Literature review for Assessing whether of mesophilic processes on a Biogas plant will reduce or eliminate *Avena fatua* (floghavre), *Echinochloa crus-galli* (hønsehirse), *Synchytrium endobioticum* (potetkreft), *Globodera rostochiensis* and *G. pallida* (potato cyst nematode)

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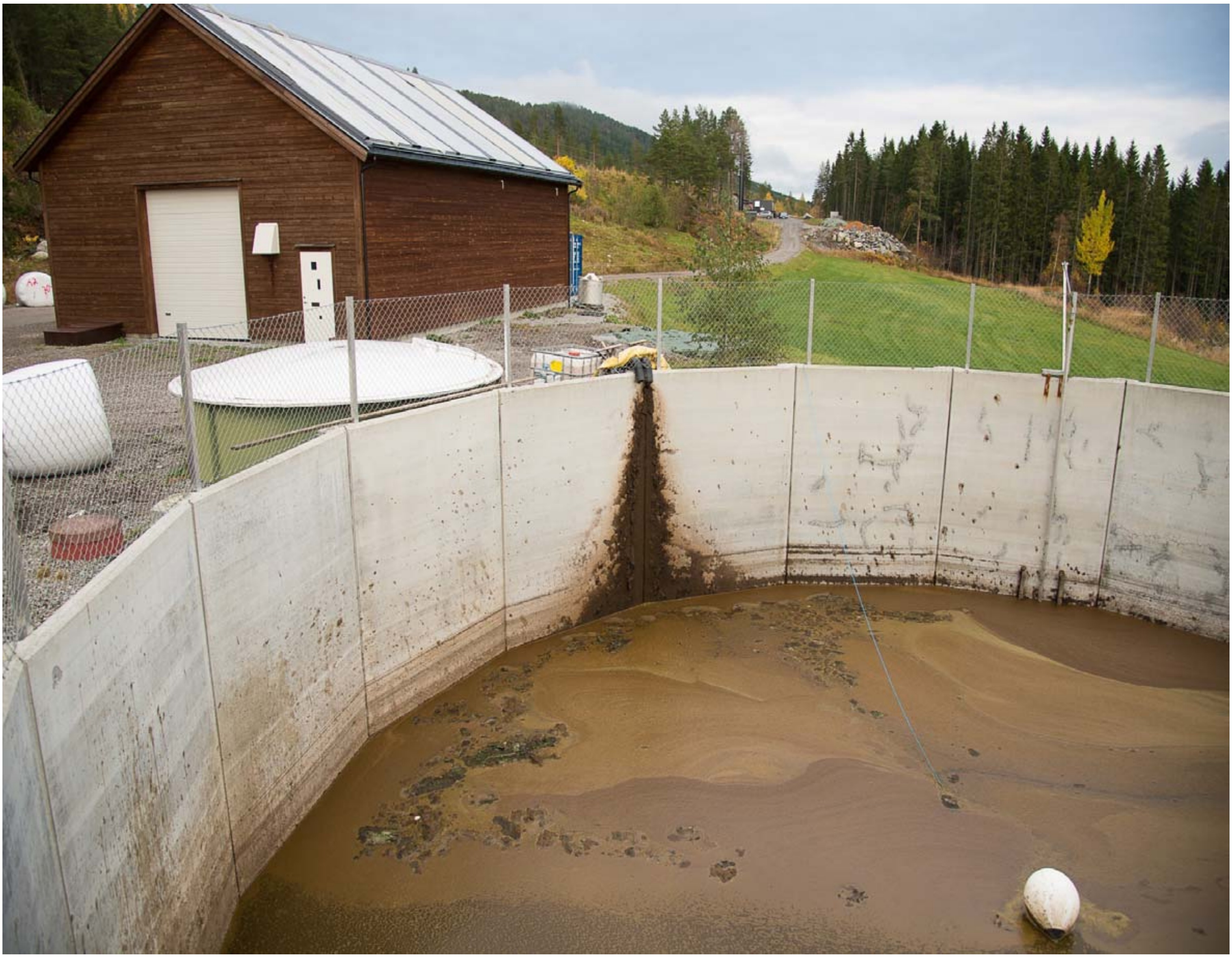

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TITTEL/TITLE

Literature review for assessing whether os Mesophilic Processes on a Biogas Plant will reduce or eliminate *Avena fatua* (Floghavre), *Echinochloa Crus-Galli* (Hønsehirse), *Synchytrium endobioticum* (Potetkreft), *Globodera rostochiensis* and *G. pallida* (Potato Cyst Nematode).

