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Reducing NH_3 emissions from cattle slurry by (biological) acidification: experimental proof and practical feasibility

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Reducing NH_3 emissions from cattle slurry by (biological) acidification: experimental proof and practical feasibility

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Authors:

Dr. Ir. D.W. Bussink (NMI)
Dr. Ing. A.M.D. Van Rotterdam- Los (NMI)
Ir. I. Vermeij (WLR)
Ir. H.J.C van Dooren (WLR)
Ir. S. Bokma (WLR)
G.J. Ouwerkerk (NMI)
Ing. H. van der Draai (NMI)
Dr. W. Wenzl (HBLFA Raumberg - Gumpenstein)

Content

Foreword

Ammonia (NH_3) emission from dairy farms has to be reduced in the near future. In a recent desk study commissioned by the Dutch Dairy Board, NMI studied the potential of biological acidification of dairy cattle slurry to reduce NH_3 emissions from stables and after application. As a follow-up this study commissioned by the Dutch Dairy Board and the Ministry of Economic Affairs focuses on small scale experiments to get more information about the potential of biological acidification and the economic perspectives. Based on these results the technical and economic feasibility of scaling up this technique is evaluated. The study was a joint effort of NMI and Wageningen UR Livestock Research. With Dr Wenzl from Raumberg Gumpenstein, where also a project about biological acidification is running, there was an information exchange.

Samenvatting en conclusies

Waarom aanzuren mest?

Het grootste aandeel (90%) van de landelijke NH_3-emissie wordt veroorzaakt door dierlijke mest. Melkveemest draagt hier het meeste aan bij (35%). Ongeveer de helft van de melkveebedrijven ligt nabij Natura 2000 gebieden. Om in de Natura 2000 gebieden de natuurdoeltypen te bereiken en te behouden is het belangrijk dat de NH_3-emissie gelijk blijft of zelfs daalt. Melkveebedrijven mogen daarom alleen uitbreiden als dit in dit gebied niet leidt tot een verhoging van de NH_3-emissie. Daarnaast is er vanuit de praktijk de toezegging dat vanaf 2014 elke melkveehouder 10 procent minder ammoniakuitstoot realiseert. Het is dus nodig om verdergaande technieken voor emissiereductie te ontwikkelen, te testen en toe te passen. Het (biologisch) aanzuren van mest is een mogelijke techniek om de NH_3-emissie uit mest te verlagen.

(Biologisch) aanzuren van mest

Drijfmest aanzuren in de stal tot pH 5,5 of lager verlaagt zowel de NH_3-emissie uit de stal (35-50%) als de emissie bij toedienen (±85%). Op boerderijniveau zijn emissiereducties van 50% mogelijk. Ook de emissie van methaan uit mest vermindert sterk (tot 100%). Wat betreft de effecten van aanzuren van mest op geur, fijn stof en lachgas zijn geen, dan wel summiere en tegenstrijdige gegevens beschikbaar in de literatuur.
In het verleden is veel onderzoek gedaan naar het aanzuren van mest met een anorganisch zuur, met name naar salpeterzuur (HNO_3) en zwavelzuur (H_2SO_4). Salpeterzuur heeft het nadeel dat het de N_2O emissie verhoogt en dat het aanzuurproces minder goed beheersbaar is. Aanzuren met zwavelzuur leidt tot hoge zwavelgehalten in mest , waardoor bij mesttoediening veel meer zwavel wordt gegeven dan het gewas nodig heeft. Dit leidt tot sulfaatuitspoeling en mogelijk tot te hoge sulfaatgehalten in het grondwater.
Een andere, veel minder onderzochte, mogelijkheid is om drijfmest biologisch aan te zuren door het omzetten van fermenteerbare koolhydraten door micro-organismen in azijnzuur en melkzuur. Dit proces wordt biologisch aanzuren van mest genoemd. De mest zelf, met name verse mest, bevat fermenteerbare koolstofbronnen (endogene C-bron) die als substraat voor de micro-organismen kunnen dienen. Het bevorderen van de productie van zuren in mest door micro-organismen kan op twee manieren door: i) direct toevoegen van zuurproducerende micro-organismen bv Lactobacillus spp. en ii) additieven toe te voegen waardoor een gunstiger mestmilieu wordt gecreëerd voor zuurproducerende micro-organismen. Deze additieven kunnen (een combinatie) zijn van:

- gemakkelijk afbreekbaar organisch substraat (exogene C-bron);
- mineralen zoals zeoliet om het reactief oppervlak tussen micro-organismen en mest te vergroten; en/of
- organisch zuur om de pH van de mest te verlagen tot gunstige condities voor een specifieke (groep) micro-organismen. Het gebruik van organische zuren heeft de voorkeur boven anorganische zuren in verband met de afbreekbaarheid van organische zuren wanneer de mest aan de bodem wordt toegediend en bovengenoemde nadelen van anorganische zuren worden vermeden.

Doel onderzoek

Het doel van dit onderzoek is om vast te stellen wat de procescondities zijn om mest zo effectief mogelijk biologisch te kunnen aanzuren (pH<= 5,5) en in hoeverre dit mede bepaald wordt door het verstrekte rantsoen en de temperatuur. De gebruikte additieven zijn (een combinatie van) zuur, C-bronnen, zeoliet en melkzuurbacteriën. In hoeverre de productie van het biogaspotentieel van mest stijgt na biologisch aanzuren is ook onderzocht. Gebaseerd op deze resultaten is de technische en economische haalbaarheid van opschaling van deze techniek geëvalueerd.
Labschaalexperimenten zijn uitgevoerd om de belangrijkste procescondities te bepalen en te kwantificeren.

Hiervoor zijn verschillende dagverse monsters van drijfmest gebruikt afkomstig van melkveebedrijven met een volledig maïs-, een gras-maïs- of een volledig grasrantsoen. Verse mest is gebruikt omdat de condities gunstiger (lagere pH en meer fermenteerbare koolstof bronnen) zijn voor microbiële verzuring dan in oudere mest. Het effect van het rantsoen is onderzocht omdat de verwachting was dat dit van invloed is op het aanzuurproces. In de experimenten is geur kwalitatief onderzocht. Fijn stof en lachgas zijn niet onderzocht.

Wat zijn de procescondities voor het (biologisch) aanzuren van runderdrijfmest?

Toevoeging van zuur

Uit de laboratoriumexperimenten blijkt dat zowel het toevoegen van anorganisch (H_2SO_4) als van organisch zuur (azijnzuur (HAc), melkzuur (LA)) aan drijfmest tot een snelle daling van de pH leidt. De daarbij benodigde hoeveelheid zuur om de mest aan te zuren is afhankelijk van de concentratie en de sterkte van het zuur en van het N-gehalte in de mest en daarmee van het N-gehalte in het rantsoen. Het verstrekte ruwvoer (gras of maïs) in het rantsoen lijkt minder van belang te zijn. Meer zuur is nodig bij een hoger N-gehalte maar de NH_3-emissiereductie is ook hoger. De temperatuur heeft grote invloed. Bij een temperatuur van 10^0C is minder zuur nodig en is de pH van de mest stabieler dan bij 25^0C.
In fed-batch experimenten is gedurende meerdere weken regelmatig een kleine hoeveelheid verse mest toegevoegd aan aangezuurde mest (pH mest <= 5,5). Het fed-batchsysteem beoogt de situatie in de stal na te bootsen. In dit systeem kon de lage pH die was bereikt door het eenmalig toevoegen van een (an)organisch zuur niet op het lage niveau worden gehouden; extra zuur toevoegen was nodig.
Het creëren van gunstige omstandigheden voor de zuurproducerende bacteriën (lage pH) heeft niet geleid tot een situatie waarin er door deze micro-organismen voldoende zuur werd geproduceerd (door het omzetten van de endogene C-substraat) om de pH op 5,5 te houden. Wel duiden de resultaten erop dat in mest die is aangezuurd door melkzuur toe te voegen, een kleine hoeveelheid endogene C is omgezet in melkzuur. Voor een gemiddelde mestsamenstelling (N-gehalte 4,1 mg /kg) wordt bij 10°C steady state bereikt wanneer ± 5,6 L (18M) H_2SO_4/m^3, ± 16 L (11,5M) HAc/m^3 of ±± 21 L (17,4M) LA/m^3 mest wordt toegevoegd.

Toevoeging van een C-bron, Lactobacillus en/of zeoliet

In de experimenten is een C-bron met een hoog suikergehalte,melasse of siroop als substraat gebruikt voor de zuurproducerende bacteriën. In het fed-batchsysteem met de juiste substraat dosering duurt het 2 - 3 weken om de streef pH van 5,5 te bereiken. De snelheid waarmee deze pH wordt bereikt en het niveau waarop de pH wordt gehandhaafd zijn afhankelijk van de toegevoegde hoeveelheid substraat, van het type drijfmest en van de temperatuur. Bij lage temperatuur (10°C) wordt meer suiker uit melasse/siroop in zuur omgezet dan bij een hoge temperatuur (25^0C). Dit is tegenovergesteld aan de meeste biologische reacties die juist sneller verlopen bij hoge temperatuur. Een mogelijke verklaring is dat bij een hogere temperatuur er meer competitie is met andere micro-organismen die het substraat niet in zuur omzetten.
Evenals bij alleen zuur toevoegen resulteert geen van de behandelingen in een systeem dat zichzelf in stand kan houden op pH 5,5 door het omzetten van endogeen C-substraat dat met de verse mest wordt toegevoegd. Na elke toevoeging van verse drijfmest aan het systeem is (uiteindelijk) steeds extra substraat in de vorm van suiker nodig om de streef-pH te handhaven. Een steady state tussen de toevoeging van verse drijfmest (N-gehalte 4,1 mg / kg) en substraat wordt bij 10° C bereikt bij toevoeging van ± 50 L siroop/m^3 drijfmest. Deze siroop bevat ± 65% suiker.

Er is geen positief effect waargenomen van het direct toevoegen van de zuurproducerende bacterie Lactobacillus spp.. Ook het toevoegen van zeoliet om het reactief oppervlak tussen micro-organismen en mest te vergroten had geen positief effect. In geen van de behandelingen leidde het toevoegen van

Lactobacillus of zeoliet tot een (extra) verlaging van de pH van de drijfmest.

Toevoeging van zuur en een C-bron

Het meest veelbelovende systeem is verse mest initieel aanzuren met een (an)organisch zuur tot pH 5,5 en vervolgens de pH te handhaven, na toevoeging van verse mest, door toevoegen van een C-bron. Door aanzuren met een (an)organisch zuur wordt de streef-pH zo goed als direct bereikt. Wanneer de pH stijgt door het toevoegen van verse mest is de streef-pH binnen 1 dag weer op het gewenste niveau door de omzetting van suiker in organische zuren. Deze snelle reactie is het gevolg van een lager zuurbufferpotentieel van de mest (weinig ureumhydrolyse) en gunstige omstandigheden in de mest voor de zuurproducerende micro-organismen. De toegevoegde suiker wordt voor ongeveer 100% omgezet in LA (homolactische gisting) wanneer mest initieel is aangezuurd met HAc of H_2SO_4. In de LA-omgeving is de omzetting iets lager. In enkele gevallen is ook heterolactische fermentatie waargenomen.
Wanneer alleen C in de vorm van siroop (65% suiker) wordt toegevoegd is ± 50 L /m^3 drijfmest nodig om de streef-pH te handhaven in een fed-batchsysteem. Bij toevoegen van C aan drijfmest die initieel is aangezuurd met HAc of LA is minder siroop (± 43 L /m3) nodig om de streef-pH te handhaven. Na initieel aanzuren met H_2SO_4 is nog minder siroop (± 28 L /m^3) nodig om de pH handhaven.

Biogasproductie

Er is een duidelijk positief effect wanneer een C-bron wordt gebruikt om mest aan te zuren; het biogasproductiepotentieel nam gemiddeld met 55% toe. Er is geen tot een kleine toename in biogaspotentieel wanneer alleen organisch zuur wordt gebruikt om mest aan te zuren (uitzondering is melkzuur in maïsrantsoen-gebaseerde mest waar het biogaspotentieel met 100% toenam). Een algemeen lineaire relatie wordt gevonden tussen biogasproductiepotentieel en drogestofgehalte van de mest. Het drogestofgehalte is afhankelijk van het verstrekte rantsoen en samenstelling en hoeveelheid toegevoegde C-bron en/ of organisch zuur. nitieel aanzuren met zuur en de streef-pH handhaven door toevoeging van makkelijk afbreekbaar C-substraat zal dan ook resulteren in een hoger biogasproductiepotentieel.

Wat zijn de mogelijkheden van biologisch aanzuren in de praktijk?

Technische en economische haalbaarheid

Vanuit economisch oogpunt is mest aanzuren met alleen H_2SO_4 het meest aantrekkelijk. Het nadeel is een risico op te hoge sulfaatgehalten in het grondwater door overbemesting met zwavel. Uit zowel milieu- als economisch oogpunt heeft daarom het gecombineerd toevoegen van zwavelzuur (H_2SO_4) en een C-bron met een hoog suikergehalte de voorkeur. Bij een systeem waarbij de verzuring voor de helft via zwavelvuur en voor de helft via het toevoegen van een C-bron geregeld wordt bedraagt de kostprijs ongeveer € 150per koe per jaar of ruim 10 euro per kg bespaarde ammoniak. Daarbij bedragen de investeringskosten en de variabele kosten respectievelijk 55% en 45% van de totale kosten.
Vanuit technisch oogpunt betekent dit dat voor ligboxenstallen met roostervloer en kelders voor mestopslag er twee containers worden geplaatst, één voor het zuur en één voor de C-bron. Via een online pH-monitoring kan de streef-pH worden gehandhaafd door tijdige dosering van zuur of een C-bron. Er zijn in Oostenrijk systemen beschikbaar waarbij de pH van mest instantaan via internet gemonitord kan worden.
In de praktijk is de verhouding waarin zuur en een C-bron gebruikt worden aan te passen aan de beschikbaarheid en de prijs van de additieven. Via een volautomatische regelunit kunnen daarbij het zuur- en het C-brongebruik worden geregistreerd evenals het pH–verloop van de mest.
Gezien de ontwikkelingen en veranderingen op de mestmarkt met betrekking tot het bewerken van mest is het interessant om na te gaan in hoeverre aangezuurde mest verder bewerkt kan worden. Zo blijkt bijvoorbeeld dat deze mest beter gescheiden kan worden in een dikke en dunne fractie.
Selectie op effectievere melkzuurproducerende bacteriestammen, het toevoegen van enzymen voor meer

fermenteerbaar substraat in de mest zelf en sturen op een efficiënte N-voeding van de veestapel zijn oplossingsrichtingen om de effectiviteit van biologisch aanzuren verder te verbeteren. Daardoor zal de benodigde hoeveelheid toevoegmiddelen afnemen en daalt de kostprijs.
In de experimenten is geur kwalitatief onderzocht maar hier is geen eenduidig beeld uitgekomen.

Conclusies

- Aanzuren van drijfmest in ligboxstallen met roostervloer kan door toevoeging van zwavelzuur, azijnzuurzuur, melkzuur of door toevoeging van een gemakkelijk afbreekbare C-bron waarbij vooral melkzuur (LA) wordt gevormd. Bij aanzuren tot een streef-pH van 5,5 of lager daalt NH_3-emissie met ±50% (op bedrijfsniveau) en CH_4-emissie met ±100%.
- De hoeveelheid zuur dat nodig is om een streef-pH van 5,5 te bereiken hangt af van de temperatuur en het N-gehalte van de drijfmest en dus van het N-gehalte van het rantsoen. Meer zuur is nodig bij een hoger N-gehalte en hogere temperatuur.
- Aangezuurde mest blijft niet uit zichzelf op de streef-pH (pH<= 5,5) (steady state) na toevoegen van verse mest. Er wordt onvoldoende C-substraat uit de vers toegevoegde mest omgezet in zuur door zuurproducerende bacteriën. Extra zuur of C-substraat is nodig om de streef-pH te handhaven.
- Het toevoegen van een zuurproducerende Lactobacillus species en zeoliet heeft in deze experimenten niet geleid tot extra zuurproductie uit in de mest aanwezig C-substraat.
- Het biogasproductiepotentieel van aangezuurde mest is, afhankelijk of er een C-bron, organisch of anorganisch zuur wordt toegevoegd, hoger dan van onbehandelde mest. Wanneer alleen siroop is toegevoegd om de mest te verzuren dan is de toename in biogasproductiepotentieel het hoogst en bedraagt ongeveer 55%.
- Uit zowel milieu- als economisch oogpunt, is het meest veelbelovende systeem een combinatie van toevoegen van zwavelzuur bij het opstarten en het handhaven van de streef-pH door een C-bron met een hoog suikergehalte toe te voegen. Alleen biologisch aanzuren kan op dit moment economisch niet uit.
- De kosten van een mix-systeem op basis van zwavelzuur en een C-bron bedragen ongeveer 10 euro per kg NH_3-emissie reductie. Per koe bedragen de kosten ongeveer 150 euro per jaar verdeeld over 55% investeringskosten en 45% variabele kosten.
- In de praktijk kan de verhouding waarin een zuur en een C-bron worden toegevoegd worden aangepast aan actuele prijzen en beschikbaarheid. Via een volautomatische regelunit kunnen daarbij het zuur- en het C-brongebruik worden geregistreerd evenals het pH–verloop van de mest.
- Het systeem op de boerderij is te implementeren in de meeste gangbare stallen (ligboxenstallen met roostervloer).
- Gezien de ontwikkelingen die momenteel optreden op de mestmarkt met betrekking tot mestbewerking kan het verder verkennen van de mogelijkheden om aangezuurde mest verder te bewerken een interessante optie zijn. Bijvoorbeeld het benutten van het verhoogde biogas potentieel van aangezuurde mest en/of de mogelijkheid dat aangezuurde mest makkelijker gescheiden kan worden in een dikke en dunne fractie. .
    - Oplossingsrichtingen om de effectiviteit van biologisch aanzuren te verbeteren en de kostprijs te laten dalen zijn: selectie op effectievere melkzuurproducerende bacteriestammen, het toevoegen van enzymen voor meer fermenteerbaar substraat in de mest zelf en sturen op een efficiënte N-voeding van de veestapel.

Summary and conclusions

Why acidify cattle slurry?

The largest share (90%) of the national NH_3 emission is caused by animal manure. Dairy manure contributes the most (35%). About half of the dairy farms are located in the vicinity of special areas of specific natural value (Natura 2000). To reach and maintain the specific nature types in these Natura 2000 areas it is important that the NH_3 emission from the surrounding farms remains constant or even decreases. Dairy farms may therefore only expand if this does not lead to an increase in NH_3 emissions in that area. In addition, the dairy sector has committed itself to a NH_3 emission reduction of 10% from dairy farms by management measures by January 1st 2014. It is thus necessary to continue to develop, test, and to apply techniques for reducing emissions. The (biological) acidifying of manure is a technique that has the potential to decrease NH_3 emissions.

(Biological) acidification of slurry

Slurry acidification in the stable to pH 5.5 or lower not only reduces the NH_3 emission from the stable (35-50%), but also the emission when the slurry is applied to the soil (± 85%). At farm level, emission reductions of 50% are possible. At this low pH the methane emissions from manure are also strongly reduced (to 100%). Regarding the effects of acidification of manure on odor, particulate matter and nitrous oxide no, or little and contradictory data are available in literature.

In the past, a lot of research has been done concerning the acidification of manure with an inorganic acid, in particular nitric acid (HNO_3) and sulfuric acid (H_2SO_4). Nitric acid has the disadvantage that it increases N_2O emissions and that the acidification process is less manageable. Sulfuric acid has the disadvantage that so much acid is needed that the amount of sulfur added to the soil through fertilization with the acidified manure strongly exceeds crop demand. This leads to sulphate leaching and possibly to high sulphate levels in the groundwater

Another, much less studied, possibility us to biologically acidify slurry through the conversion of fermentable carbohydrates by micro-organisms into acetic acid and lactic acid. This process is called biological acidification of manure. The manure itself, in particular fresh manure, contains fermentable carbon sources (endogenous C-source), which can serve as a substrate for the acid producing micro-organisms. Promoting the production of acids in manure by micro-organisms can be done in two ways: i) by direct addition of acid-producing micro-organisms, for example, Lactobacillus spp. and ii) by adding additives to manure to create a more favorable environment for acid-producing micro-organisms. These additives may be (a combination) of:

- readily degradable organic substrate (exogenous C-source);
- minerals such as zeolite in order to increase the reactive surface area between micro-organisms and manure, and / or
- organic acid to reduce the pH of the manure to create favorable conditions for a specific (group of) acid producing micro-organisms. The use of organic acids is preferred over inorganic acids in relation to the degradation of organic acids after the manure is applied to the soil and the above-mentioned disadvantages of inorganic acids are avoided.

