort (1995), colloidal support material results in the introduction of oxygen to such an extent that the circumstances in the slurry become essentially aerobic. A kind of biofilm is created when the microorganisms attach to the surface of for instance Zeolite. Researchers from TU Graz have been able to show how Zeolite interacts with microorganisms (Figure 3.4). Due to the large reactive surface area of colloidal material this thus increases the interaction surface between microorganisms and substrate. Zeolite is used in biogas plants in order to stimulate gas production. A recent publication shows that 53% more biogas is produced when using an activated Zeolite in batch cultures (Weiß et al., 2010). In Austria the Zeolite is called a migulator (mineral bioregulator).

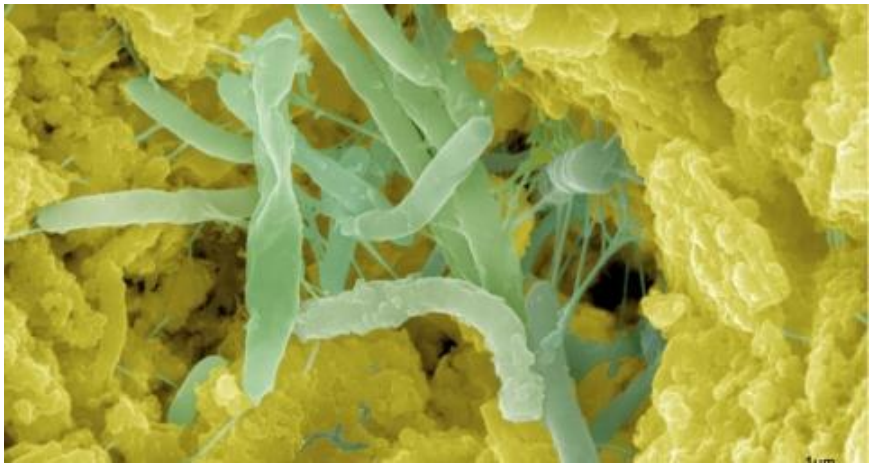


Figure 3.4. The interaction between microorganisms and zeolite (Source: http://tuaustria.at/ans licht gebracht mikroorganismen auf biogaskatalysatoren*)*.

3.4.5 Examples of varying additives from literature

As mentioned before the various studies investigating biological acidification of slurry all vary in their usage of (a combination of) additives, with varying results. In most studies a combination of acid,

microorganisms and a C source is used. Clemens et al. (2002) and Clemens and Wulf (2005) do not add an acid to decrease the pH of the starting slurry, neither is the slurry inoculated with microorganisms. They allow the endogenous microorganisms to do the work. In these studies only a C source is added to biologically acidify the slurry. As described earlier (paragraph 3.3.1) Miller & Varel (2001) do not even add an extra C source.
The different studies are inconclusive about the necessity to reduce the pH of the starting slurry and/or if the slurry must be inoculated with specific acid producing microorganisms. Adding a C source does seem to be necessary to reduce the pH of manure. The extent of this decrease depends on the type and amount of C source and on the freshness of the used slurry.

Two studies investigating a variety of C sources show that of the investigated low molecular weight C sources wheat starch, sucrose (concentration> 0.03 mol l^{1}) and glucose (concentration> 0.1 mol l^{1}) are suitable substrates for acid producing microorganisms as the pH of the slurry decreased below a pH of 5.5 within a week (Hendriks and Vrielink, 1997 and Clemens et al., 2002). The cheaper potato starch was found not to be a suitable substrate (Hendriks and Vrielink, 1997). It was however hypothesized that when treating the potato starch, the obtained cold – soluble potato starch would be a suitable substrate. In a study using both sugar and starch (both not further defined) as a C source the pH was found to decrease directly after addition, whilst for the starch it took 10 days before the pH started to decrease (Clemens and Wulf, 2005).
For all three effective low molecular weight C sources (wheat starch, sucrose and glucose) the pH increased again after reaching the minimum pH to values well above pH 6 after 0.8 - 1.3 weeks (Clemens et al., 2002) and after 4 - 8 weeks (Hendriks and Vrielink 1997). In a follow up study Clemens and Wulf (2005) found that when the initial dose was high enough, the pH could be maintained at a low level for over 40 days. This implies that when using low molecular weight substrate this must be continuously added or an initial high enough dose must be added to maintain the low pH. This can also be achieved by using a fed batch system in which day fresh slurry (and thereby fresh endogenous substrate) is added to slurry already inoculated with acid producing bacteria (Clemens and Wulf, 2005, Lameijer and Vervoort 1995).
That it takes longer for the pH of the slurry to rise again above a pH of 6 in the Hendriks and Vrielink (1997) study (4 – 8 weeks) compared to the Clemens et al. (2002) study (0.8 – 1.3 weeks) can be explained by the difference in the combination of additives used. In the system described by Hendriks and Vrielink (1997) pig slurry was first acidified to pH 6 with citric acid and inoculated with lactic acid bacteria before the substrate was added. Clemens et al. (2002) used cattle slurry and no acid and no microorganisms were added. This indicates that the effectiveness of adding a low molecular weight substrate for the longer term, increases when an acid is added to decrease the pH of the starting slurry and / or when the slurry is inoculated with microorganisms. By acidifying the initial slurry not only the circumstances become more favorable for the acid producing bacteria but also a large fraction of the acid buffer in the slurry is removed and can thus not participate in a subsequent rise in pH.

When adding larger molecular weight C sources, grounded wheat (Hendriks and Vrielink 1997) and biowaste in the form of undefined organic garbage (Clemens et al., 2002) were found to be effective substrates. Adding sugarbeet residue had only a minor effect on the pH, even at a high dose (330 g l^{-1}) and the smell of the slurry was negatively affected. Oil was also found to be an ineffective C source (Clemens and Wulf, 2005). When adding biowaste, the pH decreased slowly (~ 200 hours) to a pH of 4.7. Although the pH decreased slowly, this low pH was maintained during the remainder of the incubation experiment (Clemens et al., 2002).

It can be concluded that there is a distinct difference in pH decrease of the slurry when adding low and high molecular weight C sources. The low molecular weight C sources are direct substrate for the acid producing microorganisms. When applied in a high enough dose, the addition results in a relatively rapid (<1 week) decrease to a pH below 6. The substrate is also rapidly consumed and must thus also be continuously added to maintain the low pH. The amount of C source needed to maintain the low pH is most probably lower when an acid is added to decrease the pH of the starting slurry and / or when the slurry is inoculated with microorganisms. The continuous addition of day fresh manure in a fed batch system results in lower dosage of exogenous substrate and maintains the pH at a low level for longer (Clemens and Wulf, 2005, Lameijer and Vervoort 1995).
For the high molecular weight C sources the pH decrease is much slower because the macromolecules must first be converted into low molecular weight substrate for the acid producing microorganisms. For the longer term emission reductions and for the emission reductions during application to the soil, the use of high molecular weight C sources like bio waste seems positive based on the results that the pH was maintained at a low level after a minimum pH was reached (Clemens et al., 2002). In addition, the high molecular weight C sources are generally (much) cheaper than the low molecular weight C sources. A C source like bio waste is however a heterogeneous mixture and extra attention should be given to other components that are simultaneously added to the slurry (e.g. heavy metals and nutrients) and changes in the physical properties of the manure.