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SAMMENDRAG/SUMMARY:

Bruk av husdyrgjødsel er stadig mer brukt til å produsere biogass. Rester (bio- avfall) etter biogass prosesser, kan bli bruk som gjødsel. Hvis ugressfrø, plante patogener og nematoder overlever anaerob prosessen, bruk av bioavfall kan bli en fytosanitær risiko. Litteratur om effektene av mesofil temperatur spesielt på (to) ugress (en) plantepatogen og potetcystnematode-levedyktighet ble gjennomgått.

Ifølge den tilgjengelige litteraturen må det konkluderes at behandlinger som vanligvis brukes i mesofil prosess ikke vil være tilstrekkelige for fullstendig inaktivering av plantepatogener.

Dette refererer til patogener av potet som er oppført i norsk regulering (Matloven) og EU-direktiv 2000/29 / EF, spesielt *Synchytrium endobioticum*.

SUMMARY:

Anaerobic digestion using animal manure and crop biomass is increasingly being used to produce biogas. The leftover (bio-waste) after processing, could be returned to the field as a crop fertilizer. If weed seeds, plant pathogens and nematodes survive anaerobic digestion, the use of

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contaminated bio-waste poses a phytosanitary risk. Weed seeds, plant pathogens and cyst nematodes are likely to encounter in biogas plants. The effects, of mesophilic temperature in particular, on (two) weed seed (one) plant pathogen and potato cyst nematode viability were reviewed.

In our review, thermal inactivation was addressed. According to the available literature, it has to be concluded, that treatments usually applied in mesophilic process will not be sufficient for complete inactivation of Plant pathogens.

This refers to pathogens of potato that are listed in Norwegian regulation (Matloven) and EU Directive 2000/29/EC, particularly *Synchytrium endobioticum*.

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Preface

An increase in organic production of organic waste is expected during the next years, one of the raw materials expected to be used is livestock manure. A NIBIO report from 2017 outlines a future combination of farm / local treatment facilities and major central plants (Pettersen, et al 2017).

Biogas plants can be operated in different ways. Among other things, the temperature in the treatment tank varies. Biogas plants in Norway have around 37 ◦C in the processing tank (mesophilic proses), and approx. 55 ◦C in the processing tank (thermophilic eradication (Avfall Norge, 2010).

A mesophilic process may probably affect the survival of organisms, due to temperature, toxic gases, residence time, etc. The Norwegian Food Safety Authority therefore wishes an assessment of how this mesophilic treatment can affect the survival of the harmful organisms and in particular is essential to prevent the spreading of quarantine organisms.

As, 09.04.18

Ricardo Holgado

Content

Sammendrag

Bruk av husdyrgjødsel er stadig mer brukt til å produsere biogass. Rester (bio- avfall) etter biogass prosesser, kan bli bruk som gjødsel. Hvis ugressfrø, plante patogener og nematoder overlever anaerob prosessen, bruk av bioavfall kan bli en fytosanitær risiko. Litteratur om effektene av mesofil temperatur spesielt på (to) ugress (en) plantepatogen og potetcystnematode-levedyktighet ble gjennomgått.

Ifølge den tilgjengelige litteraturen må det konkluderes at behandlinger som vanligvis brukes i mesofil prosess ikke vil være tilstrekkelige for fullstendig inaktivering av plantepatogener.

Dette refererer til patogener av potet som er oppført i norsk regulering (Matloven) og EU-direktiv 2000/29 / EF, spesielt *Synchytrium endobioticum*.

Summary

Anaerobic digestion using animal manure and crop biomass is increasingly being used to produce biogas. The leftover (bio-waste) after processing, could be returned to the field as a crop fertilizer. If weed seeds, plant pathogens and nematodes survive anaerobic digestion, the use of contaminated bio-waste poses a phytosanitary risk. Weed seeds, plant pathogens and cyst nematodes are likely to encounter in biogas plants. The effects, of mesophilic temperature in particular, on (two) weed seed (one) plant pathogen and potato cyst nematode viability were reviewed.

