PhD project title: Environmental impacts of recycling fermentates from biogas production

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Introduction

Biogas production is the most socio-economically, cost-efficient technology for reducing greenhouse gas (GHG) emission and for recycling plant nutrients and recalcitrant carbon (Sommer et al., 2004). This reduction is due both to fossil energy substitution and to reduced potential for methane (CH_4) and nitrous oxide (N_2O) emissions from the manure at subsequent stages of the management chain (Novak and Fiorelli, 2010). The use of digested material (digestate) as fertilizers can increase the overall sustainability of biogas production. However, it is clear that the sustainability of biogas production also depends on an appropriate end-use of the digestate which should be treated, disposed of or recycled in a proper way, avoiding any negative environmental impact.

The utilization of biogas residue as soil amendments could have both negative and positive impacts on climate change (Cayuela et al., 2010). Most research suggests that the use of digestate especially the easily mineralizable carbon (C) and nitrogen (N) are digested, resulting in lower emissions of GHGs, such as CO_2 and N_2O and greater stability of C in the soil following their application (Amon et al., 2006, Marcato et al., 2009, Möller and Stinner, 2009), although others found no differences. Bertora et al. (2008) determined that anaerobic treatment strongly affects slurry composition (mainly its C, fibre and NH_4^+ content), and hence N_2O and CO_2 emission patterns as well as denitrification processes and nitrate availability. Further, the authors concluded that the solid fraction obtained after mechanical separation produced the most pronounced difference, while the liquid fraction and the anaerobically digested liquid fraction did not show significant difference with respect to the original slurry for any of the measured parameters. Schouten et al. (2012) evaluated three different agro-products and concluded that the highest losses of soil C from biological activity (CO_2 respiration) for sandy soil were observed in manure treatments (32%), followed by digestate (18%), and biochar (7%). Emissions of N_2O for this soil type ranged from 0.6% of applied N from biochar to 4.0% from manure, and the anaerobic digestate was the only by-product increasing the mineral N pool, while reducing emissions of N_2O compared with manure (Schouten et al., 2012).

Of course, soil application of biogas residue which contain high content of N under NH_4^+ form and easily degradable organic carbon that may benefit to plants uptake. It also may lead to significant emissions of N_2O, and CO_2. This is because high content of NH_4^+ in combination with a high amount of liquid makes digestates a potential source of producing N_2O due to oxygen is limited then nitrification process will be limited, while denitrification is enhanced, subsequently (Alotaibi and Schoenau, 2013). In addition, the organic C contained in the residues can be incorporated in soil organic matters (SOM) initially, but for a long period of time that may be sequestered from the soil and contributes to the increasing concentration of atmospheric CO_2. To date, it seems that the overall impact of biogas production on global C and N cycling and soil functioning have not yet well understood.

Nowadays, there is an increasing interest in the implementation of biogas production due to the demand for renewable energy, especially in livestock and agroindustry sectors where have a large amount of biodegradable wastes (animal wastes, agricultural residues and food industry wastes). In Denmark, feedstocks for the biogas industry are likely to change radically in the future. It is determined by law that at least 75% (main substrate) of the feedstock should come from animal manure. The remaining 25% (co-substrate) has traditionally come from slaughterhouse waste, but this source is limited, and a further expansion of the production would require alternative types of biomass. Futhermore, although co-subtrates are known that can enhance the energy production, for example, slaughterhouse waste, glycerine and energy crops or silage can increase the amount of biodegradable organic matter. Very little information is available regarding the effect of different types of feedstocks/biomass, retention time in biogas digestors, and application mode of digestate has on the environment when apply on agricultural soil.

Therefore, the current PhD project will focus on the environmental impacts associated with the field application of digestate using a combination of tracer studies and simple modeling. CO_2, N_2O production potential and others gasesemission (CH_4, NH_3 and NO) will be examined for varieties of digestates of different feedstocks after field application. Digestates source will be produced from various feedstocks (pig manures, slaughter household wastes, maize silge, and beet root), and differ from retention times. Both short terms and long terms laboratory incubation experiments will be conducted with defferent soil types (C_3 and C_4).

