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A literature review.

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Abstract. In many developing countries manure is anaerobically digested to produce biogas. The residue of manure digestion, bio-slurry, can be used as fertilizer for crop production and aquaculture. This study compared bio-slurry and manure as fertilizers. Nutrients in bio-slurry, especially nitrogen, are more readily available than in manure, leading to a larger short term fertilisation effect. However, risks for N losses through volatilisation and leaching are large for bio-slurry than for manure during storage, handling and application. Unfortunately, most studies that compared bio-slurry and manure exhibited methodological shortcomings that hamper an adequate comparison.

Keywords: bio-slurry, digestate, manure, biogas, fertilizer

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Preface

Asia is the centre of domestic biogas development, with the People's Republic of China having installed more than 42 million units (by the end of 2012) and India about 4.6 million units (by the end of March 2013) based on strong government support. With support by SNV Netherlands Development Organisation, market-based national biogas programmes were set-up in 18 countries in Asia, Africa and Latin America. Within these programmes and through parallel projects, more than 578,000 biogas plants were installed in these countries by the end of 2013. See www.snvworld.org for more information.

Domestic biogas plants anaerobically digest animal manure at household level into small but precious amounts of combustible gas, known as 'biogas'. This biogas can be effectively used in gas stoves for cooking and in lamps for basic lighting. The residue of the process, known as 'bio-slurry', can be easily collected and used as a potent organic fertiliser for crops and aquaculture. Most, if not all, national programmes have paid ample attention to the value of bio-slurry as a fertiliser and soil improver in the promotion, training and extension messages to the biogas households. As a result, the vast majority of these households appreciate bio-slurry, also because they are coming from a situation in which extension messages were limited to chemical fertiliser at the most, often totally ignoring the value of (undigested) animal manure. In case biogas replaces dried animal manure ('dung cakes') for the purpose of cooking, the bio-slurry has an obvious added value as it can still be used as fertiliser. In other cases, the added value of bio-slurry compared to animal manure is less straightforward and may also depend on the way the bio-slurry after digestion is being stored/processed, transported and finally applied.

Several studies on the comparative value of bio-slurry have been conducted in various countries and quite some data and information have become available up to date. However, it was not clear whether the right methodologies have been applied and hence whether the overall conclusions and recommendations of these researches have been drawn correctly. Therefore, SNV decided to commission an independent validation of researches on the value of bio-slurry compared to animal manure through a literature study. The study as reported in this publication has been executed by Alterra Wageningen UR and the Nutrient Management Institute NMI, both located in Wageningen, the Netherlands.

The literature study concludes that sound execution of research on the comparative value of bio-slurry is complicated. Most of the researches reviewed by Alterra and NMI show defects due to shortcomings in the applied methodologies. The study, however, confirms that bio-slurry - just as animal manure - can play an important role as fertiliser, but that its value will finally depend on possible nutrient losses - in particular volatilisation of ammonia and leaching of nitrogen and potassium - during storage, handling and/or application.

Based on the results of this study, SNV recommends that the national biogas programmes will continue to promote the use of bio-slurry as fertiliser (and as soil improver). At the same time, the programmes *in each country* are advised to critically review and where possible improve the various ways of its storage, handling and application to minimise nutrient losses and to accordingly update the extension messages to the biogas households. Finally, longer-term field research based on a comprehensive approach including also storage, handling and application of both animal manure and bio-slurry will provide a deeper understanding on the comparative value of bio-slurry as fertiliser and soil improver.

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Summary

Background

In many developing countries, animal manure is anaerobically digested, sometimes in combination with human excreta, to produce biogas, which is used for cooking and/or basic lighting. Besides biogas, anaerobic digestion produces a residue, the so-called digestate or bio-slurry, which contains most of the nutrients of the original manure, partially in a converted form. This bio-slurry can potentially be used as an organic fertilizer.

Numerous studies have been conducted on the comparative value of bio-slurry as a fertilizer or fish feed and its comparative value to farmyard manure. However, these studies do not provide a uniform message. The differences between the results of the studies can be due to several reasons, e.g. bio-slurry source, storage and handling, crop types, soil and climate conditions, or differences in methodologies to assess the value of bio-slurry as a fertilizer.

Aim of this study

This report contains a critical review of studies on the value of bio-slurry as a fertilizer and fish feed in developing countries and compared bio-slurry with undigested farmyard manure. This review took into account:

- The effects of digestion of manure on the amount and forms of nutrients (mainly N, P and K);
- The effects of storage and further processing of bio-slurry on amounts and forms of nutrients;
- The differences between manure and bio-slurry as fertilizer;
- The risks of the use of bio-slurry for food safety;
- The differences between bio-slurry and farmyard manure as fish feed.

The review identifies knowledge gaps in bio-slurry use and provides recommendations on future bio-slurry research and methodologies to assess the use of bio-slurry as a fertilizer and to compare bio-slurry with farmyard manure.

This review includes both non-peer reviewed reports and scientific peer reviewed papers.

Effects of anaerobic digestion on nutrients and other properties of bio-slurry

The anaerobic digestion of manure has very little to no effect on the amount of nutrients in the manure before and after digestion. Only a small part of the nitrogen (N) can end up in the biogas and some nutrients can be (temporarily) retained in the deposits in the digester. However, the form of nutrients is much more affected by anaerobic digestion. Up to 50% of the organic N is converted to ammonium (NH_4), which is a nitrogen form that is directly available for uptake by plants. The fraction of N that is converted to NH_4 depends on the N content of the feedstock and on the degree of decomposition after digestion. Because of the higher pH in bio-slurry compared to farmyard manure, the fraction of available phosphorus (P) seems to be reduced during digestion, although in most field experiments no effects of anaerobic digestion on P availability for plants were found. Further, the pH of digestate is 0.5 to 2 units higher than the pH of manure.

Nutrient losses

The high NH_4 concentration in bio-slurry can lead to volatilisation of NH_3 during storage of the bio-slurry in cases where the bio-slurry is not properly covered. This volatilisation is in most cases substantially higher than volatilisation of NH_3 from farmyard manure. More than 50% of NH_4 can be lost from uncovered bio-slurry in one month. Coverage reduces volatilisation by approximately 10 times. Another pathway for nutrient losses is leaching of the liquid fraction of bio-slurry. This is especially the case for storages without a bottom liner. As leaching depends greatly on the permeability of storage floor, no estimates of the loss through leaching could be given. For solid farmyard manure, leaching losses are generally very small.

Bio-slurry handling

As bio-slurry is a liquid when it is discharged from the digester, it is difficult to transport and thus apply it to fields not close to the digester. Therefore bio-slurry is often dried or composted to get a solid organic fertilizer. From a theoretical perspective both drying and composting can lead to losses of N through volatilisation of NH_3 , while other nutrients are normally retained in the slurry. However, analysis results for both dried and fresh bio-slurry do not provide a clear picture. N losses are in many cases not obvious, while some authors even suggest that P and K are lost during drying. Most likely the source for dried and fresh bio-slurry were different in these studies.

For composting, bio-slurry is mixed with other organic materials. Because these materials also contain N (mostly unknown amount), a clear N balance cannot be made. However, based on studies using non-manure digestates, N losses caused by composting of bio-slurry are estimated to be between 15 to 27%. These N losses are not different for composting of farmyard manure for which similar N losses have been found.

Bio-slurry as a fertilizer

The value of bio-slurry as a fertilizer depends on:

- The nutrient contents, e.g. The amount of nitrogen, phosphorus, potassium, calcium, magnesium, sulphur and micronutrients on a fresh or dry matter basis;
- The ratio between nutrients in the bio-slurry, e.g. The n/p-ratio and/or the n/k-ratio; and
- The availability of the nutrients, which is determined by the compounds that contain the nutrients.

In general, the value of bio-slurry as fertilizer is fairly high, because it contains nutrients in a readily available form. Although on a mass base the nutrient contents are much lower than in regular mineral fertilizers, which has consequences for handling and the required application rates. The nutrient contents of bio-slurry may fluctuate depending on the composition of the manure that is digested and possible N losses during the anaerobic digestion and subsequent storage and handling. These fluctuations in nutrient contents will affect the value as a fertilizer. Because N will be partly present as ammonium, which is directly available for plant uptake, and partly as organic N, the N availability will be higher than that of manure but lower than that of a regular mineral N fertilizer. The ammonium/total-N ratio will give an indication of the fertilizer N value of bio-slurry. The availability of P, K and the other nutrients is similar to that of manure and mineral fertilizers (when expressed per kg N/P/K). Ammonia volatilization after the application of bio-slurry to soil can be high, which may reduce the value of bio-slurry as an N fertilizer. To prevent N losses by ammonia volatilization the bio-slurry should be injected / ploughed into the soil, directly after its application.

Bio-slurry compared to manure

In the past, several studies have been performed that compared bio-slurry to manure. These studies do not give a consistent picture on the differences between bio-slurry and manure as a fertilizer, although N uptake is generally higher with bio-slurry than with manure. In a number of studies, bio-slurry performed equally well compared to manure. Unfortunately, most of these studies might have given a clearer message on the use of bio-slurry as a fertilizer, if they had not exhibited methodological short-comings that could have been easily overcome, like:

- The levels of only one nutrient (mostly N) are the same in treatments that were compared, while the application levels of the other nutrients (mostly P and K) are different. In this case it is not clear if eventual differences in yield are caused by properties of the fertilizers or because of differences in the levels of the applied nutrients.
- A control plot (no fertilisation) is frequently lacking. Especially when no differences are found between bio-slurry and other fertilizers, this non-difference might be a result of the availability of sufficient nutrients in the soil itself.
- The application method is often not reported. This is important because surface application of bio-slurry can lead to large N losses through volatilisation. In case yields with bio-slurry are lower than yields with manure as fertilizer, this might be because of such N losses.
- Frequently it is not clear if nutrient contents are reported as total contents or as percentages of dry matter.
- Statistics are often not reported, so it is not clear whether differences are significant.

Food safety risks

The feedstocks for bio-slurry are different types of animal manure supplemented in some cases with human excreta. These feedstocks can contain pathogens. When comparing the bacterial parameters from animal manure with bio-slurry, the hygienic quality of bio-slurry is in general equal or better than animal manure. Yet, although mesophilic biogas treatments can reduce biological hazard of bio-slurry, it is not sure that it can consistently achieve this. This holds especially for developing countries where retention times are less strictly controlled and might be too short (less than 20 days). When human excreta are included in the feedstock, stricter precautions might be required. When using relatively clean source organic materials like manure and human excreta, bio-slurry does not show problematic levels of heavy metals and pesticides. Only when other waste streams are used as feedstock, risks for contaminants in digestate may occur.

Bio-slurry in aquaculture

The use of manure for fish pond fertilization has become increasingly important in recent years by small scale fish farmers. Also, bio-slurry can effectively be used in aquaculture. Direct consumption of bio-slurry and manure by fish is not very effective, because most manures and bio-slurries contain very little compounds that can be digested by fish, because most digestible compounds have been removed by the animals that have produced the manure and for bio-slurry also by the consecutive anaerobic digestion. Mostly, the nutrients in bio-slurry and manure promote growth of algae and plankton that can serve as fish feed. Both bio-slurry and manure can lead to similar fish production as with commercial fish feeds. The differences between bio-slurry and manure as fish feed seem small. Only oxygen levels in fish ponds appear to be lower, but not severely, for manure than for bio-slurry.

Conclusions

Bio-slurry can play an important role as a source of nutrients for crop production and aqua culture. Compared to manure, nutrients in bio-slurry (especially nitrogen) are more readily available, which means that bio-slurry can have a larger fertilisation effect in short term. However, the higher N availability also leads to larger risks for N losses during storage, handling and application through volatilisation and leaching. Unfortunately, most studies that compared bio-slurry and manure exhibited methodological shortcomings that hamper an adequate comparison.

Recommendations

Several practical aspects of the use of bio-slurry have required little attention so far and should require more attention. These aspects include:

- The timing of the application of bio-slurry to the field and crops;
- The identification of crops to which bio-slurry can be applied best including the best application strategy;
- Measures to reduce the loss of nutrients through volatilisation and leaching.

Besides as a fertilizer bio-slurry may have other effects on soil quality, crop production and food safety. Therefore it is recommended that additional research is performed on:

- The effects of bio-slurry, manure and other fertilizer on the soil organic matter contents, because an adequate soil organic content is important to maintain or improve soil quality and soil fertility;
- Other possible effects of bio-slurry on crop production like pest reduction;
- The effects on food safety when human excreta are included in the feedstock for anaerobic digestion.

