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THE EFFECT OF ANAEROBIC BIOGAS PROCESS ON PLANT DISEASES

Case *Sclerotinia subarctica* and *Sclerotinia sclerotiorum*

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The effect of anaerobic biogas process on plant diseases: Case *S.subarctica* and *S.sclerotiorum*

Abstract

This research studied the survival of two *Sclerotinia* species and their reproductive capability in mesophilic anaerobic biogas process, *Sclerotinia* species cause crop losses for instance to vegetables. The digestate from the biogas process can be utilised as an organic fertiliser and it can substitute mineral fertilisers. However, there is a risk of plant pathogens and human pathogens in the digestate if it is not treated properly. In addition, plant pathogens can be really persistent and tolerant to high temperatures and other variations in environmental conditions. The aim of this study was to find out whether the mesophilic biogas process is sufficient enough for eliminating plant pathogens, especially *S.sclerotiorum* and *S.subarctica* and thus, can the digestate be utilised as an organic fertiliser without the risk of plant pathogens. This study was a follow-up on a partly EU-funded project in the Mikkeli University of Applied Sciences: ESBIO- Biogas plant as a part of the energy self-sufficient farm, which ended in 2012.

Sclerotia of *Sclerotinia sclerotiorum* and *Sclerotinia subarctica* were put into the mesophilic biogas process for 21 days and grown on PDA-agar for 14 days and the results were observed during cultivation. One batch from each experiment had a batch with pre-hygienisated sclerotia. There are no previous studies related to the survival of either of the species in the mesophilic anaerobic biogas conditions. Also the biogas and methane yield were measured alongside with anaerobic digestion assay and biodegradability of the process was analysed.

The results indicate that digestate can be utilised as a fertiliser without the risk of plant pathogen contamination. Neither *S.sclerotiorum* nor *S.subarctica* remained vital after the mesophilic biogas process according to cultivation experiments. This can be, amongst other things, due to the temperature combined with 21 days retention time, volatile organic compounds or due to anaerobic conditions. There were neither remarkable differences between the biogas and methane yield and biodegradation between pre-hygienisated batches and non-hygienisated batches.

Subject headings, (keywords)

Anaerobic digestion, mesophilic biogas process, digestate, hygienisation, plant pathogens

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CONTENTS

ABBREVIATIONS AND TERMINOLOGY LIST

Anaerobic digestion	i.e. methane fermentation. Degradation of organic material in anaerobic conditions, end products are biogas and digestate
Apothecium	Fruiting body where the spores of some fungi species are produced
Biogas	The end product of anaerobic digestion, contains mainly methane (CH_4) and carbon dioxide (CO_2)
Composting	Degradation of organic material in aerobic conditions
Digestate	Biogas residue with high nutrient content, can be used e.g. as fertiliser
HRT	Hydraulic retention time = the average length of time that a soluble compound remains in the constructed reactor
Hygienisation	i.e. pasteurisation, hygienisation of the material at least 60 minutes in 70˚C, the particle size must be less than 12 mm
Hyphae	Thread-like non-reproductive part of the fungi found in almost all fungi, mass of hyphae is mycelium
Inoculant	Bacterial population, also called inoculum
Inoculum (plant pathology)	Any part of pathogen that can cause disease
LCFA	Long-chain fatty-acids

Mesophilic digestion	Digestion which takes place in 35-37˚C
Resting spores	Spores which lie dormant, with thick cell wall that can survive unfavourable environmental conditions
Sclerotia	Singural sclerotium , dark and hard survival structure of some fungi species,
Substrate	Organic input material i.e. feed material (polysaccharides, proteins and lipids)
Susceptible host plant	Plant which is vulnerable to disease if it exposed to it
Thermophilic digestion	Digestion which takes place in 50-55˚C
Thread	i.e. Filament
TS	Total solids i.e. dry matter
TSE	Transmittable spongiform encephalopathies
VFA	Volatile fatty acids, short-chain fatty acids
VS	Volatile solids, indicates organic matter content

1 INTRODUCTION

Anaerobic digestion is becoming a more common way of treating organic wastes, as it offers a way to treat biodegradable material, renewable alternative to fossil fuels and is economically attractive. The end products of the anaerobic digestion are biogas, which is partly methane that can be utilised for instance as vehicle fuel, and digestate, the nutrient rich biogas residue, which can be utilised for instance as a substitute for mineral fertilisers. (Weiland 2010, 849, 857-858.) Materials for commercial fertilisers are limited and due to aims towards sustainability, agriculture tries to find more environmentally friendly materials and methods. The goal is to shift into use of organic digestate, such as a biogas residue, in which case the nutrient cycle is closed and the losses of nutrients are minimised. Also material efficiency increases and the greenhouse gases from the methane decrease due to the utilisation of it (compared with the uncontrolled degradation of organic material and its methane production). (Latvala 2009, 59; Holm et al. 2010, 667-669; Luste et al. 2012, 8.)

Depending on the substrate used in the anaerobic biogas process, the digestate may contain for instance human and plant pathogens, weed seeds and toxic metals. If the digestate is spread in fields untreated the pathogens and other harmful microbes and substances will be transferred to fields as well, and they may contaminate plants and further on food stuff. (Lehto et al. 2007, 35.) Also the need for fungicides increases and the crop yield is reduced. (Haraldsson 2008, 5). Soil-borne plant pathogenic fungi are able to produce spores, which can survive for instance high temperatures and pressure variations (Kumar et al. 2008, 69) and are also capable maintaining viable in soil for several years with their resting spores. (Lehto et al. 2007, 35). In order to diminish the plant pathogens from the digestate, the digestate can be pre- or post-hygienisated by heat treating or post-treated with composting. (Iivonen et al. 2013, 67.) Temperature, retention time and post digestion affects the elimination of plant pathogens. (Haraldsson 2008, 37; Noble et al. 2009, 3; Iivonen et al. 2013, 66). Thus, whether mesophilic temperature is enough to eliminate plant pathogens is not certain and if it is how long retention time is needed. (Luste et al. 2012, 40, 55).

In Finland approximately half of garden area is used for the growing of open land vegetables and the most significant plant pathogens for open land vegetables such as iceberg lettuce are *Sclerotinia sclerotiorum* and *Sclerotinia subarctica, Botrytis cinerea, Rhizoctonia solan* and for lettuce *Pseudomonas cichorii*. Bacterial infections cause losses in stuffed cabbage rolls and clubroot (*Plasmodiophora braasicae*) and *Sclerotinia* diseases appear to cause most significant losses to cabbages. (Tuomola et al. 2012, 9-14.) These plant pathogens are also most commonly found in vegetable waste. (Lehto et al. 2007, 35). Economically the most significant plant diseases regarding South Savo region of cabbage and iceberg lettuce are *Sclerotinia* diseases, club root and rot caused by bacteria. (Iivonen et al. 2013, 68.) *Sclerotinia* species are able to reproduce air borne or by sclerotia, survival structures that are able to tolerate various environmental conditions such as high temperatures, toxicity and low oxygen. (Kumar et al. 2008, 69; Haraldsson 2008, 36; Iivonen et al. 2013, 68).

The objectives of this research were to find out, whether and how the chosen plant pathogens survive the mesophilic anaerobic biogas process and emphasise finding out the methods of enhancing the possibilities of using organic and renewable fertilisers, regarding digestate from the anaerobic biogas process. As a case study the survival of *Sclerotinia sclerotiorum* and *Sclerotinia subarctica* species are studied by putting the sclerotia into laboratory scale batch reactors and observing their survival and reproductive capability in cultivations. There are no previous studies related to the survival of *S.sclerotiorum* and *S.subarctica* in a mesophilic biogas process. For *S.sclerotiorum* there have been studies related to its survival in compost, for instance by Noble et al. 2009 (elimination in 7 days in 52˚C). This study is a follow-up on a partly EU-funded project ESBIO- Biogas plant as a part of the energy self-sufficient farm, which ended in 2012.

2 ANAEROBIC BIOGAS PROCESS

In anaerobic digestion the organic substrate (biowaste, energy crops, waste water sludge, liquid cow manure and so forth) is being closed in an oxygen-free reactor, in which different types of anaerobic microbes degrade organic feed material. (Latvala

2009, 29-30). These micro-organisms can be divided into four groups and they act in different stages of the anaerobic biogas process: hydrolysing micro-organims (e.g. *Bacteriocides, Bifidobacteria, Clostridia),* fermentative bacteria, acetogenic bacteria and methanogens (e.g. *Methanosarcina barkeri* and *Metanonococcus mazei*). (Weiland 2010, 850; Haraldsson 2008, 9). The end products of anaerobic digestion are biogas (mainly CH_4 + CO_2) and digestate. Methane is produced when organic matter is being degraded. (Latvala 2009, 25.)

There are two process types: dry fermentation (TS 15-60 %) and wet fermentation (TS < 10%). Nowadays, wet fermentation processes are more common than dry fermentation processes; in Finland for instance the most common process type is one-stage mesophilic, continuously stirred tank with wet fermentation. The reactor can be either continuous reactor or batch reactor. In the batch reactor, the substrates are added in batches, which go through the fermentation, after which another batch is added. In a continuous reactor, the substrate is being added continuously as the name suggests. (Latvala 2009, 30-33.)

2.1 Stages of the biogas process

The process, in which organic material turns into biogas in anaerobic conditions, is called methane fermentation or anaerobic digestion. The methane fermentation process can be divided into four steps: hydrolysis, acidogenesis, acetogenesis and methanation (Figure 1.). In the hydrolysis phase, organic feed material (complex polymers) including polysaccharides, proteins and lipids, is degraded by micro-organisms into monomers and oligomers: sugars, amino acids and long-chain fatty acids (LCFA). In acidogenesis phase volatile fatty acids (VFA) are being produced, in acetogenesis phase acetate and hydrogen. The final phase is methanogenesis, where methane is produced by methanogenic bacteria from acetate and hydrogen. (Latvala 2009, 29; Weiland 2010, 850.)

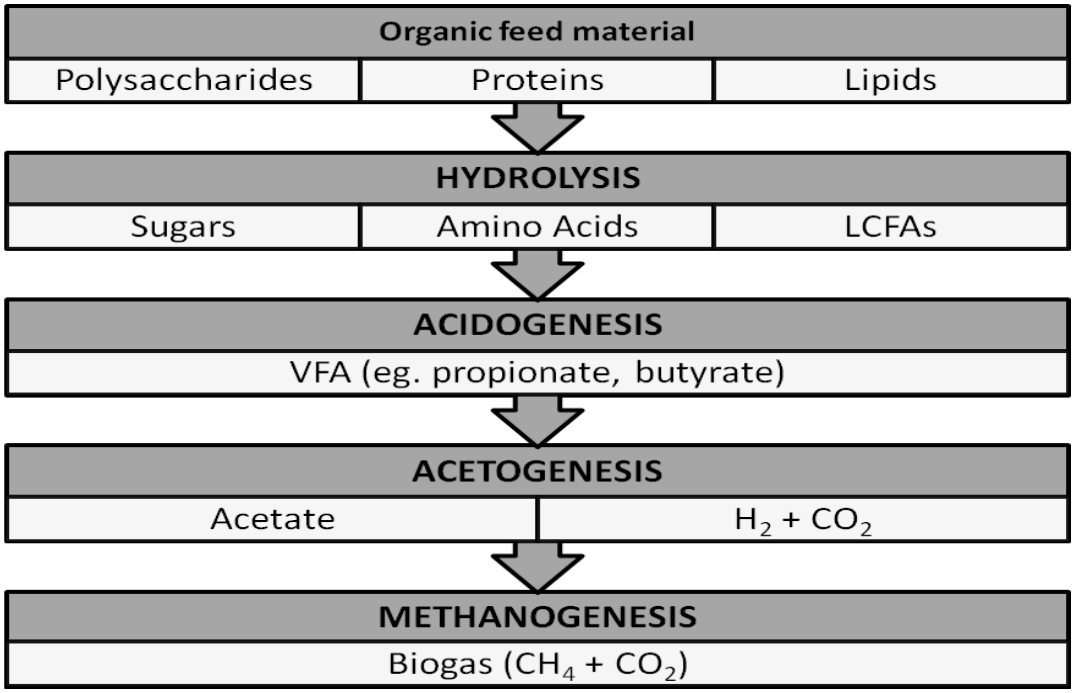


FIGURE 1. The stages of anaerobic degradation process (methane fermentation) (according to Latvala 2009 & Gujer and Zehnder 1983 [Weiland 2010])

2.2 Mesophilic/ Thermophilic biogas process

The anaerobic biogas process can be either mesophilic (optimal temperature approximately 35-37˚C) or thermophilic (optimal temperature approximately 50-55˚C). (Latvala 2009, 34). The temperature range in mesophilic temperature conditions can, however, vary from 35˚C to 42˚C and thermophilic conditions from 45˚C degrees to 60˚C. (Weiland 2010, 851). Mesophilic temperature range biogas processes are most commonly used in Finland, at the moment only a few biogas plants utilise the thermophilic biogas process (Stormossen in Vaasa, Satakierto in Köyliö). The mesophilic anaerobic biogas process requires less additional heating than the thermophilic process (approximately 10-30% less additional heating). However, the hydraulic retention time (HRT) of the mesophilic process is usually higher, depending on the substrate, for instance the recommendation for the mesophilic biogas process is 21 days. The hygienisative effect of the mesophilic process is weaker than in the thermophilic process, but longer retention time improves the hygienic condition of the digestate. However, long retention time increases heat and mixing demand. (Latvala 2009, 35.) Methanogenic diversity is most often lower in the thermophilic process than in the mesophilic biogas process, thus the thermophilic biogas process is more sensitive to fluctuations in tempera-

ture than the mesophilic process. Fluctuation of a couple of degrees (-/+ 3˚C) does not affect mesophilic bacteria or methane production remarkably, whereas the thermophilic process requires more time to adapt to the new temperature. However, methanogenic bacteria grow faster in the thermophilic process, thus the thermophilic process is more efficient: the amount of feed material can be higher and retention time shorter. Higher temperatures may increase ammonia toxicity, which can inhibit the growth of microbial population in the process. (Weiland 2010, 851.) Inhibition resulting from ammonia, increased amount of VFA and so forth usually affects biogas and methane yields. (Latvala 2009, 36).

