Beter (dan) Vergisten

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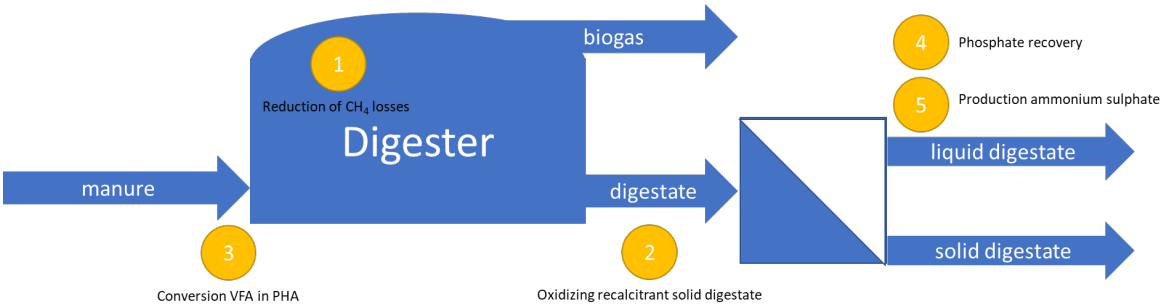
Contents

Summary

Digesters offer an efficient method to convert manure into energy. The design of existing and new digesters is based on robust and proven technology, of which it is known that the economy is only marginal, even with subsidies. In 'Beter (dan) Vergisten' several companies have teamed up with Wageningen Food and Biobased Research (WFBR) to develop new technologies that improve the economy of fermentation processes for manure digesters, by (see figure):

1. Reducing methane losses at farms by manure acidification;
2. Oxidizing recalcitrant solid digester fraction by hydrogen peroxide (and recycling);
3. Conversion of small fatty acids in liquid manure into valuable biopolymers (short-chain polyhydroxyalkanoate (PHA));
4. Phosphate recovery from acidified liquid digestate fraction; and
5. Production of ammonium sulphate from liquid digestate fraction.



1. Reducing methane losses at farms by manure acidification
In laboratory experiments biological acidification strongly reduced methane emissions from fresh cow manure. No additional fermentative microorganisms had to be added because enough endogenous microorganisms were present to reduce the pH to a level (< 5) that inhibits methane production. Lab experiments showed that the minimum amount of fermentable carbohydrate required was 5-10% (v/w). In a series of real life experiments daily addition of molasses as a carbon source to manure in cellar of a cow shed (ratio of 7% (v/w)) proved to reduce the pH from 8 to 5.8 after 21 days, while still declining. However, adding molasses is still expensive and is not expected to offer an affordable solution to the emission problem. A cheaper and workable alternative could be adding biologically produced acetic acid. In a series of real life experiments the drop in pH was smaller than expected and did not reach the level for stopping methanogenesis. It is recommended to repeat the experiments using biologically produced acetic acid whilst taking measures to improve the mixing. Recommended follow-up activities are described in the TKI-proposal MEZEM.

2. Oxidizing recalcitrant solid digestate fraction by hydrogen peroxide
The use of Fenton reagent (hydrogen peroxide and iron ions) can make the recalcitrant fibres from cow manure digestate more digestible in biogas plants, resulting in a substantial faster biogas production and higher biogas yield. However, under the conditions used, the value of the additional biogas produced is lower than the costs of the hydrogen peroxide added. To improve the economics of the process it is recommended to investigate the possibilities to reduce the costs of the Fenton treatment (lowering hydrogen peroxide dose, shorter incubation time, in-situ hydrogen peroxide production) and how to obtain a more potent bacteria consortium of bacteria. Recommended follow-up activities are described in the TKI-proposal MEZEM.

3. Conversion of VFA present in liquid manure fraction in PHA
Experimental results showed that in principle it is possible to produce, release and recover functional PHA in its native state (granular) from organisms that are fed on manure for this application. However, manure does not appear to be the most suitable green raw material to continue working with. In view of the above it is recommended to stop the research and development of a PHA production process from manure.

4. Phosphate recovery from acidified liquid digestate fraction

Phosphate can be recovered from liquid acidified manure using magnetite. To develop an economically feasible process it is important to achieve a high magnetite recovery from manure. However, on small scale, magnetite loss due to electrostatic clinging to surfaces and solids in residual manure make accurate determination of masses difficult. The principle of phosphate recovery using magnetite will be further developed in the TKI project *Recovery and valorization of phosphorus compounds from wastewater streams using Magnetic Adsorption-Desorption (MAD)*. Application of this method to wastewater is expected to be more promising in view of the magnetic separation of magnetite.

5. Production of ammonium sulphate from liquid digestate fraction

An optimization of the total process in which pretreatment, preconcentration and the TMCS process itself are included is required, because (i) in all steps heat and/or chemicals are used and (ii) all steps influence the required membrane area. Experimental work showed that pretreatment with MF/UF membranes and electrocoagulation did not lead to an increase of the surface tenson of the liquid pig digestate fraction, which would be beneficial to prevent wetting of the TMCS membrane. Experiments showed that preconcentration of the liquid phase digestate with RO before applying TMCS was possible (factor 3 was obtained with liquid pig digestate fraction), the pH should be around 6 to prevent ammonia loss. Recommended follow-up activities are described in the TKI-proposal MEZEM.

1 Introduction

Digesters offer an efficient method to convert organic waste streams such as vegetable-fruit-garden waste (GFT) and manure into energy (biogas/green gas) and a residual mineral concentrate. New digesters are in planning for the manure surplus of livestock farming (East-NL greater than 1,000,000 tonnes in 2017). The design of existing and new digesters is based on robust and proven technology (Best Available Techniques), of which it is known that the economy is only marginal, even with subsidies. In 'Beter (dan) Vergisten' several companies team up with Wageningen Food and Biobased Research (WFBR) to develop new technologies that improve the economy of fermentation processes for manure digesters, by both increasing biogas production and recovering valuable components. According to the project plan this can be achieved by (Figure 1-1):

1. Reducing methane losses at farms by manure acidification (Chapter 2);
2. Increased biogas production by improved fermentation management[1];
3. Oxidizing recalcitrant solid digester fraction by hydrogen peroxide (and recycling) (Chapter 3);
4. Conversion of small fatty acids in liquid manure fraction into valuable biopolymers (short-chain polyhydroxyalkanoate (PHA)) (Chapter 4);
5. Phosphate recovery from acidified liquid digestate fraction (Chapter 5), and
6. Production of ammonium sulphate from liquid digestate fraction (Chapter 6).

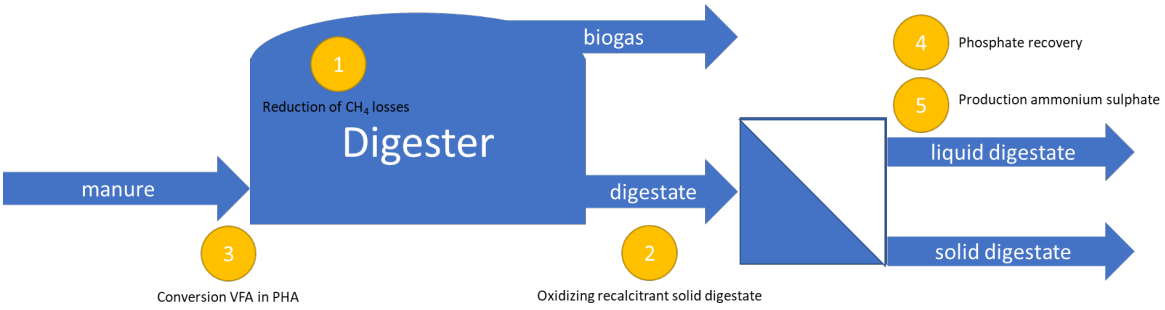


Figure 1-1 Schematic overview of a digester and the development of new technologies within 'Beter (dan) Vergisten'.

The goals pursued by the industrial partners of the project are:

- BYK-Netherlands (BYK), (www.byk.com) is one of the world's leading suppliers in the field of additives and aims at making its additives portfolio sustainable, among other things by the use of polyhydroxybutyrate (PHB) (a short-chain PHA) from manure for certain types of coatings.
- Cornelissen Consulting Services (CCS) is a consultancy firm having the ambition to remain on the forefront in the development of new technologies that benefit both the farmers' business case and the environment.
- The project goals of DMT are associated with the in-situ production of hydrogen peroxide. DMT is interested to build the apparatus.

[1] The research into the improved fermentation management was not taken up because the relevant business partner withdrew from the consortium.

2 Reducing methane (and ammonia) losses at farms by manure acidification

2.1 Introduction

Preventing or reducing methane and ammonia emissions from cowsheds is of great importance for the further development of the dairy sector in the Netherlands. Methane loss is mainly the result of methanogenesis of manure during storage or transport of the material. Reducing methane losses by inhibiting methanogenesis of manure in the preliminary phases (manure cellars, transport and storage) can be achieved by lowering the pH through the addition of acids (Fangueiro *et al.*, 2015). In Denmark sulfuric acid is used for this, but the application has a number of practical drawbacks. Moreover, addition of these chemicals is expensive and results in an extra salt load. An alternative for chemical acidification is biological acidification by organic acid-producing microorganisms in combination with fermentable substrates (carbohydrates). Addition of pure sugars to manure is too costly and therefore this project focuses on the possibility to use organic waste streams from the agro- and food industry as an affordable source of fermentable carbohydrates. Such biological acidification by addition of fermentable carbohydrates and/or effective microorganisms is common practice for e.g. silages (Muck *et al.*, 2018) but is not well studied for manure (Bastami *et al.*, 2016). In addition to preventing methane losses, acidification also results in a favourable shift in the ammonia-ammonium balance.

The composition of three types of manure is shown in Table 2.1.

Table 2-1 Composition of three manure types (ACRRES, 2015).

Manure	Dry matter (% wt)	Organic matter (% wt)	ADL (% DM)
Meat pigs	9.3	4.3	5.4
Sows	5.5	3.5	4.1
Cattle	8.5	6.4	12.2-13.0

ADL: Acid Detergent Lignin

The main components of the organic matter in manure are polysaccharides. In addition, protein, lignin and organic acids are present. Polysaccharides that are completely converted provide 620 Nm^3 biogas per tonne of organic matter (Johan van Groenestijn, personal communication). For pig manure, Kool (2005) mentions 310 Nm^3 of biogas per tonne of dry matter which equals 590 Nm^3 per tonne of organic matter, close to the theoretical maximum. Expressed in biogas yield this is 23 Nm^3 biogas per tonne of pig manure or 9.7 kg of methane per tonne pig manure. For these conversion calculations the average values of dry matter and organic matter for meat pigs and sows were used (Table 2-1), supplemented with the assumption that biogas contains 60% (v/v) CH_4. For cattle manure the numbers are lower, an estimated 21 Nm^3 biogas per tonne of cattle manure or 9.0 kg methane per tonne (Johan van Groenestijn, personal communication).

A large part of the organic matter in manure is recalcitrant, which in practise results in lower biogas production. On the average, 281 Nm^3 per tonne of organic matter can be assumed for cattle manure (Institut für Energetik und Umwelt gGmbH, 2006). Moreover, it regularly happens that half of the biogas is already produced and lost in the manure pit. The loss of methane in this way is estimated to range from 15-20% to 54% in temperate and warmer regions, respectively (Petersen, 2018). Assuming that 20% of the biogas is emitted from the manure cellar, then we can estimate the contribution to greenhouse gas emission (1 kg CH_4 corresponds to 28 kg CO_2-equivalent). The results are shown in Table 2-2, knowing that the Netherlands yearly manure production from cattle and pigs is about 55 Mtonne and 10 Mtonne, respectively (CBS, 2022).

Table 2-2 Methane emissions manure pits and Green House Warming Potential.

Manure	Methane emission from manure pit (kg/tonne manure)	GWP (kg CO_2-eq per tonne manure)	GWP (Mtonne/yr CO_2-eq for NL)
Pig	1.96	55	0.53
Cattle	1.8	50	2.77

It has been agreed in the 'klimaatakkoord' that 'agriculture and land use' will make an extra contribution of 3.5 Mtonne CO_2-eq/yr to reduce greenhouse gas emissions. From Table 2-2 it can be seen that this contribution is covered for 95% if methane emissions from the manure storages/pits are prevented. In addition, a lower pH in manure also affects the ammonium-ammonia equilibrium as is shown in Figure 2-1. At a pH<6 ammonia emissions from manure are prevented.

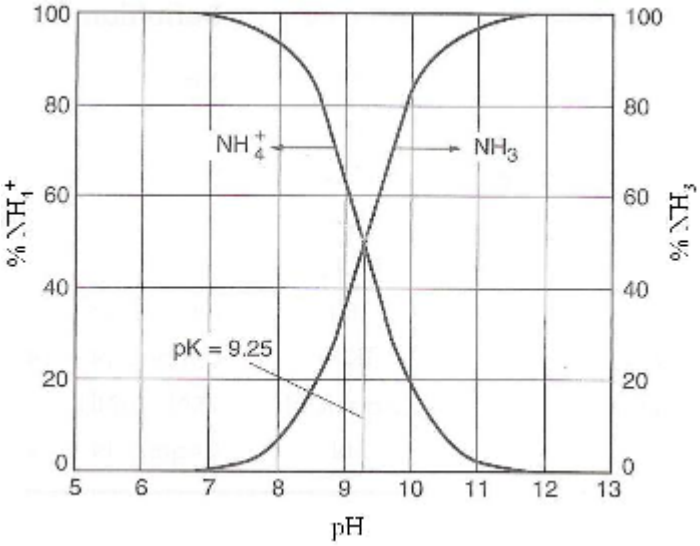


Figure 2-1 Diagrammatic representation of ammonia behaviour in water solution (Kunz and Mukhtar, 2016).

The potential for biogas production or other ways of valorisation of animal manure and organic waste is currently only partially utilized. In this chapter we first compare the effects in laboratory experiments on the undesirable biogas and methane production from cow manure after chemical acidification (with sulphuric and hydrochloric acid) or biological acidification, respectively. For the biological acidification the addition of fermentable carbohydrates in a pure form or from agricultural waste streams was studied in the presence or absence of fermentative microorganisms. The results from the laboratory were validated in a series of real life experiments using molasses as a carbon source. However, molasses is expensive and probably does not offer an affordable solution to the emission problem. A closer look at the biology of the manure processing chain showed that the addition of biologically formed acetic acid elsewhere produced, can be a cheap and workable alternative. Therefore, in a second series of real life experiments this option was explored.

2.2 Materials and methods

2.2.1 Chemical and biological manure acidification on lab scale

Chemical (H_2SO_4 and pH) and biological acidification (starter cultures and glucose)
Acidification experiments were performed on 100 mL fresh Holstein dairy cow manure in 250 mL serum bottles with rubber stoppers. Batches were incubated statically at 25°C for 23 days. Chemical acidification was done by the addition of H_2SO_4 to a starting pH of 6.5, 5.5 or 4.5. Biological acidification was done by the addition of a mixed lactic acid bacteria (LAB) starter or fresh Sauerkraut juice at a concentration of 0.5% or 5% (v/v) with or without the addition of 10% (w/v) glucose. The LAB starter was composed of the following strains in equal ratios: *Lactobacillus plantarum* DSM 20174, *Lactobacillus rhamnosus* DSM 20711, *Lactobacillus reuteri* DSM 20016, *Lactobacillus helveticus* DSM 20075. As controls fresh manure without additions or pre-acidification were included, as well as fresh manure with only the addition of 10% (w/v)

glucose. All conditions were tested in duplicate. An overview of the experimental conditions is shown in Table 2-3.

Table 2-3 Various conditions for chemical (H_2SO_4 and pH) and biological acidification (starter cultures and glucose).

Condition	Inoculum (% v/v)	Glucose (% w/v)	Acid	End pH (-)
No additions	-	-	-	7.5
Carbohydrates	-	10	-	7.5
Biological acidification				
Fresh Sauerkraut juice	0.5	-	-	7.5
	0.5	10	-	7.5
	5.0	-	-	7.5
	5.0	10	-	7.5
Mixed LAB starter	0.5	-	-	7.5
	0.5	10	-	7.5
	5.0	-	-	7.5
	5.0	10	-	7.5
Chemical acidification				
Sulphuric acid (H_2SO_4)	-	-	+	6.5
	-	-	+	< 3

Carbon source: pure carbohydrates versus agricultural side streams

Acidification experiments were performed on 100 mL fresh Holstein dairy cow manure in 250 mL serum bottles with rubber stoppers. Batches were incubated statically at 25°C for 21 days. Biological acidification was done by the addition of the pure carbohydrates (glucose or soluble potato starch (Sigma S2004)) in powder form, or agricultural side streams (grey starch or sugar beet molasses) as liquids at 10%, 5% and 1% (w/v). Grey starch contained 59.2% dm starch and lactic acid, acetic acid and butyric acid at 5.4, 2.1 and 0.7 g/L, respectively. Sugar beet molasses contained 44% sucrose and 16 g/L total nitrogen. As controls fresh manure without additions or pre-acidification was included, as well as fresh manure with chemical acidification by the addition of H_2SO_4 or HCl to a starting pH below 5.5. All conditions were tested in duplicate. An overview of the experimental conditions is shown in Table 2-4. The composition of the two side streams used (grey starch and molasses) are given in Table 2-5 and Table 2-6.

Table 2-4 Second acidification experiment of cow manure - conditions.

Condition	Organic C-source (% w/v)	Acid	End pH (-)
No additions	-	-	7.5
Biological acidification			
Glucose (monosaccharide)	10	-	7.5
	5	-	7.5
	1	-	7.5
Starch (potato)	10	-	7.5
	5	-	7.5
	1	-	7.5
Side stream 1 - Grey starch* (potato)	10	-	7.5
	5	-	7.5
	1	-	7.5
Side stream 2 – Molasses (sugar beet)	10	-	7.5
	5	-	7.5
	1	-	7.5
Chemical acidification			
Sulphuric acid (H_2SO_4)	-	+	5.0
Hydrogen chloride (HCl)	-	+	5.0

Table 2-5 Composition grey starch (Lamb Weston Meijer).

Compound	Content
Dry matter (%)	17.9
Ash (% DW)	2.9
Glucan (% DW)	66.1
of which starch (% DW)	59.2
Liquid fraction	
Lactic acid (g/L)	5.4
Acetic acid (g/L)	2.1
Butyric acid (g/L)	0.7

Table 2-6 Composition molasse (Cosun).

Compound	Content
Brix (%)	77
Sucrose (% DW)	44
pH (-)	7.5
Nitrogen (g/L)	16
Initial microbial cell count (CFU/g)	82

2.2.2 Manure acidification on pilot scale (Dairy Campus)

In the period November 15 till December 6 2021, a first real life pilot experiment was performed at the Dairy Campus in Leeuwarden (the Netherlands). The experiment was to validate the results from laboratory experiments with respect to the effect of the addition of molasses on the pH value of the manure stored in the manure cellar. The experiment was performed in department 13 of the experimental facility of the Dairy Campus which houses 16 milk cows. The department has a standard slatted floor with a manure storage underneath. The pit has an aeration system for mixing the manure but this was not used. On a daily basis 80 L molasses (8 buckets) were added on the floor from where it dripped into the pit. The molasses, with a total sugar content of 55-57%, was purchased from Cosun Sugar Company (the Netherlands). Details on the composition of the molasses are shown in Table 2-6 and in Annex 1.

In the period May 30 till July 4 2022, a second real life pilot experiment was performed, this time using 20% acetic acid purchased from 'Werken met Merken'. At the Dairy Campus the acetic acid content with water was reduced to 13% for safe use. The experiment was to compare the emissions of department 13 and 16 (16 cows each) with and without manure acidification, If we assume that 20% of the biogas is emitted from the pit, then we can estimate the contribution to greenhouse gas emission (1 kg CH_4 corresponds to 28 kg CO_2-equivalent) respectively. In department 16, 4 jerrycans with 13% acetic acid (215 L) was poured evenly over floor daily from where it dripped into the pit.

2.2.3 Analyses lab scale experiments

Methane and carbon dioxide were measured on Shimadzu GC-2010 equipment, for an injection volume of 50 µL injected at 120°C and using the carrier gas helium (5.0%) at a pressure of 0.95 bar. The separation column used was a parallel combination of Porabond Q (50 m x 0.53 mm; 10 µm; Varian; Part.no. CP7355) and Molsieve 5A (25 m x 0.53 mm; 50 µm; Varian; Part.no. CP7538), with an oven temperature of 80°C and FID detector at 150°C. Chromatograms were analysed using the Chromeleon 6.80 SR13 software.

Lactic acid was measured using the Thermo Dionex Ultimate 3000 RS equipment on a OA-1000 organic acids column (l = 300 mm; id = 6.5 mm; Alltech partnr. 9046), for an injection volume of 20 µL. The eluent used was 1.25 mM sulphuric acid, without a gradient (isocratic). The column flow was 0.6 mL/min, run time 20 min and column temperature 60°C. Organic acids were detected using a refractive index detector and chromatograms analysed using the Chromeleon v6.8 software.

Dry weight (total solids (TS)) of manure was determined after overnight incubation of ~25 g manure at 105°C and ashes weight after subsequent incubation at 550°C. Volatile solids (VS) were determined as the difference in weight between dry weight and ashes.

2.2.4 Analyses pilot scale experiments

In both real-life pilot experiments the pH, the temperature and the manure height was measured daily. Before adding the molasse or acid, measurements were done and averaged at 5 positions spread over each department. During the second real life experiment, the concentration of methane, ammonia and nitrous oxide was determined per department in the incoming and outgoing air flow by a Picarro 2508. Sampling lines and a measuring point switch (MPO) were used for this. In both ventilators, the ventilation level was measured per department and recorded. Ventilation data and data on temperature and humidity in the sections were recorded in a data logger (Campbell-Scientific CR1000X). The emission per hour was calculated per section from concentration and ventilation data.

2.3 Results

2.3.1 Chemical and biological manure acidification on lab scale

In the first experiments the influence of the starter culture (LAB versus sauerkraut) and addition of glucose to enable biological acidification was studied. These were compared to chemical acidification with H_2SO_4 at two pH's (6.5 and <3). Chemical acidification of manure with sulphuric acid to a pH < 3 reduced the biogas volume produced with no further increase after 3 days of incubation (Figure 2-2). Acidification to pH 6.5. was not sufficient to reduce biogas production. Excessive foaming was observed. As manure contains high levels of bicarbonate and rapid acidification will shift the bicarbonate-CO_2 equilibrium towards CO_2, this results in extensive release of CO_2 and thereby foaming. Simultaneous aeration during chemical acidification can reduce this excessive foaming (Fangueiro *et al.*, 2015; Muck *et al.*, 2018), but for biological acidification, aeration is most probably not required. A previous study by Bussink *et al.* (2014) indicated that for good acidification of manure it might be best to first perform a fast initial acidification using (in)organic acids, and then sustain the lower pH via biological acidification.

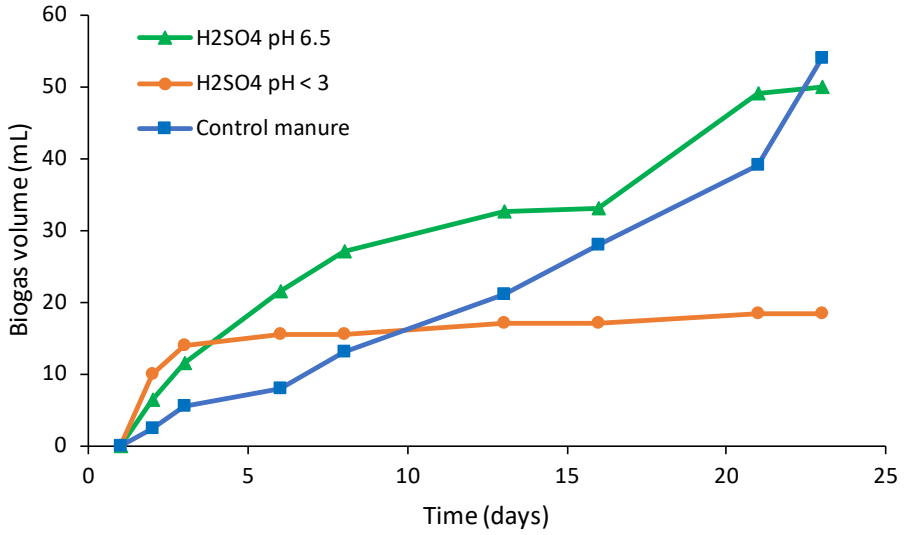


Figure 2-2 First acidification experiment - Cumulative biogas production during chemical acidification of cow manure.

Acidification inhibits methanogenesis, as has been described previously (Fangueiro *et al.*, 2015). However, the addition of a biological inoculum alone did not result in reduced biogas emission (Figure 2-3, upper graph). This indicates that, fresh manure does not contain (sufficient) readily available carbohydrates that can be converted to organic acids and that therefore an external carbon source is required. Analysis of lactic acid production confirmed indeed that lactic acid was produced upon the addition of glucose (Annex 1, Table

A1-1) and that the endogenous microbiota present in manure is sufficient to convert this glucose. In the case of an additional carbon source an increase in biogas production was observed in combination with a low LAB inoculum and high sauerkraut inoculum (Figure 2-3, lower graph). A reduced biogas production was only observed for a high LAB inoculum in combination with an easily accessible carbon source.

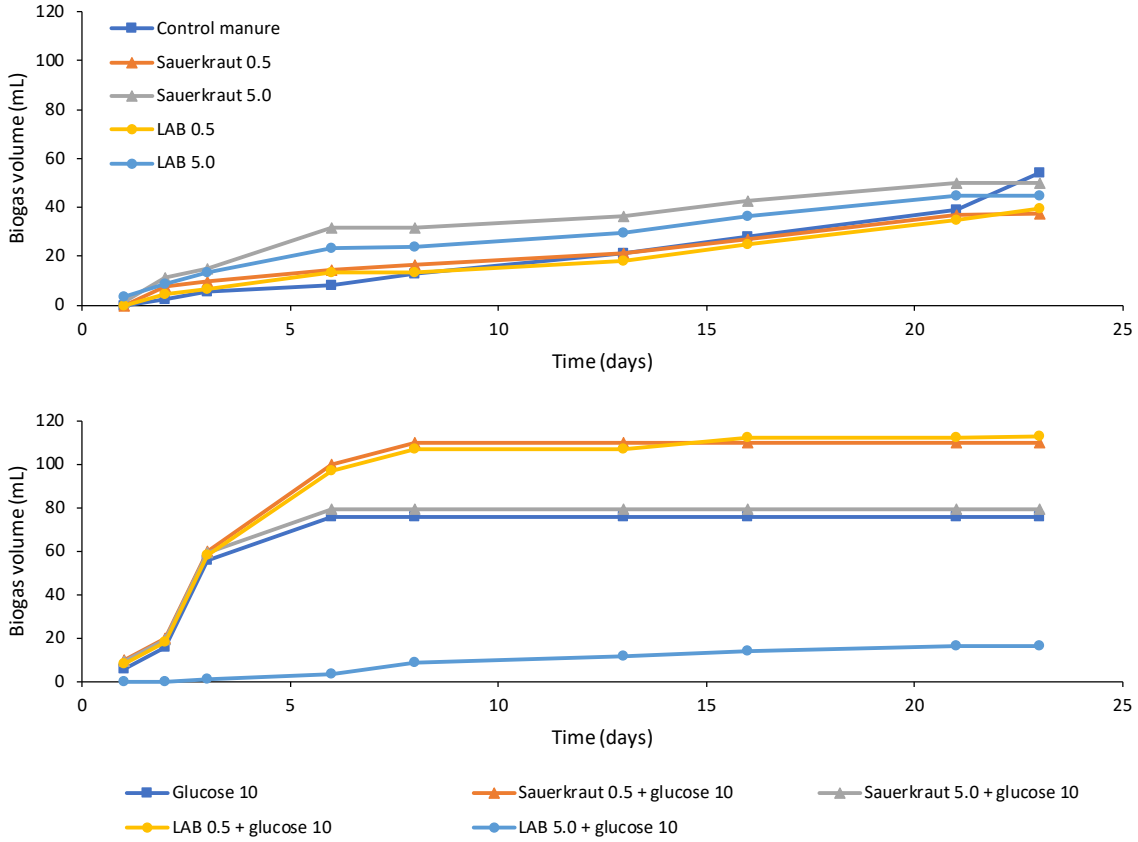


Figure 2-3 First acidification experiment - Cumulative biogas production during biological acidification of cow manure. Biological inoculum without glucose (top) and with glucose addition (bottom).

CH_4 and CO_2 content of the biogas were analysed after 16 days of incubation (Figure 2-4). Reduced methane emission was observed for both chemical acidification and biological acidification in the presence of an external carbon source. While non-acidified manure showed a gradual increase in biogas volume, a rapid initial increase in biogas volume was observed for the acidified and glucose-supplemented manure (Figure 2-2 and Figure 2-3). However, the initial rapid increase in biogas volume in acidified and glucose-supplemented incubations are likely caused by CO_2 release from bicarbonate in manure due to chemical/biological acidification.

Figure 2-4 shows that no additional effect was observed of adding fermentative microorganisms (fresh sauerkraut juice or LAB), indicating that biological acidification caused by endogenous microorganisms produces sufficient lactic acid to reduce the pH to levels that inhibit methane production (pH < 5). This confirms observations from previous studies on the limited effect of adding effective microorganisms for manure acidification (Bastami *et al.*, 2016).

The changes in biogas volume and composition were used to estimate the reduction in Greenhouse Warming Potential (GWP). The change in GWP can be calculated by assuming for CO_2 a GWP of 1 and for CH_4 a GWP of 28–36 (EPA, 2022). For glucose addition (with or without inoculum) we observed on average a ~10.5-fold reduction in CH_4 production (GWP is -10.5 x 28 = -294) and a ~2.9-fold increase in CO_2 production (GWP is 2.9 x 1 = 2.9). This could result in a net GWP reduction of -294 + 2.9 = -291.

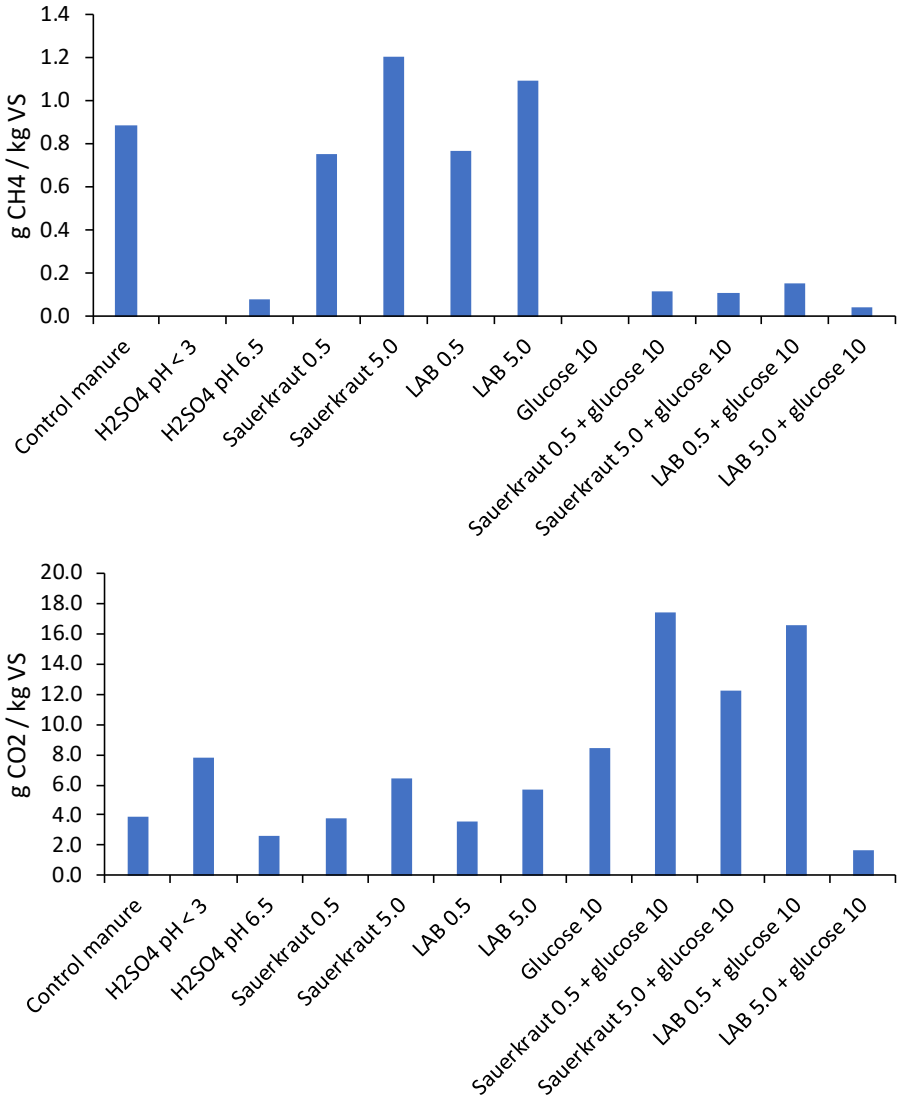


Figure 2-4 First acidification experiment - Methane (top) and carbon dioxide (bottom) emissions from cow manure after 16 days of chemical and biological acidification.

In the **second experiment** the acidification potential of pure carbohydrates and carbohydrates from agricultural side streams were compared. As shown in Figure 2-5 all substrates at 10% reduced the biogas production significantly compared to the untreated manure. Lower concentrations were in general less effective. Unexpectedly, for some substrates the addition showed a strong initial burst in biogas production, which was in particular prominent for 5% molasses. This burst in gas production might be due to a rapid conversion of sugars to CO_2 and/or release of CO_2 from manure (from bicarbonate).

The composition of the biogas was also affected, with a complete reduction in methane emission in 7 days for molasses (5% and 10%) and starch (10%) and a complete reduction in CO_2 emission for molasses and starch at 10% (Figure 2-6). Within 14 days also reduced methane emissions were observed compared to the untreated manure in experiments with glucose and starch at 5% and 10%.

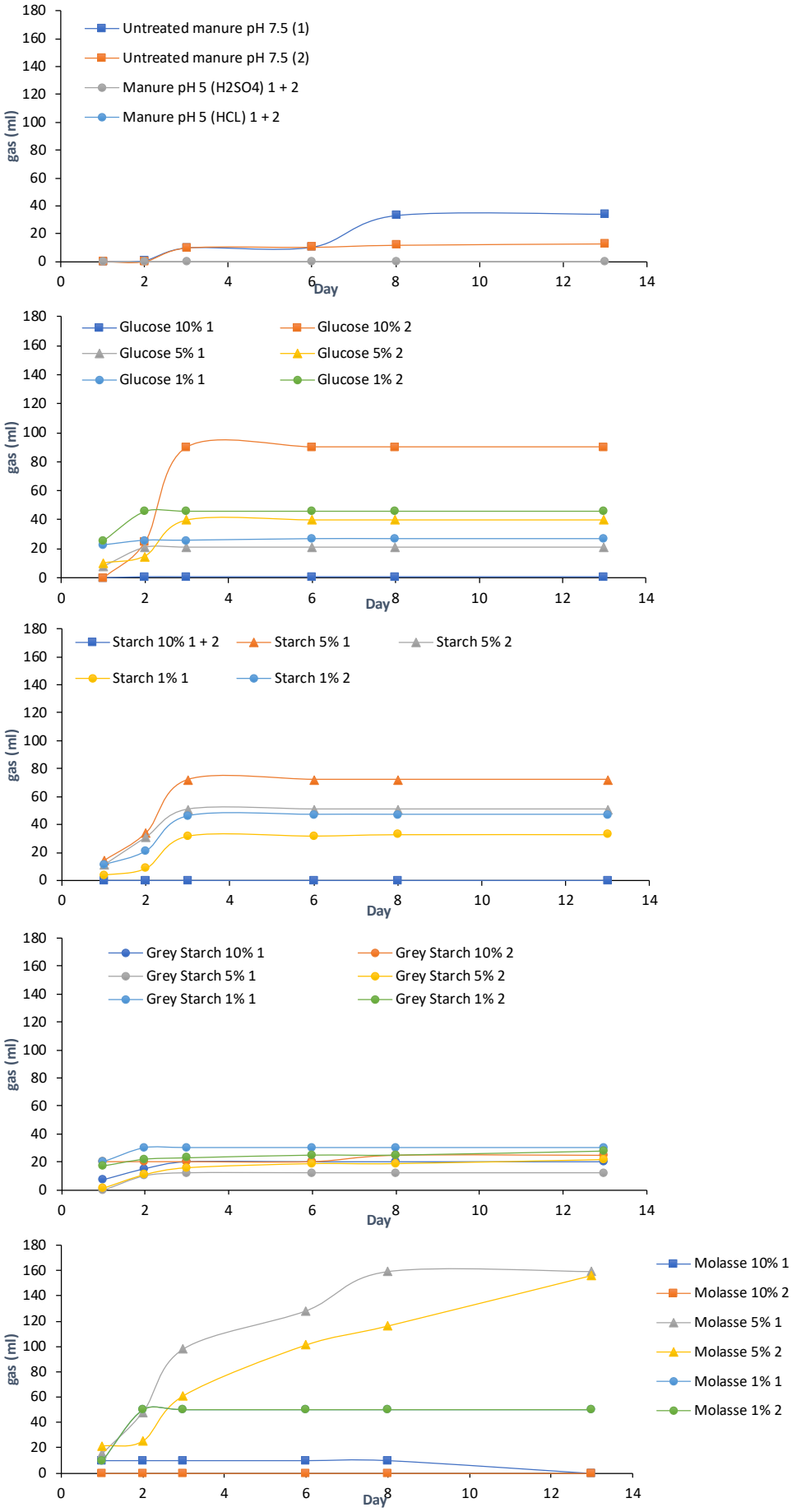


Figure 2-5 Second acidification experiment - Cumulative biogas production during chemical and biological acidification of cow manure.

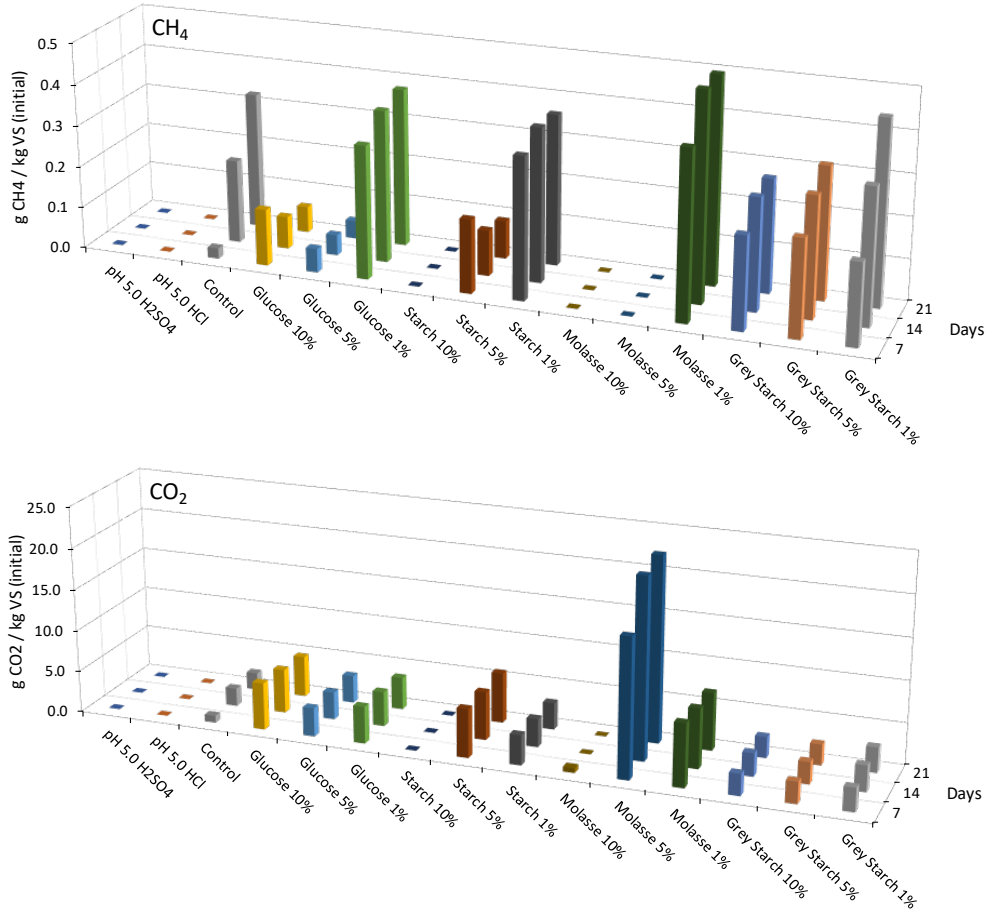


Figure 2-6 Second acidification experiment - Methane (top) and carbon dioxide (bottom) emissions from cow manure after 7-21 days of chemical and biological acidification.

After 21 days of incubation the pH reached a pH < 5 for glucose at 5%/10% and starch and molasses at 10% (Figure 2-7). Starch and molasses at 5% reduced the final pH close to 5.5 which can be assumed to be sufficient for inhibiting methanogenic activity. Lactic acid was detected in the biological acidification samples with a pH < 5 (Table A1-1, Annex 1), indicating homofermentative conversion of the sugars. For other samples with pH reduced below 6 (starch at 10, 5 and 1% and 5% molasses) no lactic acid was detected. Acidification in these incubations might have come from other organic acids produced during heterofermentative conversion of sugars, which could also explain part of the CO_2 release. In all cases, adding 1% of an external carbohydrate source was not sufficient to achieve (significant) reduction in biogas production, methane and CO_2 emissions or acidification.

The observed effects overall can be related to acidification of the manure. However, additional inhibition of methanogenesis could also come from e.g. inhibitors such as hydroxymethylfurfural (HMF) that are often present in molasses, and increased osmotic pressure due to sugar addition can also not be excluded.

It is concluded that both chemical and biological acidification of fresh cow manure strongly reduced methane emissions. For biological acidification it is required to add a fermentable carbon source, the minimum amount is 5-10%. Molasses showed a significant effect on acidification, reduction in biogas volume and emissions of methane and CO_2. No additional effect of adding fermentative microorganisms (fresh sauerkraut juice or LAB) was observed, indicating that biological acidification caused by endogenous microorganisms produces sufficient lactic acid to reduce the pH to levels that inhibit methane production (pH < 5).

