OPTIMIZATION OF THE ANAEROBIC DIGESTION PROCESS BY SUBSTRATE PRE-TREATMENT AND THE APPLICATION OF NIRS

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• Applying NIRS as a monitoring tool to assess the concentrations of ammonia (which is inhibitory to the process) in the contents of anaerobic digesters.
• Improving the BMP of materials such as cattle manure and dewatered pig manure and chicken manure by thermal pre-treatment at various temperatures between 100°C and 225°C
Results show that the NIRS method can be used to discriminate between meadow grasses with high or low BMP. In detecting the ammonia content, NIRS was shown to have the potential to be a process monitoring tool. Thermal pre-treatment proved to be most effective on dewatered pig manure which showed improvements at lower pre-treatment temperatures. Cattle manure required pre-treatment temperatures higher than 175°C to show improvement. Chicken manure did not show any improvements but instead showed a decrease in BMP at 225°C.

Keywords: Alternative energy sources, Bioenergy, Biogas, Biomass, Energy production, Environmental engineering, NIR, Manure, Pretreatment technologies, Sensor technologies, Spectroscopy, Sustainable technologies

Supervisors: Henrik Bjarne Møller & Alastair James Ward

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Abstract

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Preface

This thesis is submitted as a requirement for the completion of my PhD degree. The research work was carried out during the period of December 2008 to February 2012 at what is now the Department of Engineering, Foulum campus, Aarhus University. My work was supervised by Henrik Bjarne Møller as my main supervisor and by Alastair James Ward as my co-supervisor.

The thesis consists of a general introduction, a chapter on near infrared spectroscopy and a chapter on pre-treatment of substrates. Each of the last two chapters ends with a summary of the results that are derived from the papers that were written based on the experiments that were performed as part of the PhD study. Two of the papers have been published while the third paper has been accepted for publication.

The titles of the papers are:

1. Comparison of near infra-red spectroscopy, neutral detergent fibre assay and in-vitro organic matter digestibility assay for rapid determination of the biochemical methane potential of meadow grasses

2. NIR monitoring of ammonia in anaerobic digesters using diffuse reflectance probe

3. Effects of high temperature isochoric pre-treatment on the methane yields of cattle, pig and chicken manure

This thesis would not have been possible without the support and encouragement of both my supervisors Henrik and Alastair. I would like to thank my mum, my family, my friends and my colleagues for their love and support. Special thanks to Heidi and Britt for all the help in the lab.

Foulum, February 2012,

Chitra Sangaraju Raju

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I would like to thank all the people who made a difference in my life, to those who held me afloat when I needed it the most, to the people who had more faith in me than myself, to those who shaped me and are shaping me and to those who made this PhD possible. This thesis is for you.

Summary

Biogas production is fast gaining importance as a source of renewable energy apart from being a waste management solution. There are several areas in the biogas production process that need optimization and research, and this PhD study focused on the following areas.

1. The application of near infrared spectroscopy (NIRS) in the anaerobic digestion process
2. Pre-treatment of substrates to improve their methane yields.

The amount of methane that can be obtained from a particular substrate is usually measured by the biochemical methane potential assay (BMP) which requires at least 30 days. NIRS along with two forage analysis techniques, the in-vitro organic matter digestibility assay (IVOMD) and the neutral detergent fibre assay (NDF) were tested as methods that could be used to predict the BMP of meadow grasses in much less time. The NIRS method was most successful as a rapid and indirect method of predicting the BMP when compared to the other two methods. The NIRS method required the use of partial least squares regression, a multivariate data analysis approach, to build models that related the spectral data from the NIRS to the BMPs of the meadow grasses. The model based on NIRS had an R^2 value of 0.69 and a residual prediction deviation (RPD) of 1.75, which makes it a moderately useful model that can discriminate between high and low values of BMP for meadow grasses.

Another study where NIRS was applied to the anaerobic digestion process was to monitor the total ammonia nitrogen contents (TAN) of an anaerobic digester. Ammonia is a known inhibitor and beyond certain levels, can seriously affect the anaerobic digestion process. It is currently measured mainly by chemical analysis. The use of NIRS to measure the ammonia contents would reduce the time and the chemicals required for laboratory analysis and make real time monitoring possible. A diffuse reflectance probe attached to an NIR spectrometer was used to measure the TAN contents in the digestates of anaerobic digesters that used cattle manure as substrate. Partial least squares regression and interval partial least squares methods were used to build models of spectral data predicting the TAN concentrations. An R^2 of 0.91 and an RPD of 3.4 was obtained implying that the probe could be used for monitoring and screening purposes.

The second focus of this PhD study was to use pre-treatment methods to improve the BMP of substrates. Three types of manure, cattle manure, dewatered pig manure and chicken manure were subjected to thermal pre-treatment and the changes in their BMP were studied. The manures were pre-treated in a high temperature and pressure reactor for 15 minutes, at six temperatures between 100°C and 225°C with 25°C intervals to study the effect on their methane yield. After 27 days of anaerobic digestion, the dewatered pig manure showed improvements at much lower pre-treatment temperatures when compared

to the other two manures. All temperatures above 125°C improved the BMP of the pig manure with a maximum 29 % increase in yield at 200°C. Cattle manure showed a significant improvement in its BMP at temperatures above 175°C with the best result of a 21 % increase at 200°C. The BMP in chicken manure was reduced by 18 % at 225°C, but at lower pre-treatment temperatures there were no significant changes.

Summary in Danish

Biogas som en kilde til produktion af vedvarende energi vinder hurtigt frem da det udover at producere energi også giver en række miljøfordele og er en miljøvenlig metode til affaldshåndtering. Der er imidlertid flere områder, der kræver optimering før teknologien er fuldt udviklet og denne ph.d undersøgelse har fokus på en række områder der kan optimere teknologien herunder:

Anvendelse af nær-infrarød spektroskopi (NIRS) til process optimering og biogas udbytte måling

Forbehandling af substrater for at forbedre methan udbyttet.

Mængden af methan, der kan opnås fra et substrat måles normalt ved biokemiske metan assays (BMP), der tager mindst 30 dage. NIRS sammen med to foder analyseteknikker (in vitro-organisk stof (IVOS) og neutral detergent fiber (NDF) er blevet afprøvet som metode til at forudsige BMP af vedvarende græs. NIRS metoden var den bedste og hurtigste metode til forudsigelse af BMP i forhold til de to andre fremgangsmåder. NIRS metoden kræver anvendelse af mindste kvadraters regression og en multivariat dataanalyse tilgang til lave modeller, der vedrører de spektrale data fra NIRS til BMP af engen græss. Modellen baseret på NIRS havde en R^2 værdi på 0,69 og afvigelse (RPD) på 1,75, svarende til en anvednelig model som kan skelne mellem høje og lave værdier af BMP i eng græsser.
I en anden undersøgelse blev NIRS anvendt til at overvåge det samlede ammoniak indhold (TAN) i en anaerob udrådnings processen. Ammoniak er en kendt inhibitor, og kan påvirke den anaerobe nedbrydningsproces negativt hvis niveauet kommer over bestemte niveauer. I øjebliket måles ammoniak ved kemisk analyse der er tids- og omkostningskrævende. Anvendelsen af NIRS til at måle ammoniak indholdet vil reducere den tid og de kemikalier, der kræves til laboratorieanalyse og gøre real time overvågning muligt. En diffus reflektans probe forbundet til en NIR spektrometer blev anvendt til at måle TAN indholdet i biogas reaktorer med kvæggødning substrat. En R^2 på 0,91 og en RPD på 3,4 blev opnået som indebærer, at proben er anvendelig til overvågnings- og screening formål.
Det andet fokusområde forbehandling metoder til forbedring af BMP værdien for forskellige substrater. Tre typer af husdyrgødning, kvæggylle, afvandet svinegylle og hønsegødning blev udsat for termisk forbehandling og ændringer i BMP blev undersøgt. Husdyrgødningen blev forbehandlet i en høj temperatur og tryk reaktor i 15 minutter ved seks temperaturer fra 100°C - 225°C med 25°C intervaller og efterfølgende blev virkningen på deres methan udbytte undersøgt. Efter 27 dages anaerob udrådning var der positiv effekt på afvandet svinegødning ved lavere forbehandlings temperaturer sammenlignet med de to andre gødningstyper. Alle temperaturer over 125 ° C forbedrede BMP af svinegylle med en maksimal stigning på 29% i udbytte ved 200°C. Kvæggylle viste en signifikant forbedring i BMP ved temperaturer over 175°C med det bedste resultat på 21%

forbedring ved 200°C. BMP i hønsegødning blev reduceret med 18% ved 225 ° C, men ved lavere temperaturer var der ingen signifikante effekter.

Glossary

AD	Anaerobic digestion
BMP	Biochemical methane potential
CSTR	Continuous stirred tank reactor
DM	Dry matter
EMSC	Extended multiplicative scatter correction
FT-NIR	Fourier transform near infrared spectroscopy
HRT	Hydraulic retention time
InAs	Indium Arsenide
InGaAs	Indium Gallium Arsenide
IVOMD	In-vitro organic matter digestibility test
LFA	Long chain fatty acids
LV	Latent variables
NDF	Neutral detergent fibre
NIR	Near infrared
NIRS	Near infrared spectroscopy
MSC	Multiplicative scatter correction
OLR	Organic loading rate
PCA	Principal component analysis
PC	Principal component
PC/MR	Principal component regression
PLS	Partial least squares
PLSR	Partial least squares regression
R^2	Coefficient of determination
RMSECV	Root mean square error of cross validation
RMSEP	Root mean square error of prediction

RPD	Residual prediction deviation/ ratio of standard deviation to standard error of performance
RPM	Rotations per minute
SD	Standard deviation
SNV	Standard normal variate
TAN	Total ammonia nitrogen
VFA	Volatile fatty acids
VS	Volatile solids

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Chapter 1 - Introduction

1.1 Biogas as a renewable energy source

Anaerobic digestion (AD) is a biological process where organic matter is degraded into its most reduced form (methane) and its most oxidized form (carbon dioxide) without external electron acceptors such as oxygen, nitrates or sulphates (*1, 2*). The process occurs in nature and is one of the natural degradation pathways for organic matter. The main gaseous products of anaerobic digestion are methane and carbon dioxide, and minor quantities (< 1%) of hydrogen sulphide, nitrogen oxides, ammonia and other volatile compounds (*1*). Although there has been evidence of the use of biogas for heating in the 10th century BC, the first anaerobic digesters appeared in the mid 19th century. The initial use of digesters was mainly for sewage treatment. It was then extended to handle animal manure, municipal solid wastes and wastes from industries such as food, pharmaceutical and chemical industries. The energy crisis in the 1970's led to a significant growth in research in this area as there was an impetus to reduce the dependency on fossil fuels (*3, 4*). More recently, AD is being considered as a potential renewable energy source and not just as a waste treatment solution.

AD is a viable solution to some of the problems that the world is facing today and to those that are expected to arise in the near future. The world population is increasing and is expected to reach nine billion by the year 2040 and as a consequence the energy demand is expected to rise by 30% (*5*). Currently about 81% of the worlds energy demand is met by fossil fuels (*6*). The amount of fossil fuels available is limited and the use of fossil fuels is one of the main causes for anthropogenic greenhouse gas emissions. Greenhouse gases, as their name suggests, trap heat on to the earth's surface increasing the average global temperature, leading to serious environmental impacts such as the melting of the polar ice-caps, severe weather fluctuations, increased frequency of droughts and acidification of oceans to name a few (*7*). Energy production using fossil fuels is responsible for about 57% of the total greenhouse gas emissions (*8*). Biogas is a carbon dioxide (CO_2) neutral source of energy, in other words it uses the carbon that is already available in the existing carbon cycle and does not add to it and is hence a good substitute for fossil fuels.

Population growth will also lead to an increase in the amount of waste generated. Biogas produced from AD of wastes such as municipal solid wastes, agricultural residues and wastewater sludge, generates energy while reducing the volume of the waste. It also harnesses the methane emissions associated with the wastes; for example landfill emissions and emissions from manure storage. Methane is a potent greenhouse gas. It has a high greenhouse gas potential and is 21 times more effective (over a 100 year period) at trapping heat than carbon dioxide (*9*). 50% of the methane emissions on earth are by human activities such as livestock production, manure management, coal mining, landfills and rice production among others (*10*). AD of wastes that might have caused emissions in

landfills and from manure storage will reduce the amount of methane emitted to the atmosphere. In other words, the methane emissions from landfills and manure storage can be reduced considerably by using their organic fractions to produce methane under controlled conditions, and then collecting and utilizing the methane, effectively reducing the impact.