This PhD project is part of a larger project called BioChain. The purpose of BioChain is to provide tools that significantly contribute to achievement of a 4-fold increase in Danish biogas production, which is the target of the Danish government's energy plan.

Objectives

The overall objectives of the current project are:

- How digestate qualities affect carbon sequestration and greenhouse gases emission after field application to agricultural soil.
- Develop a simple model linking chemical composition of digestate to carbon sequestration and nitrous dioxide production in agricultural soil.

Hypotheses

We hypothesize that:

- Applying anaerobic digestated organic matters to agricultural soils could increase C sequestration and contribute to significant reduction of greenhouse gas emissions.
- Co-substrates influence C sequestration and formation of N_2O after field application of digestate.
- A simple modelling can estimate CO_2 and N_20 production after field application of digestate based on its chemical composition.

Research activities

Research activity 1: Short term and long term incubations experiments to determine effects of digestates quality on C sequestration in agricultural soil and GHGs

The characteristic of digestate are variable depend on input feedstock. The most significant property of the digestate was that a large proportion of the N occurred as inorganic forms. NH_4^+-N was reported approximately 70% of the total nitrogen in digestate incorporating pig, and 39 – 61% in digestate incorporating cattle (Alburquerque et al., 2012b). Compared to using fresh animal manure and green manure (plants and agri-products residues). Johansen et al. (2013) concluded that the recycling of digested residues to soil make a great value in terms of nutrition available for plants, also is likely a climate mitigation perspective.

However, the content of organic C has decreased significantly during the anaerobic digestion process which may causes less organic C availability for growth and activity of the soil microbial community. Also, the quantity and quantity of organic C in digestages may influence the turnover of mineral N after application to soil-plant systems. It is because less organic C is available for dynamic microbial growth, and N immobilization in microbial biomass when apply digestate compare to fresh animal manures or green manures (Johansen et al., 2013). Moreover, it seems that high availability of labile C in digestate may cause microbial depletion of O2 and also facilitate denitrification and loss of N.

Experiment 1: Short term laboratory incubation to examine GHGs emission after application of anaerobic digested residue from pig manure with beet pulp silage

Hypotheses:

- Reductions in GHGs emission from soils after application of digestates from pig manure with beet pulp silage as co-substrate compare to without beet root.
- Differences in input feedstock ratio of pig manure and beet pulp silage in biogas digesters influence GHGs emission after application of digestates to agricultural soils.

Specific objectives:

- Examining GHGs emission from soils after supplying digestates of pig manure with beet pulp silage as co-substrate.
- Comparing the decomposition rates in soils of C added from different digestates of pig manure with beet pulp silage as co-substrate.
- Determining immediate effects of digesate qualities on CO_2 and N_2O production in agricultural soils.

Material

- **Soils**
  Regular Danish agricultural soil C_3 will be taken at 0- 20 cm depth in the field experiment (field 35 in the Life experimental farm in Taastrup).

- **Digestate sources**

Digestate will be provided by Southen Denmark University from WP3 in Biochain project. Beet pulp silage and pig manure co-digestion will be tested at 5 levels (table 6) at retention time 20 days, and temperature approximately 37^{o}C.

Table 1: Retention time of digestates

Pig manure (% DM?)	Beet pulp silage (% DM?)	Digestate named
100	0	PBP_0
87.5	12.5	$PBP_{12.5}$
75	25	PBP_{25}
50	50	PBP_{50}
10	90	PBP_{90}

Experimental design

- **Treatments**

Table 2: Experimental treatments and replication experiment 1

Treatment	Soil type	Replicate
PBP_0	C3	3
$PBP_{12.5}$	C3	3
PBP_{25}	C3	3
PBP_{50}	C3	3
PBP_{90}	C3	3
Control (CT)	C3 bare soil	3

- **Incubation**

All the incubations will be in-door at control temperature 20^{o}C or 25^{o}C, and the same humidity.