3.5 Effect of biologically acidifying slurry on NH_3 emissions and N content

Different studies show a positive effect of biologically acidifying slurry on NH_3 emissions. This will be illustrated based on three studies.
In a study in which slaughter pig slurry was biologically acidified (starting slurry adapted to pH 6 with citric acid and inoculated with lactic acid bacteria, after which it was mixed with ground wheat to an amount of 83 kg per pig place per year) the pH decreased to an average of 6.1 over 15 weeks. This resulted in a NH_3 emission from the stable of ~1 kg NH_3 N animal^{-1} yr^{-1} (Hendriks and Vrielink, 1997). Compared to a conventional stable this is a reduction of approximately 88%.
In another study (Clemens et al., 2002) the NH_3 emission was found to be much lower after biologically acidified cow slurry (0,1 mol l^{-1} glucose, pH 5,9) was applied to the field compared to untreated slurry. This was especially clear during the first 20 hours after application: 81% reduction in NH_3 emission. The total cumulative emission reduction after application was 41%.
In a study in which lactic acid was added directly to slurry in incubation experiments in the lab, the pH decreased to values between 4.2 and 5.7 resulting in a reduction in NH_3 emission between 65 and 88% (Berg et al., 2006). In this study the lactic acid did not originate from the biological conversion of carbohydrates and during the experiment the pH had to be adjusted three times by adding extra acid.
These three studies illustrate the high potential of biological acidifying slurry to reduce NH_3 emissions from the stable (Hendriks and Vrielink, 1997, Berg et al., 2006) and after application to the field (Clemens et al., 2002).

3.6 Effect of biologically acidifying slurry on greenhouse gas emissions

There are no studies known to directly describe the effect of biological acidification on CH_4 , N_2O , and CO_2 emissions. In a study by Berg et al (2006) the effect of directly adding lactic acid to slurry on the CH_4 emission from slurry is investigated. Acidification to a pH varying between 4.2 and 5.7 resulted in a reduction in CH_4 emission between 91 and 98%. Methanogenesis was thus almost completely inhibited.
Several studies show that over time the pH of biologically acidified slurry may increase again. This may

result in a re-activation of the methanogenesis. In the study by Berg et al (2006), this was avoided by adding extra acid during the experiment.
The inhibition of the methanogenesis when slurry is acidified with lactic acid is consistent with the results from studies where slurry is acidified with an inorganic acid to pH levels below pH 6 (Oenema and Velthof, 1993, Ottosen et al., 2009). It thus seems that the inhibition in CH_4 production is independent of how the manure is acidified as long as the pH is (remains) below pH 6.

The methanogenesis depends on the redox potential of manure. Oenema and Velthof (1993) found a much higher redox potential (Eh 100 to 400 mV) in acidified slurry (HNO_3 pH <5) compared to untreated slurry which was highly anaerobic (Eh ± -400 mV). From the viewpoint of biogas production it is known that the redox potential should lie below -250 to -330 mV for a good methane production. It is not known what happens to the redox potential of biologically acidified slurry, nor what happens when the pH of biologically acidified slurry increases again. In the experiments in Austria Eh was monitored and results will be shown in Chapter 4. Adding colloidal support material may contribute to the reduction in CH_4 emission because according to Lameijer and Vervoort (1995) the addition of this material results in the introduction of oxygen to such an extent that the circumstances in the slurry become essentially aerobic.

By adding an additional C source to the slurry, the local C cycle in which CO_2 is captured by the crop and ultimately released by decomposition of manure, is disturbed. The pH increase in the incubation experiments indicates that the formed organic acids are broken down or evaporate (Clement et al, 2002). Adding an additional C source thus leads to an additional emission of CO_2 and/or organic acids and possibly also CH_4. In the long term, all of the extra added C will be released.
To achieve a net positive result in terms of greenhouse gas emissions, the equivalent amount of CO_2 released during decomposition of the additional C source may not exceed the CO_2 equivalents achieved by the reduction in CH_4 emissions from the acidified slurry. Because at this point the specifications concerning the most (cost-)efficient C-source are not known, this needs further investigation.

Biological acidification of slurry will increase the content of easily degradable C compounds (like acetate) in the slurry and this will potentially increase N_2O emissions (Velthof et al, 2000). Manure with a higher content of acetate affects the soil biology. Krüsel et al. (1999) suggested that the presence of acetate is beneficial for biological processes in soil. In a recent study Laughlin et al. (2009) find that the direct addition of acetate to soil enhances the formation of N_2O from NH_4^+ in soils. When additional acetate is added in the form of slurry than the effect on N_2O emissions was found to be smaller compared to when acetate is added directly to the soil. The experiments of Laughlin et al. (2009) were performed in the lab with dry soil and not in situ. It is clear that more research is needed concerning the effects of biological acidification of slurry on N_2O emissions.

If the manure is eventually used in a biogas reactor, a higher C content of the manure has advantages. More easily degradable C is present which is favorable for biogas production. The addition of potato starch to slurry was found to strongly enhance the CH_4 production in a fermenter compared to untreated slurry (Clemens et al., 2006). After field application of the fermented slurry, there was no significant difference in greenhouse gas emissions between untreated slurry and slurry to which potato starch was added.

3.7 Side effects

A side effect of acidification is that the viscosity of the manure is reduced drastically. This gives the manure better mixing capabilities resulting in a more homogenous product when applied in the field. Furthermore no foam production occurs. At many farms foam production is a problem as can be seen in Figure 3.5. Foam production means that the full storage capacity below the slats in cubicle houses can not be used. The result is that extra storage capacity is needed to overcome the period of August 1 to mid February in which no manure is allowed to be applied to the field. This is costly. This problem is even more urgent on pig farms (Starmans et al., 2009).

Figure 3.5. Foam coming through the slats (mestportaal.nl, February 3, 2012).

3.8 Summary of literature study

Similar to reducing the pH of slurry using an inorganic acid, biological acidification results in a potentially large decrease in NH_3 emission from stables and when the acidified slurry is applied to the field. The extent with which the pH decreases, and the time frame in which this decrease is maintained depends on the type and dosage of additives used. From the literature study it can not be concluded what combination and dosage of additives is minimally needed to achieve emission reductions. Process conditions thus require further investigation.
The effect of biological acidification on green house gas emissions has not been studied directly. It is expected that response to biological acidification will be similar to acidification with an inorganic acid. This however also needs further investigation.
Biological acidification seems to offer a large potential to decrease NH_3 and green house gas emissions but process conditions need further investigation. For this reason research in Austria is ongoing. The latest research results will be presented in the following chapter.

4 Biological acidification of slurry: Recent experiments from Austria

4.1 Background

Somitsch et al. (2008) and Wenzl et al. (2009) work on biological acidification by means of regulated fermentation. This approach has the starting-points that:

- the manure should be treated as fresh as possible to prevent the production of odor compounds and the loss of C substrate;
- the microbial population dynamics can be regulated via oxygen level of the slurry; and
- the pH decrease in manure by the addition of acid alters the microbial population, which affects NH_3 and CH_4 emission.

This "fermentation" approach anticipates on and makes use of the microbial population in manure in a similar way as is known from biotechnology with mixed populations of micro-organisms. The pH and oxygen are the main factors to change the manure environment in more favorable conditions for micro-organisms like Lactobacilli and Bacillus spp. which produce lactic acid, carbon dioxide, ethanol or acetic acid. They can grow under micro-aerophyllic conditions, in contrast to for example the micro organisms Eubacterium and Clostridia spp. that cause odor.

At the start the initial pH is lowered by adding acetic acid. Acetic acid is used because it is relatively cheap, has a broad working spectrum regarding microbial growth and does not corrode metals. In addition it was tested what the effect is of adding:

- an easy fermentable C source, molasses, to stimulate saccharolytic organisms;
- zeolite, which has a large surface on which micro organisms can stick. In this way the reactive surface is increased which should enhance the conversion of substrate into acid; and
- acid producing bacteria (Lactobacilli), present in the additive Kombioflor® to enhance the fermentation.