For future studies that compare bio-slurry with manure and/or other fertilizers, the following guidelines should be taken into account:

- The same nutrient levels are used for all treatments;
- The composition of all applied fertilizers is clearly reported;
- Bio-slurry and manure are from the same source;
- A control treatment is present;
- A reference treatment is present;
- Sufficient replicates are included;
- Treatments are allocated at random;
- The application method is reported;
- Statistics are reported.

1 Introduction

1.1 Background

In many developing countries, animal manure is frequently anaerobically digested, sometimes in combination with human excreta, to produce biogas. This biogas is then used for cooking or lighting, thereby replacing other fuels like firewood, dried manure and petroleum. These other fuels have often large disadvantages like increased high workload, deforestation, indoor air pollution leading to health risks, loss of nutrients, high costs and greenhouse gas emissions. Besides biogas, anaerobic digestion produces a residue, the so-called digestate or bio-slurry¹, which contains most of the nutrients of the original manure, but in a converted form. This bio-slurry can then potentially be used as an organic fertilizer.

Currently, there are several challenges in the promotion and utilisation of bio-slurry:

- Farmers in developing and some other countries frequently lack interest in the use of organic fertilizers, partly because they are not aware of the value of organic fertilizers, partly because chemical fertilizers are readily available and even subsidised in some countries. Most importantly, information, education and extension services on bio-slurry often reach a limited part of the farmers.
- Because the production of bio-slurry and the demand for nutrients for fertilisation do not always match, bio-slurry can often not be applied directly and needs to be stored. However, nutrients in the bio-slurry might get lost during the storage due to leaching or evaporation (e.g. ammonia volatilization).
- Bio-slurry generally has a high water content (more than 80%), which makes it difficult to store, handle and transport. The bio-slurry is thus in numerous cases processed further, by e.g. drying or composting. This however might lead to nutrient losses.
- The value of bio-slurry as a fertilizer may depend on several conditions like crop types, method of bio-slurry application, soil and climate conditions, etc., which makes it difficult to give a consistent message on the use of bio-slurry.
- Bio-slurry can contain pathogens and toxic compounds that originate from its feedstocks farmyard manure and human excreta. Because of this, it is not clear if bio-slurry can safely be used for crop production.

1.2 Problem definition

Numerous studies have been conducted on the use of bio-slurry as a fertilizer. However, the results of these studies do not provide a uniform message and even provide contrasting claims about the value of bio-slurry. These differences in the results of the studies can either be due to differences in conditions regarding the bio-slurry source, storage and handling, crop types, soil and climate conditions, or because inadequate methodologies have been used to assess the value of bio-slurry as a fertilizer.

To transfer the results of studies on bio-slurry to biogas extension programmes, it needs to be clear which studies have been performed adequately and consequently which results and conclusions are rightful.

¹ The term bio-slurry is ambiguous: in scientific peer-reviewed literature, the term bio-slurry is almost exclusively used for biological soil remediation in bioreactors, where the contaminated soil is diluted with water to create a slurry. In non-peer reviewed literature (e.g. reports, biogas extension programs, etc.) the term bio-slurry is also used for anaerobically digested manure in the context of biogas production in developing countries. Because this report is written in this context, we use the term bio-slurry for digested manure in this report as well (see also Section 1.5).

1.3 Aim of the project

This report contains a critical review of studies on the value of bio-slurry as a fertilizer and fish feed in developing countries and aims to compare its value with undigested farmyard manure. This review also takes into account the effects of bio-slurry storage and further processing and the risks of bio-slurry application for food safety. The review will identify knowledge gaps in bio-slurry use and provide recommendations on future bio-slurry research and methodologies to assess the use of bio-slurry as a fertilizer.

In addition to a recent critical review by the FAO on bio-slurry use (Groot and Bogdanski), this report also includes non-peer reviewed literature (reports etc.) and a review of the applied methodologies to assess the fertilizing value of bio-slurry and to compare bio-slurry with manure.

1.4 Methodology

This study on the value of bio-slurry as fertilizer and its comparison with farmyard manure has been carried out as literature review. Publications have been retrieved in two different ways:

- First, SNV has made available a set of possibly relevant documents, both from their library and through a request for publications via their network. The publications that are retrieved this way will mostly be reports and other non peer-reviewed publications.
- Further, bibliographic databases, like Scopus and Google Scholar, and the internet have been searched. The bibliographic databases cover almost all relevant scientific journals. This search will yield mostly scientific peer-reviewed publications.

The literature study is confined to publications in the English and German language. Publications in other languages are not included, which has the largest consequences for publications from China. In China smallholder biogas production is extensively applied, but publications on this are unfortunately mainly in Chinese.

Further, when referring to the (comparative) value of bio-slurry as fertilizer, we limit ourselves to the macro-nutrients nitrogen (N), phosphorus (P) and potassium (K). There are several more parameters that link to fertilizer values, like contents of micro-nutrients, the effects on soil acidity and the effects on soil organic matter. These parameters are not or only marginally addressed in this study, because data on these parameters are scarce and the differences between bio-slurry and manure are expected to be small.

1.5 Definitions

In publications several definitions of manure, bio-slurry, fertilizer etc. are used. For reasons of consistency we used in this report the following definitions:

Manure

The excreta of animals. These can be either in solid form or in a liquid form.

Farm yard manure

Solid manure, which frequently also contains straw or other organic materials that have been used in animal housing. This can be from different types of animals. When the animal type is specified, it is referred to as cattle manure, pig manure, etc.

Cattle/pig/poultry slurry

Liquid manure. This manure generally contains no additional organic material. Further, it contains water and a larger part of the animal urine compared to farmyard manure.

Digestate

The residue of anaerobic digestion of organic materials, including manure.

Bio-slurry

The residue of anaerobic digestion of manure. In some case this can also include human excreta. Bio-slurry is thus a specific type of digestate.

Fertilizer

Any organic or inorganic material of natural or synthetic origin (other than liming materials) that is added to the soil to supply one or more plant nutrients essential to the growth of plants.

Mineral fertilizer

Non-organic fertilizers. Nutrients in these fertilizers are in mineral form. Other common names for mineral fertilizer are chemical fertilizer, synthetic fertilizer, and regular fertilizer.

2 What is anaerobic digestion and what is bio-slurry?

Anaerobic digestion or fermentation is the microbial decomposition of organic materials under anaerobic conditions. Anaerobic digestion occurs wide-spread in nature in rumens of ruminants, hindguts of other animals, and in moors and paddy fields and other naturally anaerobic environments. Biogas production is the result of anaerobic fermentation.

Four major steps can be distinguished in the biogas production process (Angenent and Wrenn, 2008; Gijzen, 1987; Wilkie, 2008):

- Hydrolysis, the conversion of polymers into monomers (sugars, fatty acids and amino acids), generally the rate limiting step in the process.
- Acidogenesis, the conversion of the monomers into volatile fatty acids (VFA), alcohols, hydrogen gas, ammonia and carbon dioxide.
- Acetogenesis, the conversion of VFA and alcohols by acetogenic bacteria into acetate, hydrogen and carbon dioxide and
- Methanogenesis, conversion of acetate, hydrogen and carbon dioxide into methane and carbon dioxide by methanogenic bacteria.

Anaerobic digestion is a completely microbiological process. No higher organisms are involved, in contrast to composting.

Biogas reactors

Several types of biogas reactors exist ranging from very simple to very complex installations. Small scale installations are for instance fixed dome, floating dome and tubular digesters (see Figure 1). Small scale reactors have a size of several cubic metres (m³). They are almost exclusively fed with manure, in some cases mixed with human faeces and urine. The major advantages of these types of installations are their relatively low price and technology requirements making operation and maintenance easy for users.

The tubular plug flow digester is the simplest design.. The fixed dome and the floating cover reactor are a little more complex. The digesters consist of three elements (see also Figure 1):

- Mixing pit or inlet: where animal excrement is mixed with water before it is poured into digester chamber.
- Digester chamber: where the mix of excrement and water are fermented. Methane and other gases are produced in the chamber and push the slurry mix at the bottom of the floor into an outlet pit or expansion chamber.
- Outlet pit: where the slurry mix is collected after being pushed out from the digester.

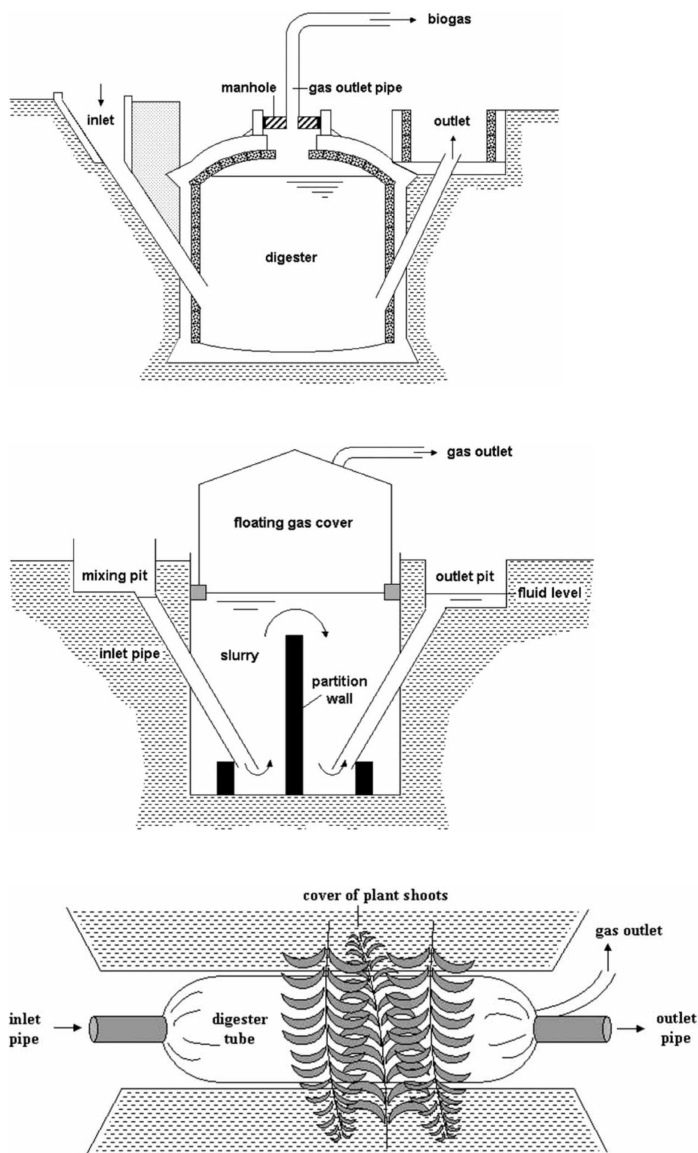


Figure 1 Types of small-scale biogas reactors. Top (Chinese) fixed dome reactor, middle (Indian) floating cover reactor, bottom tubular digester (source: Surendra et al., 2013).

Manure and water are mixed to a slurry and fed into the reactor through an inlet. Biogas is produced inside the reactor and can be collected through an outlet. Excess slurry can leave the reactor via an outlet to the outlet pit. This relatively simple technology has one major disadvantage. Biogas formation can continue in the expansion chamber and even outside the reactor. This biogas is not collected and is emitted into the atmosphere. Thereby, valuable methane disappears into the atmosphere and, maybe even worse, this system contributes to the emission of greenhouse gasses.

Modern large scale reactors may have a content of several thousands of cubic meters. In these reactors retention times are controlled through pumps, instead of the input and outflow as in small-scale digesters. These large scale reactors are not within the scope of this report and will not be discussed any further.

Feedstock

Almost all plant biomass and animal manure can be used for biogas production. The only exceptions are biomass rich in lignin, since lignin cannot be decomposed in the absence of oxygen, and highly N-rich biomass which can cause toxic effects due to the high NH_3 concentrations. Manure is the almost exclusive feedstock for the small scale reactors, sometimes in combination with human excreta and/or animal urine. Mixtures of manure and other plant and animal biomass or mixtures without manure are mostly used for large scale reactors in a process named co-digestion.

In contrast to composting, feedstock should be as fresh as possible and pre-decomposition should be prevented as much as possible, also if storage is needed. During pre-decomposition, most easily degradable components disappear, resulting in a large decrease in gas yield.

Energy production and consumption

The biogas production of several types of feedstock is given below in Table 1. Biogas production is largely determined by the degradability of the waste. Manure has a lower biogas production than many other materials due to the fact that a large fraction of the easily decomposable materials have already been removed in the intestinal tract of the animal.

Table 1

Biogas production for various manures and other feedstocks (source: Surendra et al., 2013).

