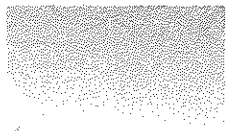
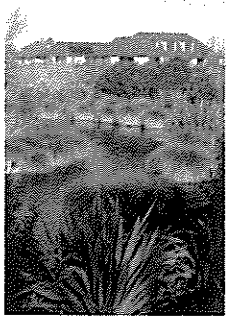
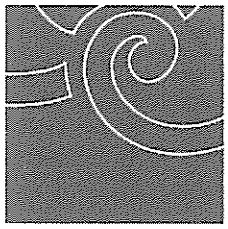
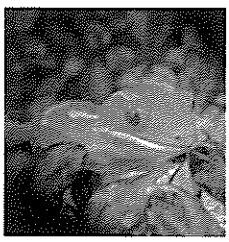


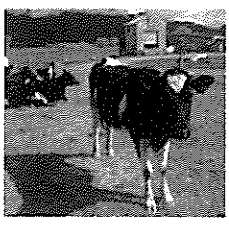


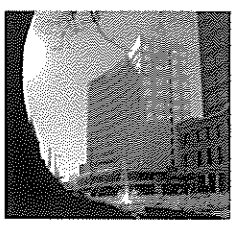


Development of novel technologies to reduce agricultural methane emissions

Contract: C09X0806







Landcare Research
Manaaki Whenua

Development of novel technologies to reduce agricultural methane emissions

Contract: C09X0806

Adrian S Walcroft, Chris Pratt, Kevin R Tate

Landcare Research

Prepared for:

Ministry of Agriculture and Forestry

Pastoral House
25 The Terrace
PO Box 2526
Wellington 6140

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Landcare Research, Massey University, Private Bag 11052, Palmerston North 4442, New Zealand, Ph +64 6 353 4800, Fax +64 6 353 4801, www.landcareresearch.co.nz

Reviewed by:	*Approved for release by:*
	David Whitehead
Dr Julie Deslippe Scientist Landcare Research	David Whitehead Science Team Leader Global Change Processes

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Contents

Summary

The project

This project investigated two novel technologies with the potential to mitigate methane (CH_4) emissions from New Zealand's dairy farms: 1) methane biofilters and 2) a clay-based rumen modifier to reduce methane production in cattle.

Methane biofilters use naturally occurring bacteria, methanotrophs, which consume CH_4 as their sole carbon source. Most of the carbon from the consumed CH_4 is oxidised to carbon dioxide (CO_2), although about 40% can be retained in the microbial biomass. Conversion of CH_4 to CO_2 in a biofilter has a greenhouse gas mitigating effect since the greenhouse warming potential (GWP) of a unit mass of CH_4 is 25 times greater than that of an equivalent mass of CO_2. By developing methane biofilter technology, it may be possible to reduce CH_4 emissions from on-farm sources such as effluent ponds and animal houses.

A rumen modifier could potentially inhibit *in vivo* methanogenesis. This work is based on the frequently observed phenomenon of geophagy – the consumption by animals of earth and soil-like substances such as clay to influence digestive microbial processes. Our hypothesis is that introducing particular clays into the rumen would inhibit CH_4 production by somehow interacting with the microbes responsible for CH_4 generation (methanogens). Preliminary *in vitro* experiments showed a significant inhibitory effect by certain clay minerals on CH_4 production from rumen contents incubated under anaerobic conditions. If this effect could be reproduced in live animals then feeding diets amended with clay minerals might be a viable CH_4 mitigation technology.

Methane biofilters

- **Objectives**

1. identify soils with an active methanotroph population;
2. develop a prototype biofilter for a dairy farm effluent lagoon/pond; and
3. determine if biofiltration is feasible for dairy cow housing structures.

- **Methods**

A variety of volcanic pumice soils showed strong CH_4 oxidation potential in previous research by the authors. These soils were placed into laboratory chambers and assessed for their capacity to oxidise high fluxes of CH_4 where it operated for a 1-year period. The soils were analysed for their methanotroph communities by a variety of molecular analyses. A series of laboratory experiments were conducted on the soils to assess their CH_4 oxidation rates as a function of inlet CH_4 concentration and gas flow rate. The results of these tests were used to determine the efficacy of biofiltration to mitigate CH_4 emissions from the two major on-farm CH_4 point-sources: animal housing and effluent ponds. Batch laboratory tests were conducted to examine the effects of temperature and moisture on oxidation. A field biofilter was constructed using one of the active soil types (from a landfill cover) identified in the laboratory trials. The filter was set-up adjacent to a dairy farm effluent pond and ran for 1.5 years. A laboratory experiment was initiated to test if the filter design could be optimised by combing the gas capture system and filter as a single structure (cover/filter), which would

result in a more cost-effective technology. Currently, this design is being tested under field conditions.

- **Results**
  - Volcanic pumice soils showed effective and sustained oxidation of high CH_4 fluxes (up to 24 g CH_4 m^{-3} h^{-1}).
  - Molecular analysis revealed that Type II methanotrophs (predominantly *Methlyocystis sp.*-related) were primarily associated with high CH_4 oxidation rates.
  - A sequence of laboratory experiments showed that a 500-m^3 soil-based biofilter could only oxidise about 50 tonnes of CO_2-equivalents/year for a typical animal housing structure. However, a much smaller biofilter (100 m^3) could oxidise about 150 tonnes of CO_2-e annually from a typical dairy effluent pond.
  - The field biofilter, treating CH_4 emissions from a real dairy effluent pond, performed strongly (removed up to 16 g CH_4 m^{-3} h^{-1}) over a 1.5 year period.
  - Preliminary results from a laboratory experiment examining a design improvement to the field biofilter (by incoporporating the gas capture unit and biofilter as a single structure) have shown that this design is effective, with a soil cover/filter able to remove up to 100% of a CH_4 influx commensurate with emissions from a typical effluent pond.
  - The biofilter work has been written-up as three scientific papers submitted to peer-reviewed international journals.

- **Conclusions**
  - The effective and sustained CH_4 oxidation rates exhbited by the volcanic soils is encourgaing for the development of biofilters as a low-cost and low-maintenance GHG mitigation technology.
  - Laboratory experiments showed that biofilters are not currently feasible for treating CH_4 emissions from housed animals, as the quantity of CH_4 removed is too small in relation to the size of the filter required. However, the results showed that biofilters are a feasible approach to mitigate CH_4 emissions from typical dairy effleunt ponds.
  - The strong performance of the field biofilter treating CH_4 emissions from a dairy effluent pond over a 1.5 year period, suggests that a 50-m^3 biofilter could effectively oxidise CH_4 emissions from a typical dairy effluent pond.
  - Our economic analysis suggests that the cost of the fiter design can be improved by merging the filter and gas capture cover into a single unit.
  - This new design appears to be potentially efficient, based on preliminary laboratory results.

- **Recommendations**
  - Longer term testing of the cover/filter design is needed to assess the stability of methanotroph populations under this system.
  - Testing of various soils (including volcanic pumice and compost) is underway to identify the most suitable substrate for use in a field cover/filter system, which will be tested in the coming year.
  - Methods for deploying the cover/filter system on effluent pond surfaces will be tested using various materials such as rubber-matting, straw and floating polystyrene cells.

- Understanding of key methanotroph strains in the studied soils will be undertaken to optimise the conditions favourable for their growth and activity, which ultimately controls filter performance.

Inhibition of rumen methanogenesis using clays

- **Objectives**

  1. Test a range of clays for their efficacy in reducing CH_4 emissions *in vitro*; and
  2. Examine key variables (clay type, loading, surface properties, rumen pH, cow) to understand the mechanism(s) of rumen methanogenisis and optimise the efficacy of clays in reducing methane production.

- **Methods**

  Twenty six clays were tested in a series of *in vitro* incubations designed to simulate a rumen digestion episode. In each incubation a known quantity and quality of herbage was added to artificial saliva and mixed with fresh cow rumen content. This mixture was incubated anaerobically with and without added clay at blood temperature (38°C) over a time period (ca. 7 h) representing one digestion cycle. Experimental variables were modified in attempts to optimise methane reduction using the two most promising clays from initial tests, and five "new" clays sampled to replace one of the two promising clays that was in short supply. Variables tested included: feed quality (e.g., grass, hay), rumen pH, clays in combination and clay surface hydrophobicity.

  One *in vivo* experiment was conducted (November–December 2008) at AgResearch *after* receiving Animal Ethics approval using a clay that was similar (though not identical) to the clay that most consistently reduced methane production in our *in vitro* experiments. A palatability trial was conducted before the main experiment. The main experiment was conducted in calorimeter rooms over 9 days and involved 8 ewes: 4 controls in which animals were fedlucerne chaffage only; and 4 tests in which animals were fed lucerne chaffage +clay.

  With the help of an experienced rumen microbial ecologist, a laboratory was set up to enable anaerobic culture of methanogens. Rumen methanogens were cultured, grown and stored at -80°C for future use, for example, in further research to establish the reasons for the variability shown to date in *in vitro* experiments.

- **Results**

  In initial *in vitro* experiments, two clays (kaolinite PC1057R and zeolite 1) significantly reduced CH_4 emissions (by up to 65%).

  Attempts to optimise these effects using these two clays, and five "new" clays showed rumen pH at the time of sampling was often, but not always, a useful indicator of the likelihood of a measurable reduction in CH_4 emissions. Overall, while some large reductions in emissions were recorded at intial rumen pHs around 6, few positive results occurred at higher pHs.

No effect of clay on rumen methanogenesis was observed in our one *in vivo* experiment However, because of limited availability of the clay that performed most consistently in our *in vitro* experiments, the clay used in the *in vivo* trial was similar but not identical to that used in *in vitro* experiments, and sheep were used, whereas the *in vitro* experiments had used cow rumen fluid. No feed palatability problems were encountered.

- **Conclusions**

  The results of this study provided evidence that in *in vitro* experiments some clays can reduce CH_4 emissions from rumen digestion. Two of the clays tested caused significant reductions in *in vitro* CH_4 emissions (up to 65%) in some anaerobic incubations, but in a few experiments no significant reductions were recorded.

  Initial rumen pH appeared to influence the efficacy of clays as mitigators of rumen methanogenesis, but the signicance of this result was not established. This may suggest that clay might be effective only at about pH 6 during the digestion cycle.

  Further research is needed to indicate why results are so variable. To avoid confounding experimental conditions, future research should include pure cultures of methanogens and clays.

- **Recommendations**

  Further research to understand the underlying mechanism(s) of reduction of methanogenesis by clays is required to indicate why results to date are so variable. To avoid confounding experimental conditions, further research should involve experiments with pure cultures of methanogens.

1 Introduction

Agriculture accounts for just under half of New Zealand's greenhouse gas emissions. The principle gas emitted is methane (CH_4), which contributes around two-thirds of agricultural emissions, with around one-third coming from nitrous oxide (N_2O) emissions from soil, and a small fraction from burning of crop residues. According to New Zealand's current inventory, approximately 97% of agricultural CH_4 emissions arise from enteric digestion processes in ruminant animals. The remaining 3% is produced by manure management, primarily in anaerobic lagoons for treatment of dairy waste.

There has been a large research investment over the past decade in providing practical mitigation technologies to enable farmers to reduce agricultural emissions. Effective and feasible technologies have been identified that have been shown to lead to reduced N_2O emissions from soil (e.g., nitrification inhibitors, grazing management, stand-off pads), but the same cannot yet be said for CH_4 emissions. The research has generated important advances in knowledge and understanding of CH_4 generation processes in the rumen that may yield an effective mitigation technology on a 5- to 10-year time horizon. However, at present the only mitigation option available to farmers to reduce CH_4 emissions is to destock, which is an effective, but clearly not feasible, option.