4.2 First trials

In Austria experiments with biological acidification of slurry started at the end of 2007 (Somitch et al., 2008). The trials were executed in the lab and in practice. In the lab trials a fermentation vessel (150 liter) with a stirring device was used, the "Gukon" (see Figure 4.2). The vessel also contained a pH, temperature and conductivity meter. For the tests in practice a Gukon of 300 liters was used. We will present a short summary of the results of these first trials before we continue with the recent experiments.

Lab experiments

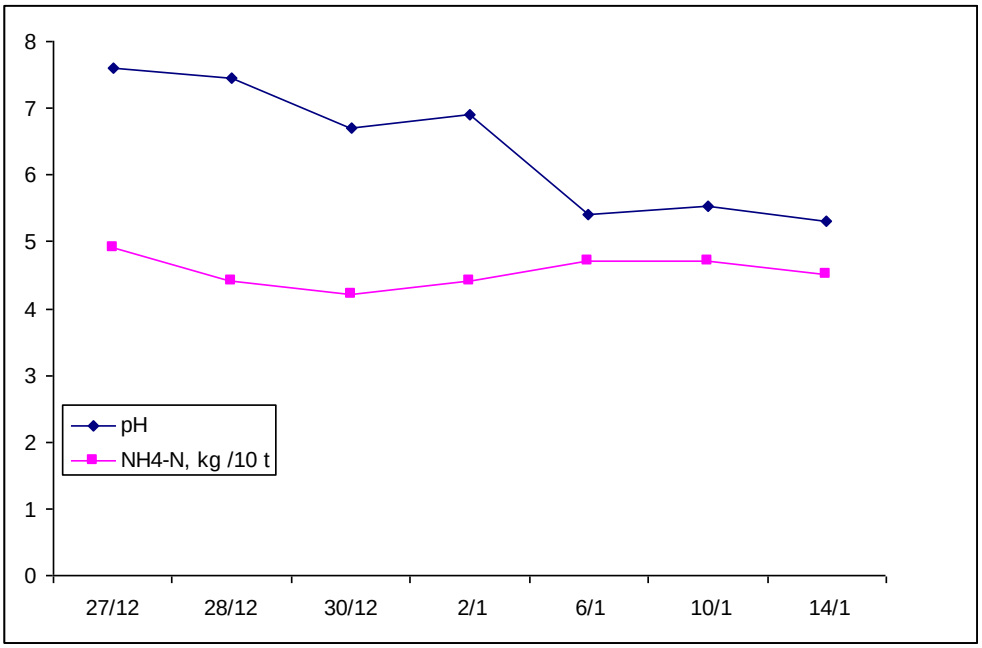
In the first trial in December 2007 120 liter cattle slurry with about 3% dry matter and with respectively 1.37 g N kg^{-1} and 0.49 g NH_4-N kg^{-1} was used. After addition of acetic acid (1 liter, 30%) the pH dropped from 7.6 to 6.7. Early January it increased slightly and once again acetic acid (1 liter, 10%) was added, causing the pH to drop to 5.3 at the end of the experiment on January 14th. Translating these results to Dutch manure with 4.1 kg N m^{-3} the amount of acetic acid used would be comparable with using 9 kg H_2SO_4.

A second trial had the same setup as the first trial, but after 18 days the slurry was stored in a separate vessel. Once a day the vessel was exposed to air for an hour via membrane aeration. The pH was 5.7 and did not change during the storage period of 60 days.

In a third trial 120 liter slurry was used with a dry matter content of 7.7% and a N content of 4.2 g kg^{-1}.

Addition of 1 liter acetic acid (30 %) lowered the pH to 6.6 after 2 days. The amount corresponds to about 2 kg H_2SO_4 m^{-3}. After 10 days the pH was 7 and remained at this level during the following 40 days. The amount of acid was not high enough to realize a pH of 6. The viscosity of the slurry decreased strongly shortly after acidification.

In a fourth trial slurry (120 liter) with about 6% dry matter, the pH was maintained at a level of 6 by continuously adding acetic acid. During two weeks 10 kg feces was added daily after removing 10 liter slurry from the Gukon (This was stored separately). In total 8 liter acetic acid was needed. This corresponds with 1 kg H_2SO_4 m^{-3}. An active slurry was observed at the bottom of the Gukon. Somitsch et al. (2008) expect that this slurry can sustain the fermentation process in a fed batch system in which fresh slurry is added to slurry that is biologically acidified.



Figuur 4.1. The pH and NH4-N content in time after addition of 1 liter acetic acid (30%) plus 150 gram Zeolite and 1 liter Kombioflor at the start of the experiment on December 27. On January 4th 1 liter acetic acid (10%) was added. The temperature of the cattle slurry was 15 ^{o}C (from Somitsch et al., 2008).

Trials in practice

During 10 days in December 2008 about 300 liters of cattle slurry was added to A gukon of 3 m^3. Three times a day, day fresh manure from a dairy barn was added. At the start of the experiment 23 liter acetic acid (60%) was added. After 6 days there was another addition of 20 liter acetic acid. Due tot the low outdoor temperatures the temperature of the slurry decreased tot 2 ^{0}C. Thereafter the Gukon was covered with straw which caused a rising of the temperature to 6 ^{0}C. The pH decreased form the initial value of 8 tot 7 and remained at that level. Addition of 25 kg molasses one day before the end of the experiment lowered the pH from 7 tot 6.5.

After the end of the experiment the slurry was stored for a while. Membrane aeration of this stored conditions made it possible to maintain the micro-aerophyllic conditions. The low temperature of the manure hampered optimal functioning of the process.

4.3 Ongoing trials

4.3.1 General

After the first trials in 2008 a follow up project was started in 2011 (MINAMMON). This project focusses on a whole farm approach starting at the cattle diet and ending with the manure. The trials are executed on 10 farms (See Annex 1). In parallel lab experiments have taken place and will take place to optimize process conditions for acidification of the manure. The lab experiments aim at a target pH between 6 and 6.5. In later experiments also methane and N_2O measurements will take place.

4.3.2 Setup of first lab trials in the MINAMMON experiment

In autumn of 2011 the first series of experiments were started to investigate the effectiveness and process conditions of biological acidification of cattle slurry. The slurry used was roughly a week old and was obtained from an organic farm where the cows feed on fresh grass (16-18 kg dry matter per day) and hay (max. 1 kg per day). The average slurry composition is shown in table 4.1. It is roughly 4 times more diluted than Dutch cattle slurry.

Table 4.1: The average cattle slurry composition (g/kg) (n=84) used in the experiment.

DM	Ash	Ca	Mg	K	P	N	NH_4-N	pH
19.6	7.0	0.57	0.19	1.73	0.21	1.13	0.51	7.3

There were four treatments (Table 4.2) differing in the (combination) of additives used to acidify the slurry. In the first treatment only acetic acid was added. In the second treatment the slurry was also inoculated with lactic acid producing bacteria (Kombioflor®). In the third treatment zeolite was also added, and in the fourth molasses was also added. The experiments are performed in two 160 l experimental units (Figure4.2).
Each experiment lasted 7 days and was repeated 4 times in 4 successive weeks, except trial 4 which was repeated twice (see Table 4.3). In each experimentation week two treatments were compared: one of them is slurry treated with one of the (combination of) additives and the other is the control in which the slurry remains untreated. Only treatment 4 was somewhat different. The last two experiments of treatment 3 (week 11 and week 12) were continued for another week and molasses was added on day 1 to Gukon 2. Each day the parameters temperature, electrical conductivity, pH, and redox potential were measured in both experimental units. The electrical conductivity (EC) is a measure for the free ion content of the slurry.

Figure 4.2. The two experimental units (GÜKON-Pilotfermenter) used to biologically acidify slurry and continually monitor pH, Eh, EC, and temperature.

Table 4.2. Overview of the type and dosage of the (combination) of additives used in the four treatments.