Method and measurement

- **Sampling**

Soil sample

Soil samples will be taken at day 0, 7, 21, 29, 41, 60 of incubation.

Gas sample

Head space gas samples are taken at day 0 (3, 10, 20, 30, and 60 minutes after incubation), 0.5, 1, 2, 3, 4, 7, 9, 11, 14, 17, 21, 25, 29, 34, 41, 47, 53, 60 during incubation time.

Expected results

- Chracteristic of digestates
- Soil organic matter composition
- Dynamics and retention of C in agricultural soil treated with diffrent digesates sources.
- Variation of GHGs emission from agricultural soil depend on digestate sources.

Experiment 2: Long term incubation for determination of GHGs emission potential and C sequestration in soil applied with digestates from different feedstocks

Hypotheses:

- Different organic carbon sources in digestates influence CO_2 and N_2O production in agricultural soils.

Specific objectives:

- Examining GHGs emission from agricultural soils applied with different digestates sources.
- Determining differences of long term C sequestration in agricultural soils between biogas production residues of different feedstocks using isotopic ^{13}C signature technique.

Material

- **Soils**

Regular Danish agricultural soil C_3 will be taken at 0- 20 cm depth in the field experiment (field 35 in the Life experimental farm in Taastrup), and C_4 soil will be takend from Africa.

- **Digestate sources**

Digestate will be provided by Southen Denmark University from WP3 in Biochain project. 4 digestate sources are produced from different feedstock at the same retention time of 20 days, and temperature approximately 37^{o}C. Primary substrate will be pig manure (PgM), and 3 co-substrate Abbatoire Waste (AbW), Maize silage (MzS), and Beet root (BtR)

Table 3: Input feedstocks to produce digesates

Digestate	Main substrate	Co-substrate
PgM	100% Pig manure (PgM)	0
PgM + AbW	75% PgM	25% AbW
PgM + MzS	75% PgM	25% MzS
PgM + BtR	75% PgM	25% BtR

Experimental design

- **Treatments**
  10 incubation treatments of soils (C_3 and C_4) with digestates, raw (fresh) pig manure, and 2 control treatments will be carried out in this experiment:

Table 4: Experimental treatments and replication experiment 2

Treatment	Soil type		Replicate
PgM	C3	C4	3
PgM + AbW	C3	C4	3
PgM + MzS	C3	C4	3
PgM + BtR	C3	C4	3
Raw PgM	C3	C4	3
Control (CT)	C3 bare soil	C4 bare soil	3

- **Incubation**

All the incubations will be in-door at control temperature 20°C or 25°C, and the same humidity.

Method and measurement

- **Sampling**

Soil sample

Soil samples will be taken at day 0, 7, 21, 30; month 3, 6, 12, 18, 24 and 30 of incubation.

Gas sample

Gas samples are taken parallel with soil sample for GHGs emission analysis.

- **Measurement**
  Isotopic ^{13}C signature technique will be used to determine the fraction of C derived from digestates.

Carbon isotope $\delta^{13}C$ values (‰) will be calculated according to the following formula:

$$\delta^{13}C\ (‰) = [(^{13}C/^{12}C_{sample}\ 2 - {}^{13}C/^{12}C_{reference})/\ {}^{13}C/^{12}C_{reference}] * 1000$$

Table 5: Measurement variables

Variable	Method
Characteristic of digestates (chemical compositions)	
Soil analyses	
Total orgarnic C (TOC) and total N (TN)	
$NH4^+$, NO_3^- ?	
Water content	
Physical composition (clay, silt, sand%)	
pH	pH-meter
Gases	
N_2O	Gas Chromatography (GC)
CO_2	GC
CH_4	GC
C fraction in digestates	isotopic ^{13}C signature

Expected results

- Chracteristic of digestates
- Soil organic matter composition
- Dynamics and retention of C in agricultural soil treated with diffrent digesates sources.
- Variation of GHGs emission from agricultural soil depend on digestate sources.

Experiment 3: Long term incubation for determination of GHGs emission potential and C sequestration in soil applied with digestates differ from retention times

Hypotheses:

- Retention time in digesters affect significantly GHGs emission after field application of digestates.