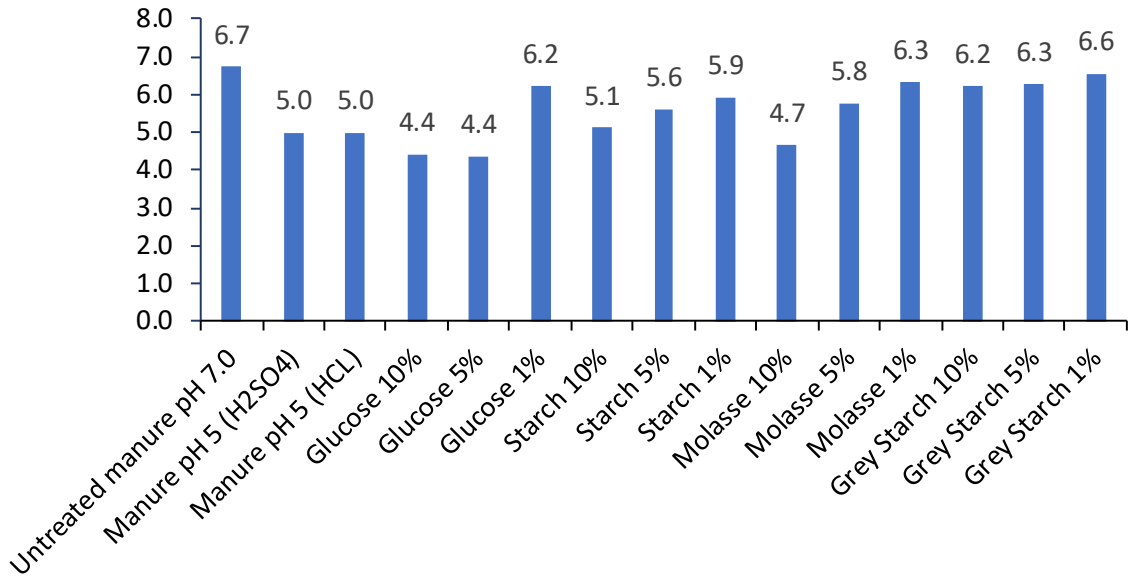


Figure 2-7 ***Second acidification experiment – Final pH at 21 days after chemical and biological acidification of cow manure.***

2.3.2 Biological manure acidification on pilot scale (Dairy Campus)

Results of the first real life experiments at the Dairy Campus are summarized in Figure 2-8. Over an period of 3 weeks, the average pH of the manure in the cellar under the stable floor dropped from 8.0 to 5.8 (standard deviation of 0.2), while the temperature remained almost constant at 15°C (standard deviation 0.4°C).

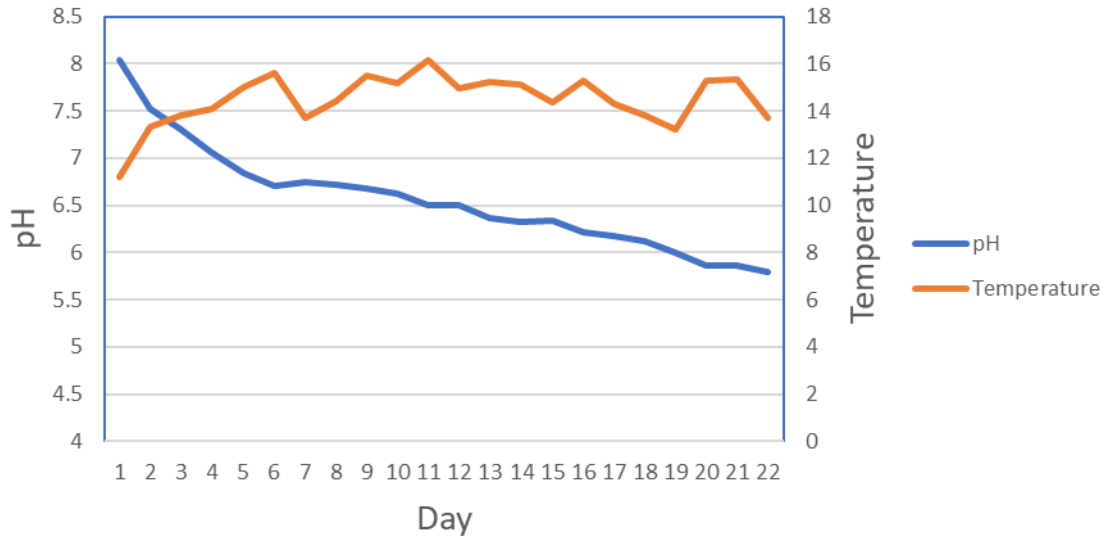


Figure 2-8 ***Average pH and temperature of the manure of the first experiment using molasse as carbon source.***

After 21 days the conditions in the cellar became such that CH_4 production from manure in the pit was hampered. Adding molasses to the manure clearly increased the bacterial activity and led to foam formation (from conversion of bicarbonate to CO_2) which made measurement of the manure volume in the cellar difficult.

2.3.3 Manure acidification on pilot scale using acetic acid (Dairy Campus)

Based on the results described in section 2.3.2, a second series of experiments was carried out, comparing process and emission data from two departments, with and without acidified manure respectively. Acidification was achieved by pouring 215 L acetic acid (13%) over the floor every day. The average pH is shown in Figure 2-9.

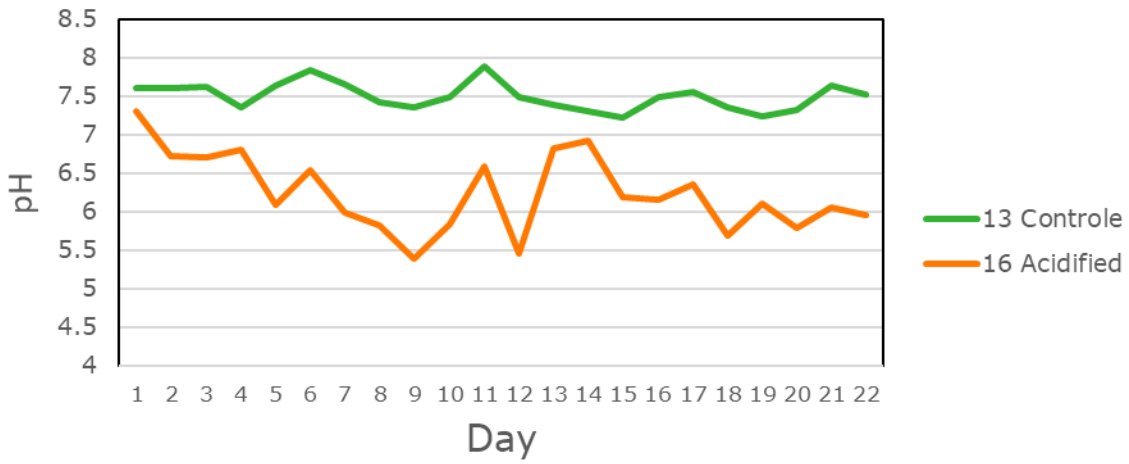


Figure 2-9 Average pH of the manure with and without acidification using acetic acid.

During the experimental period the pH of the acidified manure dropped from pH 7.5 to pH 6, the daily variations in the pH of the acidified manure being quite large which was also reflected by the standard deviation in the pH with and without acidification of 0.5 and 0.2 pH points respectively. The drop in pH was smaller than expected and did not reach the level that methanogenesis is stopped. It was concluded that this could be attributed to poor mixing and that measures are needed to improve this mixing of acetic acid.

As discussed above, no reduction in methane emissions was observed, only a reduction of 16% in NH_3 was recorded. The measured NH_3 emissions are shown in Figure 2-10.

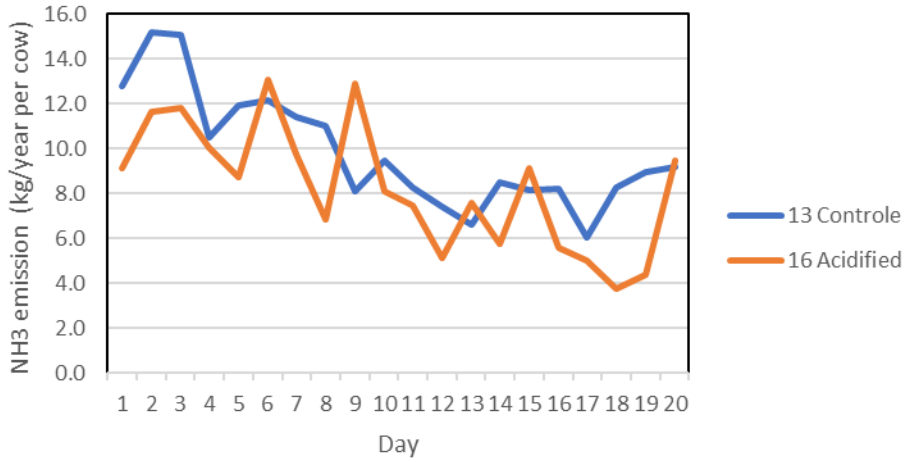


Figure 2-10 NH_3 emission with and without acidification of manure with acetic acid.

2.4 Conclusions and recommendations

In laboratory experiments starch and molasses at 5% (w/v) reduced the final pH in manure close to 5.5 which can be assumed to be sufficient for inhibiting methanogenic activity. The minimum amount of fermentable carbohydrate required for biological acidification is 5-10% (w/v). The agricultural side stream molasses showed a significant effect on acidification, reduction in biogas volume and methane CO_2 emissions.

Daily addition of molasses as a carbon source to manure in a cellar of a cow shed (ratio of 7% (w/v)) proved to reduce the pH from 8 to 5.8 after 21 days, while still declining. No additional fermentative microorganisms had to be added for this biological acidification because enough endogenous microorganisms are present to produce sufficient lactic/acetic acid that inhibits methane production.

Adding molasses is still expensive and is not expected to offer an affordable solution to the emission problem. A closer look at the biology of the manure processing chain showed that the addition of biologically formed acetic acid elsewhere produced, can be a cheaper and workable alternative. In a series of real life experiments this option was explored. However, the drop in pH was smaller than expected and did not reach the level for stopping methanogenesis. It was concluded that this should be attributed to poor mixing and that measures are needed to improve this mixing issue. Such a follow-up experiment is described in the MEZEM-proposal (Annex 6).

3 Oxidizing recalcitrant solid digestate fraction by hydrogen peroxide

3.1 Introduction

The state of the art of lignocellulosic biomass pretreatment for enhanced biogas production has been reviewed by Zheng *et al.* (2014). In 'Beter (dan) Vergisten' a combination with an advanced oxidation process is further elaborated. In this additional oxidation step, the non-biodegradable biomass is made biodegradable by cell destruction. Thereupon the residue is sent back to the digester (Figure 3-1). For the oxidation step hydrogen peroxide (H_2O_2) can be used.

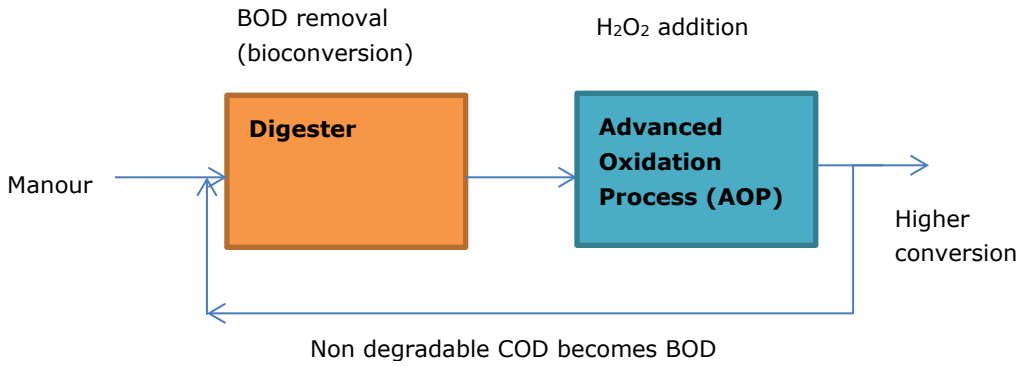


Figure 3-1 Schematic overview of technology concept of digester combined with COD to BOD conversion.

H_2O_2 has been used to reduce organics (BOD, COD, pesticides and pathogens) of wastewaters for many years. The use of hydrogen peroxide in combination with UV light is known as advanced oxidation technology (AOP) and commercial available. In existing waste water treatment plants the hydrogen peroxide is transported in bulk from chemical plants. WUR has developed an efficient process for the electrochemical production of the H_2O_2 from air (oxygen) on site (see Annex 2).

In addition to the review of Zheng *et al.* (2014) a new literature review was carried out on pretreatment of lignocellulosic material using the Fenton reagent (see Annex 2). The Fenton reagent is a mixture of H_2O_2 and Fe-ions. The following reactions occur:

$Fe_2^+ + H_2O_2 \rightarrow Fe_3^+ + HO\bullet + OH^-$

$Fe_3^+ + H_2O_2 \rightarrow Fe_2^+ + HOO\bullet + H^+$

$2\ H_2O_2 \rightarrow HO\bullet + HOO\bullet + H_2O$

The radicals produced can oxidize organic matter and it has been proven that such oxidation makes lignocellulose better digestible to bacteria.

The aim of this research was to investigate the use of the Fenton reagent (a mixture of H_2O_2 and Fe-ions) to oxidize the recalcitrant solid digester fraction and thereby enhance the biogas production in the digester.

3.2 Materials and methods

To investigate the effect of the Fenton reagent on lignocellulose and study the improved digestibility in biogas production, wheat straw was taken as a model. The favourable reaction conditions and the required amounts of peroxide and ferrous chloride were taken from literature. $FeCl_2$ was used as iron source. Tested were variation of H_2O_2 and Fe concentration and added HCl to reach pH 3. Mixtures of wheat straw and aqueous Fenton reagent solutions were incubated at 20°C. Also tested was adding H_2O_2 in 5 parts, spread over time, not all at once at the start. References without HCl and/or iron and H_2O_2 were included. After

incubation residual H_2O_2 was removed using the enzyme catalase, in some of the bottles. See Annex 2 for more information. After incubation the bottles were handed over to Opure (Ede) who carried out biogas production tests using bottles and pressure detection to assess biogas production, in triplicate at 35°C and pH 7.5, at a dry matter content of ~9% until biogas production levelled off. The biogas production from inoculant organic matter was subtracted.

In the second round of experiments the actual solid fraction of mono cattle manure digestate from a farm in Bathmen was used. Various pH and H_2O_2 concentrations and effect of washing the fibres before the pretreatment with the Fenton reagent were explored.

In the third round, again using solid fraction of mono cattle manure digestate from a farm in Bathmen, a lower dose of H_2O_2 was tested (but this time without decreasing the iron dose), a continuous addition of peroxide was tested and compared with 5 batch additions, and pH 4.5 was tested and compared with pH 3. In the subsequent anaerobic digestion another variation was introduced: using inoculant from the manure digester in Bathmen and comparing that with the inoculant from Opure. All anaerobic digestions were carried out by Opure in triplicate.

3.3 Results

The experiments on biogas production using wheat straw as a model and the Fenton reagent (as a pretreatment) yielded insight in the effect of reaction conditions used in the pretreatment. See Annex 2 for details. The anaerobic biological digestion took about 65 days. After 20 days more than half of the production was reached. The biogas contained 52% (v/v) methane. The following observations could be made:

- 20 g H_2O_2 (added in 5 parts) plus 1.15 g $FeCl_2$ (pH 3) added to 40 g wheat straw dry matter led to a faster biogas production;
- 2 g H_2O_2 (added in 5 parts) plus 0.31 g $FeCl_2$: the effect is weaker but still significant;
- Final biogas yield showed only small differences in all bottles;
- Actually the untreated wheat straw performed surprisingly well; it was not that recalcitrant.

Therefore, there were two reasons to test real digestate from a cattle manure digester next time: (1) it is more realistic and (2) wheat straw is not that recalcitrant (not a good model system). The details are given in Annex 2. Observations:

- Fenton reagent clearly makes the digestate better digestible;
- pH 3 and 20 g H_2O_2 led to a much faster digestion and higher biogas yield;
- Lower amounts of H_2O_2 at pH 3 showed no effect;
- Incubation at pH 6 showed an effect, but small;
- Unwashed material yielded lower amounts of biogas;
- Biogas contained 52-55% CH_4 in all experiment;
- The H_2S concentration in biogas ranged from 2 to 100 ppm.

While with wheat straw around 550 Nm^3 biogas/tonne OM (organic matter) was produced in all variations, the digestate produced 250 – 400 Nm^3 biogas/tonne OM, which means that digestate is more recalcitrant. The biogas yield increase depends on digestion time and the activity of the biology in the biodigester. Opure used a super inoculant and also a high concentration of inoculant. Real digesters may be less active. In real digesters the biogas may be produced less fast, which means that an acceleration caused by Fenton pretreatment will get more meaning in such a situation. Moreover, real digesters have a better chance to develop conversion activity for the new compounds formed after H_2O_2 oxidation because of adaptation in time. The biogas yield may increase in the course of months. Therefore, we did experiments using inoculant from the digester in Bathmen.

The concentrations of methane in biogas can be regarded as normal and the H_2S concentration can be regarded as low. As we have observed some effect at pH 6 and a large effect at pH 3, do we have to go down to pH 3 (costly) or is pH 4 or 5 effective as well? The effect of pH is taken into account in the next round of experiments. In addition, this round was dedicated to cost reduction. To get more insight in the economics first a conceptual process design was made. The flow sheet of the process is shown in Figure 3-2.

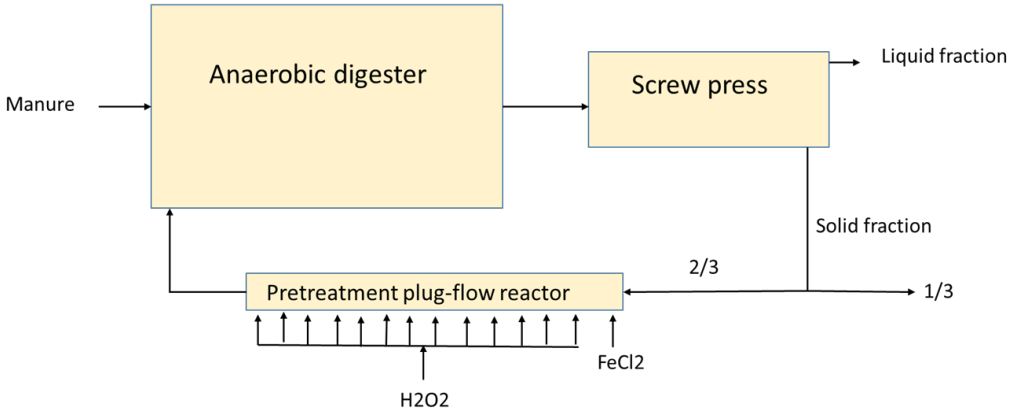


Figure 3-2 ***Flow sheet for treatment of digestate using the Fenton reagent and recycling the treated fibres to gain a higher biogas yield.***

A cost/benefit analysis was carried out in collaboration with CCS, for details see Annex 2. This analysis is for a plant that processes 700 tonnes digestate dry matter annually. The breakeven point (costs equals benefits) is at a price of H_2O_2 of € 160/tonne. However, the current H_2O_2 price is € 1000/tonne. The costs for H_2O_2 can be decreased by using a new technology: *in situ* electrochemical H_2O_2 production, which may cost € 640/tonne. To make additional biogas production by pretreatment using the Fenton reagent economically feasible, the use of H_2O_2 should be decreased. A reduction with at least a factor four is required. Another chance to reduce costs is the investment required for the reactor. If the residence time of the fibres in the reactor can be decreased from 24 h to 5 h, the reactor will be smaller and as a consequence the investment costs will be lower.

The results third round of experiments can be found in Annex 2. The most important data are summarized in Figure 3-3 and Figure 3-4.

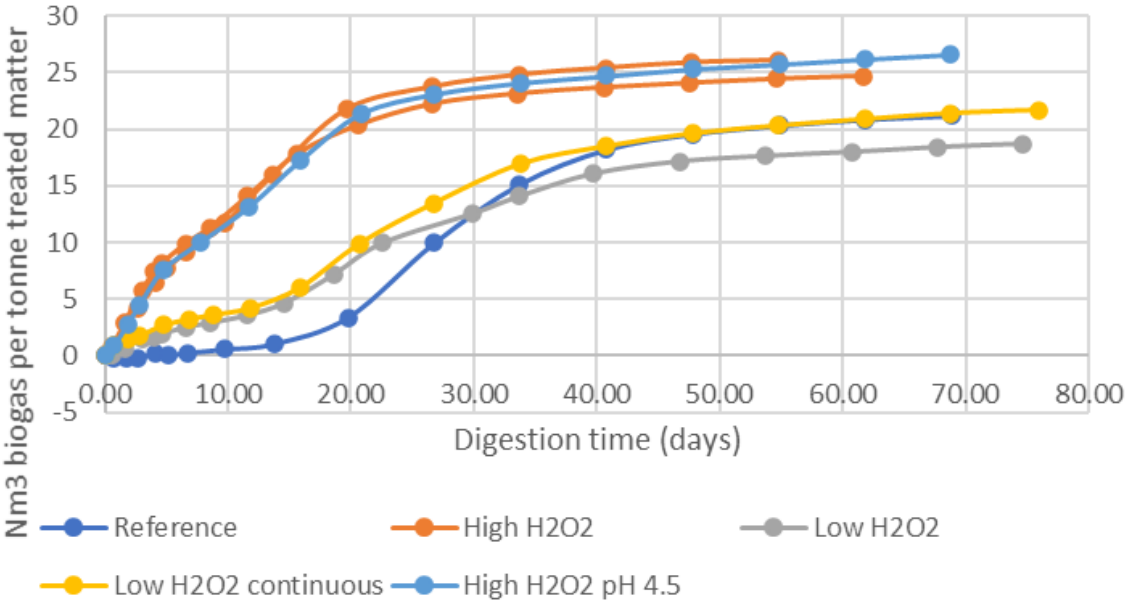


Figure 3-3 ***Biogas production after various treatments of solid fraction of manure digestate; inoculant of Opure was used.***

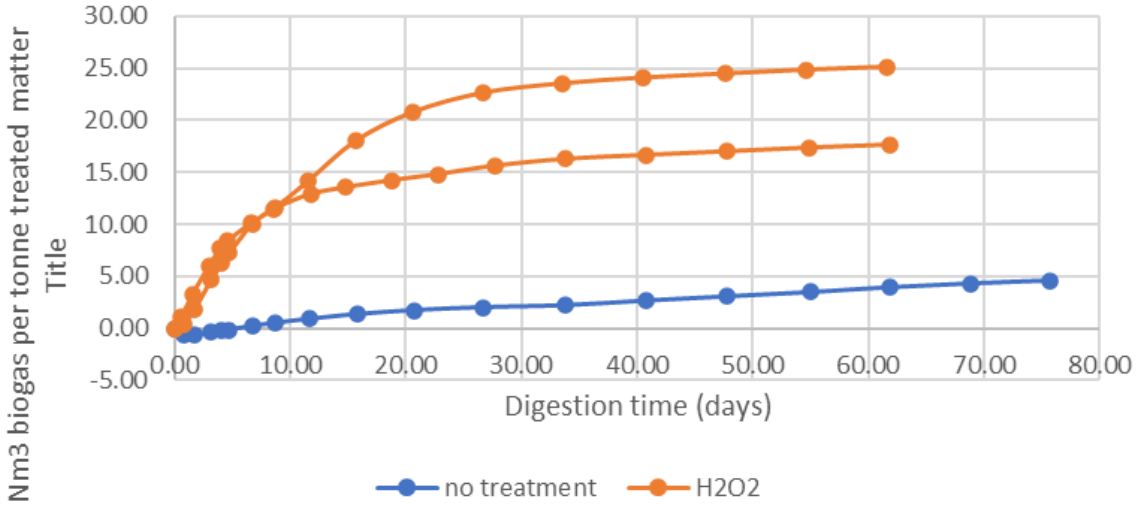


Figure 3-4 ***Biogas production after various treatments of solid fraction of manure digestate; inoculant from the manure digester in Bathmen was used.***

The following was found:

- H_2O_2/Fe addition helps to improve digestibility of digestate fibres;
- This effect is clearer when using inoculant from a manure digester;
- Pretreatment at pH 4.5 is as good as pretreatment at pH 3;
- Factor 5 reduction of H_2O_2 dose works as well, but the biogas production is lower as well;
- A continuous dose of peroxide is only slightly better than 5 shots per day.

Next we should know what will happen when using 5 times lower H_2O_2 dose and using digester inoculant. Maybe we get two times less biogas in that situation but at least the H_2O_2 costs per Nm^3 biogas will be 2.5 times reduced then. What will happen using 3 times lower H_2O_2 dose? Another way to reduce costs is an incubation with the Fenton reagent of 5 h instead of 24 h. We did not test that yet. We still have a chance this process becomes economical by using *in situ* peroxide production and if another cost reduction by at least a factor 4 can be realized.

3.4 Conclusions and recommendations

The use of Fenton reagent (hydrogen peroxide and iron ions) can make the recalcitrant fibres from cow manure digestate more digestible in biogas plants. In case an optimal inoculant is used, treatment using Fenton reagent can lead to a three times faster biogas production and after 50 days incubation a 25% higher biogas yield. In case inoculant from the original digester is used the effect is larger: without Fenton treatment hardly any additional biogas is produced upon a new incubation, but by adding Fenton reagent an order of magnitude faster biogas production and higher yield are attained. Under the conditions used, the value of the additional biogas produced is lower than the costs of the hydrogen peroxide added.

The recommendations for further research fall apart in two different strategies to improve manure fibre digestibility:

- Cost reduction Fenton treatment
    - Testing lower dose of hydrogen peroxide under conditions not yet explored: e.g. using digester inoculant instead of super inoculant
    - Shorter incubation time with Fenton reagent (leading to a smaller reactor)
    - In-situ hydrogen peroxide production
- A more potent consortium of bacteria

As an example of serendipity we found that the composition of the inoculum has a dramatic effect on biogas yield from manure fibres. The super inoculum used in the lab performed much better than the bacterial consortium in the original digester. It is hypothesized that the lab inoculum contained a much richer and

biodiverse consortium that can carry out much more biological reactions than the digester consortium. The low biodiversity in the original digester may be caused by the fact that the natural selection takes place under harsh conditions in presence of inhibitory concentrations of sulfide and ammonia. Less bacterial species are able to resist/survive these conditions. It can be recommended to prove this hypothesis and find out which conditions in the original digester should change to get a more potent bacterial consortium.

4 Conversion of VFA present in liquid manure fraction in PHA

4.1 Introduction

Manure is produced at large volumes with relatively low concentrations of organic energy sources (volatile fatty acids) and major losses of energy during storage prior to anaerobic digestion. Large parts of the available fatty acids are fermented into methane, which is subsequently emitted to the atmosphere, leading to energy loss and a major contribution to the greenhouse gas emissions (Cárdenas *et al.*, 2021). A way to prevent these energy losses and methane emissions at the farm level is the microbial conversion of volatile fatty acids (VFA's) in manure - such as acetic acid, propionic acid and butyric acid - to intracellular storage compounds such as polyhydroxyalkanoates (PHA's). Microbial biomass containing PHA's can then be transported to the biogas installations for biogas production or PHA's can be isolated and used as a polymer in materials applications.

Valorisation of manure towards PHA has been scarcely investigated and focused on the production of PHA from dairy manure fermenter liquor (digestate) (Coats *et al.*, 2016; Hanson *et al.*, 2016). PHA production directly from cow manure has not been investigated and therefore in 'Beter (dan) Vergisten' we set out to design a process for the production of PHA's from VFA's present in the liquid fraction of separated fresh cow manure. Biologically produced PHAs are stored by the microorganism as granules, which, if harvested as such, have properties that are extremely suitable for use in coatings. The aim in 'Beter (dan) Vergisten' was therefore to produce, release and recover functional PHA in its native state (granular) from organisms that are fed by manure.

While VFA's for PHA production are readily available in the liquid fraction of separated manure, the solid fraction still contains a high amount of non-fermented recalcitrant organic material that also might be converted to VFA's for PHA production. Therefore we also investigated the potential for additional production of VFA's by hydrolysis and acidogenesis of the separated solid fraction of cow manure.

Processes for selection of PHA-accumulating microorganisms in mixed microbial cultures (MMC) are often based on a feast-famine feeding regime during repetitive cycles (F/F cycling) (Koller, 2018; Oliveira *et al.*, 2017). The selection of PHA-accumulating organisms is done by imposing internal growth limitation during periods of carbon substrate excess (feast phase) and relatively longer periods of carbon substrate privation (famine phase) (Oliveira *et al.*, 2017). This is normally performed in bioreactors, often sequencing batch reactors (SBR). During the famine phase, PHA accumulators use external nitrogen and internal PHA for growth and maintenance, and those organisms that cannot store PHA are affected by the long famine period and will eventually be eliminated from the reactor. The production of PHA is further stimulated by nitrogen limitation, more specifically a certain (higher) C:N ratio is required. However, many waste streams such as waste water or manure contain high nitrogen levels (ammonia) that could prohibit efficient PHA accumulation. Processes have therefore been developed that couple PHA accumulation to the removal of ammonia, by alternating an aerobic feast phase with an anaerobic famine phase, resulting in the conversion of ammonia to nitrite in the aerobic feast phase and subsequent denitrification in the anaerobic famine phase (Frison *et al.*, 2015). This results in an increase of the C/N ratio, which may be advantageous for PHA accumulation. In this project, we applied a similar approach for the production of PHA from nitrogen-rich cow manure.

After the production of PHA, release and recovery from the biomass are the next steps. The aim in 'Beter (dan) Vergisten' was to release and recover functional PHA in its native state (granular). For this study PHA enriched sludge from a wastewater treatment plant was used. For the release mechanical cell disruption using bead mill and high pressure homogenization (HPH) were studied and for the recovery the use of the surfactant sodium dodecyl sulphate (SDS) to separate PHA from the cell debris. See Annex 3 for background information on the selection of these technologies. Recovery is studied using SDS (Ghatnekar *et al.*, 2002) and SDS at pH 11 (Xuejun, 2009). Dissolved-air flotation (DAF) (van Hee *et al.*, 2006) is an interesting

recovery option but was technically not possible to be performed at WUR facilities. The assessment of the recovery and process performance in this research were done based on PHA yield, PHA purity and microscopy (SEM). As benchmark solvent extraction with chloroform on freeze-dried sample was used.

In summary this chapter addresses:

- VFA production from solid manure fraction;
- PHA production from VFA present in liquid manure fraction;
- Release and recovery of PHA from biomass.

4.2 Materials and methods

4.2.1 VFA production from solid manure fraction

Hydrolysis and acidogenesis of the solid cow manure fraction was performed for 14 days in double-wall stirred tank batch reactors (STBR) with a 5 L work volume (6 L reactor) with pH controlled (pH 7.0) at 25°C (Figure 4-1). Continuous stirring of the reactors was obtained by anchor-type propellers. Biogas volume produced was measured using a µFlow device (Bioprocess Control, Sweden). Organic loading rates (OLR) used are shown in Table 4-1 and ranged from 3.1 to 50.2 g VS/kg.

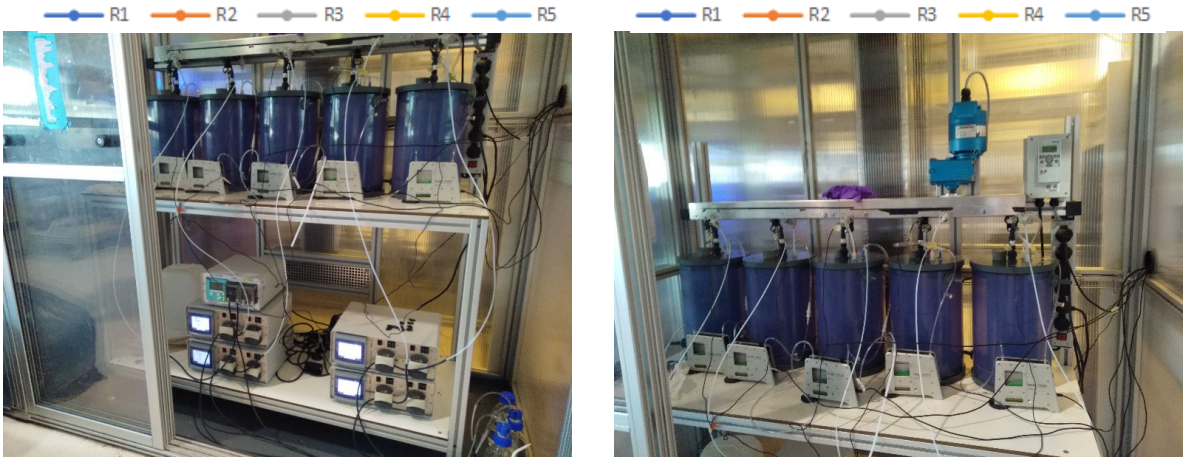


Figure 4-1 Five double-wall stirred tank batch reactors with pH control.

Table 4-1 Organic Loading Rates (OLR) of stirred tank batch reactors.

Reactor	Organic Loading Rate (g VS/kg)	% Solid fraction material (weighted-in)
R1	3.1	1.2
R2	6.3	2.4
R3	12.5	4.8
R4	25.1	9.6
R5	50.2	19.2

4.2.2 PHA production from VFA present in liquid manure fraction

A sequential batch reactor (SBR) was used for aerobic feast-anaerobic famine cycling enrichment of PHA-accumulating microorganisms on liquid cow manure fraction with concomitant nitrogen removal/reduction through nitrification/denitrification of ammonium (Frison *et al.*, 2015). As an inoculum active sludge was used from the PHARIO process for PHA accumulation from a wastewater treatment plant (Werker *et al.*, 2018).

Liquid cow manure fraction was obtained after screw press separation of manure from Holstein cows. The liquid manure fraction was further filtered over a strainer and cheese cloth to remove fibres and solids that

could block the reactor system. The fresh, filtered manure was then diluted 10-fold for its final working concentration. Reactors with a working volume of 1 L and 1.5 L total volume were filled with 500 mL filtered, diluted manure and an equal volume of untreated wastewater sludge. A 5 L bucket was filled with a 10-fold dilution of manure and connected to the reactor with an influent pump. An effluent pump was installed with an inlet at 500 mL height. The bioreactor was fitted with air stone, temperature sensor, stirrer and sample port. The SBR was run without oxygen or pH control.

The run cycle consisted of the following sequential phases (Figure 4-2 and Table 4-2):

- (i) aerobic feast phase
- (ii) anaerobic famine phase
- (iii) settling phase
- (iv) emptying phase
- (v) filling phase
- (vi) idle phase

The cycling run sequence was automated using LabVIEW software (Table 4-3).

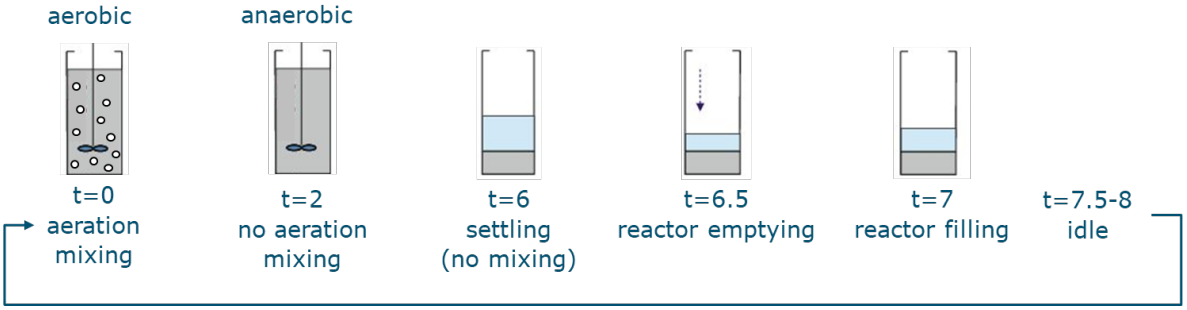


Figure 4-2 Setup PHA production from liquid manure fraction.

Table 4-2 Cycle description SBR for PHA production from liquid manure fraction.

Phase	Duration	Defining traits
Feast	4 h*	Abundant carbon, biomass growth and PHA accumulation
Famine	6 h	Carbon depleted, selection of microorganisms with internal carbon storage
Settlement	1 h	Settlement of biomass
Emptying	10 min**	Removing depleted manure
Filling	5 min	Adding fresh manure for the next cycle

**Initially, a feast phase of 2.5 h was applied. Later, this was adjusted to 4 h in an attempt to obtain a larger decrease of COD.*

***The effluent pump was kept on for twice as long as the filling phase at a higher speed to prevent potential overflowing of the reactor. Because the inlet of the hose is at a 500 mL height the reactor was never emptied fully.*

Table 4-3 Cycle conditions SBR for PHA production from liquid fraction cow manure.

Phase	Duration	Stirrer	Oxygen	Influent pump	Effluent pump
Feast	4 h	500 rpm	On	Off	Off
Famine	6 h	300 rpm	Off	Off	Off
Settlement	1 h	Off	Off	Off	Off
Emptying	10 min	Off	Off	Off	50 rpm
Filling	5 min	Off	Off	25 rpm	off

50 cycles were performed. After every 7 cycles, 10 mL samples were taken every hour of the feast phase. Samples were frozen directly awaiting analysis.

4.2.3 PHA release and recovery from biomass

The aim of these activities was to release and recover functional PHA in its native state (granular). For this study PHA enriched sludge from a wastewater treatment plant from the PHARIO was used (Figure 4-3). This biomass had a low DW (~2%) and the expected PHA content was 0.40 g PHA/g VSS (0.3 g PHA/g TSS)

(Werker *et al.,* 2018). Pretreatment was required to increase DW content and to make it microbial stable (acid treatment).

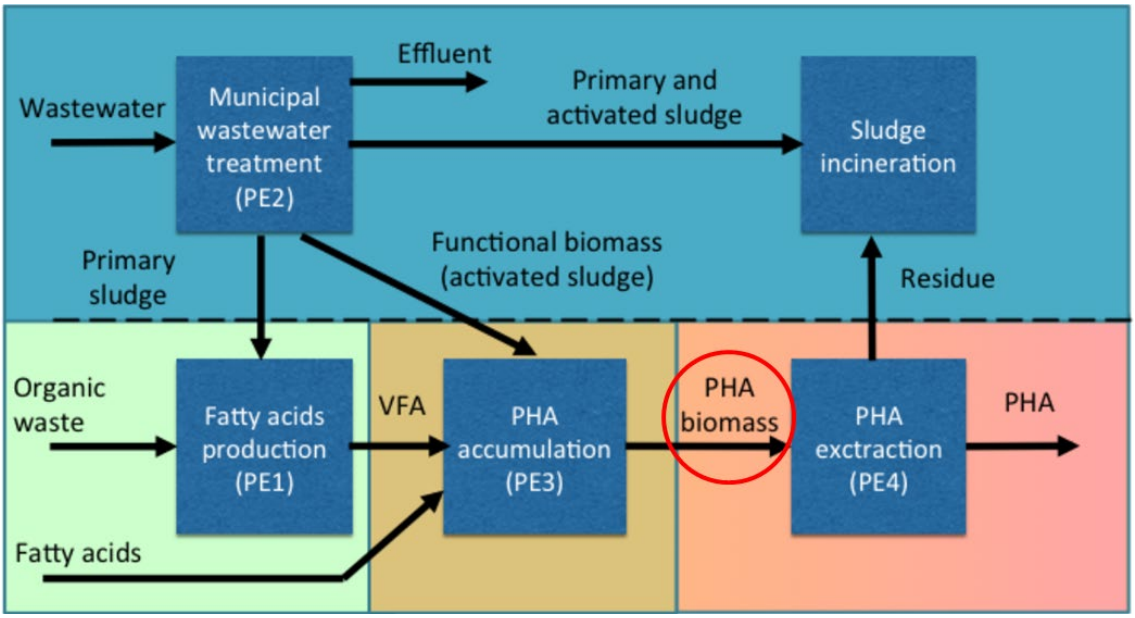


Figure 4-3 Schematic view of the PHARIO process and the origin of the PHA enriched sludge used in this study.

Three series of experiments were carried out:

1. PHA release: comparison between bead mill and HPH, both mechanical treatments
2. PHA recovery: use of SDS
3. PHA recovery: reducing SDS:sludge load

PHA release

Pretreatment of supplied sludge (~ 2% DM) by removing sand (sieving over 630 µm (2x)) and increasing dry matter content by overnight settling followed by decanting of clear liquid. This resulted in a concentrated sludge (4% DM). Centrifugation was done to further concentrate the sludge to 8% DM. Concentrated sludge and liquid phase were stored for 5-6 days at 4°C.

Bead milling was investigated using a DYNO-MILL MULTI LAB (cooled) with 0.3 L chamber, 180 mL beads (zirconium with yttrium stabilised, 0.5 mm) and operated in recirculation mode. Feed flow was 2 min/passage, feed volume per trial was 1.2 L with a DM content of 4 and 8%. Samples (45 mL) were taken at 0, 2, 4, 6, 8, 10, 20, 40 and 60 min.

Homogenization was performed using a PandaPLUS 2000 lab homogenizer (GEA) with a cooled outlet. Batch mode operation at 9 L/h, feed volume per trial 0.8 L. With 4% DM feed, pressure (250 – 500 – 1000 bar) and passes (0, 1, 2, 3, 4 and 5) were varied and 16 samples (1 + 3 x 5 (45 mL)) were taken. With 8% DM feed, pressure was constant (500 bar), passes (0, 1, 2, 3, 4 and 5) were varied and six samples (45 mL) were taken.

Assays for monitoring were Brix and EC (supernatant), protein release by Kjeldahl (supernatant) and PHA release (supernatant). More insights were obtained by determination of particle size distribution (cell clusters, cells, cell fragments) by laser particle sizer and microscopy combined with Nile red staining to visualize PHA granules.

Energy consumption of the bead mill was calculated based on Safi *et al.* (2017):

$$E_{BM} = \frac{\int_0^t P \cdot dt}{X \cdot V_L}$$

With E_{BM} specific energy consumption bead mill (kWh/kg dm), t time (h), P power (W), X dry matter concentration (g DM/L) and V_L treated volume (L).

First order kinetics for bead milling processes (i.e., cell disintegration and cell content release) have been proposed in many references (Postma *et al.*, 2015). The fraction of released water soluble proteins can be described by:

$$\frac{C_p(t)}{C_{p,biomass}} = 1 - e^{-k \cdot t}$$

With C_p (t) protein concentration in supernatant at time t (g/kg), $C_{p,biomass}$ total amount of protein inside biomass (g/kg suspension), k rate constant for water soluble protein release.