Aim of the research

The aim of this study is to determine what the process conditions are to effectively biologically acidify (pH <= 5.5) dairy slurry. The effect of diet and temperature are also taken into account. The additives used are (a combination of) acid, C-sources, zeolite and lactic acid bacteria. The extent to which the biogas production potential of slurry increases after (biological) acidification has also been studied. Based on these

results, the technical and economic feasibility of scaling-up of this technique is evaluated.
Laboratory-scale experiments have been carried out in order to determine and quantify the most important process conditions. Different day-fresh slurries were used, collected from farms where the roughage part of the diet was only corn, only grass, or a combination of grass and corn. Fresh manure is used because it is more favorable (lower pH and more fermentable carbon sources) for microbial acidification than older manure. Diet is examined because it was expected that this affects the acidification process. In the experiments the effect on odor was examined but this did not give a clear picture. Particulate matter and nitrous oxide have not been studied

What are the process conditions for the (biological) acidification of cattle slurry?
Addition of acid
Laboratory experiments show that both the addition of inorganic acid (H_2SO_4) and organic acid (acetic acid (HAc), lactic acid (LA)) to slurry results in a rapid decrease of the pH. The required amount of acid needed to acidify the slurry to a pH below the target of pH 5.5 depends on the concentration and strength of the acid and of the N content in the manure and therefore on the N content in the fed ration. The fed roughage (grass or maize) in the diet seems to be less important. More acid is needed at a higher N content but the NH_3 emission reduction is also larger. The temperature is an important factor. At a temperature of 10^0C less acid is required and the pH of the slurry is more stable than at 25^0C.
In fed-batch experiments, during several weeks a small amount of fresh manure is added to acidified manure (pH <= 5.5). The fed-batch system is used as it approximates the situation in the stable. In this system the low pH that was reached by the one-time addition of an (in) organic acid could not be maintained; it was necessary to add more acid.
Creating favorable conditions for the acid-producing bacteria (low pH) did not lead to a situation where these micro-organisms produced sufficient acid (by the conversion of the endogenous C-substrate) in order to maintain the pH at 5.5. However, the results indicate that in slurry which was acidified by adding lactic acid a small amount of endogenous C was converted to lactic acid. However, the amount was insufficient for the system to sustain itself. For an average manure composition (N content is 4.1 mg/kg) at 10°C steady state is reached when ± 5.6 L (18M) H_2SO_4/m^3 , ± 16 L (11.5 M) HAc/m^3 or ± 21 L (17.4 M) LA/m^3 manure is added

Addition of a C-source, Lactobacillus, and / or zeolite
In the experiments, a C-source with high sugar content (molasses or syrup) is used as substrate for the acid-producing bacteria. In the fed-batch system it takes 2-3 weeks to reach the target pH of 5.5 when the appropriate dosage is used. The speed with which this target pH is reached and the level at which the pH is maintained will depend on the added amount of substrate, the type of slurry and the temperature. More sugar from molasses / syrup is converted into acid at low temperature (10°C) than at a high temperature (25°C). This is the opposite to most biological reactions where conversion rates are generally more rapid at higher temperature. One possible explanation is that at higher temperatures there is more competition with other micro-organisms that do not convert the substrate into acid.
Comparable to when only acid is added, none of the treatments results in a system that can maintain the target pH when fresh slurry is added. Not enough of the endogenous C substrate that is added with the fresh slurry is converted into acid to maintain the pH. Adding fresh slurry to the system (ultimately) results in the need to add more substrate in the form of sugar in order to maintain the target pH. A steady state between the addition of fresh slurry (N-content 4.1 mg / kg) and substrate is achieved at 10°C when ± 50 L syrup/m^3 slurry is added. This syrup contains ± 65% of sugar.

No positive effect was observed from the direct addition of the acid-producing bacteria (Lactobacillus spp.).

Also, the addition of zeolite to increase the reactive surface area between micro-organisms and slurry had no positive effect. In none of the treatments the addition of Lactobacillus or zeolite resulted in a (further) reduction of the pH of the slurry

Addition of acid and a C-source

The most promising system is to initially acidify fresh slurry with an (in) organic acid to pH 5.5 and then to maintain this pH, after the addition of fresh manure, by adding a C-source. The target pH is reached almost instantly when slurry is acidified with an (in)organic acid. When the pH rises as a result of the addition of fresh manure, the target pH can be reached again within one day by adding C-substrate. This rapid reaction to the addition of C is the result of a lower acid buffer potential of the manure (little urea hydrolysis) and favorable conditions in the manure for the acid-producing micro-organisms.
Approximately 100% of the added sugar was converted to LA (homolactic fermentation) when the slurry was initially acidified with HAc or H_2SO_4. When slurry was acidified with LA, the conversion was slightly lower. In some cases, heterolactic fermentation was also observed.
If only C in the form of syrup (65% sugar) was added ± 50 L/m^3 slurry was needed to maintain the target pH in a fed-batch system. Upon addition of C to slurry that initially was acidified with HAc or LA less syrup (± 43 L/m^3) was needed in order to maintain the target pH. After initial acidification with H_2SO_4 even less syrup (± 28 L/m^3) is needed to maintain the pH.

Biogas production

There is a clear positive effect when a C-source is used to acidify slurry, the biogas production potential increased on average by 55%. There is no or a small increase in biogas potential when only organic acid is used to acidify slurry (an exception is when lactic acid is used to acidify corn-based slurry where the biogas potential increased by 100%). A general linear relationship is found between biogas production potential and dry matter content of slurry. The dry matter content depends on the fed diet and the composition and amount of added C-source and / or organic acid. Initial acidification with acid and maintaining the target pH by addition of easily fermentable C substrate will also result in a higher biogas production potential.

What are the possibilities of biological acidification in practice?

Technical and economic feasibility

From an economic point of view, acidification of slurry with only H_2SO_4 is most attractive. The downside is a risk of too high sulfate levels in groundwater by over-fertilization with sulfur. Therefore, from the combined environmental and economic point of view, the combined addition of sulfuric acid (H_2SO_4) and a C-source with a high sugar content is preferred. In a system where the acidification is half controlled by H_2SO_4 and half by adding a C-source, the cost is approximately € 150 per cow per year, or just over € 10 per kg saved ammonia. In this case the investment cost are 55% and the variable costs 45% of the total cost.
From a technical perspective this entails that in cubicle stables with slatted floor and slurry pit, two containers are placed on the farm; one for the acid and one for the C source. Through an online pH monitoring, the target pH can be maintained by timely dosing acid or a C source. There are systems available in Austria, where the pH of slurry can be monitored via the internet. In practice, the ratio in which acid and a C source are used can be adapted depending on the availability and price of the additives. Via a fully automatic control unit acid-and C-source use can be registered together with the pH of the slurry.
Taking into account the current changes in the manure market concerning the processing of manure it is interesting to investigate the possibility to process manure after it has been acidified. For instance, it seems to be easier to separate acidified slurry into a thick and liquid fraction compared to untreated slurry.
Selection of more effective lactic acid bacterial strains, adding enzymes for more fermentable substrate in the slurry and focusing on efficient N diets for cattle are possible solutions to further improve effectiveness

of biological acidification. This will decrease the required amount of additives and decrease the cost.

Conclusions

- Acidification of slurry in cubicle stables with slatted floors is achieved by adding acid and / or a readily fermentable carbon source which is converted into mainly lactic acid (LA). When acidifying slurry the target pH is less than or equal to (<=) 5.5 to ensure a sufficient reduction in NH_3 emission (± 50% on farm scale) and CH_4 emission (± 100%).
- The amount of acid required to acidify slurry to a target pH of 5.5 mainly depends on the temperature and the N content of the manure and thus on the N content of the diet. More acid is needed at a higher N content.
- It is necessary to add acid or C-substrate after adding fresh slurry to acidified slurry in order to maintain the target pH. The fed-batch system can thus not maintain the target pH of 5.5 (steady state) by the conversion of easily fermentable C added with the fresh slurry.
- The addition of acid-producing bacteria (Lactobacillus spp.) and zeolite in these experiments did not result in additional production of acid.
- The biogas production potential of acidified slurry is, depending on whether a C-source and/or (in)organic acid is used, higher than of untreated slurry. When only syrup is added to acidify slurry the increased biogas production potential is highest and amounts to approximately 55%.
- From a combined environmental and economic perspective, the combination of initially acidifying slurry with H_2SO_4 and maintaining the target pH using a C-substrate with high sugar content is the most promising system. A system based only on biological acidification is at this point not economically feasible.
- The cost of a mixed system based on sulfuric acid and a C-source is approximately 10 € per kg NH_3 emission reduction. Expressed per cow costs approximate 150 € per year divided over 55% investment costs and 45% variable costs.
- In practice, the ratio in which acid and a C-source are added can be adjusted according to current prices and availability. Via a fully automatic control unit the use of acid-and C-source can be registered together with the change in pH of the slurry.
- The system can be implemented on the farm in the most common stables (cubicle stables with slatted floor and slurry pit). The system can be similar to the system that was used 20-25 years ago or can be arranged by analogy with the Danish system in which a batch system is used.
- Given the changes and developments currently taking place in the fertilizer market regarding manure processing, further exploration of the possibilities to process acidified slurry are interesting. For example making use of the increase in biogas potential of acidified slurry and / or the possibility that acidified slurry can be separated more easily in a thick and thin fraction. .
- Possible solutions to improve the effectiveness of biological acidification and lowering the cost are: selection of more effective lactic acid producing bacteria, adding enzymes to enhance the fermentation potential of organic substrate in the slurry, and pursue a more efficient N-nutrition of livestock

Introduction

1.1 Background

Dutch policy is focused on further reducing NH_3 emissions. By far the most of the nationwide NH_3-emission is attributed to emission from animal manure (90%). The use of artificial fertilizer causes the other 10% of the NH_3-emission (Hoogeveen et al., 2010). Especially in pig- and poultry farms adjusted housing has resulted in a large decrease in NH_3-emissions. 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 (Hoogeveen et al., 2010). For dairy farms adjustment to housing is less straightforward due to the open structure. Nevertheless, several emission reducing floors have been introduced (rav).
In the vicinity of special areas of specific natural value (Natura 2000) interests of farmers and nature conservation authorities contradict as a result of NH_3-emission. 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. For these farms NH_3-emission reducing technologies are especially interesting. Furthermore, there is a commitment from the agricultural sector that all dairy farms should have reduced their ammonia emission by 10% by January 1st 2014 (Niewe Oogst 27th of May 2013).
Acidification of cattle slurry is a possible solution to reduce NH_3-emission from slurry. It not only lowers the emission from the stable but emissions are also reduced when the slurry is applied to the soil (e.g. Pain et al., 1990, Husted et al., 1991, Bussink et al., 1994, Kai et al., 2008). An additional advantage is that the emission of methane from slurry is also strongly reduced when the pH of slurry is decreased below pH 6 (Oenema and Velthof, 1993, Ottosen et al., 2009, Sørensen en Petersen et al., 2012). Concerning the effects on the smell of the slurry, particulate matter, and N_2O emission no literature or very little literature with contradicting results is available.

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 as described in Husted et al., 1991). Since then many studies have been performed on this topic and different methods have been investigated. The most straightforward way to acidify slurry is to add inorganic or organic acid. This has several downsides. For organic acids the most important downside is that they are relatively expensive. Of the inorganic acids, sulfuric acid (H_2SO_4) is the best choice but has the downsides that S applications become too high and strict safety precautions must be taken.

Biological acidification
Several studies have shown that biological acidification of slurry may be a simple but effective possibility (Lameijer and Vervoort, 1995; Hendriks and Vrielink, 1997; Clemens et al., 2002; Clemens en Wulf, 2005;

Wenzl et al., 2009; Somitsch et al., 2008). This entails the production of organic acids like lactic acid or acetic acid by microorganisms generally present in slurry. To ensure that the pH plummets, the following options are possible: i) the microbial population is modified by adding acid-producing microorganisms such as Lactobacillus spp. and ii) the manure environment is changed to create more favorable conditions for acid producing microorganisms. Possible ways to stimulate the acid producing bacteria are by 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 e.g. lactobacillus;
3. easily fermentable organic substrate as a substrate for the acid producing microorganisms; and
4. colloidal material, for example zeolite, upon which microorganisms can fix to increase the reactive surface area.

The various studies (e.g. Hendriks and Vrielink, 1997; Clemens et al., 2002; Clemens and Wulf, 2005) in which biological acidification of manure has been studied all differ in the combination and amounts of additives. For example, the quality and quantity of the added organic substrate affects the rate with which the pH decreases, the pH level that 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. At the start of the acidification process (in)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 to constantly adjust the pH. This is important, as organic acids such as acetic acid and citric acid are relatively expensive compared to sulfuric acid. From a biological perspective organic acids are preferred as they are biodegradable. From an economic perspective a ‘start-up’ with an inorganic acid may be preferred.

Relevant factors that influence the acid production by microorganisms are temperature, freshness of the manure and composition of the manure (Lameijer and Vervoort 1995). The latter is directly related to the diet of the cattle. The addition of zeolite may possibly increase the conversion rate by increasing the reactive surface between the micro-organisms and substrate.

The composition, particularly the pH and the content of fermentable organic substrates in 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 (Lameijer and Vervoort, 1995). Fresh manure has a lower pH and contains more easily fermentable organic substrate than manure that is a few days old.

In addition to the freshness of the manure, the cattle diet also affects the acidification potential of manure. In principal the acidification potential of manure is similar to the potential of manure to produce biogas. The process is the same except that in the acidification process the last step (formation of biogas by methanogenic bacteria) is inhibited by the low pH. 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 (Amon et al., 2006, Bugdahl, 2011).

Rations with much starch and little protein seem to have the highest acidification potential. Furthermore, it appears that the natural pH of cattle slurry, also the limited data of Dutch cattle slurry, can vary greatly (from 6.8 to over 8) as a result of the fed ration (pers. communication with Dr Wenzl).

Commissioned by the Dutch Dairy Board (Productschap Zuivel) NMI recently studied the potential of biological acidification of cattle slurry to reduce NH_3 emissions from stables and after application (Bussink et al., 2012). The most important conclusions of this desk study are:

- (Biological) acidification of cattle slurry in cubicle houses has the potential to be a cost efficient technique to lower NH_3 emissions on a farm scale. When the slurry pH is decreased below 5.5,

emission reductions of 55-63% are expected in the whole chain from stable to application. 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. An additional advantage is a higher N-content and N-effectiveness of the slurry when applied to the field. Cumulatively this results in 15 to 30 kg more effective N per ha from applied slurry.

- 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 with 20% at farm level and that it results in more homogenous slurry without a layer of foam 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 over costs of additives (acetic acid, C substrate and sulfuric acid).
- 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;
  - possibly zeolite and Lactobacillus spp. are regularly added;
  - possibly 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 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 biogas plant than the thick fraction of untreated slurry.

Biological acidification seems to offer a large potential to decrease NH_3 and greenhouse gas emissions but process conditions need further investigation. The extent with which the pH decreases, and the time frame in which this decrease is achieved and maintained depends on the type and dosage of additives used. From the desk study it can not be concluded what combination and dosage of additives is minimally needed to achieve emission reductions. More quantitative information of the process conditions is needed before this technique can be tested on farm-scale.

1.2 Goal

The goal of this research is to experimentally quantify process conditions for effective biological acidification of dairy slurry.

The basis forms the desk study, which was phase 1. This experimental research is phase 2. Lab-scale experiments are conducted to establish and quantify the most important process conditions. In phase 3 this may be scaled up to farm scale. Measurements then focus on validating the system. This report describes phase 2 and forms the basis for phase 3.

Phase 2 consists of three parts:

1. Experimentally determine process conditions to effectively biologically acidify slurry of dairy cows (chapter 2-5);

2. Experimentally determine to what extent the biogas production potential of manure increases after biological acidification (included in chapter 5);
3. Evaluation of the technical and economic feasibility of scaling up this technique (chapter 6).

In part 1 the process conditions are determined in four lab-scale experiments (chapter 2-5). The goal of the first exploratory experiment, chapter 2, is to become acquainted with the system. Insight is gained in the effect of variables as diet of the cow and temperature on pH and composition of the slurry. In addition, the use of artificial urine instead of real urine is investigated.
In the second experiment, chapter 3, the effectiveness of (a combination of) different additives to (biologically) acidify slurry is investigated. Both experiment 1 and 2 are batch incubation experiments.
In the third experiment, chapter 4, the effectiveness of (a combination of) different additives to (biologically) acidify slurry is investigated using a fed-batch system. In this experiment different dosages are used. In the fed-batch system fresh slurry is added 3 times a week.
In the fourth experiment, chapter 5, a more detailed insight is gained into the amounts of additives needed to achieve and maintain the pH of fresh slurry below the target pH of 5.5 in a fed-batch system. The effect on biogas potential and organic acid composition of the slurry is also investigated.
Based on the experiments the technical and economic feasibility is determined of different methods to (biologically) acidify fresh slurry. This is presented in chapter 6. In chapter 7 the conclusions are presented.

2 Experiment 1

2.1 Goal

The goal of the first experiment (conducted October 2012) is to get acquainted with the system and to get insight in the effect of dairy cattle diet and temperature on pH and composition of the slurry. The roughage part of the diet varied from only grass to a combination of grass and maize and only maize. An incubation experiment was carried out at 10^0C, 20^0C and 25^0C. In addition, the use of artificial urine instead of real urine was investigated. The latter because it is 100% fresh, easier to obtain, not contaminated with manure bacteria and it makes it more easy to standardize slurry composition.

2.2 Experimental setup

During four weeks the change in pH of slurry was monitored in 500ml flasks at three temperatures (10^0C, 20^0C and 25^0C) in duplicate. The slurry was made fresh at the start of the experiment by adding day fresh faeces to day fresh urine in the ratio 63% faeces and 37% urine. The faeces and urine were collected from three different dairy farms on which the roughage part of the diet varied from only grass to a combination of grass and maize to only maize.
Apart from making slurry from day fresh faeces and urine, slurry was also made by adding artificial urine to day fresh faeces. The ratio was also 63% faeces and 37% urine. The composition of the artificial urine is shown in Table 2.1 (Corré, 2005). The reason for investigating this option was to be able to use 'sterile' urine in following experiments.
In total the experiment counted 36 experimental units: 3 types of slurry; 3 temperatures; real and artificial urine; in duplicate. Three times a week the pH was measured. The composition of the slurry was measured at the start and at the end of the experiment.

Table 2.1. The composition of the artificial urine (Corré, 2005, author based the composition on Whitehead et al., 1989 and personal communication with Dr. Valk).

	g/L		g/L
Urea	12.9	KCl	10.5
Hippuric acid	8	$KHCO_3$	14
Creatinine	1.1	$CaCl_2$. $2H_2O$	0.4
Allantoin	2.4	$MgCl.5H_2O$	1.2
		Na_2SO_4	3.7

2.3 Composition of the slurry

At the start of the experiment the chemical composition of the slurry was measured (Table 2.2).

Table 2.2. Chemical composition of the slurries at the start of experiment 1.