- Higher retention time of digestates may increase retention of C added in soils, and reduction of GHGs emission, subsequently.
- Possible prime effects of added organic carbon on dynamic of soil organic carbon which may influence CO2 emission after the application of digestates.

Specific objectives:

- Determining variations in GHGs emission from different retention times after application of digestates to agricultural soils.
- Measuring variations of ^{13}C signature between the digestates sources.

Material

- **Soils**

Only C_4 soil which takend from Africa will be used in this experiment.

- **Digestate sources**

Digestate will be provided by Southen Denmark University from WP3 in Biochain project. Retention time will be tested at 4 levels (table 5), and temperature approximately 37^{o}C. Primary substrate will be pig manure (PgM), and co-substrate will be standard organic waste material (Fangel biogas).

Table 6: Retention time of digestates

Main substrate	Co-substrate	Retention time (day)	Digestate
PgM (?%)	Standard organioc waste (Fangel biogas)	10	T10
PgM (?%)	Standard organioc waste (Fangel biogas)	20	T20
PgM (?%)	Standard organioc waste (Fangel biogas)	30	T30
PgM (?%)	Standard organioc waste (Fangel biogas)	50	T50

Experimental design

- **Treatments**

Table 7: Experimental treatments and replication experiment 3

Treatment	Soil type	Replicate
T10	C4	3
T20	C4	3
T30	C4	3
T50	C4	3
Raw Pig manure (Pg)	C4	3
Control (CT)	C4 bare soil	3

- **Incubation**

All the incubations will be in-door at control temperature 20^{o}C or 25^{o}C, and the same humidity.

Method and measurement

Method and measurement variables will be the same in experiment 2.

Expect results

- Chracteristic of digestates
- Soil organic matter composition
- Dynamics and retention of C in agricultural soil treated with diffrent digesates sources.
- Variation of GHGs emission from agricultural soil depend on digestate sources.

Research activities 2: Measurement of oxygen distribution with planar O2 Optode to assess N20 production after field application of digestates

Distribution of oxygen in soil is well documented that influences nitrification and dinitrification process differently. In aerobic soils condition or oxic zone, nitrification will be occurred prevail over dinitrification. Ammonium is oxidised to produce nitrate which is material for bacteria respiration in soil. In anaerobic condition or anoxic zone, however, dinitrification is only happened. The spatial distribution of O_2 in soil therefore also plays an important role to drive nitrification and dinitrification processes, which lead to variation of greenshouse gases emission from soil regarding to amount of N_2O, CO_2 and CH_4. Spatial distribution of O_2 are varied upon soil types and soil structure such as water contents, size of pores geometry and soil microorganisms.

The spatial distribution of O_2 in soil influences directly soil O_2 consumption rates and nitrification rate. Many studies have been done distribution of O_2 in soil, however, most of these studies were focus on sediment, flooded soils and wetland, a few researches have been investigated spatial distribution of O_2 in agricultural soil. Thus, measurements of

O_2 transport and dynamics in organic soil are important to understand how this molecular affects decomposition of organic matter in soil, nitrification and dinitrification process with regarding to variation in greenhouse gases emission.

Optical Chemical Sensors (Optodes) have proven to be a versatile and powerful analytical tool suitable for high resolution imaging of gases and solute distributions associated with complex environments such as soil, aquatic sediments and living cells (Hakonen and Stromberg 2012). Planar oxygen optodes (O_2 optodes), which consist of an O_2 sensitve luminescent dye immobilized in a 1-10 µm thick polymeric layer on a transparent carrier (glass window), has been using to measure O_2 content in various ecosystems range from marine to agricultural systems. The method is based on sequential imaging of the O_2 dependent luminescence intensity, which is subsequently normalized with luminescent intensity images recorded under anoxic conditions (Stall et al., 2011).