In our review, thermal inactivation was addressed. According to the available literature, it has to be concluded, that treatments usually applied in mesophilic process will not be sufficient for complete inactivation of Plant pathogens.

This refers to pathogens of potato that are listed in Norwegian regulation (Matloven) and EU Directive 2000/29/EC, particularly *Synchytrium endobioticum*

1 Terms of reference as provided by the Norwegian Food Safety Authority

Assessing whether of mesophilic processes on a Biogas plant will reduce or eliminate*:*

Weeds: *Avena fatua* (floghavre) and *Echinochloa crus-galli* (hønsehirse),

Pathogen: Synchytrium *endobioticum* (potetkreft) and

Nematodes *Globodera rostochiensis* and *G. pallida* (potato cyst nematode).

2 Process Parameters

NIBIO was asked to issue an opinion on the safety of mesophilic processes concerning two weeds; one-plant pathogen and potato cyst nematodes (table 1)

Therefore, there is no validation of a specific process. The mandate does not include chemical and physical hazards, which will not be addressed.

3 Objectives

The purpose of this review is to collect information available in the literature concerning mesophilic processes and the survival of weeds *Avena fatua* (floghavre) and *Echinochloa crus-galli* (hønsehirse), pathogen *Synchytrium endobioticum* (potetkreft) and nematodes *Globodera rostochiensis* and *G. pallida* (potato cyst nematode) on bio-waste.

Table 1. Organisms requests, which could have a probability of be spread when bio-waste originating from Biogas plant is applied on agricultural fields.

Categories	Latin name	Norwegian name	English name	Registered in Norwegian regulation
Weed	*Avena fatua*	Floghavre	Wild oats	Quarantine
	Echinochloa crus-galli	Hønsehirse	Cockspur grass	Invasive alien species
Fungi	*Synchytrium endobioticum*	potetkreft	Potato wart	Quarantine
Nematode	*Globodera rostochiensis*	Gul potetcystenematode	yellow potato cyst nematodes	Quarantine
	G. pallida	Hvit potetcystenematode	Pale potato cyst nematode	

4 Biogas reactors

The Biogas production process takes place in strictly anaerobic conditions within different temperature ranges, namely psychrophilic (<25°C), mesophilic (between 30-40°C) or thermophilic (between 45-55°C). Temperature is an important factor in the production of biogas. The diverse array of natural environments in which methane-producing bacteria are found illustrates how biomethanation can take place under a wide range of temperatures, from 10°C to over 100°C, with moisture content varying from under 50 percent to over 99 percent (IEA 2005).

Anaerobic bacteria are most active at mesophilic temperatures between 30 and 40°C and in thermophilic temperatures between 50°C and 60°C (Yadvika et al. 2004).

In the Biogas digester, pH values between 7 and 8.5 prevail in general. The pH should not drop below the threshold of 6.8; otherwise, the very sensitive methanogenic bacteria are impeded in their metabolisms and hence will not be able to generate methane.

In the biogas process, organic matter is digested under anaerobic conditions. This process takes place in four steps. In the first step, called hydrolysis, macromolecules (carbohydrate, protein and fat) contained in the substrates are split into short chains compounds.

In the following steps fatty acids and acetic acid that are actually needed by methanogenic bacteria to produce a combustible biogas with a predominant content of methane will be formed.

Today, biogas plants are generally operated in a semi-continuous mode. Hereby the fresh substrate is fed to the digester one or several times a day and it overflows itself because of the hydraulic conditions in the digester. Biogas plants are equipped with one, two or three digesters and they are arranged in series (Oechsner et al., 2004). The substrate's digestion velocity depends, on one hand, on the biological environment in the digester and, on the other hand, on the substrate's structure. The substrate to be digested remains between twenty and hundred days (some time more) in the digester before it overflows in the storage tank for digested sludge. Ade-Kappelmann et al. (2004) indicated that the real retention time of microorganisms inside of a digester could be very short. It had been found that microorganisms (*Bacillus globigiia*) fed to the digester in both practical and laboratory scale, reached the outlet of the digester after 30 minutes until three days (Ade-Kappelmann et al., 2004).