Compared to other renewable energy sources AD has certain advantages and disadvantages. Almost any organic matter can be degraded (to various extents depending on the substrate properties) to produce biogas. It can thus utilize agricultural residues such as crop residues and manure, and does not have to compete for land used for food production. If energy crops are being used, biogas production uses the entire plant instead of specific plant parts, like grain in the case of first generation bioethanol (*11*). The AD process does not need pure microbial cultures (*12*) it has a multitude of microorganisms working together and these microorganisms can be supplied as an initial inoculum or can be found in the feedstock in manure substrates and will find a steady population if the substrate input remains constant. As long as the substrate supply and the process is steady, the production of biogas can be maintained at a steady rate, this is an advantage when compared to renewable energy sources such as wind energy and solar energy that depend on weather conditions. The digestate (the material remaining after AD) is nutrient rich; the total nitrogen and phosphorous nutrient content remains the same in the digestate as in the original biomass as the only significant elements removed are carbon, hydrogen and oxygen in the form of biogas (*13*). Another advantage of the biogas process is that no product separation is required; methane has very low solubility in water and readily separates and collects in the headspace of the digester (*12*). Increase in bacterial biomass is much lower in anaerobic digestion when compared to the amount of sludge produced by aerobic or anoxic processes, as the amount of energy that the microbes gain in the anaerobic process is much lower in comparison (*2, 13*).

However, a major issue with anaerobic systems is process instability, usually caused by inhibition, feed overload, inadequate temperature control or washout of biomass (*14, 15*). Some of the features that make AD an attractive option are also responsible for a lot of issues that need attention. For example, it is an advantage to have multiple groups of microbes working together, but a disturbance in the synergy between the groups could lead to process failure. Currently, a lot of research is being focussed at making the process more reliable and efficient.

The following sub-sections will give an outline of the basics behind the anaerobic process followed by the factors that affect the biogas production and then a short paragraph on substrates for the production of biogas, which all lead to the topics that were investigated as part of this PhD study.

1.2 The anaerobic process

The production of biogas from the anaerobic decomposition of organic material takes place in multiple stages: hydrolysis, acidogenesis and acetogenesis and finally methanogenesis. The two products of anaerobic digestion are biogas and the digestate. The process has one extracellular step (hydrolysis) and three intra-cellular steps - acidogenesis, acetogenesis and methanogenesis (16). These steps are carried out by different groups of microorganisms: the fermentative bacteria (hydrolytic and acidogenic), the anaerobic oxidising (syntrophic and acetogenic) bacteria and the methanogenic archaea (17). Figure 1.1 shows a flow chart of the anaerobic conversion process.

Hydrolysis:

The hydrolysis step is extracellular and is brought about by various fermentative bacteria. Hydrolysis in anaerobic terms is the solubilization of solids and is accomplished via extracellular enzymes secreted by the bacteria. Three mechanisms have been proposed for the enzymatic hydrolysis processes occurring in the AD process. One mechanism suggests that the microbes secrete the enzymes to the bulk liquid which then adsorb onto a particle and react (*1*). Another mechanism suggests that the microbes attach themselves to the particle and release enzymes into the vicinity of the particle (*1, 18*). The third mechanism also suggests that the organism attaches itself to the surface of the particle, but in this case the enzyme is also attached to the organism and apart from its enzymatic functions it also acts as a transport receptor of the hydrolysis product to the interior of the cell (*1*). Enzymes such as proteases (from proteolytic bacteria) solubilize proteins while lipases (from lipolytic bacteria) breakdown lipids and cellulases and xylanases (cellulytic and xylanolytic bacteria) solubilize complex carbohydrates, into simpler compounds and monomers (*2*). In case of lignocellulosic substrates that are difficult to degrade or particulate substrates, hydrolysis is the rate limiting step (*18*). The products of hydrolysis are simple sugars, amino acids and long chain fatty acids-LCFA (organic acids with more than 5 carbon atoms) (*1, 3*).

Acidogenesis and Acetogenesis:

In acidogenesis, the sugars and amino acids from the hydrolysis step are converted into alcohols and organic acids by fermentative acidogens releasing carbon dioxide. In acetogenesis, the products from acidogenesis such as volatile fatty acids (VFA) and alcohols and the remaining products of the hydrolysis (for example- LCFAs) are oxidised by the acetogens into hydrogen and acetic acid (*2*). The products of these steps are acetic acid, hydrogen and carbon dioxide. The acetogenic bacteria have a symbiotic relationship with the methanogens. The production of acetate results in the release of hydrogen, and

acetogens which are obligate hydrogen producers cannot function at a hydrogen partial pressure above 10^{-4} atmospheres (*19*). Methanogens utilize the hydrogen to produce methane and keep the partial pressure of hydrogen low enough for the acetogens to function. Two other processes can take place when two distinct groups of microbes, the homoacetogens and the acetic acid oxidisers are present (*20, 21*). These two groups of microbes cause the inter-conversion of acetate to hydrogen and carbon dioxide (syntrophic acetate oxidation) and the conversion of hydrogen and carbon dioxide to acetate (homoacetogenesis) (*21*).

Methanogenesis:

Methanogens belong to the domain archaea and are obligate anaerobes (*17*). The products of the acidogenesis and acetogenesis step form the substrates for the methanogenesis stage. Acetic acid (CH_3COOH), carbon dioxide and hydrogen are the main precursors used by methanogens, however, carbon monoxide (CO), formate (HCOOH), methanol (CH_3OH) and methylamine (CH_3NH_2) can also be used to form methane. Methane production is brought about mainly by two pathways, aceticlastic methanogenesis and hydrogenotrophic methanogenesis. The acetate is cleaved by aceticlastic methanogens to produce methane and carbon dioxide (Equation 1) while the carbon dioxide is reduced by the hydrogenotrophs to methane (Equation 2).

Aceticlastic methanogenesis: $CH3COO^- + H^+ \rightarrow CH_4 + CO2$ Equation 1 (*17*)

Hydrogenotrophic methanogenesis: $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$ Equation 2 (*17*)

Methane production using hydrogen results in more energy gain when compared to aceticlastic methanogenesis; however the limited supply of hydrogen in the anaerobic digester leads to the dominance of the aceticlastic pathway (*19*). Hence, 70% of the methane output is produced using acetate as the precursor and 30% is by the use of hydrogen and carbon dioxide (*17, 19*)

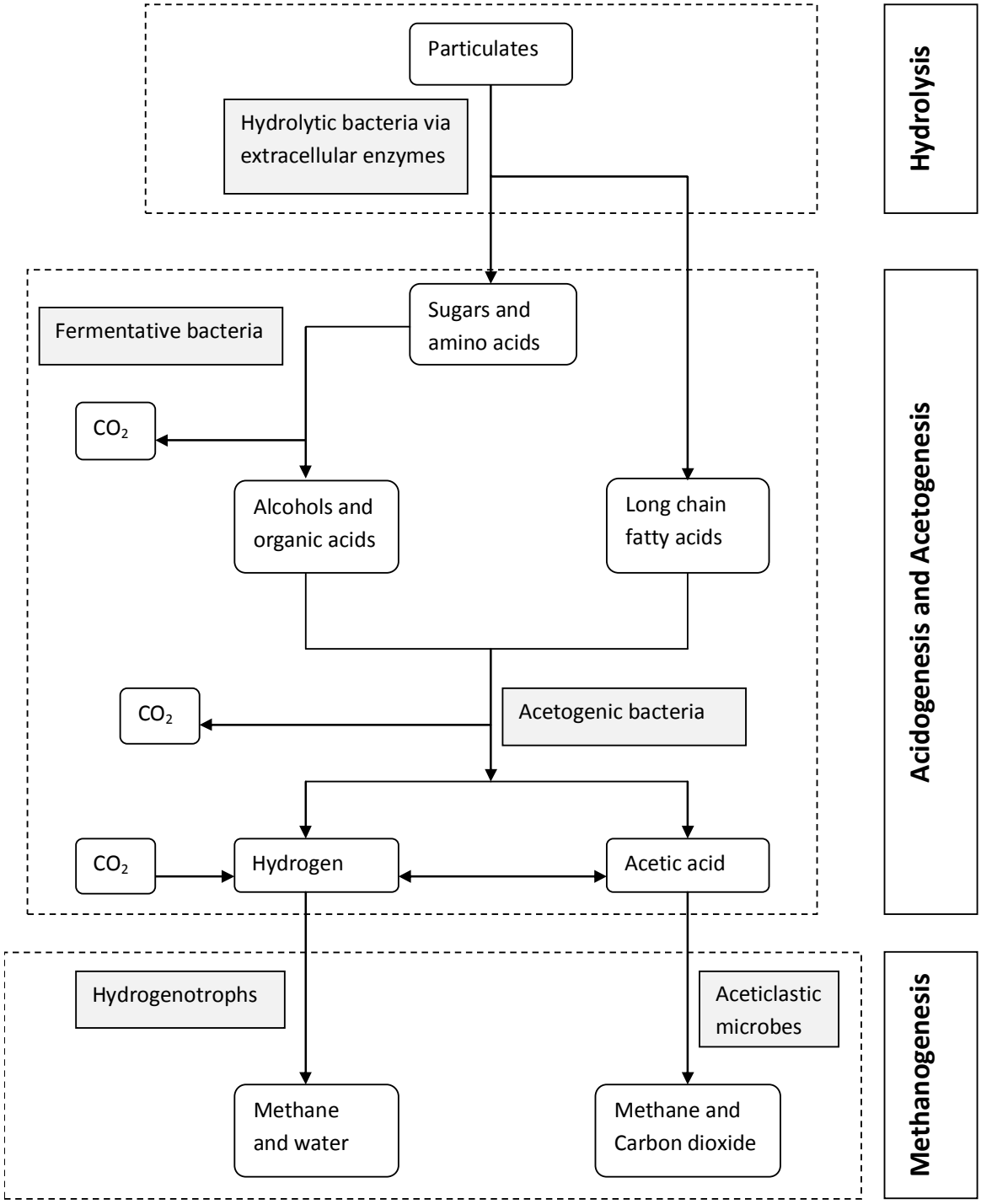

Figure 1.1: Methane production from organic substrates. Adapted from (2)

1.3 Factors affecting biogas production:

From the previous section it can be seen that anaerobic digestion is a complex process and requires the synergistic efforts of various microorganisms in various steps where the products of one step are utilized in the next one finally culminating in the production of biogas. A disturbance in one of the steps will therefore affect the entire process, and there are numerous factors that affect the process.

Parameters such as organic loading rates, temperature, pH, concentrations of nutrients and inhibitors such as ammonia and hydrogen sulphide are critical to the functioning of the AD process. Table 1.1 shows some of the parametric levels that must be maintained in digesters for optimal performance and the levels beyond which they are detrimental to the AD process.

The effect of these parameters are different on different microbial groups as each microbial group has different physiological and nutritional needs and different growth rates and it is imbalances between them that causes process instability (*22*). An imbalance in the process caused due to a disturbance in the hydrolysis stage will limit the activities in the consequent stages reducing the biogas production (*19*). A disturbance in the last stage that is the methanogenesis stage will bring about an accumulation of acids that have been formed in the previous stages (*19*). Changes in the process such as reduced biogas production, accumulation of VFAs, decrease in pH and alkalinity, increased concentrations of carbon dioxide are indicators of process instability (*19*).

Table 1.1: Factors affecting anaerobic processes.

Parameter	Optimal levels	Detrimental levels
pH	6 to7 for methanogens and acetogens 6 for acidogens (*4*)	<6 and >8.5 (*4*)
Temperature	30°C to 35°C for mesophilic reactors (*19*) 50°C to 60°C for thermophilic reactors (*19*)	Fluctuation > ± 2°C/day to 3°C/day (*19*) Fluctuation > ± 1°C / day (*15, 19*)
Organic loading rate	Depends on the composition of the substrate	> + 50% dissolved COD/day (*15*)
Free ammonia	< 0.2 g N/L (*22*)	1.7 to 14 gN/L (*22*) (Depends on the degree of acclimatization)

Changes in pH and changes in gas production or in gas composition are usually slow and while they can indicate gradual changes, they cannot be used to detect sudden changes in the process (*23*). Process instabilities lead to an accumulation of VFA which should lead to a corresponding drop in the pH. But in case of wastes that have a large buffer capacity;

manure for example, the pH change is noticeable only after the VFA concentration is very high. Thus, although pH is an easy parameter to measure and can easily be applied to online monitoring of reactors, it cannot be solely relied upon to monitor inhibition. On the other hand, VFA, which by itself in high concentrations is inhibitory to methanogens, can also be used as a process indicator (*23*). Some studies have suggested the use of individual volatile fatty acids such as propionic acid, acetic acid, butyric acid, iso-butyric acid or iso-valeric acid, as process indicators (*24, 25*). There is, however, no consensus on a general level of VFA that is inhibitory as the level depends on various factors, but unexpected increases in VFA could be seen as a sign of process imbalance that could possibly lead to process failure if the anaerobic process does not adapt to the new levels (*26, 27*).