3 USE OF BIOGAS DIGESTATE AS AN ORGANIC FERTILISER

A non-degradable organic material residue from the anaerobic biogas process is called a digestate and it contains nutrients from the substrates used in the biogas process. (Vänttinen et al. 2009, 9). These nutrients include macronutrients such as nitrogen (N), phosphorus (P), potassium (K) and micronutrients such as copper (Cu), zinc (Zn) and iron (Fe). The preferred usage of the digestate is to be used as a fertiliser, in which case the nutrient cycle is closed and the losses of nutrients are minimised to sediments, atmosphere and water bodies. The digestate can be used for agricultural purposes, for forestry, landscaping, landfills and so forth; the disposal options are presented in Figure 2. (Latvala 2009, 49; Holm et al. 2010, 667-668.)

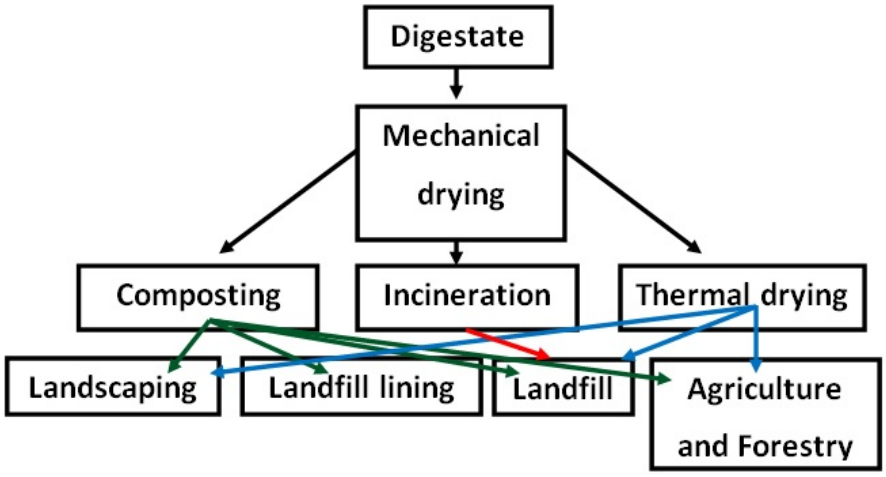


FIGURE 2. Digestate disposal options (according to Latvala 2009)

The waste characteristics when defining physical, biological and chemical characteristics of the digestate are for instance the odour and colour, particle size, water holding capacity and TS/VS content. Weed seeds, germination of seeds and pathogens affect biological characteristics of digestate, whereas heavy metals, nutrients, conductivity and organic pollutants affect the chemical characteristics of the digestate. Different parameters affect the agronomic value of the digestate, the handling of it and its fertilising value, for instance. (Holm et al. 2010, 671.)

In farm-scale biogas plants the digestate can be utilised as a fertiliser as such, if the substrates used include only wastes produced by the farm itself, which are plant or animal origin. Depending on substrates and the process used, the digestate can be spread without further treatment into the field. The water content of the digestate is decreased by mechanical drying, which can be done with centrifugal drying, filter pressing, screw pressing or with rare chamber filter pressing. (Latvala 2009, 50-52.)

In thermal drying the water content of the digestate is reduced by evaporation. Thermal drying is usually used prior to incineration, to decrease the volume of the sludge or as a final hygienisation method. The assets from the thermal drying are increased dry matter content (up to 90%), decreased volume and hygienisation of the digestate. In anaerobic biogas plants thermal drying is used often to improve the marketability and usage of the digestate. Thermally dried digestate also fulfils the hygienic requirements set for digestate. All drying activities raise the investment costs and the waste waters resulting from the drying (reject waters) must be taken care of as well. (Latvala 2009, 52.)

The digestate can also be composted, in which case the digestate is left to degrade further and become humus by aerobic microbes. The end product, compost soil, is stabile and pathogens, weed seeds and phytotoxins are eliminated or become inactive. (Latvala 2009, 54.) The elimination of pathogens depends on retention time, pH and temperature, for instance. (Holm et al. 2010, 673). The elimination of plant pathogens in compost highly depends on the plant pathogens present in the digestate, for instance the elimination of *Sclerotinia* species' resting spores i.e. sclerotia, require thermo-

philic temperatures with adequate retention time (e.g. 3 weeks in 50-70˚C) or in lower temperatures in with longer retention time. (Lehto et al. 2007, 35). Composting decreases the odour and improves the spreading of the digestate even more. The drawbacks in composting are the space requirements, odour problems, produced greenhouse gases and need for aeration. (Latvala 2009, 53.) Moreover, aeration increases the cost of composting with investment, operational and maintenance costs. The annual cost of aeration per 1 cubic meter of liquid cow manure is 1. 07-1. 79€, including operational costs, such as electricity. (Suomalainen 2007, 65-66.) Aeration can be performed through active aeration (vacuum induced aeration or forced aeration) or naturally (convection, diffusion e.g. in windrow composts) or my mixing the compost material. If active aeration is being used, vacuum induced aeration consumes more energy than forced aeration. (Krogmann et al. 2011, 541-542.)

3.1 The benefits of the digestate

The biogas process improves the fertiliser quality of the nitrogen: in the biogas process the nitrogen, which is bound into organic material, breaks down into liquid ammonium, which plants can utilise efficiently. (Svensson et al. 2004, 461; Vänttinen et al. 2009, 9). The digestate has a good short-term fertilising effect and is thus suitable for plants which require high amounts of nutrients in the early stage of their growth, such as barley. (Svensson et al. 2004, 466; Weiland 2010, 849; Iivonen et al. 2013, 58). When the digestate is being handled properly, it should have high mineral nitrogen content (ammonium), low heavy metal content and a high availability of phosphorus. (Svensson et al. 2004, 466). The digestate can be utilised also as soil amendment medium: the digestate improves the soil quality by making it airier, friable, light and water-tight (in sandy soils). (Vänttinen et al. 2009, 9; Holm et al. 2010, 668). Due to the improved properties of the digestate, the nitrogen losses (by ammonia) are decreased as the digestate penetrates the soil faster. (Weiland 2010, 857).

Digestate from the anaerobic biogas process is usually less odorous (the odours of the feedstock can be decreased up to 80%) and more viscous than the original substrate, thus making the digestate more convenient to handle and spread in fields. Also, plant pathogens, weed seeds, phytotoxic compounds and pests are known are to become

inactive in the anaerobic biogas process. (Lehtomäki 2006, 23; Weiland 2010, 857.) The digestate can substitute mineral fertilisers, which is not only good considering the decreasing amount of materials used in mineral fertilisers, but also the digestate / anaerobic digestion utilises local substrates. (Weiland 2010, 849). Furthermore, if the anaerobic treatment is sufficient enough for the elimination of plant pathogens, crop materials which are not suitable for feed or food production due to their quality can be treated anaerobically and the digestate utilised as fertiliser or into some other purpose. (Haraldsson 2008, 8).

3.2 The constraints of the digestate

As digestate usage is regulated and controlled issue, there are costs related to the possible fees and taxes, which may rise above the economic benefit gained from using the digestate. Also, the digestate cannot be transported very far afield and it is thus more cost-effective to utilise the digestate near-by, so that the transportation costs do not exceed the economic benefit. Depending whether the digestate is utilised immediately or stored, the storing may also cause extra costs. (Holm et al. 2010, 670.) Although the digestate has really high ammonium nitrogen content, as the digestate goes through the drying operations, its nitrogen content may decrease significantly (up to 90% reduction). Also, the phosphorus content in the digestate is usually low and using digestate as a fertiliser solely in intensive grain cropping is not always sufficient enough. (Svensson et al. 2004, 461-466.)

Depending on the substrate used in the biogas process the digestate may contain compounds harmful to humans, plants and environment. Digestate from urban wastes may have high concentrations of toxic metals, which may accumulate in soils and inhibit beneficial microbial growth, affect soil ecosystem and plant growth. Sewage sludge and other organic substrates (food and plant waste) may include human (viruses, bacteria and parasites) and plant pathogens (fungi, bacteria, viruses, weed seeds). (Holm et al. 2010, 670-671).

3.3 Legislation related to digestate

In Finland, if the digestate from the biogas process is used as a fertiliser, the biogas plant must have a plant approval from EVIRA (Finnish Food Safety Authority). In order for a biogas plant to get approval, it must apply the approval with application form and the prerequisites are that the biogas plant meets the requirements set in Act on Fertilizer Products (539/2006). The applicant must show that the fertiliser or raw material of the fertiliser produced in the biogas plant is safe and suitable for use. In the application, the applicant must describe the hygienisation in the biogas process, how hygienic material and raw material are stored separately in the biogas plant, the sanitation of the biogas plant, how self-monitoring is carried out and traceability. (EVIRA 2013.)

A self-monitoring plan should include with applicable parts for instance responsible people, product specific information related to the origin of raw materials and quality of them, the description of production and operational processes, the quality assurance and so forth. A record must be kept out of the implementation of the self-monitoring system, including for instance results related to the safety of processes and handling. A self-monitoring report must be drawn out of the records kept, including deviations and problems and how they were handled, for instance. (EVIRA, 2012b.) The structure of the self-monitoring includes the prerequisite programs, identification and assessment of the risks, determining tools for management control and HAACP (Hazard Analysis and Critical Control Points) program, which is merely a tool for preventing food safety hazards, and it is used with good personal hygiene programs, manufacturing practices and sanitation open standard opening procedures. (EVIRA, 2011; EVIRA, 2012c.)

The requirements can be divided into two groups: treatment requirements and product requirements. (Marttinen et al. 2013, 8). General requirements set for fertiliser products are given in Act on Fertilizer products (539/2006). It is applied if the biogas plant produces fertilisers from the biogas residue. The legislation includes fertiliser products, partly the manufacturing of them, use and transportation of the fertilisers and partly their manufacturing for one’s own usage. The fertiliser products are required to be of uniform quality, safety and suitability for intended usage. (Latvala 2009, 17.)

3.3.1 Treatment requirements

Treatments requirements are either national or if the biogas plant handles animal by-products, it must also meet requirements set in The Animal By-products Regulation (EC) No 1069/2009 (Table 1.). Animal by-products are animal derived products for other purposes than human consumption. These include dead animals, animal wastes from slaughter houses and food wastes, for instance. Animal by-products are regulated in animal by-products Regulation ((EY) N:o 1069/2009) and in Implementation Regulation (EU) 142/2011, which includes specific instructions for implementation of (EY) N:o 1069/2009. Animal by-products may include human and animal pathogens, thus these wastes must be treated in a specific way. The Animal By-products act regulates collection, transportation, storing, pre-treatment, handling, usage, disposal, marketing, exporting, importing and transit of animal by-products. The animal by-products are divided into three categories according to their origin and different categories have different treatment methods (Table 1.). (EVIRA, 2012a.)

TABLE 1. Animal by-products classification and treatment regulations (Iivonen et al. 2013; REGULATION (EC) No 1069/2009, 2009)

	Category 1	**Category 2**	**Category 3**
Material	By-products with suspected risk of TSEs (transmissible spongiform encephalopathies)-diseases, unknown risk or the material includes residues of forbidden substances or environmental toxins	By-products with a risk of other diseases than TSE-diseases or risk of residues of animal	By-products which are gained from animals and accepted for human consumption, but which are not intended for human consumption for commercial reasons
Pre– or post-treatment	Category 1 materials are not suitable for use in biogas plants	Pressure sterilisation (20 min in 133˚C at 3 bar pressure, particle size <50mm)	Hygienisation (60 min in 70˚C, particle size <12mm)
Example	Food waste outside EU	Manure, dead or	Food waste, food-

materials		slaughtered industrial animals	stuff

According to national regulations, the biogas process can be either mesophilic or thermophilic, but if the digestate is used as a fertiliser, the end product must fulfil the requirements in a way that it does not include harmful substances, organisms or impurities more than permitted level. If the substrate used in the biogas plant does not contain plant pathogens (risk-free plant material such as fodder) the treatment requirement is mesophilic or thermophilic biogas process and the end product must fulfil hygienic requirements set in legislation. (Marttinen et al. 2013, 8-9.)

If the substrates are wastes or by-products from potato and vegetable peeling processes or separately collected biowaste the treatment requirement is heat treating moist material in 70˚C for 60 minutes with particle size less than 12 mm (or other method approved by plant protection authority. Also, the plant waste can be composted in 55˚C with 40% humidity for at least two weeks. (MMM 2011, 26.) The heat treatment can be done prior or after the anaerobic digestion. (Marttinen et al. 2013, 9). It is more feasible to do the hygienisation before the anaerobic digestion, since the heat treatment degrades solid organic material into liquid form, which the bacteria can utilise better. The pre-hygienisation also increases the feed materials dry matter content, as water is being vaporized. (Luste et al. 2012, 55-56.) The hygienisation done after the anaerobic digestion is more effective, but can be prone recontamination. (Sahlström 2003, 164; Weiland 2010, 857). The hygienisation may not destroy all the spores of the fungi. In addition, the retention time has to be long enough, as the retention time of the biogas process is shown to affect the survival of plant pathogens. (Luste et al. 2012, 55.) Moreover, separate post degradation by composting (at least 55˚C temperature and 40% humidity) is used as method for eliminating plant pathogens furthermore. (Iivonen et al. 2013, 60-74). If the biogas plant uses only manure and vegetable waste as substrates and the digestate is marketed, the required hygienic level can be achieved with thermal drying or compost. (Iivonen et al. 2013, 66).

3.3.2 Product requirements

End products from biogas plants according to their type name are digestate, reject water and dry granule, for instance. Product requirements include the maximum level of pathogens, impurities (e.g. weeds) and harmful metals. (Marttinen et al. 2013, 10.) If the fertiliser is plant origin, the spreading of pathogens must be eliminated during manufacturing. (MMMa 24/11). If necessary, the plant pathogen risk must be mentioned in fertiliser's manual as the restriction of use for certain areas with susceptible host plants. (MMM 2011, 10). While national legislation (Act on Fertilizer Products) requires the monitoring of plant pathogens from the end product, Animal By-product requirement requires the confirmation of adequacy of the treatment during treatment or immediately after treatment. (Marttinen et al. 2013, 10). National legislation requires the elimination of certain plant pathogens (Table 2.).

TABLE 2. Special requirements set for fertilisers from plant origin (MMM 2011)

Pest	Maximum amount
Globodera rostochiensis	Not detected in root, beet and potato raw material or from material carried among soil
Globodera pallida	
Clavibacter michicanensis	
Ralstonia solanacearum	
Synchytrium endobioticum	
Beet necrotic yellow vein virus	
Meloidogyne spp.	
Other pathogenic quarantine pests	Not detected in plant waste derived from greenhouse production or seed beds

The use of biogas residues as fertiliser cannot endanger the food hygiene safety of the primary production. The food hygiene safety of primary production is regulated in the regulation on the hygiene of foodstuffs (EY N:o 852/2004) and in Ministry of Agriculture and Forestry's act on primary production (1368/2011). The primary production is also regulated by EU's and national legislation, which includes regulation related to food safety (EU N:o 178/2002), regulation on microbiological criteria on foodstuff 2073/2005 and Food Act 23/2006.