Sample	Dw	C Ash	OM	N-tot.	$N\text{-}NH_3$	N-org	C/N	P_2O_5	K_2O	MgO	Na_2O
Grass	73	17	56	4.2	1.7	2.5	6	1.2	4.7	0.8	1.5
Grass artificial	70	17	53	4.4	1.9	2.5	5	1.2	5.1	0.8	1.1
Grass/maize	90	20	70	5.4	2.1	3.3	6	1.5	5.8	1.3	0.8
Grass/maize artificial	88	19	69	5.0	2.1	2.9	6	1.4	5.1	1.2	0.8
Maize	82	11	71	2.7	0.4	2.3	12	1.4	1.8	1.0	0.8
Maize artificial	89	16	73	4.8	1.9	2.9	7	1.4	5.3	1.0	0.7
Dutch average	84	20	64	4.1	2.0	2.1		1.6	5.6	1.2	0.8

The slurry composition of the grass and grass/maize based diets are roughly similar, except that N content and organic matter content are slightly higher for the grass/maize diet. The slurry composition of the maize differs markedly from the other two diets. The N content, especially NH_3 content, ash and K_2O content are much lower than the other two diets and also than the Dutch average.
The composition of the slurry made from artificial urine is similar compared to the slurry made from dairy cattle urine for both the grass and the grass/maize diet but not for the maize diet. For the maize diet the slurry made from artificial urine is comparable to the other slurries and to the Dutch average but it differs largely to the natural maize slurry. This shows that the difference between the maize slurry and the other two slurry types (grass and grass/maize) is mainly caused by the difference in urine composition. This is in accordance with the findings from Sommer and Hutchings (2001) who find that urine contains the surplus of feed N that is not utilized by the cow or excreted as milk or faeces. With varying feeding strategy the excretion of faeces N is relatively constant and the TAN content in urine reflects the variation in feed.
Not only the urine composition varies with feeding strategy also the amount of urine produced is feed dependent. Duinkerken et al. (2003) found the urine production to decrease with increasing maize content in the feed. They also found the urea content in the urine to increase with increasing maize content. Our results contradict the findings by Duinkerken et al. (2003). In our results the N content of the urine from the maize diet was much lower than from the grass and grass/maize diet. The much lower N content of the slurry made from animal urine cannot be caused by a lower urine production. Because for both the natural as the artificial urine, urine and faeces were mixed in the laboratory in equal quantities for all three diets. The differences in N content are caused by the variation in both roughage and concentrate in the diet.
In the following experiments faeces and urine were collected from two other farms where the cows are fed with only maize (Table 2.3). These slurries had significantly higher total N and NH_3 contents than the slurry used in experiment 1. In experiment 2 the slurry composition from the maize farm is similar to the slurry from the grass and grass/maize farms. In the third and fourth experiment the N-total and NH_3 contents are even higher than of the slurry from the grass and grass/maize companies. In these slurries the lower N-content in the roughage part of the diet is overcompensated by the concentrate part of the diet.
It is thus clear that even when the roughage part of the diet is only based on maize, the composition of the slurry can vary widely. There does not seem to be a clear trend between maize content of the ration and N content. The most important reason for the differences in the overall composition is that apart from roughage the total dairy cattle diet is amongst others determined by concentrates.

Table 2.3. Slurry composition from three farms where the roughage part of the diet only consists of maize.

Experiment	DM	C ash	OM	N-tot.	C/N	N-NH_3	N-org	P_2O_5	K_2O	MgO	Na_2O
1	82	11	71	2.7	12	0.4	2.3	1.4	1.8	1.0	0.8
1 artificial	89	16	73	4.8	7	1.9	2.9	1.4	5.3	1.0	0.7
2	70	20	50	4.3	5	2.1	2.2	1.2	8.2	0.7	0.6
3	104	19	85	5.8	6.4	2.5	3.4	1.3	5.0	1.4	1.4
4	98	16	81	5.7	6.3	2.9	2.8	1.3	4.4	1.2	1.0

2.4 Results incubation experiment 1

The change in pH of the slurry during the four weeks of the incubation experiment are shown in Figure 2.1. For the grass and grass/maize diet the changes in pH over time are small. The pH fluctuates roughly between pH 8 and 9. For the maize diet the pH is clearly lower than the grass or grass/maize diet and it shows larger fluctuations (between pH 6.3 – 8.2).
For the slurry based on artificial urine the pH is roughly similar to the pH of the grass and grass/maize

slurry based on real urine. For the maize diet artificial urine does not seem to be an adequate replacement as the pH of the slurry is higher (7.3 – 9) compared to the slurry based on natural urine. The artificial urine does not do justice to the differences in composition of natural slurry. This composition is mainly the result of the fed diet as composed of roughage and concentrate.
Temperature seems to have a small affect on the pH. At 10^0C the pH is mostly stable during the experiment. At higher temperatures the pH is slightly lower and shows larger fluctuations. This might be caused by higher microbial activity, resulting in a higher conversion of organic molecules into organic acids at higher temperatures. Another reason might be a higher gas-exchange at higher temperature.

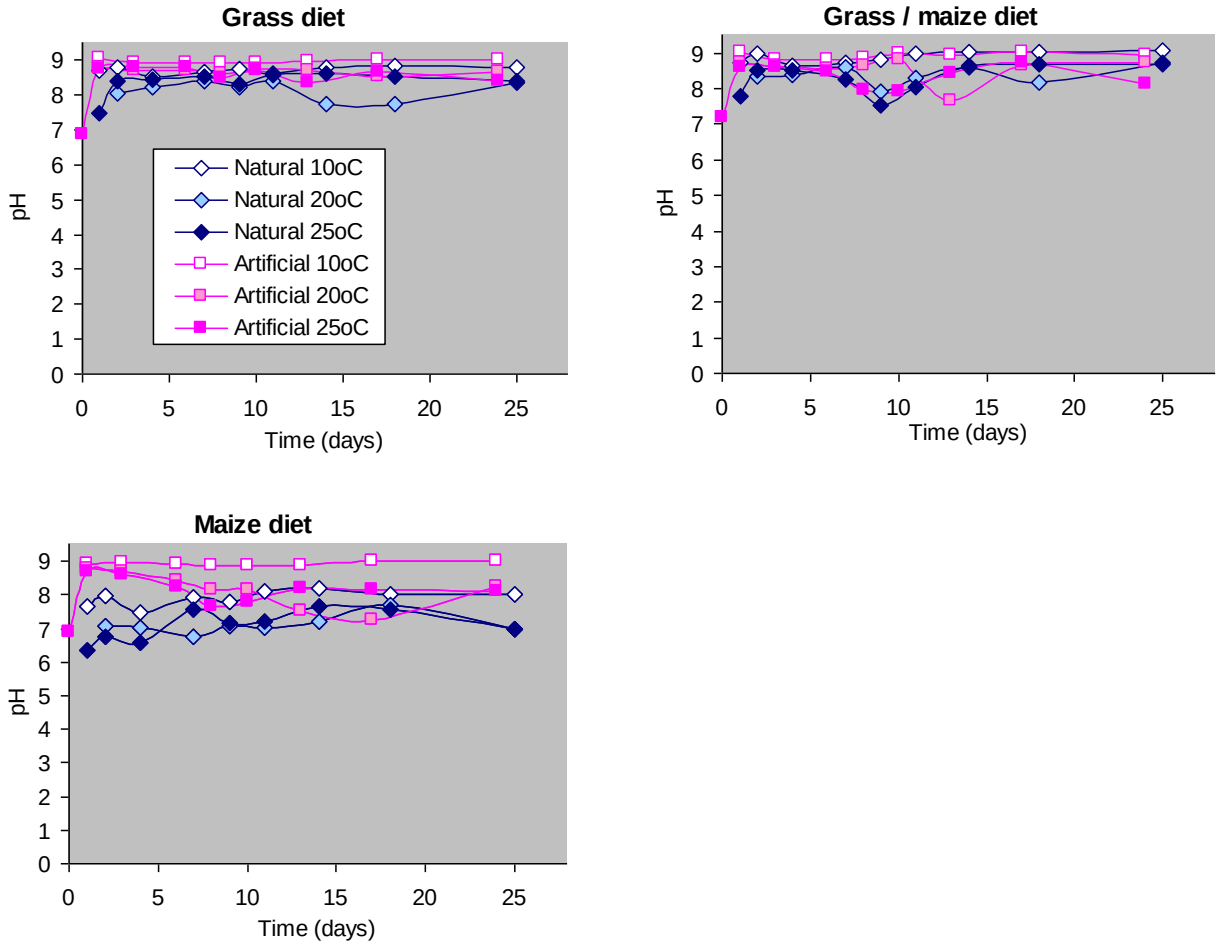


Figure 2.1. Change in pH of the slurry during experiment I for the grass diet (top), grass/maize diet (centre) and maize diet (bottom). The figures on the left hand side are an enlargement of the figures on the right.

The total N content of the slurry is not affected during the incubation experiment (Figure 2.2). Independent of the temperature, the composition after 4 weeks has hardly changed compared to the start of the experiment (t=0).
However, the NH_3 content increases during the experiment for all slurries (Figure 2.2). The relative increase is between 129% and 152% of the initial NH_3 content, except for the maize slurry which shows an increase of 225% at 20^0C and 25^0C. Overall, there seems to be a slightly higher increase in NH_3 content at higher temperatures. This increase in NH_3 content is due to mineralization of organic N. As organic N contents in the slurry decreased (data not shown) in favor of mineral N content. Total N content remained constant during the incubation (Figure 2.2).

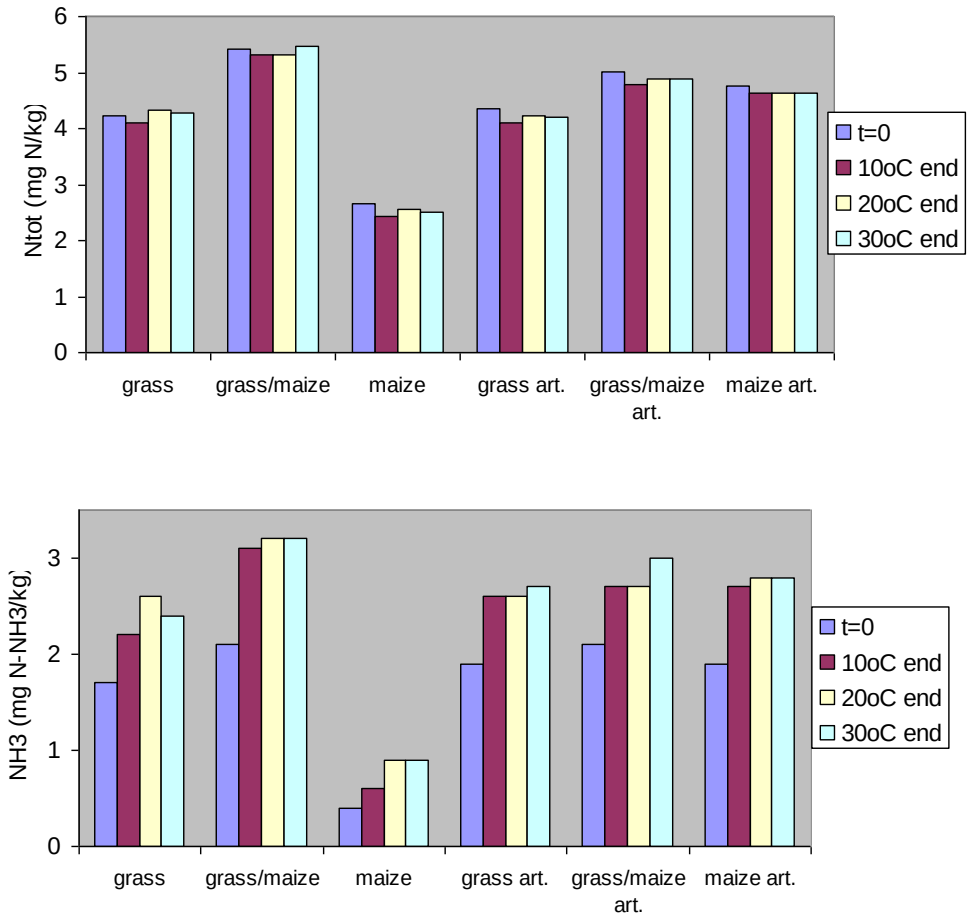


Figure 2.2. Change in composition of the slurries between the start of the experiment (t=0) and the end of the experiment (t=4 weeks).

2.5 Conclusions experiment 1

- The composition of slurry can vary widely, also when the roughage part of the diet is constant. The slurry used in these experiments represent the range in composition as found nationwide;
- In these experiments artificial urine proved not to be a good alternative to real urine;
- During the incubation of fresh slurry during four weeks the pH is relatively constant;
- At higher temperature (25^0C) the pH is slightly lower and shows larger fluctuations than at low temperature (10^0C), the more common temperature of slurry in practice.
- During the incubation only the mineral N content of the different slurries showed a marked increase after four weeks due to mineralization of organic N (which decreased). The total N content remained constant.

3 Experiment 2

3.1 Goal

The goal of the second experiment (conducted November 2012) is to investigate the effectiveness of (a combination of) different additives to (biologically) acidify slurry.

3.2 Experimental setup

The second experiment is an incubation experiment in which different additives are used with the goal to acidify slurry. Similar to experiment 1, day fresh slurry was incubated at 10 and 25^{0}C during four weeks in 500 ml flasks. The slurry was made fresh at the start of the experiment by adding day fresh faeces to day fresh urine in the ratio 63% faeces and 37% urine.
The faeces and urine were collected from three different dairy farms on which the roughage part of the diet varied from only grass to a combination of grass and maize to only maize. In this second experiment the faeces and urine with roughage based on grass and a combination of grass and maize were collected from the same farms as in the first experiment. The faeces and urine with roughage based on only maize was collected from a different farm compared to the first experiment.
The experimental setup is shown in Table 3.1. In total the experiment consisted of 86 experimental units. Three times a week the pH was measured. The composition of the slurry was measured at the start and at the end of the experiment.
In an additional experiment manure from the pit was compared to fresh manure. This experiment was only conducted for slurry based on the combination grass/maize.

Table 3.1. Experimental setup of experiment 2.

Additives	Farms	Temp 10&25	Duplicate	Total
Reference – no additives	3	2	2	12
Acetic acid (HAc)	3	2	2	12
Sulphuric acid (H_2SO_4)	3	2	2	12
Molasses	3	2	2	12
Starch	3	2	2	12
HAc + molasses + Lb[1] + zeolite	3	2	2	12
HAc+starch+zeolite+Lb	3	2	2	12
Molasses+zeolite+Lb	1	1	2	2
Total				86

[1]Lb: Lactobacillus spp.

Table 3.2. Amount of additives (expressed per L slurry) used to acidify the slurry.

	Grass and Grass/maize	Maize
H_2SO_4 (2.5M ±13.3%)	44 ml	29 ml
HAc (40%)	37 ml	25 ml
Molasses (±50% sugar)	45 g	45 g
Starch	45 g	45 g
HAc + Molasses + Lb + zeolite	37 ml/ 45 g/ 5 g/ 2.5 g	25 ml/ 45 g/ 5 g/ 2.5 g
HAc + Starch + Lb + zeolite	37 ml/ 45 g/ 5 g/ 2.5 g	25 ml/ 45 g/ 5 g/ 2.5 g
Molasses + Lb + zeolite	45 g/ 5 g/ 2.5 g	45 g/ 5 g/ 2.5 g

Based on literature different additives were used. The additives and quantities are shown in Table 3.2. The amount of H_2SO_4 used to acidify slurry to a pH below pH 5.5 is calculated based on the N content of the slurry using the N/S ratio of 1.3 as proposed by to Sørensen et al. (2009) and confirmed by Bussink (2009). Based on the amount of the thus calculated amount of H_2SO_4 needed to acidify slurry to pH 5.5, the amount of HAc is calculated. The amount of added starch, molasses, and Lb is based on literature (e.g. Hendriks and Vrielink, 1997; Clemens et al., 2002; Clemens and Wulf, 2005) and on insights gained from recent experiments in Austria by Dr. Wenzl.

3.3 Composition slurry

The composition of the slurries with varying roughage diet are quite similar (Table 3.3). The large difference found in the first experiment for the maize slurry compared to the grass and grass/maize slurry is not found in this experiment. The maize slurry was collected from a different farm than for the first experiment.
For the grass and grass/maize slurry the amount of H_2SO_4 and HAc needed to acidify slurry to a pH below pH 5.5 was calculated based on an average N content in the first experiment of 4.7 mg N kg^{-1} slurry. This assumed N content was only slightly lower than the afterwards determined actual N content in the grass slurry (4.9 mg kg^{-1}). The difference between assumed and actual N content was larger for the grass/maize slurry (actual N content 5.3 mg kg^{-1}) and maize slurry. Based on the first experiment the N content of the maize slurry was estimated to be 3.1 mg kg^{-1}. This was quite a bit lower than the actual N content (4.3 mg kg^{-1}).

In Table 3.3. Composition of the day fresh slurry at the start of the experiments

Roughage	DM	C ash	OM	N-tot.	C/N	$N\text{-}NH_3$	N-org	P_2O_5	K_2O	MgO	Na_2O
Grass	95	16	79	4.9	7	2.2	2.7	1.2	5.7	1.2	0.6
Grass/maize	88	21	67	5.3	6	2.4	2.9	1.4	6.9	1.2	0.6
Maize	70	20	50	4.3	5	2.1	2.2	1.2	8.2	0.7	0.6
Pit grass/maize	109	24	85	4.95	8	2.3	2.7	1.65	6	1.5	0.7

3.4 Results incubation experiment 2

The effect of the different additives on the pH of the slurry during the incubation experiments is shown in Figure 3.1.
Only in the grass slurry the pH is kept below the target pH of 5.5 when H_2SO_4, HAc, or a combination of HAc and a C source are added to the slurry. For the grass/maize and maize slurry the pH at the start of the experiment is below the target value but increases rapidly to values slightly lower (at 10^0C) or comparable (25^0C) to the reference slurry with no additives. The reason is that too little acid was added. As mentioned before the calculated amount of added acid was based on assumed N contents that were lower than the actual N contents of the slurry. The actual N contents were not yet available at the start of the experiment. The results will now be discussed per type of additive.

3.4.1 Addition of acid

The addition of acid only has the desired effect of achieving and maintaining the pH below 5.5 when HAc is added to the grass slurry and kept at 10^0C. At 25^0C the pH slowly increases during the experiment when HAc is added (from pH 4.6 to 7.1 after 24 days). For the grass slurry clearly too much H_2SO_4 is added. The

pH drops to pH 2.5 and this pH is maintained during the 4 weeks of incubation at 10^0C. At 25^0C the pH starts to increase after 3 weeks. This decrease to low pH value is the result of H_2SO_4 being a strong acid with pK_a values of 2 and -3. For less strong acids, e.g. HAc with a pK_a of 4.75, the pH of the slurry will not

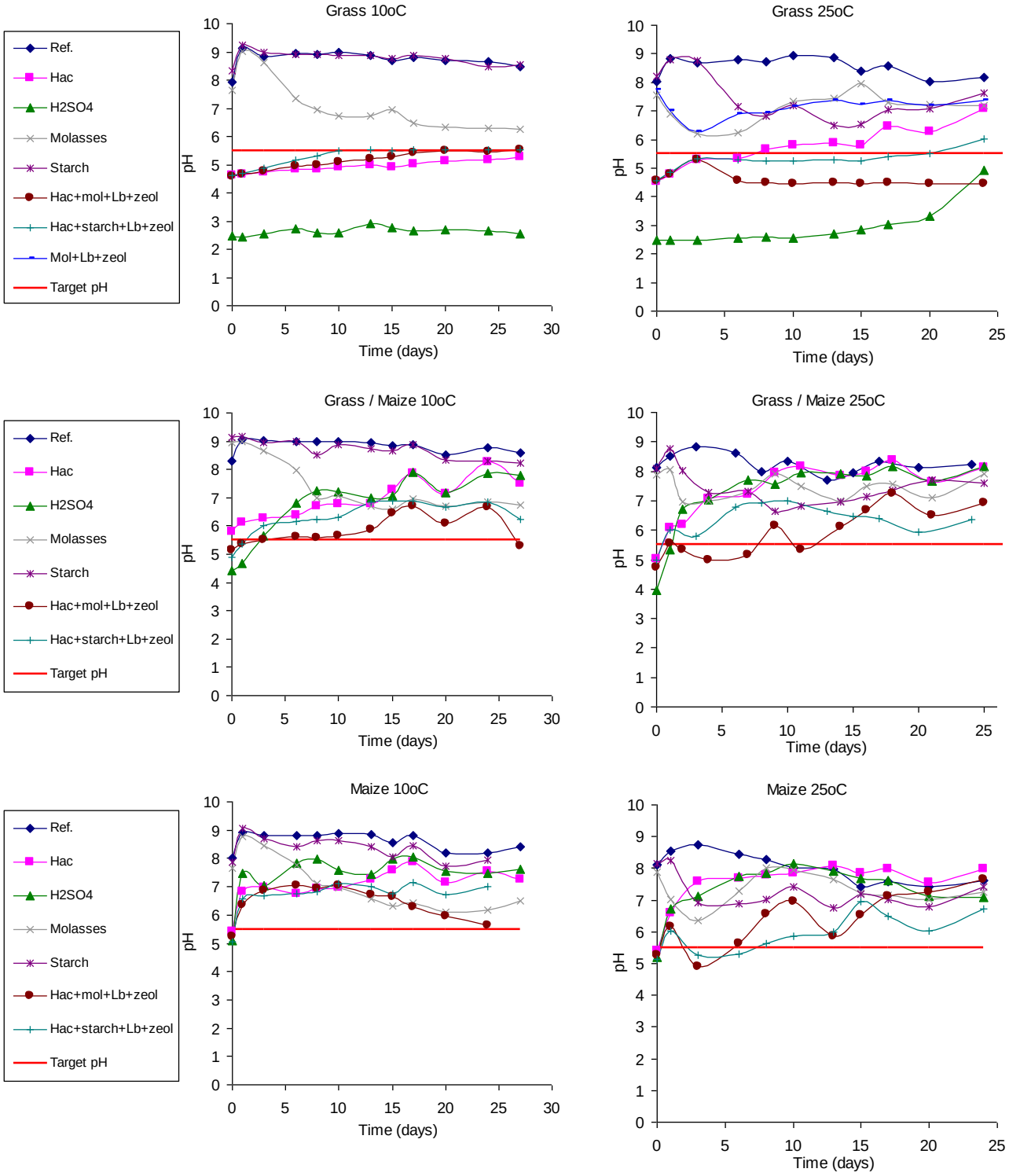


Figure 3.1 The effect of different additives on the pH of slurry during the incubation experiments.

drop to values below a pH of 4.74 when excess amounts are added.