Among a number of compounds that are toxic to the anaerobic microbes, ammonia is the most common inhibitor (*4*). However, ammonia concentrations below 200 mg/L are favourable for anaerobic microbes as it is an essential nutrient (*22*). Ammonia is present in the form of the ammonium ion (NH_4^+) and free ammonia (NH_3), of which the free ammonia (FA) is suspected to be the main cause for inhibition as it is membrane permeable and can diffuse into the microbial cells (*22*). Of all the microbes that are part of the anaerobic process, the methanogens are least tolerant to ammonia inhibition (*22*). The ammonium ion and FA exist in equilibrium, and the equilibrium depends on the temperature and pH. Increases in pH lead to an increase in concentration of FA (*28*). Anaerobic digesters are operated typically at mesophilic (20°C to 45°C) or thermophilic (45°C to 60°C) temperatures; those operated at temperatures below 20°C and are called psychrophilic reactors (*29*). Higher operating temperatures usually result in higher degradation rates and higher microbial growth rates, but also make the process more unstable and susceptible to ammonia inhibition (*1, 4, 28*). The increase in the FA concentration along with temperature makes thermophilic reactors more susceptible to ammonia inhibition compared to mesophilic reactors.

The organic loading rate (OLR) is the substrate input rate per unit volume of the reactor in terms of its organic content. The OLR is taken into consideration when deciding on the hydraulic retention time (HRT) of a reactor (*27*). In other words, each reactor with a particular HRT is designed to handle a certain organic load. Overloading will cause an initial increase followed by a decrease in biogas production and the accumulation of VFAs which if severe enough can inhibit the methanogens (*27*). Currently, feeding strategies in full scale anaerobic digesters are volumetric or gravimetric and not based on the actual quality of the input substrate (*30*). This is not an issue with farm based digesters which are usually designed to handle waste from a particular farm. In case of reactors where the substrates are mixtures of wastes or where complex substrates such as agricultural wastes whose quality can vary are used, it is important to be able to assess the substrate quality in terms of the amount of degradable solids and the amount of inhibitors if any. This is mainly in order to optimize the substrate utilization and hence the biogas production.

1.4 Substrates for biogas production:

The substrate used to produce biogas in a digester is an important aspect that determines the OLR, the amount of biogas and the methane fraction of the biogas produced (*2*). The amount of biogas that can be produced from a particular substrate can be determined in various ways. The biochemical methane potential (BMP) assay is the most common experimental way to determine the amount and the rate of methane that can be produced from a substrate (*31*). This method was proposed by Owen *et. al.* in 1979 and is a batch process (*32*). A known amount of substrate is introduced into a flask containing a known amount of inoculum(an active culture of anaerobic bacteria), and if necessary, a nutrient solution (*13*). The flask is sealed and placed into an incubator maintained at a chosen temperature and the biogas production is measured over a given time period or till the biogas production is negligible over a long time period. The results are usually expressed in unit volume of biogas or methane produced per unit weight of volatile solids that was added as substrate. Although the results of a BMP batch test cannot be directly compared to the outcome that can be expected in a full scale continuous reactor, the assay provides a basis for comparison among different substrates. The disadvantage of the batch assay is the time taken for the experiment. While 30 days is the recommended time period over which the biogas production should be measured, some studies have used up to 100 days to test the BMP of recalcitrant substrates (*32, 33*). Due to the long time period required to assess the BMP of a material, in full scale operations, substrates are fed by weight or volume based on previous experience, and not based on the actual substrate quality and thus real-time adjustments of substrate input cannot be made (*34*). A reduction in the time taken to assess the methane potential of substrates will enable real time assessment of substrates in full scale biogas plants. Rapid assessment of the methane potential of feed-stock that is to be purchased can be used to determine its monetary value.

As mentioned earlier, the advantage of AD is that almost any biomass can be used as a substrate for biogas production, thus the options to choose from are numerous. Much research has gone into identifying species and cultivars of energy crops that can produce more biogas. Germany for example uses maize as one of the main substrates in their biogas plants (*34*). In general, energy crop production has been criticised for competing with food production for arable land. Agricultural and livestock residues offer a good alternative to energy crops and have a great potential for biogas production because of the large quantity of organic matter contained in them (*3*).

Intensive animal farming generates large quantities of manure; Denmark, for example produces more than 33 million tonnes of manure per annum and many of the biogas plants in Denmark run on animal manure along with wastes from industries (*35, 36*).

However, the issue with many agricultural and livestock residues is their recalcitrance to AD. Agricultural residues such as straw, rice husk or wood chips often contain high concentrations of ligno-cellulose which is difficult to degrade. About 40 to 50% of the total

solids in manure are bio-fibres, a considerable part of which are recalcitrant to anaerobic digestion (*37*). The use of such biomass for AD may require pre-treatment to improve their degradability.

Meadow grasses are a promising source of biomass that have been shown to be a good option for AD due to various reasons such as availability, the possibility for nutrient transfer and low energy and chemical input requirements (*38, 39*).

1.5 Objectives of the PhD study:

The previous sections have outlined the importance of monitoring the factors that affect biogas production. A lot of research has been carried out in using VFA as a monitoring parameter, some of them have used near infrared spectroscopy (NIRS) as the monitoring tool (*23, 25, 40, 41*). There have also been a lot of studies on the effects of ammonia on the biogas production (*28, 42, 43*). There is however a gap in real time measurement and monitoring of ammonia concentrations in substrates, and in the contents of an anaerobic reactor.

Another interesting issue is the need to determine the methane potential of a particular substrate in a relatively short period of time as this could be used for substrate quality assessment and valuation and to determine the substrate feeding rate.

It has also been shown that certain types of substrates, although feasible in many ways, may require pre-treatment to improve the amount of methane that can be obtained from them before using them as substrates in a reactor.

Thus the objectives of this PhD study were:

1. Rapid assessment of the methane potentials of substrates
2. Determining the ammonia contents in complex mixtures such as manure or digestates from anaerobic digesters.
3. Improving the biogas potentials of agricultural and livestock residues using pre-treatments

These objectives were achieved by performing the following studies:

1. Comparing the use of forage analysis techniques such as in-vitro organic matter digestibility assay (IVOMD) and the neutral detergent fibre (NDF) assay and NIRS to determine the BMP of meadow grasses
2. Use of NIRS to assess the total ammonia nitrogen (TAN) contents of digestate using a probe that can be fitted directly onto an anaerobic reactor making it feasible for online monitoring.
3. Thermal pre-treatment of cattle, pig and chicken manure to improve their BMPs

Apart from these main experiments on which Papers 1, 2 and 3 were based, a short study on improving the sample presentation method used in Paper 2 has been presented in the form of a report.

1.6 Overview of the thesis structure:

While chapter 1 aims at giving a basic idea about biogas and some of the issues that need attention which leads to the objectives of the study and how these objectives are met, chapter 2 focuses on the use of near infrared spectroscopy to address objectives 1 and 2. Chapter 3 explains the pre-treatment of substrates to improve their BMPs. This is followed by the appendix which includes Papers 1, 2 and 3, and a short report.

1.7 List of papers:

Paper 1:

Comparison of near infra-red spectroscopy, neutral detergent fibre assay and in-vitro organic matter digestibility assay for rapid determination of the biochemical methane potential of meadow grasses

Chitra Sangaraju Raju, Alastair James Ward, Lisbeth Nielsen, Henrik Bjarne Møller

Journal - Bioresource Technology 102 (2011) 7835–7839

Paper 2:

NIR monitoring of ammonia in anaerobic digesters using diffuse reflectance probe

Chitra S Raju, Mette Marie Løkke, Sutaryo Sutaryo, Alastair J. Ward, Henrik B. Møller

Journal – Sensors 12 (2012) 2340-2350

Paper 3:

Effects of high temperature isochoric pre-treatment on the methane yields of cattle, pig and chicken manure

Chitra Sangaraju Raju, Sutaryo Sutaryo, Alastair James Ward, Henrik Bjarne Møller

Accepted- April 2012, Environmental Technology

Chapter 2 - NIRS in Anaerobic digestion:

2.1 Introduction:

Near infrared spectroscopy (NIRS) is a non-destructive analytical method that is widely used in applications where quick and efficient analysis is required, for example in industries for the rapid measurement of chemical composition or nutrient contents of materials or for quality control in pharmaceutical industries and food industries (*44, 45*). As it requires very little or no sample preparation it can be used for online monitoring and process control. Recent studies have used NIRS in the anaerobic digestion process for various purposes such as process monitoring, feed input control and early warning systems for process imbalances, to name a few (*30, 31, 40, 41, 46, 47*).

The main principle behind NIRS exploits the chemical nature of the components that are in the sample that is being measured or scanned. Organic substances include molecular groups such as, -CH-, -NH- and -OH which have characteristic absorbance patterns (*48, 49*). Molecules are in a constant state of motion and vibrate in the wavelengths associated with the infra-red region (*48*). NIR spectroscopy involves irradiating a sample with radiation of wavelength within the near infrared region (between the wavelengths 780 and 2500 nm) and measuring either the reflected energy or the transmitted energy to study the changes in the overtones and combination vibrations of molecules (*50, 51*). The energy that is absorbed by a sample, in other words the wavelength of the absorption band, depends on the molecular groups present, and hence identity of the molecular group can be determined based on the measurements from NIRS.

The basic components of an NIR spectrometer consist of a radiation source, a monochromator or an interferometer, a sample presentation accessory, and a detector (*50-53*). The radiation source is usually a tungsten halogen lamp, the detector could be silicon, lead suphide (PbS), indium gallium arsenide (InGaAs) or indium arsenide (InAs) (*48, 53*).

Fourier-transform NIR (FT-NIR) spectrometers are used for their fast measurement capability and for the advantage of obtaining a full spectrum in a single scan by measuring all frequencies simultaneously (*31, 51*). The components of an FT-NIR are shown in Figure 2.1. Radiation from the source is sent through an interferometer and then to the sample (*54*). Depending on the mode of spectroscopy (for example, transmission or diffuse reflectance), the transmitted or reflected signal is sent to the detector. The signal from the detector is amplified and converted to a digital form and transferred to the computer (*54*). In case of measurements in the diffuse reflectance mode, the spectral data obtained, is recorded as log 1/R where R is the diffuse reflectance (*55*).

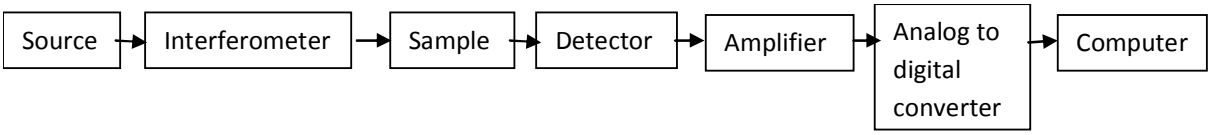

Figure 2.1: Basic components of an FT-NIR spectrometer. Adapted from (54).

Spectral data from NIRS are often noisy and measurements from samples that scatter incident light, such as ground meadow grass and digestate samples, add to the noise. It is thus important to pre-process the spectral data before building models based on it. There are various pre-processing methods that have been developed for different purposes. They can roughly be classified as scatter correction methods: multiplicative scatter correction (MSC), extended multiplicative scatter correction (EMSC), Detrend, standard normal variate (SNV) and as derivative methods: Norris-Williams and Savitzky-Golay

Building models using spectral data to predict the reference variable can be explained as follows.

Basic definitions (*56*):

- Object – the sample that is under observation (in this case either a meadow grass sample or digestate sample)
- The X-variable/ independent variable – Inexpensive or fast observation made on the object (in this case spectral data)
- Y- variable/ dependent/ reference variable – The expensive or time and labour intensive observation made on the same object (in this case BMP or TAN value for each of the sample)

The data is organised in the form of matrices to facilitate analysis. If there are '*n*' number of objects, '*p*' number of X-variables and '*q*' number of Y- variables, the data matrices could be represented as shown in Figure 2.2. The X matrix has *n* rows of spectral data and p columns of spectral variables; each object is represented by one row containing *p* number of spectral variables. Similarly the Y matrix has n rows, each row representing one object with q dependent variables.

Using multivariate analysis techniques, calibration models are built by combining the *p* measurements in X to give as good a prediction of Y as possible (*57*). This model is then used to predict the Y value of new samples based on their spectra i.e. X values (*57*). In other words, the spectral data are pre-processed if needed, and then related to a selected reference variable using multivariate analytical methods. Based on the correlations from these analyses, models to predict the reference variable are constructed.