4 PLANT PATHOGENS

4.1 Significance of plant diseases

The deleterious effects caused by plant pathogens do not only cause harm to the infected plant itself, but also to a crop yield, economic profit and environment. How vast the damages are, depend for instance on weather conditions, infecting pathogens, the host plant and control measures taken against the disease. Approximately one third of the produced plants go to waste due to plant pathogens in tropical and developing countries even more. (Valkonen et al. 1996, 16-17.) Plant diseases make approximately 10% cut to food production annually worldwide. (Jalli et al. 2010, 3). The more losses there are the more biocides are being used on them. (Waller et al. 2001, 25).

4.1.1 Quantitative and qualitative losses

Crop and yield losses are quantitative losses and can be divided into direct and indirect losses. Crop loss means the damage caused by single or several biotic factors (viruses, fungi, bacteria, nematodes etc.) causing reduction in financial return and it is measured as the difference between attainable yield and actual yield. The attainable yield means the yield that could be achieved in specific area if all the available crop protection measures would be implemented regardless of cost, thus only abiotic (environmental) factors would affect to yield. The actual yield is the yield achieved in specific area when existing crop protection measures are used. Yield loss means the damage caused by a single biotic factor, the difference between the attainable yield and the actual yield. (Nutter et al. 1993, 211-215.) Losses to the producer are mainly capital and income losses, which may in the worst case result in producer's economic difficulties. Losses during harvesting, storing and so forth usually affect the consumer process and thus the consumers suffer from the crop losses. (Valkonen et al. 1996, 16-17.)

Usually qualitative losses in products' utility value are far larger than the crop yield's quantitative losses. The decrease in quality decreases the resale value, utility value, healthiness and preservability / shelf life of the plant product, depending on the infectious plant pathogen. Some of the plant pathogens may also produce mycotoxins,

which are harmful to humans, and consumers may suffer from these losses among possible biocide residues and morbidity. (Valkonen et al. 1996, 16-17.)

4.1.2 Environmental losses

Use of pesticides and fungicides to prevent plant diseases may result in residues in environment. (Valkonen et al. 1996, 17). Also, as plant pathogens may become resistant to biocides, new and more effective pesticides and fungicides are needed. Biocides may also affect nontarget plants and animals or fungicides may destroy useful fungi species, for example. Nowadays biocides are indented to make more biodegradable, effective and more target-specific. (Klingberg 2012, 8-11.)

4.2 What is a plant pathogen?

“Plant pathology is the ’study of the suffering plants.’” (Sharma 2008, 1). Plant pathogens are divided into infectious and non-infectious diseases. The infectious diseases are called plant pathogens, which include bacteria, viruses, viroids and fungi, whereas noninfectious diseases (abiotic) are caused mainly due to environmental circumstances, mechanical damages or genetical disturbances. (Valkonen et al. 1996, 10.) Most plant pathogens kill the tissue of host plants for nutrition, thus these plant pathogens are necrotrophs. (Waller et al. 2001, 75). Plant diseases have three important components: the environment, the susceptible host plant and the pathogen, this is so called disease-triangle (Figure 3.). The occurrence of the plant disease requires the presence of all three factors. (Jalli et al. 2010, 4.) Time is considered also an important component regarding plant diseases, for instance as the time of year. Humans’ influence on plant diseases is the fifth component as it affects all earlier concepts: time of planting, the selection of cultivated crops, cultivating techniques, the use of chemical control and so forth. (Sharma 2008, 7-8.)

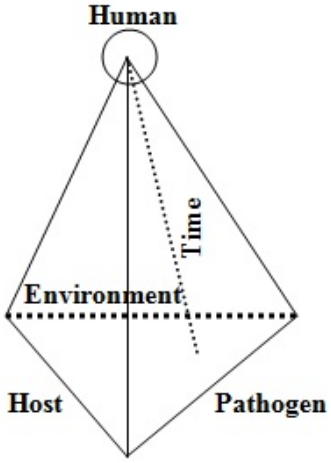


FIGURE 3. The interrelationships of different components in disease. Traditional disease-triangle includes only the susceptible host, pathogen and favourable environment (according to Sharma 2008, 8)

4.3 Stages in disease development

The chain of events which lead to the development of a disease is called disease cycle (Figure 4.). “Inoculum is any part of the pathogen that can cause infection” (Sharma 2008, 18) and at the first stage, inoculation, the inoculum gets in contact with the plant. For fungi, the inoculum is usually spores and sclerotia, for bacteria and viruses the whole entity of the bacteria of the virus. Pre-penetration is the stage prior to actual penetration. This stage includes for instance the germination of seeds and spores, the attachment of the pathogen to the host plant and recognition between the host plant and plant pathogen. The host plant or the plant pathogen may lack specific recognition factors, which result either in disease or failure in disease. After pre-penetration penetration takes place, the plant pathogen can penetrate the host plant through wounds and openings or directly through undamaged surfaces (by using enzymes). After the pathogen has penetrated the host plant infection takes place, where the plant pathogen gains nutrients from host cells or tissues. In the infection stage the pathogen also colonises the host plant where it grows and reproduces itself. The time from infection (or spore germination) to visible symptoms is called an incubation period. (Sharma 2008, 18-19.)

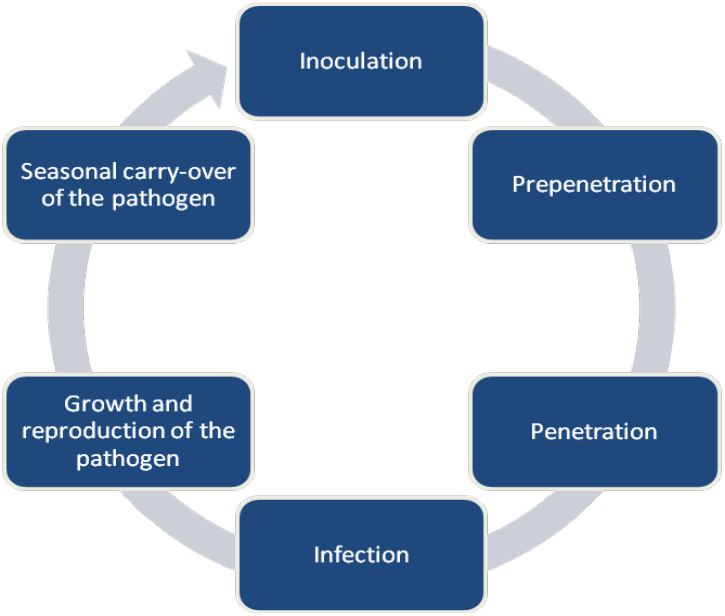


FIGURE 4. Disease-cycle (Sharma 2008, 18-19)

The disease cycle usually includes only attachment (i.e. inoculation), incubation and growth of fungi (i.e. pre-penetration), penetration and colonisation (i.e. infection). Sometimes also the dissemination and seasonal carry-over of pathogen is included in the disease cycle. Dissemination is the second spreading of the inoculum to new susceptible host plants after the pathogen has colonised the previous host plant. The seasonal carry-over of the pathogen is done for instance with resting spores, which stay in the soil through winter and attach to new susceptible plants in spring. (Sharma 2008, 20.)

4.4 Factors promoting and limiting plant pathogens in field ecosystem

The disease development requires more than just the presence of the pathogen and its susceptible host: generally environmental conditions influence whether plant pathogens occur or not. For a disease to grow optimally, earlier mentioned (Figure 3.) three factors of disease cycle must be present. The temperature is one of the environmental factors affecting plant diseases: depending on the pathogen some prefer lower temperatures and others require warmer temperatures. Moisture is another environmental factor affecting pathogens: it mostly affects the germination of fungal spores and penetration of the host by a germ tube. Moisture also increases the succulence of the host plant tissues. Powdery mildews are more severe and common in dry areas and for ex-

ample root, young seedling and tumble diseases such as *Sclerotinia* develop to be more severe on moist soils. Wind mainly promotes the spreading of pathogens e.g. fungi spores and by drying the surfaces of wet plants. Soil pH affects the plant pathogens which are soil borne and micronutrients and elements of the plant such as nitrogen, potassium and phosphorus affect to diseases as well, generally plants with balanced nutrition are more capable of protecting themselves. (Sharma 2008, 52-55.)

Resistance of plants is crucial regarding the richness of plant diseases. Breeding has increased the disease resistance of many plants species, especially gene technology has brought new ways of developing the disease resistance of plants. However, the new species of plant pathogen are also developing constantly, thus more resistant plants must be developed all the time. Intensive agriculture has lead to the cultivation of only a few plant species per area. Thus, these areas are more susceptible to epidemic plant diseases spreading due to decreased biodiversity. Hereby the change of crops and mixed plantation reduces the spreading of plant diseases. Some natural plants may also act as plant pathogen carries and their nearby presence may spread plant pathogens. (Valkonen et al. 1996, 96.)

Plant pathogens can be divided into two groups according to their prevention, the first group is those plant pathogens which produce resting spores and sclerotia and the second group includes fungi, which produce a great number of spores, and they spread efficiently long distances. The occurrence of group one plant pathogens can be estimated and prevented, whereas the second group plant pathogens usually require the use of chemical control and the plant pathogens may become resistant to biocides. Plant pathogens have to compete with other microbes and thus be able to change according to changing environmental conditions, including the presence of biocides. (Valkonen et al. 1996, 97.)

4.5 Fungi as a cause of plant disease

4.5.1 Structure of fungi

Fungi are the most significant cause of plant diseases worldwide. Fungi are eukaryotic, which means that membrane distinguishes between a nucleus and cytoplasm and fungi can be either unicellular or multicellular. Fungi lack chlorophyll particles, thus they need external energy, which they get from plants. Fungi consist of the non-reproductive part of the fungi, hypha (single) (several hyphaes comprising mycelium) and reproductive part. Hypha comprises most of the fungi, and usually there is a cell wall between the cells. The structure of the cell wall is important when the prevention of fungi is studied, as some of the fungicides prevent the growth of fungi by inhibiting the growth of cell walls. Most fungi cells' walls are composed of chitin. (Valkonen et al. 1996, 10, 20-21.)

4.5.2 Activity

Fungi cells take nutrients and remove excretion outside the cells and thread. Fungi require organic compounds from their environment, as they cannot produce those by themselves with photosynthesis. Fungi use sugars such as sucrose, maltose and glucose as a source of energy. Moreover, fungi require small amounts of inorganic compounds, water and oxygen. Simple organic compounds such as monosaccharides, amino acids and organic acids absorb through the cell membrane through diffusion, the absorption is passive and does not consume energy from fungi. Fungi can also excrete enzymes to break down larger molecules into water-soluble and small molecular composition. Different fungi species excrete only specific enzymes and hence cannot break all plant materials, in addition, the production of some enzymes is constant, but other enzymes need external stimulation. The breakdown of plant tissue is visibly seen as necrosis, rotting and decaying. Moreover, fungi can produce phytotoxins, which breakdown plant tissue, after which the fungi can utilise it as nutrition. Mycotoxins are toxins produces by fungi, which are harmful to humans and animals. (Valkonen et al. 1996, 22.)

4.5.3 Differentiation and reproduction

Fungi can produce different kinds of threads and organs, which are essential for the attachment, penetration and nutrition of fungi. Fungi can also produce structures,

which ease the survival of fungi, such as sclerotia (Picture 1.), which darken due to melanin pigmentation and become hard. Sclerotia produce apothecium when favourable environmental conditions occur. Some fungi may produce long threadlike structure, rhizomorps, which can reach long distances to find the susceptible host plant. (Valkonen et al. 1996, 24.)

The spore is a reproducing unit of fungi, which can remain viable even in unfavourable conditions. Sexual spores are produced in fruiting body and they can vary according to their emergence. *Sclerotinia* species spores, ascospores, reproduce from apothecium (Picture 1.), for instance. Asexual spores are neckling directly from thread or sporangium. The intensity and wavelength of light, temperature, humidity, pH and nutrition of fungi affect production of fruiting bodies and spores. Spores may lay dormant (resting spores) due to external circumstances (too low a temperature, too high a humidity) and germinate when favourable conditions occur. (Valkonen et al. 1996, 20-26.)

PICTURE 1. Apothecia and sclerotium of *S.sclerotiorum*. Picture: Hanna Avikainen

4.6 Spreading of fungi in nature and in farming environment

Fungi are capable of spreading in various ways; the most efficient way is via spores airborne. However, spores are vulnerable to some environmental conditions, such as

drought, low temperatures and ultraviolet radiation (UV). The spores are produced in the fruiting body of the fungi, which stays closed until there are favourable conditions for the spores to spread. Light spores spread easily with wind, but the spores should also be able to land on the host plant and stay there. (Valkonen et al. 1996, 36.)

Spores can spread via rain and splash drops. Spores flying in the air are moved in rain drops to the surface of plants and ground. The rain drops spread the existing spores on the plant leaves. Thus, not only do the rain drops spread spores, but also provide essential moisture for the spores to germinate. Some spores can also spread via irrigation water and water bodies. However, most spores are destroyed after being in water for long. Different insects spread also spores, for instance pollinator insects spread spores when they visit blooms e.g. of turnip rape and rape seed. Nematodes spread mainly viruses but they also assist further fungal infections by making puncture holes to the roots of the plant. (Valkonen et al. 1996, 36.)

Soil and plant wastes can be a source of fungi, as fungi can spread via threads. When the plant wastes are being removed the fungus spores, threads and the resting spores are moved as well. The infected plant wastes stored near-by a field can induce a significant risk of fungal infection. Spores can also spread via seedling of plant, on the surface of the seed or some fungi may grow inside the seed. Fungi also spread in bulbs, rootstocks and in cuttings. The global spreading of fungi and their diseases is mainly due to international marketing of plant seeds and seedlings. Yet, imported seeds and seedlings are inspected carefully before usage. Fungi can spread also due to cultivation techniques from field to another via maintenance equipment, shoes and vehicle tyres. In addition, when budding, cutting or tying the plants, fungi and other plant pathogens are easily spread from soil and plants. (Valkonen et al. 1996, 37.)