Feedstock	Biogas production (m ³ /ton dry solids)
Cattle manure	200-300
Pig manure	250-500
Chicken manure	310
Sheep manure	300-400
Human excreta (night soil)	380
Vegetable wastes	400
Grass lawn cuttings	700-800
Rice straw	550-620
Maize silage	600-700
Maize straw	400-1000
Kitchen waste	400-1000

In small-scale farm based digesters, biogas is used for cooking and/or basic lighting. In larger digesters, biogas can also be used to produce electricity by using a combined heat and power installation (CHP). Electricity is mostly produced in large scale digesters. Modern CHP installations have an electrical efficiency of 35-40%. The remaining energy is low-temperature heat, which may be used in case of local demand or in moderate climates for heating the digester itself.

Digestate

The remaining mixture of solution and solids after anaerobic digestion is referred to as digestate. If manure is used as feedstock, the digestate is called bio-slurry. Large scale fermenters often include a separation step to separate the solid fraction from the liquid fraction.

The liquid fraction is rich in nutrients (N, P and K), approximately half of the N is in organic form; the other half is mineral (i.e. ammonium, NH₄). During anaerobic digestion all nutrients including nitrogen remain in the reactor. Hence, in contrast to composting, no nitrogen is lost during anaerobic digestion.

The solid fraction is rich in organic matter and still contains a lot of organic N and P. The properties of the solid fraction, if added to soils, are comparable to compost with one major exception: it is relatively richer in ligno-cellulose, since lignin was not degraded during anaerobic digestion. This means that the solid fraction of digestate is more susceptible to further decomposition in the soil than compost.

3 Properties of bio-slurry in comparison with animal manure

Anaerobic digestion is a collection of processes by which microorganisms break down biodegradable material in the absence of oxygen. Chapter 2 gave a general overview of the processes in a digester. This anaerobic conversion will change the composition of the feedstock, thereby affecting its properties and its abilities for further use, e.g. as a fertilizer. This chapter discusses these changes in the composition and properties.

Decomposition of organic carbon

The fraction of organic carbon (C) that is converted to biogas varies and depends on the feedstock (e.g. type of manure) and digestion conditions. Values between 10% (Möller and Müller, 2012) and more than 70% (Massé *et al.*, 2007) have been reported. In most cases, biogas production from manure will be in the low end, because most readily available organic compounds have already been removed in the intestinal tract of the animal (see Chapter 1). The remaining organic matter in manure is usually composed of stable organic material not readily available for biological degradation (Messner and Amberger, 1987). During the anaerobic digestion process, most of the degradable organic compounds in the manure will be converted into biogas, which will result in organic compounds in the bio-slurry that are more stable and less susceptible to mineralisation than in the original manure.

Content and form of nitrogen, phosphorus and potassium

In general, the total content of nutrients is not strongly affected by the anaerobic digestion process. This means that the nutrient content of bio-slurry will be more or less the same as that of the manure that is used as feedstock. However, because part of the organic matter is decomposed during the digestion process, the nutrient content at dry matter basis will be higher in the bio-slurry than that in the manure (Möller and Müller, 2012). Because of the breakdown of organic matter during digestion, organically bound nutrients are mineralized into a directly available form. Most clearly, anaerobic digestion tends to increase the content of immediately available N, in the form of ammonium-N (NH₄-N). Möller and Müller (2012) reported NH₄-N concentrations in anaerobically digested materials of 45-80% of the total N. Gurung (1997) reported in a literature review that NH₄ levels in digested farmyard manure were about 2-2.5 times higher than in undigested farmyard manure, while total contents of nitrogen, phosphorus and potassium remained the same after anaerobic digestion.

Table 2

Variations in the composition of digestate and change with respect to the composition of undigested animal manures (source: Möller and Müller, 2012).

Parameter	Value	Change ^{a)}
Dry matter, DM (%)	1.5-13.2	-1.5 to -5.5
Organic matter (as % of DM)	63.8-75.0	-5 to -15
Total N (% of DM)	3.1-14.0	^{b)}
Total N (g/kg FM)	1.5-6.8	≈ 0
NH ₄ (% of total N)	44-81	+10 to +33
Total P (g/kg FM)	0.4-2.6	≈ 0
Water soluble P (% of total P)	25-45	-20 to -47
Total K (g/kg FM)	1.2-11.5	≈ 0
Total Ca	1.0-2.3	≈ 0
Total Mg	0.3-0.7	≈ 0
Ph	7.3-9.0	+0.5 to +2 units

a in comparison to undigested liquid manure, absolute values.

b increase with degree of degradation.

DM = dry matter.

FM = fresh matter.

Möller and Müller (2012) compared digestates and undigested manures. They concluded that digestate of various feedstocks have higher ammonium (NH₄):total nitrogen (N) ratios, decreased carbon (C) contents, reduced biological oxygen (O₂) demands, elevated pH values and lower C:N ratios (Table 2).

From this table it becomes clear that the composition of bio-slurry can vary greatly, mostly depending on type and composition of manure (see further) and also digestion conditions. Nevertheless, some general conclusions can be drawn from this table:

- The total contents of nutrients (N, P, K, Mg and Ca) in animal manure remain the same after anaerobic digestion;
- Water soluble P decreases by anaerobic digestion, probably caused by a pH increase, leading to a shift of HPO₄²⁻ towards PO₄³⁻, and subsequent precipitation of calcium or magnesium phosphates and/or struvite (magnesium ammonium phosphate). However, in most field experiments no effect of anaerobic digestion of animal manure on P availability for plants was determined (Loria and Sawyer, 2005; Möller and Stinner, 2010);
- Part of the nitrogen is converted to NH₄, although the increase in NH₄ in Table 2 is smaller than as reported by others (e.g. Gurung, 1997; Smith, 2013).

Not many data have been found about the effect of the type of manure on the composition of bio-slurry. Den Toom (2013) showed in a literature review data about the N, P and K contents of bio-slurry from cattle slurry and poultry slurry from various studies. The nutrient contents (especially that of N) were somewhat lower than those reported by Möller and Müller (2008) and N and P contents were generally higher in slurry from poultry manure than in that of cattle manure (Table 3).

Table 3

Nutrient content (in % of dry matter) of bio-slurry produced from different types of manure (Den Toom, 2013).

Type of manure (feedstock)	N	P	K
Cattle	1.3-1.5	0.3-2.8	0.3-1.4
Poultry	1.8-2.7	0.8-3.3	0.5-0.8

Gurung (1997) presented data about the composition of undigested manure of cattle, pigs and chickens and found similar data as that of Den Toom (2013).

Although the total amount of N entering the digester will equal more or less the amount of N leaving the digester with the slurry (effluent and solids), some N may temporarily not be retrievable through sedimentation of solids in the digester. This can especially be the case for batch reactors: Smith (2013) cites N-losses through sedimentation in a batch reactor to be around 30% of total-N. However, N-losses in the more common continuous digestion reactor in general tend to be smaller, especially if the digestion process and design of the digester are properly carried out.

Risk of N losses by ammonia volatilization in the digester

During the digestion process nitrogen, phosphorus, and potassium are transformed by microbial processes. Almost all nutrients will stay in the bio-slurry, if the process conditions are optimized. As already mentioned, organic N is partly converted into ammonium-N. A negligible amount of ammonia gas will escape from the digester with the biogas. This is different to most other manure management practices (e.g. composting) during which a significant part of nitrogen gets lost through volatilization.

Inhibition of anaerobic digestion by ammonium

Angelidaki and Ahring (1993) demonstrated that high levels of ammonia from manure can inhibit the digestion process. However, the ammonia levels at which inhibition occurs are higher in digesters in which the ammonia levels have gradually increased (e.g. in continuous reactors) compared to situations in which the ammonia level has suddenly increased (e.g. batch reactors). The microbial population requires a gradual adaptation period to deal with the new circumstances. Inhibition levels for thermophilic reactors are around 5 g NH₄-N l⁻¹, which are levels that can occur in well running

reactors, especially when urine is included in the feedstock. Inhibition levels for mesophilic reactors are similar, as Sheng *et al.* (2013) reported inhibition of anaerobic digestion at levels $> 3,8 \text{ g NH}_4\text{-N l}^{-1}$

Changes in pH

When manure undergoes anaerobic digestion, organic solids are converted to volatile fatty acids (VFA). As these organic acids accumulate, the pH initially tends to decrease (see Figure 2). Manure usually has enough buffering capacity to prevent the organic acids from decreasing pH too much. Methanogenic microorganisms in a next step convert the VFA's to methane, if the pH does not fall below approximately 6.5. As these acids are converted the pH of the effluent will increase due to the consumption of protons during methanogenesis (Möller and Müller, 2012) (see also Figure 2). Generally, this will result in pH values of bio-slurry > 7 . Reports on farmers in US using digester effluent on their fields have reported an increase in soil pH with a corresponding reduction or complete elimination in liming requirements (Penn State Institute for Energy and the Environment, 2013).

The digestate pH is also affected by the concentration of base cations (e.g. Ca, K); they buffer digestate pH because H^+ in the liquid phase can sorb at the solids, thereby releasing cations. The effective H^+ concentration will then decrease and pH thus increases. Simultaneously, precipitation of carbonates (e.g. CaCO_3) buffers pH between 7 and 8 (Möller and Müller, 2012).

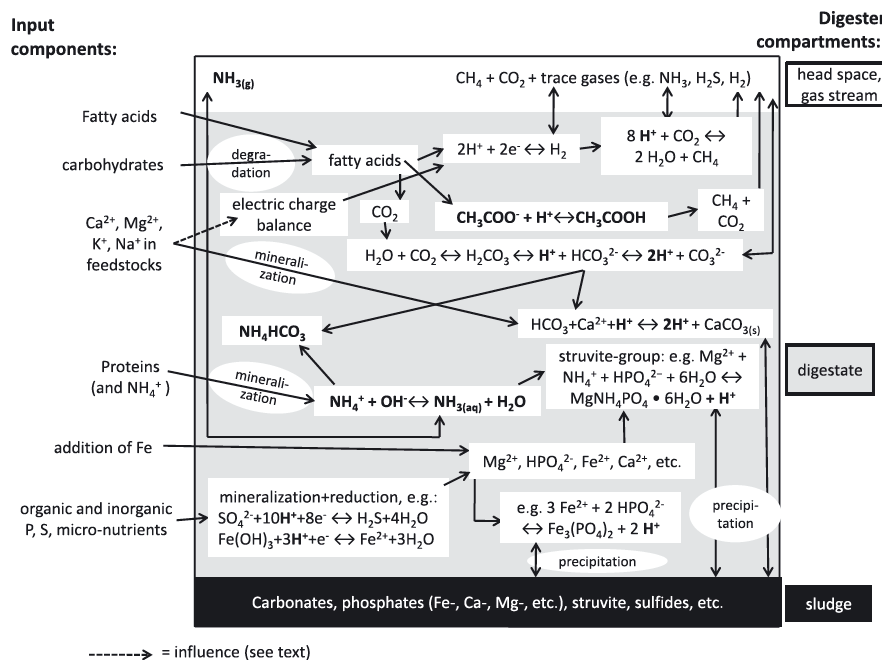


Figure 2 Factors affecting pH-value of digestates (from: Möller and Müller, 2012).

Other effects

Physically, there is a difference between farmyard manure and bio-slurry (Möller and Müller, 2012). Because small particles in the manure are broken down during the digestion process, there is a shift in distribution towards larger particles ($> 10 \mu\text{m}$) which are more resistant to degradation.

Bio-slurry is different from the original feedstock in a bio-chemical sense as they contain bioactive substances (i.e. phytohormones, nucleic acids, monosaccharides, free amino acids, vitamins and fulvic acid), with the potential to promote plant growth and to increase the tolerance to biotic and abiotic stress. Further, digestates can have higher levels of indoleacetic acid (plant hormone stimulating cell division and cell elongation) than the original manure which could only be explained by microbial synthesis during the digestion process (Möller and Müller, 2012).

4 Storage of bio-slurry

In many cases the bio-slurry from the digester cannot be directly used as a fertilizer and thus needs to be stored. This storage is mainly necessary because fertilizers should only be applied in specific periods of the growing season, while bio-slurry is generally produced continuously. Several options for storage exist, e.g. closed or uncovered lagoons, vessels and tanks. Alternatively, the bio-slurry can be separated into a liquid and a solid fraction, which are stored separately. The solid fraction can be stored dry in a similar manner as compost or animal manure. Producing a solid fraction can be less costly compared to the transportation of large volumes of digestate. Additionally, the separated fractions can be handled easier, the composition is more homogeneous, and because the nutrient contents in the two fractions are different, fertilisation can be better tuned to the plant needs.