Treatment	Additives	Dosage per 100 l slurry
1	acetic acid (40%)	0.5 l*
2	acetic acid (40%) + Lactobacillus (LB) Kombioflor®	0.5 l + 45 ml
3	acetic acid (40%) + LB Kombioflor® + Zeolite	0.5 l + 45 ml + 45 g
4	acetic acid (40%) + LB Kombioflor® + Zeolite + sugarcane molasses	0.5 l + 45 ml + 45 g + 350 g

* Recalculated for typical Dutch slurry the amount of acetic acid used equals about 17,5 liter acetic acid per m^3, which in turn equals 7.5 kg H_2SO_4.

Table 4.3. Overview of the experiment execution in time four the four treatments of table 4.2.

Week	Gukon 1	Gukon 2	Week	Gukon 1	Gukon 2	Week	Gukon 1	Gukon 2	Week	Gukon 1	Gukon 2
1	control	1	5	2	control	9	control	3	13*	control	4
2	1	control	6	control	2	10	control	3	14*	control	4
3	1	control	7	control	2	11	3	control			
4	control	1	8	control	2	12	3	control			

4.3.3 Results

General

After the addition of a combination of acetic acid , Lactobacillus, Zeolite, and Sugarcane molasses (trial 4) a violent reaction occurred, with bubbling and odor emissions during the first two days followed by a construction of a fine fibrous floating layer.

In the second trial, in which LB is added in combination with acetic acid a strong formation of bubbles was observed and a rapid reduction of the film on the slurry.

pH and redox potential

An overview of the main physical and chemical characteristics of the slurries during the four trials is shown in Table 4.4. It is clear that all treatments affect the pH and redox potential of the slurry. Depending on the trial the average pH decreases between 0.6 and 1 unit and the redox potential increases between 28 and 114 mV. A more detailed change in pH during the experiments is shown in Figure 4.3 and of the change in redox potential in Figure 4.4.

The change in pH during the experimental period of one week shows that when acetic acid is added the pH decreases from ~7.4 to a minimum of 6.6 within one day. When in addition to acetic acid also Lactobacillus (LB) are added the pH decreases to a minimum of 6.4 within one day. An additional

Table 4.4: Overview of the average main physical and chemical characteristics of the slurries. Each data point is the average of 4 replicates measured during 6 days.

	pH		Redox-Potential (mV)		Temperature (°C)		Conductivity (mS/cm)	
Trial	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
1	7.4	6.8	-474.8	-447.2	19.6	19.7	9.2	8.9
2	7.5	6.6	-461.1	-347.5	14.4	14.5	8.5	8.6
3	7.5	6.6	-461.1	-347.5	14.4	14.5	8.5	8.6
4	7.6	6.5	-446.6	-391.3	9.8	9.9	7.6	8.3

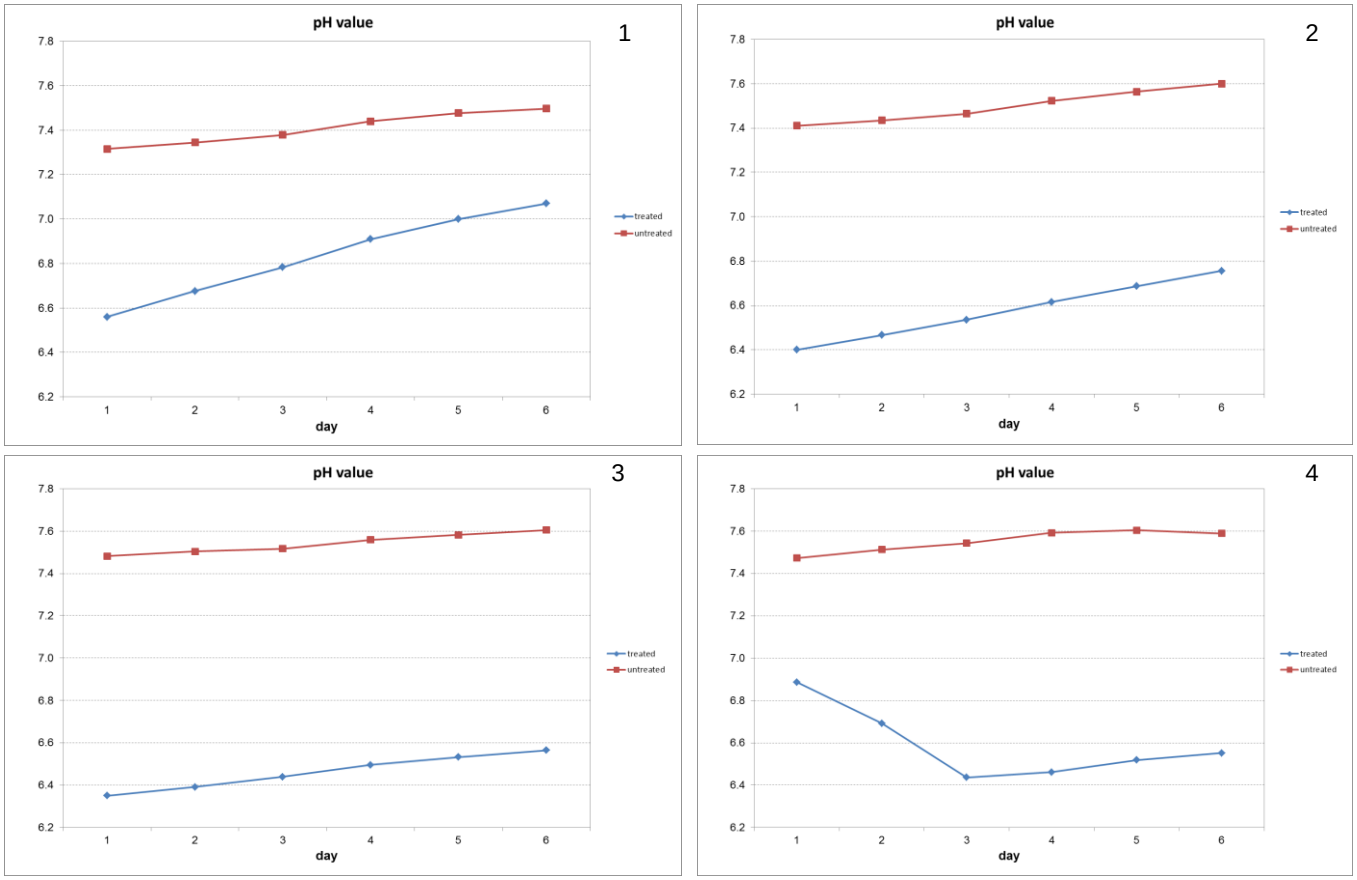


Figure 4.3: Change in pH during the experimental period of one week for the four trials. The red line is the control and the blue line is the treated slurry. For trial 1-3 each data point is an average of 4 replicates and for trial 4 of 2 replicates (Wenzl pers comm.).

addition of Zeolite does not affect this initial decrease in pH. When molasses is also added the initial decrease in pH is slower. The minimum pH is only achieved after three days. Comparable to when a combination of acetic acid and LB with or without Zeolite is added, the minimum pH is ~6.4.

During the six days of the experimental period the pH increases after the minimum pH is achieved for all four treatments, both in the treatments and controls. For the controls the pH increases slowly from ~7.4 to ~7.6 during the six days of the e experimental period. This slight increase is the result of (bio)chemical reactions. For the four treatments the pH increases fastest when only acetic acid is added (0.1 pH unit day^{-1}). After six days the pH has increased to a pH>7. When LB is also added the pH increase is slower (0.08 pH unit day^{-1}). Although the additional addition of Zeolite had no affect on the minimum pH, it does slow down the increase in pH after this minimum is achieved (0.04 pH units day^{-1}). After six days the pH still remains <6.6. The lowest pH after six days is achieved when molasses is also added. This however results from the fact that the minimum pH is achieved after three days instead of within one day, partly caused by the relative low temperature. The rate with which the pH increases after this minimum is achieved is comparable to treatment 3 (a combination of acetic acid, LB, and Zeolite).
To decrease NH_3 emission from the slurry the addition of a combination of acetic acid, LB, and Zeolite is most favorable. With the combination of these additives the initial pH decrease is fastest and the subsequent increase in pH is slowest resulting in an overall lowest pH during the experiment.