For the grass/maize and maize slurry too little acid is added because the assumed N content was lower than the actual N content. This results in a rapid increase in pH within 1 to several days. It is clear that the system is very sensitive to the amount of acid that must be added. These experiments confirm earlier

studies (Sørensen et al., 2009, Bussink et al., 2012) that the amount of acid needed to maintain the pH below 5.5 seems to be related to N content.
The pH is kept at a lower level when the slurry is incubated at 10^0C compared to 25^0C. This implies that less acid is needed when the slurry is kept at low temperatures.

3.4.2 Addition of a C source

In the experiments two C sources are added: molasses and starch. Independent of slurry type 45 g of molasses or 45 g of starch is added.
At 10^0C starch does not affect the pH in any of the slurries whilst molasses gives a substantial decrease in pH in all the slurries. Within 8-10 days the pH decreases to values below 7. In the remaining time of the experiment the pH is maintained between pH 6 and 7.
At 25^0C starch also results in a pH decrease. The response to molasses and starch is similar. After approximately a week the pH decreases to approximately pH 7. During the remaining three weeks the pH shows larger fluctuations than at 10^0C (pH fluctuates between 6 and 8). The temperature effect for starch is opposite to the temperature effect for molasses. Starch has no effect on pH of the slurries at 10^0C but shows a significant decrease at 25^0C.
The polysaccharide starch is a macromolecule consisting of a large number of glucose units. Starch must be hydrolyzed to break down into sugars. These sugars are the C source for the acid producing bacteria. It appears that the hydrolization of starch does not occur in the different slurries at 10^0C. At 25^0C the hydrolization step does occur but causes a small delay (1-5 days) in the decrease in pH compared to molasses. When using macromolecules, like starch, as a C source a high (25^0C) temperature is thus needed to hydrolyze the molecules to form sugar. This is in line with the general rule that, until a maximum is reached, biologically aided reaction rates increase with temperature.
Contrary to starch, directly adding sugar in the form of molasses results in the formation of acid and a consequent decrease in slurry pH at both 10^0C and 25^0C but the decrease is larger and more constant at 10^0C. This may be due to a higher microbial activity at 25^0C compared to 10^0C of microbes that use molasses as a C source but form other, non-acidic, products (e.g. ethanol). Presumably the higher reaction rates at higher temperatures of bacteria other than those converting sugar into acid become dominant. Nevertheless, these experiments prove that biological acidification occurs in slurry, as sugars are transformed into acid resulting in a drop in pH.

3.4.3 Addition of acetic acid, a C source, Lactobacillus, and zeolite

The addition of a C source in combination with acetic acid results in an additional decrease in pH compared to only using acetic acid. This is the case for all slurries except for the grass slurry at 10^0C. In combination with acetic acid and at the added dose, molasses is more effective than starch in reducing the pH of the grass slurry at 25^0C and the maize slurry at 10^0C. At 25^0C the positive effect of the additional C source is clear within a few days. For the grass/maize slurry this is also the case at 10^0C. For the maize slurry the additional positive effect becomes apparent after roughly two weeks.
Overall it is clear that in general the addition of a C source in combination with acetic acid increases the potential to acidify slurry. To what extent and in what time frame this additional positive effect occurs varies with slurry type (grass, grass/maize and maize) and with temperature.

3.4.4 Addition of a C source and Lactobacillus and zeolite

In a single additional experiment with grass slurry at 25^0C, Molasses and Lactobacillus (Lb) and Zeolite were added. Compared to the addition of only molasses there was no additional effect of the Lb and Zeolite at the quantities added.

3.4.5 Effect of additives on the chemical composition of slurry

At the end of the incubation experiment the different additives have a significant effect on the NH_4-N content of the slurry. There seems a linear relationship between NH_4-N content and pH for the slurries incubated at 10^0C (r^2=0.85). At 25^0C the relationship is much less pronounced (r^2=0.41). In general the NH_4-N content is lower at a lower pH of the slurry. At lower pH the speciation of N in the slurry is more in the organic form. The references show that during the incubation experiment organic N is mineralized and mineral N is formed. This process is thus inhibited when slurry is acidified.

The NH_4-N content changes during the experiment (Figure 3.2). Similar to incubation experiment 1 (Figure 2.2), the NH_4-N content of the reference increases during the 4 weeks of the experiment. The lowest NH_4-N content is found in the grass slurry where H_2SO_4 is added. In this slurry the pH dropped almost instantaneously to 2.5. The reason for the lower NH_4-N content may be twofold: i) the inhibition of the hydrolysis of urea and ii) the lower mineralization of N-organic at lower pH.
At the start of the experiment the slurry was made fresh by adding urine and faeces and straight after the additives were added. Due to the almost instant drop in pH to 2.5 when H_2SO_4 is added to the grass slurry this may have inhibited the hydrolization of urea into ammonia. Fresh urine contains a lot of urea. This urea is hydrolyzed into ammonia carbonate after which it is converted into ammonia through the reaction:

$$NH_2CONH_2 + 2H_2O \rightarrow (NH_4)_2CO_3 \rightarrow 2NH_3 + CO_2 + H_2O \qquad \text{Equation 1}$$

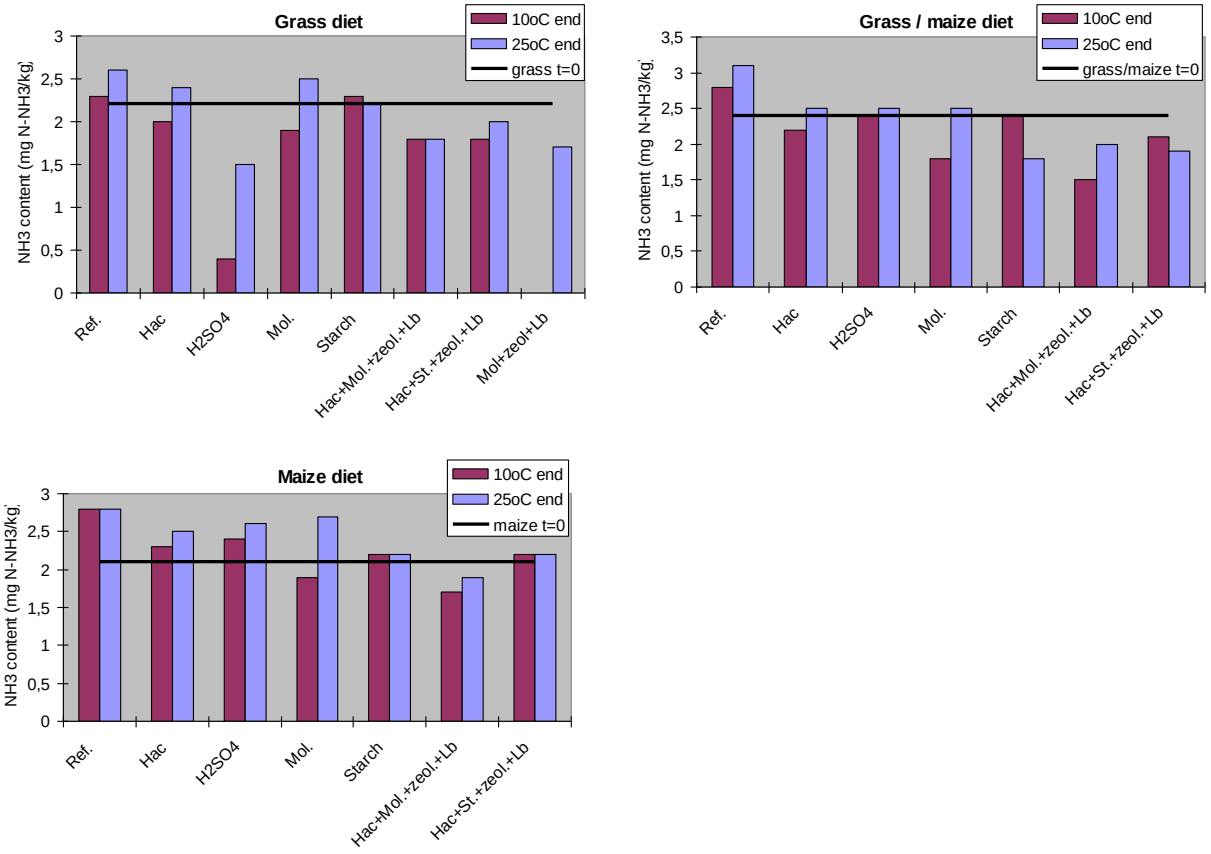


Figure 3.2. Change in composition of the slurries from the start (t=0) to the end of the experiment.

As soon as the pH rises urea is converted into NH_3. This is shown by the higher in NH_4-N content in the 25^0C slurry in which the pH increased at the end of the experiment. In the other treatments, the higher pH during the incubation experiment will have resulted in a much smaller inhibition of the conversion of urea into mineral N.
The most positive effect on the NH_4-N content (apart from adding H_2SO_4) is achieved when a combination of HAc, molasses, Lb and Zeolite are added. A positive effect is defined as a lower NH_4-N content compared to the reference slurry because a lower NH_4-N content results in lower emission potential. This effect is in all cases larger than when only HAc is added. Independent if the target pH of 5.5 is achieved, the addition of HAc has a small positive effect on the NH_4-N content.
For all additions mineralization is higher (higher NH_4-N content) at higher temperature. The only exception is in case of the addition of starch. Similar to the effect on pH, the NH_4-N content is equal or lower at higher temperature.

3.5 Conclusions experiment 2

- The system is very sensitive to the amount of acid that must be added to achieve and maintain the target pH of 5.5;
- These experiments confirm that the amount of acid needed to maintain the pH below 5.5 seems to be related to the N content;
- Less acid is needed when slurry is kept at low temperatures;
- Molasses is the most effective C source in terms of decreasing the slurry pH to pH 6 -7 and maintaining this low pH when the slurry is incubated at 10^0C; Starch is not effective at this temperature. At 25^0C both starch and molasses are effective;
- In general the addition of a C source in combination with acetic acid increases the potential to acidify slurry. To what extent and in what time frame this additional pH decrease occurs varies with slurry type (grass, grass/maize and maize) and with temperature;
- Adding Lactobacillus and Zeolite in combination with a C source does not seem to be effective at the investigated dose;
- At 10^0C the NH_4-N content of slurry seems linearly related to pH;
- For the acidification of slurry a lower temperature is better: pH and NH_4-N content of the slurry remain generally lower and less acid is needed.

4 Experiment 3

4.1 Goal

The goal of the third experiment (conducted November and December 2012) is to investigate the effectiveness of (a combination of) different additives to (biologically) acidify slurry in a fed-batch system. In this experiment different dosages are used.

4.2 Experimental setup

The third experiment was a fed-batch system. This means that the experiment started with 2L slurry and that 3 times a week (every 2-3 days) 200 ml of fresh slurry was added. Every time slurry was added, the slurry was made fresh by adding faeces and urine in the ratio 63% faeces and 37% urine. The faeces and urine were collected fresh from behind the cow once every week and stored in a fridge. Two types of slurry were used in which the roughage part of the diet consists of either maize or grass. For the third experiment the grass farm was the same as for the second experiment and the maize farm was different. On the grass farm the cows were no longer outside during the day but were permanently inside and the roughage part of the diet was grass silage from the first cut, harvested the 28th of May 2012. In an extra treatment grass slurry from the top of the pit to which HAc and molasses were added.
At the start of the experiment 2L of freshly made slurry was put into 5L buckets and additives were added according to scheme presented in Table 4.1. For the experimental units to which acid was added at the start of the experiment, the pH of the slurry was maintained around or below the target pH of 5.5 by adding extra acid and / or molasses. Extra acid and molasses were added in the ratio of the initial dosages once a week to decrease the pH below the target pH of 5.5.
The experiment was conducted at two temperatures: 10^{0}C and 25^{0}C. In total the experiment counted 62 experimental units (2 types of fresh slurries, 2 temperatures, 15 treatments, and an additional treatment with pit slurry from the grass farm at 2 temperatures).

Table 4.1. Amount of additives (expressed per L slurry) used to acidify the slurry in experiment 3 at the start of the experiment.

Slurry	H_2SO_4 (2.5M)	HAc (40%)	Molasses	Lb	Zeolite
Fresh		-			
Fresh		35 ml			
Fresh		70 ml			
Fresh		35 ml	12.5 g		
Fresh		35 ml	25 g		
Fresh		35 ml	50 g		
Fresh				2.5 g	5 g
Fresh				10 g	25 g
Fresh			12.5 g		
Fresh			25 g		
Fresh			50 g		
Fresh			12.5 g	2.5 g	5 g
Fresh			25 g	2.5 g	5 g
Fresh			25 g	10 g	25 g
Fresh	35 ml				
Pit		35 ml	25 g		

4.3 Results fed-batch experiment 3

4.3.1 Initial slurry composition

For the third experiment urine and faeces from the grass diet was collected at the same farm as in experiment 2. The N content of this grass diet based slurry had however changed considerably compared to the slurry used in the second experiment. The reason is that the cows were grazing during a part of the day when the slurry was collected for the second experiment . At the time when the urine and faeces for the third experiment was collected the cows were kept indoors and given silage from spring. This resulted in a decrease in N content of the slurry from 4.9 mg N kg^{-1} in the second experiment to 2.6 mg N kg^{-1} in the third. Part of this decrease results from a 25% decrease in DM content of the slurry. During the course of the third experiment the farmer changed the diet due to which the total N content increased slightly but the NH_3 content remained low (Table 4.2).

Urine and faeces form a maize based diet came from another farm as in experiment 2. The N content was 6.3 mg kg^{-1} of the maize based slurry at the start of the third experiment against 4.3 mg kg^{-1} in the second experiment. DM content was 108 against 70 in the second experiment. During the experiment the decrease in DM content and perhaps adjustments in the diet brought the high total N content down.

Table 4.2. Composition of the slurry at the start of the experiment.

Slurry	DM	C Ash	OM	N-tot.	C/N	N-NH_3	N-org	P_2O_5	K_2O	MgO	Na_2O
Grass 26-11	72	18	54	2.6	9	0.9	1.7	1.1	7.2	0.8	0.6
Grass 3-12	73	18	55	2.9	9	1.1	1.8	1.2	8.1	0.8	0.6
Grass 10-12	74	20	54	3.1	8	1.4	1.7	1.1	8.0	0.8	0.6
Gras 17-12	74	18	56	3.0	8	0.8	2.2	1.3	7.6	1	0.6
Grass pit 26-11	86	17	69	2.6	12	0.8	1.8	1.3	5.1	0.8	0.5
Grass pit 10-12	85	16	69	2.9	11	1.1	1.8	1.4	5.5	0.8	0.6
Grass pit 17-12	80	13	67	2.5	12	0.7	1.8	1.4	3.4	0.8	0.6
maize 19-11	108	19	89	6.3	6	2.4	3.9	1.3	6.9	1.2	0.8
maize 26-11	99	18	81	5.9	6	2.8	3.1	1.3	4.8	1.3	1.6
Maize 5-12	105	19	86	5.7	7	3.1	2.6	1.3	4.9	1.3	1.3
Maize 12-12	92	18	74	4.7	7	2.3	2.4	1.1	4.2	1.5	1.9
Standard slurry	85		64	4.1		2.0	2.1	1.5	5.8	1.2	0.7

Table 4.3 Overview of the initial and total amount of acid and molasses needed to keep the pH at or below the target pH of 5.5 during the fed-batch incubation experiments.

		Added acid (ml)			Added molasses (g)		
Slurry	Treatment	Initial	total 10^0C	total 25^0C	Initial	total 10^0C	total 25^0C
Grass fresh	HAc 140	140	140	140			
	HAc 70	70	105	155			
	HAc 70 + Mol. 25	70	70	140	25	25	50
	HAc 70 + Mol. 50	70	70	105	50	50	75
	HAc 70 + Mol. 100	70	70	70	100	100	100
Grass pit	HAc 70 + Mol. 50	70	70	70	50	50	50
Grass fresh	H_2SO_4	93	140	165			
Maize fresh	HAc 140	140	140	193			
	HAc 70	70	140	198			
	HAc 70 + Mol. 25	70	140	175	25	50	63
	HAc 70 + Mol. 50	70	105	105	50	75	75
	HAc 70 + Mol. 100	70	105	140	100	150	200
	H_2SO_4	93	150	209			

4.3.2 Addition of acid

The initial amount of added acid was calculated based on an assumed N content of 4.4 mg kg^{-1}. Based on the actual N content (Table 4.2) this would mean that too much acid was added to the grass slurry and too little to the maize slurry.

In the experiment a single dose of H_2SO_4 and two dosages (single and double) of HAc were added based on a N content of 4.4 mg kg^{-1} (Figure 4.1). At the double HAc dose (140 ml / 2L) the pH was kept at or below the target value during the entire fed-batch experiment for the grass slurry. For the maize slurry the pH rose slightly above the target pH at 10^0C. At 25^0C the pH of the maize slurry had to be corrected twice (after 2 and 3 weeks) to keep the pH below 5.5. For both the H_2SO_4 and HAc single dose, the pH had to be adjusted several times during the experiments. Table 4.3 gives an overview of the total amount of acid and molasses needed to keep the pH at or below the target pH.

In accordance with the difference in N content more acid was needed to acidify the maize slurry than for the grass slurry. Also in accordance with the conclusions from the second experiment more acid was needed to maintain the target pH of below 5.5 at 25^0C than at 10^0C.