4.1 Bio-waste originating from Biogas

The residual form biogas plants are usually called Bio-waste. This Bio-waste could be used as organic fertilizer. There is an increasing amount of bio-waste being spread on to soils used for plant production. As a result, there is increasing concern that plant diseases may originate from primary infection by plant pathogens present in waste material recycled to field crops, gardens, forests or greenhouse crops.

4.2 Possible Sources of plant pathogens

All material of plant origin that was used as forage for livestock may represent a potential risk if this material has being contaminated with plant pathogens. The composition of plant pathogens will depend on the type and species of plant material used as livestock forage.

It has been reported in published works that mesophilic anaerobic treatment for biogas production and mesophilic composting, could reduce relevant pathogens, after sufficiently long treatments. However, other published works reported an absence of effect of some mesophilic treatments.

5 Materials and Methods

Literature search was conducted in a number of literature bases, including ISI Web of Science, Scopus, CAB Abstracts (1984-2015), Agricola and Google Scholar.

Based on literature data, mesophilic treatments were considered regarding their effectiveness on the inactivation of selected weeds pathogen and potato cyst nematodes and the associated risk of their dissemination when bio-waste is used in agriculture.

6 Results

In general, anaerobic digestion in biogas plants seems an efficient way (thermophilic more efficient than mesophilic) to suppress growth/survival of weeds, some pathogen and potato cyst nematodes. In table 2 are presented the effect of mesophilic processes on a Biogas (temperature between 30-40oC).

6.1 Risks Associated with Weed Seeds

Weed seeds can enter the biogas chain either via crop biomass or via animal manure.

In Germany, 180 species of weeds have been recorded (Mehrtens et al., 2005). Several factors contribute to weed seed mortality during biogas process. The most important factors are the interaction between weed species, real residence time, temperature, and moisture (Eggley, 1990; Shiralipour and Mcconnell, 1991; Eghball and Lesoing, 2000; Larney and Blackshaw, 2003; Dahlquist et al., 2007). In general, the higher the temperature to which weeds seeds are exposed during the biogas process, the higher the weed seed mortality will be. Similarly, the longer the time of exposure to high temperature, the higher weed seed mortality will be.

Livestock may ingest weed seeds when grazing, consuming silage, or whole grain (Blackshaw and Rode, 1991). However, seeds viability could also be affected during feeding, as a mechanical process of chewing occurs, together with enzymatic digestion and exposure to acidic conditions, especially in the abomasum, this affect the viability of seeds VKM (2016a).

6.1.1 *Echinochloa crus-galli* (hønsehirse), Cockspur grass

Invasive plants as *Echinochloa crus-galli* is a challenge to Norwegian agriculture. *Echinochloa crus-galli* can reproduce rapidly, compete with desirable vegetation, and may alter ecological niches in areas where they become established (VKM 2016b).

Blackshaw and Rode, 1991 showed that ensiling completely destroyed *Echinochloa crus-galli* seeds. Similarly Westerman et al 2012 showed that viability of *Echinochloa crus-galli* seeds are reduced after ensilage process. Wiese et al., 1998 showed that seeds of *Echinochloa crus-galli* did not survive composting; seeds were killed after three days at 49^{o}C.

Survival of Echinochloa crus-galli seeds from untreated manure and slaughterhouse waste used as fertiliser is considered as improbable because it does not survive ensiling or rumen digestion of the fodder (VKM 2016a).

6.1.2 *Avena fatua* (Floghavre) Wild oats

Avena fatua (Wild oats) is listed as a quarantine pest by the Norwegian regulation (Anonym 2003). According to the Norwegian regulation on wild oats, feed should be free from *Avena fatua* before sales (LMD, 2015).

Weinhappel et al. 2010 indicated that *Avena fauta* seeds were not viable after 7 days at 37^{o}C during the mesophilic digestion in a biogas plant. Similarly, Johansen, et al (2011) showed that after 2 days at 37^{o}C seeds of *Avena fatua*, could not germinate. Blackshaw and Rode (1991) showed that viability of seed decline during passage through the digestive tract of animals.