The change in redox potential (Eh) during the experimental period of one week is depicted in Figure 4.3. All treatments, also the controls, show a maximum Eh after the first day. This will be due to the mixing with air and thus the input of oxygen at the start of the measuring period. Between the first and second day the Eh decreases again. Depending on the treatment this decrease continues or stagnates. For all treatments the (combination) of additives results in a higher Eh compared to the controls. The increase in Eh compared to the control is lowest when only acetic acid is added and highest when acetic acid and LB with or without Zeolite is added. When only acetic acid is added the Eh decreases to nearly approximate the level found in the control. For the trials in which LB is also added, with or without Zeolite the Eh remains above an Eh of -400mV after 6 days. For the trial without Zeolite the decrease in Eh seems to continue after the 6 days while for the trial with Zeolite this decrease seems to stagnate around -400mV after 5 days. When molasses is also added the Eh decreases between the first and second day of the experiment but then stagnates, also at a value around -400mV.
The Eh found in the control slurries is slightly lower compared to the Eh found in untreated slurry by Oenema and Velthof 1993. In this study slurry acidified with the inorganic acid HNO_3 (pH <5) resulted in a much higher redox potential (Eh 100 to 400 mV) compared to the Austrian experiments.
It was difficult to maintain temperature at the same level, especially in the last two experiments.

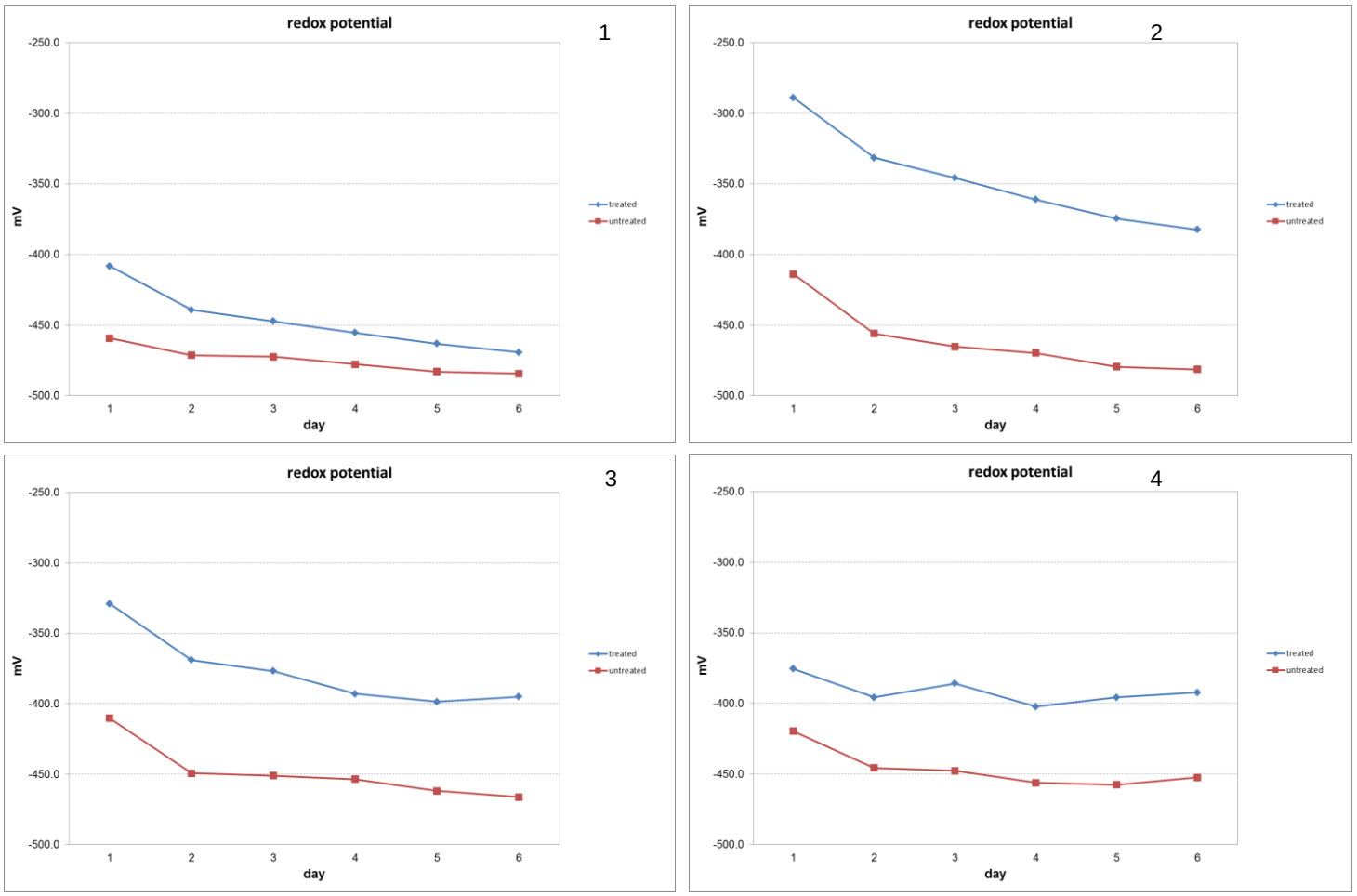


Figure 4.4: Change in redox potential (Eh) during the experimental period of one week for the four trials. The red line is the control and the blue line is the treated slurry. For trial 1-3 each data point is an average of 4 replicates and for trial 4 of 2 replicates (Wenzl pers comm).

Slurry composition

A side effect of the treatments is that the viscosity decreases strongly. Also the slurry is much more homogenous than the control and there is no film at the surface (Figure 4.5).

When acetic acid is added to the slurry the C content of the slurry increases (trial 1, table 4.5). This is because acetic acid contains C. When in addition to acetic acid also LB is added, the C content decreases. In addition the relationship between C and N content found in the controls and in the treatment with acetic acid has disappeared. In the trial with the lactic acid producing bacteria a strong formation of bubbles was observed and a rapid reduction of the film on the slurry. C is thus lost by fermentation.

Table 4.5: Overview of the average slurry composition.

	N_{tot}		NH_4		OTS %		C	
Trial	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
1	1.1	1.3	0.50	0.52	1.4	1.8	8.0	10.3
2	1.3	1.1	0.71	0.67	1.5	1.2	8.7	6.8
3	1.3	1.3	0.54	0.50	1.8	1.8	10.2	10.3
4	0.8	0.8	0.23	0.24	3.5	3.2	8.4	8.6

Figure 4.5. The control versus treatment 3 (acetic acid, Kombioflor and zeolite)

4.3.4 Conclusions

- The experiments as performed in Austria clearly show that the batch system used to perform the experiments is an effective way to study biological acidification of slurry. The pH as an indicator for NH_3 emissions and the Eh for methanogenesis could effectively be monitored during the experiment.
- The biological acidification experiments recently conducted in Austria showed a decrease in pH and an increase in redox potential when the slurry was treated with (a combination of) acetic acid, lactic acid producing bacteria (LB), Zeolite, and molasses.
- For NH_3 emission and methanogenesis to be inhibited the pH must be lower than pH 6 (Oenema and Velthof, 1993). This low pH was not reached in these experiments. This can however easily be achieved by increasing the dosage of additives which most likely is also a prerequisite to maintain the pH at a low level.
- The most effective combination of additives in terms of pH and Eh is found to be acetic acid, LB, and Zeolite. The acetic acid decreases the initial pH and increases the Eh of the slurry. Within the first day of the experiment the LB further decreases the pH and increases the Eh of the slurry. The Zeolite slows down the subsequent increase in pH and decrease in Eh during the experiment.
- The addition of molasses in combination with acetic acid, LB, and Zeolite has only limited additional value concerning biological acidification in the time-frame of the experiment (6 days).
- To be able to implement biological acidification in practice the process parameters must be studied in more detail and the batch system offers an effective way to derive these parameters.