Ideally the solid fraction can be sold as a valuable fertilizer while the liquid fraction is used relatively near to the digester. The practical means or machinery to apply these products on land is of course also relevant.

There exist several systems to store bio-slurry, e.g.:

- Cylindrical above-ground tank with coverage (e.g. In the netherlands; de geest *et al.*, 2013);
- Below ground tank (for example in china: surendra *et al.*, 2013);
- Concrete slurry store, with or without permeable (weeping) walls (e.g. In uk);
- Uncovered tanks (e.g. In italy: menardo *et al.*, 2011);
- Un-lined earth bank slurry lagoon without cover (common in developing countries, e.g. Vietnam: thien thu, 2012).

The losses of nutrients for each of these systems are different and are further dependent on environmental conditions, bio-slurry composition and storage design.

Now, this section discusses losses of nutrient by:

- Gaseous emissions;
- Leaching of digestate and pollution of groundwater and surface waters.

Further, this section discusses how environmental conditions and storage system design affect these losses.

Gaseous losses

Similar to manure, N_2O , H_2S , NH_3 and CH_4 gases can be released when storing digestate in open systems (Amon *et al.*, 2006). The emissions are relevant as some of them are greenhouse gasses but in the context of this report the loss of valuable nutrients is relevant. Losses of biogas (CO_2 and CH_4) and ammonia (NH_3) from stored bio-slurry are assumed by many authors (Balsari *et al.*, 2013; Lindorfer *et al.*, 2007; Sommer, 1997) due to its high concentration of undigested volatile solids (VS) and ammonium (NH_4). Also bio-slurry can contain significant amounts of residual biogas (Gioelli *et al.*, 2011; Menardo *et al.*, 2011). For the mostly less advanced digesters in developing countries, anaerobic digestion might not be fully complete leading to additional digestion during storage with consequent emissions of CO_2 , CH_4 and NH_3 . These gases will be released during the storage but also during the separation in a solid and a liquid fraction, and during the mixing of the bio-slurry. Especially methane (CH_4) is emitted during homogenisation of the bio-slurry before application to the field (Nicholson *et al.*, 2002).

No documents have been found that study the gaseous losses of N from bio-slurry and other digestates from various storage systems specifically. However it is likely that all techniques that decrease the loss of N from pig and cattle slurries are also relevant for bio-slurry. The amount of loss of nutrients during storage is mainly via volatilisation of NH_3 to the air. The loss of N from pig and cattle slurries to the air above the slurry is determined by its pH, temperature and the total ammonia

nitrogen (TAN), and the ammonia concentration in the air (Sommer, 1997). The pH affects NH_3 volatilisation because in the slurry the chemical equilibrium between ammonium (NH_4) and ammonia (NH_3), $\text{NH}_4^+ \rightleftharpoons \text{NH}_3 + \text{H}^+$, is shifted more towards the volatile NH_3 at higher pH values. The loss of N from stored slurry is determined by additional parameters: air refreshment, wind speed or in other words coverage of the manure. Various types of floating covers, including natural crusts, and closed covers are used to reduce gas emissions from slurries (Van der Zaag *et al.*, 2008). All types of covers are capable of reducing NH_3 emissions, odour and H_2S compared to an uncovered storage.

Table 3 shows the emissions of NH_3 as given in various papers. Emissions are given either in $\text{g NH}_3 \text{ m}^{-2} \text{ day}^{-1}$ or as percentage of total N. These can be used to estimate the loss from stored slurry if both the air-exposed surface area to volume ratio is known and the total N content. For example, assuming a surface area of $1 \text{ m}^2 \text{ m}^{-3}$ slurry, an N content of bio-slurry of 5 kg N m^{-3} , and an emission of $10 \text{ g NH}_3 \text{ m}^{-2}$, the loss is calculated estimated to be 0.2% per day or 6% per month. Sommer (1997) determined a loss of total N of 30% within one year for uncovered digested animal slurry. The loss of N is mainly via NH_3 although N_2O emission is important as greenhouse gas. Petersen *et al.* (2013) measured that 0.06-0.12% of the N was emitted as N_2O from uncovered pig slurry within 58 days.

Table 3

Emission of NH_3 from animal slurries, bio-slurries and other digestates.

Source	Emission ($\text{g NH}_3 \text{ m}^{-2} \text{ d}^{-1}$) or (% of total N)	Remarks	Reference
Uncovered pig slurry	2.0-3.1	Summer, Denmark	(Petersen <i>et al.</i> , 2013)
Pig slurry covered with straw enforced crust	0.2-0.3	Summer, Denmark	
Uncovered bio-slurry	0-25	Min. at sub-zero temp., max. in summer period, Denmark	(Sommer, 1997)
Covered cattle slurry	1.05	80 days at app. 17°C, Austria	(Amon <i>et al.</i> , 2006)
Covered bio-slurry from cattle slurry	0.25	80 days at app. 17°C, Austria	
Digestate from food waste	0.9		(Whelan <i>et al.</i> , 2010)
Non-separated digestate	2.06-4.44	Season, Italy	(Gioelli <i>et al.</i> , 2011)
Digestate liquid fraction	7.89-14.6	Season, Italy	
Covered pig slurry	25-30%	After 90 days, Vietnam	(Tran <i>et al.</i> , 2011)
Uncovered pig slurry	60-70%	After 90 days, Vietnam	
Covered bio-slurry	0.03-0.15 $\text{g NH}_3\text{-N/kWh}$	Germany	(Liebetrau <i>et al.</i> , 2013) ^a
Uncovered bio-slurry	0.2-3.7 $\text{g NH}_3\text{-N/kWh}$	Germany	

^a Units for data from Liebetrau are based on energy production of biogas plants and cannot easily be converted to more common units.

Although, most references in Table 3 are from developed countries, it is clear that coverage substantially reduces NH_3 emissions, which will also be the case in developing countries. When bio-slurry is compared to manure, Sommer (1997) found that the loss of nitrogen from bio-slurry is higher than from animal slurries (both not covered), mainly because NH_4 concentrations are much higher in bio-slurry than in the animal slurries.

Results for losses however are not always straightforward, as Amon *et al.* (2006) found that bio-slurry from cattle slurry released not more NH_3 than untreated cattle slurry during storage but released more after application. These results are remarkable taking into account the higher NH_4 contents and higher pH of digested slurry.

In the above mentioned studies, bio-slurry is only compared to liquid animal slurries. A comparison of emissions from bio-slurry with those of solid farmyard manure has not been found.

Leaching losses

Another route for the loss of nutrients during storage is via leakage. This is the case for storage of solid manure, liquid manure and bio-slurry in an un-lined earth bank storage. Massé *et al.* (2007) let the effluent of digested pig manure settle for three to six weeks. They found that most of the P was retained in the solid settled fraction, while approx. 70% of the N and K were found in the liquid

fraction. This may imply that N and K are susceptible to leaching in case the storage facilities do not have an impermeable barrier at the bottom. To prevent leakage of digestate to the groundwater, the EU has issued regulations on organic fertilizer storage included in the Code of Good Agricultural Practice in the vulnerable zones in EU countries (European Community, 1991). This code mentions the following items for storage: rules about (1) the capacity; (2) construction of storage vessels; and (3) measures to prevent water pollution. This means that the storage capacity must exceed the storage needed throughout the longest period when land application is not required (winter, outside growing season). To prevent water pollution most EU countries require that the storage of digestate is at a certain distance from water courses and wells; also the problems with odour should be minimised.

Besides storage of manure and bio-slurry also other factors, such as application, determine the loss of nutrients. A comparison between 12 farms with biogas and 12 farms without biogas was made in Vietnam (Vu *et al.*, 2012). A relatively important fraction of the manure at the non-biogas farms (16%), and an important part of the bio-slurry (60%) was discharged in the public sewage system. The high loss at the biogas farms was related to the excessive use of washing water and the common practice to discharge the liquid fraction of the digestate because of its low nutrient content (Vu *et al.*, 2012).

5 Bio-slurry handling

Fresh bio-slurry is a liquid when it leaves the digester. It can be applied as a fertilizer in this liquid form, for example by releasing into irrigation channels or manually by using buckets. This application of liquid bio-slurry is only convenient when the plots are close to the digester. Otherwise, the bio-slurry needs to be transported which is very inconvenient. Preferably the bio-slurry is then converted into a solid fertilizer. This can be done either by drying or composting of the bio-slurry.

Bio-slurry drying

In some regions, drying of bio-slurry is used to produce a solid organic fertilizer. E.g. in Bangladesh, the company Grameen Shakti markets dried bio-slurry as an organic fertilizer (Kamal *et al.*, 2011). Drying of bio-slurry can change the chemical composition and nutrient contents of the bio-slurry. Especially nitrogen contents can decrease through volatilisation of NH_3 . P and K are not volatile and are expected to be preserved during drying of bio-slurry. No specific studies have been found that looks at the effects of drying on bio-slurry quality. However changes in nutrient composition can in principle be assessed when comparing nutrient ratios for manure and dried bio-slurry. Table 4 shows some values of N:P and N:K ratios for manures and dried bio-slurries.

Table 4

N:P and N:K ratios of dried and undried organic fertilizers (Kamal et al., 2011).

Source	N:P ratio	N:K ratio
Cow dung	0.82	2.0
Dried cow dung bio-slurry	0.46	1.8
Poultry litter	0.83	2.68
Dried poultry litter bio-slurry	0.82	3.2

Table 4 does not give a clear picture on N losses. For cow dung the N:P ratio of dried slurry is much lower than the N:P ratios of cow dung. This indicates the losses of N. However, based on N:K ratios the N losses are less evident. For poultry manure the reported N:P ratios of dried bio-slurry are only marginally lower than N:P ratios of manure, while the reported N:K ratios of dried slurry are similar or even higher than ratios of manure.

Remarkably, a report by Karki (2006b) concluded that drying of bio-slurry leads to losses of P and K, which is unlikely because neither P nor K are volatile and these elements should therefore be preserved in the dried bio-slurry. This conclusion was drawn from a comparison between fresh and dried bio-slurry, however the fresh and dried bio-slurries were from different sources. In this case, it is likely that the original composition of the bio-slurry differed that much that the lower P and K in the dried bio-slurries is purely a coincidence.

The above shows that effects of drying on nutrients in bio-slurry are not very clear from currently available studies. Field results sometimes contradict theoretically expected results and consequently more research is needed on this issue.

Bio-slurry composting

An alternative for drying is composting of bio-slurry. To compost the bio-slurry, it has to be mixed with fresh organic material as agricultural residues or straw, because the bio-slurry itself contains too little degradable material to sustain the composting process.

During composting, the chemical form of the nutrients, especially N, can change and also nitrogen can be lost through volatilisation of NH_3 .

Islam (2009) reported that N contents in composted bio-slurry of both cow dung and poultry manure in Bangladesh were higher than in non-composted manure, whereas concentrations of most other nutrients were not higher. It might be that this additional N originates from the biomass which has been added to the bio-slurry for composting. Unfortunately, they did not report which biomass was used for composting. In the same report they also showed that similar results were found in a study in Nepal. Here, they concluded that the increase in N was due to the higher N concentrations in added organic material, which was evidenced by the given compositions of commonly added materials.

Also a more detailed study by Karki (2006a) in Nepal showed that N contents increased in compost relative to P and K contents. This study further showed that ammonia concentrations in composted bio-slurry are greatly reduced, either by ammonia volatilisation or by nitrogen immobilisation on the added organic material.

Table 5 gives an overview of nutrient contents in manure, bio-slurry and composted bio-slurry.

Table 5

Nutrient contents of composted and uncomposted bio-slurries (in %).

Origin	Bio-slurry			Composted bio-slurry			Reference
	N	P ₂ O ₅	K ₂ O	N	P ₂ O ₅	K ₂ O	
Cow dung bio-slurry	1.37	1.8 ^a	0.40 ^b	1.69	1.8 ^a	0.47 ^b	(Islam, 2009)
Poultry manure bio-slurry	1.84	1.8 ^a	0.58 ^b	2.60	2.1 ^a	0.96 ^b	(Islam, 2009)
Cow dung bio-slurry	1.2	0.6	1.4	1.6	1.2	0.9	(Islam, 2009)
Non-latrine attached digesters	1.76-2.02 (NH ₄ 0.12-0.2)	1.74-1.95	1.74-1.95	1.54-1.67 (NH ₄ 0.01)	0.87-1.50	0.49-0.73	(Karki, 2006a)
Latrine attached digesters	1.99-2.24 (NH ₄ 0.45-0.67)	1.26-1.29	1.22-1.61	1.64-1.70 (NH ₄ 0.01-0.12)	0.87-0.90	0.50-0.54	(Karki, 2006a)

^a Originally reported as P, recalculated to P₂O₅.