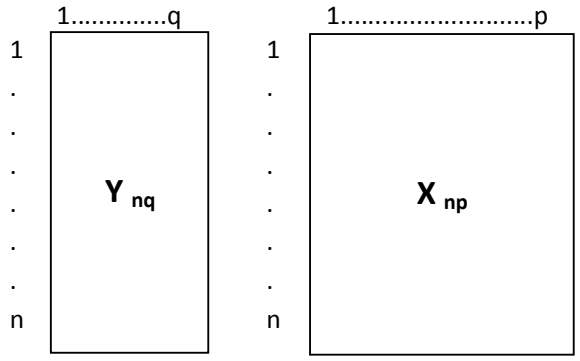

Figure 2.2: Organisation of data for multivariate analysis. Adapted from (56, 57)

Once a calibration model has been built the performance of the model can be tested by validation to evaluate its predictive ability (*56*). The model performance and accuracy is evaluated based on the following terms. The root mean square errors are a measure of prediction error depending on the type of validation used: cross validation (RMSECV) or test set validation (RMSEP) (*49*). The root mean square error has to be minimized as it gives the average uncertainty of future predictions(*50*). The coefficient of determination (R^2) is the proportion of the variance explained by the model (*58*) and is to be maximized. The number of components or latent variables is to be kept as low as possible without compromising the model quality. Too many components will lead to over-fitting with a low root mean square error of calibration (RMSEC), high R^2 but a very high RMSEP (*53, 56*). The RPD is the ratio of the standard deviation of the response variable to the RMSEP or standard error of performance of the model (*50*). An RPD greater than 1.5 is moderately useful and an RPD above 4 is considered an excellent model (*45, 59*). The RPD is particularly useful in comparing the prediction abilities between alternative models (*60*).

2.2 Application of NIR in the anaerobic digestion process:

As part of this PhD study, NIRS was used to predict the BMPs of Danish meadow grasses (Paper 1) and to predict the TAN contents in the digestate of anaerobic digesters (Paper 2).

Paper 1 was aimed at finding a faster way of determining the BMP of materials. For this, three analytical methods were tested: NIRS and two forage analysis techniques- in-vitro organic matter digestibility assay (IVOMD) and neutral detergent fibre assay (NDF). The BMP assay takes between 30 to 100 days and is the most common method used to determine the amount of methane that can be obtained from a certain substrate through anaerobic digestion (*32, 33*). There have been quite a few studies that have successfully related individual chemical components of substrates such as lignin, cellulose, hemicellulose, ADF, soluble carbohydrate, and nitrogen content among others and

combinations of these components to their BMPs (*33, 61, 62*). Compared to the BMP assay or the chemical analyses, NIRS is very quick and as mentioned earlier the NIRS method does not require the use of chemicals and can be used online and inline.

The nutrition that ruminants can obtain from forage depends on the degradation of the plant cell wall by the rumen microbes (*63*). The IVOMD which can be expressed in percentage of dry matter (%DM), indicates the percentage of material that can be digested by the ruminants and hence the nutritional value of that feed. Since the process is anaerobic, it was interesting to see if the results of this assay would correlate to the BMP.

The NDF assay measures the cell wall components and includes cellulose hemicellulose and lignin (*64, 65*). The pectic polysaccharides are not measured but since grasses have a low pectin concentration in their cell walls, NDF is considered a good estimate of cell wall contents in grasses (*65*). The aim was to see if just the cell wall contents, since it essentially represents the lignocellulosic complex, could be used to determine the BMP of a material.

Paper 2 is focused on the application of NIRS in monitoring the anaerobic digestion process. Anaerobic digesters, especially those using livestock wastes and those that operate at thermophilic temperatures are susceptible to ammonia inhibition (*42*). Some studies have successfully used NIRS to monitor VFA's (*40, 47, 66*). Paper 2 investigates the use of an NIR diffuse reflectance probe that can be directly fixed on to a reactor to predict the TAN content of digestate.

2.3 Equipment and materials:

The spectrometer was a Bomem QFA Flex Fourier transform - NIR spectrometer (Q-interline A/S, Copenhagen, Denmark). The detector that was used depended on the material that was being scanned. The InAs detector was used for the meadow grass experiment (Paper 1) while the InGaAs detector was used for the digestate samples (Paper 2).

Materials used: Dried meadow grass samples obtained from various locations in Denmark were used to relate their BMP's to their spectral data. Digestate samples from bench scale continuous stirred tank reactors (CSTR) were used for the ammonia monitoring experiment. More details regarding the materials can be found in Paper 1 and Paper 2.

2.4 Multi-variate data analysis software:

Two commercially available software, the Unscrambler ver. 9.8 software (CAMO Software A/S, Oslo, Norway) and LatentiX software ver. 2.00 (Latent5, Copenhagen, Denmark) were used for data analysis and for building models.

2.5 Data analysis:

Various multivariate analytical methods and data pre-processing methods were used to derive useful conclusions from the large amount of spectral data that was obtained from scanning the various samples. There are various multivariate analytical techniques available like multiple regression, principle component regression (PC/MR) and partial least squares regression(PLSR) (*57*). The multivariate methods used in this PhD study are described in the following subsections.

Data pre-processing:

The data pre-processing methods available in the Unscrambler ver. 9.8 were used to improve the models obtained from the spectral data. The pre-processing methods that improved the models the most are described here. In Paper 1 the best results were obtained with mean normalized data. Mean normalization is a row operation where each row of spectral data is divided by its average value. The original values describing the object are replaced by relative values (*67*). In Paper 2, standard normal variate (SNV) transformations are used to remove the effects of scatter(*68*). In SNV transformations, each spectrum is centered and then scaled by its own standard deviation (*55*).

Principal component analysis (PCA):

When dealing with large data matrices, PCA is used to reveal variables that describe some inherent structure in the data and to reduce the dimension of the data without loss of information (*44, 69*). This is done by finding a linear combination of data (a principal component) that has maximum variance, and then the next linear combination that has the second highest variance is determined and so on until most of the variance in the data has been described by those components. In other words, the first component is a vector that lies in the direction of the largest variance; the second is orthogonal to the first component and lies in the direction of the next largest variance and so on. These principal components are used to describe the data thus reducing the dimension of the data. PCA separates the data structure from noise (*66*). It also groups objects with similar characteristics together and aids in identifying outliers. PCA decomposes the X matrix into two smaller matrices called the scores and the loadings (*70*). It is possible to have an overview of the associations between objects and variables using the scores and loading plots obtained from PCA (*71*).

Partial least squares (PLS) regression and interval PLS (iPLS):

PLS is useful where a large amount of independent variables (in this case spectral data) are used to predict a set of dependent variables (BMP in Paper 1 and TAN in Paper 2). It can analyze data that is highly correlated and noisy (*72*). Ordinary multiple regression is not suited for spectral data as the spectral data are highly correlated (*57*). Unlike PC/MR, the PLS method uses information from both the spectral data set and the dependent variable data set to determine the PLS components/ latent variables and the components are obtained by maximizing the covariance between the spectra and the dependent variable (*73*). PLS assumes that the underlying set of latent variables for both the spectral data and the response variable are the same (*74*). The purpose of PLS is to build a linear model that can use spectral data to predict the dependent variable (*75*).

Interval partial least squares regression (iPLS) is a method that selects variables that are most useful for a model by building local PLS models on equidistant sub-sections of the spectrum and comparing the performance of these models in terms of RMSECV with the global model (*75*). The output is graphical and it visually represents the wavelength ranges that have been used for modelling (Paper 2). iPLS is a method that optimizes the predictive power of PLSR (*75*).

2.6 Summary of results:

The results from the studies that have been done (Paper 1, Paper 2 and the Report) show that NIRS is a promising analytical tool in process monitoring as well as feed input management.

- The NIR prediction of the BMP of meadow grasses had an RPD of 1.75 which makes it a moderately useful model that can discriminate between high and low values of the response variable (*45, 50*). The NDF and IVOMD showed very little correlation to the BMP (Paper 1).
- The study using NIRS to detect TAN in digestate was successful, and can in future be optimized to be used as a monitoring tool (Paper2).
- The importance of choosing the right variables while building a model was also demonstrated (Paper 2).

Further studies can be directed at building models to predict the BMP of other common substrates that are used for biogas production. These models can be used to classify a substrate as having a high or low BMP. By classifying the substrate and by recognizing if the material has ammonia concentrations that are inhibitory, the feeding rate of the substrate or in case of co-digestion plants, the ratio in which it is mixed, can be

controlled. As NIRS has been shown to predict TAN contents in a complex material such as digestate (Paper 2), it could be used to screen manure based substrates for their ammonia contents prior to loading into the reactor thus preventing the risk of inhibition and to maintain an optimum C/N ratio. The results from the Report show that the way the sample is presented to the NIR instrument is important, which suggests that the model in Paper 2 could be improved further. While extremely good results can be obtained in the laboratory by keeping conditions as similar as possible, it would be better to mimic practical conditions even though the quality of the models may degrade. If a model is to be applied to process monitoring it is important that the calibration contains all ranges of the response variable that might be encountered and that it includes all possible variations in conditions that might occur (*76*).

Chapter 3 – Pre-treatment of substrates

3.1 Necessity of pre-treatment

The use of agricultural and livestock waste for the production of biogas is often uneconomical due to the content of recalcitrant organic matter. The economical profitability of biogas plants that use such waste as primary feedstock depends on the addition of other substrates with high methane yields (*77*). Biogas yield of pig and cow manure is between 25 and 36 m^3/tonne of fresh mass due to a low organic dry matter content (2 to 10%) with a high fibre fraction (*78*). Manure based biogas plants in Germany add co-substrates such as energy crops, waste from food and agricultural industries, markets, canteens, and the municipal sector to improve profitability (*78*). Similarly most large scale biogas plants in Denmark use manure as feedstock along with co-substrates such as sewage sludge and industrial organic waste (*77, 79*).

Manure characteristics differ depending on various factors and therefore their methane potentials differ as well. Some of the factors that affect the manure characteristics are, the species, breed and growth stage of the animals, the feed being used, the amount and type of bedding material, the stabling system used, and the method and period of storage (*80*). The fibre fraction of manure consists mainly of undigested plant material, nutrients and often includes bedding material (*81*). About 5 to 73% of the organic matter in manure consists of lignocellulosic fibres that are recalcitrant to microbial degradation (*62*).

Agricultural residues such as straws, rice husk, and corn stover have high lignin concentrations. Wheat has a lignin content of 15 to 19% whereas corn stover has been reported to be about 19% lignin (*77, 82*). As mentioned in the introduction, the presence of lignin creates a barrier for microbial degradation of the lignocellulosic complex and a higher lignin content has been related to lower methane yields (*62*). Thus the use of agricultural or livestock waste to produce biogas has to overcome the issue of lignocellulose, to anaerobically convert as much of the volatile solids in the given timeframe as possible.

Lignocellulosic content is a term used to describe the three dimensional composite structural material in a plant cell wall, which is mainly 30 to 50% cellulose, 15 to 35% hemicellulose and 10 to 30% lignin (*82, 83*). The lignocellulosic complex varies among plant species and in addition the composition and percentages vary within the same plant species depending on age, growth stage and other factors (*84*). Lignin is a phenolic polymer and is not degradable by anaerobic processes, whereas the cellulose and hemicellulose are carbohydrate polymers (*82, 85*). The lignin, cellulose and hemicellulose are closely associated and form tight complexes, limiting the access of hydrolytic enzymes to the cellulose and hemicellulose and slowing the rate of hydrolysis. Lignin is the natural defence of plants against microbial attacks and hence some intervention is needed to break

down the lignocellulosic complex before it can be degraded anaerobically within a given timeframe. Pre-treatments are aimed at removing or changing structural and compositional constraints to improve the hydrolysis rate (*86*). An effective pre-treatment, solubilises hemicellulose thus releasing sugars, decreases cellulose crystalinity, increases the specific surface area and results in increased access of enzymes for hydrolysis, with minimum formation of inhibitors and loss of substrate (*77, 87*).

3.2 Lignocellulosic components

Lignin

Lignin is an amorphous phenolic polymer usually made of three different phenylpropane units (p-coumaryl, coniferyl and sinapyl alcohol) that are held together by different types of linkages (*88*). Lignin provides rigidity, impermeablity and resistance to microbial attacks and to oxidative stress to the plant cells (*89*). Higher proportion of lignin indicates higher resistance to chemical and enzymatic degradation and lower methane potential of susbtrates (*62, 90*). Solubilization of lignin occurs with alkaline agents and at temperatures above 180°C (*83, 91*).

Cellulose

Cellulose is a linear polymer composed of cellobiose units (a glucose-glucose dimer) and the hydrolysis of cellulose releases the individual glucose monomers: the process known as saccharification (*82*). The cellulose chains are grouped together to form microfibrils and the microfibrils are bunched together to form cellulose fibres (*89*). The amorphous and crystalline nature of cellulose is attributed to the presence of inter-chain hydrogen bonds within the microfibrils (*89*).