5 Economic analysis

5.1 Costs

From January 2014 onwards ammonia emission from dairy farms have to be reduced by 10%. Farms in the vicinity of Natura 2000 areas have extra restrictions when they want to expand their farm. In addition with the ending of the quota system in 2015 the Rabobank expects that the Dutch milk production will increase from 11.5 to 14 mln tons. This will result in extra costs to reduce ammonia emission in order to remain below the national ammonia target. Costs of measures can be very high. For instance changing to low emission dairy housing may cost 11-12 € per kg ammonia (Anonymous, 2009).
At present the cost of biological acidification can only be estimated, since there are no systems in operation yet. The costs include the costs for:

- the different additives (organic acid, C-source, zeolite);
- the apparatus needed to mix additives and slurry; and
- (the costs for extra liming).

There are also benefits due to lower costs for mineral N fertilizer and a possible higher return when the acidified slurry is used in a biogas reactor. An additional benefit is that the emission of methane (a greenhouse gas) is markedly reduced.

The costs for biological acidification are compared with the infarm system that uses H_2SO_4 to acidify slurry. In Table 5.1 an estimation of the costs is made for different systems. According to Infarm (pers communication) are the investment costs about 100.000 €. This would amount to about annual costs of €10.000. For a system making use of organic acids and organic additions it is expected that the investment costs are somewhat lower. This results in annual costs of 10.000 € for the Infarm system and 8.000 € for the other systems. Important for the total cost calculations are the costs of sulfuric acid, organic acids, the C-source and other additives. The price of sulfuric acid is variable but is estimated at 100 € per ton (Bussink, 2009) or about 5 € per kmol H+, resulting in costs of about 1€ per m^3 manure. Organic acids are mostly much more expensive (Starmans & Melse, 2011). Most convenient acids would be acetic acid or citric acid. Based on Starmans & Melse and prices mentioned on the internet for large amounts of organic acids, the price of acetic acid varies between 20 and 40 € per kmol H+ and that for citric acid between 35 and 45 € per kmol H+.
Unknown is how much organic C is needed as a substrate to feed the microbial population. In experiments with slaughter pigs (Hendriks & Vrielink, 1996) about 80 kg milled wheat was necessary per pig place (1.5-1.6 m^3 of slurry). Taken into account that pig slurry in those days contained 1.6-1.7 times more mineral N than cattle slurry nowadays, this corresponds with about 30 kg of milled wheat per m^3 cattle slurry. The milled wheat price varies between 200 € and 300 € per ton. This would amount to annual costs that vary between 25.000 -37.000 € per year. In the case that no additional C is needed these costs are zero. Furthermore, there are some costs for Kombioflor and zeolite varying between 0.25 and 0.75€ per m^3 cattle slurry. Taking into account the savings on N fertilizer than the total annual costs vary between 53 and 311 € per cow. Expressed per kg NH_3 emission reduction the costs vary between 3.5 and 21 € per kg. When expressed per kg less CH_4 emission the costs vary between 1 and 6 € per kg or between 50 and 270 € per kg CO_2 equivalents.

The costs are strongly determined by the kind and amount of additives needed to acidify the slurry. At this point it is however unclear what the optimal process conditions are. In 1997 Hendriks and Vrielink concluded that biological acidification was not ready to be implemented on pig farms due to the high

costs for the C source (2/3 of the total costs) in the form of a readily degradable C substrate. If only half of the amount of the maximum amount of C substrate is needed than the costs are comparable to the cost of low emission dairy housing, expressed per kg NH_3 (See Figure 5.1). In general the system becomes interesting for farmers if the costs remain below 10 € per kg NH_3 or 150 € per cow. In Table 2.4 it is calculated that total NH_3 emission is reduced by 60-35% depending on the slurry application system used and the pH of acidified slurry. This creates large possibilities to expand the farm with more dairy cattle even if the NH_3 emission has to be reduced by 10% in 2014. Other factors like the P and use level can than become restrictive.

At present, more research is needed concerning optimizing the process conditions to be able to give a good indication of the costs related to the biological acidification of slurry.

Table 5.1. Cost calculation of acidification using the Infarm system and the cost range for biological acidification with and without using an additional C source (energy source) for 150 cows housed indoors.

	Infarm system	Maxprice with C source	Maxprice without C source	Min price with C source	Min price without C source
equipment (investment)	100,000	80,000	80,000	80,000	80,000
amortization period	20	20	20	20	20
interest	2,500	2,000	2,000	2,000	2,000
annual amortization	5,000	4,000	4,000	4,000	4,000
maintenance cost	2,500	2,000	2,000	2,000	2,000
annual costs	**10,000**	**8,000**	**8,000**	**8,000**	**8,000**
cost of sulfuric acid	4,170				
cost of additional lime	1,893				
cost of acetic acid		3,753	3,336	2,919	1,668
cost of C source (wheat)		37,530	0	25,020	0
cost of zeolite +Kombioflor		0	3,094	0	1,031
Savings on N fertilizer	2,700	2,700	2,700	2,700	2,700
Savings on S fertilizer (20 kg S ha^{-1})	830	0	0	0	0
Optimal use of slurry storage**					
Total annual costs (€)	12,533	46,583	11,730	33,239	7,999
Total annual cost per cow (€)	84	311	78	222	53
Price per kg saved NH_3 emission*	5.57	20.70	5.21	14.77	3.56
Price per kg saved CH_4 (€)	1.52	5.65	1.42	4.03	0.97
Price per ton saved CO_2 equivalent(€)	72	269	68	192	46

* it assumed that the emission is reduced by 15 kg NH_3 and 55 kg CH_4 per cow per year (Bussink, 2009).