The energy consumption of the homogenizer was calculated based on Safi *et al.* (2017):

$$E_{HPH} = \frac{\Delta P \cdot n_{pass} / \eta_{pump}}{X \cdot 3600}$$

With E_{HPH} specific energy consumption homogenizer (kWh/kg dm), ΔP pressure (Pa), n_{pass} number of passes (-), η_{pump} pump efficiency (85%) and X dry matter concentration (g DM/L).

For high pressure homogenization, the release of components can be described by first order kinetics (Boon *et al.*, 2015a; Safi *et al.*, 2017):

$$\frac{C_p(t)}{C_{p,biomass}} = 1 - e^{-k \cdot n_{pass} \cdot \Delta P^w}$$

With c_{max} maximum release (g/L), c actual release (g/L), n_{pass} number of passes (-) and k and w fitted parameters.

PHA recovery

Pretreatment of supplied sludge (~ 2% DM with 26% PHA) by removal of sand by sieving over 630 µm (2x). Increase of dry matter content by overnight settling followed by decanting and centrifugation (8% DM). Concentrated sludge samples and liquid phase stored for 5-6 days at 4°C. Half of the concentrated sludge (8% DM, 19.4% ash) was acid washed to remove salts. The pH was adjusted from pH 6.7 to 5 with H_2SO_4, stirred for 30 min followed by centrifugation and washing sludge with water (2x). Final pH of 5.8 and 34% lower ash content (12.9%) were obtained.

Setup of the experiment is shown in Figure 4-4. Mechanical disruption was achieved by bead milling followed by mixing with SDS using a 5% SDS solution and different ratios SDS:sludge (3:1, 2:1, 1:1, 1:2, 1:3). Assessment of recovery and process performance by determination of yield (PHA), purity (DM, ash and PHA), particle size distribution and microscopy (SEM). Solvent extraction (chloroform) was done on a freeze-dried sample and used as benchmark.

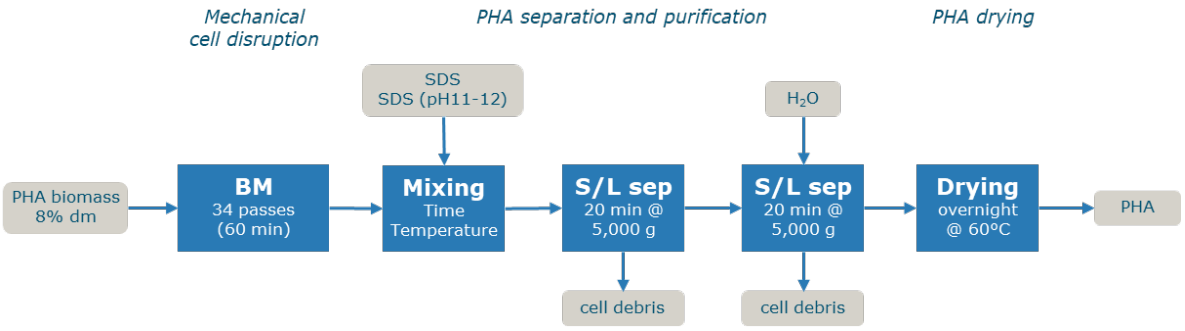


Figure 4-4 Experimental setup of experiments PHA recovery using bead milling and SDS.

PHA recovery – reducing SDS load

In these experiments the possibilities to use lower SDS doses was investigated. An overview of the experiments is given in Figure 4-5. Various combinations of bead milling (number of passes), SDS addition (amount and moment of addition (before or after bead milling), high pH and acid wash on PHA release and recovery were studied. The same pretreatment as described under *PHA recovery* was applied.

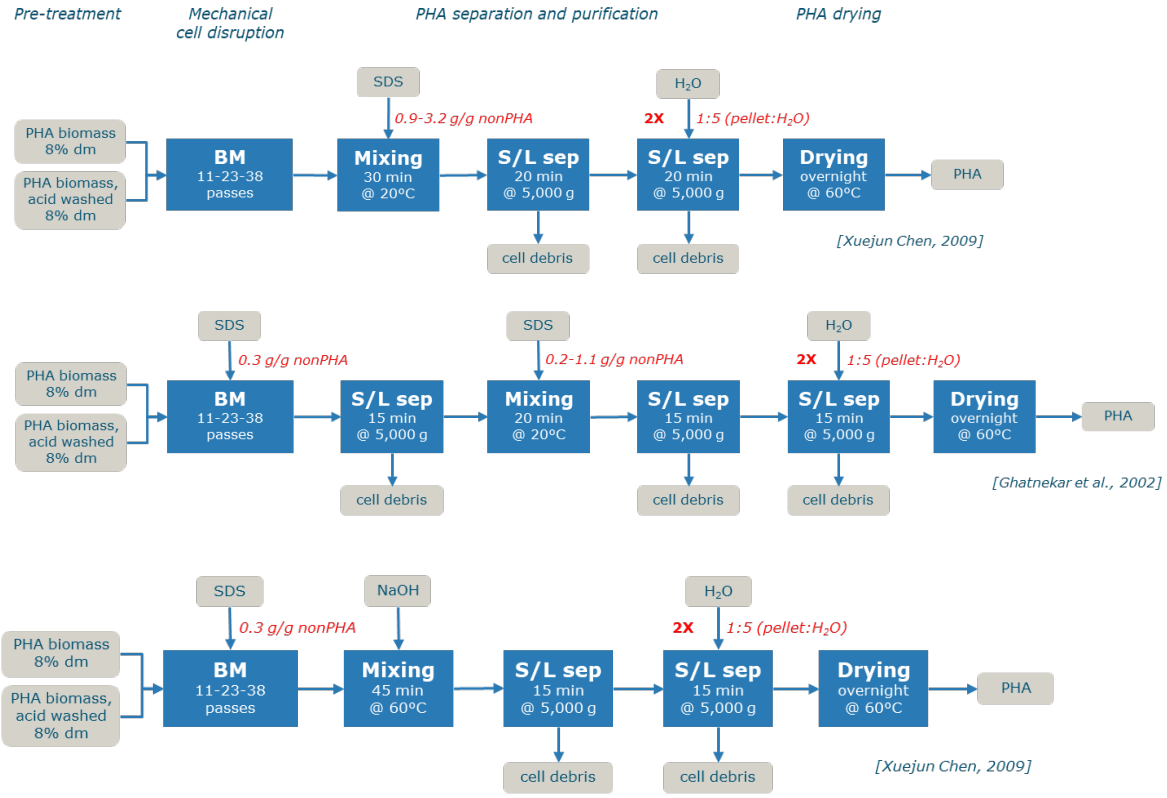


Figure 4-5 ***Overview experiments second trial to investigate the combination of bead milling, SDS addition, high pH and acid wash on PHA release and recovery.***

4.2.4 Analyses VFA production

Dry weight (total solids, TS) of manure was determined after overnight incubation of ~25 g manure at 105°C and ashes weight after subsequent incubation at 550°C. Volatile Solids (VS) were determined as the difference in weight between dry weight and ashes. COD and NH_4^+ concentrations were determined using Hach-Lange kits.

For analysis of VFA's 1.35 mL of manure sample was mixed with 0.15 mL of 0.15 M formic acid. Before GC analysis samples were centrifuged to remove precipitates, supernatant were transferred to GC-vials. Analysis was performed on a Agilent 7890B GC system equipped with a HP-FFAP column and FID detection. Chromatograms were analysed using Chromeleon 6.80 SR13 software.

Before fluorescence microscope analysis, the samples were stained using Nile Red staining, to visual HA globules directly in manure.

4.2.5 Analyses PHA production

COD was determined in fresh samples during the feast phase. Samples were diluted 5-fold to fall within the expected analysis range but not otherwise pre-treated. COD determination was done using COD cuvette test 100-2000 mg/L (Hach-Lange) according to protocol.

Being a hydrophobic substance, accumulated PHA can be detected microscopically by Nile Red fluorescence. For PHA detection by Nile Red, samples were thawed and spun down for 10 min at 3000 rpm in a swing bucket centrifuge (Eppendorf 5804R). The supernatant was transferred to a fresh tube and the pellet was resuspended in an equal volume saline. 100 µL of the resuspended biomass was mixed with 1 µL 100x Nile-red stock solution before being viewed under normal light without a filter as well as under UV light with Zeiss filter set 09 (Zeiss) where PHA rich biomass is coloured a bright orange.

For PHA analysis, 10 mL samples were spun down for 10 min at 3000 rpm in a swing bucket centrifuge (Eppendorf 5804R). Supernatant was transferred to a fresh tube and the pellet was suspended in an equal volume of water before freeze drying. 20 mg of freeze-dried biomass was weighted in glass reaction vials. 2 mL of 15% (v/v) sulphuric acid in methanol and 2 mL chloroform containing 0.5 mg/mL methyl benzoate (internal standard) were added to the samples. The solutions were heated to 110°C for 4 h whilst stirring. Solutions were cooled on ice before adding 1 mL of water and vortexing thoroughly. Samples were subsequently spun down for 5 min at 3500 rpm before the top layer (water phase) was removed. The bottom (chloroform) was dried of trace water by the addition of Na_2SO_4 after which the liquid was transferred to a GC vial and analysed using GC-MS.

4.2.6 Analyses PHA release and recovery

Protein content was determined in duplicate using the Kjeldahl method, following the protocol described by Bradstreet (1954). Samples were mixed with one catalytic Kjeltab CK (VWR), consisting of 3.5 g K_2SO_4 and 0.4 g $CuSO_4 \times 5H_2O$, 9 mL of 97% H_2SO_4 and heated for 50 min at 420°C. After cooling 75 mL water was added. The samples were then steam distilled with NaOH and the ammonium was collected in boric acid. After titration with HCl the amount of protein was calculated with a standard conversion factor of 6.25.

Particle size distribution (PSD) was measured by laser diffraction using a Mastersizer 2000 (Malvern Panalytical). Wet particle samples were introduced to the optical bench by a slurry dispersion unit. The actual light scattering pattern from a field of particles was captured by multiple detectors in the optical bench. Using the Mie theory (which accurately predicts the light scattering behaviour of materials), the software calculated the size of particles that created that pattern.

PHA was quantified by the method as described in section 4.2.5.

4.3 Results

4.3.1 VFA production from solid manure fraction

Incubation of solid manure fraction at different organic loading rates showed the production of VFA's over a 14 day period, with increasing content and yield (Figure 4-6) for higher OLRs. The maximum production achieved after 14 days were a VFA content of 4.6 g VFA/L for an OLR of 50.2 g VS/kg (R5) and a yield of 0.13 mg VFA/mg VS for an OLR of 25.1 g VS/kg (R4), respectively. The main VFA's formed were acetate (3040 mg/L) and propionate (1220 mg/L) followed by n-butyrate (150 mg/L), iso-butyrate (77 mg/L), iso-valerate (87 mg/L) and n-valerate (25 mg/L) (Annex 1, Figure A1-2). Production of biogas, which can be expected under the applied semi-anaerobic conditions, was limited and only observed for the highest OLR (855 mL in 14 d).

These results indicate that using the conditions tested a high amount of additional VFA's can be obtained from the solid fraction that could be used for PHA production. For the highest OLR a plateau in VFA production was not reached after 14 days (Figure 4-6), indicating that even higher VFA yields could be reached. Higher OLRs could also be tested still, as long as the solid fraction mixture can be stirred. Applying higher OLRs would reduce the amount of water required for diluting, which on industrial scale could reduce CAPEX and OPEX. In previous work hydrolysis and acidogenesis of fresh cow manure resulted in 0.18 mg COD_{VFA}/mg COD_{VS} at an OLR of 48 g VS/L (Coats *et al.*, 2011), while the VFA concentration from separated liquor after anaerobic digestion was 5.2 g COD_{VFA}/L (Coats *et al.*, 2016). The concentration of VFA

that was obtained in this study after hydrolysis and acidogenesis of the solid fraction manure is in the same range.

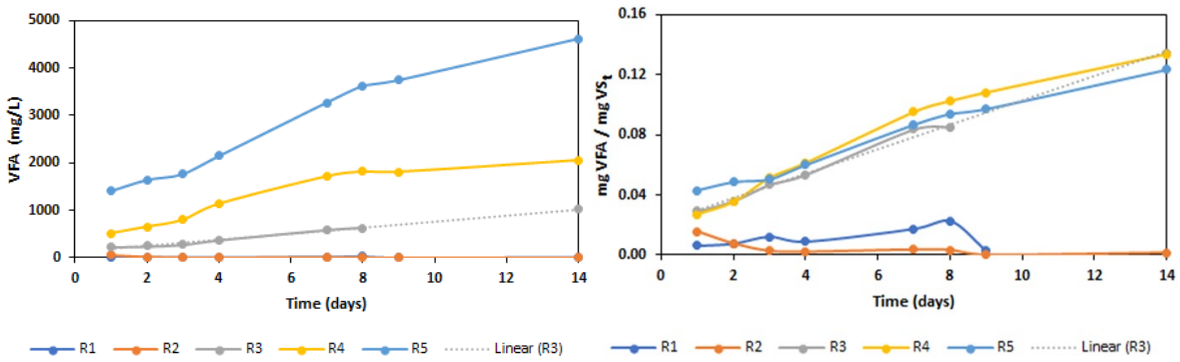


Figure 4-6 ***VFA content (right) and yield (left) from hydrolysis and acidogenesis of the solid fraction of cow manure at increasing OLRs (R1-R5, see Table 4-1).***

4.3.2 PHA production from VFA present in liquid manure fraction

Before the sequencing batch reactor was started, tests were done to settle and filter manure. Untreated manure both diluted and undiluted contained too many large solids to pump it into the reactor without clogging the tubing. Attempts were made to separate the solid and liquid fractions by centrifugation, both with and without the addition of formic acid. Cellulose was also added to determine whether this would help flocculation of the solids, but without success. The large solids in the manure were then filtered by cheesecloth. Micro- and ultra-filtration were not possible in view of the remaining solids. In order to still allow pumping of the manure and settling of the active sludge in the SBR reactor, it appeared necessary to dilute the manure 10-fold.

At the fourth feast-famine cycle in the SBR reactor, when a feast phase of 2.5 hours was applied, the COD during the feast phase was determined at 30 min intervals. No change in the COD during the feast phase was observed, only small fluctuations (Annex 3, Table A3-3). The duration of the feast phase was increased from 2.5 to 4 h. After 3 cycles the COD during the feast phase was analysed (Table A3-2) and again no detectable decrease in COD was observed. It was decided to leave the sequence unchanged to see if there were developments over time.

For analysis of PHA formation by Nile-red staining/microscopy and GC-MS analysis, samples were taken during cycle 41. It was expected that the microbiome in this cycle should already be primed for PHA production and that an increase in PHA containing biomass between 0 and 4 h should be visible.
Under normal light it was observed that the fibrous solids that were present in fresh manure were largely degraded during the feast phase (Figure 4-7).

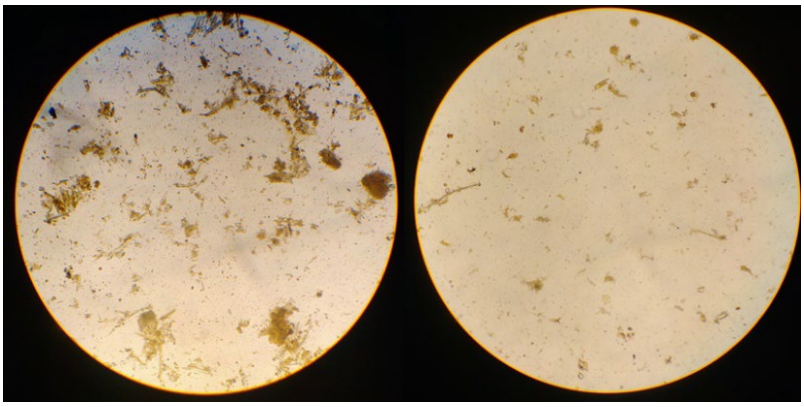


Figure 4-7 ***Manure at the start of the feast famine in cycle 41 (left) and after 4 h of feast phase in cycle 41 (right).***

Under UV light, it seemed that some non-specific staining occurred, and it was uncertain whether fluorescence could be attributed to the presence of PHA. Accordingly, the presence of any PHA in samples, taken during the feast phases of cycle 8, 27 and 41, was analysed by GC-MS, after freeze-drying, subsequent hydrolysis, and conversion of any resulting free hydroxy fatty acid monomers to methylesters. However, no PHA-derived hydroxy fatty acids were detected (not shown).

By diluting the manure 10-fold to allow settling of the active sludge the VFA concentration must have been reduced to below 1 g/L. This near-lack of VFA might have resulted in selection for a microbiome slowly degrading polymeric/fibrous carbon-containing compounds, such that there was never a real feast-famine cycling (and thus, no selection for microbiomes capable of accumulating PHA for use during the famine period), but rather a continuous slow degradation of fibrous compounds during all phases of the cycle. In addition, the ratio between (a) carbon becoming slowly available and (b) nitrogen and/or other nutrients might have been too high to support efficient PHA accumulation. It thus appears difficult to directly capture the available carbon sources in the manure in the form of PHA.

It can be concluded that it appeared to be difficult to directly capture the available carbon sources in the manure in the form of PHA, due to the composition and thickness of manure. It is therefore recommended to pre-process and separately isolate the VFA's and ammonia from the manure such that these can be used in a batch or fed-batch fermentation. Using the VFA's as a separate stream also gives more control of the carbon/nitrogen and carbon/phosphate ratios, which allows a more efficient production of PHA.

Using VFA's as a separate fraction as opposed to whole manure will probably also positively influence downstream processing of the PHA. After a feast famine cycle part of the solid fraction is degraded but many impurities will still be present at the end of every cycle. Having biomass with little impurities allows a more efficient isolation of PHA from the biomass.

4.3.3 PHA release and recovery from biomass

PHA release

The results of the bead mill are presented in this section, for the results obtained with the HPH the reader is referred to Annex 3. In Figure 4-8 the protein release (used as indicator for PHA release) is given as function of the % DM in the feed and the number of passes. A protein yield of 70% is obtained after 22 passes (4% DM) or 12 passes (8% DM). In the HPH 70% protein yield was obtained at 250 bar (4 passes), 500 bar (2 passes for both 4 and 8% DM) and 1000 bar (<1 pass) (Annex 3).

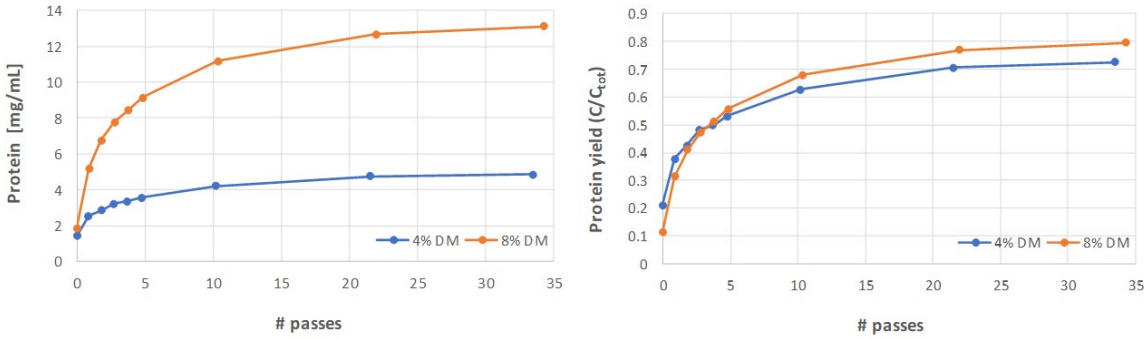


Figure 4-8 Influence of dry weight and number of passes on protein release (left) and yield (right) during beat milling (trial PHA release).

Besides protein also Brix and EC were measured in the supernatant to determine if they could be used as indicator for cell disruption and PHA release (Annex 3). PHA release was confirmed by analysis. A low PHA yield was observed due to distribution of PHA over supernatant and pellet (poor S/L separation). It was concluded that both protein and Brix can be used as indicators for PHA release.

As an indicator of PHA release the particle size distribution after several passes was determined. The results are shown in Figure 4-9. Initially mainly cell agglomerates are present (10-1000 µm). After just one pass

through the bead mill the distribution peak shifted to 10-100 µm, indicating smaller agglomerates. With increasing number of passes, the formation of particles between 0.1-2 µm is observed, which could be the single cells combined with PHA granules.

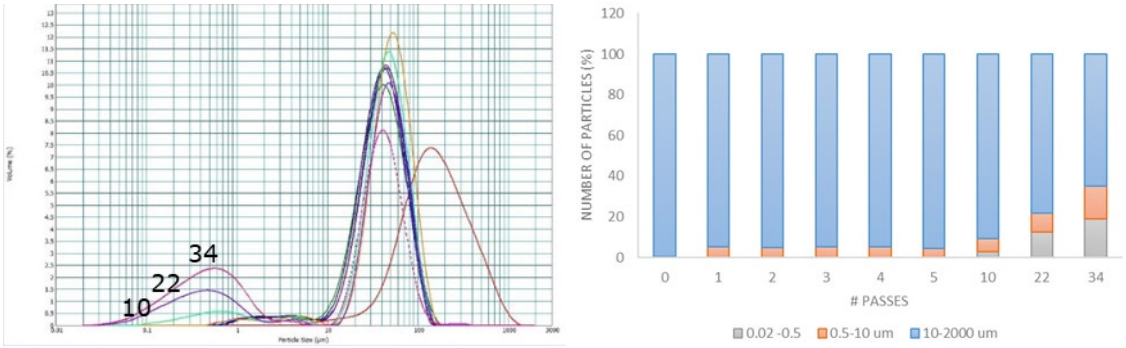


Figure 4-9 Particle size distribution after bead mill for a series of passes (8% DW). Agglomerates 10 – 3000 µm, single cells 0.5 – 10 µm) and PHA 0.1-0.5 µm.

Samples were also evaluated using microscopy combined with Nile red staining to visualize PHA granules. Typical results for the bead mill at a feed of 8% DM are shown in Figure 4-10. These results also seem to indicate a release of PHA granules at 11, 22 and 33 passes.

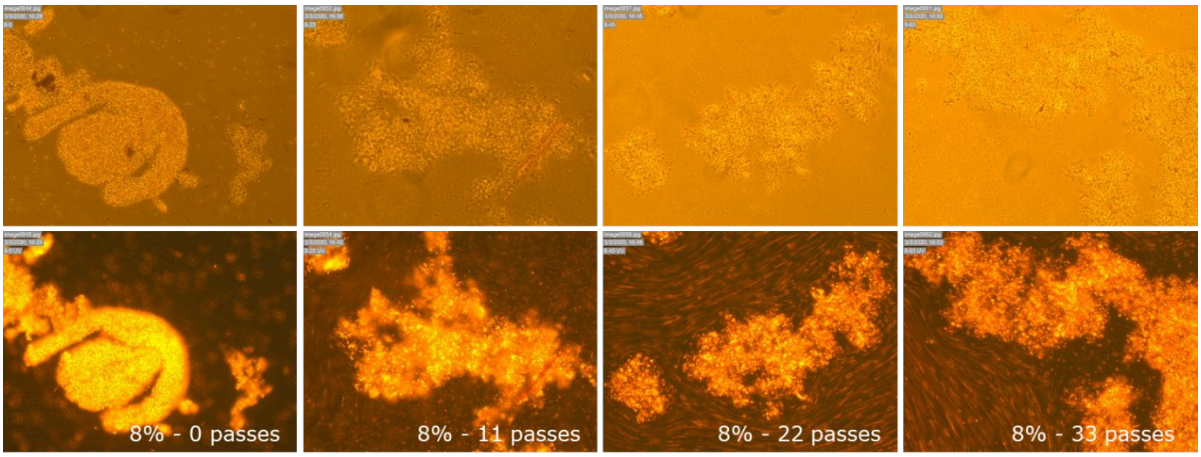


Figure 4-10 Microscopy combined with Nile red staining to visualize PHA granules.

In order to make a good comparison between mechanical disruption technologies, the energy consumption should be taken into account. For both technologies the energy consumption was estimated from the results. The details of these calculations are given in Annex 3. For a 70% protein release at 8% DM the energy consumption for the bead mill was estimated at 8.7 kWh/kg DM. The estimated energy consumption for the homogenizer for a 70% protein release, single pass at 642 bar and 4% DM, was 0.52 kWh/kg DM. These energy requirements are large, however there is a large effect of scale on the energy consumption of the bead mill. Previous work on disruption of microalgae (Boon *et al.*, 2015a) showed a large difference in idle power uptake: 40% at 20 L scale (measured) and 10% at 1150 L scale (communication Bühler). Taken idle power and the scale of the equipment into account, the estimated energy consumption for a bead mill on industrial scale is 0.1-0.5 kWh/kg DM, which is in the same range as the estimated energy consumption of the homogenizer.

Conclusions of the first trial are that pretreatment of this biomass is required to remove sand and to increase the DM content (DW > 8%). Both bead mill and homogenizer are effective for release of PHA and difference in energy consumption on large scale are small and a minor issue. The choice was made for the bead mill because it is less prone to wear and has a lower CAPEX (compared to 650 bar homogenizer). The bead mill was used in further experiments. Brix and protein release are good indicators for disruption efficiency. The analysis of PHA showed to be complicated and requires a good solid/liquid separation.

PHA recovery

PHA recovery from disrupted cells was studied adding SDS at different SDS:sludge ratios. It was observed that the addition of SDS improved the S/L separation and a more compact pellet was obtained (Figure 4-11). The highest ratio of SDS:sludge tested (3:1) resulted in a high purity of PHA 92% and yield of 63%. Chloroform extraction was used as a reference, setting the initial PHA content of the sludge (purity 29% DM) and the 100% yield. The purity of the PHA obtained with chloroform was > 100% indicating an inaccuracy probably in the PHA quantification.

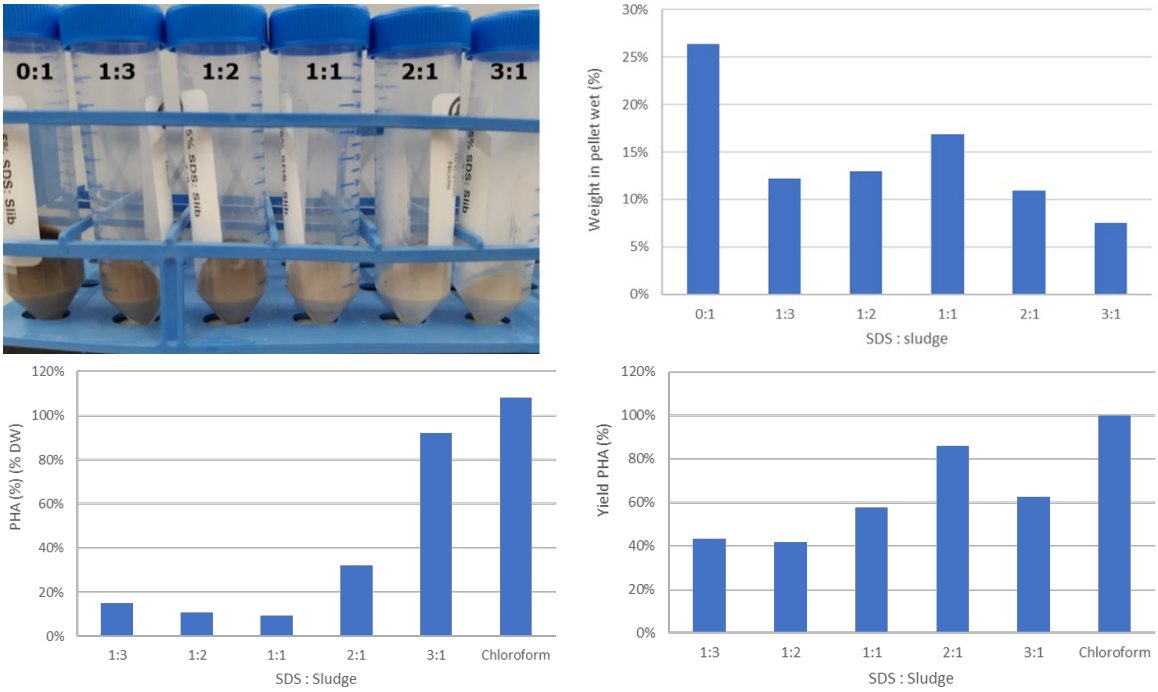


Figure 4-11 PHA recovery as function of SDS:slugde: influence on S/L separation (weight pellet), PHA purity and PHA yield.

SEM pictures were taken of two samples (SDS:sludge 3:1 and 2:1) and compared with CERAFLOUR 1000 (92% pure) (Figure 4-12). Both PHA granules and impurities are clearly visible.

The PHA yield and purity obtained were compared with data from literature (Table 4-4). Since SDS is applied to remove impurities, the SDS dose is expressed in g SDS/g non-PHA. The SDS dose is in the same range as applied in literature, but the achieved purity and yield in this research are much lower. Three plausible causes are (i) low PHA content of the starting material, (ii) relatively high fraction of inorganic impurities and (iii) less intense S/L separation conditions. The PHA content in the sludge was 29%, while this is usually 50-80% in literature. Since the PHA was produced from wastewater using mixed-culture fermentation, the starting material contained a significant amount of inorganic impurities (20% ash). In literature, batches of PHA were produced by pure culture fermentations using defined substrates and media. The applied S/L separation conditions are crucial for the PHA yield. Possibly intensive conditions (relatively high RCF and time) are used in literature, but the data is inconclusive (no rotor specifications (k factors) specified).

Based on the results a ball park figure of the SDS cost was made:

- SDS price range: 0.6-1.7 €/kg (Chembid, 2022; Bulk apothecary, 2022)
- PHA production cost: 1.8 €/kg (purification) (Vega *et al.*, 2020)
- PHA market price range:
    - 8 €/kg (market price for non-GMO virgin PHA without plasticizers and UV stabilisers) (personal communication BYK, 2020)
    - 3.5-4 €/kg (in 2025) (Bengtsson *et al.*, 2017)
    - 2.2-5 €/kg (Majone *et al.*, 2017)

Proposed SDS dose to be tested was ≤ 0.5 g/g PHA (or ≤ 1 g/g non-PHA), corresponding to 5-50% of the purification cost.

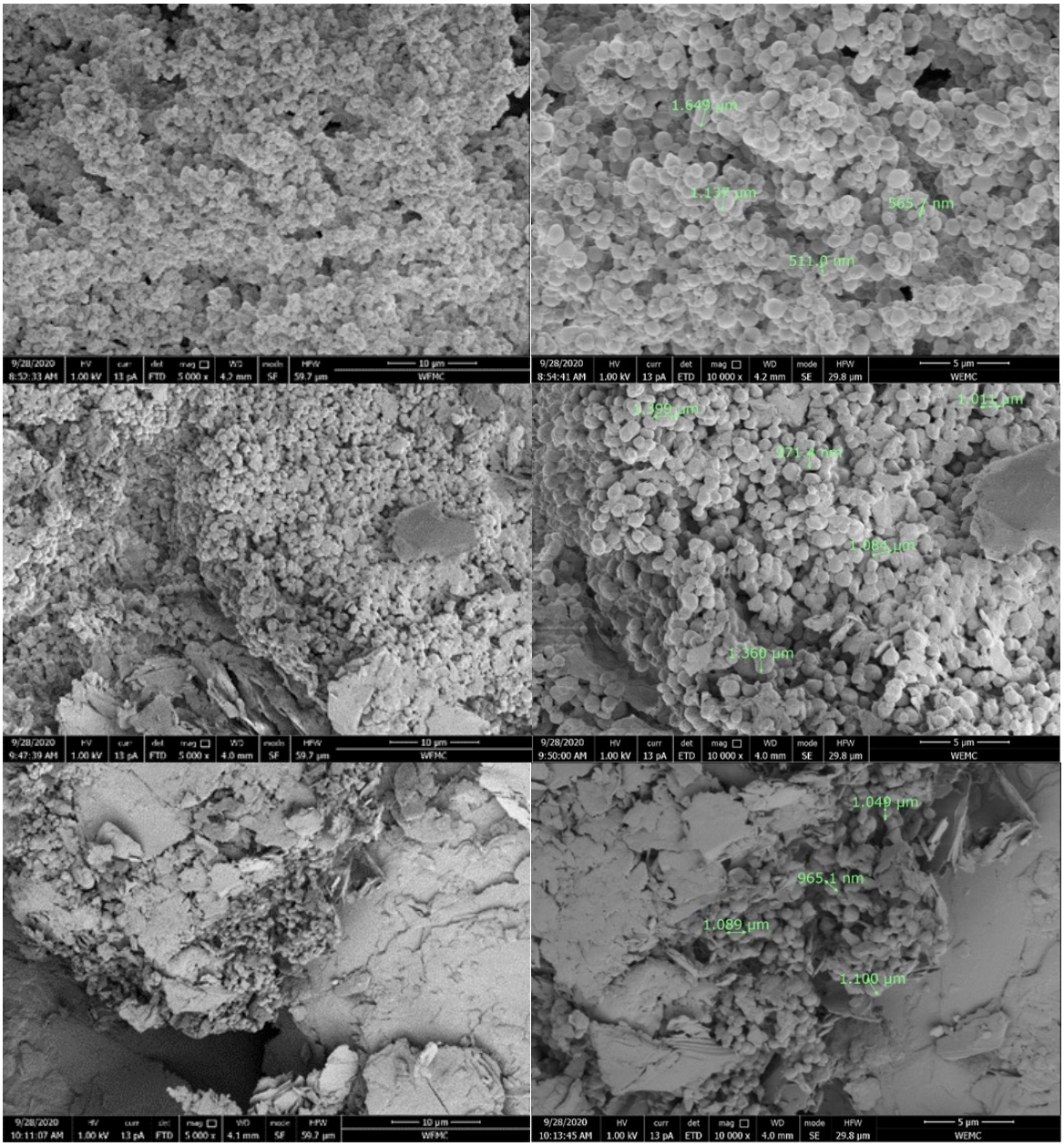


Figure 4-12 SEM pictures CERAFLOUR 1000 (92% PHA) (upper), SDS:sludge 3:1 (92% PHA) (middle) and SDS:sludge 2:1 (32% PHA) (bottom).

Table 4-4 Comparison PHA yield and purity obtained in this research with literature data. Estimation of SDS cost, using SDS price range of 0.6-1.7 €/kg. Colour indicating: light ≤ 10% of purification cost, red ≥ 100%.

Experiment	Condition	Purity	Yield	SDS dose		SDS cost [€/kg PHA]	
		[%]	[%]	[g/g nonPHA]	[g/g PHA]	low	high
1:3 (SDS:sludge)	BM - SDS - C	15	43	0.4	2.2	1.3	3.8
1:2 (SDS:sludge)	BM - SDS - C	11	42	0.6	3.4	2.0	5.8
1:1 (SDS:sludge)	BM - SDS - C	9	58	1.1	4.9	3.0	8.5
2:1 (SDS:sludge)	BM - SDS - C	32	86	2.3	6.6	4.0	11.4
3:1 (SDS:sludge)	BM - SDS - C	92	63	3.4	13.6	8.1	23.4
3% SDS - pH11	BM - SDS - C	19		0.7			
5% SDS - pH12	BM - SDS - C	67		2.3			
[Xuejun Chen, 2009]	BM - SDS (pH12) - F	98.2	85	0.4	0.1	0.1	0.2
[Jiang, 2015]	Digestion with SDS+NaOH - C	99.1	91	0.3	0.2	0.1	0.3
[Xuejun Chen, 2009]	HP - SLES (pH10) - F	96.7	82	0.5	0.2	0.1	0.4
[Dong, 2000]	Digestion with SDS+NaOCl - C	98.0	87	1.3	0.5	0.3	0.9
[Kim, 2003]	Digestion with SDS - C	95.0	97	1.6	0.5	0.3	0.9
[Xuejun Chen, 2009]	BM - SDS (pH11) - F	94.2	71	0.9	0.8	0.5	1.4
[Yang, 2011]	Digestion with LAS-99 - C	88.0	86	1.5	0.9	0.5	1.5
[Choi, 1999]	Digestion with SDS - C	98.7	89	4.3	1.5	0.9	2.5
[Xuejun Chen, 2009]	BM - SDS (pH13) - C	98.2	87	4.9	3.8	2.3	6.5
[Yang, 2011]	Digestion with SDS - C	90.0	81	8.3	4.4	2.6	7.6
[Ghatnekar, 2002]	HPH + SDS - C	95.0	98	16.7	7.3	4.4	12.5

PHA recovery – reducing SDS load

With a high SDS dose (3.5 g SDS/g non-PHA) PHA was recovered with a high purity (92%) and a 63% yield. The aim of these experiments was to reduce the SDS dose with a factor 10. The influence of acid wash and adding SDS prior to bead milling on the required SDS dose were also investigated.

The results are summarized in Figure 4-13, the underlying data are given in Annex 3. Best results were obtained when the SDS was added prior to the bead milling. This improved both the yield (>90%) and purity (>40%). The SDS dose can be reduced to 0.6 g SDS/g non-PHA. It can be argued whether the bead mill is required as no experiments were done leaving bead milling out. Also the alkaline treatment looks promising at a dose of 0.3 g SDS/g non-PHA. Further improvements on purity and SDS dose are possible by using a counter-current extraction process and possibly by performing an alkaline SDS treatment.

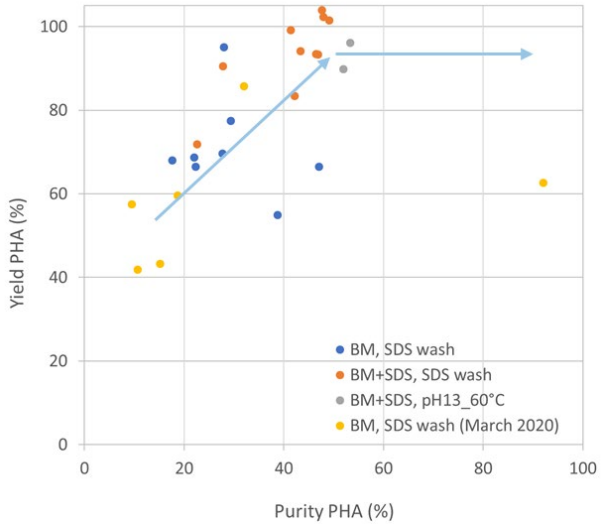


Figure 4-13 Summary of purity and yield PHA obtained using combinations of bead milling, SDS addition and elevated pH.

4.4 Conclusions and recommendations

VFA production
The solid fraction of manure still contains many undigested organic polymers, mainly in the form of fibres. This organic material is not readily available for use by many microorganisms, such as PHA accumulators, and therefore needs to be degraded into smaller building blocks such as VFA's. A higher VFA content can be obtained by hydrolysis and acidogenesis of the solid fraction. The highest OLR tested (50.2 g VS/kg) yielded 4.6 g VFA/L or 0.14 mg VFA/mg VS. These results are in the same range as reported in literature. The main VFAs formed were acetate (3 g/L) and propionate (1 g/L) as well as lower quantities of butyrate (0.2 g/L) and valerate (0.1 g/L). For the highest OLR a plateau in VFA production was not reached after 14 days, indicating that even higher VFA yields can be reached. Higher OLRs could also be tested still, as long as the solid fraction mixture can be stirred. Applying higher OLRs would reduce the amount of water required for diluting, which on industrial scale could reduce CAPEX and OPEX.

PHA production
It appeared difficult to directly capture the available carbon sources in the manure in the form of PHA, due to the composition and thickness of manure. It is therefore recommended to pre-process and separately isolate the VFA's and ammonia from the manure such that these can be used in a batch or fed-batch fermentation. Using the VFA's as a separate stream also gives more control of the carbon/nitrogen and carbon/phosphate ratios, which allows a more efficient production of PHA.

Using VFA's as a separate fraction as opposed to whole manure will probably also positively influence downstream processing of the PHA. After a feast famine cycle part of the solid fraction is degraded but many impurities will still be present at the end of every cycle. Having biomass with little impurities allows a more efficient isolation of PHA from the biomass.

PHA release and recovery
Mechanical treatment (both bead mill and homogenizer) is effective for release of PHA from biomass and energy consumption on large scale is a minor issue. Brix and protein release are good indicators for disruption efficiency. A reliable PHA analysis requires a good solid/liquid separation and the addition of SDS improves this separation. Best results are obtained when the SDS is added prior to the bead milling: yield >90% and purity >40%. The SDS dose can be reduced to 0.6 g SDS/g non-PHA. It is recommended to investigate whether the bead mill is required for cell disruption because no experiments were done adding only SDS. Also the alkaline treatment (pH 13) looks promising at a dose of 0.3 g SDS/g non-PHA. Further improvements on purity and SDS dose are possible by using a counter-current extraction process and possibly by performing an alkaline SDS treatment.

Overall
Biologically produced PHAs are stored by the microorganism as granules, which, if harvested as such, have properties that are extremely suitable for use in coatings. In 'Beter (dan) Vergisten' we showed that it is in principle possible to produce, release and recover functional PHA in its native state (granular) from organisms that are fed on manure for this application. However, manure does not appear to be the most suitable green raw material to continue working with. In view of the above it is recommended to stop the research and development of the PHA production process from manure.

5 Phosphate recovery from acidified liquid digestate fraction

5.1 Introduction

In the Netherlands, dairy-, poultry- and/or pig farming leads to locally excessive manure accumulation. Parts of the manure are used for fertilization, but this use is limited and is subjected to regulation due to its high phosphate content. The soils in the Netherlands are also already quite saturated with phosphate. A method that ensures that less phosphate enters the soil and at the same time makes phosphate available as a starting material for other processes also leads to less import of phosphate. Reducing the phosphate content of manure would allow full re-use of manure. Furthermore, natural phosphate mine (fossil) sources are at risk of becoming exhausted, re-use of the phosphate itself as "sustainable" fertilizer in agri- and horticulture is also becoming increasingly important. Further, the aim is to generate a sustainable source of phosphoric acid for the food industry (e.g. production of phosphoric acid).

Manure as a waste stream bears high concentration in elements not fit for discharge. Off the many components, nitrogen and phosphate stand out because they lead to excessive growth of microorganisms, unwanted plants and subsequently fouling, if present in excessive concentrations. At the same time, both phosphate and nitrogen are essential for all biotic growth and can form the basis of a range of products once separated from manure. This chapter focusses on the use of nano-sized (para) magnetic magnetite particles ($Fe_3O_4/Fe^{2+}(2Fe^{3+})O_4$ for phosphate (PO_4^{3-}) recovery from liquid acidified manure (see Annex 4 for background information for the selection of this technology). The liquid manure fraction is first acidified, to increase phosphate solubility. Thereupon the solubilized phosphate is adsorbed by paramagnetic magnetite particles. By applying a temporary magnetic field, phosphate containing particles can be separated from the phosphate containing source/matrix and cleaned. After desorption of the phosphate, the paramagnetic particles can be reused.