^b Originally reported as K, recalculated to K₂O.

No studies have been found that explicitly determined the loss of nitrogen through volatilisation during bio-slurry composting. Only two studies were found that quantified N losses during composting: a study by Mahimairaja *et al.* (1994) on composting of poultry manure (not bio-slurry) might give some indication of NH₃ volatilisation. Poultry manure is to some extent comparable to bio-slurries because poultry manure contains relatively high amounts of NH₄ like bio-slurry too. They found that 11-16% of the nitrogen is volatilised during composting, depending on the material that has been added. Further, a study by Bustamante *et al.* (2012) on composting of the solid fraction of digestate showed N losses between 15 and 27%. Table 5 further shows that especially the NH₄ contents of bio-slurry have diminished after composting. A similar result has been found by de la Fuente *et al.* (2013) who looked at composting of digestate from co-digestion (cattle manure + maize-oat silage). These NH₄ losses might be because of volatilisation, but also some immobilisation (i.e. incorporation into organic material) might have taken place. The reduction of NH₄ after composting has therefore greatly reduced the amount of directly available nitrogen compared to uncomposted digestates.

Additives

Another treatment of bio-slurry can be the use of additives to reduce ammonia volatilisation. Only one document has been found that reported on the use of additives (Thu, 2006). They stated that the addition of phosphate fertilizer reduced nitrogen volatilisation such that nitrogen contents were 2.45 times higher after 50 days of storage compared to bio-slurry with no additions. Remarkably, the reaction equation given in that report to explain the mechanism of ammonia retention showed calcium sulphate (CaSO₄) instead of phosphate. Consequently, it is unclear if either phosphate or sulfate (or both) can reduce nitrogen volatilisation.

6 Methods for the determination of the value of bio-slurry as fertilizer

Value of bio-slurry as a fertilizer

Principle

Because all organic manures including bio-slurry contain nutrients, they will behave more or less as a fertilizer. An important question is whether the nutrients in bio-slurry are available for crop uptake, or not. In that respect, bio-slurry will be different from mineral fertilizers, because part of the nutrients is organically bound and not directly available for plant uptake.

Nitrogen (N) in bio-slurry is partly present as the plant-available compound ammonium (NH_4^+) and as organic N. Organic N is only available for plant uptake after mineralisation to inorganic N. The N mineralisation of organic products strongly depends on the decomposability and the C/N-ratio of the organic matter in the product (Janssen, 1996). So, the availability of N from farmyard manure and bio-slurry depend on the amounts of organic and inorganic N, the stability of the organic components and the C/N ratio of the organic component. Also phosphorus may be present in many different forms in organic products, differing in the availability for plant uptake (e.g. Velthof *et al.*, 1998). This is also the case in bio-slurry (Bachmann and Eichler-Löbermann, 2013; Gungör, 2007; Möller and Müller, 2012).

The 'fertilizer value' or the 'fertilizer replacement value' of a nutrient in an organic product such as bio-slurry is generally defined as the percentage of the total amount of that nutrient that is coming available for plant uptake. This can be determined by relating the amount of a nutrient from a regular mineral fertilizer (reference) that is required to attain the same yield or uptake of that nutrient as a certain amount of the organic product (e.g. bio-slurry; see further).

Determination of the fertilizer value of bio-slurry

The value of bio-slurry as a fertilizer depends on

- Nutrient contents, e.g. The amount of nitrogen, phosphorus, potassium, calcium, magnesium, sulphur and micronutrients on a fresh or dry matter basis;
- The ratio between nutrients, e.g. The n/p-ratio and/or the n/k-ratio; and
- The availability of the nutrients, which is determined by the compounds that contain the nutrients.

In addition to the fertilizer value, bio-slurry may have positive effects on soil quality and crop growth in the long term caused by organic matter inputs.

The first step in the determination of the value of bio-slurry as a fertilizer is to determine its nutrient content. The nutrient composition of bio-slurry can be determined by a specialised laboratory. Unfortunately, measurement and reporting of nutrient contents of bio-slurry is not always done in a clear and transparent way. A common issue in reporting the composition of bio-slurry is that it is not clear whether contents are based on fresh or dry matter basis, total mass or volume basis, on total solids or on fresh feedstock material. A striking example is given in Table 6.

This table shows the contents of C and nutrients in bio-slurries in Rwanda. You can notice that for the first district, all C contents are around 50%. This suggests that these C contents are given as percentage of dry matter, because most organic materials consist of approximately 50% of C. Nutrients are then most likely also reported as percentage of dry matter. However, for the other districts, the C contents are much lower and are around 50% of the reported dry matter. This suggests that these C contents are reported as percentage of total mass. Consequently, it is most likely that nutrient contents will then be based on total mass as well.

Table 6

Nutrient contents of bio-slurry in the Rwanda country report (Fashaho *et al.*, 2013).

District	Sector	DM (%)	C (%)	Tot N (%)	Tot P (%)	K (%)	Ca (%)	Mg (%)
Nyamagabe	Uwinkingi	33.99	57.54	1.41	0.18	0.11	1.11	0.24
	Gasaka	32.46	48.16	1.71	0.16	0.06	1.23	0.55
	Mbazi	27.95	43.45	1.33	0.21	0.15	1.58	0.96
	Average	31.47	49.72	1.48	0.18	0.11	1.31	0.58
Gicumbi	Rukomo	47.08	21.61	0.54	0.22	0.07	0.87	0.37
	Shangasha	45.67	25.77	1.08	0.21	0.02	0.82	0.7
	Kageyo	44.76	32.55	0.63	0.14	0.04	0.81	1.21
	Average	45.84	26.64	0.75	0.19	0.04	0.83	0.76
Kirehe	Mushikiri	37.39	37.23	1.62	0.19	0.05	0.7	1.52
	Musaza	50.86	21.53	0.92	0.12	0.04	0.71	0.66
	Gatore	41.98	30.82	1.04	0.15	0.06	0.69	2
	Average	43.41	29.86	1.19	0.15	0.05	0.7	1.39
Musanze	Busogo	47.52	22.18	0.54	0.13	0.02	0.73	0.17
	Nyange	47.17	26.48	0.97	0.12	0.06	0.91	0.85
	Kinigi	47.51	28.94	1.29	0.13	0.04	0.88	0.76
	Kimonyi	39.8	31.91	0.98	0.22	0.07	0.66	0.51
	Average	45.5	27.38	0.95	0.15	0.05	0.8	0.57
Karongi	Murambi	46	21.85	0.61	0.13	0.05	0.7	0.23
	Rugabano	47.07	34	1.7	0.1	0.11	1.36	0.87
	Gashari	39.84	25.16	1.25	0.14	0.01	0.95	0.45
	Average	44.3	27	1.19	0.12	0.06	1	0.52
General average		42.16	32.14	1.1	0.16	0.06	0.92	0.77
Standard deviation		6.31	10.06	0.38	0.04	0.04	0.26	0.48

Nevertheless all reported values, despite their different base, are averaged in the end. For this example, the dry matter and C contents provide a clue for the reporting base. If any of these are missing, which has been found frequently, it is not clear how to interpret reported nutrient contents.

As a response to above comments the researcher indicated that all reported C contents are expressed at a dry matter basis (pers. comm. Fashaho).

A second step in determining the value of bio-slurry as a fertilizer is to determine the availability of the nutrients. The availability of a nutrient can be determined experimentally, or if that is not possible, it can be calculated. The following experimental methods are available for the determination of the availability of nutrients in organic products, such as bio-slurry:

- The use of availability indices, by which the available fraction of a nutrient is determined. An example is described by Velthof *et al.* (1998), who concluded that the content of inorganic N (which is directly available for plants and which can be extracted by 0.01 M CaCl₂-solution) in an organic product, gives a good prediction of the plant available N in products with a high inorganic N fraction. Moreover thermal fractionation and pepsin extraction provided a reasonable index for mineralisable N in organic products, because the easily degradable organic N fractions are determined with those procedures (Velthof *et al.*, 1998). Nitrogen can only be taken up by plants in mineral form (ammonium and/or nitrate), which implies that N that is present in organic form in manure or bio-slurry, should be mineralized into ammonium / nitrate, before it can be taken up by plants.
- Incubation studies, in which the nutrient release from organic products during incubation with soil is monitored during a period of several weeks / months. In general, the monitoring consists of measurements of the amounts of available nutrients in soil with and without the organic product and in soil with a reference fertilizer (e.g. Gordon *et al.*, 1987).
- Pot and/or field experiments, in which the availability of one or more nutrients of the organic product is investigated by growing plants. In general, control treatments without addition of the nutrient and reference treatments with addition of a regular fertilizer are included in the experimental setup. The availability of the nutrient can be derived from crop growth, yields and/or nutrient uptake (e.g. Raijmakers and Janssen, 1993; Velthof *et al.*, 1998).

7 Comparison of the value of bio-slurry and manure as fertilizer

Differences in properties between farmyard manure and bio-slurry

The differences between the properties of farmyard manure and these of bio-slurry have been described in Chapter 3 and can be summarized as follows:

- The organic matter content in bio-slurry is lower than that in farmyard manure.
- The organic matter is more stable in bio-slurry.
- The total N content (at fresh matter basis) will be similar, if ammonia volatilization during anaerobic digestion and subsequent bio-slurry handling is prevented.
- The ammonium content in bio-slurry is higher than that in farmyard manure.
- The pH of bio-slurry is somewhat higher than that of farmyard manure.
- The P, K, Mg and Ca contents are similar in bio-slurry and farmyard manure.

Comparing the value of bio-slurry and manure as a fertilizer

If the effectiveness of bio-slurry as fertilizer is compared to other organic or inorganic fertilizers in a pot or field experiment, the experimental set up is very important. The following aspects should be taken into account:

- The focus of the experiment should be clear and the experimental design should be in line with that focus. Because the most limiting nutrient determines crop growth and yield, it is difficult to study the availability of more than one nutrient at the same time. It should be prevented that other factors than the nutrient studied are affecting differences between treatments. E.g. if the nitrogen availability of bio-slurry is compared with that of manure, it should be prevented that differences in P and K availability between the treatments are affecting yield and/or N uptake. In this example, the N application should be the same for all treatments and should be at a suboptimal level. The availability of P, K and eventually other yield limiting nutrients should be non-limiting. If the supply of those nutrients from the bio-slurry and/or manure is not sufficient, additional amounts of those nutrients should be applied with mineral fertilizers. In that way, differences in the N availability of bio-slurry and manure can be compared.
- Control treatments should be present. E.g. if the N availability of bio-slurry is determined, a control treatment without N should be included in the set-up, to correct for N supply by the soil.
- To be able to determine the fertilizer replacement value, a reference treatment with mineral fertilizers should be present. E.g. if the aim is to determine the N fertilizer replacement value of bio-slurry, a mineral N fertilizer (e.g. urea or calcium ammonium nitrate) should be included in the set-up.
- Replicates (3 to 4) should be included to compensate for variations that will always be present.
- Treatments should be allocated at random to pots or plots.

Table 7 compiles the results of various studies that compared bio-slurry as a fertilizer to other fertilizers. This table includes whether there were any shortcoming in the methodologies that have been used in the comparison.

Table 7

Compilation of studies comparing the fertilizer value of bio-slurry with manure, evaluation of the methodology used and result of the comparison.