4.7 *Sclerotinia* species

*Sclerotinia*s have ability to infect several types of plants (for instance iceberg lettuce, cabbage (Picture 2.), oleiferous plants and so forth. (Koike et al. 2006, 23). In South Savo region, Finland, the two detected *Sclerotinia* species from fields were *Sclerotinia sclerotiorum* and *Sclerotinia subarctica*, *Sclerotinia* species are considered the most

serious plant protection problem in South Savo region. *S.subarctica* has been observed just recently in South Savo and its ability to cause diseases to different host plants is not well known at the moment. (Tuomola et al. 2012, 15-17.)

PICTURE 2. Cabbage infected with *Sclerotinia* disease. Picture: Hanna Avikainen (Tuomola et al. 2012, 12)

Sclerotinia diseases cause crop damages to oleiferous plants (e.g. turnip rape and oilseed rape) especially during rainy season. The crop yield and the oil content of the seeds decreases considerably due to *Sclerotinia*, hence it can cause the losses of hundreds of kilograms per hectare. The first symptoms of the disease occur 3-4 weeks after bloom: watery spots are developed into the stalks and stems of the plants (Figure 5). The infected parts of the plant turns white at first but later on they turn brownish. As the disease proceeds, the upper parts of the plant dry off and the development of the plant's own seeds stops, the stems also break off easily. Black sclerotia (Picture 1.) are formed inside the stem, which usually stay in the ground after harvesting. *Sclerotinia* species overwinters in the ground mainly as the survival structures, sclerotia, which can stay viable several years. (Lassi & Tulisalo 2012, 17.) The "half-time" of sclerotia (time when half of the sclerotia have perished) is 1-3 years, thus the sclerotia are persistent in soil, as they have thick wall protecting the spores from light, excess moisture and so forth. Nematodes, bacteria and unfavourable environmental conditions can eliminate sclerotia. (Valkonen et al. 1996, 38-39.)

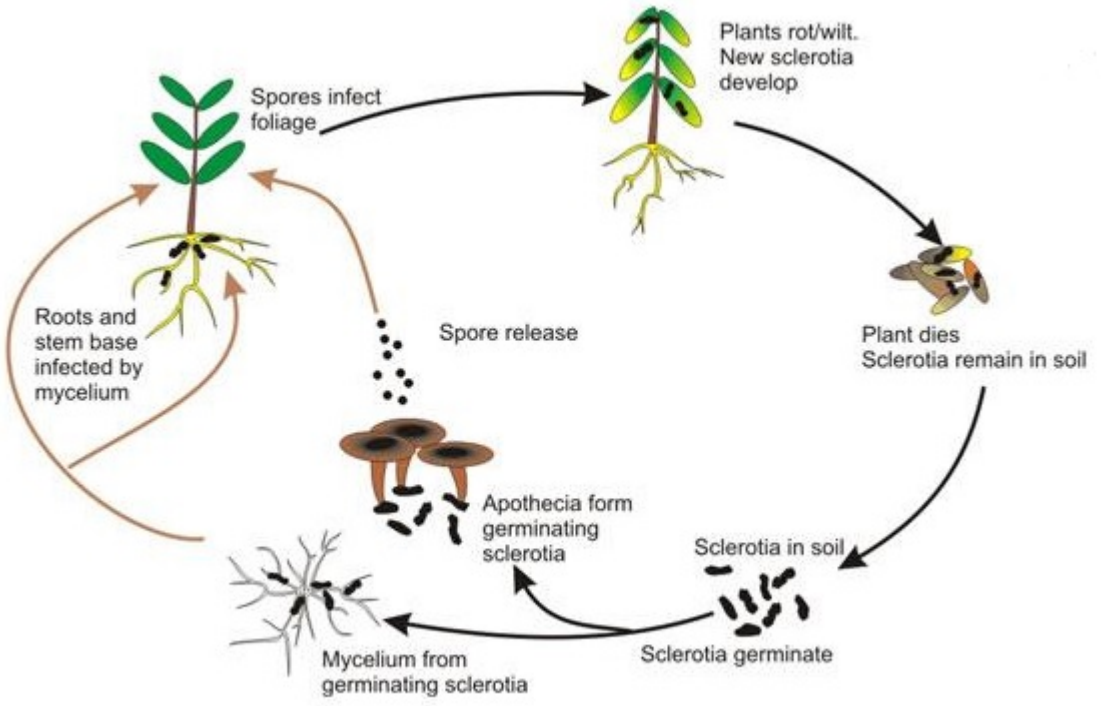


FIGURE 5. The life-cycle of *S.sclerotiorum* (HDC Factsheet 2007)

Weather conditions are vital to the spreading of *S.sclerotiorum*. The soil must be humid enough for several days for apotheciums (Picture 1.) to develop, the spores are spread from apotheciums air borne. (Lassi & Tulisalo 2012, 17-18.) *Sclerotinia sclerotiorum* can occur as asymptomatic in cabbages and carrots, but after harvesting and storaging the *S.sclerotiorum* can cause a total rotting of the vegetables. (Valkonen et al. 1996, 74). *S.sclerotinia* and *S.minor* causes Pink rot for celery, for carrots *S.sclerotiorum* and *S.minor* cause White mold, cottony rot and watery soft rot. White mold caused by *Sclerotinia* can be found also from parsley and other apiaciae plants, broccoli, cauliflower and cabbage. (Koike et al. 2006, 88-89,111-112, 127, 189-190.)

4.8 Prevention and elimination of *Sclerotinia* species

4.8.1 Cultivation practices and chemical control

In general whatever options are chosen for management of the diseases, the control measure must be effective enough to achieve acceptable levels of control. Secondly, the method chosen must be economically practical, so that using it does not cause

more losses than profit from the cultivated plants. Disease control methods must be also applicable to commercial use (some new methods have been studied, but not commercialised yet, such as some biocontrol agents). Also, the method must be suitable with other methods used, as usually the control of diseases requires more than just one method, for instance cultural practices combined with resistant plant species and fungicides (integrated plant protection). The management of plant pathogens relates to the so called disease triangle (Figure 3.): as disease development requires all three factors (susceptible host, plant disease and favourable environment) by eliminating or modifying one of them the disease can be managed. (Koike et al. 2006, 38.)

The best method for controlling *Sclerotinia* species is crop rotation, in which case the susceptible host plants for *Sclerotinia* species should not be cultivated. Turnip rape and oilseed rape are cultivated maximum at three year intervals, for instance. (Lassi & Tulisalo 2012, 17-18.) During the crop rotation non-susceptible plants for instance grain, corn and grasses can be cultivated. (Laemmelen 2001, 3).

If fungicides are used for the prevention of *Sclerotinia* diseases, the spraying of fungicides should be done in the early stage of blooming, before the symptoms occur. The fungicide makes the petals toxic for *Sclerotini*a species and thus the penetration of the pathogen is prevented. (Lassi & Tulisalo 2012, 18.) Use of fungicides has its drawbacks: they are costly, it is hard to treat large volumes of plants to eliminate the pathogens, the pathogen may become resistant to the biocide and stronger ones are needed. Also not well planned application of biocides may pollute environment and food more than necessary. The biocides usually do not only affect the intended pathogen but also other plants, beneficial microbes and animals. (Kumar et al. 2008, 69; Klingberg 2012, 8-15.) Also, for most soil borne plant diseases fungicides are not usually very effective. (Koike et al. 2006, 44-47).

Monitoring the occurrence of persistent soil borne pathogens, such as *Sclerotinia* species, is important. If seed borne pathogens are a great concern, all the materials which are infected or contaminated should be removed, so that the contamination does not spread. Seed borne plant pathogens usually derive from infected seeds, and if the seeds are cultivated the healthy plants may become infected as well, for example. Sanitation

is a practice, in which the contaminated material is being removed, for instance *Sclerotinia minor* is being sanitized by plowing the soil deeply, so that remains of sclerotia are being buried as the soil is inverted. (Koike et al. 2006, 38-48.) Drip irrigation has shown to minimise the occurrence of *Sclerotinia* species compared with overhead irrigation, as favourable conditions for *Sclerotinia* species (cool temperatures, high soil moisture, high air humidity) are being changed. (Laemmlen 2011, 3).

There have been studies showing the elimination of *Sclerotinia* species with antagonist agents. These types of biological agents act either by competing with the plant pathogen for nutrients, oxygen and space or by directly affecting the plant pathogen by antibiosis or mycoparatism. *Sporidesmium sclerotivorum* acts for instance as a parasite for *S.sclerotiorum, S.minor* and *S.trifolicum cepivorum* and it has appeared to be nearly a perfect biological control agent. (Kumar et al. 2008, 73-74.) Also *Coniothyrium minitans* have shown to have great biological control effect against *Sclerotinia* species on (*S.sclerotirum* and S.cepivorum). (Koike et al. 2006, 47; Kumar et al. 2008, 73-74). In Europe there is an accepted product for the prevention of *Sclerotinia* species, Contans®WG, which is based on *Coniothyrium minitans* fungi. The product is not registered in Finland at the moment. Some laboratory and field experiment has been done to open land vegetables with the control agent, the results were moderate and the control agent needs more investigation. (Tuomola et al. 2012, 22-25.) Also *Trichoderma* species have shown to have antagonism towards *S.sclerotiorum* and *S.cepivorum* by antibiosis, filtering toxins or competing for nutrients. (Castillo et al. 2011, 414-416; Kumar et al. 2008, 73-74).

As the use of methyl bromine as disinfectant was prohibited in Finland in 70's the use of biofumigation has increased. In biofumigation naturally occurring defence compounds of the plant itself, glycosinolates, are utilised as disinfectant agents. Glycosinolates are non toxic compounds, but when they break down due to enzymes or microbes the released ITC -compounds (icotiocyanate) are toxic to some organism in large concentrations. Biofumigation is done by mixing the biomass of brassica plants (e.g. mustard) with a seedbed. Studies have shown the reducing effect of biofumigation on *Sclerotinia* species in cultivation experiment and in field experiments combined with mulch. Biofumigation experiment was also conducted in South Savo in

2010-2011, in greenhouse conditions and in laboratory cultivation the effect of biofumigation on *Sclerotinia* species was significant, whereas in field experiment the effect was not that significant. The effectiveness of bio fumigation is influenced by the content of glycosinolates, the composition of chemical gases produced, amount of biomass used, soil texture, temperature, humidity and how fast and well the biomass material is grinded and spread to soil. (Tuomola et al. 2012, 26-38.)

4.8.2 In biogas process

Factors affecting the hygiene of the digestate depend on the pathogens present in the feed material, their amount, the temperature of the biogas process (mesophilic, thermophilic), retention time, process type, the use of the hygienisation unit and other process conditions treatment type (batch or continuous process) and available nutrients. (Sahlström 2003, 162-163; Iivonen et al. 2013, 62; Marttinen et al. 2013, 34-35). The easiest parameters to monitor regarding the elimination and the prevention of growth of plant pathogens are temperature, retention time and pH. Temperature and retention are the most important treatment parameters as sufficient temperature has shown to eliminate pathogens most likely, temperature above 50˚C inhibit the growth of most pathogens. (Marttinen et al. 2013, 35-37.) Continuous reactors are more commonly used in biogas processes. However, studies have suggested that batch reactors are better in the elimination and reduction of microbial populations and the fresh material leaving the continuous reactor may not be totally sanitised. (Ryckeboer et al. 2002, 205; Sahlström 2003, 163-164.) Batch reactors are more easily controlled than continuous reactors with regard to the temperature and time. (Sahlström 2003, 163-164).

Termorshuizen et al. conducted a study, where the survival of six pathogens in mesophilic digestion was studied, two human and four plant pathogens. In their study *Sclerotium cepivorum* survived mesophilic biogas process with 21-day retention time, whereas other plant pathogens studied (*Fusarium oxysporum, Ralstonia solanacearum, Plasmodiphora brassicaea*) were more sensitive to anaerobic mesophilic conditions. (Termorshuizen et al. 2003). The oxygen content, moisture level and the temperature have shown to affect *S.sclerotiorum* and *S.minor* in soil experiments, where especially the survival of sclerotia declined in wet soils with low oxygen concentration

(anoxic conditions). Also, almost all sclerotia lost their viability after two weeks being in soil with low oxygen (0.1%), 25˚C temperature and high moisture (20%). (Wu et al. 2008, 664.) Thermophilic biogas process has shown to eliminate some plant pathogens, and if the digestion process was continued with composting the elimination was even more effective. (Ryckeboer et al. 2002, 214). The optimum temperature for the growth of S.*Sclerotinia* is 20-25˚C, whereas four days in 30˚C resulted in poor growth and no sclerotia formation. (Cuong N.D. & Dohroo N.P. 2006, 74).

Methane and VOC produced in anaerobic digestion combined with the temperature and moisture is known to affect plant pathogens survival. (Iivonen et al. 2013, 69; Marttinen et al. 2013, 34). VFA affects the survival of pathogens, especially in low pH levels, for example low pH or too high pH (<4 or >11) may also inhibit the growth of some plant pathogens. (Marttinen et al. 2013, 35). In addition ammonia produced in the digestion process inhibits the growth of several fungal pathogens's spores germination. (Valkonen et al. 1996, 106; Marttinen et al. 2013, 34). However, it is known that some plant pathogens are able to maintain their viability even after hygienisation (for instance fungal spores and especially bacterial spores. (Haraldsson 2008, 10; Weiland 2010, 857; Marttinen et al. 2013, 34). If it is known that the feed material may contain heat tolerant plant pathogens, the hygienisation should be done in higher temperatures (74-90˚C) with longer retention time e.g. 2 hours in 80˚C or 1 hour in 90˚C. (Noble et al. 2009, 18; Iivonen et al. 2013, 69). Post-storage of the digestate has shown to minimise the amount of some fungi. Also competition for nutrients and space among different microbes in the biogas process (pathogens, other microbes and methane producing microbes) may decrease the amount of pathogens. (Marttinen et al. 2013, 34-35.)

5 MATERIALS AND METHODS

The survival of *Sclerotinia sclerotiorum* and *Sclerotinia subarctica* was studied with laboratory-scale batch biogas experiment in the environmental laboratory of the Mikkeli University of Applied Sciences. For the experiments, which were conducted in two sets, pre-cultivated sclerotia were being used. The same experiment was conduct-

ed for two *Sclerotinia* species: *S.sclerotiorum* (experiment 1) and *S.subarctica* (experiment 2). The experiment for *S.sclerotiorum* took place in the autumn 2012 and the second experiment for *S.subarctica* in the spring 2013. The duration of both of the experiments was 21 days for the batch biogas assay and approximately 14 days for cultivation experiments. The same steps applied for both of the experiments.