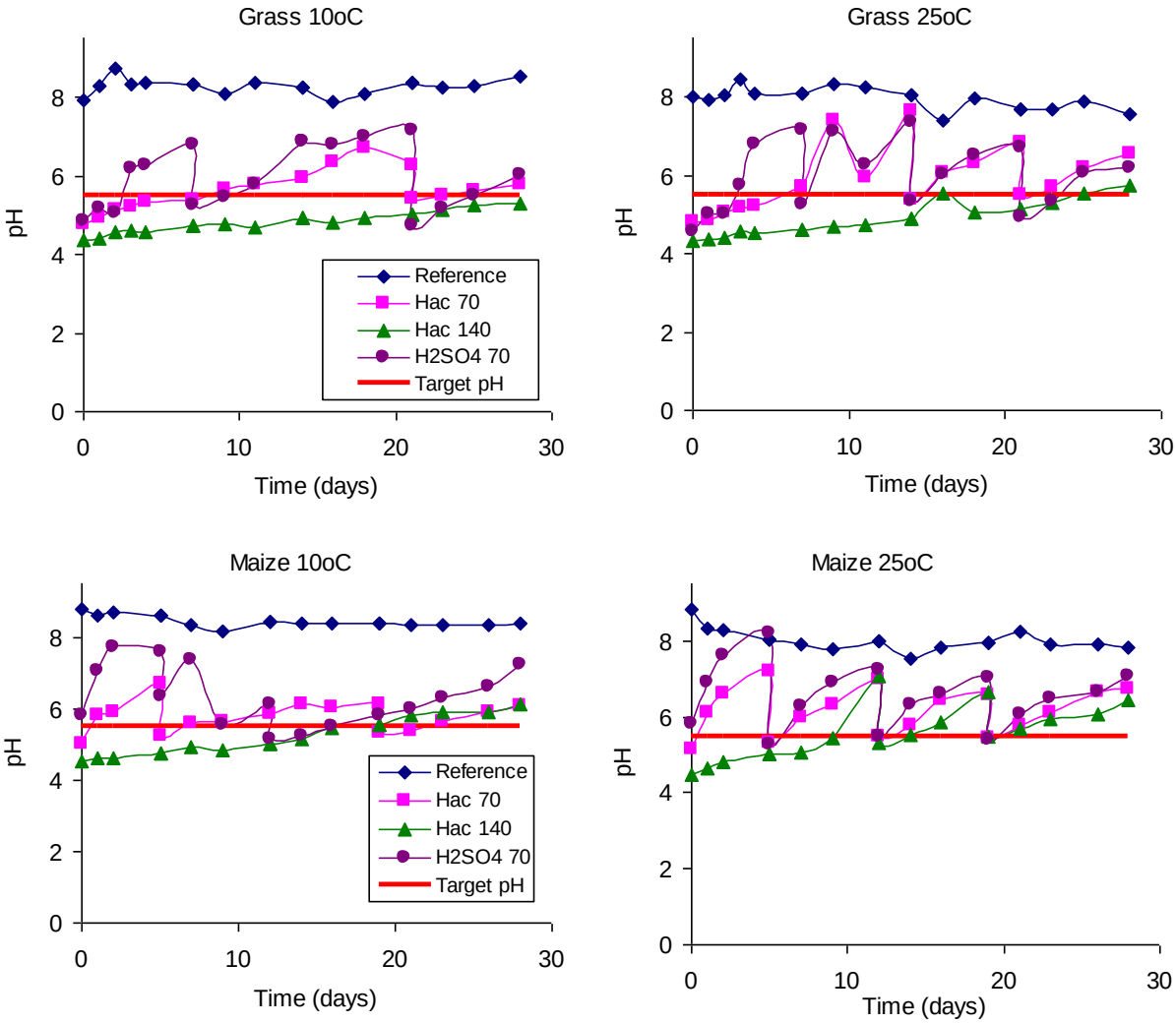


Figure 4.1. Change in pH during the fed-batch experiment after the initial day fresh slurry is acidified using either 93ml H_2SO_4 or 70 or 140 ml HAc. Every 2-3 days 200ml fresh slurry is added. Once a week the pH is adjusted to a pH below the target pH of 5.5 by adding more acid.

4.3.3 Addition of a C source

Similar to the second experiment, the addition of molasses results in a larger decrease in pH at 10^{0}C compared to 25^{0}C (Figure 4.2). At 25^{0}C the different doses of molasses initially result in a small decrease in pH but this effect decreases over time. At 10^{0}C the decrease in pH increases with dose, as expected. The dose is however not high enough to reach the target pH of 5.5. At 10^{0}C the extra input of fresh slurry every 2-3 days does on average not lead to a pH increase. In other words the pH is stays relatively constant despite the addition of fresh slurry every two days. The level at which the pH is maintained depends on the initial dose of molasses.

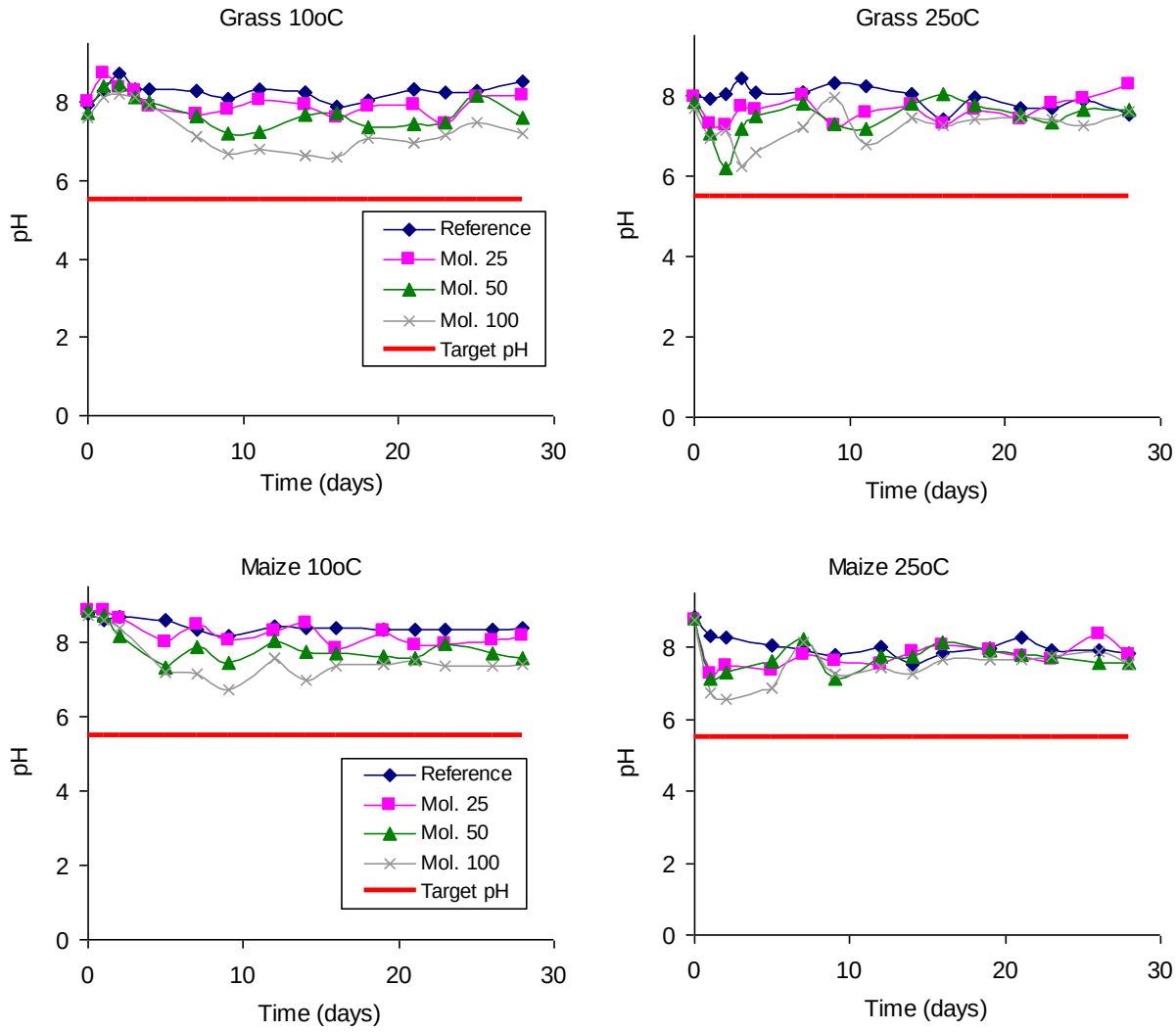


Figure 4.2. Change in pH during the fed-batch experiment after the initial day fresh slurry is acidified using either 25, 50, or 100 ml molasses. Every 2-3 days 200ml fresh slurry is added.

4.3.4 Addition of acetic acid and a C source

The first three weeks the addition of a C source in addition to HAc does not result in an additional decrease or stabilization of the pH at 10^{0}C. After 3 weeks the pH is better maintained at the target pH with the additional C source. At 25^{0}C the extra addition of a C source does seem to affect the pH, the pH however fluctuates largely. For the grass slurry the pH is maintained at a lower level during the experiment with molasses compared to without molasses. The dose must however be at least 25-50 ml L^{-1}. For the maize

slurry the variations are too large to draw a conclusion.
The addition of molasses does result in a decrease in the amount of acid needed to maintain the target pH during the fed-batch experiment (Table 4.3). A C source thus does contribute to the acidification of the slurry.

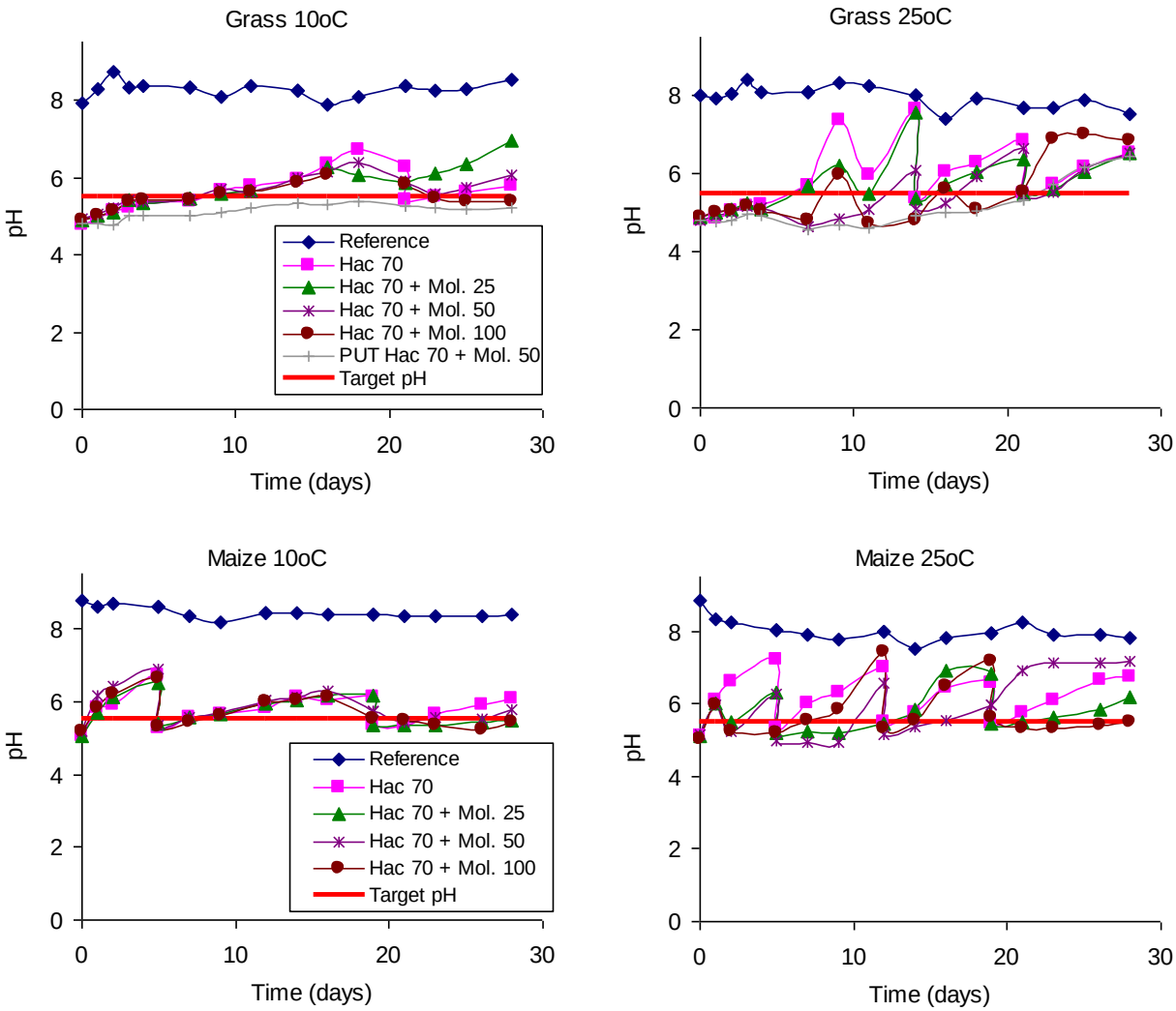


Figure 4.3. Change in pH during the fed-batch experiment after the initial day fresh slurry is acidified using either 70 ml HAc or a combination of 70 ml HAc and 25, 50, or 100 gr molasses. Every 2-3 days 200ml fresh slurry is added. Once a week the pH is adjusted to a pH below the target pH of 5.5 by adding more acid and molasses in a ratio equal to the initial addition.

4.3.5 Addition of a C source and Lactobacillus and Zeolite

At the investigated dose, Lactobacillus and Zeolite are not affective in decreasing the pH. In this experiment Lb and Zeolite are however not added to acidified slurry. At the high pH of the non-acidified slurry, Lb and Zeolite have no effect. Also not when they are added in combination with molasses.

4.4 Costs of additives

In this paragraph only the costs of the different additives are considered based on the results of the experiments described in this chapter. The costs of the total system will be presented and discussed in chapter 6.

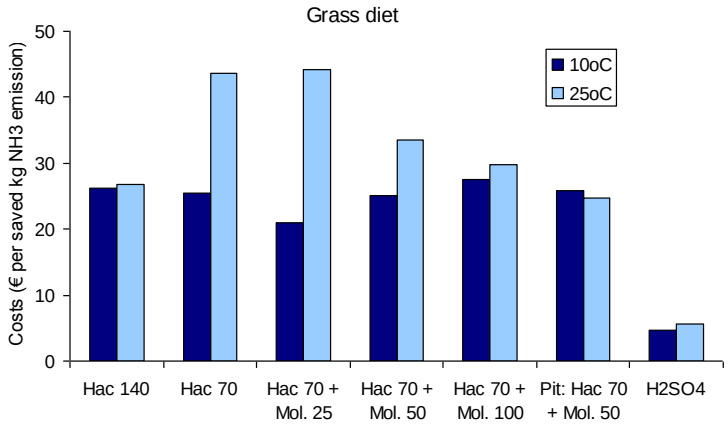
The costs of the additives acetic acid and molasses in a fed-batch experiment are much higher (5 – 10 times) than when sulfuric acid is used (Table 4.3). This is due to the low price for H_2SO_4 (±100 € ton^{-1} concentrated acid) and the relatively high price for acetic acid (±500 € ton^{-1} concentrated acid). The price for molasses is € 136,- /ton.

Based on the NH_3 content of the grass and maize slurries at the start of the experiment (Table 4.2) and the pH during the experiment the potential NH_3 emission is calculated based on the empirical relationships between pH and NH_3 emission reductions presented by Bussink et al. (1994). This includes both the emission from the stable and the emission when the slurry is applied to the soil (average clay and sand soil and the corresponding emission low technique with which slurry is applied to the field). This is only done for the treatments with acid because in the other treatments the pH was too high during the experiment to substantially reduce NH_3 emissions.

In Figure 4.4 the costs of additives (expressed in €/ kg saved NH_3 emission) are shown. It is again clear that the costs for acidifying slurry using acetic acid and molasses are much higher than when H_2SO_4 is used. At 10^0C, the price ranges between 21 – 27 € / kg saved NH_3 emission for the grass diet and between 12 – 20 € / kg saved NH_3 emission for the maize diet. This is much higher than the price of acidifying slurry with H_2SO_4 (3 – 5 / kg saved NH_3 emission.

The costs are lower at low temperatures (10^0C) because less additives are needed to acidify the slurry. In addition, the slurry pH is maintained at a lower level and is more stable at 10^0C compared to 25^0C.

The costs expressed per kg saved NH_3 emission are higher for the grass diet than for the maize diet. The higher costs for acidifying the maize slurry (Table 4.2) are thus outweighed by the gain in saved NH_3 emission. This implies that it is economically better to acidify slurry with a high N content.



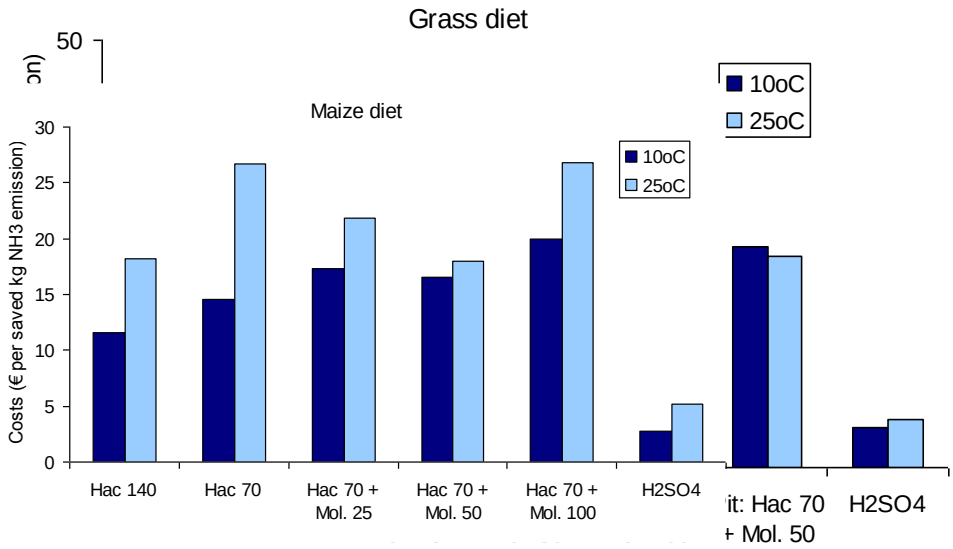


Figure 4.4 Costs of acidifying slurry using different treatments.

4.5 Conclusions experiment 3

- In a fed-batch system the pH of the slurry must frequently be corrected by adding acid / C source to maintain the target pH of 5.5;
- With the addition of only molasses the pH is maintained relatively constant despite the addition of fresh slurry every two days. The level at which the pH is maintained depends on the initial dose of molasses. At the investigated dosage the target pH of 5.5 was not reached when only molasses was added.
- Independent of dose, molasses is hardly effective at 25^0C. Molasses is effective at 10^0C.
- At the investigated dose, Lactobacillus and Zeolite are not effective in decreasing the pH. The effectiveness is not investigated in acidified slurry.
- At 10^0C, the price of additives to maintain the fresh slurry at or below the target ranges between 21 – 27 € / kg saved NH_3 emission for the grass diet and between 12 – 20 € / kg saved NH_3 emission for the maize diet.
- The costs of additives are lower at low temperatures (10^0C) because less additives are needed to acidify the slurry. In addition, the slurry pH is maintained at a lower level and is more stable at 10^0C compared to 25^0C.
- It is possible that a higher dose of an easily fermentable sugar will result in achieving the target pH at low temperatures.
- The amount of acid needed to acidify the slurry depends on the N content of the slurry. The higher the N content the more acid is needed. However, the NH_3 emission reduction is also larger at higher N-content. From a cost efficiency perspective a higher N content is favorable.
- When molasses is added in combination with an acid the total amount of acid needed to maintain the target pH is reduced. With the same amount of acid a lower pH is maintained. An optimum acid and C dose needs further investigation.

5 Experiment 4

5.1 Goal

The goal of the fourth experiment (conducted January to April 2013) is to get a more detailed insight in the amounts of additives needed to achieve and maintain the pH of fresh slurry below the target pH of 5.5 in a fed-batch system.