Reference	Manure / digestate type	Adequate methodology	Reason(s) for inadequate methodology; other shortcomings	Result of comparison, if possible
Islam, 2009	Cow manure Cow manure slurry Poultry manure Poultry litter slurry	No	Slurry and manure combined with fertilizer in treatment Possible effects of different amounts of P and/or K	
Muhammad <i>et al.</i> , 2013	Farmyard manure, liquid and dried bio-slurry, all from unreported sources	No	All treatments had same N levels, however P and K levels differed. Only reference treatment with chemical fertilizer received the recommended dose. For spinach, P levels were less than recommended dose for manure and bio-slurry	Highest yields for chemical fertilizer, then dried bio-slurry and farmyard manure, liquid bio-slurry had lowest yield. Liquid bio-slurry was applied via the irrigation water (= fertigation; possibly that has led to N losses through volatilisation)
Alburquerque <i>et al.</i> , 2012a	Cattle manure, Bio-slurry mixture of pig manure with slaughterhouse waste and biodiesel waste water	Yes	Treatment of slurry and manure are combined with fertilizer through fertigation; N from one source only, P/K from mixed sources	Slurry not significant different from FYM for both watermelon and cauliflower; N fertilizer value of bio-slurry and FYM were lower than that of mineral fertilizers
Garfi <i>et al.</i> , 2011	Guinea pig manure and digestate	No	Different application regimes Different amounts of P/K	
Morris and Lathwell, 2004	Fresh dairy manure and bio-slurry from dairy manure	Yes		No differences in plant weight maize in pot experiment
Bernal and Kirchmann, 1992	Only (fresh) pig faeces, no urine or straw, anaerobically digested and aerobically treated	Yes		Higher NH ₃ losses from bio-slurry; cumulative N mineralisation in beginning higher for bio-slurry, at 60 days not different
Fashaho <i>et al.</i> , 2013	Dairy cattle manure and bio-slurry	No	Differences between treatments in amount, timing and placement of fertilizers making comparisons between bio-slurry, dairy cattle manure and fertilizer not possible	
Chau, 1998a	Cow and pig dung and cow and pig bio-slurry	No	No control treatment; no reference treatment with mineral fertilizer	Higher yields of cassava for bio-slurry. This can be caused by the application method: before planting and after first harvest for manure, every three days for bio-slurry
Chau, 1998b	Cow and pig dung and cow and pig -slurry	No	No reference treatment with mineral fertilizer. N levels were same. P and K levels were not reported	Results for duckweed yields in ponds. Initially higher production with manure, after three weeks higher production with bio-slurry. Protein contents of duckweed were always higher for bio-slurry
Alburquerque <i>et al.</i> , 2012b	Pig- and cow bio-slurry with different amounts of co-digestion products	No, the focus was on phytotoxicity	Only the elemental composition and phytotoxicity were determined	
Akhtar, 2011	Unclear input material of bio-slurry (very likely cow dung); no FYM but compost, unclear input material compost	No	Mixing of bio-slurry and fertilizer in experiment No information on composition of bio-slurry	

Reference	Manure / digestate type	Adequate methodology	Reason(s) for inadequate methodology; other shortcomings	Result of comparison, if possible
Bachmann <i>et al.</i> , 2011	Dairy slurry and co-digested dairy slurry	No	The aim was to determine the P fertilizer value, but N supply (amount of available N) was not the same in all treatments; differences were partly caused by N	For maize no difference between two slurries. For amaranth on sandy soil no difference; on loam soil bio-slurry gave a better yield and N uptake because of higher content of available N, no difference in P-uptake
Shahariar <i>et al.</i> , 2013	Cow dung and cow dung bio-slurry. Poultry litter and poultry litter bio-slurry	No	Animal manure and bio-slurry are mixed with fertilizer in treatment. By this method the availability of nutrients from the products itself cannot be determined	
Den Toom, 2013	Various bio-slurries from cattle, pigs, goats, human faeces, manure and litter. Also combinations of fertilizer were applied	No	Each treatment received similar amounts of bio-slurry; as nutrient content varied, the amount of the applied nutrients varied.	
Singh <i>et al.</i> , 2007	Input material bio-slurry unknown; FYM; vermi-compost; sugarcane press cake	No	All treatments received equal amounts of product. Nutrient composition differs between products, hence different levels of nutrient supply	Based on available data, no difference between FYM and bio-slurry
Tani <i>et al.</i> , 2006	Cattle slurry and cattle bio-slurry (also slurry and bio-slurry mixed with bone ash and fire extinguishing chemicals)	No	Similar amounts of N, but amounts P and K are unclear. No control plot	No significant differences in corn yield between bio-slurry, slurry and chemical fertilizer
Rahman <i>et al.</i> , 2008	Bio-slurry from cattle slurry, no manure	No	This is a response trial to different amounts of slurry (NPK) not a comparison between FYM and slurry	As response trial: 0 is significant lower compared to others, 10 and 12 ton/ha were significant better than 1.4 t/ha
Möller <i>et al.</i> , 2008	Bio-slurry from dairy cattle, FYM form dairy cattle, liquid slurry from dairy cattle	Yes/no	Large experiment with different crops in rotation. N not always applied in equal amounts in every crop or on rotation basis. Report on N, unclear what status other nutrients, e.g. P or K. Significant difference for winter wheat likely due to different amounts of N. Significant difference in spring wheat may be due to significant higher levels of soil N in bio-slurry. Remark is made that bio-slurry increased yield only if soil incorporation is done shortly after field spreading.	On rotation basis no difference between FYM, undigested slurry and digested bio-slurry. In winter wheat (grain) bio-slurry significant better than FYM but non-significant to undigested slurry In spring wheat (grain) bio-slurry significant better than FYM and undigested slurry. In maize yield and N-uptake no significant difference between FYM, undigested slurry and bio-slurry. All three significant better than control. N-use efficiency in FYM significant lower than bio-slurry and undigested slurry (both are similar)
Furukawa and Hasegawa, 2006	Only bio-slurries from kitchen garbage waste and cattle manure. No FYM	No	In kitchen garbage bio-slurry 99% of total N was NH ₄ -N. In cattle bio-slurry 75% of total N was NH ₄ -N. The fertilizer value of biogas effluents appears to be accounted for by the fraction of NH ₄ -N in total N.	
Karki, 2006a	FYM, bio-slurry and composted bio-slurry Other reported experimental results did not contain FYM	No	For maize/soya/cabbage bio-slurry and FYM were applied at similar amounts. However nutrient content differed (not shown), so amounts of nutrients differed. Other reported experimental results did not contain FYM and/or were mixed with fertilizer and/or applied amounts are not given: nutrient-amount effect?	Maize: no significant difference between treatments (level of significance not shown) Soya: no significant difference between treatments Cabbage: unknown if significant difference
Dahiya and Vasudevan, 1986	Bio-slurry from cattle dung (no manure)	No	Bio-slurry is compared to chemical fertilizer. No control plot	Yields with bio-slurry as fertilizer were for most crops smaller than with chemical fertilizer

From this table the following general conclusion can be drawn:

- Most studies in which correct methodologies were used, did not show significant differences in yield between bio-slurry and farmyard manure.
- Most of the reviewed studies had shortcomings regarding their methodology for a comparison of the value of bio-slurry and manure as fertilizer. Most common shortcomings were:
 - The levels of only one nutrient (mostly N) are the same in treatments that were compared, while the application levels of the other nutrients (mostly P and K) are different. In this case it is not clear if eventual differences in yield are caused by properties of the fertilizers or because of differences in the levels of the applied nutrients;
 - A control plot (no fertilisation) is frequently lacking. Especially when no differences are found between bio-slurry and other fertilizers, this non-difference might be caused by the nutrients delivered by the soil itself. This effect can only be excluded with a control treatment;
 - Statistics are often not reported, so it is not clear whether differences are significant;
 - Bio-slurry is not always derived from manure only. In a number of cases, other waste streams are added for digestion. This hampers a good comparison between digested and undigested manure;
 - Many studies did not report the application method for both bio-slurry and manure. This is important because surface application of bio-slurry can lead to large N losses through volatilisation. In case yields with bio-slurry are lower than yields with manure as fertilizer, this might be because of such N losses.
- Great care should be taken in case of adaptive research with the involvement of many local farmers, as has been described by Fashaho *et al.* (2013). In such a research, the various research sites are considered as replicates. It requires a proper execution of the experiments at all locations and proper statistical techniques for the analysis of the results.

Results of other reviews

In recent years, some other authors have also carried out a review on the use of bio-slurry as a fertilizer. Möller and Müller (2012) showed in a literature review that the N uptake after application of bio-slurries was lower than that after application with the same amount of N via mineral fertilizer. This means that the fertilizer value for N is < 100%. However, if the application was based on equivalent amounts of the NH_4^+ -N fraction in the bio slurry, comparable apparent NH_4^+ -N recoveries of bio slurry and mineral fertilizers were reported. From those results, it can be concluded that the availability of N in bio-slurry is lower than that in mineral fertilizer, because part of the N is present as organic N. Further, they found that in pot experiments, equivalent amounts of applied N gave higher N uptakes (about 10-25%) from the bio-slurry than that from the undigested slurries. This might be explained by the higher ammonium/total N ratio of the bio-slurry.

However, this has not always been found. In some field experiments with the application of equivalent amounts of total N, the uptake of N from liquid digested animal slurry equalled that of undigested slurry after surface application, despite the higher NH_4^+ -N content of the digestate. Möller and Müller (2012) concluded that substantial parts of N might have been volatilized as NH_3 .

Recently, a review discussing the value of bio-slurry has been published (De Groot and Bogdanski, 2013). The study contained among others a discussion of the fertilizer value of bio-slurry, based on studies with field experiments. They stated that the methodology is often not specified in detail, which hampers a proper interpretation of the results. They looked at five studies in which the effect of the application of bio-slurry was compared with other organic fertilizers (slurry, farm yard manure, vermicompost and precomposted manure). Bio-slurry gave better yields in some studies, while manure did in others. Hence, also in this case no consistent picture regarding the comparative fertilizer value of bio-slurry and manure is derived. Further, they concluded that there were no studies that looked at long term effects of bio-slurry on soil fertility. This might be important as bio-slurry can affect N mineralisation or immobilisation and it can affect soil organic matter dynamics.

Summarizing

- The value of bio-slurry as fertilizer is fairly high, because it contains nutrients in a readily available form. However, the nutrient contents are much lower than in mineral fertilizers, which has consequences for handling and the required application rates. The nutrient contents of bio-slurry may fluctuate in dependence of the composition of the manure that is digested and eventual N losses

during the anaerobic digestion and/or handling. These fluctuations in nutrient contents will affect the value as a fertilizer. Because N will be partly present as ammonium, which is directly available for plant uptake, and partly as organic N, the N availability will be higher than that of manure but lower than that of a regular mineral N fertilizer. The ammonium/total-N ratio will give an indication of the fertilizer N value of bio-slurry. The availability of P, K and the other nutrients is similar to that of mineral fertilizers.

- Compared to undigested manure, the direct N availability of bio-slurry is high. At the short term, this will lead to a higher N fertilizer value of bio-slurry in comparison with farmyard manure that has equal nutrient contents. The availability of other nutrients will be similar to that of manure. However, most studies in which correct methodologies were used did not show significant differences in yield between bio-slurry and farmyard manure.
- Most of the reviewed studies had shortcomings regarding their methodology for a comparison of bio-slurry and manure.

8 Food safety risks

The feedstocks for bio-slurry are manure and in some cases also human excreta. These feedstocks can contain pathogens. This chapter looks at the extent in which these pathogens persist during, and after anaerobic digestion, and whether this can lead to food safety risks. Additional food safety risks may occur when also other waste streams are used as feedstock for anaerobic digestion. Contaminants like heavy metals and persistent organic pollutants like polycyclic aromatic hydrocarbons (PAHs) and polychlorobiphenyls (PCBs) might then enter the food chain.

Fate of pathogens during anaerobic digestion

Food-borne diseases cause a large amount of illnesses, hospitalisations and deaths each year, and this has been associated with the consumption of raw food and fruits. For example, several outbreaks of illnesses are related to lettuce-consumption and *E. coli* O157:H7. Contamination of crops, such as lettuce, can occur when manure with pathogens is used as a fertilizer (Franz *et al.*, 2008). Manure is an important reservoir for pathogens and it is therefore important to reduce the survival of pathogens in manure and, consequently the risk of contamination of plant products.

The percentage of various manure samples in Germany that contains *Salmonella* is around 0-5%, and the number of bacteria (*E. coli* and *Enterococci*) in normal animal manure varies between 10^4 - 10^6 CFU/gram. In digesters that operate at 55°C *E. coli* are not expected and have not been found. However an elevated temperature does give additional *Clostridium* and *Enterococci* reduction (Philipp and Holzle, 2012). Another important disease is paratuberculosis (Nielsen and Toft, 2009). If human excreta are added to the manure, there is a risk of pathogens similar as in sewage sludge (Horan *et al.*, 2004): *Listeria*, *E. coli*, *Campylobacter* and *Salmonella*. The use of human excreta in biogas systems is common in several countries: e.g. in a survey of pig farms in Vietnam, in the Hanoi region 82%, and in the Hue region 42% of the farmers have connected their flush toilets to the biogas system (Huong *et al.*, 2014). In case other animal by-products (other than manure) are used as feedstock for anaerobic digestion, it should be considered that these animal by-products have higher risks of containing pathogens than manure.

To reduce risks of pathogens in manure, Franz *et al.* (2008) advises a minimum storage time of animal manure of 30 days and a minimum time interval between fertilization and planting of 60 days. This is however not an option for bio-slurry, because bio-slurry contains relatively high amounts of inorganic nitrogen that are readily available for plant uptake, but also for leaching and volatilisation. Fortunately, anaerobic digestion itself might also decrease the risk of pathogen contamination of food.