This report describes the conception and development of two novel methane mitigation technologies that were funded under the Sustainable Land Management and Climate Change program. The first technology is a CH_4 biofilter that utilises naturally occurring bacteria called *methanotrophs* that consume CH_4 as their sole carbon source. Most of the carbon from the consumed CH_4 is oxidised to carbon dioxide (CO_2), although some is retained in the microbial biomass. Conversion of CH_4 to CO_2 in a biofilter has a greenhouse gas mitigating effect since the greenhouse warming potential (GWP) of a unit mass of CH_4 is 25-times greater than that of an equivalent mass of CO_2 (Forster et al. 2007). By developing methane biofilter technology, it may be possible to reduce CH_4 emissions from on-farm sources such as effluent ponds and animal houses.

The second technology is a rumen modifier that could potentially inhibit *in vivo* methanogenesis. This is based on the frequently observed phenomenon of *geophagy* – the consumption of earth and soil-like substances such as chalk and clay by animals and humans, either to render toxins in food harmless or for a medical benefit such as reducing diarrhoea. It is also based on existing knowledge of how clay particles influence soil microbial processes (Theng & Orchard 1995). The hypothesis was that introducing particular clays into the rumen would inhibit CH_4 production by somehow interfering with the microbes responsible for CH_4 generation (*methanogens*). Preliminary *in vitro* experiments showed a significant inhibitory effect of certain clay minerals on CH_4 production from rumen contents incubated under anaerobic conditions. If this effect could be reproduced in live animals then feeding diets amended with clay minerals might be a viable CH_4 mitigation technology.

2 Methanotrophs and methane biofilters

2.1 Background

Methanotrophs are bacteria unique in their ability to utilise CH_4 as a sole carbon and energy source for growth and maintenance (Hanson & Hanson 1996). They are widely found in nature, existing in soils, water bodies, swamps, rice paddies. Methanotrophs are usually present in greatest quantities near sites of CH_4 production where concentrations are very high, such as rice paddies, anaerobic ponds and landfills. They also exist as smaller populations where the atmosphere is the primary CH_4 source. There are two main groups of methanotrophs that are differentiated according to membrane structure and the metabolic pathway of CH_4 oxidation. The two groups also show different reaction kinetics. Type I methanotrophs, which proliferate near sources of high CH_4 concentration, tend to have a low affinity but a high maximum oxidation potential. Type II methanotrophs exist in areas of low CH_4 concentration, and tend to show high affinity but low maximum oxidation rates.

Methanotroph-rich soils have the potential to be used in engineered biofilters to mitigate CH_4 emissions. A biofilter is a means by which living material is used to remove unwanted pollutants from air, water or soil. The use of bacteria as the active component in biofilters is not new. For example, since the 1950s microbial biofilters have been used to remove odours due to hydrogen sulphide in air emanating from animal housing or wastewater treatment (Nicolai & Lefers 2006). A biofilter consists of a bed of porous media on which microorganisms grow forming a thin biofilm on the media surfaces. The biofilter media not only provide a surface on which to grow, but may also provide a source of water and nutrients for microbial growth, although these can also be artificially supplemented. The physical properties of the media are important as they not only control the total surface area per unit volume available for bacterial colonisation, but also strongly affect gas and liquid transport processes by diffusion and advection. The fluid containing the pollutant passes through the biofilter where it is oxidised to generally inert compounds such as CO_2 and water vapour.

The development of CH_4 biofilters is more recent and has emerged due to increasing concerns over greenhouse gas emissions and climate change (e.g., Park et al. 2002; Melse & Van Der Werf 2005). Methane biofilters are generally constructed using naturally occurring methanotroph populations from soil, compost or other material. The bacteria species present will depend on the location of the site and the CH_4 exposure history, and will most likely comprise a mixture of Type I and Type II methanotrophs. However, it may also be possible to build a CH_4 biofilter by seeding media with an artificially enriched or purified single methanotroph species that has been shown to have superior CH_4 oxidation characteristics.

Methane biofilters remove CH_4 from gas streams through biological oxidation. A proportion of the carbon that is taken up by the bacteria is retained for cellular maintenance and growth, but most is released as CO_2 back into the gas stream. Water vapour is also generated in the oxidation process:

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$$

By far the greatest research effort toward CH_4 biofilter development and testing has been aimed at the reduction of emissions of landfill gas produced by anaerobic decomposition of

refuse (e.g., Park et al. 2002; Haubrichs & Widmann 2006; Nikiema et al. 2007; Scheutz et al. 2009). Landfills are globally significant sources of CH_4. In many cases the best technology to reduce CH_4 emissions is to capture the gas and utilise it for energy and heat production by thermal combustion. This not only reduces the GWP of the emitted gas but can also offset electricity generated using fossil fuels. However, there are many situations where the quantity of CH_4 produced is not sufficient to warrant the large capital investment required for CH_4 capture and energy recovery, or the CH_4 concentration of the waste gas is too low to support thermal combustion. As a result it is desirable to have a technology that can economically mitigate emissions from small or relatively dilute sources because large numbers of these sources can contribute significant emissions at national or international scales.

Reduction of CH_4 emissions by combustion requires a point source where CH_4 can be collected and treated. In addition, thermal oxidation requires that the CH_4 concentration of the gas being treated is above about 20% to support combustion, whereas biological oxidation has no lower concentration limit. In the case of pastoral agriculture in New Zealand, enteric and animal waste sources of CH_4 are mostly diffuse since cattle and sheep normally graze outside year-round. However, on dairy farms there are usually two point sources of CH_4 that could be amenable to mitigation by thermal or biological oxidation.

The first source originates from the proportion of waste that is collected in the cowshed and yards during milking periods, which is often treated in an anaerobic lagoon under conditions that lead to biogas generation. The CH_4 content of biogas is usually above 65%, and is often as high as 85%, and is therefore suitable for thermal oxidation. Only on the largest dairy farms (>1000 cows), or on farms where extra waste is collected from stand-off areas, is it likely to be economically feasible to invest in high-temperature digestion and thermal oxidation technologies (Leiffering et al. 2008; Stewart & Trangmar 2008). A potentially lower-cost solution is to convert an existing anaerobic pond into a low-temperature digester by covering it with an impermeable membrane and collecting the biogas previously emitted to the atmosphere (e.g., Craggs et al. 2008), although significant investment in electricity generation and heat recovery technology is still required.

For most NZ dairy farms the volume of CH_4 produced from effluent treatment systems is insufficient to warrant any investment in gas capture and energy recovery. Nonetheless, the total output of CH_4 from NZ's dairy farm effluent management systems is estimated to be almost 1 million tonnes of CO_2-equivalents annually (NZ GHG Inventory 2008), clearly highlighting these waste management systems as an important GHG source. A low-cost technology based on biological oxidation in CH_4 biofilters may offer a viable mitigation solution.

A second source of CH_4 on dairy farms that could be suitable for biological oxidation is enteric-derived emissions that accumulate in animal housings. Normally there is little opportunity to oxidise enteric CH_4 once it is emitted because animals are outside and the CH_4 diffuses rapidly into the atmosphere. However, recent changes in farm management have seen increasing numbers of dairy cows housed for periods of days to months. There are multiple benefits from housing animals during inclement weather that include improved animal welfare, lower feed requirements, and reduced supplementary feed wastage. When animals are housed it is likely that the CH_4 concentration of air within the housing will be elevated, which may make it amenable to biological oxidation in a biofilter. This would require additional infrastructure such as ducting and pumps to direct air through the biofilter.

2.2 Objectives

- Establish an active methanotroph population.
  - Source and identify populations of high performing methanotrophs that are candidates for inclusion in a biofilter. Measure response to CH_4 dose rate, temperature and moisture content.
- Develop prototype biofilters for a dairy farm effluent pond.
  - Capture and quantify CH_4 emitted from the pond surface.
  - Testing of biofilter design.
    - Two-part biofilter: Separated gas capture and biofilter units.
    - Unified biofilter: gas cover/filter unit.
- Determine if CH_4 biofiltration is feasible for dairy cow housing.

2.3 Methods

2.3.1 Laboratory experiments: CH_4 dose response, temperature response, moisture response

Long-term tests on CH_4 oxidation rates by volcanic pumice soils

Previous work by Tate and Walcroft (2008) established that volcanic pumice soils covering a landfill at Taupo, in the central North Island of New Zealand, have strong CH_4 oxidation potential. Hence, these soils were tested for their long-term performance in oxidising high CH_4 fluxes under laboratory conditions. This trial aimed to assess if the soils could achieve sustained and effective CH_4 oxidation rates in engineered biofilters.

Samples were collected from three locations at the Taupo landfill site: 1) an 8-year-old soil covering refuse; 2) a 2-year-old soil covering refuse; and 3) soil from a pasture site approximately 100 m from the landfill. The soils are classed as pumice (andisols) derived from volcanic activity approximately 2000 years ago (Beets et al. 2002). Pumice soils have high macroporosity and excellent drainage properties, while simultaneously exhibiting the capacity to store large amounts of adsorbed water (Gama-Castro et al. 2000). Topsoil (0–10 cm) and subsoil (10–50 cm) samples were collected from each location. The soil from the 8-year-old cover was tested in duplicate, so in total there were 8 soil batches. Approximately 50 kg of soil was taken from several areas in each location and mixed to obtain representative samples. The soils were sieved to <5 mm to remove roots and other coarse material and then wetted to bring them to 60% of maximum water holding capacity, which is in the ideal range for optimal CH_4 oxidation rates (Humer & Lechner 1999).

Approximately 4.5 L of each soil type was loosely packed into each of 8 clear Plexiglas chambers (15 cm diameter, 40 cm height). Due to varying bulk density of the different soils, the dry soil mass in each chamber ranged from 1.7 kg (pasture topsoil) to 3.2 kg (2-year-old cover subsoil). The chambers were fitted with an inlet at the base and an outlet at the top. An artificial biogas mixture (80% CH_4: 20% CO_2) was combined with air in a mixing chamber using two mass flow controllers so that the total flow rate and CH_4 concentration of the gas mixture could be regulated. The gas mixture was then piped into the base of each chamber,

and the flow rate through each chamber was regulated at 50 ml min^{-1} for the entire experiment using individual volumetric flow controllers. This flow rate corresponded to an "empty bed residence time" (EBRT, filter volume divided by flow rate) of 90 minutes for CH_4 in the chambers. This relatively long EBRT was necessary to allow for complete oxidation of CH_4, which has a much slower biodegradability relative to volatile organic compounds (Nikiema et al. 2007).

The CH_4 inlet concentration was initially set at 1000 ppm (inlet flux of 0.5 g CH_4 m^{-3} h^{-1}), as our previous experiments had established that these landfill soils were able to efficiently oxidise CH_4 at this flux rate (data not shown). The CH_4 concentration fed into the chambers were incrementally increased to a final level of 5% (inlet flux of 24 g CH_4 m^{-3} h^{-1}). This maximum dose was selected because, at CH_4 concentrations higher than this, the gas mixture is in the explosive range. Furthermore, the ratio of O_2 (supplied by the air) to CH_4 would start to fall below the minimum level needed for complete CH_4 oxidation, since 2 molecules of oxygen are required for every molecule of CH_4 that is oxidised. Hence, this dosage represents the upper CH_4 loading rate possible to ensure complete CH_4 oxidation at the given flow rates. After each CH_4 concentration change, CH_4 oxidation was monitored until it had reached a new equilibrium level.