Hemicellulose

Hemicellulose is a carbohydrate polymer that surrounds the cellulose fibres and is made of both five-carbon pentoses (xylose and arabinose) and six-carbon hexoses (galactose, glucose and mannose) and acetylated sugars (*82, 89, 90*). Hemicellulose is highly branched and amorphous and hence is easily hydrolysed compared to cellulose (*82*). The hemicellulose composition differs in different biomasses (*89*). The solubilization of hemicellulose depends on the pH and the moisture apart from temperature and under neutral conditions solubilization of hemicellulose starts at 150°C (*88*).

3.3 Various methods of pre-treatment

The goal of pre-treatment methods applied to recalcitrant biomass is to alter the structure and chemical properties of the biomass to improve the rates of degradability (*92*). Pre-treatment methods can be classified as mechanical, thermal, chemical, biological and combinations of these.

Mechanical

Mechanical pre-treatments, which are usually size reduction techniques, aim at increasing the available surface area and reducing the cellulose crystalinity and the degree of polymerization (*88, 89*). Coarse size reduction reduces the biomass size to about 10 to 50 mm, chipping reduces the size to 10 to 30 mm while grinding and milling can reduce the sizes to between 0.2 to 2 mm (*89*). The advantages of this method are that there is no risk of formation of inhibitory compounds, and there is improvement in the methane yield due to size reduction in some cases, the main disadvantage is the high energy requirements (*88*). In certain cases though, a minimal size reduction is required to overcome heat and mass transfer problems in downstream processes (*83*).

Thermal

There are different ways of applying thermal pre-treatment to improve the hydrolysis rate of lignocellulosic biomasses. Steam treatment - where the biomass is exposed to temperatures up to 240°C and pressure for a few minutes (*88*). Steam explosion is similar to steam treatment, except at the end of the pre-treatment period the pressure is released suddenly, causing the disruption of the structure of the material (*88, 93*). Liquid hot water treatment, where the water is maintained as a liquid at high temperatures (160 to 230°C) and under high pressures (>5 MPa) (*83, 88*) or just thermal pre-treatment, an isochoric or constant volume process, where the material is placed in a sealed container and heated without applying extra external pressure (*91*). Another method is autohydrolysis, a process that hydrolyzes hemicelluloses using highly pressurised liquid hot water at 200°C (*86*).

Chemical

Acid or alkali based pre-treatments can be used alone or in combination with thermal pre-treatment. Acid based pre-treatments use either dilute or strong acids to hydrolyse the hemicellulose content and to solubilize and precipitate lignin (*88*). Degradation products such as furfurals or hydroxymethyl furfural (HMF) are formed during acid hydrolysis (*83*). Although these compounds are inhibitory to methanogens, they adapt to them after

acclimatization, to a certain extent (*88*). The choice of acid is important, using sulphuric or nitric acid introduces sulphates or nitrates into the system and reduces the methane production (*88*).

Addition of acid to thermal pre-treatment catalyses the solubilization of hemicellulose and could reduce the optimal pre-treatment temperature (*88*). In essence thermal pre-treatments by themselves behave like dilute acid hydrolysis. Water at high temperatures behaves as an acid and the hydrolysis reaction is catalysed by hydronium ions (H_3O^+), in addition acetic acid is released from the hemicellulose fraction under high temperatures adding to the effect(*83, 86*).

Alkali pre-treatment uses bases like calcium oxide, ammonia and sodium hydroxide to solubilize lignin (*83*). In alkaline hydrolysis there is an increase in internal surface area of the lignocellulosic material due to swelling induced by the alkali (*89*). This, along with saponification of the intermolecular ester bonds that link hemicelluloses to other components, leads to the separation of the lignin and the remaining carbohydrates (*86*).

Since the lignocellulosic composition varies among plant species, the efficiency of separating the lignocellulosic components can be improved by exploiting these variations. For example, the structure, composition and properties of lignocellulose from herbaceous materials like wheat straw are very different from those of softwoods or hardwoods (*94*). Alkaline pre-treatment methods are more effective on materials with low lignin contents such as agricultural residues, herbaceous crops and hardwoods than on softwood which has high lignin contents (*95*).

Biological

Biological pre-treatments mainly use fungi to degrade the lignin fraction (*83*). A wide variety of fungi and bacteria are known to degrade lignin, of which white rot fungi are thought to be the most efficient (*84, 96, 97*). Although the process is natural, and does not require the use of chemicals or energy, which reduces the costs involved, the process is slow and requires a longer residence time which is not practical for large scale applications (*83, 89*).

3.4 Pre-treatment method used in this PhD study:

Paper 3 investigated the use of thermal pre-treatment in improving the BMPs of different types of manure – cattle, dewatered pig and chicken manure. The process was carried out under isochoric conditions where a known amount of material was sealed in a high temperature and pressure reactor, and heated to the desired temperature, held at that temperature for the required amount of time and then cooled down to about 30°C. Figure

3.1 shows a typical heating and cooling curve for cow manure pre-treated at 100°C and at 200°C (Paper 3). The cooling was by a water bath with water at ambient temperature. During these pre-treatment experiments, only the temperature was constantly monitored and controlled, the pressure was not controlled.

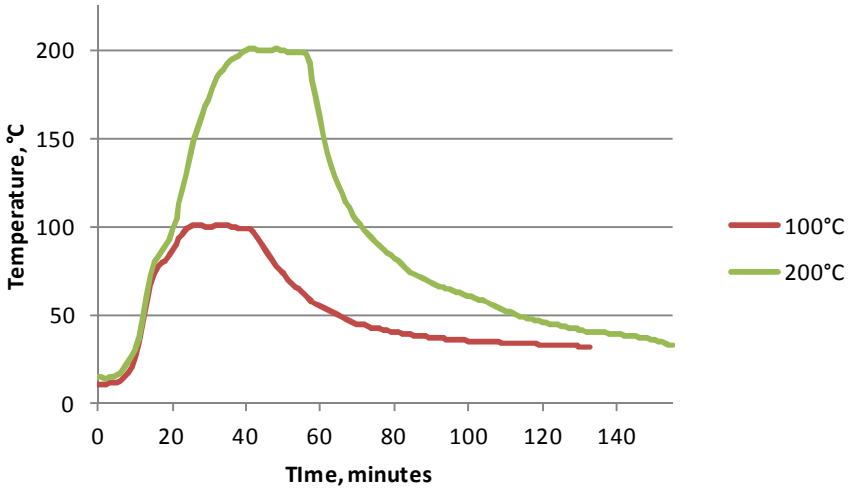

Figure 3.1: A typical heating and cooling curve for the pressure vessel.

Equipment and materials

The reactor used was a Parr high temperature and pressure reactor (Parr instrument company, USA, model -Parr 4524) and consisted of a stainless steel reactor vessel fitted with temperature and pressure measurement devices and safety valves. The reactor was also equipped with a proportional integral derivative (PID) controller that was used to monitor and control temperature, a temperature probe that was inserted into a thermo-well that extended into the pressure vessel and a mechanical stirrer with a six blade impeller and a variable speed motor. The heating was by an insulated external heating element enveloping the pressure vessel which could be raised or lowered manually. The unassembled and assembled reactor can be seen in Figure 3.2. The details of the manure samples with regards to their collection, dry matter contents, volatile solids contents can be found in Paper 3.

Figure 3.2: Parr high temperature and pressure vessel – unassembled and assembled

3.5 Severity factor

The results from Paper 3 when compared to those of other similar studies indicated that the duration of the pre-treatment also influenced the effect of the pre-treatment on the BMP along with the temperature used.

A term called severity factor is used to measure the severity of steam pre-treatment mainly in the bioethanol industry (*88*). The severity factor ($\log R_o$) combines the temperature and the duration of pre-treatment and is given by:

"$\log R_o = \log(t \times e^{((T-100)/14.75)})$"

- with 't' in minutes and 'T' in degrees Celsius (*88, 98*).

This term was applied to thermal pre-treatments to see if it could be correlated to the changes in BMP of manure. A plot of the BMP *vs.* the severity factor is shown in Figure 3.3. The data used to generate the plot along with the sources of the data is tabulated in Table 3.1.

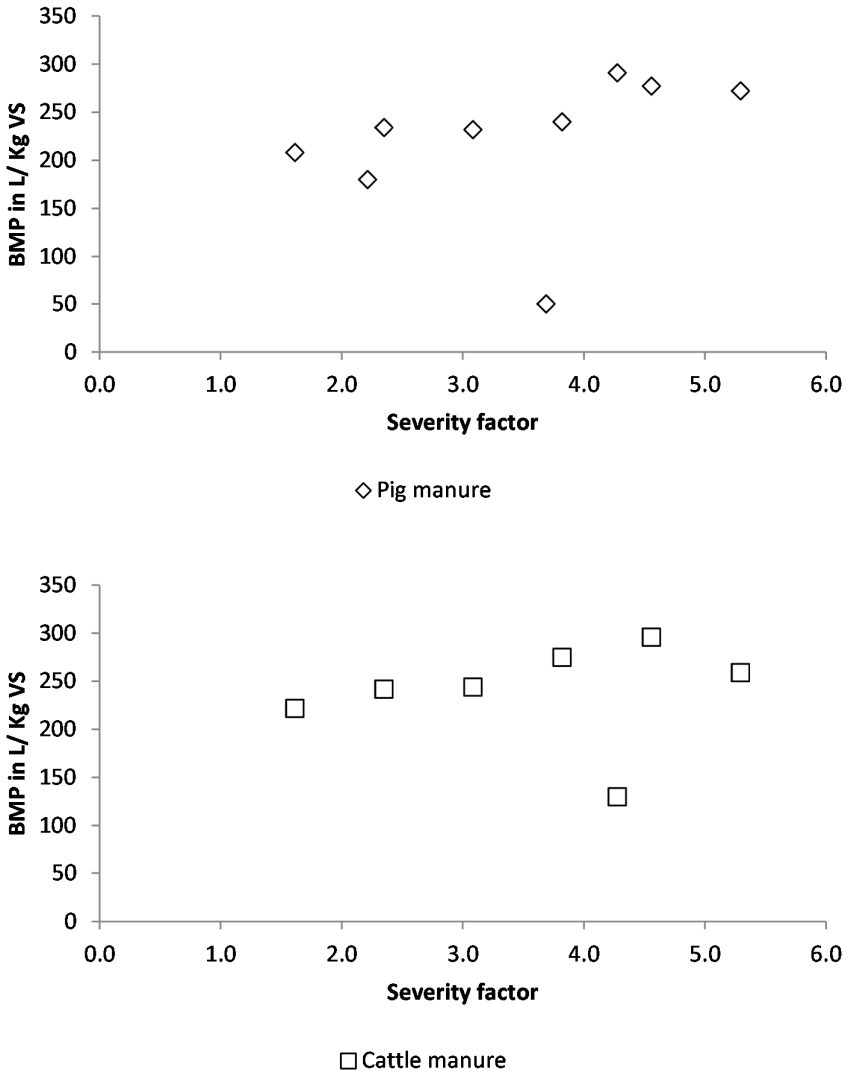

Figure 3.3: Correlation of BMP to severity factor of thermal pre-treatment for pig and cattle manure

From figure 3.3 it can be seen that other than one exception in each case, a general trend can be seen where the BMP increases along with the severity factor and reduces after a certain severity factor. The decrease in BMP is expected as at higher temperatures the formation of inhibitors or substrate degradation occurs (*86, 88*).

With more data points, a model could be made relating the severity factor to the BMP. The severity factor can then be used to decide if lower temperatures for longer pre-treatment duration or higher temperatures for shorter duration is more suitable and yet have the same effect on the BMP of that substrate. It would be expected that the BMP of each type of manure would have a different correlation with the severity factor. For example, it

would be expected that the BMP-severity factor model of straw would be different from that of dewatered pig manure.

Table 3.1: Data used to calculate the severity factor, along with the references for the sources

Reference	Manure type	Temperature in °C	Time in minutes	Severity factor	BMP in L/ Kg VS (30 days)
(*99*)	Dewatered pig manure	100	60	2.2	180
(*99*)	Dewatered pig manure	150	60	3.7	50
Paper 3	Dewatered pig manure	100	15	1.6	208
Paper 3	Dewatered pig manure	125	15	2.3	234
Paper 3	Dewatered pig manure	150	15	3.1	232
Paper 3	Dewatered pig manure	175	15	3.8	240
Paper 3	Dewatered pig manure	200	15	4.6	277
Paper 3	Dewatered pig manure	225	15	5.3	272
(*100*)	Pig manure	170	60	4.3	291
Paper 3	Cattle manure	100	15	1.6	222
Paper 3	Cattle manure	125	15	2.3	242
Paper 3	Cattle manure	150	15	3.1	244
Paper 3	Cattle manure	175	15	3.8	275
Paper 3	Cattle manure	200	15	4.6	296
Paper 3	Cattle manure	225	15	5.3	259
(*100*)	Cattle manure	170	60	4.3	130

3.6 Summary of results

The effect of thermal pre-treatment was more pronounced in some manure types than others. The effect mainly depended on the type of substrate, the pre-treatment temperature and the pre-treatment duration.