Table 8 summarizes the effects of various conditions during anaerobic digestion of manure and other organic materials on pathogens and also on weeds. Besides the temperature and time in the digester also the type of process (batch or continuous) can be important (Aitken *et al.*, 2005). Single-stage primary swine waste lagoons already reduce *Salmonella*, *E. coli*, *enterococci* and *coliphages* but not *C. perfringens* spores (Hill and Sobsey, 2003). In anaerobically stored cattle slurry, *M. paratuberculosis* can survive for 98 days at 15°C and 252 days at 5°C (Olsen *et al.*, 1985). The increased temperature during anaerobic digestion has a distinct influence on the inactivation of various bacteria, especially on *E. coli*, and to some extent on *Enterococcus faecalis*. Higher temperature have much less or no effect on *C. perfringens* and *Bacillus cereus* (Olsen and Larsen, 1987).

Table 8

Effect of temperature and time on human, animal, or phytopathogens, fungi and viruses under anaerobic conditions. In most cases the input material was spiked* with pathogens and weeds and not indigenous.

Pathogen	Input of digester	Mortality/ability to germinate/ after in digestate	References
<i>E. coli</i>	Sewage sludge	98% in 21 days at 35°C	Horan <i>et al.</i> , 2004
<i>Listeria monocytogenes</i> *		99.5% in 21 days at 35°C	
<i>Campylobacter jejuni</i> *		No effect in 21 days at 35°C	
<i>Salmonella senftenberg</i> *		99.5% in 21 days at 35°C	
<i>Acscaris suum</i>	Cattle manure	No effect of 2 days at 37°C 100% effect after 10 days at 37°C 100% effect after 2 days at 55°C	Johansen <i>et al.</i> , 2013
<i>E coli</i> O157:H7	Cattle manure	Survival during 6 months at 16°C	Semenov <i>et al.</i> , 2011
<i>M. paratuberculosis</i> *	Cattle manure	100% in 28 days at 35°C 100% in 2 days at 53-55°C	Olsen <i>et al.</i> , 1985
<i>M. avium subsp. paratuberculosis</i>	Mixture of animal manure, various plant crops	Free after 2 months at 41-42°C	Slana <i>et al.</i> , 2011
<i>E coli</i>	Cattle manure	99.7% in 5 days at 37°C. 100% in 40 min at 55°C 98.3% in 10 sec at 70°C	Watcharasukarn <i>et al.</i> , 2009
<i>Enterococcus faecalis</i>		98.3% in 15 days at 37°C 98% ₀ in 2 days at 55°C 100% in 1 day at 70°C	
<i>C. perfringens</i> *		95% in 5 days at 37°C <90% in 40 min at 55°C <90% in 10 sec at 70°C	
<i>Acscaris suum</i>	Untreated mixed bio-waste, including manure, fat, blood, food waste	100% effect after 15 min at 55°C	Sahlstrom <i>et al.</i> , 2008
Bacteria except <i>C. perfringens</i>		100% effect after 60 minute at 70°C	
<i>C. perfringens</i>		No effect after 60 minute at 70°C	
<i>Salmonella phage 28B</i>		No effect after 30 or 60 minutes at 70°C	
<i>Porcine parvovirus (PVV), swine vesicular disease virus (SVDV)</i>		Reduction after of PVV and SVDV after 30 min at 55°C, complete reduction of SVDV at after 60 minutes at 70°C	
<i>E coli</i>	Pig slurry	90-99% reduction	Quynh Huong <i>et al.</i> , 2014
Seeds of 5 plants species	Mixture of pig slurry, various plant crops	90% reduction between 1,5 and 20 days at 40-41°C	Westerman <i>et al.</i> , 2012
Seeds of 7 plants species	Cattle manure	0-1% germination after 1 week at 55°C	Johansen <i>et al.</i> , 2013
Seeds of 6 plant species	soil	100% reduction after: 0.17-0.67 hours at 70°C, 0.25-3 hours at 60°C, 0.25-3 hours at 60°C, 4-113 hours at 50°C, partly affected at 46°C, 42°C and 39°C	Dahlquist <i>et al.</i> , 2007
Phytopathogens	Mixture of cattle slurry, various plant crops	After 138 hours at 35-42°C the digestate was sanitized	Bandte <i>et al.</i> , 2013
Six different fungi	Organic household waste	90% reduction within 0.8-10 days at 37°C 90% reduction within 0.02-0.05 days at 55°C, however no reduction for thermophilic fungi	Schnurer and Schnurer, 2006

Another indication of potential food safety risks of bio-slurry application are the regulations set in many developed countries on digestate application. In most developed countries, where the use of bio-slurry and other digestates as a fertilizer is regulated, the regulator has set process requirements, requirements about the presence of pathogens, rules about impurities and the presence of weeds, and application rules (IPTS, 2013). For example, the process requirements in the EU, in case of any animal by-products is a pasteurisation step of at least 60 minutes at 70°C (European Union, 2009), except for farmyard manure, animal slurry, bedding and biomass of plant origin. The process requirements, in case of anaerobic digestion for materials not containing any animal by-products are certain time-temperature profiles, for example: digestion at 55°C for at least 24 hours and a retention time of at least 20 days. These regulations agree with the findings in Table 8 on pathogen reduction during anaerobic digestion.

A comparison of the bacterial parameters from manure with the output from a commercial continuously running digester during one year (Bonetta *et al.*, 2011) showed that the hygienic quality of the digested products (solid and liquid fraction) is equal or better than that of manure. The Scientific Panel on Biological Hazards of the European Food Security Authority (EFSA) (The Scientific Panel on Biological Hazard, 2007) has concluded that although mesophilic biogas treatments can reduce biological hazard of bio-slurry (as also shown in Table 8), it is not sure that it can consistently achieve this. This holds especially for developing countries where retention times are less strictly controlled and might be shorter than recommended.

Fate of pathogens after application to the soil

The effect of bio-slurry or manure addition to soil on pathogenic bacteria (*Escherichia coli*, *Salmonella*, *Listeria*) after the introduction to the soil (Goberna *et al.*, 2011) shows that anaerobic digestion sanitizes the material except for *Listeria*. In a period of three months, the pathogenic bacteria in the soil, added by manure, and also the *Listeria* by digestate, have returned back to levels before the fertilisation, probably due to the indigenous soil microbiota.

Also for bio-slurry, an indication of food safety risks can be obtained by looking at regulations for digestate application in developed countries. The use of digestate as a fertilizer for crops, such as grass for animals, or vegetables for human consumption, is allowed if the digestate complies with the regulations and Good Agricultural Practices (GAP). In the UK, GAP states that bio-slurry and other digestates, once pasteurised, are safe to use although livestock should not be grazed, or forage crops cut, within three weeks of application, or within two months for pigs, in accordance with legislative and good practice requirements for the use of all organic materials (WRAP, 2012).

In case human excrements are included as feedstock, the Good Agricultural Practices for sewage sludge can be a guide. In the UK, sewage sludge should be treated such that at least 99% of the pathogens are destroyed (Department for Environment, 2013). If these conventionally treated sludges are used as a fertilizer, a period of 30 months between planting of salads, and 12 months for other vegetables and crops is used. If used as a fertilizer on grassland or other forage a period of three weeks of no grazing applies, and the fertilizer should be injected.

Also the phytotoxicity of various digestate products might be an issue (Albuquerque *et al.*, 2012a; McLachlan *et al.*, 2004). From germination tests with standard plant seeds and various digestate products in comparison with various composts, it appears that the soluble salt content determines the phytotoxicity (Albuquerque *et al.*, 2012a; McLachlan *et al.*, 2004). This is especially relevant when digestate products are considered as a substitute for peat in nursery and/or horticulture.

Food safety risk from contaminants

When using relatively clean source organic materials like manure and human excreta, digestate does not show problematic levels of heavy metals and pesticides (Govasmark *et al.*, 2011). Only when other waste streams are used as feedstock, risks for contaminants in digestate may occur.

In most developed countries strict regulations apply for the use of digestates as organic fertilizer. Frequently, the regulation only allows the use of digestate as a fertilizer if the feedstock materials are regulated materials. However, due to high costs for waste treatment in these countries there is risk of discharging non-regulated, sometimes contaminated wastes in digestion (Neve, 2012).

Waste types that might be considered to be used in anaerobic digesters and that can contain elevated contents of contaminants are sewage sludge (e.g. Aparicio *et al.*, 2009), the organic fraction of municipal solid waste (OFMSW), and various industrial wastes (e.g. leather waste). Industrial waste, for example oil, can contain heavy metals and organic contaminants such as PAH, PCB, dioxins which can cause risks for plant, animal and human health. However, the digestion process does not seem to decrease the content of PCBs and PAHs in waste whereas composting is successfully applied as a remediation strategy (Brandli *et al.*, 2007a; Brandli *et al.*, 2007b; Hellstrom *et al.*, 2011).

Besides contaminants, also the occurrence of steroid hormones can be an issue, as it was shown that these can occur in the digestion liquid, which at least shows that these substances are not completely removed by the process (Rodriguez-Navas *et al.*, 2013).

Concluding

A storage time and elevated temperature decreases the number of pathogens in bio-slurry, but does not affect all pathogens. On the basis of the number of pathogens it is likely that the risks of pathogen contamination of food grown is lower for fresh bio-slurry than for fresh farm yard manure. However, storage and the earlier application to the field of farm yard manure will also reduce pathogens in it, so pathogen risks are probably similar for bio-slurry and manure. The addition of human excreta as feed stock might give additional risks for bio-slurry, although literature does not provide a definitive conclusion on that.

9 Bio-slurry in aquaculture

The integrated livestock-fish culture approach sees the integration of fish farming with livestock husbandry in a design allowing wastes from one system to be used as inputs in another system (Ogello *et al.*, 2013). Fish-poultry integration is popular across Africa and Asia. However, fish-cattle integration is not as popular. The high cost of pelleted fish feeds makes integrated fish farming a viable option for the reduction of the cost of feeding fish while simultaneously increasing the yield. This can lead to higher economic returns. Livestock manure as source of biogas production and organic fertilizer for fish ponds can help to reduce cost of investment in purchasing inorganic fertilizers. Ogello *et al.* (2013) concludes that livestock-fish integration is one of the most practicable solutions to food insecurity and malnutrition in East African countries.

The use of organic manure for pond fertilization has become one of the most important management operations in recent years by small scale fish farmers, basically to increase biological productivity of fish ponds (Chanda and Maska, 2012). In general, 50 kg of manure produces 1 kg of fish (Spliethof, 1988). Direct consumption of manure by fish is not very effective. From the point of view of energy intake, most types of manure are inferior to other food. The metabolisable energy of cow manure ranges from 600-800 Kcal kg⁻¹ while zooplankton contains 3000-4000 Kcal kg⁻¹. Very likely bio-slurry contains even less energy because easily degradable compounds have been removed during the anaerobic digestion. Also, the protein in the manure cannot be assimilated by fish in the form present in the manure.

Alternatively, organic manure can be converted into nutrients through decomposition by micro-organisms. These nutrients can be absorbed by the food-web in the pond. The application of manure results in growth of plankton, which is a food source for a large number of fish species. There is a close relationship between the amount of manure applied and the kind of plankton that will predominate. A large amount of manure will make green and blue algae predominate. These algae are difficult to digest for fish. When small amounts of manure are applied, other plankton species will dominate which are easier to digest by fish (Spliethof, 1988).

Aspects of water quality in aquaculture

Water quality parameters are of concern in fish culture. They can cause stress and reduce growth rate or cause mortality of fish. Water quality limitations reduce the profitability of fish production, because the reduction in water quality that leads to stress and the deterioration of fish health reduces growth and increase the risk of disease and loss of fish. The water quality criteria required for maintaining a healthy and rapid growth environment are the basis for designing and managing aquaculture systems, and the parameters of primary concern are (Wheaton, 1999):

1. Oxygen
2. N and P compounds
3. Carbon dioxide
4. Solids
5. Total gas pressure
6. Chemotherapeutants (e.g. systemic fungicides)
7. Pathogens

The reduction or production of above parameters can lead to concentrations that affect the growth and health of the fish and that may require treatment of the water. For instance the total gas pressure is related to the gas bubble disease, a non-infectious, environmentally-physically induced trauma in fish. Chemotherapeutants can be used to treat diseases, but special considerations are required if the water is to be discharged, reused in serially for fish culture or treated in biological filters (Wheaton, 1999). Probably parameters 1-5 are of direct importance in relation to the use of bio-slurry. The table below shows some water quality guidelines.