Methane, CO_2, and nitrous oxide (N_2O) concentrations in the gas entering and exiting the chambers were measured in triplicate, twice weekly. pH, Olsen-P, total C, total N, ammonium (NH_4^+)-N, nitrate (NO_3^-)-N and soil moisture were measured for each top and subsoil in the chambers at the start (0 months), during (8 months), and at the end (12 months) of the experiment. A 50-g sample from each chamber was obtained by mixing portions of soil from the middle of the profiles.

Assessment of CH_4 inlet dose and residence time on oxidation rates

A series of experiments were conducted to assess the effect of changing inlet CH_4 concentration and residence time on CH_4 oxidation rates in biofilters. The goal was to assess CH_4 oxidation in response to the variety of inlet concentrations and flows that would be expected to be associated with emissions from diverse sources such as dairy effluent ponds (low flow of high CH_4 concentration) and animal housing (high flow of low CH_4 concentration).

The topsoil from the pasture site away from the landfill was chosen as one filter substrate for this test because it had exhibited slightly higher CH_4 oxidation rates than the other volcanic soils during the long-term laboratory trial. In addition, recent tests on a green waste compost soil have shown very promising oxidation rates (up to 2 times higher than the volcanic soils) leading to our inclusion of this material as a second substrate for assessment. The compost soil was approximately 6 months old and was collected from a green waste processing site in the lower North Island of New Zealand.

Seven replicate 4.5 L-batches of the volcanic pumice soil and compost soil were placed into clear Plexiglass chambers. The chambers were fitted with a gas inlet at the base and an outlet at the top. Controlled flows of air and CH_4 were mixed in an empty chamber and this gas mixture was directed into the base of the chambers. CH_4 was initially fed into the chambers at 5000 ppm and a gas flow rate of 50 ml min^{-1} over an 8-week acclimatisation period. Subsequently, all the soils were exposed to 5 different CH_4 inlet concentrations: 150 ppm, 5000 ppm, 1%, 5% and 8%. The flows of gas into the seven soil chambers were set to

produce residence times of 600, 300, 120, 60, 30, 15, and 5 minutes. These residence times encompass the range of gas flow rates and filter sizes that are likely to be practically encountered with CH_4 biofilter systems. The soils experienced each inlet concentration for a period of 4 days prior to switching inlet dose. Methane, CO_2 and N_2O concentrations in the gas entering and exiting the chambers were measured daily in triplicate for each inlet CH_4 concentration.

Laboratory batch tests were conducted on each of the soils to determine the effects of moisture and temperature on CH_4 oxidation, as these are two of the most significant environmental parameters affecting field biofilter performance (Nikiema et al. 2007). To test the influence of temperature, four 100-ml soil batches were placed into four 1.8-L airtight glass jars and 10 ml of CH_4 was injected into each jar. The soils were wetted to 60% moisture (by dry weight). The jars were stored at four different temperatures: 5°C, 15°C, 25°C and 37°C. Samples were drawn from each jar at regular intervals over a 2.5 hour period and analysed for CH_4 and CO_2 concentrations. At the end of the test the jars were opened and the experiment was repeated on the same soils. The same experimental configuration of batch testing was conducted to assess the impacts of moisture on CH_4 oxidation. Moisture levels of 0%, 15%, 40%, 50%, 60%, 75% and 100% (by dry weight) were evaluated. The jars were kept at 25°C for the moisture tests.

2.3.2 Prototype biofilters: pond cover, field filter, "floating" filter

Field filter set-up: initial two-component design

A field biofilter was constructed using a 1-m length of 350-mm diameter stormwater pipe mounted vertically. The filter media comprised a 70-L 1:1 (volume basis) mixture of expanded perlite and topsoil from the 8-year old landfill cap at Taupo. Expanded perlite is a light-weight material and was used to decrease the soil bulk density and improve aeration. Producing a light-weight filter media is envisaged to be beneficial for full-scale applications, as it will reduce the burden of the filter support structure and associated costs.

The filter was set-up beside the effluent pond of the Number 4 Dairy farm of Massey University where approximately 450 cows are milked twice daily, between July and May. The effluent pond is 29 m × 32 m with sloping sides to centre depth of 4.6 m, giving a surface area of 928 m^2 and an overall volume of 2000 m^3.

Biogas was captured from the pond surface by a 4 m^2 gas-tight floating cover. The rate of biogas emission from the pond surface (ml min^{-1} m^{-2}) was recorded using a purpose-built volumetric flow meter, based on a design described by Smith and Stöckle (2008). The biogas was pumped into the base of the biofilter, and a separate air pump provided oxygen to the filter at a rate sufficient to ensure no oxygen limitation to the rate of CH_4 oxidation. Gas exited the filter through a vent pipe on the top of the filter.

Gas samples were collected periodically from the headspace of the closed filter and from the biogas inlet pipe. Samples were collected in duplicate and sampling times covered working hours between 9:00 a.m. and 5:00 p.m. In order to assess if CH_4 oxidation rates varied outside of these hours, automated sampling was conducted hourly over 2 full day periods (from 15 March 2010 to 17 March 2010) using a robotic sampler.

Gas samples were analysed for CH_4, CO_2, N_2O and O_2. Hydrogen sulphide (H_2S) and NH_3 concentrations were analysed in the biogas and the gas exiting the biofilter over 2 days in the

middle of the trial. Temperature probes were fitted into: the biofilter (top 5 cm); the headspace under the pond cover; the pond surface water (top 5 cm); the air; and the sludge layer at the bottom of the pond, and recordings were made half-hourly using a data-logger. This field filter trial ran for 16 months.

The soil/perlite mixture from the field filter was analysed for pH, Olsen-P, total C, total N, NH_4^+-N, NO_3^--N and moisture content. Analyses were conducted before and at the end of the experiment. At the completion of the field trial, the filter was dissected into three layers (top third, middle third, bottom third) and approximately 200 g of biofilter media were sampled to examine whether any depth profile of pH and/or soil nutrients had developed. Sulphate-S was also measured to assess the effect of H_2S from the biogas at different strata in the biofilter. Hydrogen sulphide in biogas oxidises in the presence of water and air to produce sulphuric acid. A pH reduction, caused by H_2S oxidation, can inhibit methanotroph growth and activity (Nikiema et al. 2007). All analyses were performed in duplicate.

Effect of H_2S on field biofilter CH_4 oxidation

Laboratory batch tests were conducted on media from each layer to determine CH_4 consumption rates. In the batch tests, 200 ml of soil/perlite mixture from each depth layer of the filter were placed in a 1.8-L air-tight glass jar and 4 ml of CH_4 was injected into each jar. Samples were drawn from each jar at regular intervals over a 2.5 hour period and analysed for CH_4 and CO_2 concentrations. The experiment was performed in duplicate.

Pond cover/filter design and testing

The original effluent pond biofilter described above was a combination of two separate structures: 1) a pond cover to capture biogas from the pond's surface; and 2) a remote biofilter on the pond's bank that receives biogas piped from the cover. A new experiment was conducted to assess the feasibility of merging the pond cover and the biofilter as a single structure overlying the pond. This cover filter design could reduce the costs associated with installing separate gas collection and filter units. The design differs fundamentally from the first design in that oxygen requirements are met solely by diffusion through the filter media rather than being pumped through the filter. This is achieved by arranging the media as a thin layer above the pond through which biogas diffuses upward and air diffuses downward.

A scaled-down cover filter design was initially tested in the laboratory using compost soil which had exhibited the highest CH_4 oxidation rates of the soils tested to date. The apparatus consisted of a 50-L plastic container with surface dimensions of 33 × 48 cm. The bottom 10 cm of the container was filled with water and methane was bubbled through the water at a rate commensurate with emissions from a typical New Zealand dairy effluent pond (18 ml m^2 min^{-1}; Craggs et al. 2008). Eight litres (3.3 kg dry weight equivalent) of the compost soil was placed on a fine stainless steel mesh, which was perched on a frame 5 cm above the water level. The plastic container was sealed and air was vented across the top of the biofilter media at a sufficient rate to ensure the oxygen concentration inside the chamber approached that of the ambient air. Gas was sampled from an outlet vent above the soil layer and gas samples were analysed for CH_4, CO_2 and N_2O. Oxygen levels were measured every 2–3 weeks below the soil layer to assess whether the rate of O_2 diffusion through the soil was sufficient to meet the O_2 requirements of methanotrophs through the soil profile.

Pond cover filter field experiment

An experiment has recently been set-up to test the cover filter design under field conditions. For convenience of measurement, the filter is located on the bank adjacent to the pond and receives emissions from the 2 × 2 m cover floating on the pond surface. The construction of the experiment is the same as for the laboratory system described above, but scaled-up to a 2 × 2 m unit. In addition, the filter unit is composed of stainless steel to provide protection against the elements. The experiment was just beginning at the time of writing this report.

2.3.3 Animal housing: CH_4 concentration measurements

Gas samples were collected from a wintering barn in Southland on the South Island of New Zealand and from a covered feedlot in the Manawatu on the North Island of New Zealand. The wintering barn houses 350 cattle permanently over the winter months. The barn has 3 open sides. Gas samples were collected from 22 locations in the roof space of the building on 3 occasions over a 2-day period from 28 to 29 July 2010.

The covered feedlot was also open-sided and approximately 100 cattle were present in the building for two 2-hour daily feeding periods. Samples were collected from 10 locations in the roof space of the covered feedlot on 6 occasions from 13 to 26 July 2010. Gas samples from the wintering barn and the covered feedlot were analysed for their CH_4, CO_2 and N_2O concentrations. The feasibility of using biofiltration to offset CH_4 emissions from these housing structures was assessed by modelling CH_4 oxidation rates expected for the ambient CH_4 concentrations in the buildings and the expected air flow rates through animal housings reported in the literature.

2.3.4 Gas and soil analyses

Gas

Methane, CO_2 and N_2O concentrations in gas samples were measured gas chromatography (GC) (Varian CP-3800 instrument). Methane was measured by a flame ionization detector (FID), CO_2 by a thermal conductivity detector (TCD) and N_2O by an electron capture detector (ECD). The detectors were calibrated periodically using standard gases with concentrations that extended beyond the range of measured concentrations. Oxygen concentrations in the gas samples were measured by a hand-held probe (Apogee, Model 201). Hydrogen sulphide (H_2S) and NH_3 concentrations were analysed using a portable VRAE multi gas monitor (model PGM–7800).

Soil

Unless otherwise stated, data are expressed on an oven-dry (105°C) weight basis. Soil moisture was determined by drying the samples for 24h at 105°C and pH was measured in a 1:2.5 water suspension (Blakemore et al. 1987). Total C and N were measured by combustion in a FF-2000 CNS analyser (LECO Corporation, St. Joseph, MI, USA). Olsen-P was determined by extraction with bicarbonate (0.5 M sodium bicarbonate, pH 8.5, 1:20 soil:extractant, 30 minutes shaking). Phosphorus concentrations were measured in the extracts by the ascorbic acid/ammonium molybdate/ antimony potassium tartrate colorimetric method on a Lachat FIA 8000. Nitrate-N and NH_4^+-N were extracted using 2M KCl (1:10 soil:extractant, 1 h shaking), and measured colorimetrically on a Lachat QuickChem FIA 8000. Sulphate-S was measured by KH_2PO_4 extraction and ion chromatography.