Setups for magnetite recovery, recovery rates of phosphate and magnetite (magnetite recovery from manure) were investigated. Further a comparison was made for the phosphate analysis between an optical test kit and segmented flow analysis, carried out by an external laboratory. This work builds on work executed earlier by Bussmann and Wichers (KB-project *Fosfaten uit organische mest verwijderen* (KB 33-003-004)).

5.2 Materials and methods

5.2.1 Materials

Cow manure was obtained from a private contact. A mixture of solid manure and urine was acidified with two different acids: lactic acid and sulphuric acid (Table 5-1). The two different acids were selected to determine whether inorganic acids are required (H_2SO_4 is used in Denmark) or whether an organic acid, which can be produced from fermentation of manure fractions (Chapter 2) can be used.

Table 5-1 Preparation of acidified manure.

Preparation	Lactic acid	H_2SO_4
Manure (g)	1203	1201
Urine (g)	801	804
Acid added (mL)	49 (lactic acid)	11 (H_2SO_4)
pH_{end} (-)	4.55	4.64

The final mixture was centrifuged at 2395 rpm for 20 min and decanted. Both liquid acidified manure fractions were used in further experiments.

A photometric assay was used for phosphate quantification. "High Range" 0 – 50 ppm (mg/L), HANNA instruments, nr. H138061 (Nieuwegein, The Netherlands). Magnetite powder type "synthetic" was used for all experiments (Inoxia Ltd, Cranleigh, UK).

5.2.2 Magnetite recovery from acidified liquid digestate fraction

3 g of magnetite was added to 30 mL of liquid acidified manure in a 50 mL centrifugation tube and mixed on a roller bed for 30 min. The content was emptied in three manners:

a) Decantation after centrifugation
b) Decantation after sedimentation (30 min)
c) Decantation while subjected to magnetic field

Experiments a) and b) were conducted using only manure acidified with LA. Experiment c) was conducted with both LA and H_2SO_4. An attempt was made to leave the magnetite manure slurry in the centrifugation tube and decant it with the magnet, and also to discharge the slurry from the tube into an aluminium tub (commonly used for drying samples), which was laid on the flat surface of the magnet, providing a greater surface for the magnetite to sediment with the magnetic field. The magnet was switched on for 1min before decanting the liquid. The remaining solids were dried overnight and their mass determined. From that mass the recovery of magnetite was calculated.

5.2.3 Phosphate recovery from acidified liquid digestate fraction

3 g of magnetite were added to 30 mL of manure (both LA and H_2SO_4, both in duplicate) and mixed on a roller bed for 30 min. The content was emptied into a flat aluminium tray with a diameter of around 5 cm. The tray was placed on a magnet and after 1 min the liquid was decanted, while the magnet stayed turned on and in contact with the underside of the tray. The magnetite was resuspended in 30 mL demineralized water and the pH adjusted to 10, using NaOH. The resuspended magnetite was centrifuged for 10 min at 9.000 x *g* at 4°C. The pH was adjusted to pH 5 using HCl. All samples, the supernatants from decanting and the resuspended magnetite, were centrifuged for 30 min at 9.000 x *g* and 4°C. Then the supernatant of all samples were diluted by a factor 1:100 and the H_2PO_4 content was measured via photometric assay.

5.2.4 Phosphate quantification in acidified liquid digestate fraction

For the phosphate determination a calibration curve was made. Stock solution was 100 mL 5 mM KH_2PO_4. 0.06841 g was weighed, put into volumetric bottle and filled up to 100 mL with milli-Q water to make the stock solution. The stock solution was diluted with milli-Q water as detailed in Table 5-2.

Table 5-2 Calibration curve starting with stock solution of 5 mM KH_2PO_4.

Samples and dilution	Concentration (mM)	Adsorption at 710 nm
Stock	5.000	0.980
Stock 1:8	0.625	0.795
Stock 1:16	0.310	0.585
Stock 1:32	0.160	0.317
Stock 1:64	0.080	0.200
Milli-Q water	0.000	0.049

Manure was diluted in demineralised water as described in Table 5-3. All samples were centrifuged for 1 h at 9,000 ×g at 4°C after dilution. Only the supernatant was used for PO_4^{3-} measurement. A new calibration line was made for the phosphate measurements.
Phosphate in manure was quantified via two different methods and the results were compared. The first method was the photometric assay (PA), that was described and used before. The second method was via

sequential flow analysis (SFA), carried out through the laboratories of Soil Biology Group at WUR. This method detects the free orthophosphate after aqueous extraction. Analysed were the same samples as described in 5.2.3.

Along with the free orthophosphate, also total phosphorus content was measured after the samples were digested with H_2SO_4, Se and H_2O_2.

5.3 Results

5.3.1 Magnetite recovery from acidified liquid digestate fraction

Recovery of solid magnetite from liquid manure proved to be difficult. This in partly due to the nature of magnetite, which clings to plastic surfaces through electrostatic tension and is difficult to recover from the viscous liquid manure because of its small diameter. All figures show the average of duplicate experiments, the difference was generally in the range of 1%. Recovery of magnetite from water was 98% (Figure 5-1). Upon centrifugation of magnetite suspended in manure, followed by decanting, recoveries of 107% and 108% were recorded. This is explained with the sedimentation of residual solids in the manure, which were not washed off the magnetite. Sedimentation did not serve for separation. It was not possible to separate magnetite from manure via decanting after sedimentation, magnetite was lost during decanting (recovery 82% and 78%).

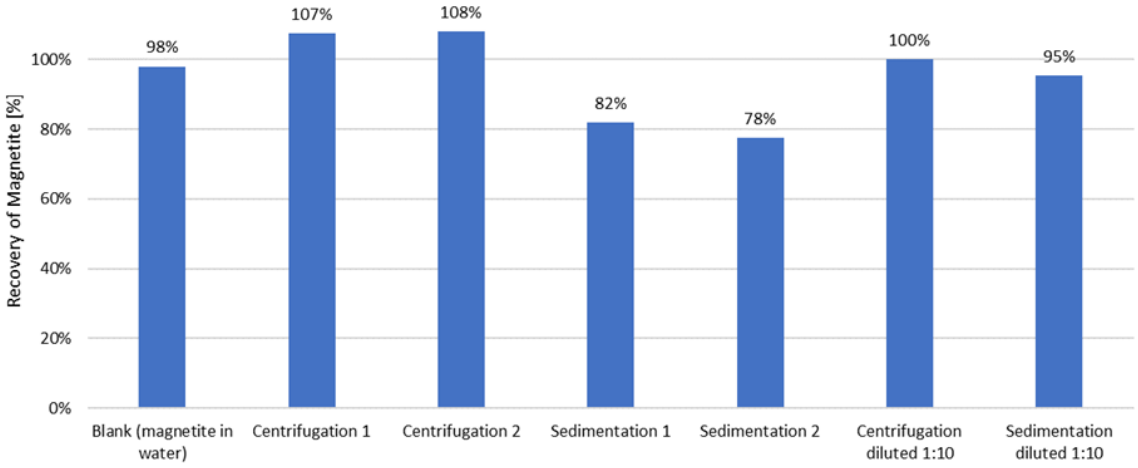


Figure 5-1 Recovery (%) of magnetite added to liquid fraction of acidified manure using centrifugation and sedimentation.

Once the manure was diluted 1:10, sedimentation and centrifugation led to recoveries of 100% and 95% respectively. This is most likely due to the reduced viscosity and better solid/liquid separation after dilution. However, also in these experiments, results were not clear as to what percentage of solids from the manure fraction were recovered with the magnetite. Magnetite itself was not quantified, only the weight after decanting and drying.

Next magnetite was recovered using a magnetic field to retain magnetite while decanting the liquid manure (Figure 5-2). The data shows that recovery in a falcon (centrifugation) tube is possible, but only if the manure is diluted. Using an aluminium tub, magnetite settles quickly. As in Figure 5-1 recoveries were above 100%, due to the solids remaining in the residual liquid manure.

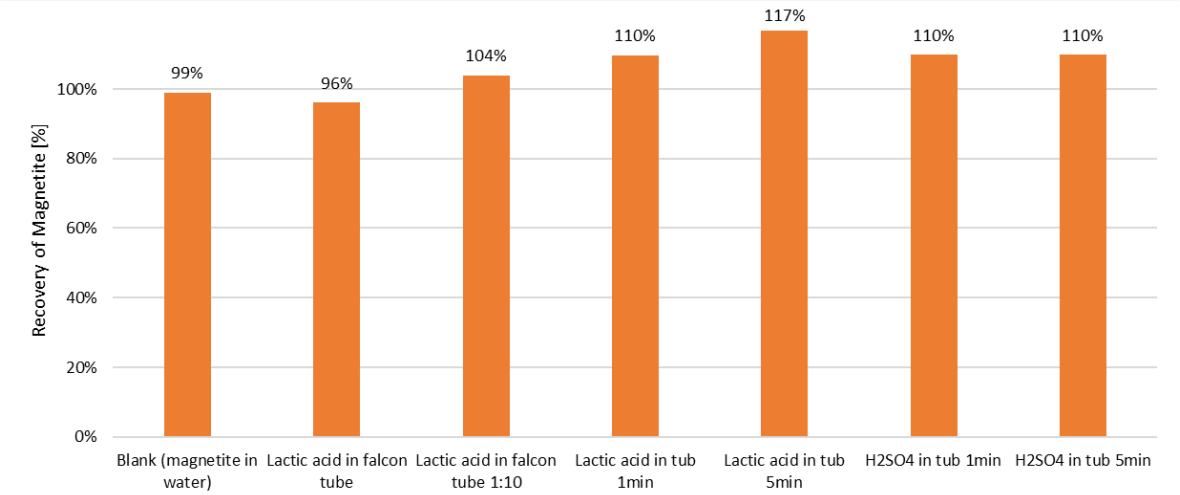


Figure 5-2 Recovery (%) of magnetite added to liquid fraction of acidified manure using magnetic field.

It is possible, that another technology, such as immobilization of magnetite, leads to lower magnetite loss. Magnetite recovery is critical for the economical design of the process and must be kept very high throughout.

5.3.2 Phosphate recovery from acidified liquid digestate fraction

Phosphate concentration was measured in supernatant, after magnetite was recovered via decanting over a magnetic field, and recovered phosphate after desorbing and resolubilizing from magnetite. The phosphate amounts (mmol) are given in Figure 5-3. Due to the rather large deviations between the duplicate measurements, results of both measurements are shown, detailed as 1 and 2.

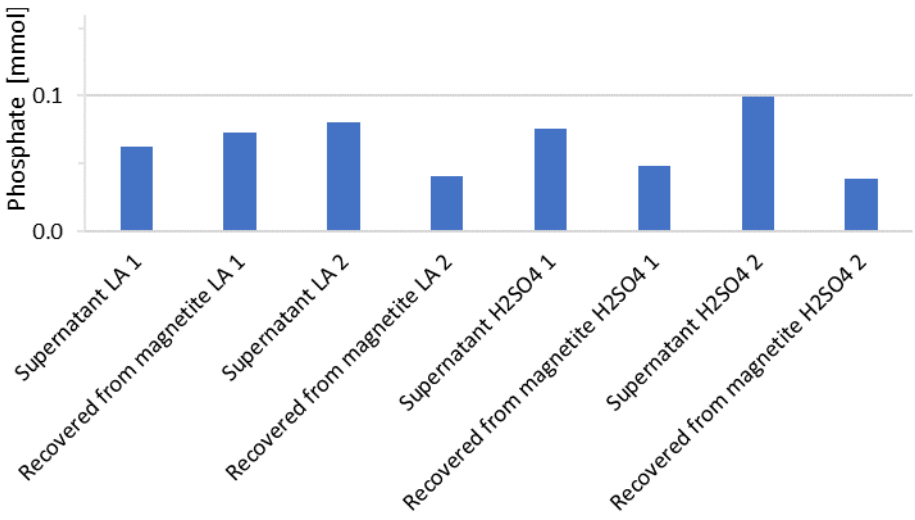


Figure 5-3 Phosphate in liquid phases from supernatant and recovered from magnetite.

While the deviation in distribution between solid and liquid phase between the experiments is rather large, in sum (supernatant and recovered), the four experiments had a small deviation of less than 6% (average 0.19 mmol ± 5.6%).

5.3.3 Phosphate concentration in acidified liquid digestate fraction

Liquid manure is turbid and viscous. It does not appear an ideal liquid to use in a photometric assay. While the photometric assay offers a quick tool to measure phosphate concentrations, a more accurate measurement can be achieved via sequential flow analysis. The measurements of phosphate concentration with the photometric assay were compared to the measured concentrations using sequential flow analysis (see Annex 4). In conclusion, the photometric assay can be used to measure phosphate concentration in the lab. It gives reasonably accurate results. It is much more practical than hiring external laboratories to execute more accurate analyses. A limiting factor of the photometric assay is its sensitivity to dilution and therefore the same dilution factor should be used throughout. The phosphate concentrations presented here were determined using the photometric assay.

After centrifugation, the supernatants were clearer, but not clear. A small pellet was observed. Concentration of phosphate was calculated based on slope and intercept from calibration line in Figure A4-2 and is shown in Table 5-3 and Figure 5-4.

Table 5-3 Manure samples for phosphate measurements, the dilution factor (DF) and the measured concentration multiplied with DF.

Samples and dilution	Adsorption at 710 nm	DF	Concentration (mM)
Lactic acid - 1:10	0.591	10	3.5
Lactic acid - 1:10	0.805	10	4.9
Lactic acid - 1:20	0.669	20	8.0
Lactic acid - 1:20	0.722	20	8.7
Lactic acid - 1:50	0.36	50	9.9
Lactic acid - 1:50	0.344	50	9.4
Lactic acid - 1:100	0.205	100	9.6
Lactic acid - 1:100	0.212	100	10.1
Sulphuric acid – 1:10	1.058	10	6.6
Sulphuric acid – 1:10	0.973	10	6.0
Sulphuric acid - 1:20	0.77	20	9.4
Sulphuric acid - 1:20	0.758	20	9.2
Sulphuric acid - 1:50	0.334	50	9.1
Sulphuric acid - 1:50	0.334	50	9.1
Sulphuric acid - 1:100	0.189	100	8.6
Sulphuric acid - 1:100	0.191	100	8.7

Phosphorus was measured coming from orthophosphate or total phosphorus and the results are shown in Figure 5-5. The data shows that the majority if phosphorus is present in manure in forms different than orthophosphate. The phosphate concentration in untreated manure (LA and H_2SO_4) appears about as large as the combined concentration in coupled samples (e.g. Supernatant LA1 + Recovered from magnetite LA1, etc). However, the total phosphorus concentration is much lower in both untreated manure samples, than in the combined coupled samples. An explanation for this has not been found.

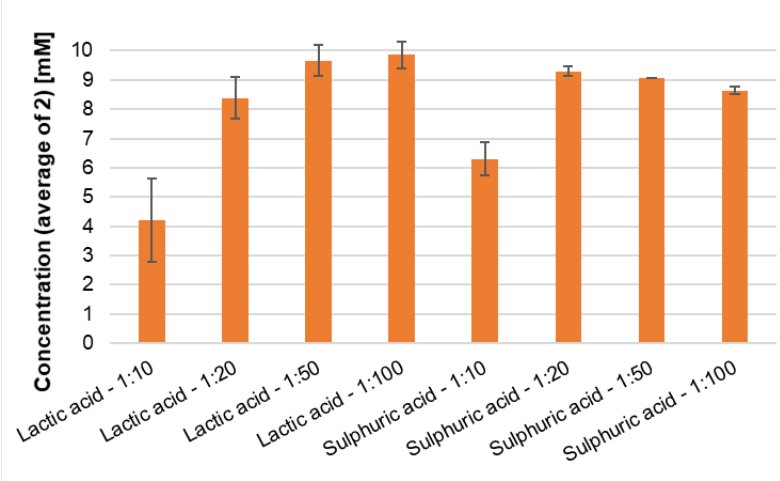


Figure 5-4 ***Average value of double measurements of phosphate concentration in different dilutions of manure. Error bar shows the difference between two measurements.***

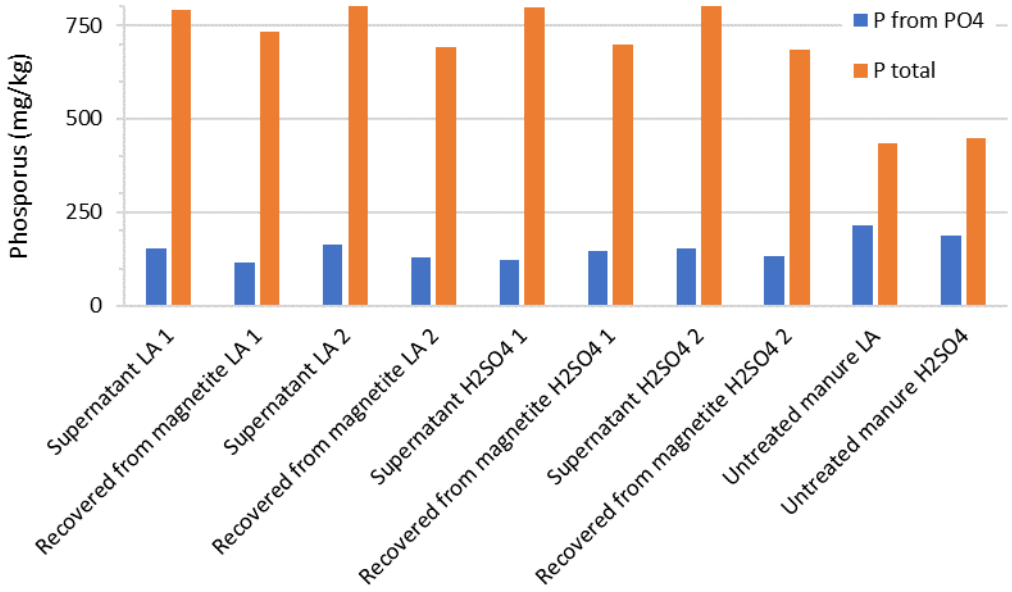


Figure 5-5 ***Comparison of phosphorus from orthophosphate and total phosphorus.***

5.4 Conclusions and recommendations

The data shows that phosphate can be recovered from liquid acidified manure using magnetite, which is recovered via decanting over a magnetic field. Handling magnetite and manure proves to be difficult, as the former acts like a powder influenced by electrostatic forces when dry and the latter is viscous and turbid. Assessing the exact recovery of magnetite from manure is important to develop an economically feasible process, if the loss of magnetite is too high, processing costs will likely be unfavourable. However, on small scale, magnetite loss due to electrostatic clinging to surfaces and solids in residual manure make accurate determination of masses difficult.

The phosphate concentration in manure can be determined using a photometric assay and appropriate dilutions. This is potentially time and cost saving, if the alternative involves external laboratories. However, the phosphate concentrations comparison does not offer an exact match. This may in part be due to inaccuracy of either method, difficult handling of manure and magnetite (i.e. pipetting, due to viscosity, is not always accurate) and errors in handling of the samples in the lab.
It is recommend to:

- Change to another type of magnetite with a smaller particle size distribution (= higher adsorption area) and of higher purity in order to increase adsorption capacity.
- Assess the amount of possible consecutive adsorption/desorption rounds before the level of adsorption drops off.
- Assess possible interaction/interference of "other" anions with the adsorption process (e.g. CO_3^{2-}, NO_3^-, etc.).

The phosphate concentrations measured in the liquid acidified manure were in the range of ten times higher than in the model solutions used in previous work. In order to apply previous findings, phosphate concentration in model solutions should be matched to concentrations found in manure. This will be important to asses process economics, as dilution of manure on large scale should most likely be avoided.

Further, it is recommended to investigate methods of handing magnetite, i.e. via immobilization on (porous) carrier materials. As shown in previous work, small particle size for magnetite is required to maintain large surfaces and thus large adsorption capacities. However, with the small size of magnetite (nanometre range), handling becomes increasingly difficult and improvements on laboratory an process scale should be investigated.

6 Production of ammonium sulphate from liquid digestate fraction

6.1 Introduction

One possible step in the treatment of manure is anaerobic digestion. This process results in a thick fraction and a liquid fraction, containing most of the ammonium and potassium. Aim of the activities described in this chapter is the recovery and concentration from the liquid fraction of both potassium and ammonium. One of the options to recover and separate ammonium and potassium is using transmembrane chemosorption (TMCS). The principle of TMCS is shown in Figure 6-1.

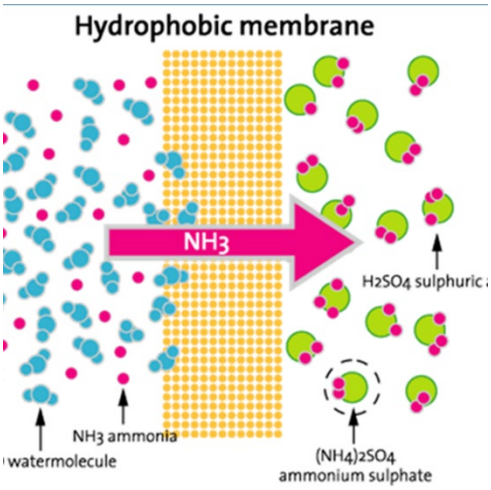


Figure 6-1 Principle of transmembrane chemosorption (TMCS).

The liquid digestate fraction is fed to a membrane which acts as a contactor between two phases: an aqueous feed phase containing ammonium and potassium and an aqueous acid phase (sulphuric acid or nitric acid). The membrane is made from a hydrophobic material and only water vapour and ammonium vapor can pass the membrane. Liquid water is rejected. The pH and temperature at the feed side have to be adjusted in such a way (higher pH and/or higher temperature) that the ammonium is present in the volatile NH_3 form (ammonia). Ammonia will pass the membrane to the acid solution where it is converted to an ammonium salt $(NH_4)_2SO_4$ or NH_4NO_3. Due to this conversion the driving force for NH_3 vapour transport will remain maximal and the ammonium will be removed from the feed liquid and concentrated at the other side of the membrane as an ammonium salt. At the meantime, potassium will remain in the feed liquid and is thus separated from ammonium salt and can be further concentrated by e.g. reverse osmosis. An option is to do the concentration with reverse osmosis before the TMCS process instead of after the TMCS process. Two options for RO concentration before TMCS have been explored.

The activities were focused on the ammonium recovery step. Based on available mass transfer parameters for the NH_3 transport and NH_3/NH_4^+ equilibrium data, the required membrane surface area has been calculated, depending on degree of ammonium recovery, pH and temperature. A preliminary economic evaluation (not optimized) has been made for the TMCS process for a 75% removal of ammonium from the liquid digestate fraction. Moreover, experimental work has been executed to investigate the effect of pretreatment of the liquid digestate fraction on wetting of hydrophobic membranes because that will affect the performance of the membranes.

6.2 Materials and methods

6.2.1 Effect of pretreatment of manure digestate on TMCS

The aim was to elucidate what factors have a significant impact on the wettability of the membranes, e.g., fouling or surface tension of the feed solution. In this project only the surface tension of the liquid was considered. A reduced surface tension will lead to unwanted wetting of the membranes at a lower feed pressure. Different pretreatment methods were applied to determine the effect of pretreatment of the digestate on the surface tension.

Pretreatment using membranes was carried out in the dead-end mode using a high-pressure stirred cell that can hold up to 300 mL of liquid (Figure 6-2). The characteristics of the MF and UF membranes that were tested are summarize in Table 6-1.

Figure 6-2 The Sterlitech HP4750X stirred cell. Active membrane area 14.6 cm^2.

Table 6-1 Main characteristics of the MF and UF membranes that were tested.

Method	Membrane type	Material	Characteristics
Microfiltration	Alfa Laval -FSM0, 15pp	Fluoro polymer	Pore size: 0.15µm
Ultrafiltration	Nadir UF-PES-050H	PES	MWCO* (nominal): 50 kDa

*MWCO: molecular weight cut-off

Pretreatment using electrocoagulation (EC) was performed in batch-mode under galvanostatic conditions. The system comprised a pair of aluminium electrodes (6.0 cm x 7.5 cm), and a glass vessel filled up with the liquid digestate fraction.

The surface tension of the liquid digestate fraction, the permeate solutions of the MF and UF process, as well as the solution obtained after coagulation were measured using a force surface tensiometer Sigma 700 based on the Du Noüy ring method (Figure 6-3).

Figure 6-3 Sigma 700 Surface tension equipment.

6.2.2 Preconcentration with reverse osmosis

An option is to concentrate the liquid digestate fraction with RO before applying TMCS. Preliminary concentration experiments with the liquid fraction of pig manure digestate were executed at two different pH conditions. The experiments were executed in an AlfaLaval labstak M20 unit (Figure 6-4), in which flat RO membranes were mounted. The type of RO membrane used was Filmtec BW30XLE.

Figure 6-4 AlfaLaval Labstak M20.

6.2.3 Calculation of required membrane surface area and cost evaluation

The required membrane area for ammonia removal with TMCS is depending on the following parameters:

- % N-removal
- pH
- Temperature
- Flux (depending on mass transfer)

Based on previously determined experimental parameters (mass transfer coefficients) and theoretical equilibrium data, the membrane area was calculated for different conditions regarding % N-removal, pH and temperature. The costs were based on a previous case study carried out by the technology supplier in the TKI-project *Meerwaarde Mest & Mineralen*.

6.3 Results

6.3.1 Effect of pretreatment of manure digestate on TMCS

An overview of the experiments is given in Table 6-2. Cow digestate that was used was pre-filtered over a nylon filter in experiment 3, 4 and 5.

Table 6-2 Overview pretreatment experiments.

Exp.	Digestate	Membrane	Conditions	Filtrate (g)
1	Cow digestate	MF Alfa Laval-FMSO	1.5 bar, 29 h	12.7 (brown)
2	Cow digestate	UF UF-PES-05OH	2.5 bar, 64 h	18.3 (brown)
3	Pre-Filtered Cow digestate	MF Alfa Laval-FMSO	1.5 bar, 25 h	12.2 (brown)
4	Pre-Filtered Cow digestate	UF UF-PES-05OH	2.5 bar, 20 h	11.8 (brown)
5	Pre-Filtered Cow digestate	RO- SW30XLE	40 bar, 3.5 h	31.1 (clear)

The results from Table 6-3 shows that UF/MF filtration in the stirred Sterlitech cell is very hard. Fluxes are very low and too low for practical applications. Even prefiltration of the cow digestate over a nylon filter does not make difference for the MF/UF filtration performance. Filtration of the prefiltered cow digestate in a RO filtration experiment gives a much better flux performance. This indicates that there is plugging of the pores in the UF/MF filtration experiment, which will not occur in the RO experiment with much denser membranes (total rejection of components, so not suitable for pretreatment).

In Table 6-4 the results of the surface tension of the different samples is shown. The higher the surface tension, the better the feed is for treatment with TMCS. As shown in the table, the surface tension of water is the highest, followed by the permeate of the RO experiment, which is expected because of the high retention for all the components in the digestate. The rest of the results are not quite in line with the expectations. It was expected that by membrane filtration a part of the organic components should be rejected resulting in a higher surface tension of the permeate (filtrate). This is not observed in these results. The untreated cow digestate shows about the same surface tension as treated fractions. Also, the permeate of the UF does not show a higher surface tension than the permeate of the MF. It seems that MF/UF pretreatment is not useful to improve the surface tension of the digestate.

Table 6-3 Surface tension of digestate with and without pretreatment.

Sample	Surface tension (mN/m)	Description
H2O1	72.7	Blank water
Dig-fltr1	54.8	Cow digestate pre-filtered
Dig-Centri1	50.1	Cow digestate pre-filtered & centrifugated
FSMO250621	50.1	Pre-filtered cow digestate after filtration over FSMO (MF) membrane
PES28/6/21	52.5	Pre-filtered cow digestate after filtration over PES (UF) membrane
FSMO290621	62.7	Pre-filtered & centrifugated cow digestate after filtration over FSMO membrane
PES300621	55.6	Pre-filtered & centrifugated cow digestate after filtration over PES membrane
SW30XLE	71.9	Pre-filtered & centrifugated cow digestate after filtration over SW30XLE (RO) membrane

EC experiments (Electro Coagulation) were executed with pig digestate instead of cow digestate. Several conditions were tested, i.e., applied current, electrode separation, time, and electrode-digestate volume ratio. The separation of the electrodes had a bigger effect on the surface tension than the other variables. The results are shown in Figure 6-5.

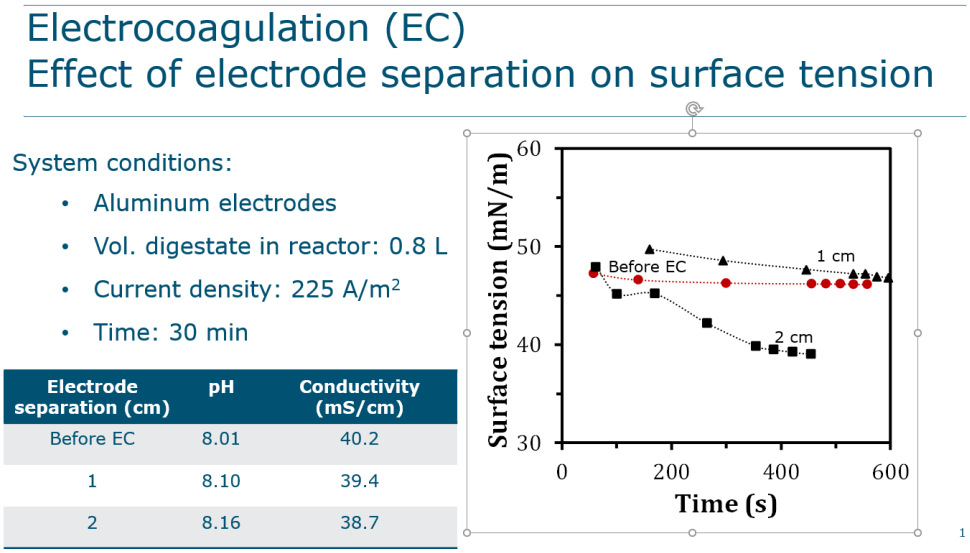


Figure 6-5 Effect of EC treatment on the surface tension of pig digestate.

The EC results in Figure 6-5 show that EC treatment has a negative effect on the surface tension instead of a positive effect. EC will introduce metal ions in the digestate which apparently have a negative effect on the surface tension and the longer the experiment runs, the more ions will be released. The surface tension

appears to be lower than in the membrane pretreatment experiments, but this can be explained by the fact that cow digestate is used in the membrane pretreatment, while pig manure digestate is used for the electrocoagulation experiments.

6.3.2 Preconcentration with reverse osmosis

Two experiments were performed at different pH. The results are given in Table 6-4. These results show that the concentration factor based on electrical conductivity (on salt concentration) is effected by the pH. This is to be expected, because at pH of 9 (experiment 1), the ammonia is largely in the NH_3 form and will pass the RO membrane, resulting in more salt loss. At pH 6 (experiment 2) most of the ammonia is in the NH_4^+ form and is rejected by the RO membrane, so that hardly any loss of salts occurs during the RO concentration.

Table 6-4 Results of two RO experiments to preconcentrate the liquid manure fraction.

	Experiment 1	Experiment 2
pH pig manure digestate (-)	9.05	6*
Starting EC (mS/cm)	32.2	32.2
Pressure (average) (bar)	50	50
Concentration factor based on volume reduction (-)	3.7	3.3
EC concentrate (mS/cm)	77.7	103.2
EC permeate (mS/cm)	4.2	2.4
Concentration factor based on EC (-)	2.4	3.2

* Acidification with HCl

It can be concluded that preconcentration before TMCS is only useful if the pH is adjusted to a lower pH, otherwise there will be loss of ammonia. This, of course, this incurs additional costs for pH adjustment and an optimization must be performed to determine whether concentrating before TMCS is justified.

6.3.3 Calculation of required membrane surface area and cost evaluation

Figure 6-6 shows how pH and temperature have an effect on the ammonium/ammonia (NH_4^+/NH_3) equilibrium. The more nitrogen in the NH_3 form, the better for N-removal by TMCS. Figure 6-7 shows the effect on the required membrane area (in this case at 75% N-removal and a feed solution of 1 m^3/h and a NH_3 concentration of 2500 mg/l NH_3). The effect of percentage N-removal is shown in Figure 6-8.

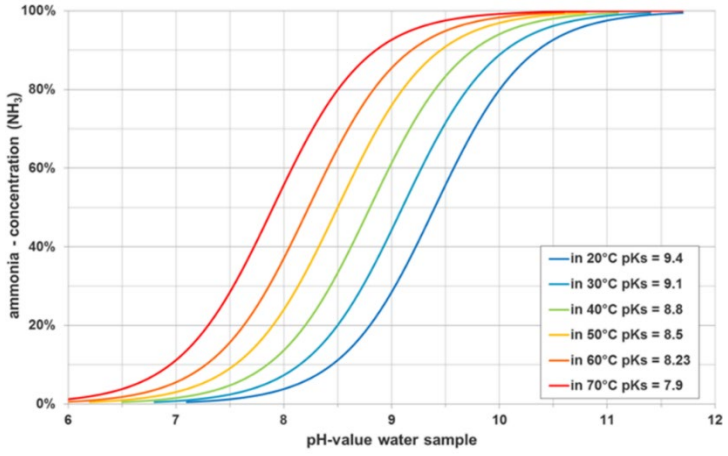


Figure 6-6 Ammonium/ammonia (NH_4^+/NH_3) equilibrium as function of pH and temperature.

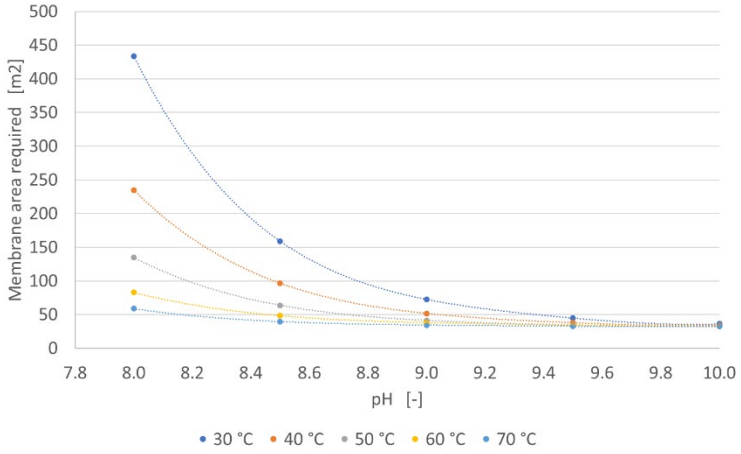


Figure 6-7 Membrane area required for 75% N-removal as function of pH and temperature (1 m^3/h, 2500 mg/L NH_3).

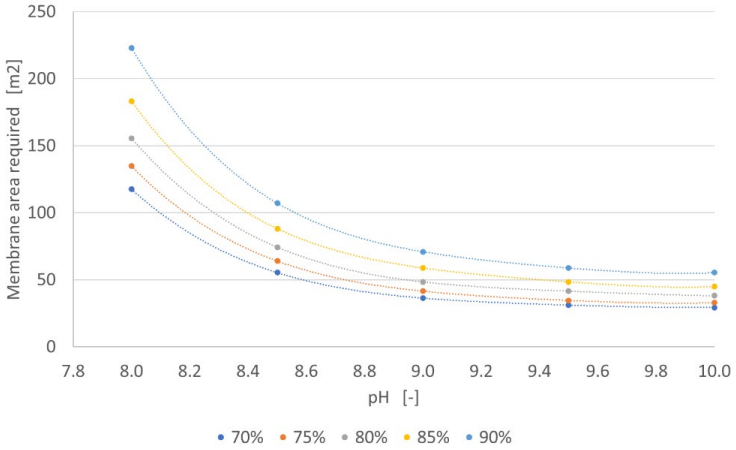


Figure 6-8 Membrane area as function of % N removal and pH (50°C, 1 m^3/h, 2500 mg/L NH_3).

In a previous TKI-project *Meerwaarde Mest & Mineralen*, an economic evaluation was made by the technology supplier Bluetech (see Annex 5 for details). These data were used as base case for the cost estimation.

Base case

For a base case with the following data, a preliminary cost evaluation for TMCS was executed:

- Feed stream: 15 m^3/h
- Ammonia feed concentration: 4500 mg/L
- T = 30°C
- 75% ammonium removal
- Absorption liquid: H_2SO_4
- Product: 25 wt.% ammonium sulphate

The estimated membrane area is 480 m^2, resulting in an estimated CAPEX of € 580,000.

Estimated OPEX for the base case (€ 1.211,500/year)

- Energy: € 7900/year
- Membrane replacement: € 35,100/year (every 4 years replacement)
- Sodium hydroxide: € 616,600/year
- Sulfuric acid: € 517,400/year
- Depreciation and interest: € 34,500/year

Value produced ammonium sulphate: € 334,500/year. The value of the potassium stream was not taken into account.

Scaling to other capacities

Assuming that the costs for control are ca. 8% of the investment costs, these amount to € 50,000. These costs remain constant independent from capacity/membrane area. For the calculation of the CAPEX of another required membrane area, the following assumption was made:

CAPEX(Area) = $(Area/480)^{0,8}$ x 530,000 + 50,000

For OPEX the following scaling rules were used:

- Energy: (capacity/15) x € 7900
- Membrane replacement: (Area/480) x € 35,100
- Sodium hydroxide: these costs are made to increase the pH at the feed side to produce NH_3 and are related to the amount of N and the buffering capacity of carbonate present. It is assumed that the required amount is only related to the amount of N present:
  (capacity x mg/L ammonia in feed)/(15 x 4500) x € 616,600
- Sulfuric acid: (capacity x mg/L ammonia in feed x fraction removal)/(15 x 4500 x 0.75) x € 517,400
- Depreciation and interest: CAPEX(Area)/€ 580,000 x € 34,500

Value produced ammonium sulphate:
(capacity x mg/L ammonia in feed x fraction removal)/(15 x 4500 x 0.75) x € 334,500

Case at the farm

For TMCS at farm level we used the following data:

- Feed stream: 1 m^3/h
- Ammonia feed concentration: 2500 mg/L
- T = 30°C
- 75% ammonium removal
- Absorption liquid: H_2SO_4
- Product: 25 wt.% ammonium sulphate

For a feed flow of 1 m^3/h, the required membrane area is which is ~30-35 m^2. This corresponds with a pH of around 10 (Figure 6-7 and Figure 6-8).
CAPEX = $(36/480)^{0.8}$ x 530,000 + 50,000 = € 116,730.

OPEX (€/year):

- Energy: 1/15 x 7900 = € 530
- Membrane replacement: 36/480 x 35,100 = € 2630
- Sodium hydroxide: (1 x 2500)/(15 x 4500) x 616,600 = € 22,840
- Sulfuric acid: (1 x 2500 x 0.75)/(15 x 4500 x 0.75) x 517,400 = € 19,160
- Depreciation and interest: 116,730/580,000 x 34,500 = € 6,940

OPEX: € 52,100

Value produced ammonium sulphate:
(1 x 2500 x 0.75)/(15 x 4500 x 0.75) x 334,500 = € 12,390.

Total yearly net costs: € 39,710. If a full continuous process is assumed (8000 h/y), the cost per m^3 of treated digestate is € 4.96.

One of the major costs is the cost of chemicals. The costs of NaOH can be reduced by increasing the temperature of the feed (Figure 6-7) and decreasing the pH to 8-8.5. This means additional costs for heating and extra membrane surface. For heating, (waste) heat from the digester might be used. Doubling the membrane surface area will mean that the investment cost will increase to €166,180 and the yearly depreciation and interest costs will increase to € 9,890. This is worthwhile if the use of NaOH can be reduced substantially.

6.4 Conclusions and recommendations

Aim of the research was to investigate the possibilities and options for ammonia recovery with Trans membrane chemo sorption (TMCS). Different issues concerning TMCS have been studied.

1. Effect of pretreatment on surface tension of liquid phase manure digestate. Increasing the surface tension will prevent wetting of the hydrophobic TMCS membranes under pressure conditions. Pretreatment with MF/UF membranes do not show changes in the surface tension of the digestate. Electrocoagulation of the digestate leads to an unwanted decrease in surface tension.
2. Preconcentration of the liquid phase digestate with RO before applying TMCS. RO experiments on native pig manure digestate show that a volume concentration factor of at least 3 is possible, but that there is loss of ammonia due to the hight pH (mainly NH_3 form). By decreasing the pH to around 6, most of the ammonia is in the NH_4^+ form and will be rejected by the RO membrane. Than both a volume and salt concentration of at least a factor 3 (with pig manure) are possible.
3. Required membrane surface area for TMCS process. The required membrane area depends to a large extend on the driving force for ammonia removal, being the vapor pressure of ammonia in the feed, and the required N-removal. The ammonia pressure can be influenced by concentration, temperature and pH. Both temperature and pH have an effect on the NH_3-NH_4^+ equilibrium, effecting the concentration NH_3 and thus the driving force and required membrane area.
4. Economics of the TMCS process. The economics of the process are mainly determined by the required membrane area, the chemicals needed (caustic and acid) and the market potential of the product produced (ammonium salt). This requires a optimization of the total process in which pretreatment, preconcentration and the TMCS process itself are included, because in all the steps heat and/or chemicals are required. For example, the pH in TMCS can be reduced (less caustic costs) by increasing the temperature (more heat costs) or the opposite way. Is it worthwhile preconcentrating, while extra acid is required?

Considering these conclusions it is recommendable to execute this optimization study as a next step to determine the feasibility of the TMCS process.

7 Conclusions and recommendations

Reducing methane losses at farms by manure acidification
In laboratory experiments chemical and biological acidification both strongly reduced methane emissions from fresh cow manure. No additional fermentative microorganisms have to be added for biological acidification because enough endogenous microorganisms are present to produce sufficient lactic acid to reduce the pH to a level (< 5) that inhibits methane production. Lab experiments showed that the minimum amount of fermentable carbohydrate required for biological acidification is 5-10%. The agricultural side streams molasses showed a significant effect on acidification, reduction in biogas volume and methane and CO_2 emissions.

Daily addition of molasses as a carbon source to manure in a cellar of a cow shed (ratio of 7%) proved to reduce the pH from 8 to 5.8 after 21 days, while still declining. Adding molasses is still expensive and is not expected to offer an affordable solution to the emission problem. A closer look at the biology of the manure processing chain showed that the addition of biologically formed acetic acid elsewhere produced, can be a cheaper and workable alternative. In a series of real life experiments this option was explored. The drop in pH was smaller than expected however and did not reach the level for stopping methanogenesis. It was concluded that this can be attributed to poor mixing and that measures are needed to improve this mixing issue. Such a follow-up experiment is described in the MEZEM-proposal (Annex 6).