5.2 Experimental setup

The fourth experiment is a fed-batch system. Similar to the third experiment the experiment started with 2L slurry and 3 times a week (every 2-3 days) 200 ml fresh slurry was added. Just before fresh additions in the fed-batch system, the slurry was made fresh by adding faeces and urine in the ratio 63% faeces and 37% urine. The faeces and urine were collected fresh from behind the cow once every week and stored in a fridge. Two types of slurry were used in which the roughage part of the diet consisted of either maize or grass. The faeces and urine were collected from the same dairy farms as used in the third experiment.
At the start of the experiment 2L of freshly made slurry was put into 5L buckets and additives were added according to the scheme presented in Table 5.1. The C source was a syrup with a sugar content of 65%. Similar to molasses this source of sugar is readily available for Dutch farmers. It was used in these experiments because it is easier to work with as the viscosity is lower of molasses. Additionally the sugar content is higher (65%) than of molasses (50%). Lactic acid (LA) was added as an acid to these experiments as it was suggested that this might be a more favorable environment for lacto bacillus bacteria than other acids (pers. Communication with Dr. Smit van NIZO food research BV).
The experiment was only conducted at 10^0C. Due to failure of one of the climate cells the experimental units with maize slurry were not kept at a constant 10^0C. For these units the average temperature was around 15^0C.
The experiment consisted of two parts: 4A and 4B.
In experiment 4A the total experimental units counted 44 (2 types of slurries, 1 temperature, 22 treatments Table 5.1). For the treatments to which an acid or a combination of acid and syrup was added the target pH was maintained by adding acid during experiment 4A whenever the pH rose above pH 5.7. Experiment 4A continued for 28 days.
In experiment 4B, a part of experiment 4A was continued (28 – 63 days) in order to fine tune how much acid or C source is needed to maintain the target pH in a fed-batch system. In experiment 4B per slurry type 9 treatments of experiment 4A were split into two (2x2L) resulting in 36 units in experiment 4B.
Seven treatments to which initially acid or a combination of acid and syrup was added were split. In the first unit the pH was maintained by adding acid, in the second unit the pH was maintained by adding syrup. To all these second units 50 ml syrup was added after 28 days. For the two treatments continuing in experiment 4B to which initially only syrup was added, the experimental units were also split; to one part 200 ml fresh slurry was added 3 times a week and to the other this was increased to 300 ml. In addition, the two reference units and in total 6 other units (2 grass and 4 maize) were continued after the 28 days of experiment 4A. An additional experiment was added to experiment 4B in which H_2SO_4 and H_2SO_4 + C source were added to both slurry types. In total experiment 4B counted 48 experimental units.

Three times a week the pH and redox were measured in each experimental unit. If necessary extra additives were added to adjust the pH and the pH was measured again. After 28 days the biogas potential of 12 samples was determined in an incubation experiment at Biogas-Labo laboratory in Gent (Be).
After 60 days the organic acid composition was measured of 20 samples at the NIOO laboratory.

Table 5.1. Amount of additives (expressed per L slurry) initially added to acidify the slurry in experiment 4A. HAc is acetic acid (7M), LA is lactic acid (3.9M), C is carbon in the form of syrup (65% sugar), Lb is Lacto Bacillus, and zeol. is Zeolite.

	Grass diet					Maize diet				
	HAc	LA	C	Lb	Zeol	HAc	LA	C	Lb	Zeol
Reference										
HAc	50					75				
LA		64					64			
LA		91					91			
C			100					150		
C			200					300		
C + Lb			100	10				150	10	
C + Lb			200	2.5				300	2.5	
HAc + C	35		40			50		60		
HAc + C	50		20			75		30		
LA + C		64	40				64	60		
LA + C		91	20				91	15		
HAc + C + Lb	35		40	2.5		50		60	2.5	
HAc + C + Lb	35		40	10		50		60	10	
LA + C + Lb		64	40	2.5			64	60	2.5	
LA + C + Lb		64	40	10			64	60	10	
LA + C + Lb		91	20	10			91	30	10	
HAc + Lb	35			10		50			10	
HAc + Lb	50			2.5		75			2.5	
LA + Lb		64		10			64		10	
LA + Lb		91		2.5			91		2.5	
LA + C + Lb + zeol.		64	40	10	50		64	60	10	50

5.3 Results fed-batch experiment 4

The results of fed-batch experiment 4 will be discussed per type of additive.

5.3.1 Addition of acid

The addition of solely acid results in an immediate drop in pH. This is accompanied by strong foam formation. The foam subsides within a few hours. After the initial pH drop it is necessary to keep on adding extra acid to maintain the target pH of 5.5 in the fed-batch system. This is shown for the grass slurry in Figure 5.1. For the maize slurry the results are similar. Figure 5.1 also shows that over time a steady state is achieved between the amount of fresh slurry added and the amount of acid needed to maintain the target pH. This is independent of the amount of acid added at the start of the experiment. For example, at the end of the experiment (63 days and 6.8 L slurry) the total amount of added LA was 357 ml 3.9M LA when 127 ml was initially added and 346 ml when initially 181 ml was added.

In grass slurry the steady state fluctuates for concentrated (17.4M) HAc around 14 +/- 1 L m^{-3}, for concentrated LA (11.5M) around 18 +/- 1 L m^{-3}, and for concentrated (18M) H_2SO_4 around 5.1 +/- 0.4 L m^{-3} (Table 5.2). When expressed in added protons (H^+) per m^3 slurry most H^+ is added using HAc, and least using H_2SO_4. This reflects the strength of the acids, i.e. how readily they protonate. Expressed by the pK_a value of the acid, HAc is the weakest acid with a of pK_a=4.75, followed by LA with a pK_a of 3.85. H_2SO_4 is a strong acid with pK_{a1}=1.92 and pK_{a2} = -3.9. The effectiveness of the added acids is highest for H_2SO_4. When we assume the effectiveness of H_2SO_4 as being 1, the effectiveness of HAc is 0.73 and of LA 0.86.

The effectiveness of HAc and LA are less than can be expected from the dissociation constant. This is possibly caused by the low temperature (10^0C) or because some of the added acid is consumed by microorganisms.

The higher acid dose for maize slurry compared to grass slurry is in accordance with the higher N content of the maize slurry. Additionally, the temperature was higher (±15^0C instead of 10^0C) in the maize slurry treatments than in the grass slurry treatments. Previous experiments (exp. 2 and 3) show that more acid is needed at higher temperature. Sørensen et al. (2009) propose that the amount of H_2SO_4 that is needed to acidify slurry to a pH of 5.5 is based on a fixed N/S ratio of 1.3. For the N content of the grass (3.2 mg kg^{-1}) and maize slurry (5.7mg kg^{-1}) this would correspond with respectively 4.3 and 7.8 L H_2SO_4 m^{-3} slurry. For the grass slurry the amount needed is slightly higher (5 L m^{-3}) and for the maize slurry slightly less H_2SO_4 is needed (6.7 L m^{-3}) than expected based on this ratio. Overall however the amount of H_2SO_4 roughly corresponds with previous findings.

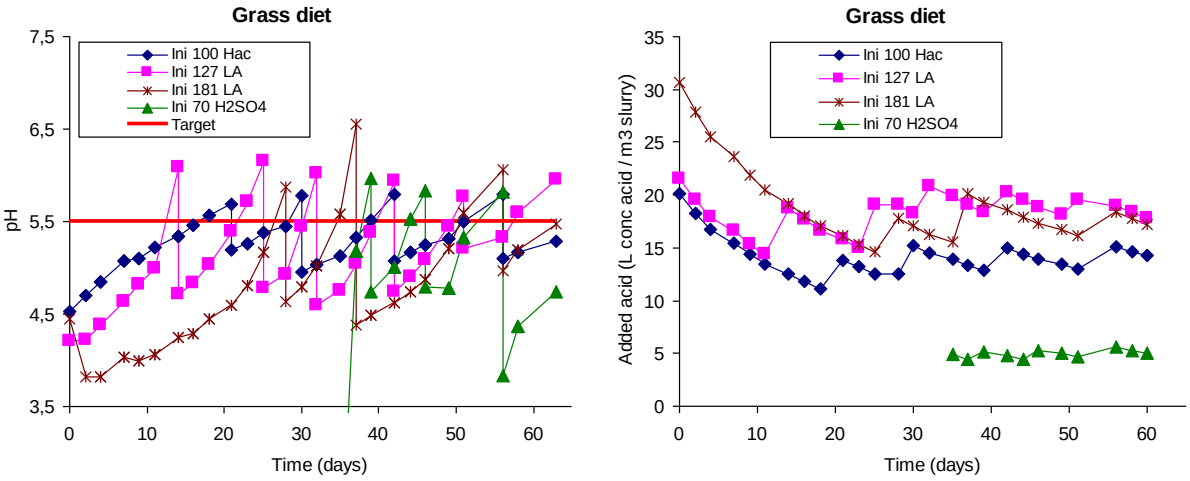


Figure 5.1 Change in pH (left figure) and in added amount of acid (right figure, expressed in L concentrated acid / m^3 slurry) during fed-batch experiment 4. The initial day fresh slurry was acidified using either 100 ml HAc, 127 or 181 ml LA, or 70 ml H_2SO_4. Every 2-3 days 200ml fresh slurry is added and if necessary the pH was adjusted by adding more acid.

Table 5.2 Overview of amount of acid (7M HAc, 3.9M LA, and 2.5M H_2SO_4) added at the start of the experiment to 2L slurry and the total amount added to 6.8L slurry except the H_2SO_4 treatment which is added to a total of 4.8L. The level at which steady state is achieved between the addition of acid and slurry is expressed as L concentrated acid per m^3 slurry (concentrated acid is 17.4M HAC, 11.5M LA, and 18M H_2SO_4).

		Added acid (ml)		Steady state L conc acid/m^3		mol acid/m^3
Slurry	Addition	Ini	Total	Avg	SD	Avg
Grass	Ini 100 HAc	100	241	14	0.9	244
Grass	Ini 127 LA	127	357	19	0.9	215
Grass	Ini 181 LA	181	346	18	1.2	199
Grass	Ini 70 H_2SO_4	70	145	5.0	0.4	80 (179 mol H^+)
Maize	Ini 150 HAc	150	329	20	0.9	347
Maize	Ini 127 LA	127	482	25	1.2	281
Maize	Ini 181 LA	181	497	25	1.1	286
Maize	Ini 70 H_2SO_4	70	184	6.7	0.9	122 (243 mol H^+)

The organic acid content and composition was measured in 20 representative slurries at the end of experiment 4B (Table 5.3). In the treatments in which only HAc is added to maintain the pH, the total organic acid content is ±16 mg/kg of which the greater part is HAc and only a small part LA. In the treatments in which only LA is added, the total organic acid content is ±27 mg/kg of which the greater part is LA and only a very small part HAc. The low value for the H_2SO_4 treatment is because only organic acids are measured and not H_2SO_4. In accordance with the higher dosages, the organic acid content is higher in the maize slurry than in the grass slurry.

The HAc concentration measured in the slurry (Table 5.3) corresponds well with the steady state HAc concentration deduced from the fed-batch experiments (Table 5.2). For the grass slurry a steady state is achieved when 244 mol HAc per m^3 slurry is added. The measured concentration is 259 mol HAc per m^3 slurry. For the maize slurry a steady state is achieved at 347 mol HAc per m^3 slurry and the measured concentration is 381 mol per m^3. This confirms that the system reaches a steady state between the input of HAc and fresh slurry.

The LA concentration measured in the grass slurry (267/298 mol/m^3, Table 5.3) is higher than the steady state LA concentration deduced from the fed-batch experiments (215/199 mol/m^3, Table 5.2). The measured concentration is thus 22-44% higher than the concentration expected from the steady state between adding LA and slurry. A possible explanation may be that some of the readily degradable C in slurry is converted into LA. However, the amount of converted C is small and does not lead to a self-sustaining system.

When sugar is added to slurry acidified with LA, the LA concentration does not change. This confirms that the system reaches a steady state between input of acid and slurry. However, the steady state concentration is slightly higher than expected from the added LA.

Table 5.3 Concentration (mg/g) of total organic acids and LA, HAc, propionic acid, and ethanol in several representative slurries at the end of experiment 4B (63 days). Formic acid and butyric acid were not present in detectable amounts. LA and HAc are also expressed as mol acid per m^3 slurry.

		Organic acids (mg/g)					mol acid/m3	
Slurry type	Additive	Total	LA	HAc	prop. acid	ethanol	LA	HAc
Grass	Reference	2	n.a.	1	0.3	0.2	n.a.	23
Grass	Ini 100 Hac	16	1	16	0.2	0.3	8	259
Grass	Ini 100 Hac; 4B + C	22	12	10	0.3	0.8	136	161
Grass	Ini 100 Hac + 40 C; 4B + C	22	17	4	0.2	1.1	190	75
Grass	Ini 70 Hac + 80 C	17	5	12	n.a.	0.3	55	206
Grass	Ini 70 Hac + 80 C; 4B + C	22	17	5	n.a.	1.2	186	88
Grass	Ini 127 LA	25	24	1	0.2	0.4	267	18
Grass	Ini 127 LA + 80 C; 4B + C	29	26	2	0.2	1.1	293	36
Grass	Ini 181 LA	28	27	1	0.2	0.3	298	22
Grass	Ini 181 LA + 40 C; 4B + C	26	24	2	0.3	1.0	264	30
Grass	Ini 70 H2SO4	2	1	2	0.2	0.3	6	27
Grass	Ini 70 H2SO4 + 80 C	17	13	3	0.2	1.0	150	49
Maize	Reference	7	n.a.	6	1.1	1.5	n.a.	104
Maize	Ini 150 Hac	26	3	23	0.6	1.0	28	381
Maize	Ini 150 Hac; 4B + 50C	38	25	12	0.7	1.6	276	207
Maize	Ini 127 LA + 120 C; 4B + 50C	43	39	4	0.9	1.4	428	59

5.3.2 Addition of a C source

The treatments to which only syrup (sugar content of 65%) is added show the potential of the bacteria in fresh slurry to convert sugar into acid (Figure 5.2). This conversion takes time during which the slurry foams. During the decrease in pH the slurry separates in a liquid and thick phase. Before each pH was measured the slurry was mixed. After the target pH was achieved the slurry no longer separated into two phases but remained stable.

The difference between the grass and maize based slurry is striking. In the grass slurry the conversion is slower (±3 weeks) and the equilibrium pH is higher (±pH 5.5) compared to the maize slurry (respectively ±2 weeks and equilibrium pH ±4.7). The rate of conversion in both slurry types is not limited by the amount of syrup present; a double dose does not increase the conversion rate. It is thus the (microbial) composition of the slurry that determines the rate of sugar conversion. Temperature may also play a role. In the maize slurry the higher conversion rate may (partly) be the result of the higher temperature (±150C) compared to the grass slurry (100C). It is probably also the microbial community that determines at what pH the system reaches a steady state. The microbial community in slurry is known to depend on the cows diet (Van Vliet et al., 2007). Other management related factors unique to each farm, may also affect the microbial community in the slurry.

The organic acid measurements give some more insight and show some interesting differences (Table 5.4). The total acid content is slightly higher in the maize compared to the grass slurry and in both slurries mainly consists of LA. The higher total organic acid content in the maize slurry is mainly due to a higher acetic acid content. Also the propionic acid content is slightly higher in the maize slurry. This indicates that in the maize slurry the microbial community responsible for the conversion of sugar into acid is more active and more diverse than in the grass slurry.

Although these results clearly show that slurry can biologically acidify, the system cannot be maintained at or below the target pH for an indefinite time. Fresh sugars must be added to maintain the target pH in de fed-batch system. Fresh slurry also contains C. However, this C source does not seem to be readily available in the sense that it is converted into acids. The fed-batch system can thus not sustain itself. Not even when the low pH is achieved and the microbial population is altered to actively transform sugars into acid. For grass the steady state between input of fresh syrup and slurry is achieved at an average of 44 +/- 3 L syrup / m^3 slurry. For the maize slurry this is 63 +/- 4 L/m^3.

In principle 1 sugar molecule can be converted into 2 lactic acid molecules through homolactic fermentation:

$$C_6H_{12}O_6 \text{ (sugar)} \rightarrow 2\ C_3H_6O_3 \text{ (lactic acid)} \qquad \textit{Equation 2}$$

With 100% conversion adding 1 kg of syrup equals 650 g sugar which equals a potential LA formation of 7.2 mol/L. For the steady state situation this means that for the grass and maize slurry respectively 317 and 454 mol LA per m^3 slurry is potentially added. For the grass slurry this amount corresponds very well with the measured LA concentration (333 and 233 mol/m^3, Table 5.4). This implies that all the added sugar is converted through homolactic fermentation into LA. Another explanation may be that the homolactic conversion is less than 100% but that some of the easily degradable C from the slurry is also converted into LA.

For the maize slurry the measured LA concentration is slightly lower (300 and 377 mol/m^3) than expected from the steady state addition of LA (454 mol/m^3). The reason is that apart from LA also HAc and some propionic acid is formed.

Table 5.4 Concentration (mg/g) at the end of experiment 4B of the measurable organic acids in the treatments to which only a Syrup was added.

			Organic acids (mg/g)						mol acid/m^3
Pot	Slurry	Additives	Total	LA	HAc	Formic acid	Prop. acid	Ethanol	LA
5B	Grass	Ini 200; 4B + 300 ml	32	30	1.9	n.a.	0.2	1.5	333
6B	Grass	Ini 400 C; 4B + 300 ml	26	21	2.3	2.2	0.2	1.7	233
27	Maize	Ini 300 C	35	27	6.9	n.a.	1.1	2.6	300
28	Maize	Ini 600 C	41	34	5.6	n.a.	1.4	1.6	377

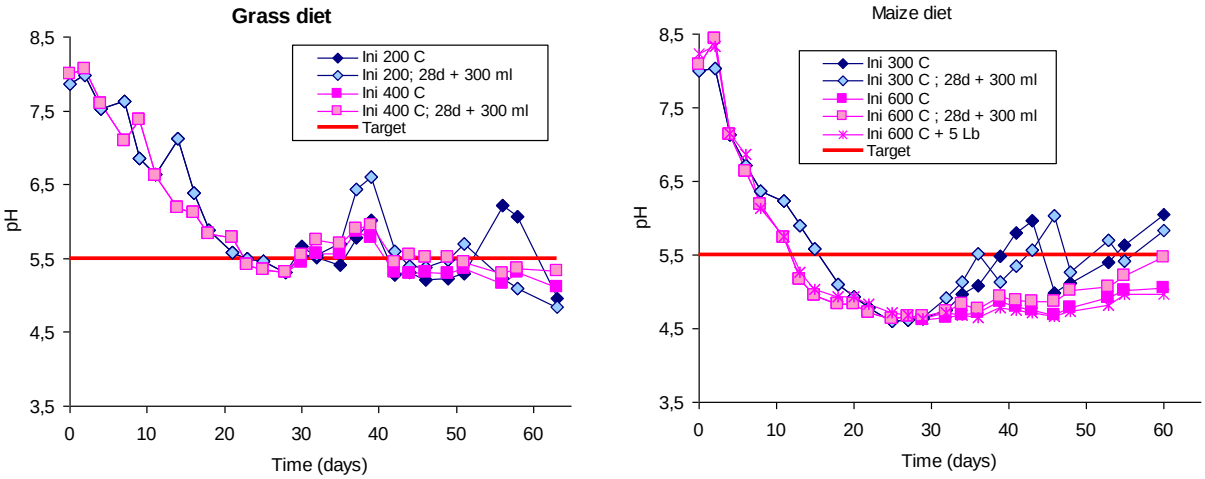


Figure 5.2 Change in pH during the fed-batch experiment after the initial 2L of day fresh slurry was acidified with either 200 or 400 ml Syrup in the grass slurry and 300 or 600 ml in the maize slurry. Every 2-3 days 200ml fresh slurry was added. In the second part of the experiment (4B) the experimental units were split; to one part 200 ml fresh slurry was added 3 times a week and to the other this was increased to 300 ml.

5.3.3 Addition of a combination of acid and a C source

The chosen experimental setup shows several different C – acid interactions. In experiment 4A syrup was added in combination with acid at the start of the experiment. With time, the target pH was maintained by adding more acid (not C). In experiment 4B a number of treatments from exp. 4A were split into two. In part one the target pH was maintained by adding acid. In part two the pH was maintained by adding syrup. Figure 5.3 is a representative example of the change in pH for the combination LA and LA/syrup.