The surface area of the soils was estimated using the moisture factor technique (Churchman & Burke 1991). These authors reported a strong positive correlation (r^2=0.8) between specific surface area, as determined by ethylene glycol monoethyl ether (EGME) retention, and moisture factors for several soils collected within New Zealand. Moisture factors are calculated by dividing the air-dry mass of soil by its oven-dry (105°C) mass. The surface area of the soils was quantified by applying the relationship between EGME-derived surface area and soil moisture factors reported by Churchman and Burke (1991). Equations defining the upper and lower envelopes of the relationship were used to determine the minimum and maximum surface area for each soil. Particle size distribution was determined as described by Claydon (1989), while particle density, bulk density and porosity were calculated following the techniques described by Gradwell (1972).

Molecular analyses

Microbial molecular analyses were performed on topsoils and subsoils taken from the 8-year-old landfill cap to investigate the species of bacteria in the soils responsible for oxidising the high CH_4 concentrations in the initial laboratory chamber tests. The *pmo*A and *mmo*X genes from methanotroph DNA were amplified. Methanotrophs from the top (A), middle (B) and bottom (C) of the chambers were cultured in a nitrate-mineral-salt-medium, and their activity in oxidising CH_4 was assessed. Populations of methanotrophic bacteria were studied by genetic analysis of soil *pmo*A and *mmo*X similar to a previous study conducted on forest soil in Canada (Jugnia et al. 2006).

DNA was extracted from each soil sample (Top A, B, C, and Sub A, B, C) using the Power Soil DNA kit (Mo Bio, Carlsbad, CA, USA) according to the manufacturer directions but with the cell lysis step accomplished using a bead beater instrument (Retsch, Haan, Germany). *pmo*A genes were amplified using a touch-down PCR protocol (Horz et al. 2001) and forward (F) and reverse (R) primers (F: 5'-ggngactgggacttctgg; R: 5'-gaasgcngagaagaasgc). *mmo*X genes were amplified using the same thermocycler protocol and the primers mmoX-F (5'-ccgctgtggaagggcatgaa) and mmoX-R (5'-cactcgtagcgctccggctc) (Horz et al. 2001). As a positive control PCR amplification 16S rRNA genes were targeted using universal primers (F1: 5'-gagtttgatcctggctcag; R13: 5'-agaaaggaggtgatccagcc) (Dorsch and Stackebrandt 1992). The PCR amplicons were gel-extracted, purified with a QIAquick gel extraction kit (Qiagen, Maryland, USA) and cloned into pGem-T vectors (Promega, Madison, WI, USA). Clones were randomly selected for sequencing from each library. Sequencing results were analyzed using BioEdit and Mega4 software. *pmo*A sequences were aligned to closest matched sequences retrieved from Genbank after BLAST analysis. Pair-wise distance coefficients between the various sequences were calculated using the Jukes-Cantor method. A neighbour-joining tree was then derived from the distance matrix, which included *pmo*A genes of all well-characterized strains.

2.4 Results

The biofilter work has been written up in the following three scientific papers submitted to peer-reviewed international journals: *In vitro methane removal by volcanic pumice soil columns over one year*; *Biofiltration of methane emissions from a dairy farm effluent pond*; and *Assessing the net efficacy of biofiltration to mitigate methane from sources of variable methane concentration and flow.* Another manuscript is currently being prepared discussing the methantroph populations detected in the landfill pumice soils.

2.4.1 Establishing upper CH_4 oxidation rates in biofilter soils

Long-term tests on CH_4 oxidation rates by volcanic pumice soils

The first biofilter experiments conducted in this research examined the abilities of various volcanic soils to effectively oxidize elevated CH_4 fluxes over a long-term period (>1 year). Establishing whether natural soils can efficiently operate over long periods with minimal maintenance is seen as the first step in assessing the feasibility of biofiltration to mitigate on-farm CH_4 emissions.

Soil CH_4 oxidation rates for the long-term tests on the volcanic soils are shown in Figure 1. Variation between replicated data points were all less than 3% of the reported values, so only mean values are shown. All topsoils strongly oxidised CH_4 when inlet concentrations ranged between 0.5 and 17 g CH_4 m^{-3} h^{-1}. By contrast, the subsoils exhibited a much lower capacity to oxidise CH_4 as the inlet concentration was increased to 17 g CH_4 m^{-3} h^{-1}.

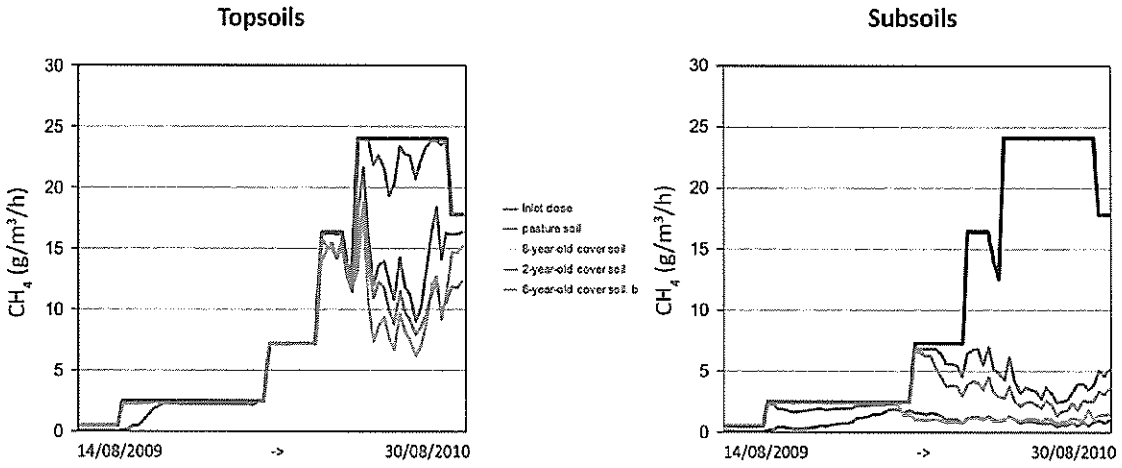


Figure 1 CH_4 uptake rates for different soils and inlet concentrations. The 8-year-old cover soils labeled 'b' were subjected to high inlet CH_4 doses (17 g CH_4 $m^{-3}h^{-1}$) in a previous experiment. All values are corrected to CH_4 density (0.669 g l^{-1}) at 20°C.

Methane oxidation in all soils was variable at the highest CH_4 inlet flux (24 g CH_4 m^{-3} h^{-1}). The CH_4 oxidation rates in the subsoils generally continued to decline with respect to time (below 7 g CH_4 m^{-3} h^{-1}), whereas the topsoils all achieved removal efficiencies greater than 7 g CH_4 m^3 h at the 24 g CH_4 m^{-3} h^{-1} feed dose (Figure 1). The pasture topsoil was clearly able to oxidise CH_4 more efficiently than all other soils, eventually reaching a stable removal rate of 100% at 24 g CH_4 m^{-3} h^{-1} (Figure 1). The high oxidation rate exhibited by this soil, which was collected away from the CH_4 source (i.e. the decomposing refuse), indicates rapid activation of the soil's methanotroph population to the high CH_4 fluxes. From a practical perspective, this is very encouraging for developing biofilters because it suggests that acclimation periods for biofilter start-up can be very short.

At the conclusion of the trial, the inlet concentration was reduced to 17 g CH_4 m^{-3} h^{-1}. The landfill cap topsoils and the pasture topsoil were able to oxidise 70–100% of the incoming

CH_4 at this dose level. The subsoils, by contrast, could only remove 5–30% at the 17 g CH_4 m^{-3} h^{-1} inlet dose (Figure 1).

Carbon dioxide (CO_2) concentrations in the gas exiting the chambers provided a strong indicator of CH_4 oxidation, as CO_2 is a by-product of biological CH_4 oxidation. Elevated CO_2 concentrations were recorded in the exit gas from all chambers, confirming that the observed reduction in CH_4 levels through the soils was due to biological oxidation rather than adsorption to or leakage from the containers. Methane/CO_2 ratios in the exit gas from the chambers correlated very strongly with CH_4 oxidation rates (r^2=0.92) suggesting that CH_4 and CO_2 concentrations alone can provide a robust technique for quantifying CH_4 oxidation rates in methanotroph-rich soils in cases where flow rates are not known, and hence, fluxes cannot be measured. However, in such cases verification is needed that methanotrophs are the principal soil organisms responsible for producing CO_2.

The maximum CH_4 removal rates (24 CH_4 g m^{-3} h^{-1}or 65 μg^{-1} g^{-1} h^{-1} or 160 g m^{-2} d^{-1}) exhibited by the soils in this study were high compared with values reported for other active soils. Kettunen et al. (2006) reported a maximum CH_4 oxidation rate of 13 μg^{-1} g^{-1} h^{-1} for a compost and sand substrate. Kightley et al. (1995) documented a similar oxidation rate (16 μg^{-1} g^{-1} h^{-1}) in their work on CH_4 removal by porous, coarse sandy soils. In their global review of CH_4 oxidation, Scheutz et al. (2009) reported a maximum CH_4 oxidation rate of 173 μg^{-1} g^{-1} h^{-1}. However, typical removal rates for methanotroph-rich soils are much lower, between <1 and 40 μg^{-1} g^{-1} h^{-1} (Scheutz et al. 2009). In a review on CH_4 oxidation rates by landfill cover soils, Chanton et al. (2009) reported an average CH_4 oxidation rate of 72 g m^{-2} d^{-1}. Clearly, the high CH_4 removal rates recorded in our investigation indicate the presence of very active methanotroph populations in these volcanic soils.

Soil physical properties are known to affect CH_4 oxidation rates (Kettunen et al. 2006), hence particle-size distribution profiles, air-filled porosity, surface area, and bulk density were measured for all soils assessed in this study. The particle-size distribution profiles for all soils were very similar (Table 1). The pasture topsoil had the highest proportion (62%) of fine-grained particles (i.e. silts and clays), while the 2-year-old cover topsoil had the lowest fraction of fine material (43%). All the soils exhibited very high silt/clay contents compared with other media assessed in previous studies. For example, Kettunen et al. (2006), in their work assessing CH_4 oxidation rates by landfill soils, tested a coarse biofilter substrate comprising <1% silts and clays. Park et al. (2002) also used a coarse soil (17% silt/clay fraction) in their work on CH_4 uptake by engineered biofilters. Similarly, Kightley et al. (1995) concluded that soils composed predominantly of large particles favour CH_4 oxidation compared to fine materials. Nikiema et al. (2007), in their review of CH_4 biofiltration by landfill soils, considered the ideal particle size for CH_4 biofilters is between 0.5 and 2 mm, and that particle sizes smaller than this tend to restrict gas movement through filters. Clearly, our findings differ from the conclusions of these previous studies. This work has demonstrated that fine-grained volcanic soils can achieve very high oxidation rates.