- Dewatered pig manure showed improved BMP from pre-treatment temperatures of 125°C onwards
- Cattle manure needed pre-treatment temperatures above 175°C for improved BMP
- Chicken manure showed no improvement in the BMP due to thermal pre-treatment but showed a decrease at 225°C
- In cases where significant increases were seen due to the pre-treatment, the biggest difference was seen within the first 30 days. Increases in initial methane production rates are important when considering full scale biogas plants.
- For practical applications, it seems thermal pre-treatment can be applied to dewatered pig manure, as, among the three manure types tested, it improves significantly at lower temperatures in comparison to the other two manures. A proper cost benefit analysis will be required to see if the pre-treatment can improve the economic profitability of the biogas plant.

One main aspect that is to be considered with thermal pre-treatment is the energy balance. Thermal pre-treatments have been applied to full scale operations to improve their economic profitability (*100, 101*). It is important to ensure that the use of energy for the pre-treatment is justified by more than an equivalent increase in energy in terms of methane yields. Thermal pre-treatments can be justified where waste heat is available and where the heat used for pre-treatment can be recycled or where the energy gain due to the pre-treatment is much higher than the extra energy input that is required.

Appendix

Paper 1

Comparison of near infra-red spectroscopy, neutral detergent fibre assay and in-vitro organic matter digestibility assay for rapid determination of the biochemical methane potential of meadow grasses

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Paper 2

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Article

NIR Monitoring of Ammonia in Anaerobic Digesters Using a Diffuse Reflectance Probe

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Abstract: The feasibility of using a diffuse reflectance probe attached to a near infrared spectrometer to monitor the total ammonia nitrogen (TAN) content in an anaerobic digester run on cattle manure was investigated; as a previous study has indicated that this probe can be easily attached to an anaerobic digester. Multivariate modelling techniques such as partial least squares regression and interval partial least squares methods were used to build models. Various data pre-treatments were applied to improve the models. The TAN concentrations measured were in the range of 1.5 to 5.5 g/L. An R^2 of 0.91 with an RMSEP of 0.32 was obtained implying that the probe could be used for monitoring and screening purposes.

Keywords: NIRS; biogas; ammonia; inhibition; monitoring; manure; PLS; iPLS

1. Introduction

Intensive farming methods generate large amounts manure that need safe disposal. In Denmark, more than 33 million tonnes of manure are produced per annum [1]. Current manure management strategies involve spreading of manure on agricultural fields to recycle the nutrients, aerobic treatment, separation of the solid and liquid fractions, composting and anaerobic digestion among others [2].

Denmark has many full-scale biogas plants that use livestock manure as substrate along with organic wastes from industries [3]. Livestock wastes contain ammonia, which is inhibitory to anaerobic digestion, and contain compounds like urea and proteins that will degrade into ammonia [4]. Ammonia is present in the form of the ammonium ion (NH_4^+) and free ammonia (NH_3), of which the free ammonia (FA) is suspected to be the main cause for inhibition [5]. The ammonium ion and free ammonia exist in equilibrium, and the equilibrium depends on the temperature and pH. A decrease in pH reduces the amount of free ammonia. When a process is inhibited by free ammonia, the methanogens are affected, and consequently the volatile fatty acids (VFA) accumulate reducing the pH, this in turn reduces the free ammonia concentration [4]. This leads to a stable condition but at a sub-optimal level called an inhibited steady state. Total ammonia nitrogen (TAN) levels of more than 4 g N/L were found to cause inhibition; levels beyond this showed stable biogas production after an initial adaptation period but this biogas yield was lower than that of uninhibited reactors [4]. Livestock manure can often have more than 4 g N/L of ammonia, especially in the case of swine manure and poultry manure [4], the ammonia concentrations can also be high in anaerobic co-digestion plants that mix high protein wastes to their substrates. Thus, monitoring the ammonia content of the slurry in anaerobic digesters is an important aspect of process control and in managing the substrate feeding rate.

Ammonia content is usually measured and monitored by laboratory analysis such as colorimetry. This procedure involves the use of reagents, is time consuming and is not practical for process control. Near infrared (NIR) spectroscopy has been used to monitor various process indicators in the anaerobic digestion process. Earlier experiments using Trans-flexive NIR Spectroscopy (TENIRS) has shown good results in predicting the ammonia contents in an anaerobic digester [6]. Another study that showed good results in applying NIR spectroscopy to predict ammonia, used the polyethylene bag method where a sample of cattle manure was filled into a polyethylene bag and then pressed on to the surface of the scanning window of the NIR spectrometer [7]. However, the TENIRS requires the use of a macerator to reduce the size of the slurry particles to below 3 mm before the sample can be sent through it. The polyethylene bag method requires the sample to be taken out of the reactor and then analysed elsewhere.

A previous study used a reflectance probe to monitor the propionate contents of a small continuously stirred reactor successfully [8]. It has also been shown that the probe can be directly fitted on to a reactor without major changes to the reactor body [9]. The aim of this study was to investigate the feasibility of predicting the ammonia content of manure using a diffuse reflectance probe. The reflectance probe also offers easy maintenance and unlike transmission spectroscopy does not depend on the transmission path lengths. A drawback of the reflectance probe is that more scatter is expected, and therefore more noise will be added to the spectra. This study describes the first step which is to determine if the diffuse reflectance probe can actually be used to determine the total ammonia nitrogen (TAN) concentrations in a complex material such as slurry from an anaerobic digester and if feasible, future studies can be directed at fitting the probe onto a full scale anaerobic reactor and test its performance. The slurry samples were scanned offline, using the diffuse reflectance probe. The spectral data was analysed using multivariate analysis and models relating the spectral data to the TAN contents were developed. Manure or slurry samples are a matrix of particles of different sizes and as a consequence, measurements based on reflectance mode will have variation in light scattering (*i.e.*, wavelength dependent path length variation) between samples which can negatively affect the

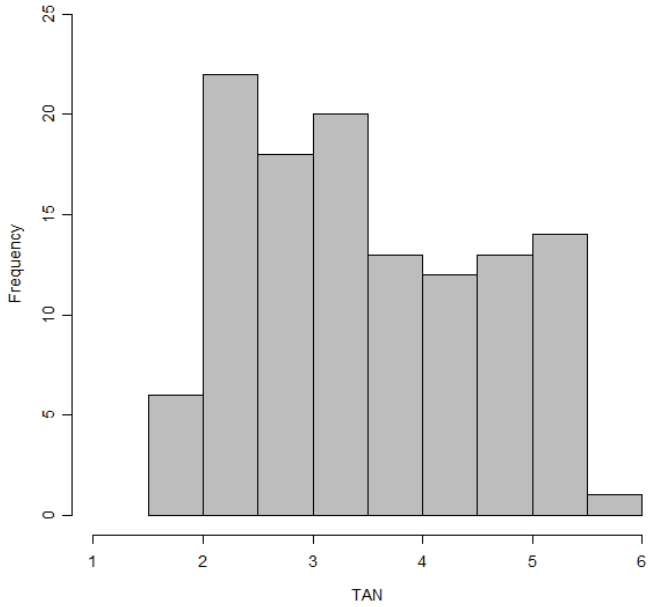
modelling process. The effects of scatter can be corrected mathematically using data pre-processing methods; such methods were used to improve the models.

2. Experimental Section

2.1. Sample Collection

Five bench scale continuous reactors were run on cattle manure that was collected from dairy cattle farms located in the Research Centre–Foulum (Denmark). The reactors had a working volume of 7 L and were operated at a thermophilic temperature of 50 °C. Four of the reactors were used to test the effect of ammonia inhibition on the methane yield while the remaining one served as the control. Urea (crystallized Ph. Eur Cat. No. 2880.362) at concentrations of 0.175, 0.350, 0.525, 0.700% w/w were added to the four reactors to induce ammonia inhibition. The reactors had a retention time of 14 days. About 200 g of digestate was collected from each of the reactors twice a week, and the total ammonia nitrogen (TAN) content of the samples in g/L was measured by colorimetry at 690 nm, using the Spectroquant ammonium test 1.600683(EPA 350.1) and a Merck® spectrophotometer After the TAN analysis the samples were frozen in 250 mL polyvinyl chloride (PVC) containers until the NIR scanning was performed. The TAN values were spread between 1.5 to 5.5 g/L and included many samples that had TAN levels more than the 4 g N/L above which process inhibition is said to occur [4]. These ranges were useful to see if the probe could detect ammonia at levels that are inhibitory and also at levels that are acceptable. Figure 1 is a histogram of the TAN values showing the spread of the reference data points. The effect of the ammonia inhibition on the methane yield of the manure will be published in a separate paper.

Figure 1. Histogram depicting the spread of the TAN values.



2.2. NIR Scanning

The NIR scanning was performed using a Bomem QFA Flex Fourier Transform spectrometer fitted with an InGaAs detector (Q-interline A/S, Copenhagen, Denmark). The diffuse reflectance probe that was used (QIA2050, also from Q-interline A/S) had a stainless steel body with a 5 mm sapphire window embedded into it and scanned in the range of 833.3 nm to 2,500 nm (12,000 cm^{-1} to 4,000 cm^{-1}). The probe is specifically optimized for materials with high scatter like slurry from anaerobic digesters.

The NIR scanning, as mentioned earlier, was performed offline. The frozen digestate samples were first brought to room temperature (19 to 20 °C) by thawing at room temperature overnight. The NIR probe was rinsed with de-ionized water, wiped clean with a tissue and then placed into the PVC container containing the digestate sample and clamped into position using a laboratory clamp stand such that there was at least 2 cm of sample beneath it, ensuring that the position was the same for every sample. An agitator was immersed parallel alongside the probe and the digestate sample was mixed at 190 rotations per minute (rpm) to make sure that enough sample passed in front of the scanning window of the NIR probe. The speed of the agitator was optimized by trying different rpm settings to ensure that there was no bubble formation which would negatively impact the scan while ensuring the sample did not settle. For each sample, the measurement took about 80 s and consisted of 256 scans which were then averaged for that particular sample. A total of 119 manure samples were scanned in a time-span of two days. The background scan was measured against a white spectralon disk.

2.3. Model Calibration and Validation

NIR spectra are often noisy [10] due to various reasons, including instrument noise and high absorbing materials, and detector performance. The entire spectra, obtained from the NIR spectrometer amounted to 1,006 spectral variables. A lot of the variables were noisy due to high absorbance in wavelengths above 1,800 nm and due to low detector sensitivity to wavelengths below 900 nm. These areas were consequently cropped. There is often offset and slope variation between NIR spectra of samples that have equal analyte concentration but different light scattering properties. Light scattering differences in spectra can be minimized by data pre-processing.

Data pre-processing is therefore a necessary step before modelling and can be classified into two main types. The first are scatter correcting methods such as multiplicative scatter correction (MSC), extended MSC (EMSC), standard normal variate (SNV), de-trending, baseline offset correction (BOC) and normalization. The second are spectral derivative pre-processing methods such as Norris-Gap (NG) and Savitzky-Golay (SG) polynomial derivatives. The data pre-processing methods available in the Unscrambler Version 9.8 software were applied to the spectral data and using the pre-processed data, models to predict the TAN content were developed.

Two modelling methods: Partial least squares regression (PLS) and interval partial least squares (iPLS) were used to relate the spectral variables obtained from the NIR to the reference variable (the measured TAN values).

The commercially available Unscrambler Version 9.8 software (CAMO Software A/S, Oslo, Norway) was used to develop the PLS models. The PLS is based on the regression method developed by Herman Wold [11]. Each model was validated by both full cross validation and test set validation. Full or

leave-one-out cross validation is a model validation method where one sample is left out iteratively and a calibration model is built, and then the sample that was left out is predicted using this model. The iteration is continued until all samples are left out of the calibration set once. For the test set validation the data set was divided into a calibration dataset and a validation dataset that both covered the range of ammonia levels: the data was listed according to the ammonia level, and every fourth sample was added to the test set (29 samples) and the rest of the samples were included into the calibration set (90 samples).