5.1 Cultivation

The sclerotias were obtained from Helsinki University's Ruralia institute, the cultivation conditions were based on the publication "Biofumikaatio jäävuorisalaatin viljelykierrossa – menetelmän mahdollisuudet ja heikkoudet pahkahomeen torjunnassa" by Iivonen et al. 2012. The sclerotia were cultivated into PDA medium, with 40 mg/l streptomycin (to prevent bacterial growth in the medium). The sclerotia were grown approximately 14 days in dark and room temperature.

After there were enough sclerotia, they were transferred into small net bags, 10 sclerotia were transferred into each bag. For experiment 1. 8 net bags were used, one for each glass bottle. For second experiment, 10 net bags were used, one for each glass bottle. Four of the net bags with sclerotia (2 of *S.sclerotiorum* and 2 of *S.subarctica*) were pre-hygienisated with heat-treatment, which was done by putting the net bags into +70˚C water and keeping them in an incubator in 70˚C for 60 minutes.

5.2 Inoculum, substrate and anaerobic biogas assay

The biogas batch experiment was conducted in 2-litre glass bottles. The substrate used was liquid cow manure (stored in cold storage room, approximately +4˚C) from Juvan Muumaa GP, Juva and inoculant from Juvan Bioson Ltd, Juva (the plant operates on liquid cow manure, dry chicken manure and vegetable waste). The liquid cow manure and inoculants were mixed with 50:50 VS-ratio in batches 3 & 4 (31.3 gVS) (exp. 1), and batches 4 & 5 (33.3 gVS) (exp. 2). The total volume of the inoculants and substrate with water was 1500 ml, where the proportion of inoculants was 750g for both of the experiments and the proportion for liquid cow manure was 388 g for exp 1. and 390g for exp 2. The pH was measured before and after the experiment, the determina-

tions were done according to standard SFS 3021: 1979. If necessary, the pH of the batch was adjusted approximately to 7 with 1M HCl before the biogas assay. The determination of total solids (or dry mass) and volatile solids were based on standard SFS 3008: 1990. Total solids were determined by drying the biomass in the oven in 105˚C for approximately 24 hours, total solids include biologically degradable organic material and non-degradable material such as lignin. Volatile solids were determined by incinerating the dry material (TS) in 550˚C approximately 2 hours, until the samples were turned to ashes (to determine anaerobically degradable material). There were three parallels to each sample.

2-liter bottles were attached to aluminium bags via siphon (Figure 6.) and a sample bottle for gas measurements was attached to the siphon (from which methane determinations were done). All the batch bottles were put into a heated closet, the temperature in the closet was 36±0.5˚C in experiment 1 and 35±0.5˚C in experiment 2. The temperature in the closet was observed with digital temperature meter and the results were recorded the same time as biogas measurements were conducted. The net bags with sclerotia were added in all of the bottles. Batches 1 A&B (hygiene reference) in both experiments and 2 A&B in experiment 2 (hygiene reference with aeration) were left open, other ones were closed with fitting silicone stoppers and flushed with nitrogen gas (N_2) approximately 3 minutes to obtain anaerobic conditions. The reactor bottles were shaked daily to prevent sedimentation, improve hydrolytic decomposition and maintain constant digestion. (Soininen et al. 2007, 13).

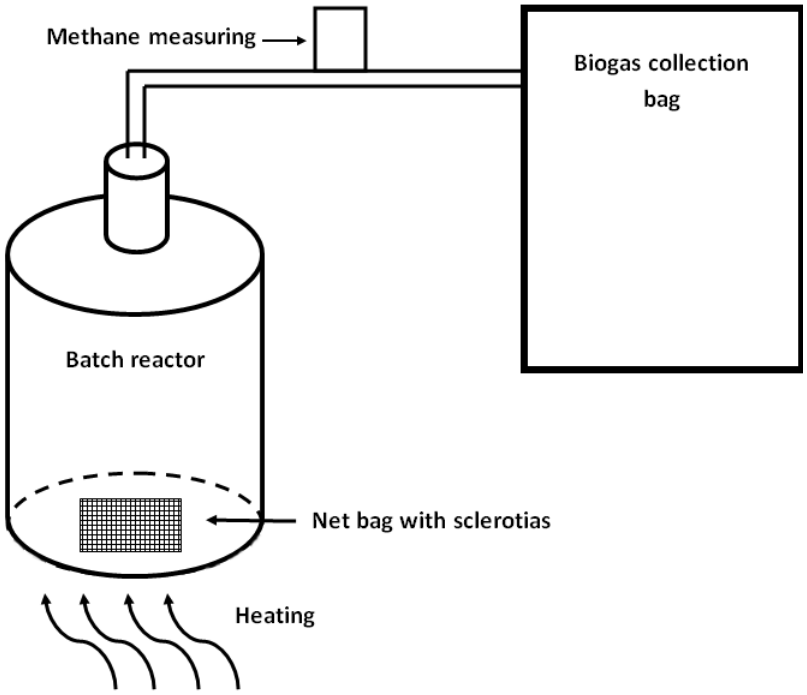


FIGURE 6. Illustration out of the experimental set up

For experiment 1 (*S.sclerotiorum*), there were 4 different batches with the parallel batch (A&B) and with different treatments (Table 3.). For experiment 2 (*S.subarctica*), there were 5 different batches with the parallel batch (A&B), with different treatments (Table 3.). The treatments were the same for both of the experiment, except that experiment 1 did not include a batch with aeration (batch 2 in experiment 2).

TABLE 3. Different batches with description, A&B are parallels (experiment 1 & 2)

Batch number	Description
1 A & B Hygiene reference (sclerotia + water)	Sclerotia were put in distilled water (1500 ml), without any substrate or inoculant. There were no caps in the bottles and thus no biogas collection
2 A & B (only experiment 2) Hygiene reference with aeration (sclerotia + water + aeration)	Same as batch 1, except with aeration, to increase oxygen content in the water.
2 A & B (exp.1), 3 A & B (exp. 2) Biogas reference	750 g of inoculant, filled with distilled water till 1500 ml

(sclerotia + inoculant + water)	
3 A & B (exp. 1), 4 A & B (exp. 2) (sclerotia + inoculant + liquid cow manure + water)	750g of inoculant, 388g (1)/390g (2) of liquid cow manure, filled with distilled water till 1500 ml
4 A & B (exp. 1), 5 A & B (exp. 2) (pre-hygienisated sclerotia + inoculant + liquid cow manure + water)	Same as for batch 3/4, except sclerotia were put in sterile 70˚C hot water for 60 minutes and kept in incubator in 70˚C for 60 minutes

The anaerobic biogas experiment lasted 21 days, during which the biogas and methane yield was measured every 1-5 days in exp. 1 and in exp. 2 every 1-4 days, in the beginning when the biogas yield was high, more frequently. The biogas yield volume was measured using volumetric measurement, a volume displacement device (Picture 3.); the principle was based on the displacement difference of water with gas. The biogas yield measurements were made in room temperature (approximately +20˚C). The methane content from biogas was analysed with a gas chromatograph (HP Agilent 6890 GC: PerkinElmer Elite-Alumina column 30 m x 0,53 mm, flame ionisation detector 225˚C, oven 100˚C, inlet 225˚C, carrier gas helium 10ml/min, split ratio 35:1, injection volume 100µl). The time of the experiments was determined to be 21 days, since according to previous laboratory batch assays longer retention time would be more efficient regarding the stabilising of methane and biogas production. However, some of the slowly degrading material (e.g. lignin content materials) may not have been degraded during the process.

PICTURE 3. Biogas yield measurement using a volume displacement device. Picture: Sari Seppäläinen

5.3 Determination of DO in batch 2 (exp.2)

The second batch with aeration in experiment 2 was made in order to test, whether the oxygen concentration in the water affects the growth of sclerotia (in other batches anaerobic conditions prevailed). For batch 2, in experiment 2, the theoretical diluted oxygen in the bottle was calculated with an air flow rate (approximately 2 l/min), knowing that oxygen content in the air is approximately 21%, and oxygen dissolution 6.95 mg/l in 35˚C (SFS-EN 25814:1992). The diluted oxygen concentration for 1.5 litre volume water is 10.4 mg and the aeration pumps 3 mg of oxygen in a minute into the bottle, thus the oxygen concentration should be sufficient. However, the aeration's position was changed during the experiment, to confirm the sufficient level of oxygen: first the net bag was at the bottom of the bottle with the aeration device, but the net bags were moved later on above the aeration device. As there was no organic material

present (other than sclerotia) in the batch reactor the biological oxygen demand of the microbes is not very high, and the level of diluted oxygen should be sufficient.

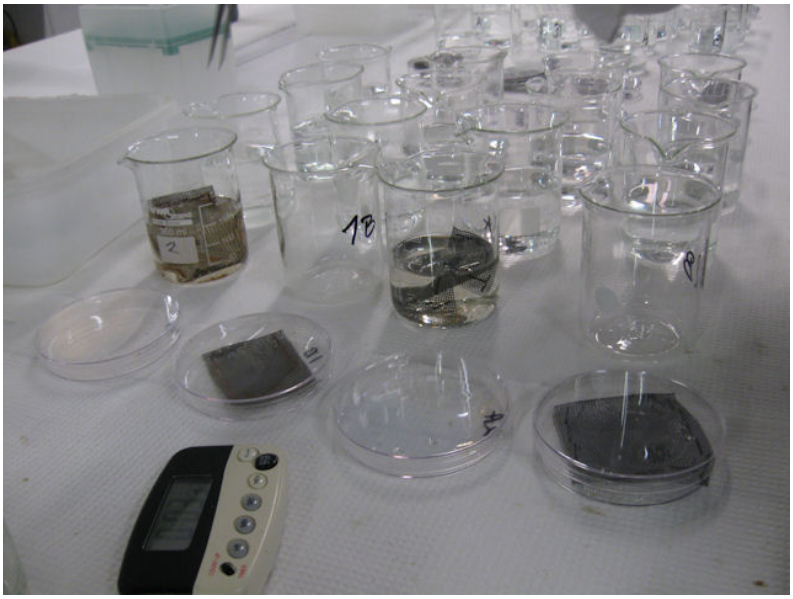
5.4 Unloading and washing

The batched were unloaded after 21 days of anaerobic digestion. Total solids, volatile solids and pH were measured alongside with unloading (Picture 4.). The same methods for the determination of pH, TS and VS were used as in Section 5.2., results are shown in Section results 6.2, pH was approximately 7 for both of the experiments.

PICTURE 4. pH measurement alongside with unloading (exp. 2). Picture: Sari Seppäläinen

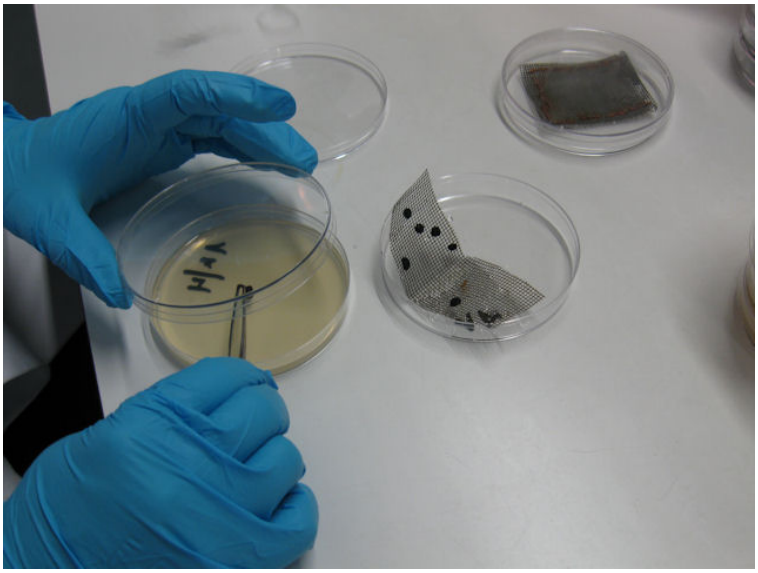
After unloading the sclerotia net bags from the reactor bottles, the net bags were washed from substrate (Picture 5.), in order to diminish bacteria and other fungi. The sclerotia were washed 5 minutes in 70 % ethanol, and then rinsed 3 times in sterile water. In order to make sure that washing the sclerotia with disinfectant had not affected or inhibited the growth of the *S.subarctica* and *S.sclerotiorum* the same washing operation was done to both species in the same method as with washing in unloading, but without putting them into the mesophilic anaerobic biogas process. There were two sets from both species with two different kind of treatment to each one of the species with five parallels, five sclerotia from each species was cultivated without any

treatment and five sclerotia were cultivated after treating with alcohol and sterile water.



PICTURE 5. Unloading and washing of sclerotia (exp.1). Picture: Tuija Ranta-Korhonen

After washing, the sclerotia were arranged in the middle of the Petri dish, using tweezers (Picture 6.), one sclerotium per one Petri dish and grown in the dark approximately 14 days in room temperature. When opening the net bags, it was noticed that some of the sclerotia were damaged, especially in batch 4 (exp.1) and 5 (exp. 2), which were pre-hygienisated. The sclerotia may have also damaged during the anaerobic biogas process or during the washing process. The results were observed every 1-3 days to see whether sclerotia have survived. The diameter of the fungi was measured in millimetres using a calliper.



PICTURE 6. Cultivation of sclerotia after unloading (exp. 1). Picture: Tuija Ranta-Korhonen

6 RESULTS

Neither the sclerotia of *Sclerotinia sclerotiorum* nor *Sclerotinia subarctica* survived the anaerobic mesophilic biogas process. All the Petri dishes were either empty (containing only the initial sclerotia), had some yeast growth or some other, dark green, fungi growing on them. Pictures out of the results are shown in Appendix 1 (Pictures 7-9). The alcohol treatment did not inhibit the growth of sclerotia though it slowed down the growth of the treated sclerotia at the beginning for a few days compared with untreated sclerotia. Pictures from the confirmation test are shown in Appendix 2 (Pictures 10 & 11.)

6.1 Methane and biogas yield

6.1.1 *S.sclerotiorum* (exp. 1)

The proportion of methane out of produced biogas was approximately one third (in batch 2 and 3) and approximately half in batch 4 (Table 4.). Cumulative methane yields were calculated without inoculant using the biogas reference batch (2). The methane content in pre-hygienisated batch was notably higher compare with non-

hygienisated batch, whereas the biogas production was relatively higher in non-hygienisated batch.