The porosities of our volcanic soils were exceptionally high (69–82%), while their bulk densities were very low (0.39–0.74 kg l^{-1}), compared with values reported in previous studies, where porosity is typically between 10% and 40% and bulk density is approximately 1 kg l^{-1} (Kightley et al. 1995; Humer & Lechner 1999; Kettunen et al. 2006). The combined effects of fine particle size and high porosity have been shown by Gama-Castro et al. (2000) to improve aeration and adsorption in pumice Andisols, which facilitate enhanced gas transfer processes (Kettunen et al. 2006). Our data suggest that porosity, which more directly controls gas

movement through a soil, is a more important parameter in controlling the CH_4 oxidation capacity of a soil than is its particle size distribution,

Table 1 Physical properties of studied soils

	‡Particle size distribution (%)			Porosity (%)	Surface area (m^2g^{-1})	Bulk density ($kg\ m^{-3}$)
	Clay	*Silt*	*Sand*			
Topsoils						
Pasture topsoil	3	59	38	82	10–45	390
8-year-old cover topsoil	3	45	52	78	10–30	510
2-year-old cover topsoil	3	40	57	75	10–30	570
†8-year-old cover topsoil b	1	56	43	75	10–40	580
Subsoils						
Pasture subsoil	1	51	48	78	5–20	510
8-year-old cover subsoil	8	41	51	70	5–25	720
2-year-old cover subsoil	4	40	56	68	2–15	740
†8-year-old cover subsoil b	7	41	52	70	5–25	730

†The 8-year-old cover soils labelled 'b' were subjected to high inlet CH_4 doses (17 g CH_4 m^{-3} h^{-1}) in a previous experiment.
‡clay = <0.002 mm, silt = 0.06–0.002 mm, sand = >0.06 mm.

Specific surface area (SSA) can also play an important role in CH_4 oxidation. Although not normally assessed in CH_4 oxidation studies, SSA can provide a useful indicator of a soil's CH_4 oxidation potential. Soils with high SSA favour CH_4 oxidation by providing many surfaces for colonization by methanotrophs and for soil nutrient adsorption. The SSAs for the soils assessed in this study (Table 1) were typical for pumice soils (5–45 m^2 g^{-1}). Gama-Castro et al. (2000) reported an average SSA of 50 m^2 g^{-1} for pumice andisols in Mexico. By contrast, average SSAs for sandy and silty soils are generally in the range of 0.1–1 m^2 g^{-1} (White 1997). Overall, the comparatively high SSAs of the soils studied in this work, in conjunction with their high porosity and high proportion of fine particles, suggest they have excellent physical properties to support a large and active methanotroph population.

Chemical parameters were monitored over the course of the experiment and are presented for T=0, 8 months and 12 months in Table 2. Soil pH values decreased in all the chambers over

the course of the experiment, with a minimum value of 4.9 recorded for the pasture topsoil, and the 8-year-old cover top and subsoil at the end of the experiment (Table 2). The reduction in pH probably resulted from nitrification/ammonification, known acid-forming processes, and this is supported by the NO_3^- and NH_4^+ data. However, these declines in pH had little impact on the ability of the soils to oxidise CH_4 as evidenced by the high CH_4 removal rate of the pasture topsoil (Figure 1) that also exhibited the lowest pH value (Table 2). The pH of all soils was below the optimal range (7–7.5) for CH_4 oxidation for the majority of cultured methanotrophs (Hanson & Hanson 1996), indicating that methanotrophs in our soils may be more tolerant of low pH than other reports suggest. In this experiment we used synthetic biogas (80% CH_4, 20% CO_2) that did not contain trace gases like H_2S and NH_3 that generally occur in biogas derived from anaerobic decomposition of organic matter. Hence, under field conditions the control of biofilter pH may be challenging. Gravimetric moisture contents of the soils remained relatively constant over the course of the trial and were largely within the optimal range of 15–50% for CH_4 oxidation (Hanson & Hanson 1996; Nikiema et al. 2007). Although dry biogas was used, soil moisture contents actually increased; particularly in the topsoils (Table 2). This resulted from generation of water during CH_4 oxidation since two molecules of water are produced for every molecule of CH_4 oxidised. The results suggest field-based biofilters should have no problem maintaining sufficient moisture levels for CH_4 oxidation, particularly as 'real' biogas contains much high moisture levels than artificial biogas. Soils may become too wet for efficient CH_4 oxidation when they approach their water-holding capacity (Park et al. 2002; Powelson et al. 2006), but this could be controlled by active or passive ventilation of air through the filter.

Topsoil C contents were higher than those of subsoils, and generally increased during the trial (Table 2). Increases in C concentrations were likely due to increased C turnover from a larger methanotroph population. Olsen-P concentrations changed little over the initial 8 months but decreased in all soils at the conclusion of the study (Table 2). High soil available-P favours CH_4 oxidation rates (Kightley et al. 1995). Nikiema et al. (2010) showed that increasing P levels in soils, up to approximately 250 mg P kg^{-1} soil (wet weight), resulted in an increase in CH_4 oxidation. The low Olsen-P levels recorded at the end of the study (Table 2) may simply reflect temporary P sequestration by the actively growing microbial population. Over time this P should be recycled. Nonetheless, the results of our study suggest close monitoring of P, including microbial P, will be necessary for field-scale filters. Our results suggest available-P levels can remain stable under *in vitro* conditions for at least 8 months.

Soil N levels were largely stable and generally increased slightly in the chambers over the course of the experiment (Table 2). While NO_3^--N concentrations generally decreased in the soils over the trial, the consistently high CH_4 oxidation rates exhibited by the topsoils indicate that the methanotrophs were not N-limited at any stage. Variation in soil NO_3^--N and NH_4^+-N contents are expected in an experiment of this duration, and certain strains of methanotrophs can fix their own N (Nikiema et al. 2007). However, over longer time frames, N availability to methanotrophs may become limited and adjustments might be necessary in the form of nutrient additions.

Table 2 soil chemical properties and moisture contents over the course of the trial

Soil type	Sampling episode	pH	Moisture (wt % dry weight)	Total C (wt % dry weight)	Total N (%)	NO_3^--N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})	Olsen P (mg kg^{-1})
Pasture topsoil	Start	5.61	‡ (47.4) ‡61.8	6.94	0.51	1.6	4.1	87
	During (8 months)	5.69	55.6	7.00	0.54	22.0	1.4	104
	Finish (12 months)	4.90	72.0	8.60	0.60	0.4	16.0	50
8-year old cover topsoil	Start	5.49	‡ (35.9) ‡46.2	3.48	0.29	11.2	6.8	21
	During (8 months)	4.43	40.1	3.67	0.32	177	5.2	31
	Finish (12 months)	4.90	46.3	4.60	0.36	0.3	6.9	12
2-year old cover topsoil	Start	6.14	‡ (38.8) ‡49	4.12	0.30	4	0.9	19
	During (8 months)	5.29	39.9	4.14	0.32	149	0.3	28
	Finish (12 months)	5.10	51.6	5.40	0.37	0.4	10.1	10
§8-year old cover topsoil: b	Start	5.48	‡ (42.5) ‡46	3.87	0.29	117	2.7	11
	During (8 months)	5.46	55.9	4.06	0.33	90.3	3.1	12
	Finish (12 months)	5.20	63.5	5.20	0.35	0.5	10.3	7
Pasture subsoil	Start	6.03	‡ (36.0) ‡39.9	0.79	0.05	0.3	3.1	8
	During (8 months)	6.46	30.4	0.93	0.06	<0.1	3.3	6
	Finish (12 months)	5.80	22.7	0.96	0.06	0.3	5.0	4
8-year old cover subsoil	Start	4.97	‡ (31.1) ‡39.5	0.59	0.08	22.8	11.7	5
	During (8 months)	4.87	30.4	0.54	0.07	57	2.5	5
	Finish (12 months)	4.90	23.4	0.51	0.07	62.7	55.9	4
2-year old cover subsoil	Start	6.26	‡ (27.8) ‡34.8	0.55	0.04	2.2	0.7	5
	During (8 months)	6.05	27.8	0.93	0.06	<0.1	0.4	4
	Finish (12 months)	5.20	29.2	1.50	0.08	0.6	11.7	2
§8-year old cover subsoil: b	Start	6.35	‡ (22.0) ‡39.6	0.51	0.05	2.4	3.6	3
	During (8 months)	5.82	30.9	0.94	0.06	<0.1	1.3	3
	Finish (12 months)	5.40	30.0	1.30	0.06	0.3	6.6	2

‡Figure in brackets is initial moisture content of soil; ‡adjacent figure is moisture content of soil after wetting to 60% WHC;
§The 8-year-old cover soils labelled 'b' were subjected to high inlet CH_4 doses (17 g CH_4 m^{-3} h^{-1}) in a previous experiment.

Generally, high soil NO_3^--N levels are not inhibitory to soil methanotrophs and, in fact, support effective CH_4 oxidation, as discussed above. By contrast, NH_4^+-N can interfere with CH_4 oxidation processes (Hanson & Hanson 1996; Hilger et al. 2001; Price et al. 2004) but the NH_4^+-N concentrations in all soils assessed in this study are below the inhibitory threshold of 222 mg kg^{-1} reported by Price et al. (2004).

It is clear from Figure 1 that the topsoils assessed in this study were far more efficient at oxidising CH_4 than the subsoils. The topsoils exhibited higher porosities and SSAs than the subsoils: properties favourable for CH_4 oxidation (Kettunen et al. 2006; Park et al. 2002; Tate et al. 2007). Furthermore, pools of key nutrients (total N, P) and C were lower in the subsoils than the topsoils. The particularly low and diminishing Olsen-P contents of the subsoils and the ability of volcanic soils to electrostatically adsorb P onto charged iron hydroxide surfaces may account for their decreased CH_4 uptake rates over time.

In addition to physical and chemical parameters, a greater biological diversity in the topsoils may have contributed to their stronger CH_4 uptake compared with the subsoils. At a methanotrophic level, the topsoils do not appear to be more diverse than the subsoils. Preliminary molecular analyses of the 8-year-old landfill cap soils revealed the presence of both pmoA and mmoX genes in both topsoils and subsoils. However, the 8-year-old landfill subsoils host a greater diversity of methanotrophs (including Type Is such as *Methylococcus capsulatus* and Type IIs such as *Methylocapsa acidiphila, Methylosinus trichosporium, Methylocystis sp*-related) than the corresponding topsoils (Figure 2). The results apparently suggest that the difference in CH_4 oxidation capacity between the top and subsoils is not simply related to the presence of a key methanotroph group in the topsoils being absent in the subsoils. Yet at higher trophic levels, the topsoils do appear to be more biologically diverse than the subsoils.

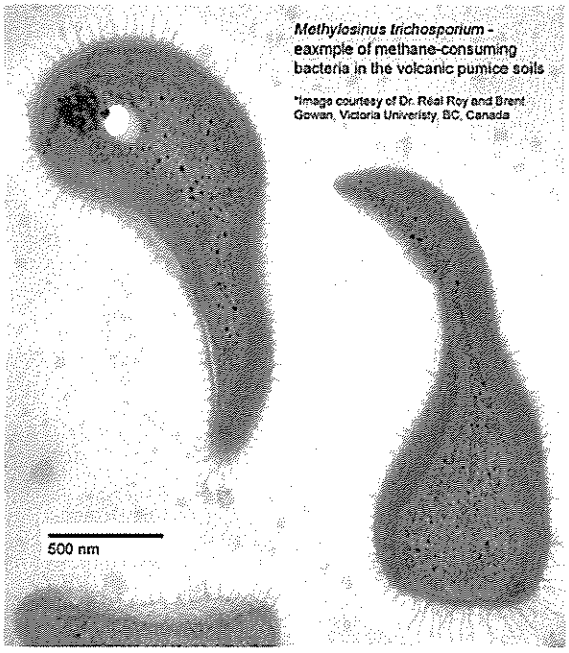


Figure 2 Type II methanotroph in the landfill volcanic topsoils.