The other method used for modelling was the iPLS which is a graphically oriented local modelling procedure [12]. The iPLS builds local models on sub-intervals of the whole spectrum and selects the optimum sub-intervals in the spectral data to give precision prediction models [12,13]. Each sub-interval contains a selected number of spectral variables. The iPLS models were built using the PLS toolbox Version 6.2.1 (Eigenvector Research Inc., Wenatchee, WA, USA) in Matlab Version 7.12 (MathWorks, Natick, MA, USA). The iPLS was run using forward selection on raw and on SNV pre-processed spectra with a sub-interval size of 30 variables and a maximum of four PLS components (or latent variables) were allowed. To decide upon the number of components that could be used, the number of components was varied and looking at the RMSEP of the full model it was found that there was no advantage in using more than four components. For validation during iPLS optimization, full cross validation was used. When the optimum interval combination was found, the model was validated using the test set.

The prediction performance of the models was evaluated based on their modelling parameters: the coefficient of determination (R^2), the root mean square error of prediction (RMSEP) and by their residual prediction deviation (RPD) which is the ratio of the standard deviation to the RMSEP [14]. The number of principal components used to construct the model was also used as an indicator. High R^2 and RPD values, minimum number of components possible and low RMSEP values indicated a good model.

In general, eliminating redundant variables and basing models on the variables that are significant will give lower estimation errors [15]. The iPLS automatically provides the variables that correlate the most to the reference variable. In the case of PLS, once the best possible model was built, the number of spectral variables was reduced by using Marten's uncertainty test function [16] to see if the model could be improved further by removing variables that are not important to the model. The uncertainty test function is available in the Unscrambler Version 9.8 software and uses the jack-knifing method to separate the unimportant variables from the useful ones hence simplifying the model. The reduced set of variables was used to build a new model and then the uncertainty test was once again used to reduce the variables further. This iterative approach was continued till the modelling parameters began to deteriorate. The uncertainty test was also applied on the entire spectral range to investigate if the results, after removing the unimportant variables, would be comparable to those of the best models obtained by other methods.

3. Results and Discussion

The validation statistics including the modelling parameters of selected models are listed in Table 1. Models 1 to 6 are the PLS models while models 7 and 8 are from iPLS. From the Figure 1 it can be seen that the frequency of the TAN values is not even. An even spread of values would give a more robust calibration model [17].

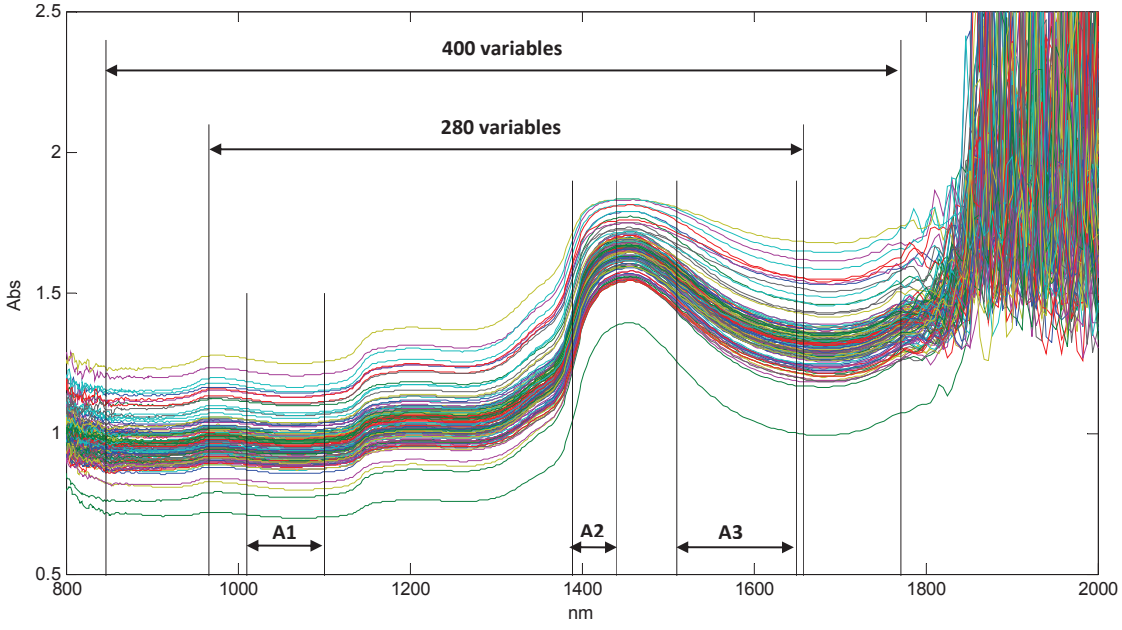
Table 1. Validation statistics.

Model number	Method	Data pre-processing	Number of spectral variables	Spectral range (nm)	RMSECV	R^2 (CV *)	Number of PCs	RMSEP	R^2 (TS **)	RPD
1	PLS	raw	400	847.2 to 1,770.8	0.66	0.63	6	0.56	0.72	1.93
2	PLS	raw	280	967.3 to 1,657.6	0.36	0.89	11	0.34	0.90	3.17
3	PLS	SNV	280	967.3 to 1,657.6	0.38	0.88	9	0.32	0.91	3.43
4	PLS	SNV	73	-	0.36	0.89	6	0.37	0.88	2.91
5	PLS	SNV	117	1,010 to 1,100, 1,390 to 1,440 and 1,510 to 1,650	0.45	0.83	16	0.50	0.77	2.17
6	PLS	raw	43	-	0.54	0.76	6	0.46	0.81	2.34
7	iPLS	raw	119	1,127.2–1,333.6 and 1,525.0–1,634.7	0.55	0.74	7	0.46	0.81	2.33
8	iPLS	SNV	119	1,127.2–1,333.6 and 1,525.0–1,634.7	0.43	0.84	5	0.32	0.91	3.39

***** Cross validation; ****** Test set validation; Model number 4 is obtained by reducing the number of variables used in model 3; The spectral ranges for models 4 and 6 are not mentioned as they consist of many discontinuous intervals.

Figure 2 is a spectral plot of the absorbance *vs.* the wavelengths (in nanometers) for all scanned samples and gives an overview of the various spectral regions used for constructing models in this study. The raw data obtained from the NIRS included noise, and the noisy sections of the spectra were removed after visual inspection and reduced to 400 variables in the region of 847.2 nm to 1,770.8 nm. At the same time, the plot of the raw spectra showed one scan that seemed very different from the others, and this scan was removed as an outlier. Three other samples with high residuals and Hotelling T^2 values were also excluded. An example of the results plot obtained from the PLS modelling done using the Unscrambler Version 9.8 software, is shown in the supplementary section.

Figure 2. Spectral regions used for developing the models. A1, A2 and A3 represent the regions associated with the NH_4^+ group.

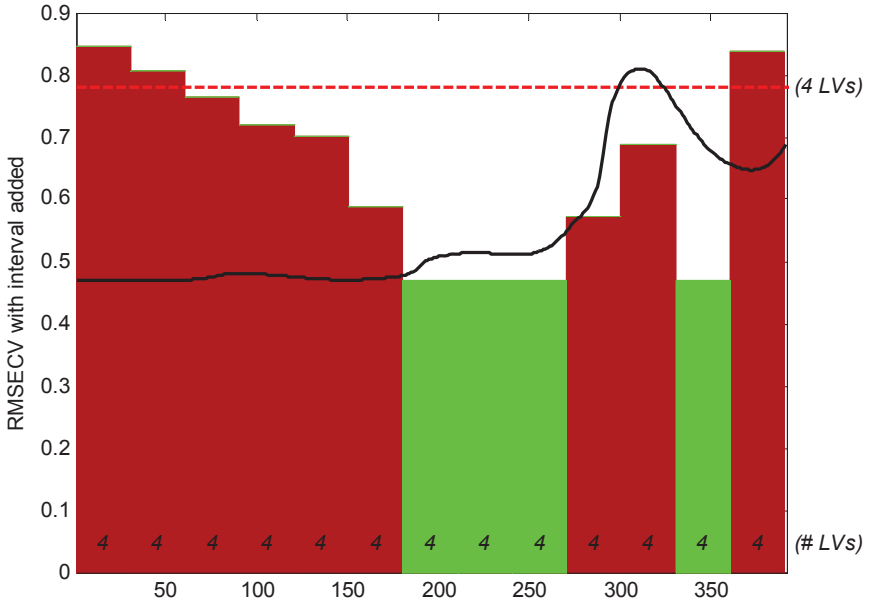


Model 1 is based on the 400 variables, the relatively low R^2 and high RMSEP showed that there was scope for improvement. Various continuous sections within this selected spectral region (with 400 variables) were investigated and the region that gave the best correlation (model 2) was selected for further data pre-processing. The spectral region that gave the best correlation to TAN was between 967.3 nm to 1,657.6 nm and included 280 variables. This region includes most of the regions associated with the NH_4^+ group. The wavelengths in NIR spectroscopy associated with the NH_4^+ group are: 1,010 to 1,100 nm, 1,390 to 1,440 nm, 1,510 to 1,650 nm (represented as regions A1, A2 and A3 respectively in Figure 3) and 2,330 to 2,400 nm [18]. However, model 5 which was built by selecting only the specific spectral regions associated with the NH_4^+ group (excluding the last range which was noisy) and correlating it to the reference variable did not perform better than model 2 or model 3. In NIRS, anharmonicity, interactions between the constituents [18] and overlapping absorption bands make it difficult to ascribe a particular component to a certain wavelength region. The use of chemometrics and especially multivariate variable selection methods such as jack-knifing and iPLS make it possible to overcome this by identifying the variables that are most relevant. A model based on all the 1006 spectral variables available, showed extremely low R^2 and extremely high RMSEP which was expected as a lot of noisy variables had been used. But reducing the number of variables to 43 by using the uncertainty test iteratively, improved the prediction capabilities of the model based on the entire spectral range considerably (model 6) again emphasizing the importance of choosing the right variables.

While using PLS modelling on the 400 variables dataset, pre-processing of the data improved the R^2 and RMSEP of the models only slightly. Pre-processing the spectral data (280 variables) by the SNV method (model 3) improved the model slightly more than other pre-processing methods. SNV is used to remove slope variation and to correct for scatter effects. This is a mathematical transformation method, where each spectrum is corrected individually by first centering the spectral values, and then the centered spectrum is scaled by the standard deviation calculated from the individual spectral values [10,19]. Although the improvement in the model was small, the number of components used for the modelling decreased by 2. Reduction of the number of components used in modelling increases the robustness of the model and makes the model less sensitive to noise [15]. Lowering of the number of components indicates reduction of noisy variables that are included in the calibration model. This was similar with the use of the uncertainty test to reduce the number of variables (model 4). Although model 4 did not change much in terms of R^2 and RMSEP compared to model 3, the number of components was reduced by 3 and the number of variables used to build the model were reduced considerably.

Figure 3 is a sample of the graphical output from the iPLS modelling, which is a plot of the RMSECV *vs.* the variable number (model 8). It visually presents the variables that were most relevant for the modelling process by selecting the intervals that have a low RMSECV and indicating them in green and the redundant ones in red. The dotted line at the top of the plot represents the RMSECV of a model built on the entire variable range. A plot of the mean spectra is also given as a black line which aids in identifying the regions of the spectra that are important.

Figure 3. The output of iPLS; RMSECV with intervals *vs.* the selected variables (represented as variable numbers, not wavelengths). The selected variables are in green and the omitted variables are in red. The number of latent variables (LV) used are shown as well.



The iPLS procedure was used as another method for selecting the optimum variables regardless of knowledge of the assignment of the NH_4^+ group in the NIR region. The iPLS models used the 400 variables region to iteratively search for variables that gave the least RMSECV. The iPLS models improved in terms of R^2, RMSEP, and the number of components when data pre-processed by SNV (model 8) was used compared to the iPLS model using data that was not pre-processed (model 7). Interestingly the noise reduction due to the pre-processing step had a more pronounced effect on the model statistics in iPLS than in the PLS models that were based on the larger spectral range.

The iPLS model was based on a combination of the spectral intervals 1,127.2 to 1,333.6 nm, and 1,525 to 1,634.7 nm. The first range of optimal selected spectra does not correspond to the wavelengths normally associated with ammonia, but the selected spectral variable range 1,525 to 1,634.7 nm lies within the range of 1,510 to 1,650 nm which is associated with ammonia [18]. Comparing the modelling parameters obtained from PLS models and the iPLS models it can be seen that except for the number of components which are much lower in the iPLS model, the R^2 and the RMSEP are quite close to each other.