Oxidizing recalcitrant solid digestate fraction by hydrogen peroxide
The use of Fenton reagent (hydrogen peroxide and iron ions) can make the recalcitrant fibres from cow manure digestate more digestible in biogas plants, resulting in a substantial faster biogas production and higher biogas yield. However, under the conditions used, the value of the additional biogas produced is lower than the costs of the hydrogen peroxide added. To improve the economics of the process it is recommended to investigate the possibilities to reduce the costs of the Fenton treatment (lowering hydrogen peroxide dose, shorter incubation time, in-situ hydrogen peroxide production) and how to obtain a more potent consortium of bacteria.

Conversion of VFA present in liquid manure fraction in PHA
It is possible to increase the VFA content in the liquid manure fraction by hydrolysis and acidogenesis of the solid fraction. The highest OLR tested (50.2 g VS/kg) yielded 4.6 g VFA/L or 0.14 mg VFA/mg VS after 14 days. Even higher VFA production can probably be reached at higher ORL (limited by mixing) and longer processing time. We did not succeed in producing PHA from VFA in manure in a feast/famine setup. This was due to (i) unfavourable composition (low C:N) and (ii) thickness of manure (need for dilution). PHA was recovered from PHA-enriched waste water sludge using 0.6 g SDS/g non-PHA (yield >90%, purity >40%). The need for a mechanical pretreatment is not conclusive. Possibilities to reduce the SDS dose are alkaline treatment and counter-current extraction process.

Biologically produced PHAs are stored by the microorganism as granules, which, if harvested as such, have properties that are extremely suitable for use in coatings. We showed that in principle it is possible to produce, release and recover functional PHA in its native state (granular) from organisms that are fed on manure for this application. However, manure does not appear to be the most suitable green raw material to continue working with. In view of the above it is recommended to stop the research and development of the PHA production process from manure.

Phosphate recovery from acidified liquid digestate fraction
Phosphate can be recovered from liquid acidified manure using magnetite. To develop an economically feasible process it is important to achieve a high magnetite recovery from manure. However, on small scale, magnetite loss due to electrostatic clinging to surfaces and solids in residual manure make accurate determination of masses difficult. The principle of phosphate recovery using magnetite will be further developed in the TKI project *Recovery and valorization of phosphorus compounds from wastewater streams using Magnetic Adsorption-Desorption (MAD)*. Application of this method to wastewater is expected to be

more promising in view of the magnetic separation of magnetite. Furthermore, the combination with electrodialysis minimises the use of chemicals which are required for the regeneration of magnetite.

Production of ammonium sulphate from liquid digestate fraction

An optimization of the total process in which pretreatment, preconcentration and the TMCS process itself are included is required, because (i) in all steps heat and/or chemicals are used and (ii) all steps influence the required membrane area. Experimental work showed that pretreatment with MF/UF membranes and electrocoagulation did not lead to an increase of the surface tenson of the liquid pig digestate fraction, which would be beneficial to prevent wetting of the TMCS membrane. Experiments showed that preconcentration of the liquid phase digestate with RO before applying TMCS was possible (factor 3 was obtained with liquid pig digestate fraction), the pH should be around 6 to prevent ammonia loss.

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Annex 1 Reducing methane losses at farms by acidification of manure

Table A1-1 First acidification experiment - Lactic acid production after 23 days of biological acidification of cow manure.

Condition	Lactic acid (g/L)	End pH
No additions	-	7
10% glucose	35.5	4-5
0.5% Sauerkraut + 10% glucose	32.3	
5.0% Sauerkraut + 10% glucose	30.0	4-5
0.5% LAB + 10% glucose	35.4	
5.0% LAB + 10% glucose	31.2	4-5

Table A1-2 Second acidification experiment - Lactic acid production after 21 days of biological acidification of cow manure.

Condition	Lactic acid (g/L)	End pH
No additions	-	6.7
10% glucose	53	4.4
5% glucose	64	4.4
1% glucose	-	6.2
10% starch	-	5.1
5% starch	-	5.6
1% starch	0.14	5.9
10% molasses	53	4.7
5% molasses	-	5.8
1% molasses	-	6.3
10% grey starch	-	6.2
5% grey starch	-	6.3
15% grey starch	-	6.6

Cosun Beet COMPANY

PRODUCT SPECIFICATION

Molasses

Article number	753039
Version number	3
Version date	1-1-2020
Date of edition	3-3-2021

This product complies with the EU General Food Law, Dutch Commodities Act and Codex Alimentarius.

Product description
A syrup obtained from the production of sugar

Composition
Molasses

Recommended labelling
Molasses

Country of origin
The Netherlands

Specifications

1. Organoleptic characteristics

	Value	Analytical method
Consistency	viscous	Visual
Form	liquid	Visual
Colour	dark brown	Visual
Odour	natural to molasses	Organoleptic test
Taste	bitter, sweet	Organoleptic test

2. Chemical/physical norms

	Measuring unit		Value	Analytical method
Brix	° :		80.0 - 81.0	Refractometric, ICUMSA GS4/3/8-13
Saccharose	% :	indicative	37 - 47	ICUMSA GS4/3-9 (followed by calculation)
Total sugar	% :		55 - 57	ICUMSA GS4/3-9
Reducing sugar	% :	indicative	10 -20	Luff Schoorl before inversion
Ash content	% on dry matter :	max.	8 - 11	Sulphated ash ICUMSA GS3/4/7/8-11
Sulphur dioxyde	mg/kg :	max.	10	Monier Williams, AOAC 962.16, HPIC
Specific gravity	kg/l :	ca.	1.44	ICUMSA SPS-4

3. Microbiological norms

	Measuring unit		Value	Analytical method
Mesophilic bacteria	cfu/10g :	max.	100000	ICUMSA GS2/3-41
Yeasts	cfu/10g :	max.	100	ICUMSA GS2/3-47
Moulds	cfu/10g :	max.	100	ICUMSA GS2/3-47
Salmonella	cfu/25g :		Absent	NEN-EN-ISO 6579

4. Contaminants

Complies to Regulation (EC) No 396/2005 (maximum residue levels of pesticides), Regulation (EC) No 1881/2006 (maximum levels for certain contaminants in foodstuffs), Dutch Commodities Act and Codex Alimentarius.

Cosun Beet COMPANY

PRODUCT SPECIFICATION

Molasses

Article number	753039
Version number	3
Version date	1-1-2020
Date of edition	3-3-2021

Nutritional value (per 100 gram)	Measuring unit		Value	Method
Energy	kJ/kcal :	ca.	1088/256	Calculated
Fat	g :		0	
Saturated	g :		0	
Carbohydrates	g :		56	
Sugars	g :		56	
Protein	g :		8	
Salt	g :	max.	1.5	

Allergen information

The product contains no allergens as mentioned in Regulation (EU) No 1169/2011 (Annex II).

Shelf life

After production, under the below-mentioned storage conditions, the shelf life is at least 3 years.

Storage conditions	Measuring unit		Value
Relative humidity	% :		N.A.
Temperature	°C :	ca.	20

Do not store at a temperature > 40 ºC.

The product must be stored in a closed and clean container, drum, pail or tank.; Condensation should be avoided.
We recommend to use the 'first in, first out' stock management principle.

Packaging

Container 1.400 kg
Integrated plastic pallet 100 x 120 cm

Legislation & Statements

Non-GMO

Only raw materials obtained from traditionally propagated plant species are used to produce this sugar. During the production no use is made of processing aids produced by means of genetic modification. According to Regulation (EC) No. 1829/2003 and Regulation (EC) No. 1830/2003 no GMO labelling is required.

Ionisation

During the production no ionising radiation as described in Directive 1992/2/EG is applied to the product.

Food contact materials

The materials in contact with the product are in compliance with the relevant food contact materials legislation; Regulation (EC) No 1935/2004, Regulation (EC) No 10/2011 (in case of plastic materials) and Regulation (EC) No 2023/2006.

Certifications

BRC Global Standard for Food Safety
ISO 22000 Food Safety Standard
Kosher
Halal

Other information

Suitable for vegetarians and vegans

Annex 2 Oxidizing recalcitrant fractions by peroxide

Pretreatment of lignocellulosic material using the Fenton reaction - Literature search
After anaerobic digestion (biogas production) not all organic compounds from manure, co-substrates and source separated organics (SSO, Dutch: GFT) are converted. Important recalcitrant fractions are lignocellulose and to a lesser extent lignin and humic acids. In SSO it may be plastics. The lignocellulosic fibres are inaccessible to hydrolytic enzymes. By breaking the lignocellulosic complex the material can become accessible and as a consequence digestible. The treated material can be returned to the digester and yet converted into biogas. Such lignocellulose pre-treatment can be carried out using high temperatures, acids, alkali, mechanical forces or oxidative chemicals/enzymes. In this project the route using oxidative chemicals is explored, more specifically H_2O_2 produced in situ. Only H_2O_2 will have little effect on lignocellulose. The presence of a transition metal greatly improves the oxidative power and the most well-known is the combination of H_2O_2 and Fe^{2+} which can carry out the Fenton reaction, which generates various types of radicals. These radicals react with lignocellulose and breaks the complex.

Michalska *et al.* (2012) used grass type of biomass and carried out the Fenton reaction using 50 g biomass DM, 1 g Fe^{2+} and 25 g H_2O_2 per L water. The reaction time was 2 h. The untreated material could not be hydrolysed after addition of hydrolytic enzymes (e.g. cellulase) while the treated material showed a glucose yield of 17-22% (mono-glucose/initial glucan) upon enzymatic hydrolysis. Treated and untreated biomass were subjected to anaerobic digestion (biogas production). The untreated material did not produce any biogas while the treated biomass produced more: 25 L biogas per kg biomass DM (which still is not much).

Bhange *et al.* (2015) used garden biomass and experimented with various concentrations of Fe^{2+} and H_2O_2. The authors found that 250 ppm Fe^{2+} in combination with 10,000 ppm H_2O_2 in a suspension of garden biomass caused a high cellulose degradation (without enzymes). The reaction was carried out at 30°C.

Jung *et al.* (2015) used rice straw and Fenton. Enzyme cocktails (cellulase) were used to test the material. A treatment using 0.03 M $FeCl_3$ and 2.25 M H_2O_2 in a suspension with 10% biomass DM, 25°C, 24 h, worked best. After treatment 93% of the glucans could be converted into glucose. Interestingly, double amounts of $FeCl_3$ and H_2O_2 yielded only 88% glucose. Shorter reaction times (4 h) yielded slightly lower amounts of glucose (73%).

Li *et al.* (2016) used a two-step pretreatment of corn stover. First an acid hydrolysis to degrade hemicellulose and to recover xylose, second a Fenton reaction to disconnect lignin and cellulose. The aim was to make cellulose accessible to enzymes. The Fenton reaction was carried out using 1 g $FeSO_4 \cdot 7H_2O$ and 30 g H_2O_2 per L at 20°C. The reaction time was 12 h. 71% of glucose could be recovered as monosaccharide.

Kato *et al.* (2014) mixed 10 g biomass DM with 100 mL solution containing 250 mg $FeCl_2 \cdot 4H_2O$ and 100 mL 6% H_2O_2 solution. According to Kato *et al.* (2014) 1.25 mmol Fe^{2+} per 10 g biomass DM was optimal. Higher or lower amounts were less effective. A reaction time of 120 h was required to get 5 times higher glucose production after addition of enzymes. Unfortunately, no absolute values for glucose yield are given in this paper. After a Fenton reaction using a suspension of 5% Miscanthus DM the TOC values of the filtrate (no enzymes used) increased from 117 to 1922 mg/L. This is still low, the question is to what extent the remaining solids can be digested. The authors tried to investigate that question by microbial fermentation (clostridia, no methane bacteria) of grasses that were treated using the Fenton reaction. Three times more gas (carbon dioxide and hydrogen (?)) was produced in treated samples. Again, unfortunately, no absolute values were given.

Oxidation of lignocellulose can also be carried out using manganese. Takagi *et al.* (1987) tested various transition metals in combination with H_2O_2. Manganese was the best. Rice straw, MSW and newspaper were treated overnight at room temperature using $MnSO_4$ and H_2O_2 in a mol/mol ratio of 1:100. In suspensions of 20% newspaper the presence of 2-4% H_2O_2 was sufficient. The addition of manganese improved

monosaccharide production after enzymatic hydrolysis 1.5 times. Not really dramatic. Monosaccharide production was not at all impressive in this research work.

Hydrogen peroxide can also be used in combination with alkali to pre-treat lignocellulose. Su *et al.* (2015) carried out a pulping of corncobs at 50°C using 2% H_2O_2 and sufficient NaOH to create a pH of 11.5. Hemicellulose and lignin dissolved. The residue (cellulose) was left as a solid and could be enzymatically hydrolysed easily (80% glucose yield). This method looks very much like soda pulping. Due to the high pH and large amounts of alkali used this is not interesting to pre-treat digestate.

These papers give a first impression of the concentrations Fe and H_2O_2 used. However, the effect varies and is sometimes disappointing. More successful examples are required to create more confidence and to learn some more tricks. A paper that reports H_2O_2 use with and without Fe (and observe the difference) would be welcome. More information on Fenton is available in the field of sludge digestion.

In-situ peroxide generation and oxidation

H_2O_2 has been used to reduce organics (BOD, COD, pesticides and pathogens) of wastewaters for many years. The use of hydrogen peroxide in combination with UV light is known as advanced oxidation process (AOP) and is commercial available. In existing waste water treatment plants the hydrogen peroxide is transported in bulk from chemical plants. The introduction of the on-site production of hydrogen peroxide could solve the issue of:

- The low stability of oxidation chemicals, which cannot be stored for longer periods;
- Restrictions on the storage of large quantities of oxidation chemicals resulting from the hazard potential of concentrated substances.

WUR has developed an efficient process for the electrochemical production of the H_2O_2 from air (oxygen). Oxygen is reduced electrochemically to H_2O_2 in a multi-compartment electrolyser, thereby obtaining H_2O_2 concentrations up to 10%, which is much higher than commonly used in AOP (ppm levels). Compared to the industrial process no hazardous compounds such as hydrogen and organic solvents are required. An experimental setup is available at WFBR (Figure A2-1).

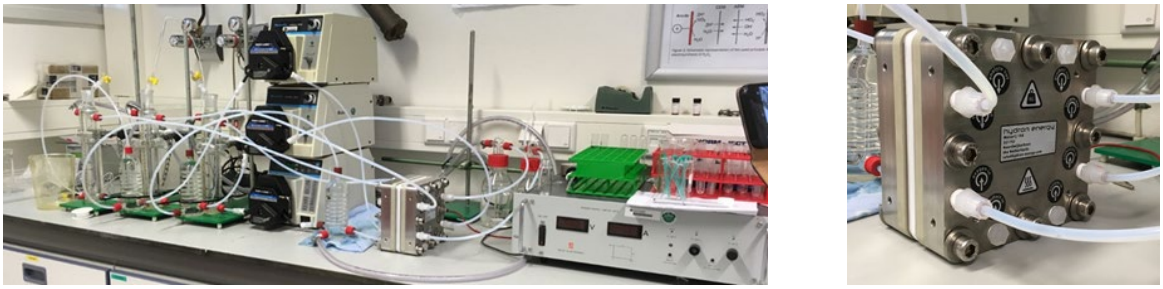


Figure A2-1 Electrochemical setup for the electrochemical production of H_2O_2 (left), 100 cm^2 prototype electrolyser (right).

At present on-site production of H_2O_2 is not in use in combination with digesters. In that case the use of AOP technology together with on-site produced H_2O_2 is still only TRL 5 (Technological Readiness Level) and has not been applied in practice before.

UV with (bulk purchased) H_2O_2 is an upcoming technology and considered as a promising and reliable technology option. Details of the performed work have been summarized in power point presentations and are shown below.

WP4: Hydrogen peroxide

Total bioconversion of COD in digesters by in-situ peroxide generation and oxidation

8-10-2019, Roel Bisselink, Johan van Groenestijn, Mark Roghair, Jiayang Liao (Amber)

Work package 4

- Task 4.1: Assessment of AOP with on-site produced H_2O_2 for selected digesting case (Techno-Economic)
- Task 4.2: Experimental verification and pilot plant design (contractual, financial and practical)

2

Economic potential of increased digestion

- Twence case: 500,000 ton/yr manure (swine)
  - Dry matter$_{av}$ = 7.4%, organic matter = 3.9%
  - Digestibility = 70%
  - Non-digested organic matter = 5850 ton/yr
- Hydrogen peroxide (H_2O_2) treatment assumptions:
  - Digestibility increased to 90% (10% lignin assumed)
- Additional biogas production:[1]
  - $2.4 \cdot 10^6$ m^3 biogas = $56 \cdot 10^6$ MJ = $16 \cdot 10^6$ kWh

Additional turnover prospect due to enhanced digestion:
290 kEUR (excl. SDE) and 970 kEUR (incl. SDE)[2]

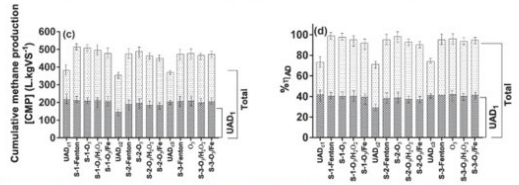
What about H_2O_2 costs?

[1] 620 Nm^3/ton and 23.3 MJp/m^3 biogas (RVO Omrekeningsfactor)
[2] based on: Correctiebedrag: 0.0185 EUR/kWh & Basis bedrag: 0.062 EUR/kWh

3

Advanced Oxidation Process (AOP)

- Not all organic compounds from manure, co-substrates and source separated organics (SSO, Dutch: GFT) are converted.
  - Recalcitrant fractions (lignocellulose) can be broken down by hydroxyl radicals ($OH^{\cdot}$)
- Up to a ratio of 0.5 (H_2O_2 : DM) has been used.
- Recent publication showed great potential at relatively low ratio (0.05):



UAD$_{C2}$ = 25% Sheep dung, 15% Cow dung, 20% Wood dust and 40% waste water

Almomani *et al.*, Fuel 253 (2019), 964.

Hydrogen peroxide usage

- Twence case:
  - Assuming ratio of 0.05 (H_2O_2 : DM)
  - Non-digested organic matter = 5850 ton/yr = 23350 ton/yr DM
  - Required amount of H_2O_2: 879[1] - 1168 ton


740 EUR/ton


1700 EUR/ton

  - H_2O_2 costs: 650 – 864 kEUR
- Net result:
  - Excl. SDE: -574 - -361 kEUR (+144 kEUR (incl. Lignin))
  - Incl. SDE: +106 - +320 kEUR (+485 kEUR (incl. Lignin))

[1] Compensated for amount of organic matter

5

Validation of economic potential

- Planned work (2019) for digestate treatment:
  - Validation of assumed ratio H_2O_2 : DM of 0.05 on Twence 'type' digestate
  - Insight on influence of ratio on digestability

6

What about on-site H_2O_2 production

- On-site H_2O_2 has advantages as handling, transport and storage are avoided / minimized.[1]
- For now, costs of on-site H_2O_2 production will be compared with bulk delivery


740 EUR/ton

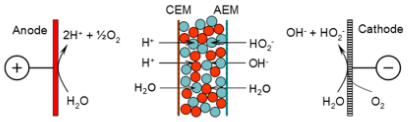

1700 EUR/ton


? EUR/ton

[1] these costs/advantages are difficult to monetize

7

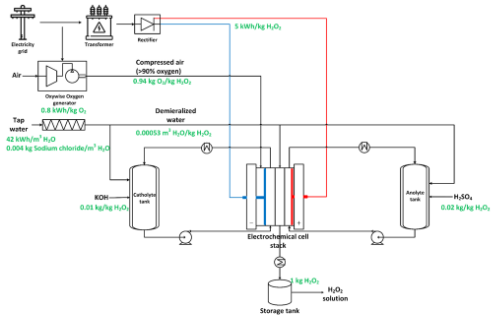
Principle H_2O_2 production



- Advantages on-site (local) production of H_2O_2:
  - Salt-free production of ~10 wt.% H_2O_2
  - Higher concentrations possible (other membranes)
  - Only water, oxygen (air) and electricity as reactants
  - High current efficiency
  - Safety (no/reduced handling, transport and storage)
  - Modular technology

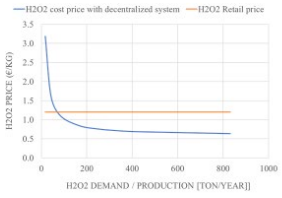
8

H_2O_2 production process



Initial economic evaluation on-site H_2O_2

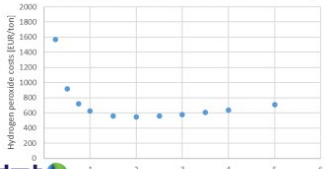
- Production capacity H_2O_2: 50 kg/h = 416 ton/yr
- **Production costs: 0.69 EUR/kg H_2O_2**
  - CAPEX 39 kEUR/yr
  - OPEX 248 kEUR/yr



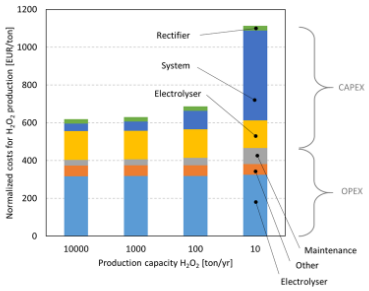
Detailed costs determination

- Calculation of process flows, mass transport and energy usage and dissipation:

- Optimal costs determination:



Relative costs of on-site H_2O_2 production



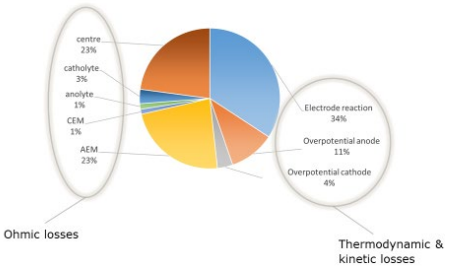
- Costs:

Twence case, savings:

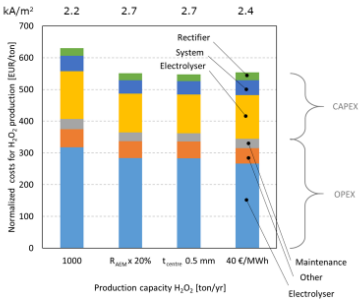
97 – 128 kEUR/yr

Economic improvements

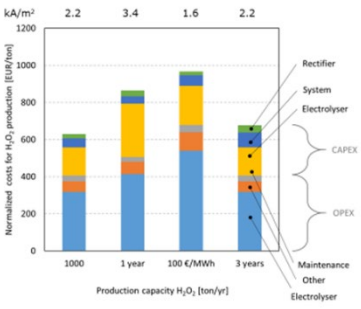
- Electricity consumption electrolyser



Electricity consumption electrolyser



Electricity consumption electrolyser



Future work

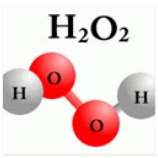
- Validation economic potential digestate treatment (2019):
  - Validation of assumed ratio H_2O_2 : DM of 0.05 on Twence 'type' digestate
  - Insight on influence of ratio on digestability
- Cost model optimisation
  - Improving process and cost parameter estimation (e.g. anode stability, PSA oxygen recovery, AEM resistivity, ...) (2019)
  - Selection of critical components to determine lifetime expectancy (2019)
  - Determination of lifetime expectancy (2020)

Experiments – First round

Biogas production from lignocellulosic biomass after treatment with H2O2

Beter (dan) Vergisten

Johan van Groenestijn, Bart Aerts and Roel Bisselink



Aim

- The ultimate goal is to develop a process for pretreatment recalcitrant fibres (lignocellulose) in manure digestate using H2O2 produced in situ. This means treatment at pH 8. Fibers will be recycled to the digester.
- As a result of the pretreatment the material becomes more fermentable.
- We will first conduct an exploratory experiment to see whether the principle works.
- We use wheat straw as a model substrate and just H2O2 from a bottle (not produced with electrodes). We will first follow the conditions recommended in literature

Method: pretreatment

- Use FeCl2
- Variation of H2O2 and Fe concentrations
- Add HCl to reach pH 3.
- 24 h incubation at 20°C
- Also adding H2O2 is 5 parts. Spread over time, not all at once at start.
- References without HCl and/or iron and H2O2
- Duplicates
- Residual H2O2 was removed using catalase, in some of the bottles

Pretreatment in bottles

- With 40 g wheat straw DM and 500 g liquid and additions

Experiment nr	Gram H_2O_2 per bottle	Gram $FeCl_2$ per bottle	
1	0	0	Reference with only water and HCl.
2	20	1.15	Everything added at start
3	20	0	Everything added at start
4	2	0.31	Everything added at start
5	2	0	Everything added at start
6	0.4	0.062	Everything added at start
7	20	1.15	Addition of H_2O_2 in 5 parts
8	2	0.31	Addition of H_2O_2 in 5 parts
9	20	0	Everything added at start; no HCl addition
10	0	0	Reference with only water; no HCl.

Method: anarobic digestion (biogas)

- Carried out by Opure
- Using bottles and pressure detection to assess biogas production.
- In triplicate
- 35°C, pH 7.5
- At dry matter of about 9%
- Until biogas production levels off
- Biogas production by inoculant is subtracted

Results: pretreatment

- If initial pH 2.7-2.9 and low amounts of iron: pH increases during incubation (up to 5)
- If no HCl: pH 5.6 – 6.3

Results: anaerobic digestion

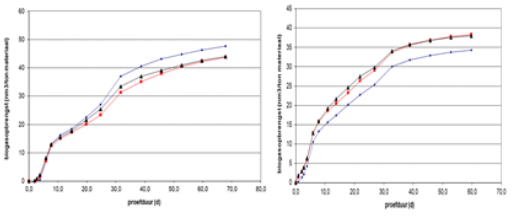
- Digestion took about 65 days
- After 20 days more than half of the production was reached
- Biogas contained 52% (v/v) methane

Results: biogas production (duplicates)

nr	Gram H_2O_2 per bottle	Gram $FeCl_2$ per bottle		N-liter biogas/ kg organic matter (20 days)		N-liter biogas/ kg organic matter (end)	
1	0	0	Ref. plus HCl	275	270	554	527
2	20	1.15		269	333	471	586
3	20	0		340	344	553	531
4	2	0.31		316	287	547	498
5	2	0		280	296	532	521
6	0,4	0.062		252	289	505	558
7	20	1.15	Additions in 5 parts	358	364	560	586
8	2	0.31	Additions in 5 parts	295	311	549	553
9	20	0	No HCl	327	391	519	606
10	0	0	Ref no HCl	232	235	499	510

Bottle 10 (ref) and bottle 7 (max H2O2 and Fe)

Nm3 biogas per ton of slurry (wet material)



Degradation of the organic matter

nr	Gram H_2O_2 per bottle	Gram $FeCl_2$ per bottle		% organic matter degraded	
1	0	0	Ref. plus HCl	71	67
2	20	1.15		61	76
3	20	0		70	68
4	2	0.31		72	66
5	2	0		69	67
6	0.4	0.062		65	73
7	20	1.15	Additions in 5 parts	71	75
8	2	0.31	Additions in 5 parts	71	72
9	20	0	No HCl	68	79
10	0	0	Ref no HCl	63	65

Conclusions

- 20 g H2O2 (added in 5 parts) plus 1.15 g FeCl2 (pH 3) added to 40 g wheat straw dry matter led to faster biogas production
- 2 g H2O2 (added in 5 parts) plus 0.31 g FeCl2: effect is weaker but still significant
- Final biogas yield: only small differences
- Actually the untreated wheat straw performed surprisingly well; it is not that recalcitrant
- Higher pH and only H2O2 should get a second chance

Next: pretreatment and digestion

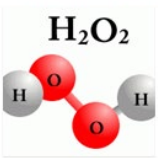
- Take material that is more recalcitrant: digestate (fibres): effects will be more significant.
- Try procedures 7, 8 and 9. Also try pH 3 – 8.
- Again estimate costs
- Third year: other feedstocks, prepare for larger scale, proces design and economic evaluation.

Experiments – Second round

WP2: Biogas production from lignocellulosic biomass after treatment with H_2O_2

Beter (dan) Vergisten

April 13 2021, Johan van Groenestijn, Bart Aerts

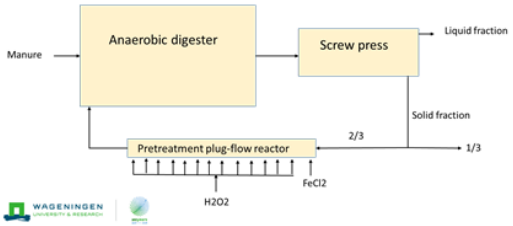


Aim

- The ultimate goal is to develop a process for pretreatment recalcitrant fibres (lignocellulose) in manure digestate using H_2O_2. Fibres will be recycled to the digester
- As a result of the pretreatment the material becomes more fermentable

Flow sheet

- Recycling and pretreatment (using H_2O_2) of recalcitrant material from digestate



Work carried out Nov 2019- Feb 2020

- First exploratory experiment to see whether the principle works
- Wheat straw as a model substrate. First follow the conditions recommended in literature. H_2O_2 from a bottle.
- Outcome: treated wheat straw slightly faster biogas production, but biogas yields are the same as untreated straw. pH3.
- Surprisingly high biogas production from straw. Straw is not that recalcitrant.

December 2020 – March 2021

- Use solid fraction of mono cattle manure digestate
- From farm in Bathmen
- Try various pH, various H_2O_2 concentrations
- Explore effect of washing the fibres

Conditions in bottles

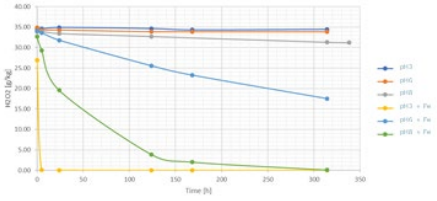
Nr	Gram H_2O_2 per bottle	Gram $FeCl_2$ per bottle	Material and pH
1	0	0	Washed fibre pH 8
2	20	1.15	Washed fibre pH 3
3	20	1.15	Washed fibre pH 6
4	20	1.15	Washed fibre pH 8
5	20	1.15	Unwashed fibre pH 8
6	5	0.6	Washed fibre pH 3

Method: pretreatment and anaerobic digestion

- Add HCl to reach pH 3
- 24 h incubation in bottles at 20°C
- 40 g biomass DM used in 500 mL liquid in bottles
- Also adding H_2O_2 is 5 parts. Spread over time, not all at once at start
- Duplicates
- Residual H_2O_2 was removed using catalase, in some of the bottles

- Anaerobic digestion by Opure: bottles in triplicate

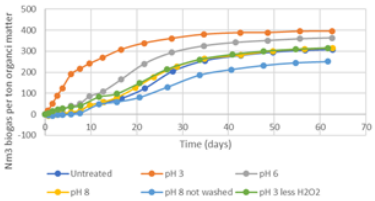
First: stability of H_2O_2 without biomass



Outcome

- H_2O_2 is sufficiently stable at all tested pH, but with Fe(II) the Fenton reaction starts and this reaction runs faster at low pH. At pH 8 the reaction occurs, but slowly.
- Colour: blue turns into brown: Fe(II) turns into Fe(III)
- $FeCl_2$ complete soluble at pH 3. Not at 6 and 8.
- Adding biomass: a lot of gas bubbles

Results test with digestate



- Average of 6 biodigester bottles
- Biodigestion under optimum (adjusted) pH and temperature

Results: percentage of organic matter degraded in the biodigesters

nr	Content bottle	% organic matter degraded (duplicates)
1	Untreated	38/40
2	pH 3	53/55
3	pH 6	50/49
4	pH 8	41/38
5	pH 8 not washed	32/31
6	pH 3 less H_2O_2	38/44

Results

- Fenton reaction clearly makes the digestate better digestable
- pH3 and 20 g H_2O_2: much faster digestion and higher biogas yield
- Lower amounts of H_2O_2 at pH3: no effect
- pH6: an effect, but small
- Unwashed material: less biogas
- Biogas contained 52-55% CH_4 in all experiments
- H_2S in biogas: 2 – 100 ppm

Results: dry and organic matter after the H2O2 treatment (dupicates)

nr	Content bottle	Dry matter (%)	organic matter (% of dry matter)
1	Untreated	8.3/8.5	89.1/89.1
2	pH 3	7.1/7.0	77.7/77.0
3	pH 6	7.1/7.0	85.8/86.2
4	pH 8	7.3/7.0	86.9/87.1
5	pH 8 not washed	7.2/7.4	85.5/85.3
6	pH 3 less H_2O_2	7.2/7.4	87.0/86.2

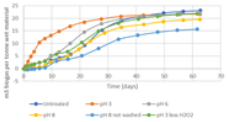
- pH 3: organic matter lost by oxidation?

Discussion

- Wheat straw: around 550 Nm^3 biogas/ton OM (all variations)
- Digestate: 250 – 400 Nm^3 biogas/ton OM: more recalcitrant
- Biogas yield increase depends on digestion time and the activity of the biology in the biodigester.
- Opure used super inoculant and high concentration inoculant
- Real digesters may be less active: slower: all curves from graphs above may be stretched along the X-axis.
- Real digesters have a better chance to develop conversion activity for the new compounds formed after H_2O_2 oxidation. Adaptation in time.

Discussion (2)

- pH6: some effect and pH3 great effect
- Do we have to go down to pH3 or is pH4 or 5 effective as well?
- Do we loose organic matter at pH3 and 20 g H_2O_2?
- If so: additional final biogas yield per ton slurry not much better than reference.
- pH3 lower H_2O_2 also lower Fe. What is the bottle neck?



Next

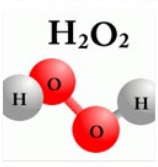
- First make economic calculations
- Based on these calculations: which direction for improvement? Which new experiments?
- Possibilities:
- Use biology from manure digesters in biogas production experiments.
- Explore pH 4 and 5.
- Organic matter really disappearing during treatment?
- Try to lower H_2O_2 addition.
- Continuous addition of H_2O_2: more effective? Lower H_2O_2 use?
- Unwashed material in combination with low pH.

Experiments – Third round

WP4: Biogas production from lignocellulosic biomass after treatment with H_2O_2

Beter (dan) Vergisten

March 1 2022, Johan van Groenestijn, Bart Aerts

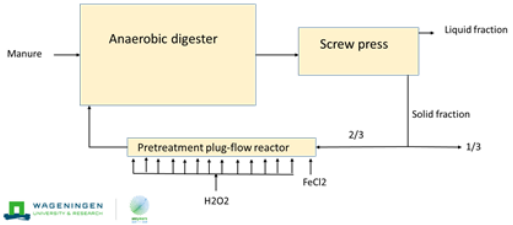


Aim

- The ultimate goal is to develop a process for pretreatment recalcitrant fibres (lignocellulose) in manure digestate using H_2O_2. Fibres will be recycled to the digester
- As a result of the pretreatment the material becomes more fermentable

Flow sheet

- Recycling and pretreatment (using H_2O_2) of recalcitrant material from digestate



December 2020 – March 2021

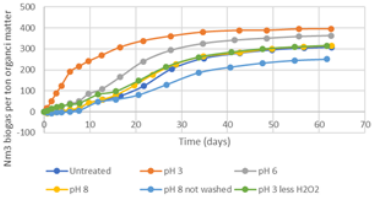
- Use solid fraction of mono cattle manure digestate
- From farm in Bathmen
- Try various pH, various H_2O_2 concentrations
- Explore effect of washing the fibres

Method: pretreatment and anaerobic digestion

- Add HCl to reach pH 3
- 24 h incubation in bottles at 20°C
- 40 g biomass DM used in 500 mL liquid in bottles
- Also adding H_2O_2 is 5 parts. Spread over time, not all at once at start
- Duplicates
- Residual H_2O_2 was removed using catalase, in some of the bottles

- Anaerobic digestion by Opure: bottles in triplicate

Results test with digestate, early 2021



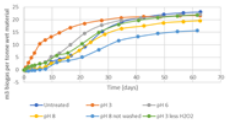
- Average of 6 biodigester bottles
- Biodigestion under optimum (adjusted) pH and temperature

Discussion April 2021

- Digestate: additional 250 – 400 Nm^3 biogas/ton OM
- Biogas yield increase depends on digestion time and the activity of the biology in the biodigester.
- Opure used super inoculant and high concentration inoculant
- Real digesters may be less active: slower: all curves from graphs above may be stretched along the X-axis.
- Real digesters have a better chance to develop conversion activity for the new compounds formed after H_2O_2 oxidation. Adaptation in time.

Discussion (2)

- pH6: some effect and pH3 great effect
- Do we have to go down to pH3 or is pH4 or 5 effective as well?
- pH3 lower H_2O_2 also lower Fe. What is the bottle neck?



Economy

Break even at a price of H2O2 of € 160/tonne

Current H2O price: € 1000/tonne

In situ electrochemical H2O2 production may cost € 640/tonne

Options to reduce costs

- Use less H2O2
- Smaller pretreatment reactor (now residence time 24 hours; how about 5 hours?)

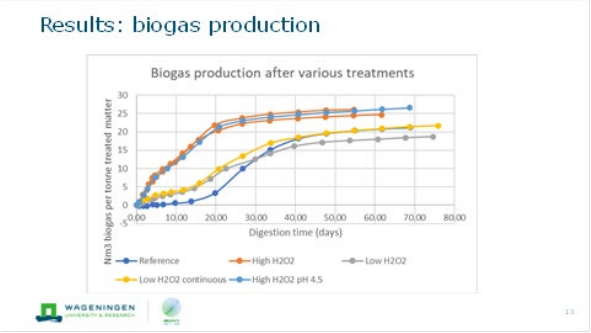
- If we can reduce reactor costs 50% and H2O2 use with factor 3 then we can reach break-even.

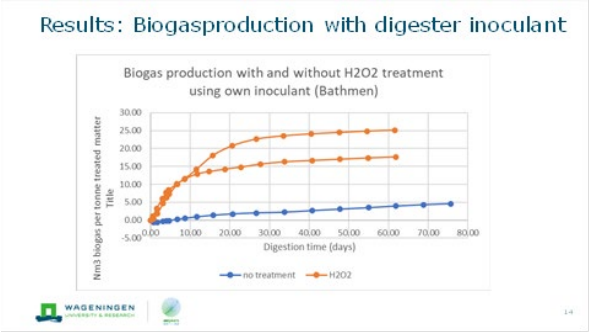
New experiments Nov 2021 – February 2022

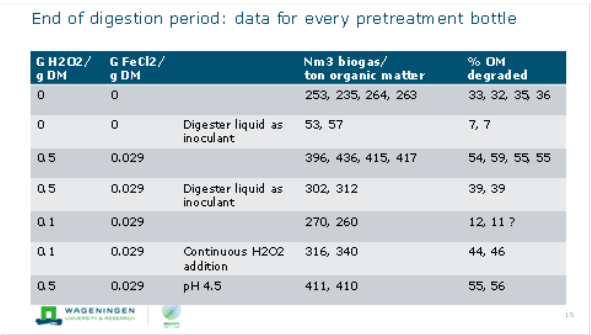
- Again use digestate solid fraction from mono cattle manure digester in Bathmen
- Reduce H2O2 dose but not iron dose
- Try continuous dose of H2O2 instead of 5 shots
- Try pH 4.5 instead of pH 6.
- Opure uses super inoculant in biogas tests; try Bathmen digester content as inoculant

Pretreatment set up; all in duplicate

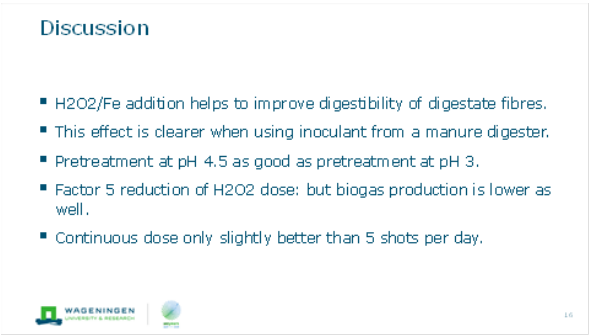
G H2O2/g biomass dry matter	G FeCl2/ g biomass dry matter	Deviation from standard procedure
0	0	
0	0	Digester liquid as inoculant
0.5	0.029	
0.5	0.029	Digester liquid as inoculant
0.1	0.029	
0.1	0.029	Continuous H2O2 addition
0.5	0.029	pH 4.5

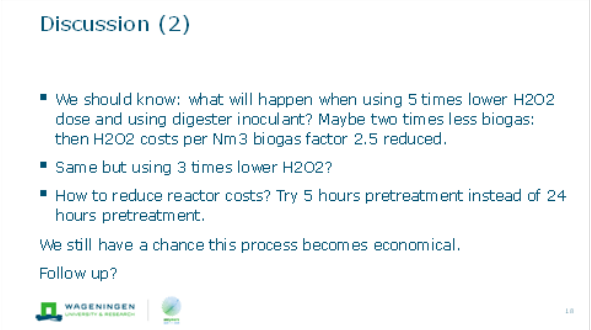






G H2O2/ g DM	G FeCl2/ g DM		Nm3 biogas/ ton organic matter	% OM degraded
0	0		253, 235, 264, 263	33, 32, 35, 36
0	0	Digester liquid as inoculant	53, 57	7, 7
0.5	0.029		396, 436, 415, 417	54, 59, 55, 55
0.5	0.029	Digester liquid as inoculant	302, 312	39, 39
0.1	0.029		270, 260	12, 11 ?
0.1	0.029	Continuous H2O2 addition	316, 340	44, 46
0.5	0.029	pH 4.5	411, 410	55, 56





Cost – benefit analysis additional biogas production by peroxide treatment of digestate

Benefits

Biogas has a value. Market value € 0.0185/kWh-HHV. Basis value (value including SDE subsidy) € 0.062/kWh-HHV (PBL, 2018. Tabel 9.3)[2]:

- 22.4 normal litres per mol gas
- Biogas contains 54% methane
- Methane HHV is 55.5 MJ/kg

It can be calculated that 1 Nm^3 biogas has a value of € 0.37 including subsidy. The results of the experiments in this study were: 22 m^3 biogas can be produced per tonne wet mixture (8% DM digestate unseparated). About 10 m^3 is the result of peroxide treatment. This is 275 m^3 biogas/tonne digestate dry matter, of which 125 m^3 by peroxide treatment. This 275 m^3 can also be reached without peroxide but taking a long time (at least two months) for digestion. In case there is not much time, peroxide gets more important.

Benefit:

- 275 m^3 biogas is € 101 per tonne digestate dry matter
- 125 m^3 biogas is € 46 per tonne digestate dry matter

The investment in a solid fraction digestate recycle stream (with H_2O_2 addition) will serve the production of 275 m^3 additional biogas.