** not quantified

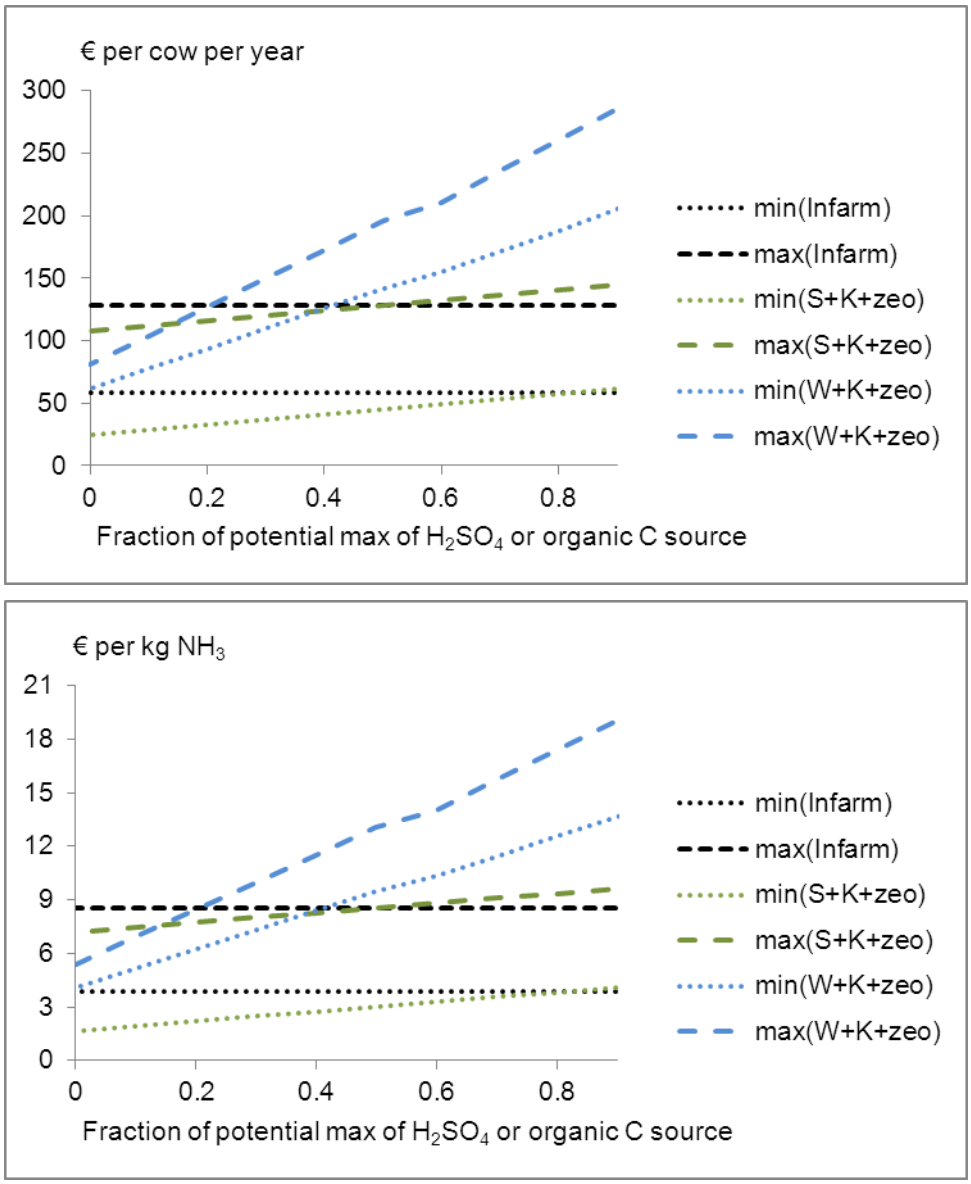


Figure 5.1. The costs of the Infarm System (H_2SO_4 price varies between 100 (min) and 300 (max) € per ton) and of biological acidification using varying amounts of H_2SO_4 (S+K+zeo) or organic C (W+K+zeo). The upper graph expressed as annual costs per cow and the lower graph expressed as € per kg saved NH_3.

6 General discussion and outlook

6.1 The system

Biological acidification

With acidification not only NH_3 emission from the stable is lowered but also emissions are reduced after the acidified slurry is applied to the soil. It was calculated that depending on the application technique, on a farm level NH_3 emission is reduced by 55-65% or 35-45% when cattle slurry is acidified to respectively pH=5.5 or pH=6.0. Acidification is possible by adding inorganic acids like H_2SO_4 or by biological acidification that makes use of the self acidification power of manure in combination with the addition of organic acids and or C substrate.

The results in literature and recent Austrian experiments show that biological acidification is a promising technique to reduce manure pH in order to reduce NH_3 emission but also CH_4 emission. To make it a successful and cost efficient technique for practical applications it is important that the pH can be maintained below a target value (pH=6 or 5.5) with a minimum input (a combination of):

- organic acid;
- C substrate;
- micro organisms like solutions containing Lactobacillus spp for inoculation; and or
- zeolite to increase the reactive surface of the microbial population.

Experimental data suggests that the pH remains stable for a longer time when the pH is below 6 and provided that the Eh remains in the right range to inhibit methane production (around -400 mV). This can be achieved by micro-aerophyllic aeration (Somitsch et al., 2008). Increasing the Eh stops methanogenesis or prevents it from starting. This means that the lactic and acetic acid produced by bacteria is not transformed into methane. When methane is formed this causes the pH to increase again This is also caused by volatilization of volatile fatty acids and degassing of CO_2. In cases where the target pH is above 6 generally a slow rise in pH is observed. This means that extra acid or C substrate is needed to maintain the pH at the desired level. A pH above 6 also results in a smaller reduction in NH_3 emission compared to when the target pH is below 6. In order to get a maximum reduction in NH_3 emission with minimal additions of C substrate to maintain the pH, a pH of 5.5 is proposed for Dutch circumstances.

Recent Austrian experiments, where cattle slurry was acidified to pH 6.4, showed that the addition of zeolite in combination with Lactobacillus spp. gave the lowest increase of the pH after the start of an experiment. Due to the large reactive surface area of zeolite the interaction surface between microorganisms and substrate is increased thereby stimulating the production of acid and/or methane depending on the environmental condition. (A recent publication showed that 53% more biogas was produced when using an activated Zeolite in batch cultures, Weiß et al., 2010).

Data from literature also showed that it is important to use fresh manure or else much more acid is needed to reach the target pH. In addition, fresh manure itself is an important substrate. Clemens and Wulf (2005) and Lameijer and Vervoort (1995) showed that the continuous addition of day fresh manure in a fed batch system resulted in lower dosage of exogenous substrate and maintains the pH at a low level for longer. In some cases even no exogenous substrate is necessary (Miller& Varel, 2001). Another factor of importance is temperature. At low temperature the turnover of C substrate is much lower, causing a slower decrease in pH when fresh substrate is added.

Based on the results so far it seems most likely that the highest cost efficiency for biological acidification can be achieved in a fed batch system where:

- at the start fresh slurry is immediately acidified to pH 5.5-6;
- zeolite and Lactobacillus spp. are regularly added;
- C substrate is added or organic acid in case that the quality of the fresh manure is not sufficient as a C substrate to maintain the target pH; and
- the temperature remains above 10^{o}C.

At the moment however it is not clear how much C substrate (besides the fresh manure) is needed after the start up. Also the quality of the substrate is important, because it determines how efficient it can be used by microorganisms. These parameters (the amount and the quality) determine the cost efficiency to a large extent as becomes clear from Table 5.1 and Figure 5.1. They determine if the technique can compete with other techniques that lower the emission from the stable. This requires further testing on a semi lab scale before testing in practice takes place in the Netherlands. In addition, it is also important to address the effect of temperature and animal diet. Does a higher temperature decrease the need for additional C substrate thereby making the process more cost efficient? Is there a large difference between cattle manure from maize or grass based diets? An additional question could be if it is useful to store the acidified slurry separately or not. It is expected that in a separate storage tank the air exchange can be controlled much better (better anaerobic conditions), giving better conditions for maintaining the target pH without addition of acid or C substrate.

Biological acidification in combination with H_2SO_4 acidification

It is expected that biological acidification is the route to go. However a mix of the Infarm system and the biological system is thinkable. In that case biological acidification is the basic concept and using the Infarm mixing system. With such an approach it becomes possible to add H_2SO_4, acetic acid and or C substrate. The advantages of such an approach are:

- risk avoiding; the market price of H_2SO_4 or acetic acid or the C substrate determines what is added to the slurry;
- that the acidifying potential present in the easy fermentable C in slurry is used first. This causes a certain pH decrease. A further pH decrease, to obtain the target pH, is possible either by using H_2SO_4, acetic acid or a cheap C substrate. It is expected that if only H_2SO_4 is used much less H_2SO_4 is needed than in the present Infarm system; and
- that the time to get an operational system will be shortened, because it is always possible to get the pH at the target level (in the unfavorable situation with a large amount of H_2SO_4). It is not necessary to wait until the slurry fermentation system is fully developed. In the long term knowledge development of slurry fermentation will cause that less auxiliary additives become necessary, causing an improvement of the economic perspectives (hypothesis).