Thus, based on all the models seen in Table 1, it is indicative that the spectra provided by the diffuse reflectance probe can be correlated to the TAN content. The iPLS model based on the data pre-processed by SNV gave an R^2 of 0.91 and an RPD of 3.39, which is considered a successful model [20,21]. Future research is needed to test the probe in-line in the reactor and with an independent test set. Comparing this with other reported results; the TENIRS system uses trans-flexion, a combination of transmission and reflectance and requires the use of a transmission vial for the scanning process [22]. Transmission is usually used for spectral analysis of liquids while solids are scanned by reflectance [22]. Since manures and slurries are a combination of both liquids and solids, the inclusion of both transmission and reflectance could be an important factor for the R^2 of 0.98 in the

TENIRS experiment. One disadvantage of using a transmission vial for the measurements is that it is susceptible to clogging and to the formation of deposits. These lead to inaccuracies, due to a change in the transmission path length which is vital to the calculations involving the received signal. The diffuse reflectance probe does not have this problem.

High VFA concentrations cause inhibition, as the methanogens are sensitive to pH changes. Changes in VFA concentration is also indicative of process imbalances, as any inhibition of methanogens will lead to VFA accumulation. Previous studies using the diffuse reflectance probe in an anaerobic digester have shown that it can also be used to predict the VFA concentrations [8,9] and can thus be used to monitor VFA along with TAN. Apart from a monitoring system that could indicate inhibitory levels of TAN content, the NIR probe could also be used to screen manure based substrates for their TAN contents prior to loading into the reactor thus preventing the risk of inhibition. It can be used to maintain an optimum C/N ratio, between 20/1 and 30/1, which is another way of preventing ammonia accumulation and improving digester performance [23]. The use of NIR in predicting the amount of TAN will also aid in feed input management especially while dealing with feedstock that is high in protein and TAN content. In a previous study, NIR spectroscopy has been used to predict the biochemical methane potential (BMP) of meadow grass based substrates [24]. If further studies indicate that the diffuse reflectance probe can be calibrated to predict the BMP of manure based substrates, the probe could serve multiple purposes in the process control of anaerobic digesters.

4. Conclusions

The models obtained were successful and thus the diffuse reflectance probe is promising as an online ammonia monitoring tool for materials such as manure and digestates from anaerobic digesters. Based on the spectra obtained from the probe, PLS and iPLS gave similar models, except iPLS used lesser number of components indicating a more robust model. Pre-processing of the data also reduced the number of components in the models when compared to models that were based on raw data. Selecting the correct range of spectra that would be used in the model, however, proved to be very important in this process.

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Paper 3

Effects of high temperature isochoric pre-treatment on the methane yields of cattle, pig and chicken manure

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Report

Optimizing the sample presentation method - (unpublished data)

The experiment:
A small experiment was carried out to see if the sample (digestate in this case) presentation method used in Paper 2 could be improved upon. A few of the manure samples that were used in Paper 2 were selected at random and scanned in different ways. Each scanning was repeated 5 or 10 times. Then these 5 or 10 replicate spectra were analysed using PCA. The replicate scans of the same sample tend to lie very close to each other on a score plot obtained from PCA. PCA is mainly used to detect the main variations and if the same scanning method is used on a particular sample then the variation between the different scans should be very low and hence the points representing those scans should lie very close to each other on a PCA plot. LatentiX software ver. 2.00 was used for the PCA analysis. In all cases the samples were brought to a temperature of 20°C (±2°C) before scanning to minimize the effects of the temperature change. The depth of the tip of the probe into the sample was kept constant, ensuring that the sample in front of the probe was at least 2 cm thick. The probe collects information from material that is within 2 mm of its tip, but 1 cm is recommended as a safety margin (*102*).

The various factors that were thought to affect the scans were:

1. Day to day changes; A sample was scanned on two consecutive days to see if there was a change
2. Changing the speed of mixing: Two mixing speeds; 80 rpm and 120 rpm were used to see if reducing the speed influenced the scatter.
3. Scanning an undisturbed sample at the upper and lower strata: Complex samples that contain particles of mixed size such as manure and digestate tend to settle over time forming an upper stratum with very few large particles (especially in case of digestates) and the lower strata with larger settled particles. Scanning in the upper strata could reduce some of the scatter effect, and scanning in the lower strata should produce a larger scatter effect compared to the upper strata. The distinction between upper and lower strata was purely on the basis of visual observation. The opaque part of the sample was considered as the lower strata.
4. Another possibility was to mix the material and then scan every minute for a total period of ten minutes while allowing the settling to continue or to scan after mixing for 30 seconds and letting the particles settle for precisely 1 minute and then scan for each repetition.

Results:

1. Day to day changes: The first principal component easily separated the scans according to the day they were scanned although the same sample was used (Figure A. 1). One reason could be due to sample degradation over the two day period of scanning, but the degradation would be minimal as the sample was refrigerated between the scans. Nevertheless, there is a variation and one way of building this variation into a model would be to scan the samples on at least three different days and then use the averaged spectra to build the model.

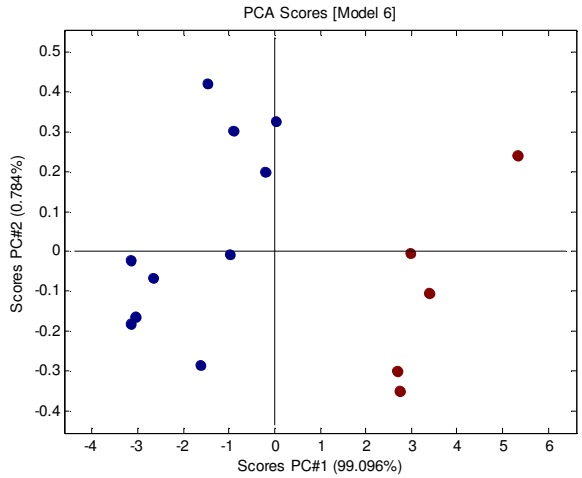

Figure A. 1: Day to day variation. The scans from day 1 are in dark blue while the scans from day 2 are in dark red.

2. Changing the mixing speed: There was no clear distinction between the two mixing speeds (Figure A.2).

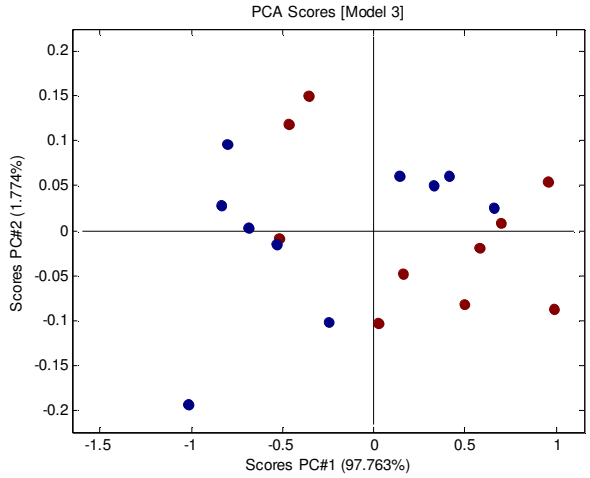

Figure A. 2: Mixing speed at 80 rpm is represented in dark blue while 120 rpm is represented by points in dark red.

3. Scanning an undisturbed sample, upper and lower strata: Figure A.3 shows a clear difference in the scans done at the upper strata and those done in the lower strata. As expected, the large amount of settled particles in the lower strata induces more scatter effects and thus the points are scattered in the plot. The scans in the upper strata however are grouped together and indicate much less scatter effects than the lower strata.

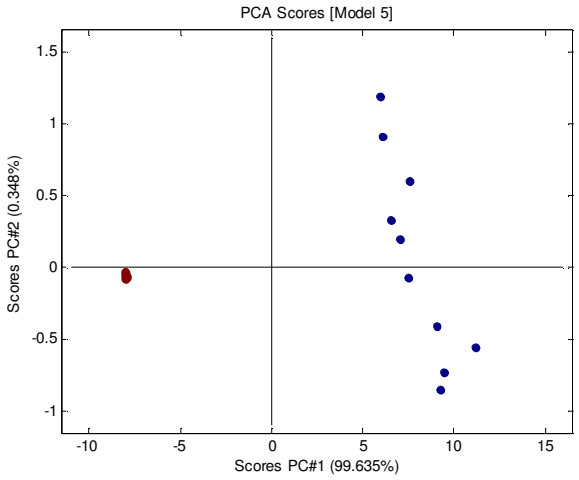

Figure A. 3: Scans performed on the upper strata are in dark red, while those in dark blue represent scans in the lower strata.

4. Effect of mixing and settling: The mixing and settling method resulted in grouping that was much better than that of the lower strata but not better than the upper strata scans (Figures A.4 and A.5).

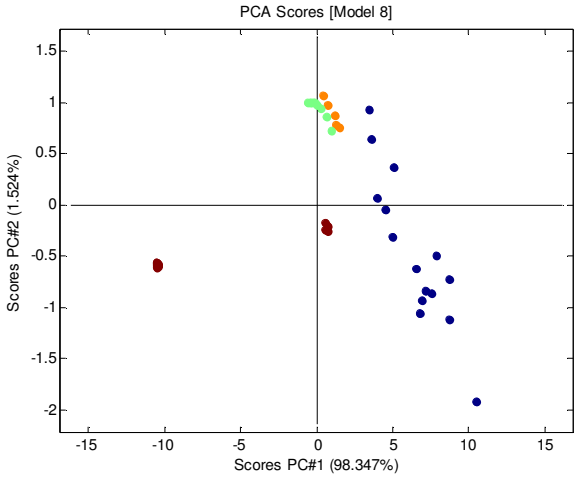

Figure A. 4: The green and orange are the samples that were mixed and then allowed to settle. Dark red points represent upper strata and the dark blue is for the lower strata

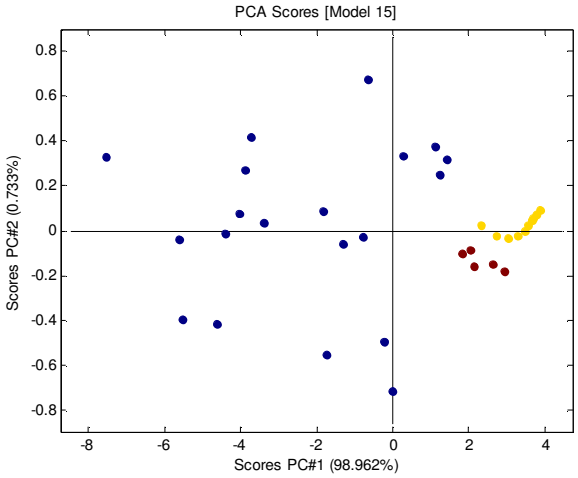

Figure A. 5: Difference between the mixing types and scans in the lower strata. Dark blue represents the lower strata, yellow and dark red represent mixing and then settling.

The results from this preliminary test show the importance of sample presentation. In conclusion, the best grouping was with the samples scanned in the upper strata of the undisturbed sample (Figure A.3). But this condition is difficult to emulate in anaerobic digesters where the contents have to be mixed to ensure good contact between the microbial population and the substrate, especially in case of CSTRs. The next best option was to mix and allow the solids to settle for a while before scanning (Figures A.4 and A.5), this can be recreated in a large scale reactor (Figure A.6) where samples can be run out of the reactor in pipes as described in (*47*). The sample can be pumped through the pipe for a period long enough to remove any remnants of old samples in the pipe. Instead of the vertical option described in (*47*), the NIR probe can be affixed to a horizontal portion of the sampling pipe where the fresh sample can now be allowed to settle for a short while and then the relatively clear upper strata can be scanned by the NIRS. But, this method is difficult to define with precision, as settling times with substrates such as digestate is hardly ideal and vary depending on a lot of factors.

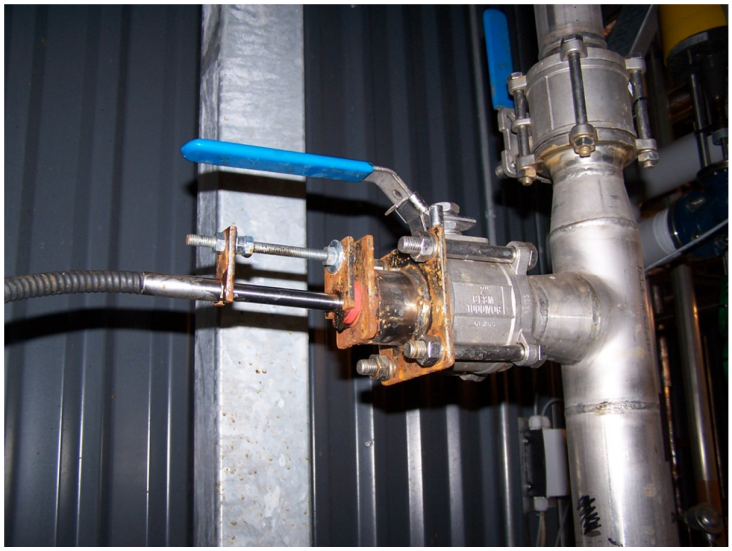

Figure A. 6: An NIR - diffuse reflectance probe affixed to a pilot-scale scale reactor.

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