[2] In 2022 for mono manure digesters < 400 kW the value has risen to 0.111 €/kWh (including subsidy).

Costs

Hydrogen peroxide is the most important cost. Costs for iron is negligible. H_2O_2 costs € 1000/tonne (Kemcore, 2022). We now use 20 gram H_2O_2 per 40 gram digestate dry matter. Therefore, costs is € 500/ tonne digestate dry matter.

The reactor (Figure 3-2) may be fed with solid fraction of digestate. That has a dry matter content of 26%. A pipe with a screw is required (like a screw elevator). We still need to find out if we have to dilute this matter in order to mix it with peroxide. In our experiments we used 8% dry matter. In the experiments the reaction took 24 h, but we did not test if this time can be shortened. For our calculations we assume a full scale plant with a feed of 26% dry matter and a residence time of 24 h.

Suppose a large cattle farm with a digester. This farm may produce 700 tonnes digestate dry matter per year. If the concentration is 26% the volume is 2692 m^3/year. But this matter is passing two times the peroxide reactor (Figure 3-2). The volume of the reactor should be 2692 x 2/365 = 14.8 m^3. Costs of a screw conveyor is € 70,000 (DACE, 2020). The Lang factor in a an existing plant is about 3.5. Investment costs are € 245,000. Annual costs: 12% for depreciation and interest and 3% for maintenance: € 36,750. This is € 53/tonne digestate dry matter.

Discussion

In the calculations above the costs are higher than the benefits. Options to reduce cost are:

- Minimize H_2O_2 addition. Remaining promising options are (1) lower peroxide dose without decreasing the iron dose and (2) lower peroxide dose in a continuous addition (instead of five batches in 24 hours)
- The use of higher digestate dry matter concentration at the same H_2O_2 concentration may be equal effective but reduces the peroxide costs per tonne digestate;
- Reaction time of 24 h may be unnecessarily long. Decrease to 5 h will decrease the reactor costs dramatically;
- In the process flow sheet the digestate is recycled twice instead of once. This may yield more biogas;
- Opure used an own inoculant. But this inoculant has never seen the peroxide generated products before. In a real plant the bacterial flora in the biodigester has the opportunity to adapt to the new products and increase the biogas production.

Annex 3 PHA production and isolation from manure

VFA production from solid manure fraction

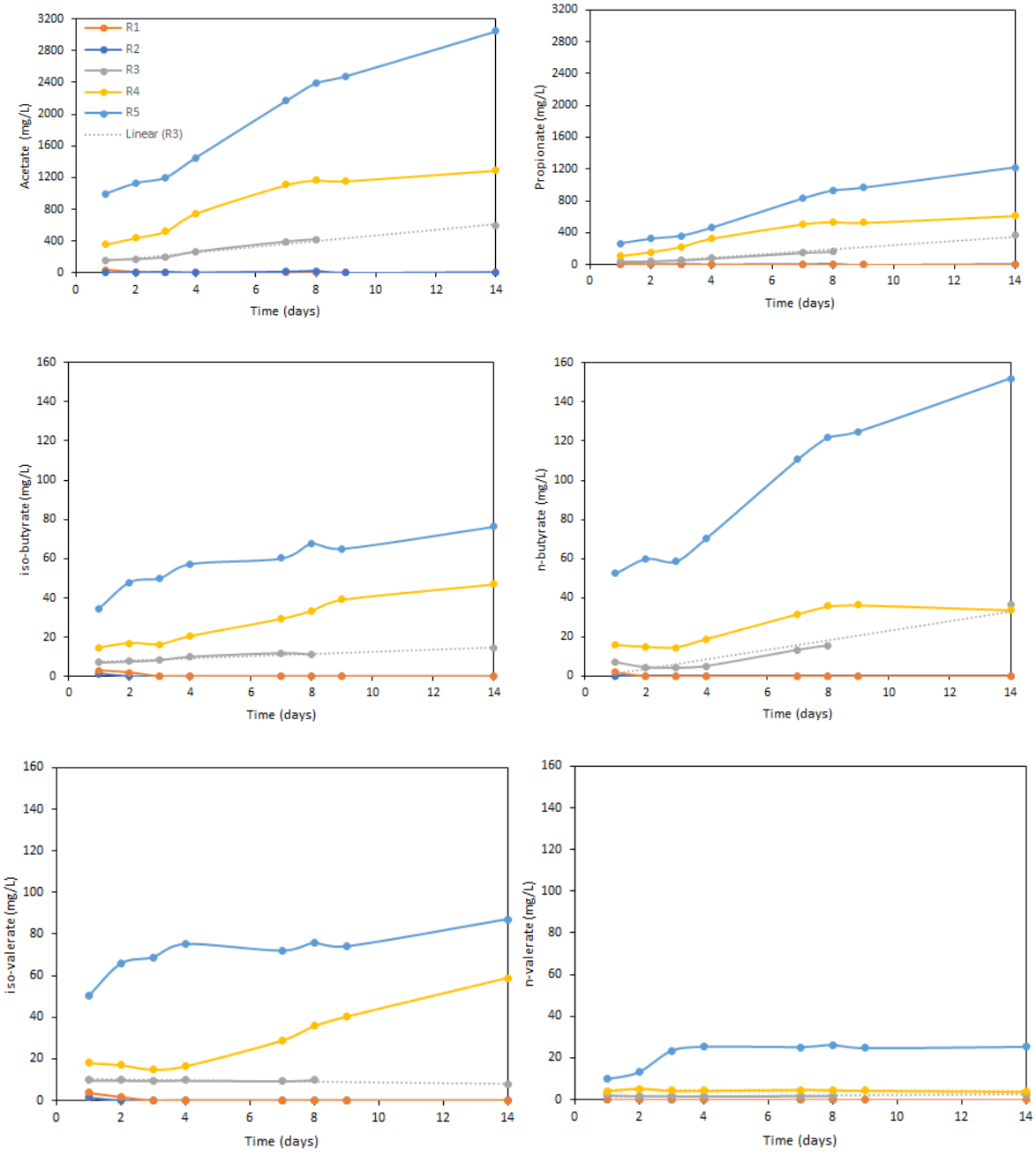


Figure A3-1 VFA's produced from hydrolysis and acidogenesis of the solid fraction of cow manure at increasing OLRs (bioreactors R1-R5).

PHA production from VFA in liquid manure fraction

At the fourth feast-famine cycle in the SBR reactor, when a feast phase of 2.5 hours was applied, the COD during the feast phase was determined at 30 min intervals (Table A3-1). There was no change in the COD in the fourth feast cycle. Some fluctuations in the concentration can be explained by small solids still being abundant in manure, causing issues with pipetting as well as quickly settling after diluting the 10% manure even further. To lengthen the time for COD consumption, the feast phase was increased from 2.5 to 4 h.

Table A3-1 Development of COD during fourth feast phase.

Time point in feast phase	COD (g/L)
Start	8.32
1 h	8.30
1.5 h	8.18
2 h	8.76
2.5 h	8.52

After increasing the duration of the feast phase to 4 h, the COD was determined again during the feast phase in the third cycle: samples were taken at the beginning and end of the feast phase and analysed (Table A3-2). There was still no detectable decrease in COD observed. It was decided to leave the sequence unchanged to see if there were developments over time. COD had increased compared to the 2.5 h feast cycle. This could be explained by the large amount of small solids that can settle along with the biomass during the settlement phase, increasing COD over time.

Table A3-2 Development of COD during feast phase (third feast phase after increasing duration feast phase to 4 h).

Time point in feast phase	COD (g/L)
Start	10.53
4 h	10.24

Literature overview PHA release and recovery

PHA are deposited intracellularly in the form of inclusion bodies ("granules"). Size is ~100–500 nm, composition: 97.7% PHA, 1.8% protein and 0.5% phospholipids. Typical Mw 200 – 2000 kDa. For a schematic overview see Figure A3-2.

There are several overview articles written (Jacquel *et al.*, 2008; Koller *et al.*, 2013; Kosseva and Rusbandi, 2018; Kunasundari and Sudhesh, 2011). Research focusses on production, although DSP significantly affects the overall process economics. DSP should be environmentally friendly because PHA is an eco-friendly material. Criteria for selection DSP (cell disruption followed by PHA recovery) within this project are quality (native state), yield and purity, OPEX and CAPEX, environmental friendliness.

Technologies described for cell disruption and PHA recovery are amongst others solvent extraction, chemical digestion, enzymatic digestion, mechanical disruption, supercritical fluids and combinations:

- Solvent extraction (Kunasundari and Sudhesh, 2011) is the most common adopted method. It is carried out in two steps. First modification of cell membrane permeability thus allowing release and solubilisation of PHA, followed by a non-solvent precipitation. In the PHARIO project a one-step extraction (patented) is used with 2-butanol (~103°C), followed by a cooling down during which a gel is formed. Main drawback of solvent extraction is the loss of the native state of PHA. In this project it will be used as benchmark.
- Enzymatic extraction (Kunasundari and Sudhesh, 2011) typically entails three steps: (i) heat treatment to inactivate biomass, (ii) enzymatic hydrolysis (proteases) which should be very selective and (iii) washing with surfactant. There is experience on lab-scale at WFBR and a method is available.
- Mechanical disruption can be performed by bead milling, homogenization or ultrasonication and has to be followed up by a recovery technology. The main advantage (and criteria within this project) is that it maintains the native state PHA. Literature is limited and no mention is made of obtaining native state PHA. Mechanical treatment must be followed by an isolation method that also doesn't influence the native state. An example is air flotation, which makes use of the existing PHA granules and the interaction of particles with the gas/liquid interface.

An overview of the literature is given in Table A3-3.

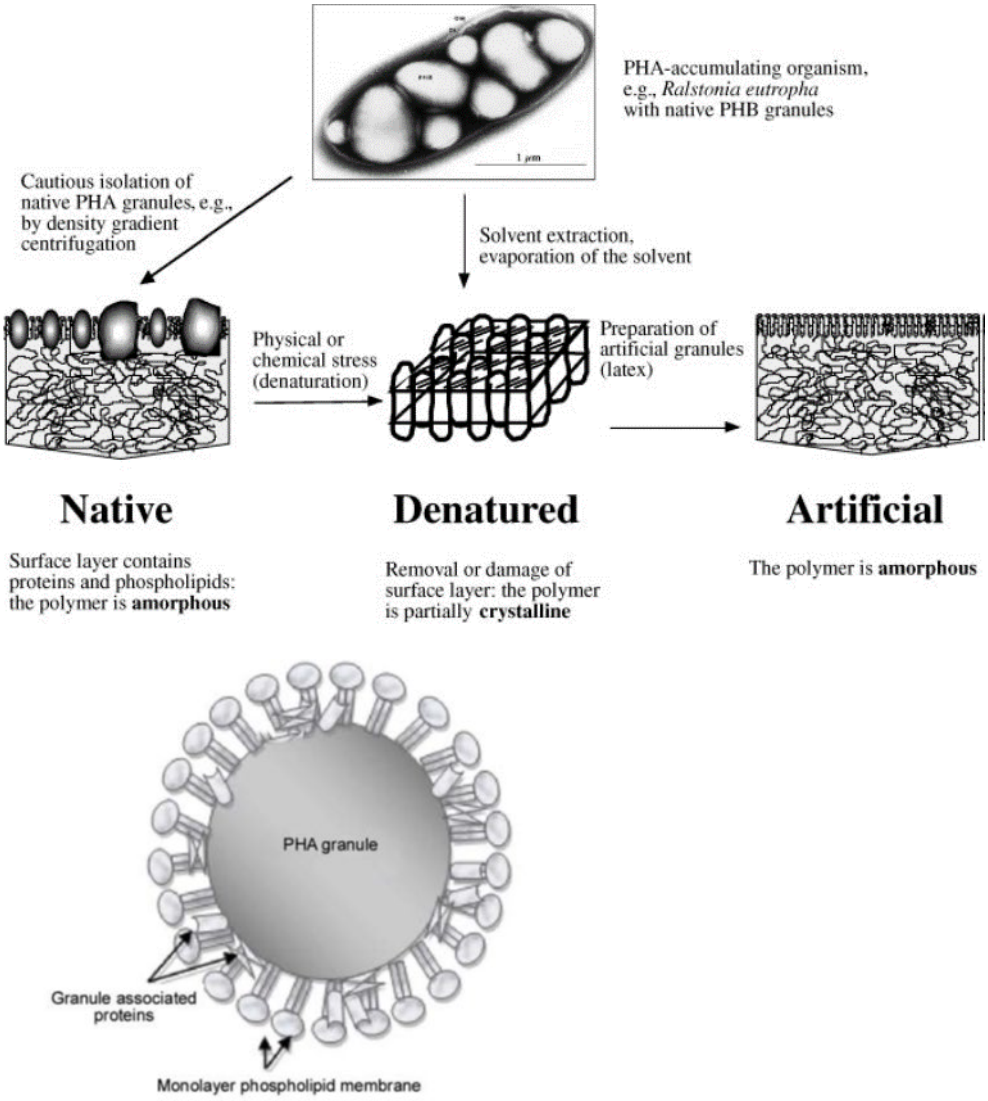


Figure A3-2 Schematic overview PHA (Jendrossek and Handrick, 2002).

Table A3-3 Literature overview release and isolation PHA from biomass.

Isolation method	Isolation conditions	Microorganisms	Polymers	Purity	Yield	Reference	Comments
Homogenization	90-95 MPa, 25°C	*A. latus*	P(βHB)	n.d.	n.d.	Tamer et al [1998]	Measured protein release as indicator
Homogenization	Two cycles, 800 kg/cm2, 1-6% CDM	*C. necator*	scl-PHA	n.d.	97-99	Koller et al. [2013]	Counting viable cells as indicator
Homogenization + centrifugation	Homogenization, centrif., NaOCL (0.085 %, 1 h), centrif.	*Escherichia coli*	PHA	98.5	n.d.	Wegen van et al. [1998]	
Homogenization + centrifugation	Homogenization, NaOCL (1.5 %), centrif., PBS buffer, centrif.	*Escherichia coli*	P(3HB)	94	97	Ling et al. [1997]	
Homogenization + SDS	SDS (5%), two-stage, 1-3 passes	*Methylobacterium sp*	P(3HB)	95	98	Ghatnekar et al. [2002]	
Bead mill	Two beads sizes (500 and 950 um), bead loading 75-85%	*A. latus*				Tamer et al. [1998]	Measured protein release as indicator
Sonification		*Haloferax mediterranei*	P(βHB)	n.d.	n.d.	Hwang et al [2006]	Granules observed with SEM
Supercritical fluids	sCO_2, 200 atm, 40°C, 0.2 mL CH_3OH, 100 min	*Ralstonia eutropha*	P(βHB)	n.d.	89	Hejazi et al. [2003]	--
Supercritical fluids	sCO_2, 200 bar, 30°C, 1% (v/v) toluene, 40 min, 2 times	*Ralstonia eutropha*	P(βHB)	n.d.	n.d.	Khosravi-Darani et al. [2004]	For these optimal conditions protein release was used as indicator
Supercritical fluids	sCO_2, 140-620 bar, 40-100°C,	*Pseudomonas resinovorans*	PHA	n.d.	none	Hampson & Ashby [1999]	Used for lipid extraction
Dissolved-air flotation	pH 3.5, 5 bar, 7 min	*Pseudomonas putida*		86	n.d.	Hee van et al. [2006]	SEM pictures
Air classification	Sonification, freeze-dried, pulverized to 2 um, PHA in fines	*E. coli*	P(3HB)	97	90	Noda [1998]	
Air classification	Sonification, freeze-dried, pulverized to 5 um, PHA in fines	*Alcaligenes eutrophus*	P(3HB)	95	85	Noda [1998]	

Pretreatment PHARIO sludge

An impression of the amount of sand present in the sample and the settling overnight are given in Figure A3-3.

Figure A3-3 Impression of the pretreament of the sludge: removal of sand and overnight settling.

PHA release using high pressure homogenizer (HPH)

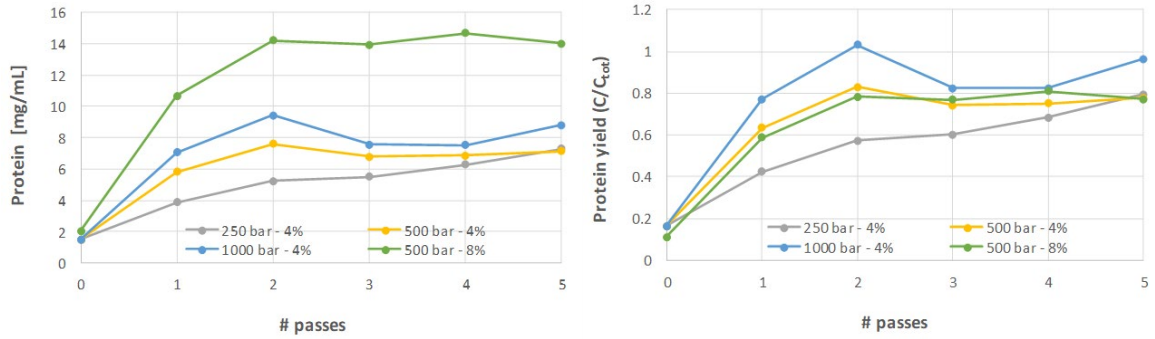


Figure A3-4 Influence of dry weight on protein release and yield during HP treatment (first trial).

With HPH 70% protein yield obtained at 250 bar (4 passes), 500 bar (2 passes for both 4 and 8% DM) and 1000 bar (1 pass).

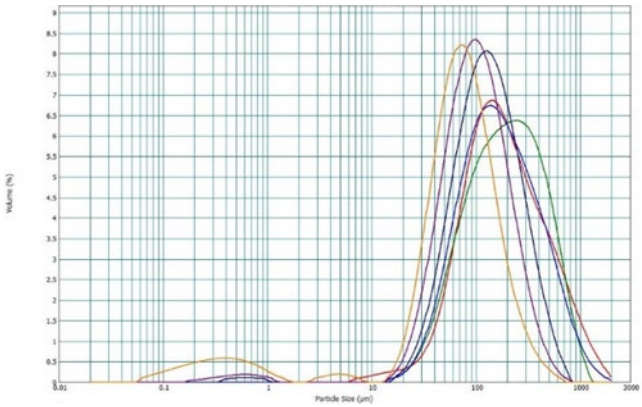


Figure A3-5 Particle size distribution HPH at 8% DM and 500 bar as function of number of passes (0 till 5).

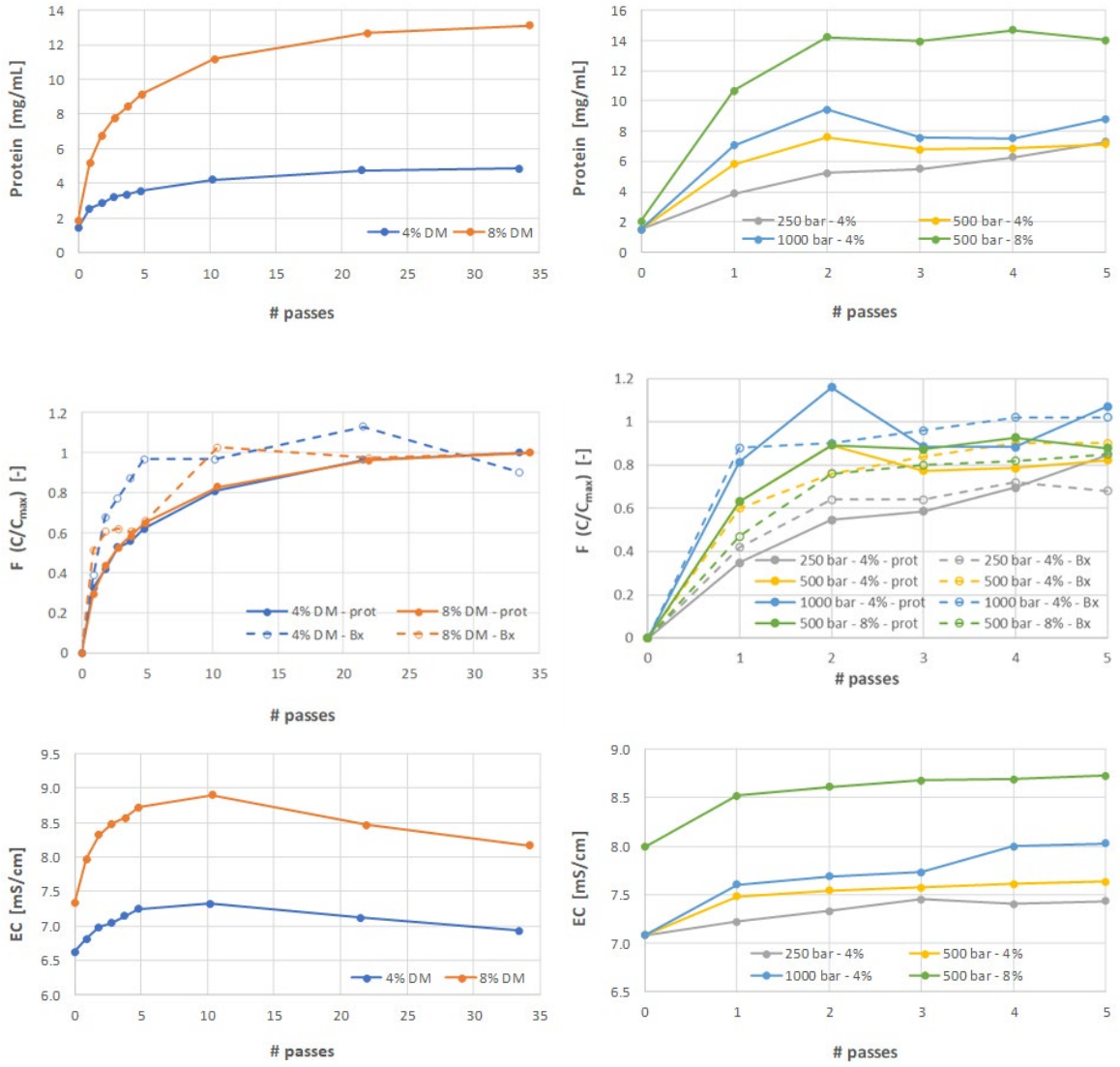


Figure A3-6 Protein, °Brix and EC measurements in the supernatant during bead milling and HP treatment (first trial).

Estimation of energy consumption mechanical disruption

For bead milling the first-order rate constant for protein release was estimated at $1.7 \cdot 10^{-3}$ 1/s (Figure A3-7), this value was used to estimate the energy consumption. The energy consumption is lower at a higher DM and increases with a higher targeted protein release. For a 70% protein release at 8% DM the energy consumption is 8.7 kWh/kg DM.

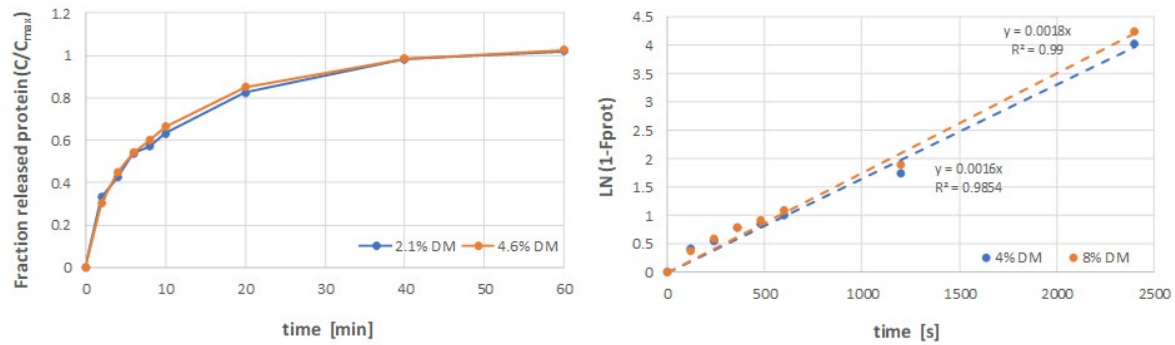


Figure A3-7 Estimation of first-order kinetics protein release for calculation energy consumption bead mill (first trial).

Table A3-4 Energy consumption bead mill based on protein release.

Y_{prot} [-]	DM [%]	BM filling [%]	d_{beads} [mm]	Speed [rpm]	u_s (agitator) [m/s]	Time [min]	Capacity [kg DM/hr]	E_{BM} [kWh/kg dm]
0.7	4	60	0.5	2400	8	50	0.06	30
0.7	8	60	0.5	2400	8	24	0.20	8.7
0.5	4	60	0.5	2400	8	8.4	0.34	5.1
0.5	8	60	0.5	2400	8	7.5	0.64	2.7

For HPH the first-order rate constant was estimated at $1.69 \cdot 10^{-13}$ 1/s and *w* equals 1.646 (Figure A3-8). These values were used to estimate the pressure at which the protein release is 70% (4% DM, η_{pump} 85%), resulting in 642 bar. The calculated energy consumption at this pressure is 0.52 kWh/kg DM.

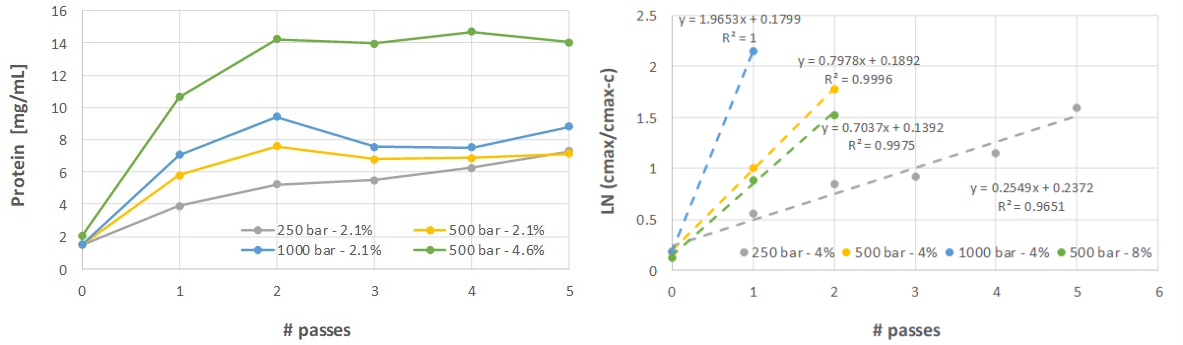


Figure A3-8 Estimation first-order rate constant protein release for HPH.

It should be noted that there is a large effect of scale on the energy consumption of the bead mill. This is based on previous work on disruption of microalgae (Boon *et al.*, 2015a and 2015b). This is due to the large difference in idle power uptake: which is 40% at 20 L (measured) and 10% at 1150 L (communication Bühler). This results in an estimated energy consumption on industrial scale for bead mill of 0.1-0.5 kWh/kg DW. There is no reason to assume that this will not also apply to the disruption of PHA containing biomass.

PHA recovery using SDS at high pH

Combination SDS and high pH (based on patent producer China (Xuejun, 2009)), at pH 11, 3% SDS addition, 10 min at room temperature. At pH 12, 10% SDS addition, 1 h at 70°C. Results are shown in Figure A3-9. 10% SDS with pH 12 resulted in purity of PHA 66%. No results for the yield are available. Filtration (as described in patent) was not successful in this lab trial, therefore centrifugation was used. This experiment was repeated.

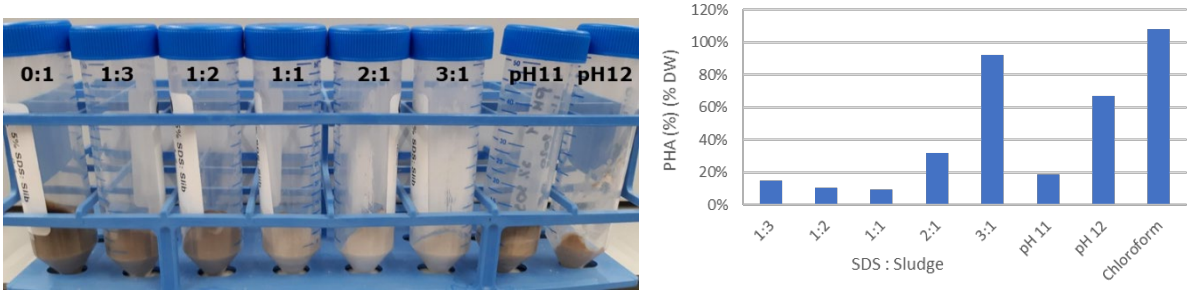


Figure A3-9 PHA recovery as using SDS at elevated pH.

PHA recovery – reducing SDS dose

Figure A3-10 shows the influence of an acid wash and the addition of SDS during bead milling on protein release. SDS addition prior to bead milling showed cell disruption at 0 passes and a limited increase in protein release during bead milling. For the acid washed sludge a slightly lower yield was found. Based on these results it may be concluded that the addition of SDS is possibly sufficient to release PHA and no bead

milling is required. It should be noted that the measured protein releases were lower than the 80% PHA yield obtained in the experiment with a high SDS dose.

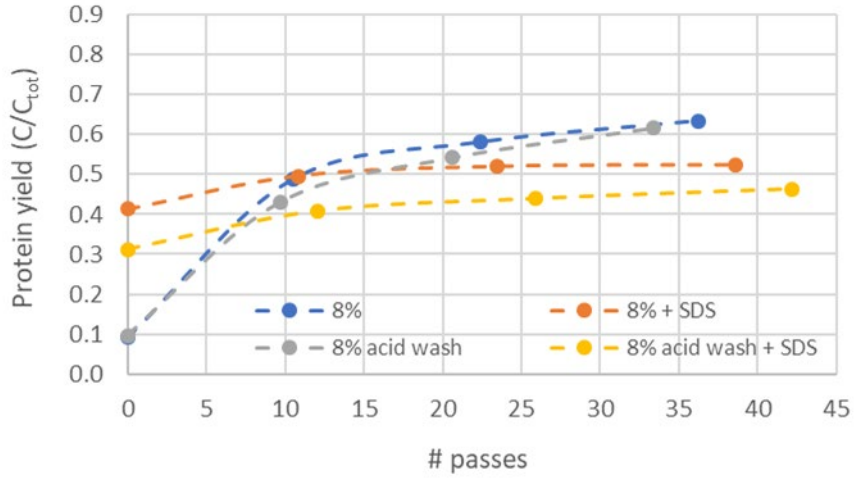


Figure A3-10 Influence of acid wash and addition of SDS on protein release during bead milling.

Changes in particle size distribution during mechanical disruption showed that there were a limited number of particles produced in the required range of 1-10 um (size of native PHA granules) (Figure A3-11). The most promising results were obtained with acid wash and addition of SDS before bead milling.

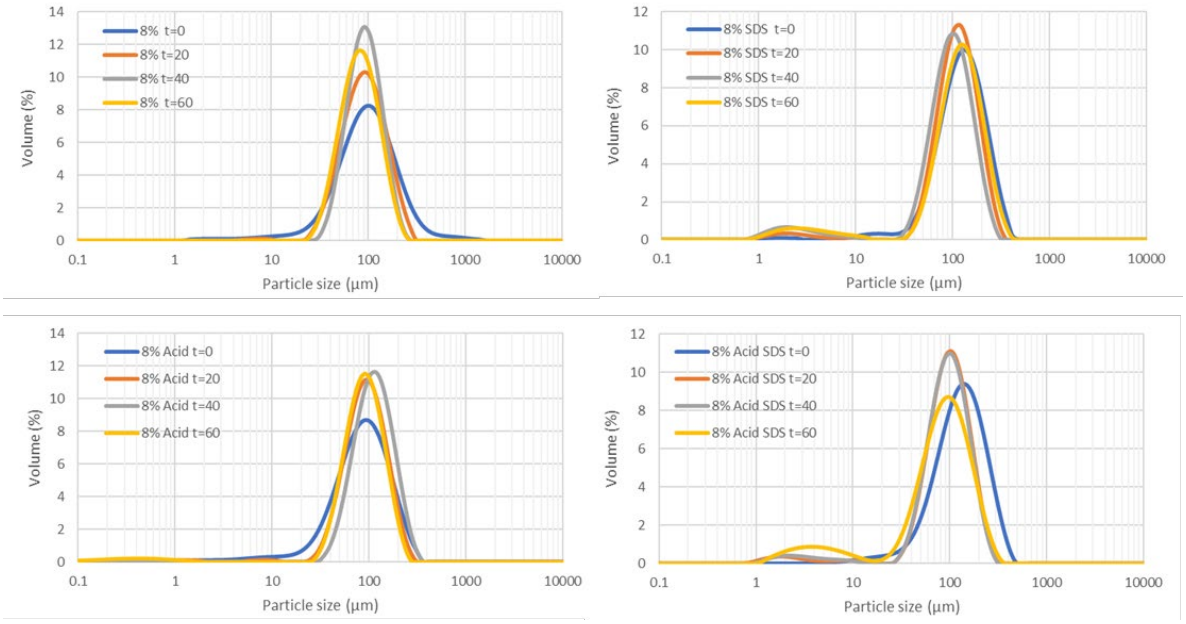


Figure A3-11 Influence of acid wash, addition of SDS and time on the particle size distribution during bead milling (8% DM).

PHA yield and purity were determined for the conditions in which the SDS was added after bead milling (upper scheme in Figure 4-5). Higher yield and lower purity were obtained compared to the trial performed at 3.5 g SDS/g non-PHA (Figure A3-12). The main PHA losses were due to a difficult S/L separation. Slightly higher yield and purity were obtained for acid washed biomass. The best results (high yield (94%) in combination with highest purity (28%)) were obtained at 0.9 g SDS/g non-PHA, the lowest addition investigated.

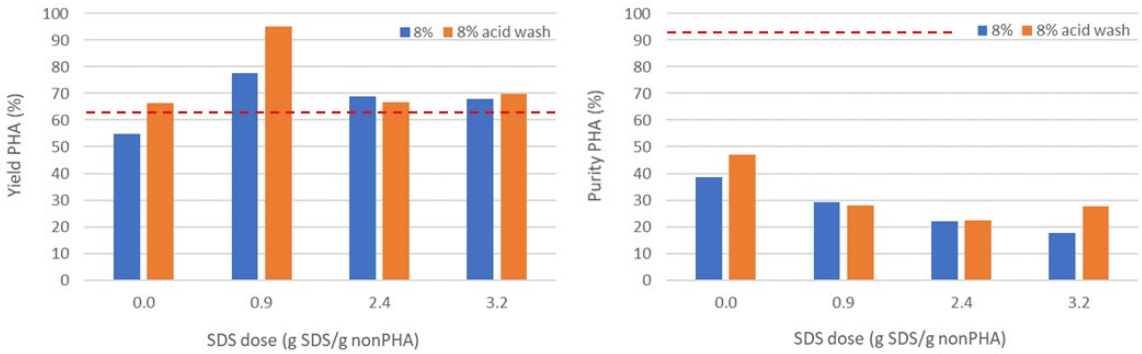


Figure A3-12 Yield and purity PHA after bead milling followed by SDS wash. Dashed lines indicate result at dosage of 3.5 g SDS/g non-PHA.

PHA yield and purity were determined for the conditions in which the SDS addition was combined with bead milling, followed by an SDS wash afterwards (middle scheme in Figure 4-5). Higher yields and higher purities were obtained compared to bead milling in absence of SDS (Figure A3-13). The S/L separation improved by the SDS addition during bead milling. Slightly higher yield and purity were obtained for acid washed biomass. The best results (high yield (100%) in combination with high purity (48%)) were obtained at 0.6 g SDS/g non-PHA, the lowest addition investigated.

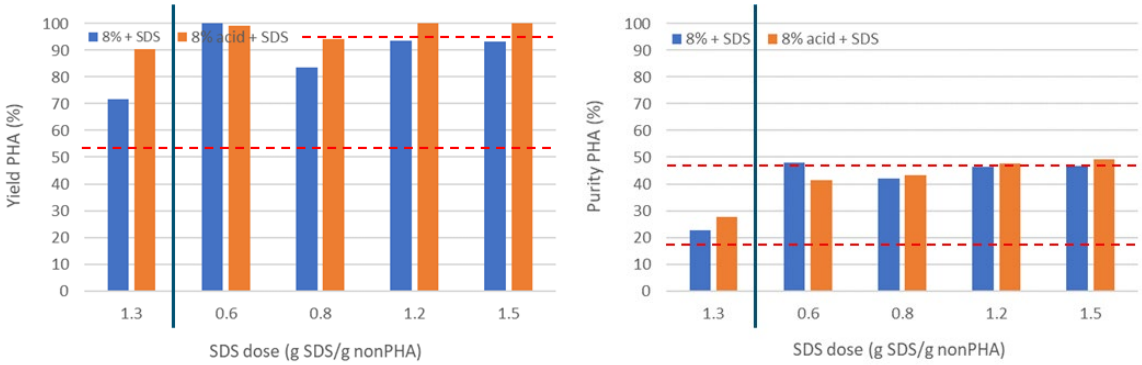


Figure A3-13 Yield and purity PHA after combined bead milling and SDS wash (second trial). Dashed lines range results second trial bead milling followed by SDS wash.

In the third combination (lower scheme in Figure 4-5) SDS and bead milling were followed by a high pH treatment. The PHA recovery was high (>90%) with comparable PHA purity (53%) at a dose of 0.3 g SDS/g non-PHA. Again a slightly higher yield for acid washed biomass was obtained.

Annex 4 Phosphate recovery from manure

Background information technology selection

In literature several techniques to separate phosphate from manure are mentioned, each having its specific drawbacks:

- Recovery of phosphate from the manure matrix by precipitation as (insoluble) struvite $NH_4MgPO_4.6H_2O$. Disadvantages are:
  - Difficult separation/extraction;
  - Not exactly known what happens when struvite is used as fertilizer in the environment (e.g. plant availability, fertilizer efficiency);
  - Application still limited due to legislation;
  - Economically hardly profitable.
- Adsorption of phosphate to iron powder enclosed in filter bags. Disadvantages are:
  - Limited exposure to reaction surface;
  - Limited capacity;
  - Not relevant for phosphate industry.
- Synthetic polymers capable of selective ion adsorption using an electric field. Disadvantages are:
  - Still in early development;
  - Complicated.
- Combustion. Nitrogen evaporates, phosphate remains in the ash. Disadvantages are:
  - Expensive;
  - Energy use is high because of high moisture content manure (heating value of manure is negative).
- Waterloo Biofilter EC-P system. Low energy electrochemistry releases iron ions into septic systems or ditch water to remove phosphorus as inert, crystalline iron phosphate minerals (vivianite). Disadvantages are:
  - Complex;
  - No feasibility of iron salts as fertilizer.

Potential advantages of using magnetic magnetite nano particles compared to the above techniques are that its approach is straightforward, easy to perform and sustainable, due to reused magnetite and products (phosphate depleted manure and phosphate). In literature there is quite some work done on vivianite using a similar principle. However, in contrast to magnetite, desorption of phosphate from vivianite requires stoichiometric quantities of acid, making the process expensive and less attractive. Magnetite ad- and desorbs phosphate based on pH. In a proof of principle, it was established that in a model solution carbonate is not in competition with phosphate. In this work, experiments were executed with real manure. Beginning with the quantification of recovery of magnetite from the liquid fraction of manure using different methods of recovery, followed by phosphate recovery from liquid acidified manure using magnetite for adsorption of phosphate and a pH swing to desorb phosphate. Finally, phosphate concentrations in manure were determined and compared using different measurement methods.

Phosphate quantification in manure

Phosphate in manure was quantified via two different methods. The first method was the photometric assay (PA), the second sequential flow analysis (SFA), carried out through the laboratories of Soil Biology Group at WUR.

For the photometric assay a calibration curve was made to verify the useable concentration range and it showed good linearity up to a concentration of around 0.3 mM. The entire curve is shown in Figure A4-1 on the left, the linear part is shown in more detail on the right. Slope and intercept of the linear region (blue dotted line, Figure A4-1 (right)) were used to calculate phosphate concentrations based on adsorption measurements.

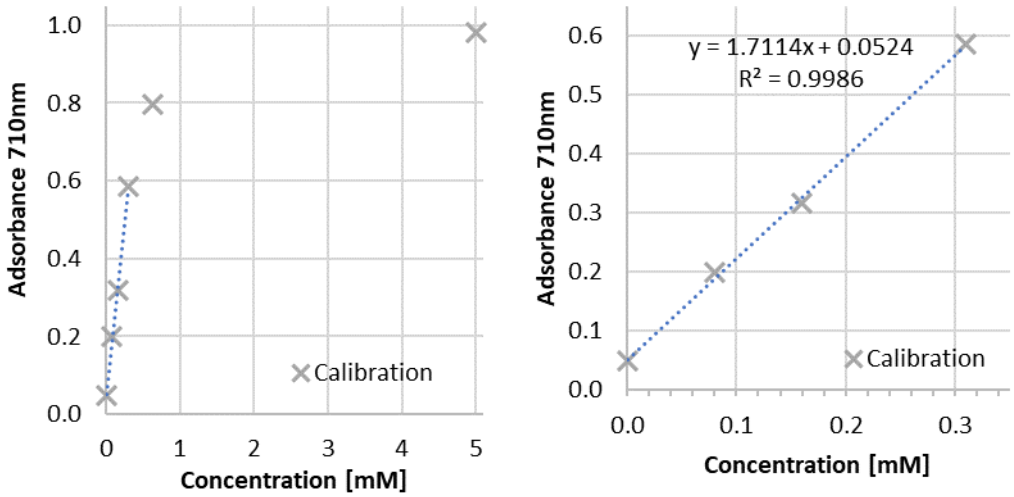


Figure A4-1 Calibration measurements. All measured points (left) and zoomed-in on the linear part, which was used as calibration curve (right).

For the measurement of phosphate in manure a dilution series of liquid manure and a new calibration line was made. The calibration line, made on the same day as the dilutions and measurements, had a slope of 1.52 AU/mM and intercept of 0.06 AU (with AU standing for adsorption units) and was linear in the range of 0.05 to 0.5 mM (Figure A4-2).

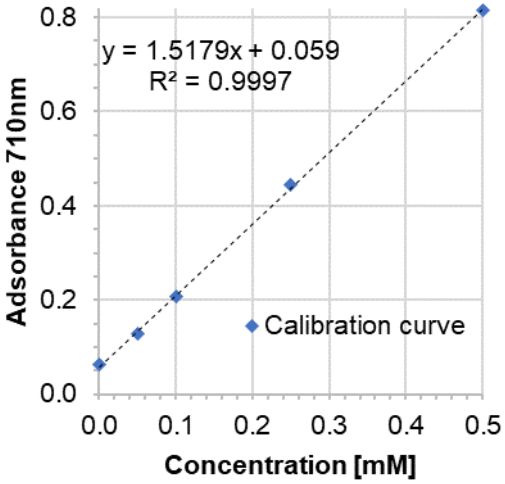


Figure A4-2 Calibration line of phosphate in milli-Q water.