Experiment 4: Oxygen distribution in soil after applying digestates differ from feedstocks

Hypotheses:

- Availabilities of labile carbon in digestates may increase O_2 consumption and N_2O formation in soil.
- Application models of digestates to soil influence spatial and temporal O_2 distribution and GHGs emission.

Specific objectives:

- To investigate effects of digestates qualities on dynamic of O_2 distribution in soil which influence N_2O formation in agricultural soils.
- To examine how the application models of digestates to soils affect spatial and temporal O_2 distribution and GHGs emission.

Material and method

Soil:

- Regular Danish agricultural soil C_3 will be taken at 0- 20 cm depth in the field experiment (field 35 in the Life experimental farm in Taastrup).

Digesates sources:

- Digestates are used the same with the sources from experiment 2 (activity 1), which will be provided by Southen Denmark University from WP3 in Biochain project.

Experimental method:

- A mesocosm incubation soil with digestates will be carried out, and planar oxygen optodes with multiple transparent glass chambers (instead one chamber in the original planar O2 optodes) will be used in this experiment.

Experimental design and set up

Treatments

Table 8: Experimental design for Experiment 4

Application model* / Treatment	Well-mixed	Layered	Surface sprayed	Total replications
PgM	3	3	3	9
PgM + AbW	3	3	3	9
PgM + MzS	3	3	3	9
PgM + BtR	3	3	3	9
Raw PgM	3	3	3	9
Control (CT)	3	3	3	9
Total replications	18	18	18	54

*: the ways of applying digestates to soil

Set up

- The experiment will be considered set up based on previous experiments of (Kun et al., 2013)[1] with adjustment of soil and digestates sources.
- The planar O_2 optodes systems using, therefore, will be the same.

Sampling and measurement

- Gas sample:
  - Headspace gas sampling at 0h, 10 minutes, 20 minutes, 0.5, 1, 2, 3, 4, 5, 10, 12, 24, 48 hours after the application of digestate to soil.
  - GHGs emission will be measured by Greenhouse Gas Analyser (450-GC, Bruker, Germany) at KU soil lab.

[1] KUN, Z., SANDER, B., MORTEN, L., RONNIE, N. & LARS, S. J. 2013. Knowledge about spatial heterogeneity of O2 in soil is important for understanding N2O emissions - A novel approach using planar optodes. University of Copenhagen.

- Soil sample:
  - Soil samples will be taken at the beginning and the end of experiment to analyze soil organic matter and pH.
- Optodes images:
  - For each treatment, a series of 48 optode images were recorded with a 30 minutes interval between each image.

Expected results

- The significant difference in spatial and temporal O_2 distributions in agricultural soil after applying digestates differ from feedstocks or retention times.
- N_2O formation/production process information in soil after application of digestates.
- GHGs emission variations which influenced by the application models.

Experiment 5: Oxygen distribution in soil after applying digestates differ from retention times

Hypotheses:

- Retention time of digestates in biogas digesters influence O_2 consumption and GHGs emission significantly after applying to agricultural soils.

Specific objectives:

- To determine how retention times of digestates in biogas digesters effects O_2 depletions and N_2O formation in soil.

Experimental method:

Method is the same using in experiment 4.

Experimental design and set up

Treatments

Table 9: Experimental design for Experiment 5

Application model* / Treatment	Well-mixed	Layered	Surface sprayed	Total replications
T10	3	3	3	9
T20	3	3	3	9
T30	3	3	3	9
T50	3	3	3	9
Raw Pig manure (Pg)	3	3	3	9
Control (CT)	3	3	3	9
Total replications	18	18	18	54

*: the ways of applying digestates to soil

Experimental set up:

The same experimental set up with experiment 4 is considered.

Sampling and measurement

Sampling method and variables measurement are conducted the same in experiment 4.

Expected results

- The significant difference in spatial and temporal O_2 distributions in agricultural soil after applying digestates differ from retention times in biogas digesters.
- N_2O formation/production process information in soil after application of digestates.
- GHGs emission variations which influenced by the application models.

Research activities 3: Develop a simple model linking chemical composition of digestate to carbon sequestration and nitrous dioxide production in agricultural soil

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