6.2 Risks Associated with Fungi

Several factors could contribute to eradication of plant pathogens, during composting. Noble and Roberts (2004) indicated that heat generated during the active phase of the composting process, and production of toxic compounds such as organic acids and ammonia, lytic activity of enzymes etc.

produced during compost acts as microbial antagonistic, including the production of antibiotics. Correspondingly pathogens naturally lose their viability. However heat generated during the active phase of the composting process appears to be the most important in pathogen destruction.

However the recommendations by EPPO (2008) are that bio-waste or other residual products should be treated with wet heat at 74°C for 4 h, 80°C for 2 h or 90°C for 1 h waste contaminated by quarantine or heat-tolerant pests. Compared with mesophilic digestion the temperatures recommended by EPPO (2008) are higher.

6.2.1 *Synchytrium endobioticum* (potetkreft) potato wart disease

Potato wart, caused by the fungus *Synchytrium endobioticum*, is a listed as quarantine pest in Europe and in Norway (anonymous 2003).

Resting bodies (sori) of the potato wart disease fungus are able to survive for over 30 years in soil and are resistant to extremes temperature and microbial antagonism and competition (EPPO, 1990, Nobel and Roberts 2004).

Survival of *Synchytrium endobioticum* in water at 60°C for 2 hours has been reported (Nobel and Roberts 2004).

Steinmöller and Müller (2012) demonstrated that winter spores of *Synchytrium endobioticum* survived composting, for 70 days at 30–45°C, for 21 days at 50–55°C and for 12 days at 60–65°C. As well viable winter sporangia could be extracted after pasteurisation for 90 min at 70°C and heating in a water bath at 80°C and in a dry oven at 90°C for 8 hours.

Steinmöller and Müller (2012) concluded that winter sporangia might survive exposures even at higher temperatures.

On the other hand, there are studies showing contradicting results e.g. Glynne (1926) reported that wet winter sporangia were inactivated at 70°C for 1 hour. Weiss and Brierley (1928) reported that winter sporangia in dried wart material resisted dry heat at 100°C for 12 hours and heating to 60°C for four to seven days with apparently undiminished viability.

At the present information concerning *Synchytrium endobioticum* comportment during composting is very limited.

Today, *Synchytrium endobioticum* is almost eradicated from Norwegian potato fields by means of resistant potato cultivars. The last detection in Norway was in 1994 *Synchytrium endobioticum*. (Hermansen et al., 2012).

6.3 Risks Associated with nematodes

Nobel and Roberts (2004) found that seven plant parasitic nematode species affecting plants during composting were eliminated within 1 day at temperatures of at least 52°C. These were: *Globodera pallida*, *Globodera rostochiensis*, *Heterodera schachtii*, *Meloidogyne hapla*, *Meloidogyne incognita*, *Meloidogyne javanica*, *Pratylenchus penetrans*.

Results presented by Ryckeboer et al. 2002 indicate that some cyst forming species such as the beet cyst nematode (*Heterodera schachtii*) has the ability to survive in compost at lower temperatures than 31°C for long periods.

Elsdon-Dew in 1953 reported that *Heterodera* eggs were found in numerous instances in the faeces of hospital patients in South Africa, and in one instance was traced to infected potatoes. The eggs appear to be resistant to digestive juices.

6.3.1 *Globodera pallida* and *Globodera rostochiensis* Potato cyst nematodes (PCN)

The cysts of *Globodera rostochiensis* and *Globodera pallida* can survive many years in soil without host plants. Turner (1996) in North Ireland extracted viable PCN from fields without potato cultivation for the period of 25 to 40 years. In the same way in Norway Holgado et al., (2015) recovered viable *Globodera rostochiensis* from a field without potato production for 32 year.

Nishinome et al., (1996) reported 40°C for 10 days or 50°C for 5 days are the lethal temperature for PCN. According Nishinome et al., (1996) PCN was also killed in potato processing sludge when temperatures were below the lethal temperature, at only 34°C. Bollen, (1985) indicated that mortality of PCN may occur due to toxicity of the sludge.

Steinmöller and Müller, 2012 reported that *Globodera rostochiensis* were killed by composting for 7 days at temperatures between 50°C to 55°C, and by pasteurisation for 30 min at 70°C. Bøen et al. (2006) observed a reduction of cysts by 99.9% after 8 days of composting at 50°C.