The topsoils were darker coloured, hosted a number of visible larger organisms (earthworms and insects) and exhibited uniformly elevated C contents (3–7 wt%). These factors suggest a thriving biological community, including methanotrophs and predators that will accelerate the turnover of cells and the nutrients they contain. In addition, other microorganisms may supply growth factors, such as vitamins, essential for methanotroph activity (Hanson & Hanson 1996). By contrast, the subsoils were lighter coloured, contained no obvious macroscopic organisms and had low C concentrations (<1 wt%). These features indicate a smaller, less diverse biological population that would have restricted methanotrophic growth and activity. Overall, it is likely that a combination of chemical, biological, and physical factors may account for the stronger CH_4 oxidation rates exhibited by the topsoils than the subsoils.

Assessment of CH_4 inlet dose and residence time on oxidation

Clearly, the results of the long-term oxidation experiment demonstrated that natural soils have the ability to efficiently oxidize elevated CH_4 emissions under *in vitro* conditions for extended periods of time. The next step in the research was to examine the feasibility of biofiltration as a CH_4 mitigation tool for dairy farms by assessing CH_4 oxidation rates in response to the CH_4 concentrations and flows associated with on-farm CH_4 sources (namely effluent ponds and animal housings).

For this part of the work, two soils were chosen: one was the volcanic soil from the pasture site which demonstrated the highest CH_4 oxidation rates in the long-term *in vitro* experiment; the other was a green waste compost soil that has recently shown very promising potential to rapidly consume CH_4. The CH_4 oxidation efficiencies of the soils as a function of inlet CH_4 concentration and residence time are presented in Figure 3. As expected, oxidation efficiencies increased with respect to increasing residence time and decreasing inlet concentration. The compost soil exhibited higher removal rates than the volcanic soil.

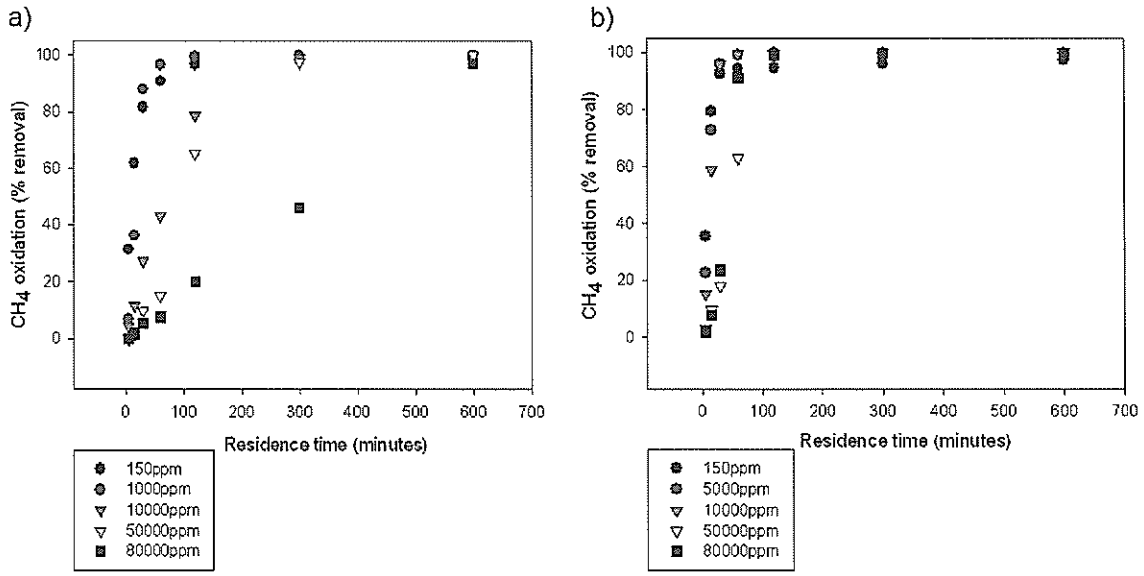


Figure 3 Effect of CH_4 inlet concentration and residence time on CH_4 oxidation efficiency of: a) volcanic pumice; and b) compost soil. Values are means of 4 replicate measurements.

The higher oxidation rate exhibited by the compost soil compared with the volcanic pumice may relate to differences in methanotrophic populations between the substrates. We are yet to analyse the microbial community of the compost soil, but plan to do so shortly. Differences in performance between the soils may also relate to their physiochemical properties. The soils' physical properties (porosity = 82–83%, bulk density = 0.39 kg^{-1} m^{-3} and moisture = 62–73%) were similar. The high porosity and low bulk density exhibited by the soils are favourable for enhanced gas transfer and CH_4 oxidation (Gama-Castro et al. 2000; Kettunen et al. 2006). The soils' moisture contents were higher than the ideal range (20–35% wt/dry wt) reported by Hanson and Hanson (1996) for CH_4 oxidation. Yet, as will be shown later in this document, the optimal moisture contents for CH_4 oxidation by the soils assessed in this study are higher than this reported range.

In contrast to the soils' similar physical characteristics, their chemical properties varied markedly. The pH of the volcanic pumice soil was slightly acidic (5.2) whereas the compost exhibited neutral pH (7.1). Hanson and Hanson (1996) reported an optimal pH range of 7–7.5 for CH_4 oxidation in soils and sediments. Additionally, Nikiema et al. (2007) note that low pH is potentially inhibitory for methanotroph activity. Hence, the higher pH of the compost soil may have enhanced CH_4 oxidation rates relative to the volcanic soil. Soil C (16.9%), N (1.7%), and Olsen (bioavailable)-phosphorus P (417 mg kg^{-1}) levels were much higher in the compost soil than the volcanic material (C = 7.85, N = 0.54% Olsen-P = 23 mg kg^{-1}). These elements are critical for methanotroph growth and activity (de Visscher et al. 1999; Hilger et al. 2000; Nikiema et al. 2007) and may account for the higher CH_4 oxidation rates observed in the compost soil compared with the volcanic soil.

Despite the difference in performance of the volcanic and compost soils observed in this study, they both demonstrated high oxidation rates compared with other biofilter media reported in the literature (maximum oxidation rate = 11 g CH_4 m^{-3} h^{-1} or 28 μg CH_4 g^{-1} h^{-1} at a residence time of 120 minutes and an inlet concentration of 50000 ppm for volcanic soil, maximum oxidation rate = 49 g CH_4 m^{-3} h^{-1} or 125 μg CH_4 g^{-1} h^{-1} at a residence time of 60 minutes and an inlet concentration of 80000 ppm for compost). Clearly, the soils assessed in this study host very active methanotroph populations and are well-suited for use in engineered biofilters to treat anthropogenic CH_4 emissions.

The results in Figure 3 can be used to construct a contour plot depicting CH_4 oxidation as a function of CH_4 inlet concentration and residence time in biofilters (Figure 4). This contour plot can be used as a tool to assess the feasibility of biofiltration to mitigate emissions from the two major on-farm CH_4 sources: 1) enteric rumination and 2) effluent ponds. For economic considerations, a sealed biofilter unit with a volume of 100 m^3 is estimated to cost US$25K, based on information presented by Melse and Van Der Werf (2005). However, the economics of CH_4 biofilters will also depend on incentives such as those provided by carbon trading systems.

1) ***Ruminant animal housing*** – in this first case, a hypothetical dairy cattle housing unit is assessed, with a ventilation rate of 250 m^3 LU^{-1} h^{-1} as reported by Ngwabie et al. (2009). This is typical of a wintering barn in Sweden. For this analysis it is assumed that the cattle are in the barn for half of the year and that the average CH_4 concentration in the barn is 150 ppmv (Ngwabie et al. 2009), which is consistent with measurements we have made in a European-style wintering barn in New Zealand (data not shown). The herd size in the hypothetical housing unit is 100, which is typical for European dairy farms (Melse & Van Der Werf 2005). Ambient air from the housing unit is directed through the hypothetical

biofilter at the same rate as the ventilation flow (i.e. 250 m^3 LU^{-1} h^{-1}). The volume of the biofilter would need to be 500 m^3 to achieve a CH_4 residence time of >1 minute for this scenario. If the filter size is any smaller, it will result in much shorter residence times and, based on the contour plots in Figure 4, would be unlikely to permit significant CH_4 oxidation.

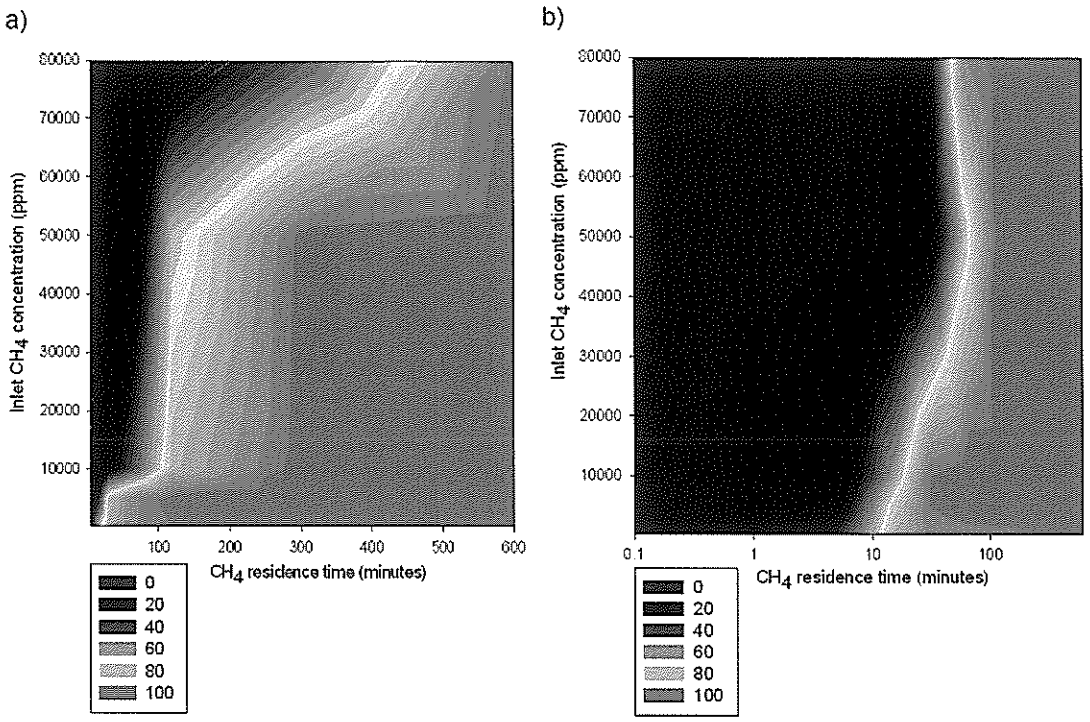


Figure 4 Contour plot showing CH_4 oxidation (% removal) as a function of residence time and inlet CH_4 concentration for: a) volcanic pumice; and b) compost soil. Experiments were performed at 25°C and 60% (wt/dry wt) soil moisture content. Note the x-axis for the compost soil is a logarithmic scale.

The contour fields (Figure 4) show that for a CH_4 inlet concentration of 150 ppmv and a residence time of 1.5 minutes, only a 20% CH_4 oxidation rate can be achieved, even by the very active compost soil. For the theoretical wintering barn described for this study, this only equates to an offset of approximately 2.2 tonnes of CH_4/year (assuming a density of 0.67 g CH_4 L^{-1}), or 50 tonnes of CO_2-equivalents (assuming a global warming potential of 23x for CH_4 relative to CO_2 over a 100 year time frame; Melse & Van Der Werf 2005). Higher CH_4 oxidation efficiencies could be achieved by constructing a larger filter but then the economics and logistics of the technology become unviable. For example, if the filter volume was increased to 5000 m^3 (approximately the same size as the barn), about 7 tonnes of CH_4 could be oxidised annually, or 150 tonnes of CO_2-e. This equates to an offset of approximately US$4K annually, based on a CO_2-e pricing of US$25/tonne, which is only marginal compared with the construction and operating costs of the biofilter. It may be possible to achieve successful biofiltration of dairy cattle housing emissions by developing an artificial biofilter medium saturated with active methanotroph strains that are able to oxidise CH_4 much more rapidly than methanotroph communities present in soils and sediments. This would allow for a much smaller and more practical biofilter. However, based on the results of our study, offsetting CH_4 emissions from dairy cattle housing is currently considered impractical using soil-based biofilters, particularly when it is considered that the soils assessed in this work exhibited very high CH_4 oxidation rates compared with other biofilter soils reported in the literature.