With such an approach it is likely that an operational system becomes available for practice in a relatively short time.

Biological acidification and biogas production

It is thinkable that the acidified slurry is used in a biogas facility. As shown in Figure 3.1, biological acidification of slurry is equal to the first two steps of biogas production. The third step, the production of methane, is inhibited by the conditions in the acidified slurry. When conditions are adapted in the biogas reactor, methanogenesis will transform the fatty acids into biogas (CH_4).

With a successful biological acidification the biogas output will be higher than from conventional slurry due to i) the fact that in a fed batch system fresh manure is acidified directly without undesired losses, ii)

the presence of an activated biofilm (on Zeolite basis) and iii) the addition of some exogenous substrate. This can be tested with a standard test like with the Hohenheimer Biogasertragstest (Helffrich&Oechsner, 2003).

At present most biogas plants use slurry in combination with co products. From the point of view of maximum valorization of the nutrients in slurry, mono digestion of the thick fraction will be the route to go (Peeters et al. 2011). The thick fraction is obtained after slurry is separated mechanically in a liquid fraction and a thick fraction. This thick fraction contains most of the organic matter, the source for biogas (Table 6.1). Due to its lower viscosity, the acidified slurry can be separated much easier in a liquid and a thick fraction. Separation of conventional cattle slurry will result in an increase of the organic matter content by a factor 2.5. For acidified slurry this factor may even be higher.

Table 6.1. Estimated composition of cattle and mechanically separated cattle slurry (combination of Dutch literature sources)

	Dry matter %	Organic matter %	Total N g kg^{-1}	Nmin g kg^{-1}	P_2O_5 g kg^{-1}	K_2O g kg^{-1}
Cattle slurry	8.5	6.4	4.1	2.0	1.5	5.8
Liquid fraction of cattle slurry*	4.8	3.1	3.9	2.0	1.2	5.9
Thick fraction of cattle slurry*	19.8	16.3	4.5	2.0	2.3	5.4

*) assumed is a separation into 25% thick fraction and 75% liquid fraction.

The dimension and outlay of a biogas plant can become much smaller when only the thick fraction is used without expensive co substrate. This will reduce the production costs of biogas markedly. In addition, it is expected that the financial return of acidified slurry will be higher than from conventional slurry due to a higher gas output.
If a system in which biologically acidified slurry is separated into a thick fraction for biogas production and a liquid fraction for direct application as a fertilizer, becomes operational in the near future than the advantages of lowering ammonia emissions from the stable and from application of the liquid fraction remain. Furthermore a part of the nutrients (mainly the P_2O_5) can be cost-effectively removed from the farm in the form of the residue of the thick fraction after biogas production. This could mean that a somewhat higher stocking density is possible because less (N and) P_2O_5 is available.
An additional possible advantage could be that the residual heat that is produced in the biogas plant can be used to warm up the fed batch system in order to realize a lower pH and at a faster rate.

The use of acidified slurry, with or without separation into a liquid and thick fraction theoretically offers perspective to use in a biogas plant. It seems worthwhile to further investigate this possibility.

Whole farm approach

The final blue print when considering the whole farm would be:
- optimized cattle feeding with respect to milk yield and optimum slurry quality for
- acidification with a minimum of additives for

- minimizing NH_3 and CH_4 emissions from the stable;
- more easy separation of the acidified slurry in a liquid and thick fraction with a higher biogas potential than the conventional thick part for:
    - more cost efficient biogas production;

  - heat production to enhance the biological acidification process;
  - removing part of the P from the farm, which may result in a higher stocking density; and
- minimizing NH_3 losses during application.

In the ideal situation a triple win-win situation may occur:

- less NH_3 losses;
- less CH_4 losses; and
- (higher) energy production on a farm scale.

6.2 Research questions

Short term

The most promising technique seems biological acidification based on a fed batch system that starts with a rapid initial acidification with acetic acid followed by inoculation with Lactobacillus spp. and addition of zeolite. This system needs further testing on a semi lab scale to get more quantitative information about the optimal process conditions in order to get more quantitative information about the amount of C substrate needed, the effect of temperature and the effect of the dairy cow diet (grass based versus maize based). In addition the biogas production potential of the thick fraction of successfully acidified slurry should be estimated. This information is essential to make a more precise cost calculation and to determine if biological acidification is possible for less than 150 € per cow or 10€ per kg saved NH_3. These prices seem to be the upper limit for a system to be implemented in practice.

Longer term

If the answer of the short term research questions are positive than the following points remain:

- testing of the system under practical conditions by making a test facility on farm scale whether or not based on the Infarm system;
- validation of the reduction in ammonia (methane) emission in the cubicle housing and after application in the field;
- investigating the effect of biological acidified slurry on soil quality (hypothesis: positive);
- testing how easily biologically acidified slurry can be separated in a liquid and thick fraction and what the biogas potential of the thick fraction is; and
- developing an integrated system of fed batch acidification in combination with biogas production.

7 Conclusions and recommendation

7.1 Conclusions

- When the slurry pH is decreased below 5.5, emission reductions of 54-66% are expected.
- Biological acidification of cattle slurry in cubicle houses has the potential to become a cost efficient technique to lower NH3 emission on a farm scale. The estimated costs for biological acidification vary between 4 and 20 € per kg NH_3 saved (or 50 to 310 € per cow). This price range is to a large extent determined by the amount of C substrate needed. It is expected that the costs can be maintained below 10 € per kg NH_3. Additional lab testing is needed to get more quantitative information about optimal process conditions for biological acidification in order to make more precise cost calculations.
- Positive side effects of acidification are that it reduces methane emission and that it results in more homogenous slurry without a foam film on top. The latter results in a more efficient use of the storage capacity in cubicle houses possible.
- For the short term (in order to make a quick start) an acidification system based on a mix of biological and inorganic acidification seems to be attractive from the viewpoint of risk distribution between costs of additives (acetic acid, C substrate and H_2SO_4).
- For the long term the highest cost efficiency for biological acidification is expected to be a fed batch system. In such a system fresh manure is frequently added to manure that has already been acidified and where::
  - at the start fresh slurry is immediately acidified to pH 5.5-6;
  - zeolite and Lactobacillus spp. are regularly added;
  - a limited amount of C substrate or organic acid is added in case that the quality of the fresh manure is not sufficient as a C substrate to maintain the pH; and
  - the temperature remains above 10^{o}C.
- It is expected that it is more profitable to use the thick fraction of biologically acidified slurry as a mono substrate in a bio gas plant than the thick fraction of untreated slurry. The reason is twofold. First, due to a lowering of the viscosity of the slurry after biological acidification, the slurry is separated more easily in a thick and liquid fraction. Secondly, during biological acidification all parameters are optimized to produce slurry containing a maximum amount of compounds that are direct substrate for methane producing bacteria. In the acidification procedure methane production is inhibited by pH and Eh conditions. Once the conditions are adapted to favor methanogenesis in the biogas reactor it is expected that the biogas potential is higher compared to the untreated slurry.

7.2 Recommendations

It is recommended to test biological acidification more detailed on a (semi) lab scale to get more quantitative information about the optimal process conditions needed to acidify slurry to a target pH below 6. Process parameters that need optimizing are the amount of C substrate, the effect of temperature. The effect of dairy cow diet (grass based versus maize based) also need further investigation. In addition, the biogas production potential of the thick fraction of successfully acidified should be estimated.

This information is essential to make a more precise cost calculation and to determine if biological acidification without or with biogas production is possible for less than 150 € per cow or 10 € per kg NH_3 emission reduction. These prices seem to be the upper limit to be an interesting technique to implement in practice.

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