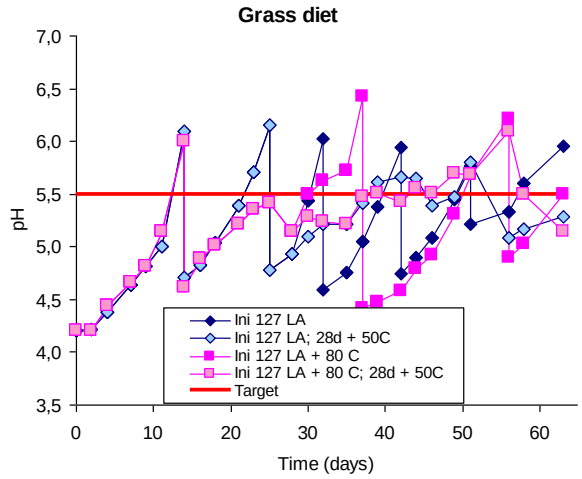


Figure 5.3 Change in pH during the fed-batch experiment after the initial 2L of day fresh slurry was acidified with either 127 ml LA or 127 ml LA & 80 ml syrup and the pH was maintained during the first 28 days (exp. 4A) by adding LA. After 28 days these two treatments were split and the pH was maintained by adding either acid (dark symbols) or syrup (light symbols). Every 2-3 days 200ml fresh slurry was added.

After the slurry has been acidified and the pH rises, the system can be readily acidified by adding a syrup. This is contrary to the system to which only syrup is added. In this system it takes 2-3 weeks to reach the target pH (Figure 5.2). This rapid effect of the added C on the pH is presumable the combined effect of the fact that the already added acid has reduced the buffer potential of the slurry (Lameijer and Vervoorn 1995) and that the environment in the slurry is favorable for the acid producing microorganisms to convert sugar into acid. Adding syrup is an effective way of maintaining the target pH of slurry.
In accordance with previous results, none of the treatments result in a system that can sustain itself by converting the C added with the fresh slurry into acid. To all treatments either acid or C must be added to maintain the target pH in the fed-batch system. Table 5.5 shows the amount of acid or syrup that is needed for steady state between the input of fresh slurry and the input of acid or C to maintain the target pH. This is calculated from experiment 4B. As mentioned before (§5.3.1) steady state is reached for grass and maize slurry when respectively 14 / 20 L HAc/m^3 is added, 18 / 25 L LA/m^3, or 5 / 6.7 L H_2SO_4/m^3. When only C in the form of syrup (65% sugar) is added respectively 44 / 63 L syrup/m^3 is needed to maintain steady state. When C is added to grass and maize slurry acidified with HAc, respectively 42+/-17 / 46+/-8 L syrup/m^3 is needed to maintain the target pH.
When slurry has been acidified with LA respectively 37+/-7 / 55+/-10 L syrup/m^3 is needed to maintain the target pH when fresh slurry is added. When C is added to slurry that has already been acidified an approximately equal amount of syrup is needed to maintain the pH compared to treatments to which only syrup is added. The only exception is when slurry is acidified with H_2SO_4. For this treatment slightly less syrup is needed to maintain the pH.

Table 5.5 Amount of additives needed to maintain the target pH of 5.5.

			Additives to maintain pH (L /m^3 slurry)	
Slurry type	Slurry acidified with (exp. 4A)	pH maintained with (exp. 4B)	Avg	SD
Grass	HAc	Hac	14	1
	LA	LA	18	1
	H_2SO_4	H_2SO_4	5.0	0.4
	syrup	syrup	44	3
	HAc	syrup	42	17
	LA	syrup	37	7
	H_2SO_4	*syrup**	*28 (40)*	*7 (6)*
Maize	HAc	Hac	20	1
	LA	LA	25	1
	H_2SO_4	H_2SO_4	6.7	0.9
	syrup	syrup	63	4
	HAc	syrup	46	8
	LA	syrup	55	10
	H_2SO_4	*syrup**	*28*	*7*

* treatment acidified wit H_2SO_4 and pH maintained with syrup had not yet achieved steady state, less syrup is needed than stated here. For the grass slurry the values of the maize slurry are stated and of the grass slurry between brackets.

The different acids may affect the effectiveness with which the microorganisms transform sugars into acid. In principle 1 sugar molecule can be converted into 2 lactic acid molecules (equation 2). This reaction is

called homolactic fermentation. With 100% conversion adding 1 kg of syrup equals 650 g sugar which equals a potential LA formation of 7.2 mol/L. This is very high: the pH would, in the absence of any buffer, drop to pH -0.9. Part of the sugar can also be converted by microorganisms other than Lacto Bacillus into other molecules. Heterolactic fermentation for instance yields carbon dioxide and ethanol in addition to lactic acid.

The organic acid measurements (Table 5.3) confirms the biological conversion of sugars into LA. In the LA environment there is no clear difference in the organic acid concentrations between maintaining the target pH with syrup or LA. The total organic acid concentration fluctuates between 25 and 29 mg/g and more than 90% is LA. In the HAc environment there is a clear change in organic acid composition when the pH is maintained using syrup instead of acid. In the treatments where the pH is maintained using syrup the total organic acid concentration increases and HAc concentration decreases in favor of LA compared to the treatments where the pH is maintained with HAc. The added syrup is thus converted into LA.

The effectiveness of the microbial conversion of sugar into lactic acid is compared based on a conversion factor (c.f.). The conversion factor is calculated by comparing the total added H^+ in treatments with only acid to treatments to which both acid and syrup are added. For instance, 357 ml LA is just as effective as 237 ml LA + 85 ml syrup. For the syrup the conversion factor is calculated based on a maximum H^+ concentration of 7.2M ($[H^+]_{sugar}$) using equation 3:

$$Vol_{acid} \times [H^+]_{acid} \text{ (acid treatment)} = Vol_{acid} \times [H^+]_{acid} + Vol_{syrup} \times c.f. \times [H^+]_{sugar} \text{ (acid + C treatment)} \quad \textit{Equation 3}$$

The H^+ concentration ($[H^+]$) for the different acids is HAc = 7M, LA = 3.9M, H_2SO_4 = 2*2.5 = 5M. The conversion factors are shown in table 5.6. Without distinguishing between slurry type, all of the added sugar is converted into LA in the HAc environment and in the H_2SO_4 environment. In the grass slurry the steady state between input of H_2SO_4 / syrup and fresh slurry has not yet been achieved. This means that the calculated c.f. is an underestimate. In the LA environment the c.f. is lower than in the HAc environment. For the microorganisms the HAc environment thus seems slightly more favorable to convert sugar into LA than in the LA environment. The reason for this is not clear.

Table 5.6 Conversion factor representing the effectiveness of the microbial conversion of sugar into lactic acid in slurries that have been acidified with HAc, LA, or H_2SO_4.

	Average	Grass slurry	Maize slurry
HAc	0.97	0.93	1.00
LA	0.66	0.60	0.72
H_2SO_4	0.99	(0.38)	0.99

5.3.4 Addition of a combination of acid, a C source and Lacto Bacillus and / or zeolite

No positive effect was observed from the addition of Lacto Bacillus. Initially the effect was even negative. The reason was that the carrier material of the Lacto Bacillus was calcium carbonate which buffers acid. In a small additional experiment with another Lacto Bacillus source especially designed for slurry, also no positive effect was observed.

5.4 Effect of different additives on biogas production potential

A positive side-effect of biologically acidifying slurry is that this might increase the biogas potential. Results of the anaerobic fermentation experiments at 38^0C (method Innolab) are shown in Table 5.7. These results

are obtained at the end of experiment 4A after 28 days.

A clear positive effect is found when syrup is added to slurry: the biogas potential increases with 47% in grass slurry and 61% in maize slurry. Not only does the total biogas potential increase, also the biogas production is more rapid when C is added compared to the reference slurry (Figure 5.4).

Overall, biogas potential shows a linear relationship with dry weight content of the slurry (r^2=0.9, Figure 5.5A). Dry weight content of slurry increases when additives are added. Based on the volume and molecular weight of added acid and syrup the theoretical increase in dry weight compared to the reference is calculated. This is presented in figure 5.5B. The biogas potential thus increases with the addition of easily degradable additives. The only exception seems to be LA in the maize slurry. For this treatment the dry weight content increased more than expected based on the amount of additive. The biogas potential follows the measured increase in dry weight. At this point this cannot be explained.

Table 5.7 Biogas potential and results from anaerobic biogas incubation experiments at 38^{0}C.

		Biogas potential (Nm³/ton)	Methane (%)	H_2S (ppm)	Residence time (d)
Grass	Reference	31	65	71	29
Grass	Ini 100 Hac	28	68	14	27
Grass	Ini 70 Hac + 80 C	32	67	65	29
Grass	Ini 127 LA	35	69	34	29
Grass	Ini 127 LA + 80 C	35	66	28	30
Grass	400 C	46	66	81	28
Maize	Reference	47	66	36	14
Maize	Ini 150 Hac	53	66	89	29
Maize	Ini 100 Hac + 120 C	55	64	17	27
Maize	Ini 127 LA	91	64	71	30
Maize	Ini 127 LA + 120 C	56	67	39	28
Maize	Ini 600 C	75	64	21	29

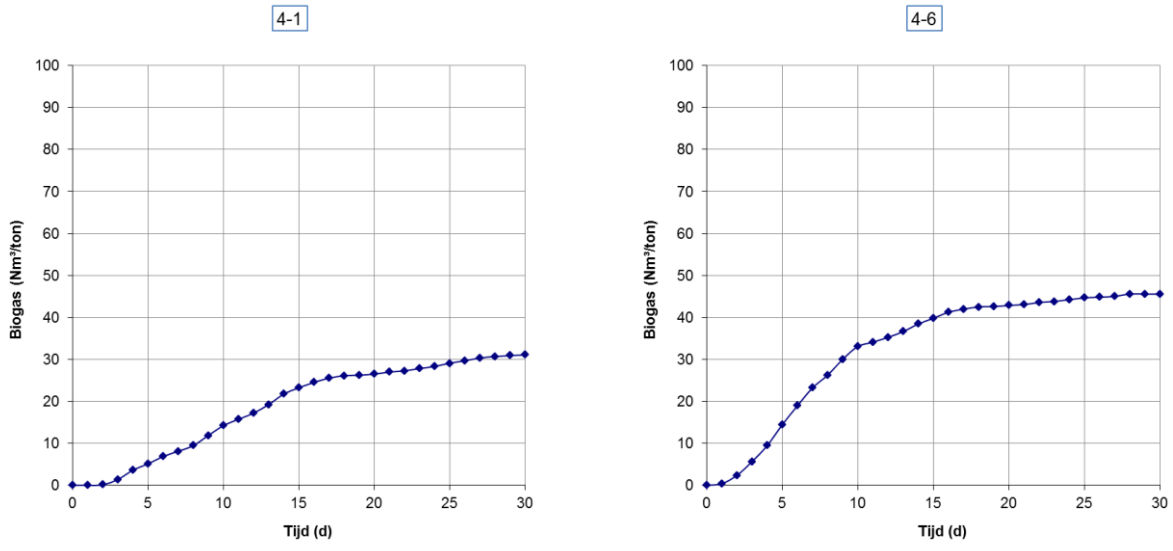


Figure 5.4. Increase in biogas potential (Nm^3/ton) over time (d) for the reference grass slurry (4-1) and grass slurry treated with Syrup (4-6).

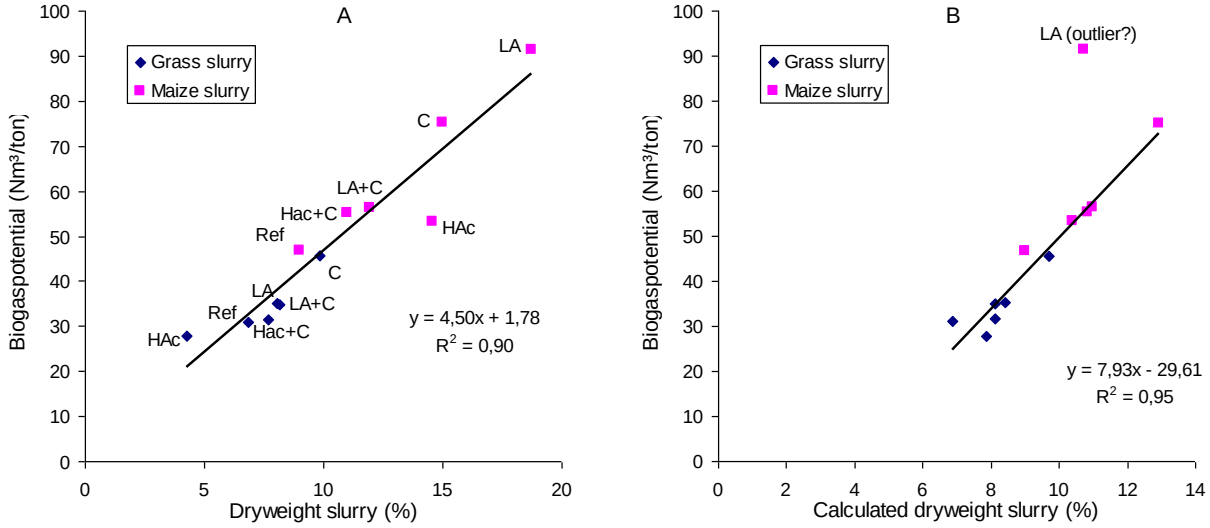


Figure 5.5 Relationship between biogas potential and dryweight of the slurry (%).

When organic acid is used to acidify slurry the increase in biogas potential is 0 to 21% compared to the reference. As mentioned, an exception is the treatment of maize slurry with LA, where the biogas potential shows an increase of almost 100% compared to the reference slurry. For HAc there is no clear increase in biogas potential. LA has a more positive effect than HAc on the biogas potential. In the circumstances of the biogas experiment (anaerobic and 38^{0}C) the LA environment is thus more favorable for the fermenting bacteria than the HAc environment. LA is transformed into CH_4 whilst this is not the case for HAc.
When in combination with organic acid, syrup is also added at the start of the experiment the biogas potential is very slightly higher compared to the treatments where only organic acid is used. Because these samples were taken at the end of experiment 4A there was no sample of a treatment where syrup is used to maintain the target pH. In all treatments where initially organic acid or organic acid plus syrup was added, the pH was maintained by adding organic acid. It is thus conceivable that when syrup is used to maintain the target pH after the slurry is initially acidified with an acid, the biogas potential will be higher than when acid is used to maintain the target pH. This is also in line with the linear relationship between biogas potential and dry weight content (figure 5.5) because syrup adds more than acid to dry weight content.
The maize slurry has a higher biogas potential than the grass slurry. This is in accordance with previous results where the pH in the maize slurry dropped faster and further compared to the grass slurry when syrup was added. In addition, the conversion factor of sugar to LA in the fed-batch experiments was also higher for the maize than for the grass slurry (Table 5.6). The dry weight content of the maize slurry is also higher than of the grass slurry.

5.5 Conclusions experiment 4

- Irrespective of the additives and their effect on the environment in the slurry the fed-batch system cannot maintain the target pH of 5.5 without additional acid or sugars;
- The system reaches a steady state between the input of fresh slurry and the input of acid and/or C source needed to maintain the target pH of 5.5. For the addition of only acid steady state is reached for grass and maize slurry when respectively 14 / 20 L HAc/m^3 is added, 18 / 25 L LA/m^3, or 5 / 6.7 L H_2SO_4/m^3. When only C in the form of syrup (65% sugar) is added respectively 44 / 63 L syrup/m^3 is needed to maintain steady state. When C is added to slurry acidified with HAc, respectively 42 / 46 L syrup/m^3 is needed to maintain the target pH. When slurry has been acidified with LA respectively 37 /

55 L syrup/m^3 is needed to maintain the target pH.

- When only a C source is added, the microbial community responsible for the conversion of sugar into acid in the maize slurry is more active and more diverse than in the grass slurry. This may (partly) also be caused by a higher temperature.
- The added sugar is mainly converted into LA by homolactic fermentation. Only when syrup is added to maize slurry or to slurry that has been acidified with LA some heterolactic fermentation seems to occur.
- HAc and H_2SO_4 seem the best environment for the conversion of sugar into LA.
- The biogas potential linearly increases with increase in dry weight content due to adding either C and / or acid. When only syrup is added the increase is approximately 55%.

6 Technical and economic feasibility of biological acidification

In the previous chapters the results of a number of lab experiment have been described. Although a validation of the most promising (combination of) additive at farm scale is to be done the costs of such a system can already be estimated based on previous experiences and literature. To be able to calculate different scenarios a possible system for biological acidification must be defined.

6.1 Technical feasibility

Acidification of slurry with H_2SO_4 has been introduced in Denmark (Infarm system) in the first years of this century. The Infarm system is the starting point for the technical feasibility of a system for biological acidification. Such a system can be described by a functional diagram as shown in figure 6.1.

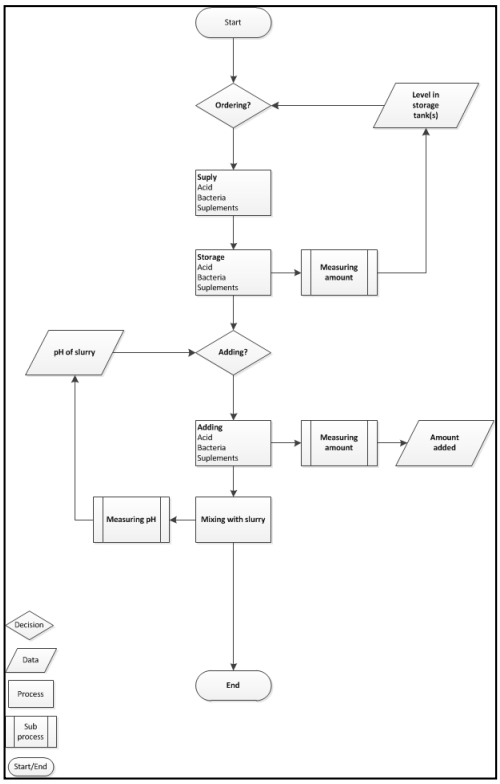


Figure 6.1 Functional diagram of biological acidification.

Main processes are adding of acid and/or C source to the slurry, mixing the slurry and constantly measurement the resulting pH. The measured actual pH can lead to the addition of more acid and/or C source. Additives are stored on the farm. When levels in the storage tank(s) reach a critical minimum level extra additives have to be ordered. The amount of additives used is measured and can be part of a (legal) control system.

The Infarm system was used in pig and dairy farms with slurry storage under the building. This is also the common housing and storage system in The Netherlands although the pits are generally deeper (and contain therefor more slurry) than in Denmark and have not only a function for collecting and transporting the slurry but also have a storage function. The feasibility of the Infarm system under Dutch circumstances was tested at Dairy Campus (formally known as Nij Bosma Zathe) the dairy research farm of Wageningen UR Livestock Research. This test proved that there were no major obstacles. The amounts of acid needed to stabilize the pH at a low level were larger than in the Danish situation but it was possible to keep the pH at the desired level. Only during the initial phase when pH dropped from the common value of 7-8 to the

aimed value of 5-5.5 foam caused some inconveniences. As soon as the pH level was reached the foam disappeared.

Using H_2SO_4 leads to several precautions as H_2SO_4 is a strong acid and under anaerobic conditions there is a changes of H_2S formation which is a lethal gas at low concentration. The functional diagram of figure 6.1 can be translated in a schematic outline (figure 6.2). This outline is made with a 'standard' dairy barn in mind. This means that the cows are housed on a concrete slatted floor and with a slurry storage partly in pits under this floor. Modern farms have pits under the whole barn including cubicles and feeding lane. Older barns sometimes only have pits under the walking alleys. The pits consist of two or more circuits in which the slurry can be mixed. The mixing is generally done with a tractor PTO driven mechanical mixer or in a some cases with an electrical driven one. The mixer openings for the mechanical mixer (one for each circuit) are mostly situated at one of the end walls of the barn. This could also be the place where acid or a C source are added to the slurry.