2) ***Dairy effluent ponds*** – these waste treatment systems are another major on-farm CH_4 source. Capturing and burning biogas from effluent ponds to produce energy is not economically viable for most dairy farms, which have herd sizes of approximately 100–350 cattle (Melse & Van Der Werf 2005; Craggs et al. 2008). Hence, these waste storage ponds represent CH_4 source that may be amenable to biofiltration. The CH_4 yield from effluent ponds on typical-sized dairy farms is approximately 26 m^3 d^{-1} (Craggs et al. 2008). Methane comprises about 60% of the biogas emitted from effluent ponds. Therefore, to be effectively oxidised in a biofilter this biogas will need to undergo about a 10-fold dilution with air to provide enough oxygen for methanotrophs to complete the stoichiometric oxidation reaction (considering that air contains about 20% O_2):

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$$

Based on this background information, a biofilter with a volume of 100 m^3 would effectively provide a residence time of 550 minutes for CH_4 emitted from a typical dairy effluent pond. On mixing with air at 10-fold dilution, the CH_4 concentration in the biogas becomes 6%. According to the contour plots in Figure 4, a biofilter comprising either the volcanic pumice soil or the compost soil could achieve almost 100% CH_4 oxidation at this residence time and CH_4 inlet concentration. This corresponds to 7 tonnes of CH_4 (150 tonnes CO_2-e) that could be oxidised by a single effluent pond, annually. Economically, this represents an offset of approximately US$4K, for a 100 m^3 filter. Hence, biofiltration using soil media appears to be a promising technology for treating emissions from dairy effluent ponds, because a much smaller filter volume can remove the same amount of CH_4 than for animal housing applications. The feasibility of the technology for this application will depend on the payback period for the biofilter being deemed acceptable by the filter operator.

Nitrous oxide emissions

Fluxes of N_2O produced by the soils were monitored over the course of the experiment because N_2O is a much stronger greenhouse gas than CH_4. Given the high concentrations of N in both soils it is possible that N_2O could be released by biological processes. This is important because N_2O production could markedly reduce the efficacy of a CH_4 biofilter (Melse and Van Der Werf 2005). N_2O emissions from the compost soil were higher than emissions from the volcanic soil. This is expected, as the N content of the compost soil was much higher than that of the volcanic soil. The maximum N_2O emission from the volcanic and compost soil was 0.18 mg m^3 h^{-1} and 3.4 mg m^3 h^{-1}, respectively. Maximum N_2O emissions for both soils occurred at low CH_4 inlet concentrations and short residence times. These conditions were associated with low CH_4 oxidation rates and perhaps represent conditions more favourable for ammonia-oxidising bacteria.

Total N_2O fluxes themselves do not reveal much information regarding the impact of N_2O production on CH_4 oxidation. Rather, it is the production of N_2O relative to CH_4 oxidation that is most meaningful in terms of overall biofilter performance. Hence, ratios of N_2O production to CH_4 oxidation were plotted over the various CH_4 inlet concentrations and residence times assessed in this study. The fluxes of N_2O emitted and CH_4 oxidised were both converted to CO_2-equivalents before calculating ratios. The results are presented in Figure 5.

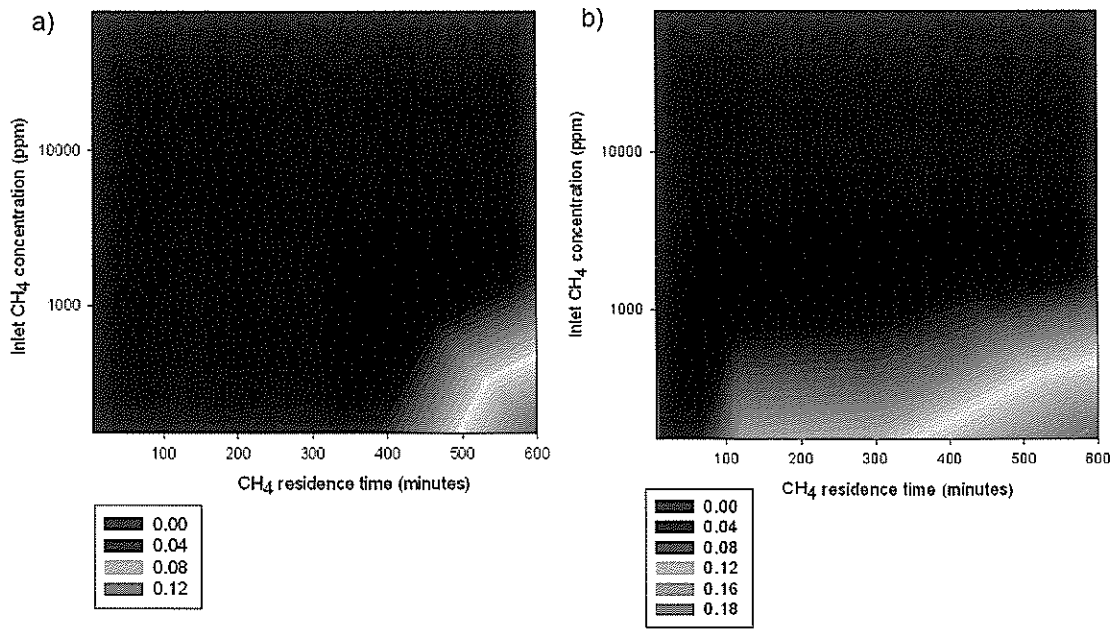


Figure 5 contour plot showing ratios of N_2O production/CH_4 oxidation as a function of residence time and inlet CH_4 concentration for: a) volcanic pumice; and b) compost soil.

It can be seen that the ratio of N_2O production to CH_4 oxidation was greatest at long residence times and low inlet CH_4 concentrations. The maximum N_2O emission effect was observed for the compost soil, where N_2O emissions represented almost 20% of CH_4 oxidised at a residence time of 600 minutes and an inlet CH_4 concentration of 150 ppm (Figure 5). For sources of low CH_4 concentrations biofilters are likely to have very short CH_4 residence times, otherwise they will be unfeasibly large. Hence, N_2O impact is unlikely to overshadow CH_4 oxidation in practical biofilter applications. Nonetheless, N_2O production by biofilter soils, particularly N-rich organic soils, clearly has the potential to compromise their efficiency at reducing total GHG emissions. Nitrous oxide fluxes from any engineered biofilters should be closely monitored as it is possible that a particular soil with lower CH_4 oxidation capacity than another soil may in fact be more effective at reducing total greenhouse gas emissions.

Effect of soil moisture content and temperature on CH_4 oxidation

Temperature and moisture are two of the most significant environmental parameters affecting CH_4 oxidation by soils. Hence, the effects of both these factors on CH_4 oxidation rates by the volcanic and compost soil were assessed. The optimal temperature for CH_4 oxidation by both soils was 37°C and CH_4 oxidation rates increased almost linearly with increasing temperature (Figure 6). While it is possible that higher oxidation rates would be observed at temperatures beyond 37°C, it is not envisaged that field biofilters would need to operate at such high temperatures, or conversely at very low temperatures (below 0°C).

It can be seen in Figure 6 that CH_4 oxidation rates at 25°C are 2–4 times higher than at 15°C. At 5°C, oxidation rates were nearly zero (Figure 6). This highlights the need to adjust the contour fields shown in Figure 4 in response to varying temperature depending on the expected operating temperature of a biofilter. This highlights the need to adjust the contour

fields shown in Figure 4 in response to varying temperature depending on the expected operating temperature of a biofilter.

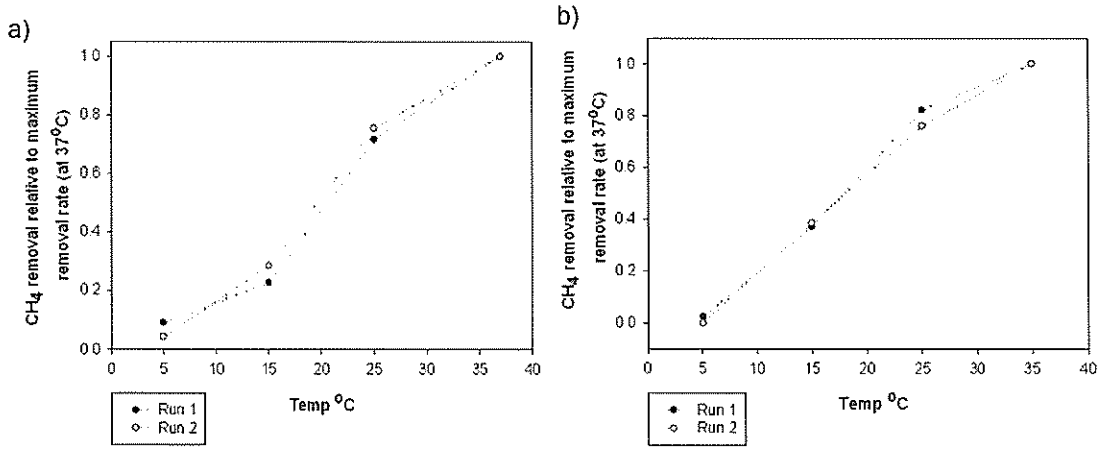


Figure 6 Effect of temperature on CH_4 oxidation rates by a) volcanic pumice soil; and b) compost soil. Experiments performed at 60% (wt/dry wt) soil moisture.

The optimal moisture content for the volcanic soil was 30–50% (wt/dry wt) and 60–80% for the compost soil (Figure 7). Moisture contents below 30% resulted in rapid decrease in oxidation efficiency for both soils. At the other end of the scale, a 100% moisture content was only inhibitory for the volcanic pumice soil (Figure 7). Unlike temperature, soil moisture levels may be easier to manage in engineered biofilters. As discussed earlier, the moisture contents of soil fed dry air and CH_4 remained consistently high (50%) over a period of a year. It is envisaged that as long as the biofilter structure is sealed, the filter media should be able to maintain its initial moisture content.

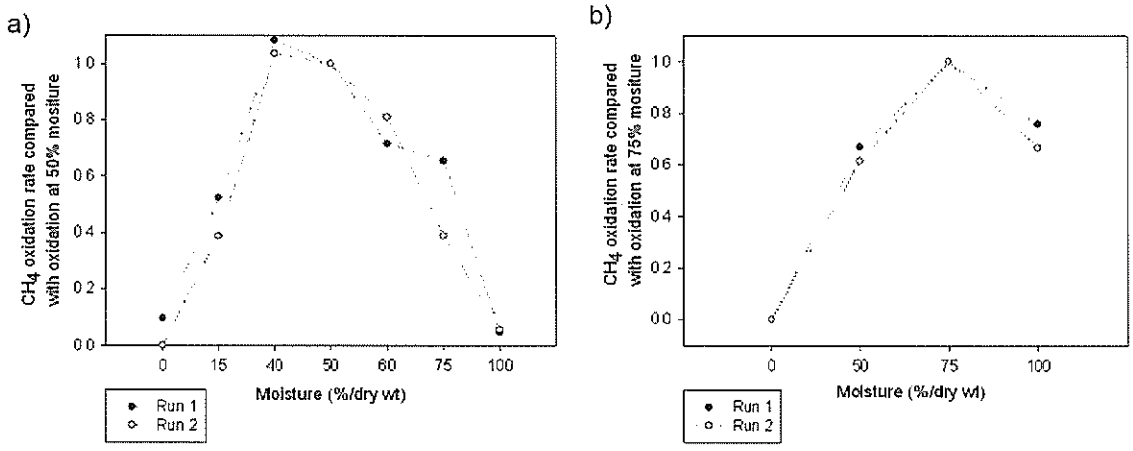


Figure 7 Effect of soil moisture content on CH_4 oxidation rates by a) volcanic pumice soil, and b) compost soil. Experiments performed at 25°C.