Next, the photometric assay was benchmarked against a more laborious, but more accurate method to determine phosphate concentration. The same samples were analysed by the photometric assay and sequential flow analysis, and the results were compared. Comparison shows that the photometric assay and sequential flow analysis deliver results in the same order of magnitude (Figure A4-3). The photometric assay (PA) is based on a near instant measurement after appropriate sample dilution and mixture with a reactant of the assay kit. The sequential flow analysis (SFA) relies on hired technology through an external laboratory. In general it appears that SFA measured slightly higher phosphate concentrations than PA in almost all samples, with the exception of the untreated samples.

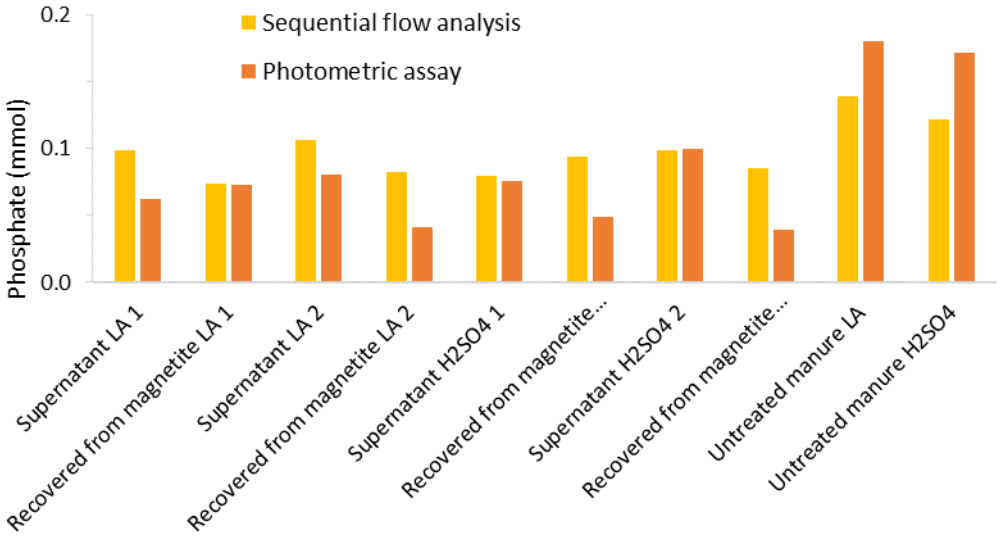


Figure A4-3 Comparison of phosphate content measured via photometric assay and sequential flow analysis. For sample description, refer to 5.2.3.

Annex 5 Cost calculation production ammonium sulphate

Kostenschatting voor Hoge druk NF en Ammonia Membraan Strippen

bluetec

16-12-2020

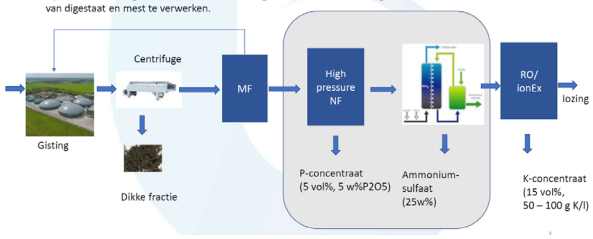
Hoge druk NF en Ammonia Membraan Strippen

BLUE-tec heeft voor de WUR onderzoek gedaan naar Hoge Druk Nanofiltratie gevolgd door Ammonium Membraan Strippen op het Microfiltratie permeaat van Groot Zevert.

WUR heeft gevraagd of BLUE-tec een inschatting kan maken van investeringskosten en operationele kosten voor deze processtappen, wanneer deze bij Groot Zevert geïmplementeerd zouden worden.

Process schema Groot Zevert

Op basis van de uitgevoerde testen en bevindingen stelt BLUE-tec het volgende schema voor om dunne fractie van digestaat en mest te verwerken.



Uitgangspunten voor ontwerp AMS

Ingangsparameters

• Nominaal	15,2 m3/h
• NH_4-N	4.500 mg/l
• T	30°C

Procescondities

• N-verwijdering	75%
• Absorptievloeistof	96% H_2SO_4
• Product	25 w% ammonium sulfaat

CAPEX en OPEX AMS

CAPEX

Investeringskosten	€ 580.000,-
Inclusief:	
- Levering complete installatie	
- Doseerstations en opslag tanks	
- In bedrijfname	
- Automatisering	
Exclusief:	
- Aansluiting op locatie	

OPEX

Energie	€ 7.900,-/j
Membraanvervanging (4 jaar)	€ 35.100,-/j
Natronloog	€ 616.600,-/j
Zwavelzuur	€ 517.400,-/j
Ammoniumsulfaat	-€ 334,500,-/j
Rente & afschrijving	€ 34.500,-/j
Totaal	€ 877.100,-/j (€ 6,26/m³ MF permeaat)

www.blue-tec.nl

bluetec

MEMBRANE TECHNOLOGY FOR A SUSTAINABLE FUTURE

Annex 6 MEZEM project proposal

Format versie juni 2022

Titel PPS-voorstel: MEZEM Mest Zonder EMissies

Aanvraagnummer: LWV22071

Inzenden uiterlijk 11 september 2022 via de indienlink op https://kia-landbouwwatervoedsel.nl/regelingen/.

Algemene informatie

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Het PPS-voorstel draagt bij aan missie:
- ☐ A. Kringlooplandbouw
- ■ B. Klimaatneutrale landbouw en voedselproductie
- ☐ C. Klimaatbestendig landelijk en stedelijk gebied
- ☐ D. Gewaardeerd, gezond en veilig voedsel
- ☐ E. Duurzame en veilige Noordzee, oceanen en binnenwateren
- ☐ F. Nederland de best beschermde en leefbare delta

Of aan Sleuteltechnologie:
- ☐ ST1. Smart Technologies in Agri-Horti-Water-Food
- ☐ ST2. Biotechnologie en Veredeling

Of aan:
- ☐ Internationalisering
- ☐ Cross-over met TKI LSH
- ☐ Cross-over met TKI Logistiek

Als hierboven een missie is gekozen, bij welk MMIP binnen die missie past het voorstel? B1

Korte samenvatting van het PPS-voorstel

MEZEM ontwikkeld technologie om enerzijds op de boerderij methaan- en ammoniakemissies uit mest verregaand te reduceren en anderzijds om tussen- en eindproducten uit mest te produceren voor optimaal gebruik buiten de landbouw. Het gaat daarbij om (extra) biogas; een ligninerijk halffabrikaat voor gebruik in de wegenbouw; constructiematerialen; en aqua ammonia (ammoniakwater) voor gebruik in de chemische industrie en/of als DeNoxing-middel. Onderzoek en innovaties in mestverwerking zijn tot op heden veelal gericht op de ontwikkeling van producten voor gebruik binnen de agrarische sector zelf. De huidige mesthoeveelheden in Nederland zijn hiervoor echter te groot en alternatieven buiten de landbouw zijn gewenst. MEZEM ontwikkelt hiervoor een alternatieve route

Ontvangen advies in fase 1: ■ positief ☐ neutraal ☐ negatief

PPS-voorstel

Bijlage 1: beschrijving state-of-the-art en deliverables
Bijlage 2: uitgebreid meerjarig werkplan

1. Doel en beoogde resultaten

Het doel van het MEZEM-voorstel is technologieontwikkeling om daarmee:

1. Methaan- en ammoniakemissies te voorkomen in de mestverwerkingsketen, te beginnen bij de mestopslag in stallen.
2. Meerwaarde te creëren uit verschillende fracties van mest door de productie van extra biogas, lignine-rijke constructiematerialen, en chemische intermediates.

De technologische interventies die MEZEM nastreeft zijn in onderstaand schema weergegeven (I t/m V). Het beoogde resultaat is een positieve business case voor emissiereductie en mestverwerking.

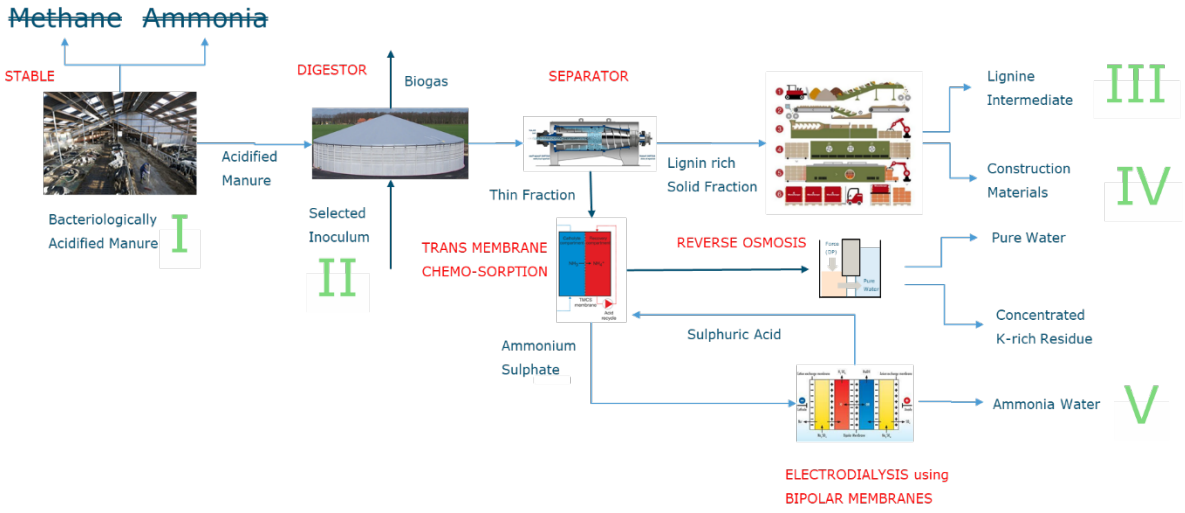


Figure 1: MEZEM-interventies (I t/m V, zie Bijlage 1) in de mestverwerkingsketen

Zowel grondgebonden als niet-grondgebonden landbouw moeten produceren binnen de grenzen van bestaande milieuregelgeving. Grondgebonden landbouw is onlosmakelijk verbonden met Nederland vanwege de gunstige agro-ecologische omstandigheden, terwijl niet-grondgebonden landbouw een belangrijke rol speelt bij het sluiten van kringlopen door waarde toe te voegen aan bijproducten uit de voedselverwerkende industrie. Het overschot aan mest dat daarbij ontstaat, zorgt voor tal van milieuproblemen, maar wordt met MEZEM verwerkt tot grondstoffen die ook buiten de landbouw kunnen worden ingezet.

MEZEM richt zich dus op het ontwikkelen van verwerkingsroutes om ongewenste emissies te voorkomen en mest om te zetten in een bio-grondstof voor waardevolle producten buiten de landbouw. Uitgangspunt hierbij is dat verse mest in de mestkelder biologische wordt verzuurd, waarmee de vorming en uitstoot van ammoniak en methaan wordt voorkomen en optimaal gebruik kan worden gemaakt van mest als bio-grondstof. MEZEM genereert daarmee extra inkomsten voor boeren en industrie.

Het project bouwt direct voort op resultaten van eerdere PPS-projecten zoals ‘Beter dan Vergisten’. In het MEZEM-project zal nauw worden samengewerkt met het lopende TKI-AF project Biovalor. Biovalor richt zich echter op technologieën voor C-, P- en N-producten binnen de landbouw. De technologie ontwikkeling (o.a. terugwinning ammoniak) in dit project zal bijdragen aan Biovalor en vice versa. Uiteraard zullen de activiteiten in applicatieontwikkeling verschillen.

De projectpartners profiteren van MEZEM als volgt:

- De agrarische sector krijgt toegang tot technologie om ammoniak en methaan emissies verregaand te reduceren;
- Mestverwerkingspartners wordt de mogelijkheid geboden om intermediates (lignine, aqua ammonia) buiten de agrarische sector af te zetten;
- De chemische industrie en de bouw wordt een bio-based grondstof geboden;
- De technologieleveranciers krijgen inzicht in de haalbaarheid van innovatieve procestechnologieën;

- De ingenieursbureaus en systeemleveranciers kunnen geavanceerde technologieën aan hun gereedschapskist toevoegen.

2. Passendheid binnen de KIA en bijdrage aan het portfolio

- **Passendheid binnen de KIA**

Dit project sluit naadloos aan bij de hoofddoelstelling van MMIP B1: *reductie van emissies uit de veeteelt* door verse mest in de mestkelder/silo te verzuren. Dit betreft hier zowel broeikasgasemissies (CH_4, CO_2, N_2O), maar in belangrijke mate ook ammoniakemissies. Tegelijkertijd biedt deze voorverzuring een betere uitgangspositie om fracties uit mest om te zetten in waardevolle biogrondstoffen; het hoofddoel uit MMIP B6.

In Missie B van de KIA is de ambitie voor landbouw opgenomen om te komen tot een reductie van 6 Mton aan broeikasgas equivalent. Deze missie is uitgewerkt in MMIP B1 E11A: *Emissiereductie methaan veehouderij*. We schatten dat het verzuren van verse mest en het daarmee voorkomen van methaanemissies (ruwweg 50% van de fermenteerbare vaste fractie in mest) potentieel een reductiebijdrage in de veehouderij kan leveren van 2.9Mton/yr CO_2 equivalent. Het voorstel sluit daarbij aan bij de geformuleerde onderzoeksprioriteiten.

Het project sluit aan bij MMIP B6 E12B: *Productie en gebruik van Biomassa*. In het bijzonder het deelprogramma*: Vaste biomassa als constructiemateriaal*. Door gebruik te maken van mestresiduen in bouwstoffen en constructiematerialen wordt CO_2 opgeslagen zolang het materiaal in de toepassing aanwezig is (negatieve C-emissie).

Onderzoek naar het gebruik van mest is momenteel vooral gericht op hergebruik in de landbouw als meststof of bodemverbetering. Dat is begrijpelijk, maar met de huidige onbalans in met name stikstof in Nederland is het gebruik in andere toepassingen nodig om de mineralenbalans te herstellen. Dit project draagt bij aan het opwaarderen van mest in meerdere hoogwaardige toepassingen buiten de landbouw.

- **Bijdrage aan het huidige portfolio**

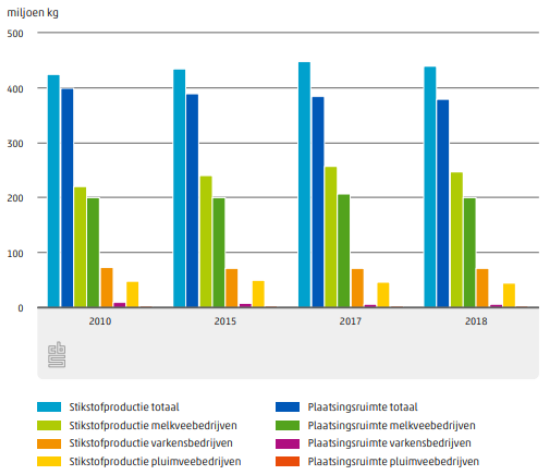
In Nederland zijn technische maatregelen om methaan- en stikstofemissies uit stallen te reduceren vooral gericht op stalsystemen (innovatieve vloeren, snelle mestscheiding, luchtwassers). Dit project daarentegen exploreert een alternatieve route namelijk het verzuren van mest in de mestkelder/silo, een methode die in Denemarken zorgt voor een substantiële emissiereductie. In Denemarken wordt daarbij zwavelzuur gebruikt wat die methode in Nederland ongeschikt maakt. MEZEM verzuurt mest echter met organische zuren, direct biologisch geproduceerd in de mestkelder/silo dan wel als reststroom uit andere sectoren (waterzuivering). Daarnaast worden nieuwe producten ontwikkeld (aqua ammonia en lignine rijke bouwstoffen) en wordt extra biogas geproduceerd.

3. Impact

- **Impact op de klimaatuitdaging**

MEZEM ontwikkeld technologie ter voorkoming van methaan- en ammoniakemissies. Uit het CBS-rapport *'Dierlijke Mest en Mineralen 1990-2018'* blijkt dat het landbouwareaal dat beschikbaar is om alle geproduceerde mest te recyclen simpelweg te klein is (zie onderstaande figuur voor de stikstof fractie). Het voorgestelde project lost het probleem op door ongewenste emissies te voorkomen en (tussen-)producten te produceren die buiten de landbouwsector kunnen worden gebruikt.

5.4.1 Stikstofproductie in vergelijking tot de plaatsingsruimte voor dierlijke mest



De C-fractie

MEZEM maakt optimaal gebruik van de koolstofrijke vezelfractie in mest, door te voorkomen dat deze fractie in de mestkelder/silo wordt omgezet in methaan met daarmee samenhangende emissies. Hierbij bouwt het voort op de resultaten van onder meer de PPS 'Beter dan Vergisten' waarin is aangetoond dat (biologische) verzuring in de mestkelder de vorming van methaan voorkomt. Tabel 1 laat zien dat daarmee in Nederland een potentiële reductie aan broeikasgasequivalent (GWP) van de sector is te realiseren van meer dan 3 miljoen ton CO_2-equivalent per jaar. Dit is 11% van de jaarlijkse CO_2-eq. uitstoot van de Nederlandse landbouw en 1.8% van de totale Nederlandse CO_2-uitstoot[3]

Tabel 1: Methaanemissies en het potentiële broeikasgasreductie-equivalent.

Manure	Production 10^6 ton/yr (CBS)	Biogas potential Nm^3/ton Manure	Methane emissie from pit (kg/ton manure)	GWP (Mton/yr CO_2 eq)
Pigs	9.1	23	1.94	0.49
Cattle	51.2	21	1.78	2,55

De deelnemers aan *de Landbouw en Landgebruik-tafel* hebben de ambitie geformuleerd om de uitstoot van broeikasgassen in Nederland in 2030 met 6 Mton CO_2-eq te verminderen (Klimaatakkoord 28 juni 2019). Op basis van de gegevens uit Tabel 1 kan worden geconcludeerd dat met het voorkomen van methaanemissies op de boerderij, 50% van deze ambitie wordt afgedekt. Daarnaast hebben genoemde deelnemers de uitdaging geformuleerd om koolstof uit mest te binden aan materialen, hetgeen met de lignine fractie één van de hoofddoelen van MEZEM is.

De N-fractie

Op boerderijen in Nederland wordt jaarlijks circa 51 miljoen ton rundermest en circa 9 miljoen ton varkensmest geproduceerd (zie tabel 1). 81% van de rundveebedrijven heeft een overproductie van stikstof en voor varkensbedrijven is dit zelfs 97%. De overproductie aan stikstof uit de veesector is ongeveer 20% en een reductie in emissies naar het milieu van 50% is vereist. Mestverwerking met de winning en afzet van mestproducten uitsluitend binnen de Agri-sector is dan ook geen oplossing voor deze overproductie en de ongewenste emissies. Binnen MEZEM wordt dit probleem aangepakt door aqua ammonia (ammoniakwater) te produceren als intermediate voor de chemische industrie.

- **Impact op duurzame hulpbronnen**

De C-fractie

De ongewenste, voortijdige omzetting van de koolstofrijke vezelfractie in de mestkelder wordt voorkomen door deze biologisch te verzuren onder toevoeging van een koolstofbron aan de mest (resultaat PPS Beter dan

[3] Welke sectoren stoten broeikasgassen uit? (cbs.nl)

Vergisten'). De kosten die hiermee gepaard gaan, worden terugverdiend door de productie van extra biogas (oplopend tot wel 3x zoveel) in een vergister. Daarnaast heeft WFBR (in samenwerking met Opure) in de afgelopen vijf jaar ontdekt dat ogenschijnlijk recalcitrant organisch materiaal vergaand tot biogas kan worden omgezet indien de toegevoegde micro-organismen (die in de vergister de vergisting uitvoeren) een rijk mengsel vormen van vele soorten. Methoden zullen worden ontwikkeld om de soortenrijkdom in een mestvergister te vergroten zodat er een flora ontstaat die de organische stof meer volledig kan omzetten waardoor meer biogas wordt geproduceerd. Het digestaat dat na optimale vergisting overblijft, kent een aanzienlijk ligninegehalte en is daarmee een potentiële goudmijn van hernieuwbare aromaten. Voor een goede evaluatie van dit potentieel moeten zowel het gehalte als de structuur van de lignine nauwkeurig worden bepaald. In wegverhardingen wordt thans bitumen gebruikt als bindmiddel op fossiele basis maar lignine is een veel belovend 'groen' alternatief.

MEZEM:

- Voorkomt ongewenste voortijdige omzetting en emissies van de vezelfractie in mest;
- Vergroot de biogasopbrengst door:
    - toevoeging van een koolstofbron aan de mestkelder en
    - de soortenrijkdom van micro-organismen in de vergister te vergroten;
- Ontwikkelt lignine uit mestdigestaat voor toepassing in weg- en bouwtoepassingen.

De N-fractie

Bij de industriële verwerking van mest wordt ammoniak momenteel teruggewonnen als een ammoniumzout. Het proces vereist volumineuze kolommen, wat de winning duur maakt. Elektroscheiding is een snel opkomende technologie waarmee in een zeer compacte membraaninstallatie selectief ammoniak uit mest kan worden teruggewonnen en geconcentreerd. In een volledig elektrisch aangedreven proces wordt de stikstof teruggewonnen als ammoniakwater waarmee de verkoopwaarde en de bruikbaarheid van het product wordt vergroot. Noodzakelijke chemicaliën worden in het proces gerecycled, er hoeven geen chemicaliën van buiten worden toegevoegd.

MEZEM:

- ontwikkelt een volledig elektrisch aangedreven membraanproces zonder toevoeging van chemicaliën voor de productie van hoogwaardig ammoniakwater uit mest.

- **Impact op de veestapel**

Door het realiseren van een economisch en milieutechnisch aantrekkelijke afzetmarkt van producten uit mest wordt een groot deel van de nadelen van de omvang van de veestapel in Nederland (CO_2- en NH_3-emissies) weggenomen. Boeren krijgen hierdoor niet alleen een aanvulling op hun inkomen (of in ieder geval eliminatie van kosten van mestafvoer), maar mest wordt ook duurzaam ingepast in de Nederlandse economische infrastructuur.

- **Kennis implementatie**

MEZEM bouwt voort op de uitkomsten van de PPS 'Beter dan Vergisten' waarin is aangetoond dat emissies op de boerderij aanzienlijk kunnen worden verminderd wanneer de pH van de mest in de mestkelder lager dan 5.8 is. Emissies van ammoniak (grotendeels) en methaan (in mindere mate) worden dus voorkomen.
De kennis die binnen dit project wordt gegenereerd, wordt op drie manieren gedeeld en geïmplementeerd:

1. De industriële partners, zowel technologieleveranciers als eindgebruikers, hebben de intentie om de resultaten te gebruiken om te commercialiseren in hun productportfolio's;
2. De resultaten worden tijdens het project besproken en gedeeld met boeren en samenwerkingsverbanden van bedrijven die boeren ondersteunen zoals LTO, ZLTO, Stichting Biomassa (coöperatie in de Achterhoek), Mestbioraffinage en mineralencentrale Groot Zevert, Proefboerderij De Marke, evenals provincies.
3. De resultaten worden door WUR gebruikt om duurzame processen en circulaire producten verder te ontwikkelen om de circulaire en toekomstbestendige landbouw te versterken. Dit begint bij een goede samenwerking met het eerder genoemde project Biovalor.

4. Aanpak van het project

MEZEM kent vijf werkpakketten waarvan vier technisch van aard en een vijfde waarin niet-technische zaken worden geadresseerd (Voor details wordt verwezen naar bijlage 2). Bij het opstellen van MEZEM hebben we ondervonden hoe de Nederlandse stikstofcrisis nieuwe initiatieven op allerlei vlak frustreert. Om die reden hebben

we het totaal budget van MEZEM verlaagt en de grootschalige pilotplant activiteiten buiten het project gelaten. Deze activiteiten worden wel beschreven maar vormen geen onderdeel van MEZEM. Voor deze activiteiten zal aanvullende financiering worden gezocht.

WP1. Emissiereductie van methaan en ammoniak en behoud van vergistbare drogestof door biologisch aanzuren van mest. Onderzoek op laboratorium- en pilotschaal naar maatregelen om de pH in de mestkelder/silo te verlagen, daarmee de methanogenese van mest te remmen en ammoniakemissie te reduceren. Het werkpakket kent als deliverables:

- D1.2 Rapport Emissie en verzuringsexperimenten
- D1.3 Geïnstalleerde melasse-mest menger
- D1.5 Rapport biogas productie verzuurde mest (p.m.)

WP 2. Optimale biogasopbrengst uit recalcitrant materiaal in mest. Onderzoek op laboratoriumschaal: is soortenrijkdom van belangrijk bij de biogasopbrengst en remt het mestmilieu de ontwikkeling van een soortenrijk mengsel? Het werkpakket kent als deliverables:

- D2.2 Rapport invloed soortenrijkdom en mestmilieu
- D2.3 Rapport Verbeteringsmaatregelen vergister: experiment grote schaal (p.m.)

WP3. Lignine uit mest een potentiële goudmijn? Zorgvuldige karakterisering van lignine uit mest, procesontwikkeling en applicatieonderzoek voor het gebruik als bindmateriaal in asfalt cq als lijm in constructiematerialen. Het werkpakket kent als deliverables:

- D.3.1 Best performing lignin fractionation technology.
- D.3.2 Structure-function relationships lignin fractions.
- D.3.3 lignin sample fractions.
- D.3.4 Test report lignin fractions (asphalt or adhesive).
- D.3.5 Report potential lignin from manure.

WP4. Aqua Ammonia uit mest een groene intermediate! Onderzoek naar een economisch winning van de ammoniak uit mest met een innovatief proces van Trans Membraan Chemo Sorptie (TMCS) in combinatie met Electrodialyse met Bipolaire Membranen (EDBM). Het werkpakket kent als deliverables:

- D4.1 Beste ontwerp en operatiecondities TMCS + EDBM
- D4.2 Beste ontwerp en operatiecondities pretreatment
- D4.3 Beste ontwateringstechnologie en locatie (p.m.)
- D4.4 Geïntegreerd systeem TMCS + EDBM

WP 5. ‘De ondersteunende activiteiten’. Projectmanagement, niet technische aspecten (LCA, wetgeving mestgebruik buiten de sector) kennisverspreiding.

De onderstaande tabel geeft een beeld van de planning van het project. Na ieder jaar worden per werkpakket go-/ no-go beslissing genomen.

Acitiviteiten	jaar 1				jaar 2				jaar 3			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
WP1: Emissiereductie en behoud drogestof: biologisch aanzuren												
1.1 Emissie experimenten met verzuurde mest Dairy Campus	xxx	xxx										
1.2 Lab. experimenten met alternatieve koolstofbronnen			xxx	xxx								
1.3 Menging koolstofbron aan mest					xxx	xxx	xxx	xxx				
1.4 Methaan productie bij vergisten van verzuurde mest									xxx	xxx		
1.5 Potentieel van alternatieve azijnzuurroute (pro memorie)											xxx	xxx
WP2: Optimale biogasopbrengst: soortenrijkdom												
2.1 Lab. Expl soortenrijkdom en biogasopbrengst		xxx	xxx	xxx								
2.2 Verbetering van de mestvergisting op labschaal					xxx	xxx	xxx	xxx				
2.3 Proef op grote schaal (pro memorie)									xxx	xxx	xxx	xxx
WP3: Lignine uit mest: Een ggoudmijn?												
3.1 extraction and purification of lignin from manure/digestate	xxx	xxx	xxx	xxx								
3.2 Analysis lignin-rich fraction for application		xxx										
3.3 Upscaling for manure/digestate lignin fractionation					xxx	xxx	xxx	xxx				
3.4 Manure derived lignin functionality testing in asphalt binders									xxx	xxx		
3.5 Manure derived lignin functionality testing in adhesives									xxx	xxx		
3.6 Determine overall potential												xxx
WP4: Aqua Ammonia: Cascade TMCS + EDBM												
4.1 Cascade TMCS + EDBM: zonder zuurconsumptie		xxx	xxx	xxx	xxx	xxx	xxx					
4.2 Pretreatment: vermijden baseconsumptie					xxx	xxx	xxx	xxx				
4.3 Ontwateren: minimaliseren van waterige stromen (pro memorie)					xxx	xxx	xxx	xxx				
4.4 Integraal Systeem									xxx	xxx	xxx	xxx
4.5 Demo op locatie (pro memorie)												

5. Organisatie

Het MEZEM consortium bestaat uit één academisch kennisinstituut en meerdere particuliere industriële partners die de gehele waardeketen bestrijken.

Tussen de projectpartners wordt een Consortium Agreement overeengekomen, die de basis zal vormen voor de samenwerking, en voor de interne en externe communicatie. Het project zal worden begeleid door een stuurgroep bestaande uit één senior vertegenwoordiger van elke partner. Deze stuurgroep komt elk kwartaal via teleconferentie bijeen, waarvan minimaal tweemaal per jaar in persoon.

De dagelijkse uitvoering van het project wordt geleid door senior onderzoeker en projectleider van WFBR. Hij/zij wordt ondersteund door de werkpakketleiders voor elk van de afzetgebieden (C-, en N-fracties). De voortgang en eventuele knelpunten worden bewaakt door de verspreiding van voortgangsrapportages die worden samengesteld door het onderzoekskernteam. Deze rapporten worden besproken in de vergaderingen van de stuurgroep.

6. Kennisvalorisatie en -disseminatie

De projectresultaten zullen door de industriële partners worden gebruikt in hun bestaande bedrijfsvoering en in overeenstemming met hun motivatie om aan dit project deel te nemen. Verspreiding van kennis buiten dit consortium zal altijd plaatsvinden na instemming van alle partners en na (eventuele) aanvraag van octrooien die nieuw gecreëerde IP beschrijven en beschermen. Het consortium zal de kennis verspreiden via de volgende kanalen om de volgende gemeenschappen te bedienen:

- Kennis wordt verspreid onder de wetenschappelijke gemeenschap door publicatie in wetenschappelijke tijdschrift(en) en door het geven van lezing(en) op geschikte conferenties en/of symposia.
- Kennis wordt verspreid naar de professionele gemeenschap door posts op LinkedIn en per artikel(en) in relevante vakbladen en op de websites van de consortiumpartners.
- Kennis wordt verspreid onder het grote publiek door een nieuwsbericht te publiceren via een geschikt, algemeen kanaal, zoals een krant.

Dit in aanvulling op de onder hoofdstuk 3 genoemde kennisimplementatieactiviteiten.

7. Financiering en begroting

Wordt gereserveerde PPS-toeslag ingezet?

■ Nee

☐ Ja, geef aan welk TKI het betreft: ……

Is de begrote cofinanciering reeds definitief toegezegd door de betreffende partners?

☐ Ja

■ Nee, Geef aan welk deel van de cofinanciering (bedrag) nog niet definitief is toegezegd:

Van welk percentage private cofinanciering gaat het voorstel uit?

■ minimaal 50% (standaard)

☐ minimaal 30% (uitzondering).

Bij 30%: in fase 1 is deze beoordeeld als ☐ redelijk ☐ neutraal ☐ niet redelijk

Tabel 1. Samenvatting kosten en financiering.

Costs	2023	2024	2025	2026	2027	Total
	Amounts in k€ (excl. VAT).					
Cost knowledge institutes (from **Table 2a**)	135.0	273.0	273.0	135.0	-	816.0
Cost other (In-kind) project partners (from **Table 2b, 3a, 3b**))	45.0	91.0	91.0	45.0	-	272.0
Overall Costs	180.0	364.0	364.0	180.0	-	1,088.0
Financing						
Co-financing	**2023**	**2024**	**2025**	**2026**	**2027**	**Total**
In-kind contribution private partners (from **Table 3a**)	45.0	91.0	91.0	45.0	-	272.0
In-kind contribution public partners (from **Table 3b**)	-	-	-	-	-	-
Cash contribution private partners (from **Table 3c**)	45.0	91.0	91.0	45.0	-	272.0
Cash contribution public partners (from **Table 3d**)	-	-	-	-	-	-
Total co-financing	90.0	182.0	182.0	90.0	-	544.0
Requested public contribution	**2023**	**2024**	**2025**	**2026**	**2027**	**Total**
WR capacity requested or TO2 contribution (Table 3e)	90.0	182.0	182.0	90.0	-	544.0
PPP grant requested (from Table 3e)	-	-	-	-	-	-
Total public contribution requested	90.0	182.0	182.0	90.0	-	544.0
Total financing	180.0	364.0	364.0	180.0	-	1,088.0

Handtekening(en) voor akkoord:

Kennisinstelling: Wageningen Food and Biobased Research

Naam: ir. E. (Edwin) Hamoen (Program Manager Biorefinery)

Handtekening:

Datum: 9 September 2022

Private trekker:

Naam en bedrijf/organisatie:

CCS Energie-Advies

Dr.ir. R. (René) Cornelissen

Handtekening:

Datum: 10 September 2022

Bijlage 1: State of the Art en deliverables

Het doel van MEZEM is enerzijds om ongewenste ammoniak- en methaanemissies op de boerderij verregaand te reduceren en anderzijds om afzetgebieden buiten de landbouw te ontwikkelen van producten uit zowel runder- als varkensmest. De maatregelen die worden voorgesteld zijn:

- Het biologisch aanzuren van mest;
- Biogas uit recalcitrant materiaal;
- Lignine uit mestdigestaat;
- Aqua Ammonia uit mest met Trans Membraan Chemo Sorptie (TMCS) in combinatie met Electrodialyse met Bipolaire Membranen (EDBM).

1. Emissiereductie en behoud van vergistbare drogestof door biologisch aanzuren van mest

1.1 State of the art

Voor een uitgebreide beschrijving van de state-of-the-art van het aanzuren van mest wordt verwezen naar een recent verschenen rapport van Puente-Rodriguez et al 2022. In de samenvatting stellen zij dat het aanzuren een kansrijk principe is om methaan- en ammoniakemissies uit mest te reduceren. Het aanzuren heeft in Nederland tot nog toe geen voet aan de grond gekregen, onder andere door (i) de veronderstelde hogere kosten ten opzichte van emissiearme vloersystemen; (ii) de noodzaak van een externe mestopslag; (iii) de zorgen over de mogelijke risico's op verzuring van de bodem; en (iv) verhoogde concentratie zwavel in bodem en in grond- en oppervlaktewater. De haalbaarheid van het aanzuren lijkt echter te veranderen en de genoemde studie identificeert de kansen en belemmeringen voor de implementatie in Nederland.

Zoals gesteld, wordt het aanzuren van mest thans gezien als een kansrijke maatregel die, afhankelijk van het zuur en de toegevoegde hoeveelheid, leidt tot:

i. een emissiereductie van methaan uit mest van 65-90% bereikt kan worden (Petersen et al., 2012; Petersen et al., 2014; Sommer et al., 2017; Habtewold et al., 2018), en
ii. substantiële vermindering van de ammoniakemissie, zowel in de stal als tijdens het uitrijden. Bij aanzuren in de stal (melkveehouderij) wordt in Denemarken een ammoniakemissie reductie van rond 40% aangehouden en bij aanwenden van 49%.

In Denemarken is het aanzuren van mest al meer dan tien jaar als emissie-reducerende maatregel toegestaan en gecommercialiseerd. Daarbij wordt op de boerderij de pH van mest met zwavelzuur aangepast (JH AGRO A/S - Environmental Solutions for Animal Production). Volgens een VERA-statement leidt deze techniek tot een reductie van de ammoniakemissie van 64% in de varkenshouderij. (VERA-Urkunde006_JH-Forsuring-NH4.indd (vera-verification.eu)). Momenteel zijn er in Denemarken 75 melkveebedrijven en 76 varkensbedrijven voorzien van dit systeem. In Denemarken wordt mest ook aangezuurd tijdens aanwending.

Ten aanzien van emissies uit mest lag en ligt in Nederland de focus met name op ammoniak. Als gevolg van klimaatverandering en daaraan gekoppelde nationale en internationale klimaatdoelstellingen is daar de noodzaak bijgekomen om methaanemissies in de landbouw te beperken. Puente-Rodriguez et al 2022 stellen dat in samenhang met de hardnekkige stikstofcrisis en de behoefte aan integrale oplossingen, de balans ten aanzien van het aanzuren van mest mogelijk zal omslaan.

Methaan (CH_4) in mest wordt gevormd bij de afbraak van organische stof (OS). Het wordt in mest geproduceerd door methanogene micro-organismen onder aerobe omstandigheden. De huidige praktijk om mest gedurende langere tijd in mestkelders/silo's op te slaan zorgt nu echter voor de juiste omstandigheden voor deze micro-organismen om zich te vermenigvuldigen en methaan te vormen. Het aanzuren van mest remt deze activiteit van methanogene micro-organismen.

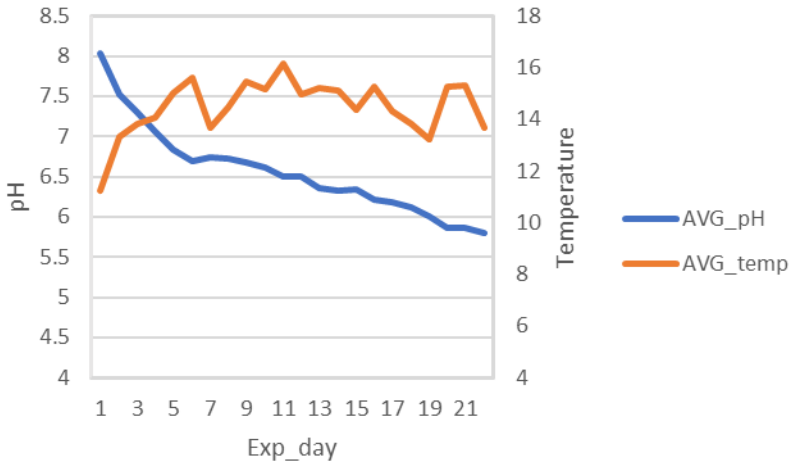
Feces en urine bevatten geen ammoniak. Ammoniak ontstaat als ureum in de urine wordt omgezet met behulp van het enzym urease dat aanwezig is in de feces en in de biofilm op oppervlakken als een bevuilde vloer. Urease heeft een optimale activiteit bij pH 7.4 waarbij uit ureum zowel ammoniak als kooldioxide ontwijken. Een lage zuurgraad beïnvloedt niet alleen de urease-activiteit maar ook het evenwicht tussen ammonium en gasvormig ammoniak. Bij een pH <5,5 is ammoniakemissie uit de mest bijna nul (Bussink et al., 2012).

De conclusie is dat met een pH in de mestkelder/silo <6 en bij uitrijden <6,5-7, significante reducties in ammoniak- en methaanemissie worden bereikt. Dit maakt aanzuren een interessante maatregel voor de verdere verduurzaming van de veehouderij. Op basis van de inzichten beschreven in Puente-Rodriguez et al 2022 wordt geconcludeerd dat aanzuren van mest het meest effectief is bij een integrale aanpak van emissies. Logischerwijs zal de belangstelling voor het aanzuren van mest toenemen naarmate emissie-eisen worden aangescherpt.

1.2 De Vernieuwing

Drogestof-verliezen uit mest op de boerderij kunnen worden voorkomen door de pH in de mestkelder/silo te verlagen en daarmee de methanogenese van mest te remmen en, parallel daaraan, ammoniakemissie te reduceren. Toevoeging van een mineraal zuur (bijv zwavelzuur) is echter duur en kent landbouwkundige en milieutechnische bezwaren. Een alternatief voor chemische verzuring is biologische verzuring met behulp van nature aanwezige organische zuurvormende micro-organismen, in combinatie met vergistbare substraten (koolhydraten). Toevoeging van pure suikers aan mest is kostbaar en daarom zijn in de PPS Beter dan Vergisten de mogelijkheden bestudeerd om organische reststromen uit de agro- en voedingsindustrie in te zetten als betaalbare bron van vergistbare koolhydraten. Een dergelijke biologische verzuring door toevoeging van fermenteerbare koolhydraten en/of effectieve micro-organismen is al wel gebruikelijk bij het inkuilen (3) maar is voor mest nog niet goed bestudeerd (4).

In de PPS 'Beter dan Vergisten' is aangetoond - eerst op laboratoriumschaal en daarna in een stal van de Dairy Campus (Leeuwarden) - dat mest biologisch kan worden verzuurd onder toevoeging van melasse als koolstofbron. Onderstaande figuur laat zien hoe de zuurgraad in de mestkelder afneemt van pH>8 op dag 1 naar pH<5.8 na drie weken, aan het einde van de proefperiode en onder dagelijkse toevoeging de koolstofbron.



In de state-of-the-art wordt geconcludeerd dat bij een pH <6 sprake is van een substantiële emissiereductie. In het experiment op de Dairy Campus werd deze emissie helaas niet bemeten. Om die reden zullen in MEZEM deze aanvullende metingen als nog worden uitgevoerd, zodat later over het reductiepotentieel geen verwarring ontstaat.

In de PPS Beter dan Vergisten is met succes melasse gebruikt als koolstofbron voor de verzuring van mest. Er zijn echter andere restproducten uit de agroketen beschikbaar als alternatief (zie o.a. Positieve lijst co-vergisting). In MEZEM worden de mogelijkheden van deze alternatieven nader in ogenschouw genomen en getest. In de uitgevoerde experimenten op de Dairy Campus is dagelijks een hoeveelheid melasse met gieters over de stalvloer uitgegoten. In experimenten is een dergelijke manier van werken nog acceptabel maar in de praktijk is dat niet zo en is verregaande automatisering vereist. MEZEM zal in samenwerking met de industrie hiervoor een systeem ontwerpen.

De toevoeging van melasse als koolstofbron resulteert in de mestkelder in de biologische productie van vetzuren, met name azijnzuur. Indien deze vetzuren vervolgens in een vergister worden omgezet in methaan (1 mol azijnzuur geeft in 1 mol methaan), dan resulteert dit in een aanzienlijke verhoging van de biogasproductie (bij 7% melasse toevoeging is op papier sprake van 2-3x meer biogas opbrengst). Kosten voor de aankoop van melasse kunnen op deze wijze worden gecompenseerd met extra gasopbrengst. In MEZEM wordt de economie van deze verwerkingsroute verder uitgewerkt.