TABLE 4. The percentage of methane out of biogas, cumulative biogas and methane yield and the amount of biogas and methane produced from 1 g of VS in experiment 1 *(S.sclerotiorum*)

Batch	Biogas (%)	Cum. Biogas (ml)	Cum. Biogas (ml/gVS)
2 A & B (biogas reference)	100	3600	110
3 A & B	100	13000	420
4 A & B (hygienisated)	100	10300	330
	CH4 (%)	**Cum. CH4 (ml)**	**Cum. CH4 (ml/gVS)**
2 A & B (biogas reference)	27	980	31
3 A & B	28	3800	120
4 A & B (hygienisated)	45	4500	150

There were some differences between the pre-hygienisated batches (4 A & B) compared with non-hygienisated batches (3 A & B) (Figure 7.), especially in the cumulative biogas yield and methane content. The proportion of methane from biogas was higher in batch 4 (pre-hygienisated batch), whereas the biogas content was higher in batch 3 (non-hygienisated batch).

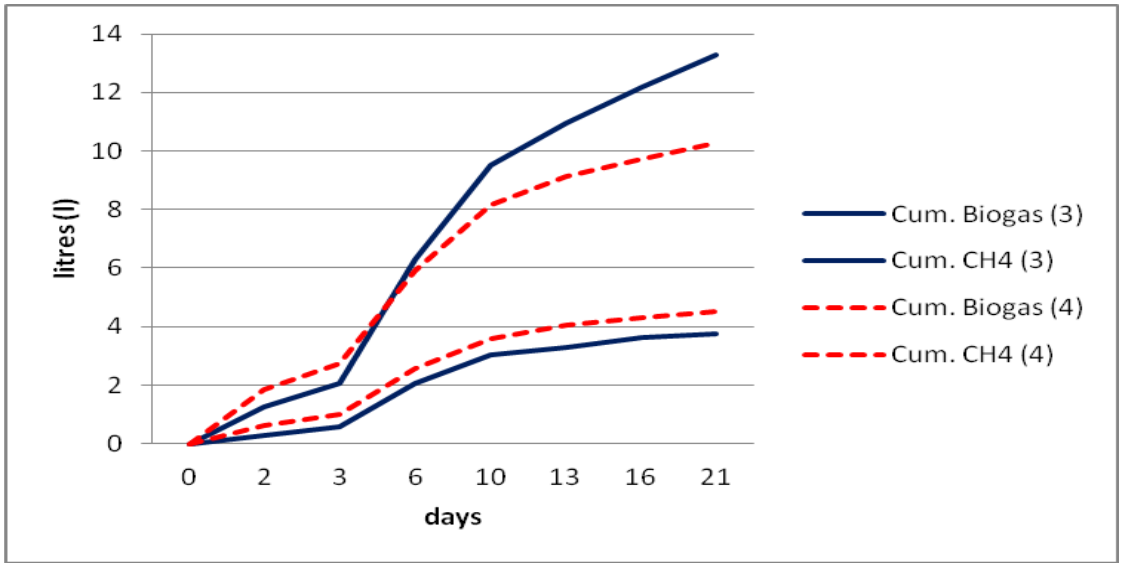


FIGURE 7. The cumulative biogas and methane of experiment 1, batch 3 & 4 (pre-hygienisated)

6.1.2 *S.subarctica* (exp. 2)

The proportion of methane out of produced biogas was approximately one third in all batches (Table 5.). Cumulative methane yields were calculated without inoculant using the biogas reference batch (3). There were no remarkable differences between the pre-hygienisated batches (5 A & B) compared with the non-hygienisated batches (4 A & B) (Figure 8). The cumulative methane yield does not differ either remarkably between the batches.

TABLE 5. The percentage of methane out of biogas, cumulative biogas and methane yield and the amount of biogas and methane produced from 1 g of VS in experiment 2 (*S.subarctica*)

Batch	**Biogas (%)**	**Cum. Biogas (ml)**	**Cum. Biogas (ml/gVS)**
3 A & B (biogas reference)	100	8800	250
4 A & B	100	13000	380
5 A & B (hygienisated)	100	13400	402
	CH4 (%)	**Cum. CH4 (ml)**	**Cum. CH4 (ml/gVS)**
3 A & B (biogas reference)	30	2600	69
4 A & B	39	5400	160
5 A & B (hygienisated)	35	4700	140

In experiment 1 the biogas production started after 3 days of starting the biogas process (Figure 7.), whereas in experiment 2 the biogas production started smoother (Figure 8.). Experiment 2 had fresher inoculant than experiment 1 and the delay in biogas production is because the process in experiment 1 needed a longer time to adapt.

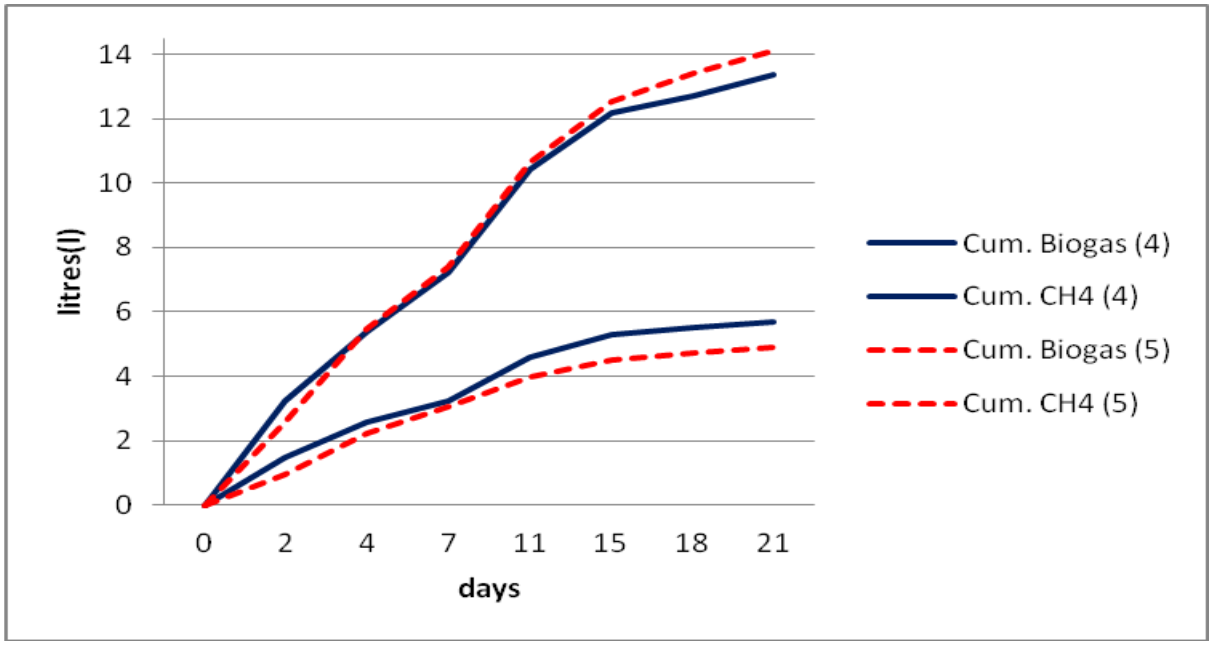


FIGURE 8. The cumulative biogas and methane of experiment 2, batch 4 & 5 (pre-hygienisated)

6.2 TS/VS/Biodegradation

Total solids and volatile solids were determined before and after the batch assay; the results of the first determination are shown in Table 6. Liquid cow manure for exp.1 and exp. 2 was obtained the same date (25th October 2012) although due to different time of determination of TS and VS, the values vary from each other slightly.

TABLE 6. Values for total solids (TS) and volatile solids (VS) in percentages for experiment 1 (*S.sclerotiorum*) and experiment 2 (*S.subarctica*) and amount of organic dry material is grams (gVS) before mesophilic batch biogas assay

Materials	Obtained (date)	TS (%)	VS (%)	gVS
Inocculant (1)	22.11.2012	5.7	4.2	31.3
Inocculant (2)	10.1.2013	6.1	4.4	33.3
Cow manure (1)	25.10.2012	9.6	8.1	31.3
Cow manure (2)	25.10.2012	10.0	8.6	33.3
Inocculant :& Cow manure 50:50 (1)		7.6	6.1	
Inocculant :& Cow manure 50:50 (2)		8.1	6.5	

Figure 9. shows the biodegradation (indicated by VS removal) in percentages and standard deviations, the initial values for VS are shown in Table 6. The amount of VS had reduced approximately by half in both experiments in both batches (non-

hygienisated and pre-hygienisated). The reduction of VS was approximately one third in biogas reference batches.

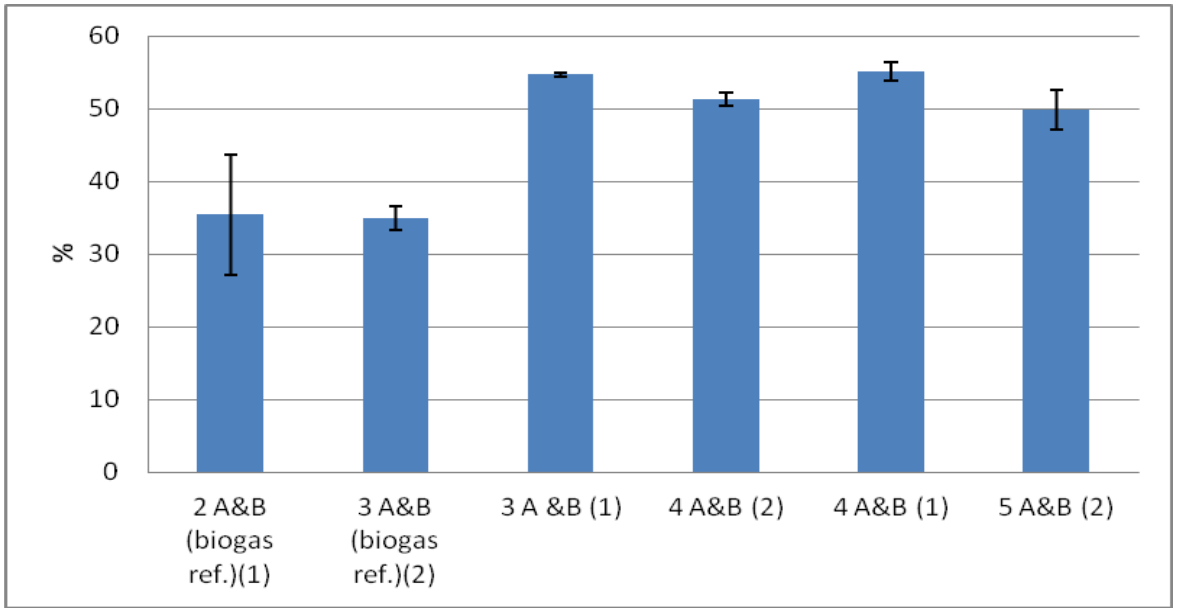


FIGURE 9. The biodegradation of organic material in percentages in different batches, exp. 1 (1) and exp. 2 (2)

7 DISCUSSION

The purpose of this study was to find out the utilisation possibilities of digestate as an organic fertiliser, which can substitute mineral fertilisers, by studying its plant pathogenic properties after the mesophilic anaerobic process with 21-day retention time. Based on the results the digestate can be utilised as an organic fertiliser, without the risk of *Sclerotinia* species contamination. *Sclerotinia* species are able to produce hard covered, persistent, resting spores, which are the most likely organisms to survive composting and other waste treatment processes. (Valkonen et al. 1996, 24; Noble et al. 2009, 3). The survivals of *S.sclerotiorum* and *S.subarctica* in the mesophilic biogas process have not been studied before.

Based on the results the mesophilic anaerobic digestion with 21-day retention time was sufficient enough for the elimination of both *Sclerotinia* species viability and reproductive capability. Therefore, if plant wastes with possible plant pathogens are

treated with mesophilic anaerobic digestion, *Sclerotinia* species should not remain their reproductive capability and hence the digestate can be utilised as an organic fertiliser. Nevertheless, if sclerotia have persistent hard-covered structure and are heat-tolerant and the sclerotia did not maintain their viability after the anaerobic digestion, perhaps other plant pathogens may not survive it either. Certainly, this is only an assumption and would require long-term field experiments with numerous plant pathogens.

Whether the sclerotia were pre-hygienisated or not, did not seem to affect their growth: the digestion process itself was sufficient enough for eliminating the pathogen. Therefore, the hygienisation is not necessary considering the outcome although it makes the elimination of *Sclerotinia* species from digestate more guaranteed (in case of flaws in the reactor system and so forth). Nevertheless, it was noticed during unloading that pre-hygienisated sclerotia were more damaged outside than the non-hygienisated sclerotia. Moreover, before the digestate is being utilised, it goes through different steps: hygienisation, anaerobic digestion, post-digestion and storage (aerobic). The post-digestion degrades the material further on, the rest of the methane is stored and the hygienic condition improves. Thus, the pathogens must survive all these different steps with different environmental conditions. Therefore, it is also unlikely that fungi could adapt to different environmental conditions and remain viable. (Haraldsson 2008, 37.)

The temperature may have affected the survival of sclerotia, especially with 21-day retention time, as longer retention time improves the hygienic condition of the digestate. (Latvala 2009, 35). Plant pathogens are known to become inactive in the anaerobic biogas process or at least their survival is minimised. (Lehtomäki 2006, 23; Weiland 2010, 849). Anaerobic conditions may have affected the survival of the sclerotia. Second batch in experiment 2 had aeration without any substrate to test whether lack of oxygen has affected the results of experiment 1, where the sclerotia did not stay viable either. On the one hand, the diluted oxygen should be sufficient enough - on the other hand, it is unknown whether sclerotia can utilise diluted oxygen or how they generally survive in water. Sclerotia did not remain viable in soil with low oxygen content (0.1%) and high moisture (20%). (Wu et al. 2008, 664). Thus, it can be

assumed that anoxic or anaerobic conditions may have been one of the reasons for the sclerotia not remaining viable. Moreover, the reason behind non-surviving sclerotia was not either in the washing process done when unloading, as it was confirmed with a separate test.

Volatile organic compounds are being produced during anaerobic digestion, these VOCs may inhibit the growth the *Sclerotinia* species. Biofumigation is based on the effect of VOCs and in laboratory and greenhouse tests has shown to be effective against *Sclerotinia* species. Also some antagonist species produce VOCs, which are harmful to specific fungi or other pathogens. VOC produced in anaerobic digestion combined with the temperature and moisture is known to affect plant pathogens survival. (Iivonen et al. 2013, 69). Ammonia produced in the digestion process inhibits the germination of several fungal pathogens' spores, for instance. (Valkonen et al. 1996, 106).