However, viable PCN cysts have been recovered from sewage sludge after anaerobic digestion although viability was reduced (Turner et al., 1983). Similar studies by Catroux et al. (1983) showed that almost 100% of the cysts of *Globodera rostochiensis* and *Globodera pallida* were killed during anaerobic sewage digestion. Composting with temperatures rising to more than or equal to 40°C most of the PCN were killed whereas aerobic digestion destroyed only a small proportion of the cysts. PCN could survive up to 3 months of storage in the outer layer of sludge. In limed sewage sludge at pH 10 or more, the cysts were killed in 14 days.

Table 2. Survival of Mesophilic processes on a Biogas (temperature between 30-40°C)

Categories	Latin name/Norwegian name/English name	Temperature/ days necessary for Eradicate	survival
Weed	*Avena fatua* Floghavre /Wild oats	7 days at 37° C	unlikely
	Echinochloa crus-galli Hønsehirse / Cockspur grass	Not survive ensilage	unlikely
Fungi	*Synchytrium endobioticum* Potetkreft/ Potato wart	Not enough temperature for elimination	likely
Nematode	*Globodera rostochiensis* Gul potetcystenematode/ yellow potato cyst nematodes *G. pallida* Hvit potetcystenematode /Pale potato cyst nematode	40°C for 10 days	unlikely

7 Conclusions

Concerning mesophilic processes during biogas production current scientific knowledge is insufficient to identify with confidence the safety regarding survival of plant pathogens taking account of only temperature and exposure time.

For some pathogens, varying results can be found in the literature concerning the time and temperature needed for complete inactivation, due to the complexity of the organisms and tests.

The variability in the results may be due to difficulties to recover the organism and pathogens from the tested substrates.

The mechanical process of chewing, together with enzymatic digestion and exposure to acidic conditions, especially in the abomasum, reduces the viability of seeds.

Seeds of *Avena fatua*, and *Echinochloa crus-galli* are uncertain to survive passage through the digestive tract of animals. Additionally seeds of *Avena fatua*, and *Echinochloa crus-galli* will lose their viability in manure storage. In manure storage in which temperature reaches 55°C, seed survival is unlikely.

Globodera rostochiensis and *G. pallida* are moderately likely to survive and remain infective after storage in cattle manure. With reference to temperature on mesophilic process, if high temperature is reach (between 40^{o}C to 55^{o}C) and lasting for 10 to 7 days survival of *Globodera rostochiensis* and *G. pallida* will be unlikely.

According to the available literature, it has to be concluded, that treatments usually applied in mesophilic process will not be sufficient for complete inactivation of plant pathogens. This refers to pathogens of potato listed in Norwegian regulations (Anonym 2003 /Matloven) and EU Directive 2000/29/EC, particularly *Synchytrium endobioticum*, the agent causing potato wart disease.

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Nøkkelord:	Biogass, overlevelse, karantene, Floghavre, Hønsehirse, potetkreft, potetcystenematode
Key words:	Biogas, survival, quarantine, Wild oats, Cockspur grass, potato wart, potato cyst nematode
Andre aktuelle publikasjoner fra prosjekt:	

Norsk institutt for bioøkonomi (NIBIO) ble opprettet 1. juli 2015 som en fusjon av Bioforsk, Norsk institutt for landbruksøkonomisk forskning (NILF) og Norsk institutt for skog og landskap.

Bioøkonomi baserer seg på utnyttelse og forvaltning av biologiske ressurser fra jord og hav, fremfor en fossil økonomi som er basert på kull, olje og gass. NIBIO skal være nasjonalt ledende for utvikling av kunnskap om bioøkonomi.

Gjennom forskning og kunnskapsproduksjon skal instituttet bidra til matsikkerhet, bærekraftig ressursforvaltning, innovasjon og verdiskaping innenfor verdikjedene for mat, skog og andre biobaserte næringer. Instituttet skal levere forskning, forvaltningsstøtte og kunnskap til anvendelse i nasjonal beredskap, forvaltning, næringsliv og samfunnet for øvrig.

NIBIO er eid av Landbruks- og matdepartementet som et forvaltningsorgan med særskilte fullmakter og eget styre. Hovedkontoret er på Ås. Instituttet har flere regionale enheter og et avdelingskontor i Oslo.

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