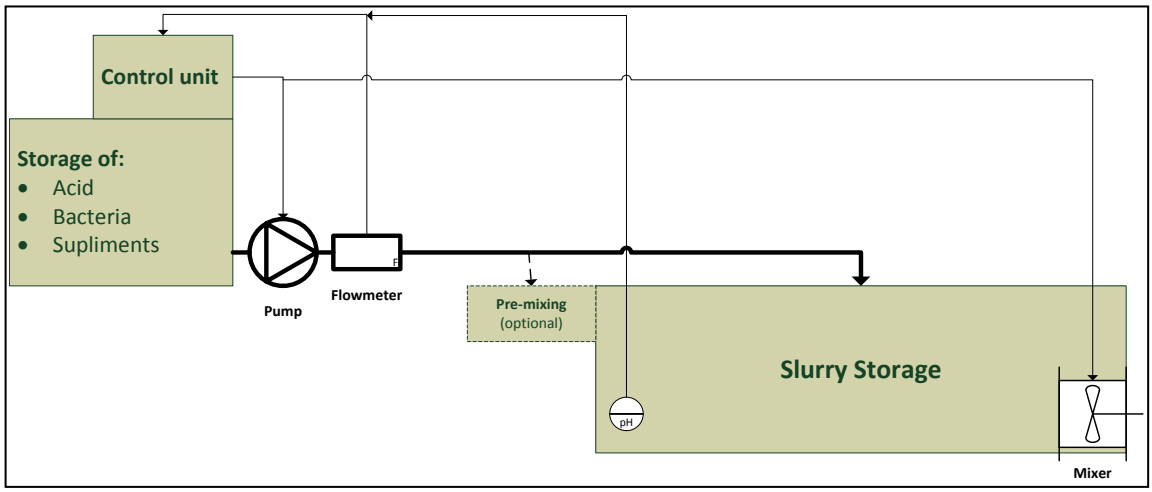


Figure 6.2 A schematic outline of a biological acidification system at farm level.

Such a system contain one or more storage tanks for the additive(s) depending on the number of additives used. Centre of the system is a control unit that regulates the frequency and amount supplied to the slurry storage based on information from at least one pH meter in the slurry. The acid and/or C source can be added with a pump directly into the pits or added in a small pre-mixing storage where it is mixed with a small amount of slurry before this mixture is added to the main storage. When small amounts of additives are used this can contribute to an even distribution of the additive in the pits. The pits should have at least one mixing unit depending on the size and number of pits or mixing circuits. A flow meter measures the amount of additives and can together with the level in the storage tanks be the basis for legal reporting or ordering of additives.

6.2 Economic feasibility

The economic feasibility is calculated based on a farm scale system. This involves the costs of the additives and also the costs of installing and maintaining the technical system as shown in figure 6.2. The following starting points are used:

Milking cows	150	
Milk production	9,000	Kg/cow/year
Feed ration		Summer feeding
Interest	4.5	%

Manure production	26.9	m^3/cow/year	
Stable emission	11.0	kg/cow/year	
Emission reduction			
stable	50%	5.5 kg/cow/year	Source: Report 645, Smits et al. (2013)
manure application	85%	7.3 kg/cow/year	

Previous chapters have shown that with the addition of fresh slurry, additives must also be added to keep the slurry in the pit at the target pH. The amount of additives mainly depend on slurry type and temperature. The costs are calculated for an average cattle slurry with a N content of 4.1 mg/kg. The costs are calculated for four scenario's based on the addition of: 1) a C source, 2) H_2SO_4, 3) HAc, and 4) LA. Scenario's 2, 3, and 4 are split up into scenario a where only acid is used and scenario b where acid and C are used in a specific ratio. The costs of the additives are based on the amount of additives needed per m^3 slurry to maintain the target pH of an average cattle slurry (Table 6.2) and the costs of the additives (Table 6.1).

Table 6.1 Overview of the costs per additive when bought in bulk (concentrated acid expressed per ton) and expressed in € per L concentrated acid / syrup as added in the experiments.

	Costs bulk (€/ton)	Date prices	Concentration (M)		Density (g/cm^3)	Costs €/L
			Used in exp.	Concentrated		
Acetic acid	500.-	(2007/2011)	7	17.4	1.05	0.525
Lactic acid	1000.-	(2007/2011)	3.9	11.5	1.20	1.20
Sulfuric acid	100.-	(2007/2011)	2.5	18	1.84	0.184
syrup	136.-	(2008/2009)			1.30	0.177

The prices of the different additives are summarized in table 6.1. The amount of additives needed to maintain the target pH of 5.5 is calculated for the average N-content of Dutch dairy slurry (table 6.2).

Table 6.2 Amount of additives needed to maintain the target pH of 5.5 for average Dutch cattle slurry (N content 4.1 mg/kg).

		Additives (L /m^3)	
Slurry acidified with	pH maintained with	Avg	SD
HAc	Hac	16	1
LA	LA	21	1
H_2SO_4	H_2SO_4	5.6	0.6
syrup	syrup	51	4
HAc	syrup	43	14
LA	syrup	43	8
H_2SO_4	syrup	28	7

The investment costs differ per system as a result of the number (1 or 2) and capacity of the storage containers, and what is stored. Storing H_2SO_4 is for instance more expensive than storing a C source because it is very corrosive. The investment costs are lowest when only a C source is added (42 €/cow/yr) and highest when a combination of H_2SO_4 and a C source are used (84 €/cow/yr, Table 6.3).
Additional costs result from an additional use of lime ($CaCO_3$). Lower NH_3 emission results in a higher N content. Additional benefits arise from higher yields as a result of higher N content in the slurry. This is valued at approximately 3 € per kg saved N. Assumed NH_3 emission reduction is 12.3 kg NH_3/cow/yr, based on 50% emission reduction from the stable and 85% during manure application. For the treatments

to which H_2SO_4 is added, a benefit is that no additional S fertilization is needed. When only H_2SO_4 is used to acidify slurry the amount of S added exceeds by far crop demand leading to the environmental disadvantage of a buildup of S in the soil forming a potential threat to the water quality of adjacent water bodies (Bussink 2009).

Overall the most expensive system is when slurry is acidified with LA due to the high costs of LA (€1000,- per ton). The costs decrease considerably when the slurry is initially acidified with LA and the pH is maintained by adding syrup. Although more is needed, syrup is much cheaper than LA (Table 6.1). When the ratio in which acid and C source are used is low (10% acid in the example presented in Table 6.3) the costs decrease from 723 €/cow/yr to 272 €/cow/yr. The costs are however too high when LA is used.
From a cost perspective HAc is much more favorable than LA. The costs to initially acidify slurry with HAc are approximately equal to the costs to maintain the target pH when using syrup. From an economic perspective varying the ratio in which acid and C source are used thus has only a small effect on the total costs. An advantage of using syrup is that the biogas potential increases and this may have an extra economic return. This extra economic return is not incorporated in the calculations.
H_2SO_4 is by far the cheapest acid of the three and least is needed per m^3 slurry. This results in very low costs (67,- €/cow/yr) when only H_2SO_4 is used. As mentioned before this leads to high additions of sulphur to soils which forms a potential threat to the water quality of adjacent water bodies (surface- and groundwater, Bussink et al., 2009). The price for syrup and concentrated H_2SO_4 are approximately equal

Table 6.3 Economic effects of different scenarios for (organic) acidifying of manure for an average cattle slurry.

Scenario	1 C	2a H_2SO_4	2b H_2SO_4+C	3a HAc	3b HAc+C	4a LA	4b LA + C
Variable costs (€/cow/year)							
Steady state Acid		28	28	227	227	668	668
Steady state Carbon	240		132		159		158
Ratio acid / C			**0.6**		**0.4**		**0.1**
Average costs	240	28	70	227	186	668	209
Energy	5.5	5.5	5.5	5.5	5.5	5.5	5.5
Investment (€/cow)	327	427	600	490	563	500	573
Investment (€/cow/year)	42	62	84	73	80	74	81
Additional costs / benefits €/cow/year)							
Yield increase due to higher N content grass	-32	-32	-32	-32	-32	-32	-32
S manure		-3.5	-3.5				
Extra costs $CaCO_3$	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Net costs							
(€/cow/year)	264	67	132	282	248	723	272
(€/m^3 manure)	9.7	2.5	4.9	10.5	9.2	26.9	10.1
(€/kg NH_3 emission reduction)	21	5.2	10.2	22	19	56	21

but more syrup is needed than H_2SO_4 to maintain the target pH. For this reason maintaining the pH with syrup increases the total costs. In the example presented in Table 6.3 the ratio acid / C source of 0.6 was chosen which leads to total costs of 132 €/cow/yr. In practice this ratio can be adapted depending on the availability and price of additives. An advantage of using syrup is that the biogas potential increases and this may have an extra economic return.

- From a combined environmental and economic perspective the combination of initially acidifying slurry with H_2SO_4 and maintaining the target pH using syrup is the most promising system.

- In practice, the ratio in which acid and a C source are used can be adapted depending on the availability and price of additives.

6.2.1 Sensitivity analysis

The sensitivity analysis has been made for the starting points which lead to the highest costs per cow. These are the carbon price, HAc, and LA price. In next table the results presented in table 6.3 are repeated with 50% reduction of the C, HAc, and LA prices. Only when the prices for HAc and C decrease substantially, they will compete with H_2SO_4. LA will never be compatible with H_2SO_4 unless the ratio LA over C source is very low.

Table 6.4 Sensitivity analysis by decreasing the price for C, HAc, and LA by 50%.

	1 C	2a H_2SO_4	2b H_2SO_4 + C	3a Hac	3b HAc + C	4a LA	4b LA + C
Ratio acid / C			0.6		0.4		0.1
Net costs							
(€/cow/year)	143	67	105	168	155	389	167
(€/m^3 manure)	5.2	2.5	3.9	6.2	5.8	14.5	6.2
(€/kg NH_3 emission reduction)	11	5.2	8.2	13	12	30	13

Instead of using the extra N maintained in the slurry for extra grass production the farmer could also decide to reduce the mineral fertilizer input with the same amount. This would result in a cost increase of ±11 €/cow/yr assuming a reduction in NH_3 emission of 12 kg NH_3/cow/yr.

6.2.2 Comparison with other systems

NH_3 emission in stables for dairy cattle can also be reduced by different floor systems. Systems with a closed sloped floor can reduce NH_3 emission by 22% and a slatted floor with a convex rubber top layer by 33%. In table 6.5 the costs of some systems are compared with the costs of (biologically) acidifying slurry. Considering the costs per kg NH_3 reduction, the system with a closed sloped floor is comparable with the H_2SO_4 scenario. The investment costs for the floor system are much lower, but also the reduction % is much lower. The floor with a convex rubber top layer is comparable with the H_2SO_4 + C scenario. The differences are between the variable costs and the NH_3 reduction %, which are much lower for the floor system.

Table 6.5 Comparison between (biologically) acidifying average Dutch cattle slurry and two emission reducing floor systems. System 1 is a closed sloped floor with rapid removal of slurry using a slurry shovel, the second system is has a convex rubber top layer.

	H_2SO_4	H_2SO_4 + C	Floor system 1	Floor system 2
Variable costs (€/cow/year)	28	70*	1	1
Investment (€/cow/year)	62	84	16	42
Net costs				
(€/cow/year)	67	132	17	43
(€/m^3 manure)	2.5	4.9		
(€/kg NH_3 emission reduction)	5.2	10.2	7.1	12

* Ratio acid over syrup of 0.6

One of the reasons that existing farm scale biogas installations are not profitable is the increased cost of co-substrates. The demands for such co-substrates (high energy, low volume) are the same as those for a

possible C source used in biological acidification. When biological acidification is implemented on a large scale the cost of the C source can increase considerably as extra competition is introduced on the market. A possible solution that needs further investigation, is adding enzymes or a combination of macromolecular C sources in combination with enzymes. In this study only very limited evidence was found that the endogenic C sources (present in the slurry) also contribute to the acidification potential of the slurry. As proposed by Lameijer and Vrielink (1995) addition of enzymes that breakdown macromolecules to sugars may result in endogenic substrate for the acid producing bacteria. Suárez Quiñones et al. (2012) indeed found that the addition of enzymes that breakdown macromolecules to sugars increases the biogas potential of manure, and thus also possibly the acidification potential. In the study by Suárez Quiñones et al. (2012) a cocktail of enzymes were added containing a.o. cellulase, hemi-cellulase, xylanase, pectinase, xylan esterase, pectin esterase, lipase, amylase glucosidase and protease.
Lameijer and Vrielink (1995) also suggest that adding a macromolecular C source in combination with enzymes that breakdown this substrate into sugars may be an effective way to biologically acidify slurry. The advantage of this approach is that the macromolecular C source is much cheaper than a sugar source and gives more flexibility in what substrate is used. This also needs further investigation.

6.3 Additional remarks

- One of the reasons in the past that despite low costs and high reduction of ammonia emission, the use of H_2SO_4 was not recognized as an official reduction system, was due to limitations concerning legal control. It is very important that a possible biological acidification system has a well-developed and recognized tracing and tracking system. Constant logging of the slurry pH and/or amount of additives used are possibilities that need further development.
- The increased biogas potential is not included in the cost calculations. When a biogas installation is present this benefit can be ‘cashed’. A higher biogas potential will not lead to higher methane emissions from slurry during storage and application as long as the pH remains below 6.
- One of the reasons that existing farm scale biogas installations are not profitable is the increased cost of co-substrates. The demands for such co-substrates (high energy, low volume) are the same as those for a possible C source used in biological acidification. When biological acidification is implemented on a large scale the cost of the C source can increase considerably as extra competition is introduced on the market. This may be solved by adding enzymes or a combination of cheap macromolecular C source in combination with enzymes. This however needs further investigation.
- As all biological processes are vulnerable for antibiotics and anti-septic compounds farmers with a biological acidification system should be very aware of not to add cleaning water from the milking equipment, used food baths containing for example formalin or milk from cows treated with antibiotics to their slurry storage.
- New markets sometime lead to supply of product of inferior quality. Farmers ordering acids or a kind of C source should be aware of this and take appropriate control actions.
- The storage and use of H_2SO_4 brings extra safety regulations and at a larger scale will possibly lead to difficulties getting an environmental permit. In that case central acidification can be an alternative for an on farm system. But also in other scenarios where HAc and/or a harmless C source is added centralized biological acidification can bring scale advantages. In such a system the farmers slurry is collected and brought to a central acidification unit where it is stored. Possible advantages of increased biogas potential can be harvested easier here.
- In an intermediate scenario a farmer may transport a certain amount of slurry to a central acidification unit only in the startup phase and get the same amount of acidified slurry back. He then only has to maintain the lower pH which brings less costs. When extra acid is needed this procedure can be

repeated. This scenario has to be investigated on issues like legislation, safety, transport and possibilities to build such a unit to be sure that this is a feasible scenario.

6.4 Conclusions

- Acidification with only H_2SO_4 is the cheapest option but cannot be considered as biological acidification.
- A reduced amount of H_2SO_4 in combination with a C source (syrup) is economically second best. When expressed in € per kg NH_3 emission reduction, the costs are comparable with emission reducing floor systems.
- Treatments with HAc, LA +C, and only a C source do not differ much in total costs. The costs are approximately twice as high as the H_2SO_4 + C system.
- The options with only LA is by far the most expensive.
- Although costs are calculated at the farm level the step between the earlier described lab experiments and the full scale system is rather large. An intermediate step on semi-practical scale is recommended.

7 Conclusions and recommendations

In this phase of the study the perspectives to biologically acidify dairy cattle slurry were investigated on the basis of ab-scale research . The research focused on 3 issues:

1. Experimentally determine process conditions to effectively biologically acidify slurry of dairy cows (Chapter 2-5);
2. Experimentally determine to what extent the biogas production potential of manure increases after biological acidification (included in Chapter 5);
3. Evaluation of the technical and economic feasibility of scaling up this technique (Chapter 6).

7.1 Conclusions

The lab-scale study results in following conclusions:

- The composition of slurry can vary widely, also when the roughage part of the diet is constant;
- Slurry can be acidified by adding acid and/or by adding an easily degradable C source.
- These experiments confirm that the amount of acid needed to maintain the pH below 5.5 depends on the slurry composition, mainly expressed by the N content. More acid is needed at a higher N content. However, the NH_3 emission reduction is also larger at higher N content;
- Slurry can be biologically acidified by the conversion of a C source into predominantly lactic acid (LA). Optimally sugar is added and the slurry is kept at a low temperature (10^0C). Starch is not effective at this low temperature. At 25^0C both starch and sugar are effective, but sugar is less effective than at 10^0C;
- When only sugar is added to slurry, the rate with which the pH decreases and the level at which the pH is maintained depends on:
    - added dose;
    - slurry type;
    - temperature.
- The added sugar is mainly converted into LA by homolactic fermentation. Only when syrup is added to maize slurry that is stored at 15^0C instead of 10^0C or to slurry that has been acidified with LA some heterolactic fermentation seems to occur.
- Adding Lactobacillus and Zeolite has not found to be effective;
- Temperature is an important parameter. At lower temperature less additives are needed to acidify slurry. In addition, the slurry pH is maintained at a lower level and is more stable at 10^0C compared to 25^0C.
- In a fed-batch system the pH of the slurry must frequently be corrected by adding acid / C source to maintain the target pH of 5.5. The fed-batch system can thus not maintain the target pH of 5.5 by the conversion of easily fermentable C added with the fresh slurry. This is irrespective of the additives and their effect on the environment in the slurry;
- The system reaches a steady state between the input of fresh slurry and the input of acid and/or C source needed to maintain the target pH of 5.5. For the addition of only acid, steady state is reached for an average cattle slurry (N content 4.1 mg/kg) at 10^0C when ±5.6 L H_2SO_4/m^3, ±16 L HAc/m^3, or ±21 L LA/m^3 is added. When only C in the form of syrup (65% sugar) is added 51 L syrup/m^3 is needed to maintain steady state. When C is added to slurry that has already been acidified 28 (H_2SO_4) or 43 (HAc and LA) L syrup/m^3 is needed to maintain the target pH.
- The biogas production potential linearly increases with increase in dry weight content due to adding

either C and / or acid. When only syrup is added the increase biogas production potential is approximately 55%.

- From a combined environmental and economic perspective the combination of initially acidifying slurry with H_2SO_4 and maintaining the target pH using syrup is the most promising system.
- Expressed per kg NH_3 emission reduction the price of the combined H_2SO_4 – C system is comparable to low emission floor systems.
- In practice, the ratio in which acid and a C source are used can be adapted depending on the availability and price of additives.
- Technically there are different options to setup the system. The on-farm set-up can be implemented in most cubicle housing systems

7.2 Recommendations

- The results show that biological acidification is an interesting technique to reduce ammonia emissions. It is recommended to continue the research with focus on scaling up the experiments to semi practical conditions as an intermediate before it is tested/applied in practice. The reason is that the effect of different diets, environmental circumstances and the technical set-up can be tested. This could either be in lager vessels (up to 200 liter) or using the facilities used for earlier testing of slurry acidification at Dairy Campus. The four available units of 15 cows each offer opportunities to test feeding strategies and at the same time measure ammonia and methane emissions.
- It is recommended to develop and or test techniques to add additives to slurry in a controlled way.
- At a farm level the system can be setup in different ways that need further investigation. For instance the slurry can be acidified centrally in the vicinity of the farm, or some slurry can be acidified centrally and can be used as a starting slurry for the rest of the slurry pit.
- At this stage a system that makes use of organic acids or C-substrate in combination with sulphuric acid is most attractive to implement in practice. It is recommended to focus further on the reduction of the required amount of additives by lab-scale experiments. This can be done in three ways:
  - By using enzymes which can decompose organic matter that is present in slurry in the form of e.g. lignin, hemi-cellulose cellulose into sugars (endogenic C-substrate) which in turn can be converted by the microbial population into organic acids.
  - Selection on more efficient Lactobacillus spp. is another possible route to improve the acidifying potential of slurry.
  - By adding a macromolecular C source in combination with enzymes that breakdown this substrate into sugars. The advantage of this approach is that the macromolecular C source is much cheaper than a sugar source and gives more flexibility in C substrate.
- A high dietary N efficiency will also result in a lower amount of additives. In fact this requires a whole farm approach.
- The results show that biological acidification also increases the biogas potential of slurry. As a side effect it could be investigated if mono fermentation of slurry for biogas production is attractive.
- It is not clear if biological acidified slurry has positive side effects on soil. This needs further investigation.
- Taking into account the current changes in the manure market concerning processing manure it is interesting to investigate the possibility to process manure after it has been acidified. For instance, Lameijer and Vervoort (1995) suggest that separating slurry into a thick and liquid fraction is easier with acidified slurry compared to untreated slurry.

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nutriënten management
instituut nmi bv
postbus 250
6700 ag wageningen
binnenhaven 5
6709 pd wageningen
tel. (088) 876 1280
internet www.nmi-agro.nl