Hygienisation degrades solid organic material into liquid form, which the bacteria can utilise better and thus the biogas and methane production of hygienisated materials is usually higher in comparison with non-hygienisated. (Luste et al. 2012, 55-56). In Table 4. it can be seen that the methane content in exp.1 in pre-hygienisated batches was notably higher than in non-hygienisated batches (45% compared with 26%). Methanogens are sensitive to fluctuations in the process and other unfavourable conditions (Weiland 2010, 851), thus it can be that the hygienisation assisted the growth of the most sensitive bacteria – methanogens, e.g. by eliminating competing microbes, which resulted in high methane content. Otherwise biogas production was relatively high in the non-hygienisated batch, which indicates that degradation pathway has occurred before methanogenesis.

The methane potential for cow manure given in literature is 175m^3/tVS (Latvala 2009, 25) and the methane potential in previous determinations with the same cow manure has been 190m^3/tVS (Luste 2013). Therefore, the methane content in these experiments was somewhat lower in comparison with previous determinations and literature value. The material may have contained slowly degrading material, which resulted in lower methane yield and thus the retention time was not long enough regarding the

degradation of all the organic material. The methane yield could have been higher, if there would have been more than one substrate used, as usually a higher methane yield is achieved when various different substrates are co-digested. (Haraldsson 2008, 10). Also, as the inoculants were obtained from Juvan Bioson Ltd and the biogas plant uses liquid cow manure, dry chicken manure and vegetable wastes as substrates, the methane yield could have been higher if the same substrates were used in the experiments, as the microbes in the inoculant are adapted to these substrates. In this experiment, only liquid cow manure was used as a substrate, and it may have affected the methane yield as it takes some time for the microbes to adapt only degrading cow manure. In exp. 2, the difference in methane content was not remarkable between the batches; this can be due to different *Sclerotinia* species, for instance.

Biodegradation describes how well dry material is degraded by microbes. The differences between hygienisated and non-hygienisated batches are not remarkable (Figure 9.). Therefore, it can be concluded that sclerotia did not affect biogas and methane yield notably (except methane content in exp. 1) and neither biodegradation. However, it is assumed that in batches 4 (exp. 1) & 5 (exp. 2) the sclerotia were eliminated during pre-hygienisation.

As continuous reactors are more commonly used (in Finland) than batch reactors, further studies could be made by repeating similar experiment in the continuous reactor, as batch reactors are known to be easier to control regarding pathogens than continuous reactors. The prevention of recontamination in full-scale reactors is more difficult when compared with laboratory-scale reactors, as laboratory circumstances are relatively easy to maintain. (Sahlström 2003, 163.) Therefore, the study could be also made in full-scale reactor. These types of studies would demonstrate the results of these experiments furthermore.

8 CONCLUSIONS

Neither *Sclerotinia sclerotiorum* nor *Sclerotinia subarctica* remained vital after the mesophilic biogas process. This can be due to the temperature combined with 21-day

retention time, volatile organic compounds produced in the digestion process or due to anaerobic conditions, for instance. There were neither remarkable differences between the biogas and methane yield and biodegradation between pre-hygienisated batches and non-hygienisated batches. Based on the results, the digestate can be utilised as an organic fertiliser to substitute mineral fertilisers without the risk of *Sclerotinia* species.

BIBLIOGRAPHY

Castillo, F.D.H., Padilla, A.M.B., Morales, G.G., Siller, M.C., Herrera, R.R., Gonzales, C.N.A. & Reyes, F.C. 2011. *In Vitro* antagonist of *Trichoderma* Strains Against *Sclerotinia sclerotiorum* and *Sclerotium cepivorum*. American Journal of Agricultural and Biological Sciences 6(3): 410-417 (2011). PDF document. http://thescipub.com/pdf/10.3844/ajabssp.2011.410.417. Referred 21.3.2013.

Cuong, Nguyen Duc & Dohroo N.P. 2006. MORPHOLOGICAL, CULTURAL AND PHYSIOLOGICAL STUDIES on *Slerotinia sclerotiorum* CAUSING STALK ROT OF CAULIFLOWER. Omonrice 14 71-77 (2006). PDF document. http://www.clrri.org/lib/omonrice/14-09.pdf. Referred 22.3.2013.

EVIRA, 2011. HACCP. WWW document. http://www.evira.fi/portal/fi/tietoa+evirasta/asiakokonaisuudet/omavalvonta/haccp/. Updated 2.3.2011. Referred 9.3.2013.

EVIRA, 2012a. Eläimistä saatavat sivutuotteet. WWW document. http://www.evira.fi/portal/fi/tietoa+evirasta/asiakokonaisuudet/elaimista_saatavat_sivutuotteet/. Updated 20.12.2012. Referred 9.3.2013.

EVIRA, 2012b. Lannoitevalmisteet. WWW document. http://www.evira.fi/portal/fi/tietoa+evirasta/asiakokonaisuudet/omavalvonta/lannoitevalmisteet/. Updated 20.2.2012. Referred 9.3.2013.

EVIRA, 2012c. Omavalvonnan rakenne. WWW document. http://www.evira.fi/portal/fi/tietoa+evirasta/asiakokonaisuudet/omavalvonta/omavalvonnan_rakenne/. Updated 22.3.2012. Referred 9.3.2013.

EVIRA, 2013. Lannoitevalmistelaitosten hyväksyntä. WWW document. http://www.evira.fi/portal/fi/kasvit/viljely_ja_tuotanto/lannoitevalmisteet/laitoshyvaksynta/. Updated 18.1.2013. Referred 9.3.2013.

Haraldsson, Lena 2008. Anaerobic digestion of sugar beet – fate of plant pathogens and gas potential. Examensarbete 2008:4. Institutionen fär mikrobiologi Sveriges lantbrukuniversitet. PDF document. http://ex-epsilon.slu.se:8080/archive/00002798/01/Exjobb_Haraldson_nr_4_08.pdf. Referred 23.4.2013.

HDC Factsheet 2007. HDC Factsheet 09/07. Soil disinfestation options for cut flower growers. WWW document. http://herbs.hdc.org.uk. Referred 1.4.2013.

Holm, Peter E. & Jensen, Lars Stoumann & McLaughlin, Michael 2010. Utilization of Biologically Treated Organic Waste on Land. In the book Christensen, Thomas (ed.) Solid Waste Technology and Management. Wiley: 2011. Pages 665-681. ISBN 9780470666968.

Iivonen, Sari, Avikainen, Hanna & Kivijärvi, Pirjo 2012. Biofumikaatio jäävuorisalaatin viljelykierrossa – menetelmän mahdollisuudet ja heikkoudet pahkahomeen torjunnassa. Maataloustieteen päivät 2012 (internet publication).Suomen Maataloustieteellisen Seuran julkaisuja no 28. Editor: Nina Schulman & Heini Kauppinen. Published 15.1.2012. PDF document.
http://www.smts.fi/Vihannesviljelyn%20haasteet/Iivonen_Biofumikaatio.pdf. ISBN 978-951-9041-56-8.

Iivonen, Sari, Jäppinen, Eero, Laihanen, Mika, Luste, Sami, Nykänen, Arja, Ranta-Korhonen, Tuija, Seppäläinen, Sari, Seuri, Pentti, Soininen, Hanne, Tontti, Tiina & Väisänen, Hanna-Maija 2013. Energiaomavarainen maatila. Julkaisuja 29. University of Helsinki, Ruralia-institute. PDF document.
http://www.helsinki.fi/ruralia/julkaisut/pdf/Julkaisuja29.pdf. ISBN 978-952-10-6531-6 (PDF)

Jalli, Marja, Hannukkala, Asko, Laine, Peppi & Parikka, Päivi 2010. Tulevaisuuden kasvinsuojeluongelmat – kasvitaudit, 32 pages. MTT. PDF document.
http://www.maaseutu.fi/attachments/newfolder_92/5uovIcKsL/Marja_Jalli.pdf. Referred 4.4.2013.

Klingberg, Ninni 2012. The effect of fungicide treatment on the non-target foliar mycobiota of Pinus sylvestris- seedlings in Finnish forest nursery. Master's Thesis. Helsinki University. PDF document.
https://helda.helsinki.fi/bitstream/handle/10138/34669/Ninni%20Klingberg%20Master%27s%20thesis.pdf?sequence=1. Referred 22.2.2013.

Koike, Steven T., Gladders, Peter & Paulus, Albert O. 2006. Vegetable Diseases: A Colour Handbook. Manson Publishing Ltd. 449 pages.

Krogmann, Uta, Körner, Ina & Diaz, Luis.F. 2011. Composting: Technology. In the book Christensen, Thomas (ed.) Solid Waste Technology and Management. Wiley: 2011. Pages 533-568. ISBN 9780470666968

Kumar, M.R, Sudheer, M.J. Reddy, B.C. & Srinivasulu, B., 2008. Biological control of plant pathogens, Weeds and Phytoparasitic Nematodes. Global Media. 231 pages. ISBN 9788178001579

Laemmlen, Franklin 2001. *Sclerotinia* Diseases. University of California. Publication 8042. PDF document. http://anrcatalog.ucdavis.edu/pdf/8042.pdf. ISBN 978-1-60107-220-7. Referred 25.3.2013.

Lassi, Kati & Tulisalo, Unto 2012. Öljykasvinviljelijän opas. MTK (Maa- ja metsätaloustuottajien Keskusliitto) &
Elintarviketeollisuuden Öljynpuristamoyhdistys. PDF document.
http://www.agronet.fi/rypsi2000/printtiversio.pdf. Updated 23.4.2012. Referred 7.3.2013.

Latvala, Markus 2009. Paras käytettävissä oleva tekniikka: Biokaasun tuotanto suomalaisessa toimintaympäristössä. Syke. Suomen ympäristö 24|2009. Helsinki: Edita Pri-

ma Ltd. ISBN 978-952-11-3497-5. PDF document. http://www.ymparisto.fi/download.asp?contentid=106756&lan=FI. Referred 15.2.2013.

Lehto, Marja, Salo, Tapio, Sorvala, Sanna, Kemppainen, Riitta, Vanhala, Petri, Sipilä, Ilkka & Puumala, Maarit 2007. Peruna- ja juureskuorimoiden jätteet ja jätevedet. MTT, Maa- ja elintarviketalous 94. 70 pages. ISBN 978-952-487-083-2. PDF document. www.mtt.fi/met/pdf/met94.pdf. Referred 10.3.2013.

Lehtomäki, Annimari 2006. Biogas Production from Energy Crops and Crop Residues. University of Jyväskylä. 91 pages. PDF document. https://jyx.jyu.fi/dspace/handle/123456789/13152. ISBN 951-39-2559-5. Referred 25.2.2013.

Luste, Sami 2013. Email-discussion 18.4.2013. Research Manager. Mikkeli University of Applied Sciences

Luste, Sami, Soininen, Hanne, Ranta-Korhonen, Tuija, Seppäläinen, Sari, Laitinen, Anne & Tervo, Mari 2012. Biokaasulaitos osana energiaomavaraista maatilaa. A: Research reports, 75. Mikkeli University of Applied Sciences. ISBN 978-951-588-352-0.

Marttinen, Sanna, Paavola, Teija, Ervasti, Satu, Salo, Tapio, Kapuinen, Petri, Rintala, Jukka, Vikman, Minna, Kapanen, Anu, Torniainen, Merja, Maunuksela, Liisa, Suominen, Kimmo, Sahlström, Leena & Herranen, Mirkka 2013. Biokaasulaitosten lopputuotteet lannoitevalmisteina. MTT, Raportteja 82. MTT Jokioinen: 2013. PDF document. http://www.mtt.fi/mttraportti/pdf/mttraportti82.pdf. ISBN 978-952-487-432-8. Referred 5.4.2013.

MMM 2011. Maa- ja metsätalousministeriön asetus lannoitevalmisteista 24. 27 pages. PDF document. http://www.mmm.fi/attachments/elo/newfolder/lannoiteaineet/61fAl8BFZ/MMMMa_24_11_lannoitevalmisteista_FI.PDF. Referred 11.4.2013.

Noble, R.,Elphinstone, J.G., Sansford C.E., Budge, G.E. & Henry C.M. 2009. Management of plant health risks associated with processing of plant-based wastes: A review. Bioresource Technology, vol.100, issue 14, p. 3431-3446.

Nutter, Forrest W.JR., Teng, Paul S. & Royer, Matthew H. 1993. Terms and Concepts for Yield, Crop loss, and Disease Thresholds. Plant Disease, Vol.77, No. 2, p. 211-215. The American Phytopathological Society. PDF document. http://www.apsnet.org/publications/plantdisease/backissues/Documents/1993Articles/PlantDisease77n02_211.PDF. Referred 13.4.2013.

REGULATION (EC) No 1069/2009, 2009. The European Parliament and the Council of the European Union. Official Journal of the European Union. PDF document. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:300:0001:0033:EN:PDF]. Referred 3.3.2013.

Ryckeboer, Jaak. Cops, Stef & Coosemans, Jozef 2002.The fate of plant pathogens during anaerobic digestion and composting. Published in BioCycle April 2002, vol.43, no. 4, p.50.

Sahlström, Leena 2003. A review of survival of pathogenic bacteria in organic waste used in biogas plants. Bioresource Technology 87(2003) 161-166.

SFS-EN 25814. 1992. Water quality – Determination of dissolved oxygen – Electro-chemical probe method (ISO 5814:1990).

SFS 3008. 1990. Determination of total residue and total fixed residue in water, sludge and sediment. 2nd edition.

SFS 3021. 1979. determination of pH-value of water. 2nd edition.

Sharma, P.D. 2008. Plant Pathology. Global Media, 185 pages. ISBN 9789350437889.

Soininen, Hanne, Kiukas, Iiro & Mäkelä, Leena 2007. Biokaasusta bioenergiaa etelä-savolaisille maaseutuyrityksille. Mikkeli University of Applied Sciences. Research Reports 24.

Suomalainen, Marcella 2007. Naudan lietelanan käsittelymenetelmien taloudellinen vertailu. Master's Thesis. Lappeenranta University of Technology. PDF document. http://www.doria.fi/bitstream/handle/10024/29758/Naudan%20lietelannan%20k%C3%83%C2%A4sittelymenetelmien%20taloudellinen%20vertailu.pdf. Referred 15.4.2013.

Svensson, K, Odlare, M & Pell, M. 2004. The fertilizing effect of compost and biogas residues from source separated household waste. Journal of Agricultural science (2004), 142, p. 461-467.

Termorshuizen, A.J.,Volker, D., Blok, W.J., ten Brummeler, E., Hartog, B.J., Janse, J.D., Knol, W. & Wenneker M. 2003. Survival of human and plant pathogens during anaerobic mesophilic digestion of vegetable, fruit and garden waste. European Journal of Soil Biology 39 (2003) p.165-171.