Table 9

Water quality parameters for fish health in fresh water (El-Sayed, 2006; Wheaton, 1999; University of Arizona, 2013).

Parameter	Recirculation systems	Trout and salmonides	Tilapia
Maximum concentrations:			
Carbon dioxide (mg l ⁻¹)	20	10	20
NH ₃ (mg l ⁻¹)	0.02-0.5	0.0125	3.4
Nitrite (mg l ⁻¹)	0.5-5.0	0.1	small fish 81, large fish 8
Nitrate (mg l ⁻¹)	1000	3.0	
Total suspended and settleable solids (mg l ⁻¹)		80	2-2000
Optimum ranges:			
pH	6.0-9.0	6.5-8.0	5-11
Dissolved oxygen (mg l ⁻¹)	>6.0	>5.0	>4
Salinity EC (mg l ⁻¹)			5-10

At high levels, NH₃ is toxic to fish (for channel catfish LD50=3.10 mg l⁻¹). At lower concentrations (0.05-0.2 mg l⁻¹) it causes a significant reduction in growth. In water, the NH₃ concentrations are dependent on the NH₄ concentrations, pH and temperature. NH₃ concentration will increase with higher NH₄, pH and temperature.

Nitrite (NO₂⁻) is an intermediate during the biological oxidation of ammonium (NH₄⁺) to nitrate (NO₃⁻). The toxicity of nitrite depends on the concentration of Ca- and Cl-ions; an increase in these ions can increase the tolerance to nitrite. The toxicity of nitrite depends upon species and life stage. With a shift towards lower pH values there is an increase of nitrous acid from nitrite. Aqueous nitrite is in equilibrium with nitrous acid. Nitrous oxide is more toxic to aquatic organisms. The equilibrium is dependent upon pH. At normal culture pH, equilibrium favours essentially only the nitrite ion (Wheaton, 1999).

Suspended and deposited solids may affect reproductive behaviour, gonad development and the survival of egg, embryo and larval stages of warm water fishes. In certain intensive production systems suspended solids have been cited as one of the major production limiting factors, by e.g. causing direct damage to the gills of fish (Wheaton, 1999).

Aqueous carbon dioxide reduces the capacity of fish blood to transport oxygen and contribute to the deposition of calcium in the kidneys with higher levels of CO₂ in the blood. Actual toxicity of CO₂ depends on fish species, fish age, and water quality parameter such as O₂ concentration, alkalinity and pH.

Nguyen *et al.* (2011) showed that both the use of fresh or digested pig slurry affected the dissolved oxygen (DO) levels in the fish pond compared to the use of fish feed pellets. In the morning, the DO-levels were lower with the use of pig manure or digestate than that with pellets. DO-levels in the afternoon were higher with use of pig slurry or digestate. Both findings indicate that slurry and digestate stimulated growth of algae and phytoplankton (increasing DO during daytime) and increased decomposition of organic matter (decreasing DO during night time) in the fish pond. However, too much stimulation causes the DO-levels to drop below minimum requirements especially in the morning, as shown by Nguyen *et al.* (2011). There was little difference between the use of pig slurry or digestate although there is a tendency that pig slurry reduced DO-levels in the morning more and increased in the afternoon less than digested pig slurry. There was also little difference in ammonium levels between slurry and digestate but were higher than that in the control treatment; in both treatments ammonia levels remained well below the critical level of 1.0 mg l⁻¹. Turbidity measurements showed that in the beginning the control treatment (fish feed pellets only) had higher visibility than the treatments with slurry or digestate. Further on in the experiment, there was no difference between the treatments which was attributed to the feeding behaviour of the stocked fish. The results of the study showed that the growth of fish (Tilapia and various carp species) was higher with the use of pig slurry than with the use of feed pellets.

Use of manures and slurries in aquaculture

Also Chanda and Maska (2012) demonstrated that manures (poultry and cattle manures) under Zambian circumstances stimulated the primary production of phytoplankton and nutrient availability of P thereby stimulating production of different plankton eating fish (e.g. carp and tilapia).

Muendo (2006) showed that a Tilapia aquaculture system with the use of organic manure can be as productive as systems with the use of pellets as fish feeds. In both systems, phytoplankton dominated the food-web and served as fish feed. Muendo concluded that more research is required into fish feeding in ponds.

Fallahi *et al.* (2012) found that the application of digested cattle slurry decreased the transparency of ponds during the culture period compared to use of fresh cattle dung. This is mainly due to its enriching features that stimulate bacteria, phytoplankton and zooplankton production making it a more suitable fertilizer for fish production compared to raw cattle dung. Higher harvests were obtained for carps in the slurry treatment (1.858 tons/ha) compared to harvests (1.573 tons/ha) in the control group.

Tilapia (*Tilapia aurea*) (indirectly) fed with digested cow slurry (liquid form with 12% solids and dried digestate) combined with commercial low protein pellets (50%) can grow at the same rate as tilapia fed solely on commercial high protein pellets (Degani *et al.*, 1982). Fish fed with 50% sun dried digestate combined with 50% low protein pellets had a significant slower growth rate. The replacement of 50% of the feed by digested slurry caused a rise in the quantity of chlorophyll a (measure of the quantity of algae) and in primary production, from which the tilapia seem to have benefitted enough to make up for the feed otherwise obtained by the pellets. The dried slurry did not cause a rise in chlorophyll a, and the primary production was lower. It is very likely that during the drying of the digestate substantial losses of nutrients, notably N, has occurred (see also Chapter 5).

Das *et al.* (1994) showed that the co-digestion of the aquatic plant *Azolla pinnata* with cow dung can increase the energy output of the digester and increase fish production. Digested cow slurry-Azolla maximized the growth of phyto and zoo-plankton, showing its potential as pond fertilizer. Das *et al.* (1994) found that conventional fish-feed (20%) plus the digested cow dung-Azolla slurry was the most suitable as fish-feed.

The primary production value of digestate may also be enhanced through the use of other agricultural-industrial by products. Felix *et al.* (2006) reported on the use of sugar industry by-products as plankton boosters and yield enhancers in carp culture systems. The products favoured plankton production within optimum water quality parameters and did not cause excessive plankton blooms.

Concluding remarks

Bio-slurry acts as a fertilizer and stimulates the primary production of algae and micro-organisms in a fish pond. The algae and other micro-organisms serve as feed in the production of fish. Direct consumption of bio-slurry is not effective from both energy and protein point of view.

Results from studies described in the scientific literature indicate that bio-slurry can be used as productively as commercial feed for fish that feed on phytoplankton dominated food-web systems. Examples are given for Tilapia and carp cultures. However it is also remarked that more research is required on fish feeding in ponds.

10 Which aspects of bio-slurry use as a fertilizer require more research?

10.1 Recommendations for future research topics

From many studies, it is clear that the use of bio-slurry can play an important role as fertilizer, especially in developing countries. However, there are still several issues related to research on bio-slurry as a fertilizer that have not had sufficient attention in studies so far.

Practical aspects of bio-slurry application

- The properties of bio-slurry are different in some aspects when compared to manure. For example, a much larger part of the N in bio-slurry is in the ammonium form (NH_4). This is directly available to plants, but also susceptible to leaching and volatilisation. For manure, most N is organic N which will only be available for plants after mineralisation. This means that bio-slurry should be applied to the soil much closer in time before sowing or planting than manure application. However, we have found no studies that included these differences in the timing of the application of bio-slurry and manure.
- Because the direct N availability of bio-slurry is higher than for manure, bio-slurry might be better suitable for some crops than for other crops. Therefore it is recommended to determine for which crops it is the most suitable in order to develop practical guidelines for practical applicability of bio-slurry. This could be investigated by comparing the nutrient supply of bio-slurry at the one hand and the nutrient requirement of various crops at the other hand.
- Related to this is the use of bio-slurry within the complete fertilization strategy. Because bio-slurry is liquid it can much easier be applied as a side dressing compared to solid manure. It is recommended to determine how bio-slurry can be used optimally within whole fertilization plan.
- The ammonium in bio-slurry can be lost through volatilisation, during storage, handling and after application. In order to optimise the value of bio-slurry as an N fertilizer this NH_3 volatilization should be prevented. Very little research has been done so far on measures to reduce volatilisation. Possible measures are:
 - Covering of the bio-slurry storage;
 - Separating of the bio-slurry in a liquid and a solid fraction;
 - Incorporation or injection of bio-slurry in the soil;
 - Acidification of the bio-slurry.

These measures should be investigated under various conditions in developing countries.

Aspects not covered in this report

- The availability of nutrients is not the only factor that determines soil fertility. In the long run, the application of bio-slurry, manure or mineral fertilizers can affect the soil organic matter content. An adequate soil organic matter content is important, because it will improve the water holding capacity of the soil, increase the nutrient retention and prevent nutrient leaching, and it can stabilise the soil to prevent erosion. This study did not include the effects of bio-slurry application on soil organic matter. It is recommended to undertake an assessment of long-term soil fertility effects on soil organic matter and differences between fertilizer types (bio-slurry, manure, compost, mineral fertilizer, ...).
- Besides being a fertilizer, bio-slurry can also have other effects on crop production. For example, de Groot and Bogdanski (2013) also report that bio-slurry application reduces pests. It is unclear how consistent these effects are and what are the mechanisms behind it.

Food safety risks

- Several studies have shown that food safety risks for bio-slurry application are equal or smaller than for manure application. Only when human excreta are included in the feedstock, the consequences for food safety risks are not well known and should require more research.

10.2 Recommendations for future comparative studies

In this report we reviewed several studies that compared bio-slurry as fertilizer with manure and other fertilizers. Unfortunately, a number of these studies exhibited shortcomings that could have been easily overcome. These studies might have given a clearer message on the use of bio-slurry as a fertilizer. Consequently for future studies or demonstrations that compare bio-slurry with manure and other fertilizers it is recommended that:

- Levels of all nutrients are the same for the bio-slurry application and the manure applications. E.g. if the nitrogen availability is investigated, it should be prevented that differences in P and K availability between treatments are affecting yield and/or N uptake. For that reason, P and K availability should be the same in all treatments.
- Control treatments should be present. E.g. if the N availability of bio-slurry is determined, a control treatment without N should be included in the set-up, to correct for N supply by the soil.
- A reference treatment should be present. E.g. if the N availability of bio-slurry is determined, a mineral N fertilizer (e.g. urea or calcium ammonium nitrate) should be included in the set-up.
- Sufficient replicates (3 to 4) should be included to compensate for variations (e.g. soil properties) that will always be present.
- Treatments should be allocated at random to pots or plots.
- Statistics should be reported, to make clear whether observed differences are significant.
- Bio-slurry and manure should be from the same source. In a number of cases, other waste streams are added for digestion. This hampers a good comparison between digested and undigested manure.
- The application method for both bio-slurry and manure should be reported. This is important because surface application of bio-slurry can lead to large N losses through volatilisation. In case yields with bio-slurry are lower than yields with manure as fertilizer, this might be because of such N losses.

The value of bio-slurry as a fertilizer can to a large extent be derived from the composition of the bio-slurry. For this it is crucial to clearly report on its composition, as total content, as percentage of dry matters or otherwise.

10.3 Recommendations for the optimal use of bio-slurry

To optimize the use of bio-slurry in practice, the following activities are recommended:

1. Formulation of guidelines or best practices for the application of bio-slurry in practice. These best practices may consist of the following elements:
 - The composition of the bio-slurry should be known, because it can strongly vary. Ideally, the composition is determined in a laboratory. Alternatively, it should be estimated in another way.
 - Based on its composition and nutrient supply of the bio-slurry (N, P, K, etc.), bio-slurry should be applied to the most suitable combination of crop and soil.
 - In the ideal situation, the nutrient supply by the bio-slurry meets the nutrient requirement of a crop in its environment (soil type, composition, etc.). However, it could be necessary to apply mineral fertilizers in addition to the bio-slurry to meet the crop requirement.
 - The level of application of application should be optimized by taking into account crop requirements and nutrient supply by the soil.
 - The optimal time of application is determined by the pattern of nutrient uptake by the crop and the pattern of the nutrient release by the bio-slurry. These should be synchronized.
 - The bio-slurry should be added to the soil in such a way that the nutrient uptake by the roots is favoured and ammonia volatilization is prevented.
2. Establishment of demonstration fields in various regions, to increase the awareness amongst potential users of bio-slurry. In those field experiments, the potential value of bio-slurry as a fertilizer should be demonstrated. It should be shown i) that bio-slurry contains valuable nutrients that are available for plant uptake, ii) that similar effects can be obtained as with mineral fertilizers and iii) how it should be used in a proper way (as part of a fertilization strategy).

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