De productie van vetzuren kent ook belangstelling van partijen die thans afvalwater zuiveren en GFT-verwerken. We verwachten dat langs deze weg in de nabije toekomst grote stromen goedkoop azijnzuur op de markt komen. Als alternatieve verzuringsroute zou azijnzuur dan direct aan de mest kunnen worden toegevoegd; zoals bij het gebruik van melasse (Beter dan Vergisten) of zwavelzuur (Denemarken). Bij het vervolgens vergisten van mest draagt het toegevoegde azijnzuur ook bij aan een extra opbrengst aan biogas en de economie, dit in contrast met het toevoegen van zwavelzuur. Ook de alternatieve azijnzuurroute zal in MEZEM in een experimenteel programma op de Dairy Campus worden uitgewerkt.

1.3 Referenties

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Sommer, S.G., Clough, T.J., Balaine, N., Hafner, S.D., & Cameron, K.C. (2017). Transformation of Organic Matter and the Emissions of Methane and Ammonia during Storage of Liquid Manure as Affected by Acidification. Journal of Environmental Quality, May-June, 514-521. doi:10.2134/jeq2016.10.0409.

Uitvoeringsregeling Meststoffenwet, geldend van 01-01-2022 t/m 15-04-2022: Regeling van de Minister van Landbouw, Natuur en Voedselkwaliteit van 4 november 2005, nr. TRCJZ/2005/3295, houdende regels ter uitvoering van de Meststoffenwet (Uitvoeringsregeling Meststoffenwet)

2. Hogere biogasopbrengst uit recalcitrant materiaal

2.1 State of the art

Biogas bestaat uit methaan en koolstofdioxide en kan o.a. gebruikt worden om groen gas te produceren dat in het gasnet kan worden afgeleverd. In Nederland wordt dat vaak gedaan, bijvoorbeeld door bedrijven die mest vergisten (omzetten in biogas). De stand van de techniek is dat de organische stof in de huidige mestvergisters onvolledig wordt omgezet in biogas. Dat is vooral het geval met rundermest, dat veel lignocellulosehoudende vezels bevat die ook al in de pens van de koe zijn vergist. Wat er overblijft (de mest) is daardoor moeilijk nogmaals te vergisten. Een studie van Eneco noemt een opbrengst van 210 Nm^3 biogas per ton mest organisch stof, terwijl het theoretisch maximum 500-600 Nm^3/ton organisch stof is (Haalbaarheids-studie Bergambacht, 2010). Alfa et al. (2021) heeft 160 Nm^3/ton organisch stof gevonden voor puur rundermest (dus geen covergisting).

Volgens Khan en Ahring (2021) wordt de lage biogas opbrengst veroorzaakt doordat de organische stof recalcitrant is voor vergisting en dat dat vooral komt doordat rundermest voor 40-60% bestaat uit lignocellulosehoudende vezels. Bekend is dat dat soort vezels maar voor 20-30% worden omgezet in een vergisting. Lignocellulose is een stevig complex van cellulose, hemicellulose en lignine. Vandaar dat de oplossing vaak wordt gezien in het opbreken van de lignocellulose. In een recent artikel (Khan en Ahring, 2021) wordt eerst een overzicht gegeven wat onderzoekers hebben geprobeerd aan voorbehandeling van mestvezels: mechanische kracht (wet explosion), inweken in ammoniumhydroxyde, verhitten en koken met loog. Bij deze methoden is het dan slim om niet de verse mest te nemen maar de vaste fractie van het digestaat en die te onderwerpen aan bovengenoemde ontsluitingsmethoden. Zo konden onderzoekers 136% meer methaan halen uit vergiste mestvezels, na nogmaals vergisten t.o.v. onbehandelde vergiste mest (Khan en Ahring, 2021). Khan en Ahring (2021) hebben zelf geëxperimenteerd met vergiste mestvezels en die onderworpen aan verhitting bij 180°C met en zonder 3% NaOH. De combinatie van loog en verhitting leverde 127% meer methaan na vergisting. Echter het toedienen van loog en het verhitten (hoge druk nodig) is een methode die lastig is uit te voeren. WFBR heeft in het TKI project ‘Beter (dan) Vergisten’ mestvezels uit digestaat uit een rundermestvergister behandeld met het sterk oxidatieve Fenton reagens (ijzer en waterstofperoxide). Dat is een iets schonere en mildere methode. Na 30 dagen vergisten leverde dat 60% meer biogas, maar na 60 dagen liep het verschil tussen behandelde en onbehandelde mestvezels terug tot 30%. De

kosten van het gebruikte peroxide zijn vooralsnog hoger dan de waarde van het geproduceerde biogas. Ook de andere voorbehandelingsmethoden hebben een kostenprobleem.

Wereldwijd worden dan ook geen voorbehandelingsmethoden gebruikt om meer biogas te halen uit mest. Ook het gebruik van enzymen heeft niet doorgezet. Bij de vergisting van rioolwaterslib en GFT worden hier en daar thermische methoden gebruikt (hoge druk hydrolyse), maar niet bij mest. Bij de vergisting van mest is er dus nog een groot onbenut potentieel, maar een eenvoudige betaalbare methode om dat potentieel aan te boren ontbreekt nog. Een betere omzetting van organisch materiaal naar biogas heeft niet alleen het voordeel van de productie van meer hernieuwbare energie, ook het digestaat wordt dunner, terwijl ammonium en fosfaat beter vrijkomen, wat gunstig is bij het winnen van stoffen uit het digestaat. Ook zal het ligninegehalte van de vaste fractie hoger zijn, waardoor het winnen van lignine ook eenvoudiger wordt.

2.1 De vernieuwing

WFBR (in samenwerking met Opure) heeft in de afgelopen vijf jaar ontdekt dat ogenschijnlijk recalcitrant organisch materiaal toch vergaand in biogas kan worden omgezet indien de toegevoegde micro-organismen (die de vergisting moeten uitvoeren) een rijk mengsel vormen van vele soorten. Zo hebben we de dikke fractie van rundermestdigestaat (dus zeer recalcitrant) vergist met entmateriaal bestaande uit een mengsel van verschillende digestaten o.a. zuiveringsslib, gft vergister en mest/co-vergister en dat vergeleken met entmateriaal uit een rundermestvergister. Met het entmateriaal uit de rundermestvergister werd 60 Nm^3 biogas per ton organische stof geproduceerd en met het mengsel 250 Nm^3 biogas per ton organische stof. In een andere serie konden we zelfs 300 Nm^3 halen: dat is extra biogas nadat de eerste vergisting al is geweest, dus in een tweede vergisting. Deze recalcitrante vezels kunnen dan nog eens 39% worden afgebroken (39% van de organische stof wordt omgezet in biogas). Experimenten met tarwestro (bijna puur lignocellulose) leverde met het boven beschreven rijk entmateriaal 550 Nm^3 biogas per ton organische stof, nagenoeg het theoretisch maximum.

Een clou wat hier aan de hand zou kunnen zijn wordt gegeven door recente experimenten van Alfa et al. (2021) met vergisting van rundermest, paardenmest en mengsels van die twee. De vergisting werd uitgevoerd in batches van 37 dagen zonder additioneel entmateriaal (de mest zelf moest de micro-organismen leveren). Lage biogasopbrengsten werden gevonden wanneer alleen maar rundermest werd vergist of alleen maar paardenmest, maar in mengsels werd per gram organische stof tweemaal zoveel biogas geproduceerd. De auteurs overwegen dat dit wel eens zou kunnen komen door het bredere spectrum van micro-organismen in het geval van mestmengsels. Meer soorten geeft meer kansen. Elke soort heeft zijn eigen specialisatie in het afbreken van organisch materiaal en aangezien het organisch materiaal uit vele verbindingen bestaat, in verschillende configuratie/ordening, zijn er waarschijnlijk honderden soorten micro-organismen betrokken bij de omzetting van mest in biogas. De hypothese is dat de huidige mestvergisters te arm zijn in soortenrijkdom (biodiversiteit) zodat bepaalde omzettingsroutes niet goed verlopen. De Nederlandse microbioloog/botanicus Baas-Becking stelde “alles is overal, het milieu selecteert”. Volgens zijn postulaat ontstaat in en mestvergister (zuurstofloos, afbreekbaar materiaal, 1,5 maand tijd) vanzelf een microbiële flora die de organische stof omzet in biogas. De meest geschikte micro-organismen zullen zich ontwikkelen uit een keuze van miljoenen soorten. Maar in een mestvergister waarin ook remmende stoffen zoals sulfide en ammonium aanwezig zijn moeten de micro-organismen niet alleen organische stof kunnen afbreken maar ook kunnen overleven in een ongunstig milieu en dan wordt het keuzeproces anders; de spoeling wordt dan dun.

In dit project gaan we onderzoeken of de biogasopbrengst iets te maken heeft met de soortenrijkdom en hoe we de soortenrijkdom in een rundermestvergister kunnen vergroten zodat er een flora ontstaat die de organische stof meer volledig kan omzetten (waardoor meer biogas wordt geproduceerd). Dat zou kunnen leiden tot een aanbeveling om iets aan de vergisting te veranderen, bijvoorbeeld de verlaging van de vrije sulfideconcentratie (door ijzer toe te voegen) of door continu een co-substraat toe te voegen waarin veel bruikbare micro-organismen aanwezig zijn.

2.3 Referenties

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3. Lignine from manure

3.1 State-of-the art

After grass, corn silage is the main crop used for the production of cattle and general ruminant feeds (Van Schooten et al, 2018). As such, cattle and ruminant diets are relatively rich in plant cell wall material in the form of lignocellulose, an intricate complex comprised of cellulose, hemicellulose and lignin. The former two are polysaccharides and the latter is a heterogeneous aromatic polymer, particularly recalcitrant towards conversion and digestion (Himmel et al., 2017). In general, herbaceous secondary cell walls are composed of 30-40% w/w cellulose, 20-40% w/w hemicellulose and 15-30% w/w lignin (Sun & Cheng, 2002).

To ensure future food security, competition for arable land between food and feed ingredients should be minimized. Hence, feed producers are encouraged to increasingly incorporate food waste and agricultural side-streams in animal diets (FAO, 2011), which is further driven by sustainability and circularity ambitions. This is not limited to ruminants, but also includes pig and poultry feeds. Examples of conventional side-streams targeted for animal feed application are wheat straw, wheat bran and brewer's spent grain, but the scope is expected to expand to lower value heterogeneous side-streams like prunings and leaf litter in the near future as well (see TKI LWV22.115 FEFO). Due to their lignocellulose-rich nature, the incorporation of said side-streams will increase the fibre fraction in the diet, and concomitantly also increase the content of lignin.

Being recalcitrant against conversion and fermentation, substantial amounts of lignin accumulate and end up in manure (Hills & Roberts, 1981). Manure lignin contents can amount up to 30% w/w of the dry matter (Mafongoya et al., 2000) (Møller et al., 2004). Lignin is also largely recalcitrant to anaerobic digestion and hence, lignin contents increase even further going from manure to the solid digestate (Häfner et al., 2022) (Teater et al. 2011). Likewise, ash contents increase in the digestate compared to manure. It must be noted, though, that lignin contents heavily depend on the diet and, importantly, on the analytical method used for lignin determination.

Given the substantial lignin content, manure and digestate could be very low value resources and potential goldmines of renewable aromatics. Currently, lignin is mainly obtained as a side-stream in the pulp and paper industries and lignocellulosic biorefinery industries. At present, however, these lignin-rich side-streams are mainly used for internal energy generation, but higher value application is necessary if future economic profitability and true circularity are strived for (Figure 1).

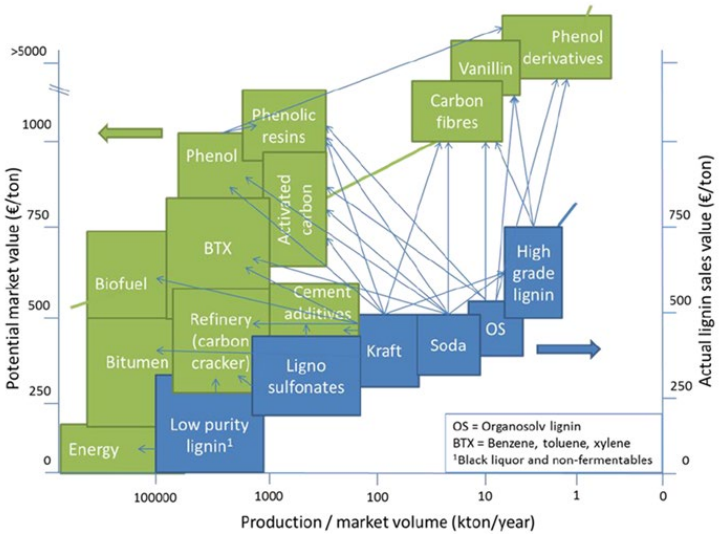

Figure 1: Current available lignin types and potential applications: volumes and values (Gosselink, 2011).

Such higher-value applications are targeted in the running TKI project 'More Reactive Lignin' (LWV20.136), through the development of mild lignin fractionation technologies and targeted modification of the fractionated lignins, as alternative to the traditional pulping methods used industrially. These mild fractionation technologies are currently being developed on various feedstocks, including miscanthus, pine and eucalyptus, and it will be crucial for manure lignin valorization to assess the use and efficiency of these technologies on manure as well.

Expected challenges include the manure and digestate high ash contents, buffering capacity and intimate interaction of lignin with other recalcitrant, undigested polymers in the feces matrix. Important in this sense is that the residual fraction will likely be of fundamentally different composition than when biomass itself is targeted, due to the fact that the original polysaccharides are largely converted and/or removed already and recalcitrant biopolymers and ashes are particularly accumulated. Hence, also the valorization of this residue will be a specific target of the MEZEM project, where ultimate road and construction applications are envisaged.

3.2. The Innovation

Manure/digestate lignin valorization: application potential

For proper evaluation of the potential of lignin in manure and digestate, both its content and structure need to be determined accurately. However, the issues that affect lignin fractionation highlighted above, likely also impact lignin analysis. Hence, the MEZEM project will make use of highly sophisticated analytical tools for lignin analysis, both contentwise and structure wise. Amongst these tools is the recently developed quantitative pyrolysis-GC-MS platform (Van Erven et al., 2017) (Van Erven et al., 2021), which in close collaboration with WUR Food Chemistry is currently being developed further to accommodate animal feed and derived faecal samples (NWO LiFeTracer, 17874).

Fractionated manure lignins will be analyzed with an extensive analytical suite, including molecular weight through SEC, subunit composition and linkages through 2D NMR, hydroxyl type and content through ^{31}P NMR (Constant et al., 2016). Guided by these structural insights, and greatly relying on the expertise built in lignin structure-function relationships, specific application and valorization routes will be decided upon. MEZEM can clearly benefit from the available expertise and infrastructure in this direction at WFBR.

Promising technologies for manure/digestate lignin fractionation will be upscaled for actual application testing to 2L and 20L scale, for which reactors and separation equipment is available at WFBR. Examples of such applications include fossil-based binder replacement in the direction of asphalt, adhesives, ink and phenol-formaldehyde resins.

Manure/digestate residual fibre fraction valorization: road and construction application

In road pavements bitumen is used as the fossil-based binder. Currently, a strong focus is given to the development of sustainable asphalt binders for the future. Here, lignin plays an important role as this biogenic carbon source and binder in nature has a strong impact on the substantial lowering of the greenhouse gas emissions capitalized by Moretti et al. (2022) as a result of the Dutch funded project CHAPLIN TKI BBEG and CHAPLIN XL. Anno 2022 more than 30 demonstration roads have been paved with a lignin-bitumen binder in which the substitution level is up to 50% of lignin (Landa and Gosselink, 2019). As wood lignin from Kraft pulping is expected to be more expensive than for example lignin in side streams like manure or digestate this could be very interesting for this application. Asphalt binders represent a very large market of 90 Millions of tonnes per year globally, so a large potential is there. The challenge will be to fractionate and purify the manure's lignin stream, in order to act as a bitumen substitute for asphalt application. We expect that the lignin content in this manure stream should be upgraded to more than 60%, so partial removal of other constituents is needed. Said road pavements also contain substantial amounts of filler material, a function that could potentially be fulfilled by the ash fraction of the residue.

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4. Aqua Ammonia uit mest met Trans Membraan Chemo Sorptie (TMCS) en Electrodialyse met Bipolaire Membranen (EDBM)

Ammoniak uit mest wordt in de praktijk teruggewonnen uit een gasfase van mest die wordt gewassen met zwavelzuur of salpeterzuur, waarbij zwavelzuur/salpeterammoniumzout als meststof wordt gevormd. Het proces vereist volumineuze kolommen, wat het duur maakt in relatie tot de waarde van de meststof.

Elektroscheiding is een snel opkomende technologie waarmee in een zeer compacte membraaninstallatie selectief ammoniak uit mest (dunne fractie) kan worden teruggewonnen en geconcentreerd. In een volledig elektrisch aangedreven proces wordt de stikstof teruggewonnen als aqua ammonia (ammoniakwater) en niet als ammoniumzout. Dit vergroot het verkoopvenster en de bruikbaarheid van het product, voorkomt het gebruik van extra chemicaliën en dus het ontstaan van zoute afvalstromen. In dit werkpakket wordt hiertoe een volledig elektrisch aangedreven membraanproces ontwikkeld waarin transmembraan-chemosorptie (TMCS) wordt gekoppeld aan Elektrodialyse men bipolaire membranen (EDBM) (zie ook schema op p2).

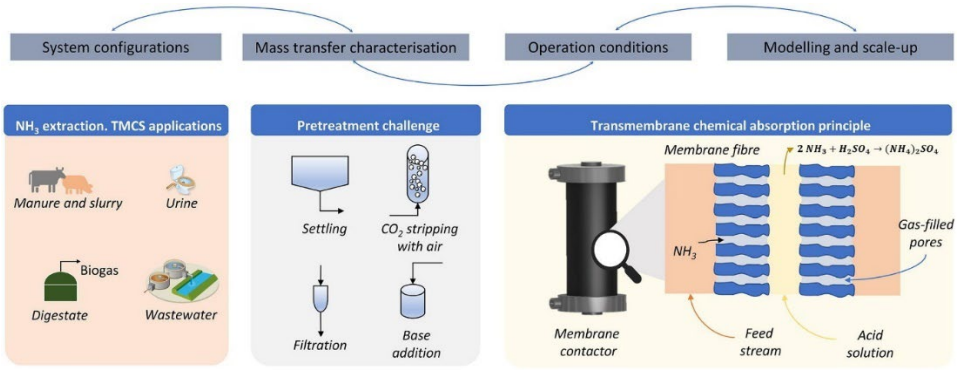
4.1 State of Art TMCS

Recent is een reviewartikel gepubliceerd dat een goed overzicht geeft wat betreft de toepassingen en uitdagingen van TransMembraan-Chemosorptie (TMCS, ook wel membraanstrippen genoemd) voor het terugwinnen van ammonium-uit afvalwater (Gonzalez-Salgado et al., 2022), zie Figuur 1.

Van TMCS zijn drie commerciële toepassingen bekend voor het terugwinnen van ammonium (NH_4^+) uit een digestaat van een afvalwaterzuivering en het maken van kunstmest in de vorm van ammoniumsulfaat $(NH_4)_2SO_4$. In die toepassingen wordt een afvalwaterstroom gestript van ammonia (opgelost NH_3) in een hollevezelmembraancontactor (van LiquidCel Membrana GmbH-3M) met gebruikmaking van een 0.1 – 0.5 M zwavelzuuroplossing ($\rightarrow$ dit leidt tot consumptie van H_2SO_4).

Een goede voorbehandeling (pretreatment) van de voeding is bepalend voor de business case (CAPEX/OPEX) en essentieel om het (niet-vluchtige ion) ammonium om te zetten in ammonia (dat als gasvormig ammoniak door de membraanporie stroomt). De omzetting kan op een aantal manieren:

- temperatuurverhoging van de voeding (van 20 naar 40°C → extra energiekosten);
- pH-verhoging door CO_2-strippen met lucht (→ extra energiekosten);
- toevoegen van een base (in de praktijk NaOH → extra kosten voor chemicaliën).



Figuur 1: Toepassingen, uitdagingen en principe (van links naar rechts) van Trans Membraan Chemosorptie (ref).

Kentallen voor de consumptie van H_2SO_4 en NaOH voor de behandeling van een concentraat van een anaerobe vergister van een afvalwaterzuivering zijn respectievelijk: 3.5 en 4.5 kg per kg verwijderd NH_3 en 1.5-2 kg per m^3 behandelde voeding (Gonzalez-Salgado et al., 2022). Uit pilotexperimenten met de vloeibare fractie van ruwe (dus niet vergiste) varkensmest met een totaal stikstofgehalte (TAN) van 2774 mg/L (Molinuevo-Salces et al., 2020) is gevonden dat meer dan 70% van het ammonium kan worden verwijderd, waarbij een 3.2 wt% $(NH_4)_2SO_4$ concentraat wordt verkregen. De proceskosten zijn ongeveer 2.1 € per kg teruggewonnen stikstof.
Voordelen van TMCS zijn:

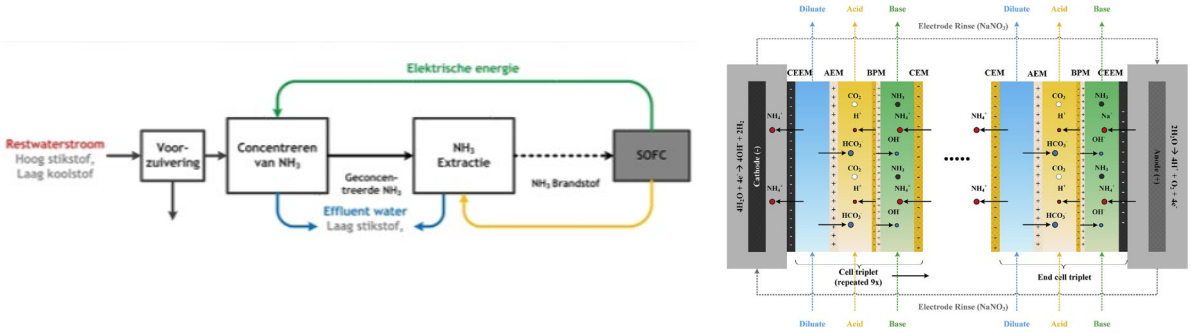
- Fractionering van de voeding in een kaliumrijke en in een ammoniumrijke fractie is mogelijk (alleen het ammonium wordt in de vorm van vluchtig ammoniak afgevoerd door het membraan)
- Afvalwater kan worden gezuiverd van ammonium en er kunnen diverse (qua samenstelling en concentratie) toepasbare ammoniumgebaseerde kunstmestvarianten gemaakt worden. Afhankelijk van de toegevoegde zure stripoplossing zijn dit: ammoniumsulfaat $(NH_4)_2SO_4$ (uit zwavelzuur), ammoniumnitraat NH_4NO_3 (uit salpeterzuur), of ammoniumfosfaat als $NH_4H_2PO_4$ of $(NH_4)_2HPO_4$ (uit fosforzuur)
- Diverse afvalwaterstromen (met een verschillend N-gehalte) kunnen worden behandeld zoals urine, mest, afvalwater en digestaat
- TMCS is een modulair-schaalbare membraantechnologie met een hoge TRL (3 commerciële toepassingen, diverse pilot applicaties)

Nadelen van TMCS zijn:

- Niet geschikt voor de productie van Aqua ammonia (ammoniawater, NH_4OH) omdat de stripoplossing zuur moet zijn en dus geen OH- zal bevatten;
- De voorbehandeling is relatief complex en duur (verwijdering van deeltjes en organische verontreinigingen, alkalisatie door CO_2-strippen, temperatuurverhoging en/of dosering van base. Daarnaast moet een relatief grote voedingsstroom behandeld worden vanwege het hoge watergehalte hiervan);
- Consumptie (inclusief transport en opslag) van chemicaliën zoals zuur (H_2SO_4) en base (NaOH), mede als gevolg van het relatief hoge watergehalte in de te behandelen stromen.

4.2 State of the Art EDBM

Electrodialyse met Bipolaire Membranen (EDBM) is een technologie waarmee een zout elektrochemisch kan worden omgezet in een zuur en zijn geconjugeerde base. Dit proces is gebaseerd op het principe dat ionisatie van water in H^+ (zuur) en OH^- (base) optreedt bij het bipolaire membraan en dat deze ionen door het elektrisch veld in respectievelijk een zuur- en basekanaal worden getrokken. Door het in combinatie toepassen van elektrodialyse worden relatief geconcentreerde zuren en basen verkregen. Een groot voordeel van EDBM is dat er geen extern aangevoerde base (NaOH) of zuur (H_2SO_4) nodig is maar dat dit onsite gemaakt kan worden (mits een lokaal beschikbaar zout aanwezig is). Recentelijk is aangetoond (Van Linden et al, 2020, zie Figuur 2) dat EDBM ook gebruikt kan worden om een ammoniumzout zoals NH_4HCO_3 om te zetten in een zuur (H_2CO_3, dat in evenwicht is met het opgeloste gas CO_2) en de base NH_4OH (dat in evenwicht is met ammonia, dus het opgeloste gas NH_3).



Figuur 2: Totaalproces voor terugwinning en concentratie van ammonium (en omzetting in een brandstofcel, links, ref) en de productie van opgelost ammonia door EDBM uit een waterige NH4HCO3 voeding (rechts, ref). Voor het maken van kunstmest valt EDBM onder “Concentreren van NH3” en TMCS voor “NH_3 Extractie” (de Solid Oxide Fuel Cel SOFC vervalt voor deze kunstmestapplicatie).

Zoals opgemerkt door Van Linden et al, 2020, kan EDBM gebruikt worden als alkalisatie/voorbehandeling voor Trans Membraan Chemosorptie (TMCS) om ammonium om te zetten in ammonia in het basekanaal van EDBM en dit vervolgens met TMCS te strippen. Dit gepatenteerde EDBM concept wordt gecommercialiseerd door het bedrijf MEZT, een spin-off van TU-Delft (https://mezt.nl/) maar is nog niet toegepast in de praktijk. EDBM is een modulair-schaalbare membraantechnologie van TRL 3-4 (ref). Er bestaan geen (openbare) technische performance gegevens voor de geïntegreerde hybride combinatie EDBM+TMCS waarbij EDBM de voorbehandeling is voor TMCS. In principe (niet verder uitgewerkt in de literatuur), kan het gemaakte zuur bij EDBM (na CO_2-verwijdering uit de voeding en mits in de juiste vorm) ook als zure stripoplossing gebruikt worden bij TMCS.

Voordelen van EDBM zijn:

- Er wordt aqua ammonia (ammoniawater, NH_4OH) gemaakt in het basekanaal van EDBM (maar dit bevat tevens andere zouten zoals KOH bij digestaat als voeding) en dit is relatief geconcentreerd (bv. van 1 naar 10 g/L, zie ref);
- Er kunnen hoge concentraties van zuur en base gemaakt worden (tot wel 1 M);
- Diverse afvalwaterstromen kunnen behandeld worden met zowel anorganische als organische zouten;
- Er zijn geen externe aangevoerde (inclusief transport en opslag) chemicaliën zoals zuur (H_2SO_4) en base (NaOH) nodig vanwege de onsite produktie van zuur en base uit water.

Nadelen van EDBM zijn:

- Het geproduceerde ammoniawater is niet zuiver: fractionering van de voeding in een kaliumrijke en in een ammoniumrijke fractie is niet mogelijk (zowel ammonium als kalium worden getransporteerd naar het basekanaal van EDBM);
- Behalve ammoniawater kunnen geen andere ammoniumgebaseerde kunstmestvarianten gemaakt worden (bij standalone operatie);
- De voorbehandeling is relatief complex (verwijdering van deeltjes en organische verontreinigingen, verwijdering van meerwaardige ionen zoals Ca en Mg die anders scaling geven).

4.4 De vernieuwing

De vernieuwing betreft de hybride technologiecombinatie Pretreatment + TMCS + EDBM waarbij EDBM als nabehandeling van TMCS toegepast wordt op de geproduceerde ammoniumsulfaatstroom. Deze geïntegreerde technologie combineert diverse voordelen cq elimineert diverse nadelen van de standalone technologieën

- Er worden drie productstromen gemaakt:
  - 1. Zuiver en tevens geconcentreerd ammoniawater (NH_4OH) dat als intermediate kan worden ingezet.
    - Hoge zuiverheid: door scheiding van ammonia van kalium of andere stoffen in de TMCS.
    - Hoge concentratie: door gebruik van geconcentreerd zuur in de TMCS dat in hoge concentratie gemaakt kan worden in de EDBM.
  - 2. Een kaliumrijke stroom die geen of nauwelijks ammonium bevat en ook als meststof kan worden ingezet.
    - Hoge zuiverheid: door afscheiding van ammonia van de kalium in de TMCS.

        - Hoge concentratie: na verdere concentrering (door Reverse Osmosis RO of Forward Osmosis FO). Dit is mogelijk omdat de osmotische druk van de kaliumstroom relatief laag is door verwijdering van het ammonium.
    - 3. Water (als product van RO of na terugwinning van de drawoplossing bij FO) dat mogelijk hergebruikt kan worden in het integrale proces.
- Er worden geen of weinig chemicaliën geconsumeerd (geen transport en opslag, minder veiligheidsrisico's):
    - Zuur
        - Zwavelzuur, of een andere zuur naar keuze, fungeert slechts als een (hoog geconcentreerde) werkvloeistof tussen TMCS en EDBM maar wordt netto niet verbruikt.
    - Base
        - In principe kan een gedeelte van het geproduceerde ammoniawater (NH_4OH) gebruikt worden als basedosering bij de voorbehandeling van TMCS. Dit is een te optimaliseren trade-off want dit gaat uiteraard ten koste van de productiecapaciteit, maar kan wel toevoeging van extern aangevoerde NaOH (en contaminatie van de kaliumstroom) voorkomen. Daarnaast is, zoals bij standalone TMCS, temperatuurverhoging of CO_2-strippen ook een middel om te besparen op het verbruik van NaOH.
- Beperkte voorbehandeling:
    - Er is geen aparte voorbehandeling nodig voor EDBM.
        - De ammoniumsulfaatvoedingsstroom is zeer zuiver omdat alleen het vluchtige ammonia gestript wordt in de TMCS en dus contaminatie door deeltjes, organische componenten, tweewaardige ionen als Ca en Mg of andere ionen op kan treden.
    - De voorbehandeling voor de hybride combinatie is zeker niet meer maar mogelijk zelfs minder dan voor standalone TMCS.
        - Dit laatste is het geval als een gedeelte van het ammoniawater wordt gebruikt als basedosering in de voeding van de TMCS. Zoals hierboven beschreven is dit een belangrijke trade-off omdat de business case voor standalone TMCS bepaald wordt door de hoge kosten van de voorbehandeling.

WUR bouwt met MEZEM voort op zowel het toegepaste onderzoek van TMCS zoals verworven in het project Nitrocycle voor circulair N (DFI-AF-18011, deelprogramma: winnen van nutriënten uit mest, reststromen en afvalwater) als op het EDBM onderzoek zoals ondermeer ontwikkeld in het lopende project SOLIDARITY voor Process efficient Solid & Liquid Dewatering and Drying (TSE-20-22-04 - MOOI Industrie).

Bijlage 2: Uitgebreid meerjarig werkplan

WP 1: Emissiereductie en behoud van vergistbare drogestof door biologisch aanzuren van mest.

Bij het voorkomen van emissies zijn maatregelen aan de bron naar verwachting het meest (kosten)effectief. Het in een vroeg stadium verzuren van verse mest is zo'n maatregel; het heeft een aangetoond positief effect op de ongewenste en ongecontroleerde omzetting van de fermenteerbare koolhydraatfractie uit mest naar methaan. De plannen uit bijlage 1 zijn uitgewerkt in onderstaand activiteiten overzicht.

Activiteiten, *Deliverables (italic*) en Planning

1.1	**Emissie experimenten met melasse verzuurde mest Dairy Campus**	**maand**
1.1.1	Bepaling stalemissie niet verzuurde mest gedurende 1 maand	2-4
1.1.2	Bepaling stalemissie met verzuurde mest gedurende 1 maand	4-6
1.1.3	Evaluatie	
1.2	**Laboratorium experimenten mestverzuren met alternatieve koolstofbronnenl**	
1.2.1	Verzuringexperimenten met reststromen van de 'Positieve lijst co-vergisting'.	6-8
1.2.2	Kosten/baten-analyse van alternatieven. Is er een business case?	8-12
D1.2	*Rapport Emissie en Verzuringsexperimenten*	
1.3	**Menging koolstofbron aan mest**	
1.3.1	Analyse stroming in mestkelder/silo	13
1.3.2	Evaluatie commercieel beschikbare mengsystemen	14-15
1.3.3	Ontwerp mengsysteem	16-20
1.3.4	Experiment stal Dairy Campus	20-24
D1.3	*Geïnstalleerde melasse-mest menger*	
1.4.	**Methaan productie bij vergisten van verzuurde mest**	
1.4.1	Laboratorium experiment methaan opbrengst verzuurde mest	25-27
1.4.2	Experiment vergister Dairy Campus met verzuurde mest.	28-30
1.4.3	Kosten/baten analyse	30
1.5.	**Potentieel van alternatieve azijnzuurroute (p.m)**	
1.5.1	Marktanalyse partijen die thans afvalwater zuiveren en GFT-verwerken en vetzuren produceren.	6-8
1.5.2	Laboratorium experiment met azijnzuur verzuurde mest	31-33
1.5.3	Experiment Dairy Campus met daar geïnstalleerd mengsysteem (acitiviteit 3)	34-36
1.5.4	Kosten Baten analyse	36
D1.5	*Rapport biogas productie verzuurde mest*	

WP 2: Hogere biogasopbrengst uit mest

Activiteiten, *Deliverables (italic)* en Planning

2.1	**Experimenten op laboratoriumschaal: is soortenrijkdom belangrijk bij de biogasopbrengst en remt het mestmilieu de ontwikkeling van een soortenrijk mengsel?**	**maand**
2.1.1	Proeven met verdunde mest (geen remming) en verschillende entmaterialen met verschillende soortenrijkdom in flesreactoren; meting biogasopbrengst en soortenrijkdom (DNA analyse). Is er een verband?	3-5
2.1.2	Proeven met onverdunde mest: meting acute effecten (minder biogas, afname soortenrijkdom) en langetermijn effecten (na maandenlange voeding en incubatie): is het mestmilieu ongunstig voor de handhaving van breed spectrum aan micro-organismen?	5-7
2.1.3	Verschil verdunde en onverdunde mest in langdurige incubatie en voeding, startend met rijk entmateriaal: meting biogasopbrengst en soortenrijkdom: is het ongunstige mestmilieu een concentratie-effect?	7-9
2.1.4	Experimenten met het toevoegen van sulfide, ammonium, zouten en andere mogelijke veroorzakers van een ongunstig mestmilieu: meting effect op biogasproductie en soortenrijkdom	9-11
2.2	**Verbetering van de mestvergisting op labschaal**	
2.2.1	Uit activiteit 1 volgt een meest waarschijnlijke oorzaak van de soorten-armoede in mestvergisters. Ideeën worden gegenereerd om die oorzaak weg te nemen, door het veranderen van de vergisting.	11
2.2.2	Het testen van het effect van deze maatregelen in vergisting van rundermest: meer biogas?	12-20
2.2.3	Kosten/baten-analyse van deze maatregelen en extra biogasopbrengst. Is er een business case?	21
D2.2	*Rapport met de bevindingen (in hoeverre soortenrijkdom belangrijk is bij de biogasopbrengst of het mestmilieu de ontwikkeling van een soortenrijk mengsel remt, en resultaten omtrent de verbetering van de mestvergisting)*	
2.3	**Proef op grote schaal (p.m)**	
2.3.1	Implementatie van de maatregel bij een rundermestvergister: meting van biogasopbrengst, soortenrijkdom en andere relevante parameters	22-33
D2.3	*Rapport met de bevindingen (proef op grote schaal: implementatie van een verbeteringsmaatregel bij een rundermestvergister met het effect op de biogasopbrengst, soortenrijkdom en andere relevante parameters) (p.m.)*	

WP 3: Lignine from manure

Activities, *Deliverables (Italic),* Planning:

3.1	**Development and evaluation of mild fractionation technologies for the extraction and purification of lignin from manure/digestate**	**month**
3.1.1	Alkaline fractionation and GVL fractionation technologies will be applied for the selective fractionation of lignin from manure/digestate. Optimization on solid/liquid ratio, temperature, pressure, time will be performed.	2-6
3.1.2	Both fractionation technologies will be evaluated in terms of effectivity, yields, costs, upscaling potential	7-10
3.1.3	Selection of the best performing fractionation technology for the upscaling work under 3.3.	10-12
D.3.1	*Best performing lignin fractionation technology.*	
3.2	**Detailed analysis of the resulting lignin-rich fraction to predict the potential for application**	
3.2.1	State-of-the-art analytical techniques will be applied to fully characterize the composition, structure, functional groups of the lignin fraction derived from manure/digestate. HSQC NMR for structural linkages, hydroxyl type and content through 31P NMR, quantitative pyrolysis-GC-MS platform, SEC for molar mass, purity analysis (ash, nitrogen content) etc.	5-8
3.2.2	Establish structure-function relationships to indicate the potential for the use of these lignin-rich fractions	5-8
D.3.2	*Structure-function relationships lignin fractions.*	
3.3	**Upscaling promising technologies for manure/digestate lignin fractionation**	
3.3.1	Fractionation technology will be tested and evaluated at 2L and 20L scale including downstream processing to purify the resulting products	12-18
3.3.2	Suitable lignin fractions will be produced at several 100 gram scale to be tested in the applications under 3.4.	18-24
D.3.3	*Lignin sample fractions.*	
3.4	**Manure derived lignin functionality testing in asphalt binders)**	
3.4.1	Evaluation of lignin in asphalt binders by blending with bitumen and/or biobased ingredients	24-25
3.4.2	Detailed testing of blends on compatibility, homogeneity, stability, and rheological behavior	26-27
3.4.3	Promising manure lignin samples can be tested by the Asphalt Knowledge Centre on asphalt specimen level (outsourcing AKC 25 k€)	28-30
or		
3.5	**Manure derived lignin functionality testing in adhesives**	
3.5.1	Evaluation of lignin in resins / adhesives by reaction with a biobased crosslinker	30-31
3.5.2	Detailed testing of resins on crosslinking temperature and density, stability, rheological behavior	32-33
3.5.3	Promising manure lignin samples will be tested in a resin applied to wood fibres to produce labscale panels. Overall applicability, temperature and curing behavior, moisture resistance and mechanical properties will be determined. Comparison with a commercial product will be made.	34-36
D.3.4	*Test report lignin fractions (asphalt or adhesive).*	
3.6	**Determine the overall potential for lignin derived from manure/digestate in asphalt binders and resins**	
3.6.1	Evaluation and Reporting	36
D.3.5	*Report potential lignin from manure.*	

WP 4: Aqua Ammonia uit mest met Trans Membraan Chemo Sorptie (TMCS) en Electrodialyse met Bipolaire Membranen (EDBM)

Activities, Deliverables (Italic), Planning:

4.1	**Cascade TMCS + EDBM: aqua ammonia productie zonder zuurconsumptie**	**maand**
4.1.1	Conceptueel Ontwerp & Basic Modelleren. Diverse EDBM configuraties, diverse zuren als werkvloeistof zoals zwavelzuur of azijnzuur als proceseigen zuur of fosforzuur of salpeterzuur.	4-8
4.1.2	Labtesten van Diverse Concepten & Evaluatie. (i) Zuurverlies bij circulatie over TMCS en EDBM? (ii) Trade-off voor optimale zuurconcentratie tussen TMCS (lage pH) en EDBM (hoge pH want dan minder conversie nodig). (iii) Welke ammoniaconcentratie en produktiesnelheid is haalbaar cq nog te verbeteren? (iv) Wat is het energieverbruik?	9-21
D4.1	*Deliverable: beste ontwerp- en operatiecondities en KPI's voor cascade TMCS + EDBM*	
4.2	**Pretreatment: vermijden/minimaliseren van baseconsumptie**	
4.2.1	Conceptueel Ontwerp & Basic Modelleren van diverse voorbehandelingsopties. Is CO_2 verwijdering nodig gezien de pH van de voeding. Deeltjesverwijdering moet voorafgaan aan de eerste membraanscheiding in de flowsheet plaatsvinden. Dit kan dus ook bij de RO (als eerst ontwaterd wordt). Verschuiving evenwicht NH_4^+ naar NH_3 (trade-off: hoe meer NH_3, des te duurder de voorbehandeling, maar des te compacter/goedkoper de TMCS; minimaal/geen base verbruik lijkt het beste voor de business case): (i) Temperatuursverhoging (niet gewenst). (ii) Verbeterde TMCS module met tegenstroom ipv dwarsstroom (operatie bij relatief lage verhouding NH_3/NH_4^+ mogelijk met verschuiving van evenwicht door continue afvoer van NH_3). (iii) Geproduceerde NH_4OH gebruiken voor aanlogen. (iv) Voorafgaande waterverwijdering zodat minder loog nodig is.	12-16
4.2.2	Labtesten van Diverse Concepten & Evaluatie	17-24
D4.2	*Deliverable: beste ontwerp- en operatiecondities en KPI's voor Pretreatment*	
4.3	**Ontwateren: minimaliseren van waterige stromen (p.m.)**	
4.3.1	Conceptueel Ontwerp & Basic Modelleren. RO zo vroeg mogelijk in de flowsheet om met minimale stromen te kunnen werken. Verder ontwateren met andere technieken voorbij de RO-grens bv Electrodialyse	12-16
4.3.2	Labtesten van Diverse Concepten & Evaluatie	17-24
D4.3	*Deliverable: beste ontwateringstechnologie en KPI's en de beste locatie hiervan voor het integrale proces **(p.m.)***	
4.4	**Integrale Systeem: Pretreatment + TMCS + EDBM: aqua ammonia productie zonder consumptie van zuur en base en met minimale waterstromen**	
4.4.1	Conceptueel Ontwerp van Geïntegreerde Systeem	25-26
4.4.2	Procesmodelleren, Optimalisatie & Opschaling	27-32
4.4.3	Technisch-Economische Analyse. Pretreatment is bepalend voor de business case. EDBM kan mogelijk ook in 2 kanaalssysteem (goedkoper) of 4 kanaalssysteem (hogere ammoniaconcentratie mogelijk)	33-36
D4.4/M4.	*Deliverable & Milestone: Geintegreerde Systeem inclusief beste ontwerp- en operatiecondities & technisch/economische KPI's*	
4.5	**Demo op locatie (p.m., valt buiten het project)**	
4.5.1	Pilotontwerp en productie	
4.5.2	Locatie preparatie	
4.5.3	Demonstratie & Evaluatie	
4.5.4	Technisch-Economische Analyse	
D4.5	*Technisch-economische KPI's voor Geintegreerde Systeem*	

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