Tuomola, Juha, Avikainen, Hanna, Iivonen, Sari, Li, Hao-Xi, Anne, Piirainen & Pirhonen Minna 2012. Vihannesviljelyn taloudellisen kannattavuuden kehittäminen tautien ja lannoituksen hallinnalla. Raportteja 80. University of Helsinki, Ruralia-Institute. PDF document. www.helsinki.fi/ruralia/julkaisut/pdf/Raportteja80.pdf. Referred 18.2.2013.

Valkonen, Jari, Bremer, Katri, & Tapio, Eeva 1996. Kasvi sairastaa – oppi kasvitaudeista. Helsinki: Yliopistopaino. ISBN 951-570-261-5.

Vänttinen, Veli-Heikki, Tähti, Hanne, Rasi, Saija & Rintala, Jukka 2009. Biokaasuteknologian alueellinen hyödyntämispotentiaali Keski-Suomessa. University of Jyväskylä. PDF document.

www.biokaasufoorumi.fi/GetItem.asp?item=digistorefile;150443;... Referred 20.3.2013.

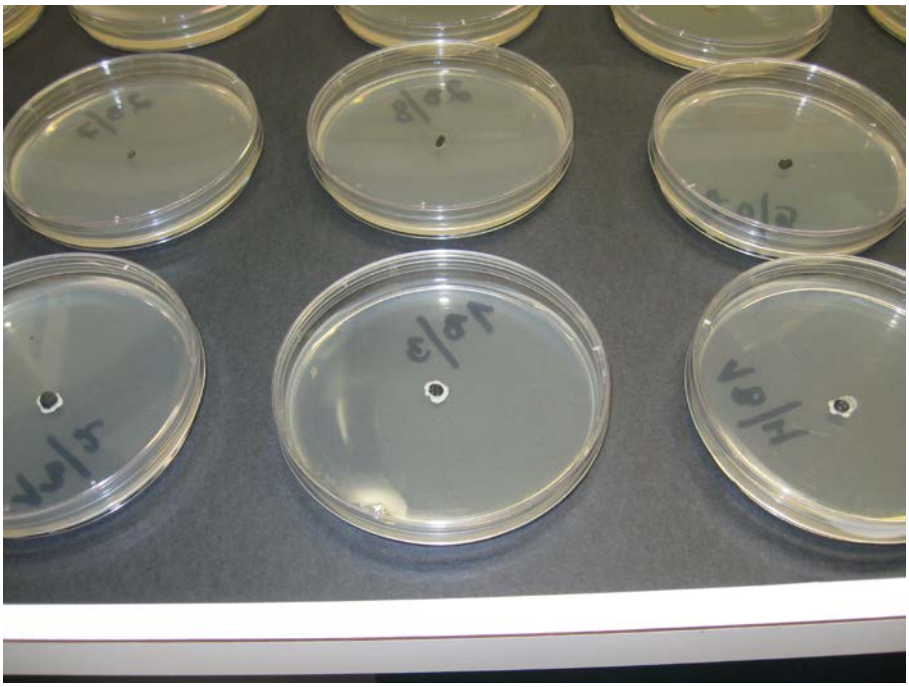
Waller, J.M, Lenne, J.M. & Waller S.J. 2001. Plant Pathologist's Pocketbook. CABI Publishing. 526 pages. ISBN 9780851998909.

Weiland, Peter 2009. Biogas production: current state and perspectives. Appl. Microbiol. Biotechnol (2010) 85: 849-860.

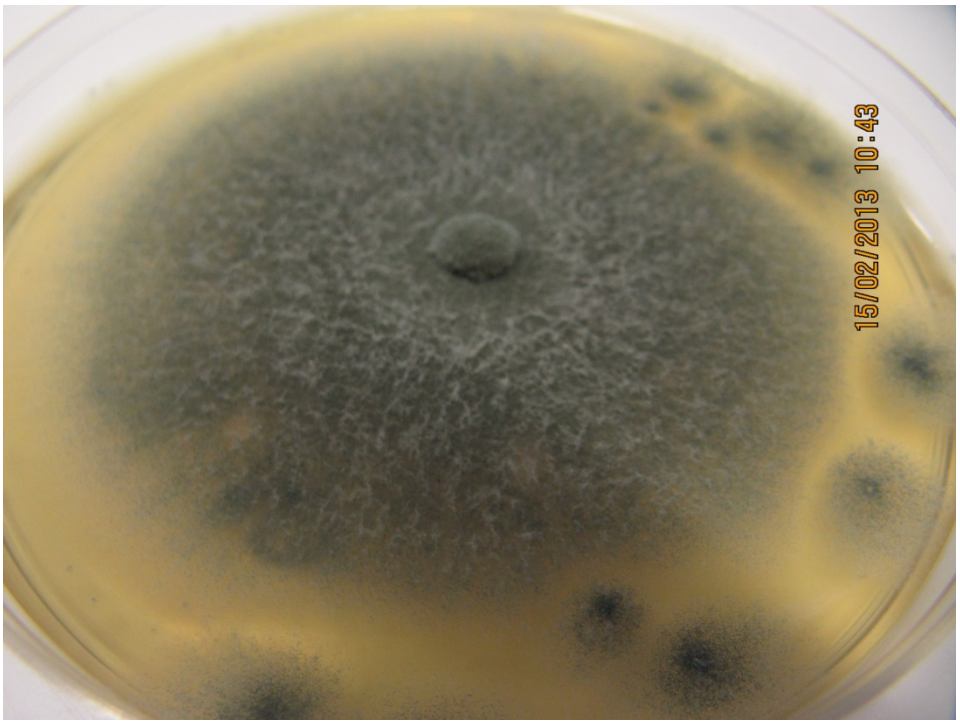
Wu, B. M., Subbarao, K. V., and Liu, Y.-B. 2008. Comparative survival of sclerotia of *Sclerotinia minor* and *S. sclerotiorum*. Phytopathology 98:659-665.

APPENDIX 1(1).

Pictures from cultivations



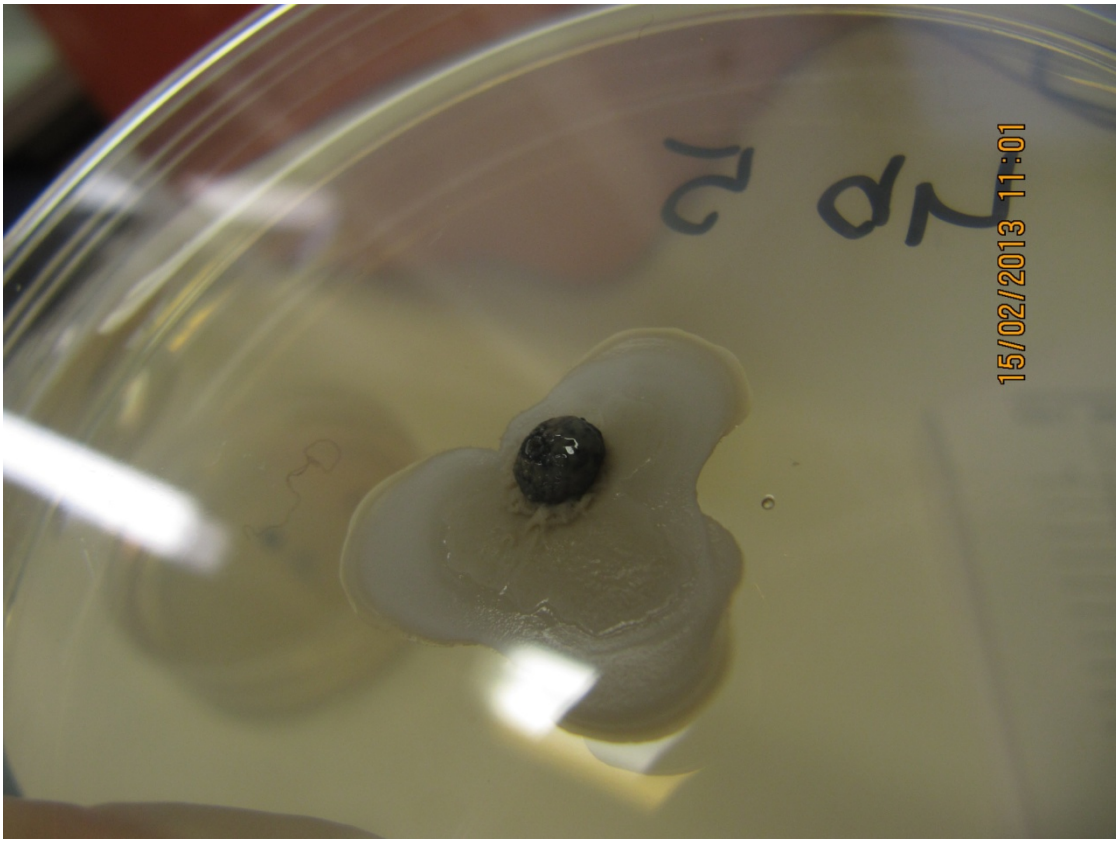
PICTURE 7. Cultivations of *S.sclerotiorum.* Picture: Tuija Ranta-Korhonen



PICTURE 8. *Sclerotinia subarctica* –incubation experiment, after 11 days of incubation. The Petri dish was filled with some other fungi (Batch 2 with aeration)

APPENDIX 1(2).

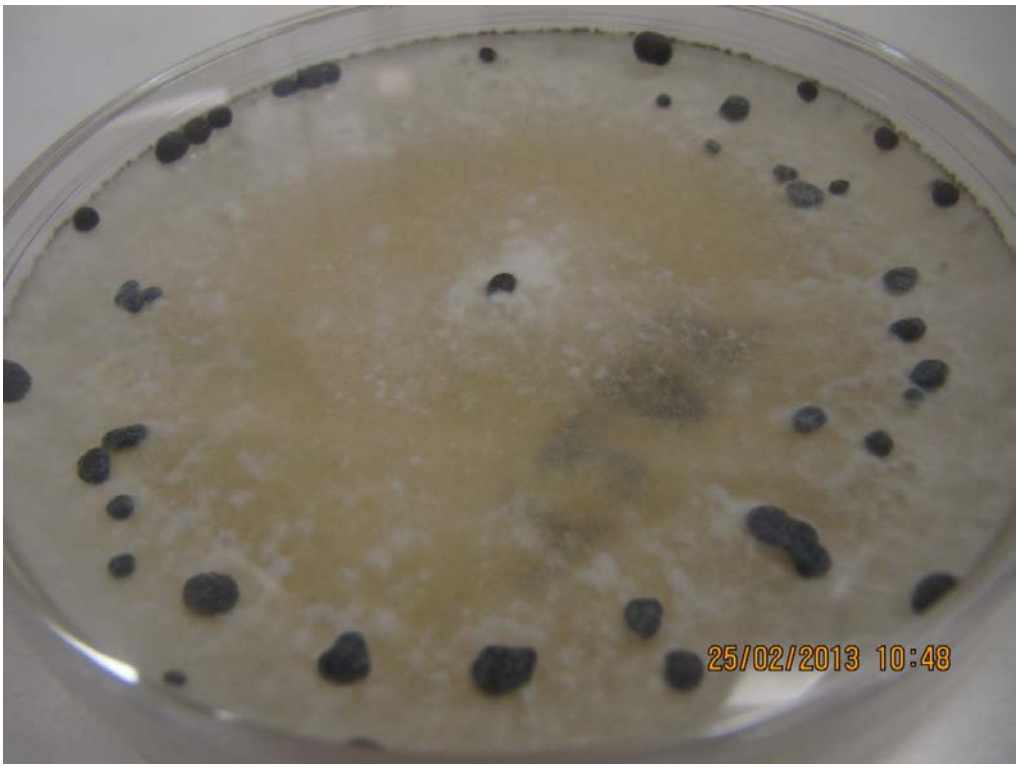
Pictures from cultivations



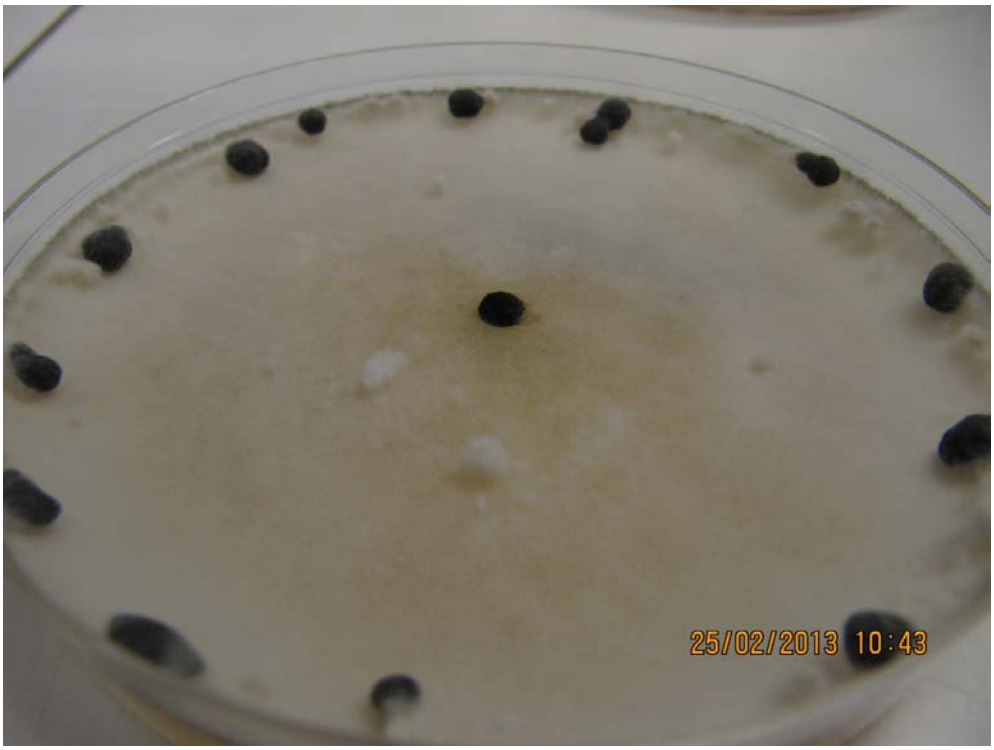
PICTURE 9. *S.subarctica* after 11 days of incubation (Batch 5)

APPENDIX 2.

Pictures from confirmation test



PICTURE 10. *S.sclerotiorum* after 12 days of incubation (untreated)



PICTURE 11. *S.subarctica* after 12 days of incubation (treated)