In general, the optimal temperature and moisture contents for the studied soils are similar to those reported by other authors examining various biofilter soils. For example, Mor et al. (2006) reported an optimal temperature range of 15–30°C and moisture content of 45–110%

(wt/ dry wt) for CH_4 oxidation by food and garden waste compost soils. Nikiema et al. (2007) found that CH_4 oxidation rates in compost-based soils are highest at 30°C with a soil moisture content of 25–50% (wt/dry wt).

Overall, temperature and moisture can potentially impact on biofilter performance In cold, temperate environments, an average operating temperature of 10°C might be expected. In these cases, CH_4 oxidation rates would certainly be lower than those reported in this study. By contrast, a biofilter operating in a warm tropical location might experience average temperatures of >25°C and the expected biofilter performance would be higher than that reported in this work. Clearly, local temperature needs to be considered when assessing the performance of a specific biofilter.

2.4.2 Prototype filters for effluent ponds

Field filter performance

The laboratory experiments discussed above revealed that volcanic pumice soils are able to effectively oxide elevated CH_4 fluxes over long time frames. It was also shown that while enteric emissions are logistically difficult to treat by biological oxidation, CH_4 emitted from dairy effluent ponds may be effectively mitigated by biofiltration. This section of the work recounts our experiences in using soil filters to offset CH_4 emitted from an effluent pond on an operational dairy farm. First, the results from a field prototype filter are discussed and this is followed by the results of our work examining techniques to optimise the initial filter design.

The performance of the field-scale prototype filter was monitored for approximately 1.5 years, from December 2009 to April 2011. Methane influx rates into the filter and oxidation efficiency are shown in Figure 8. Methane oxidation rates were calculated by comparing the differences between the recorded exit CH_4 concentrations from the biofilter and the predicted exit CH_4 concentrations if no oxidation was occurring. Because of the pond's variable biogas emission rates, average CH_4 inflow rates during the residence time of the biogas in the filter were used to determine fluxes. The accuracy of this method for estimating oxidation rates was tested by filling the filter with sterile media and measuring flux rates. Measurements were made on four occasions during this control trial. The observed CH_4 fluxes for the control tests were within <10% of the predicted theoretical fluxes, indicating that the adopted method for measuring oxidation rates was sufficiently accurate.

Methane oxidation rates exhibited by the field filter reached 16 g m^{-3} h^{-1} (53 μg g^{-1} h^{-1}) in summer 2011 (Figure 8). Carbon dioxide concentrations in the gas exiting the biofilter were higher than inlet values, providing a good indicator of biological oxidation of CH_4. The highest CO_2 concentration in the biofilter exit gas was 6.4% on 21 February 2011. The theoretical CO_2 concentration (i.e. if no biological activity occurred in the filter and CO_2 was only sourced from the biogas) in the same gas sample was 1.4%. By comparison, the observed CH_4 concentration in the biofilter exit gas at this interval was <0.01% and the theoretical CH_4 value was 4.4%. Hence, the CO_2 production corresponded very closely with the CH_4 reduction suggesting that CH_4 oxidation was the principal biological process occurring in the filter by the end of the trial. Exit gas N_2O concentrations from the filter were in the range of atmospheric levels (0.32 ppm) and, hence, N_2O fluxes from the filter were deemed to be negligible.

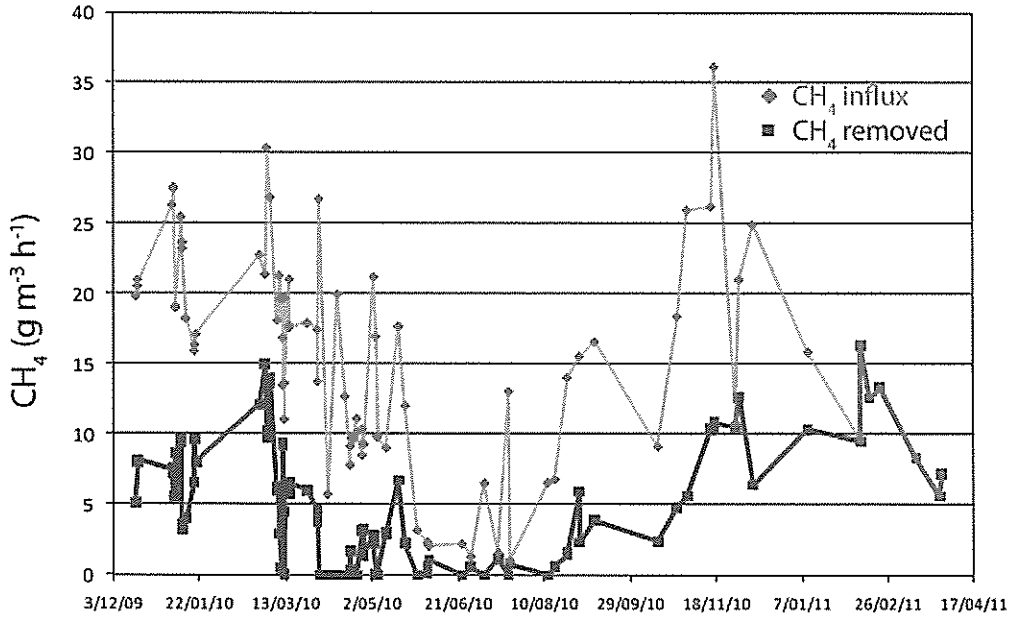


Figure 8 CH_4 oxidation rates in the field filter over the 1.5 year trial. All duplicate values for CH_4 removal are included in the dataset.

Diurnal variations in CH_4 influx and oxidation rates in the filter were recorded using an automated robotic sampler in March 2010 (Figure 9). The data show that CH_4 influx rates fluctuate over the course of a day. This is not unexpected, as pulses of biogas eruptions are frequently observed on dairy effluent pond surfaces. Figure 9 also shows that CH_4 oxidation rates respond to this variability in influx with higher oxidation observed at times of higher influx rates. This positive relationship between CH_4 influx and oxidation rates has been reported by other researchers (e.g., Melse & Van Der Werf 2005). In this study, the highest CH_4 oxidation rates were observed around midday and in the evening from 6:00 p.m. to midnight. Methane oxidation rates were lowest in the morning around 9:00 a.m. The sampling times for the 1.5 year field trial ranged between 9:00 a.m. and 5:00 p.m. and, based on the data in Figure 9, are considered to reasonably encapsulate the daily variations in CH_4 influx and oxidation rates in the biofilter.

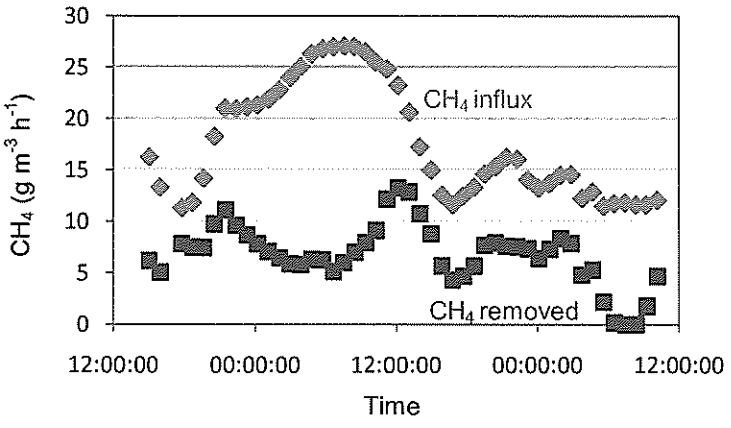


Figure 9 Hourly variations in CH_4 influx and oxidation rates in field filter over 2-day period (15–17 March 2010).

The CH_4 consumption rates exhibited by the filter (16 g m^{-3} h^{-1} or 53 μg g^{-1} h^{-1}) were commensurate with the CH_4 removal rates observed in the earlier laboratory experiments and were high compared with oxidation rates reported for biofilter media assessed in other studies. For example, Melse and Van Der Werf (2005) documented a maximum CH_4 degradation rate of 8 g m^{-3} h^{-1} for a compost/perlite biofilter treating piggery effluent emissions. The strong CH_4 oxidation rates exhibited by the field filter in this study are reflective of the presence of a thriving methanotroph community capable of rapid CH_4 consumption. As discussed previously, our research has shown that the volcanic landfill cap soil used in the filter contains Type II methanotrophs (*Methylocystis* sp.-related). *Methylocystis* sp. is characterised by a low CH_4 affinity/high oxidation rate (Scheutz et al. 2009) and has been detected in highly-concentrated CH_4 environments such as landfill caps (e.g., Uz et al. 2003; Kumaresan et al. 2009).

In addition to an active methanotroph population, the physiochemical properties of the filter media undoubtedly played a pivotal role in the observed efficient CH_4 oxidation rates. The physiochemical properties of the soil/perlite mixture are presented in Table 3. The high porosity, high moisture content, elevated available P, total C and N as well as low bulk density in the filter media are all conducive to enhanced methanotroph growth and activity (Hanson & Hanson 1996; Price et al. 2004; Nikiema et al. 2007, 2010). By the end of the trial, no slumping or compaction of the filter media was apparent, indicating that the filter's physical properties (bulk density and porosity) are suitable sustained and effective CH_4 oxidation (Table 3). Moisture levels (40–60%) were generally ideal for CH_4 oxidation (Nikiema et al. 2007), although there were signs of potential water-logging at the base of the filter by the end of the experiment (Table 3). Moisture build-up at the base of the filter could be mitigated by increasing the ventilation area at the filter's top. The only reason the filter was sealed in this study was to assist with regular gas flux measurements, and this would not be necessary for on-farm biofilters.

Table 3 Physiochemical parameters of field filter media

		Porosity (%)	Bulk density (kg m^{-3})	Moisture (%)	pH	Total C (%)	Total N (%)	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	Olsen P (mg kg^{-1})
Start		84	310	*(29.6) 38.1	5.5	2.8	0.24	9.24	5.59	17
Finish (12 months)	Top 1/3rd	84	310	43.9	4.6	5.1	0.39	6.8	0.1	9
	Middle 1/3rd	84	310	65.3	4.5	4.9	0.35	1.2	0.1	8
	Bottom 1/3rd	84	310	85.3	3.9	5.2	0.39	2.5	0.1	13

*Figure in brackets is initial moisture content of soil, adjacent figure is moisture content of soil after wetting to 60% WHC.

Total carbon levels increased throughout the filter profile over time (Table 3), indicating C fixation by methanotrophs. The total N content of the filter media also increased over the course of the experiment (Table 3), indicating effective N immobilisation by the microbial

population within the filter. Ammonia-N levels decreased slightly over the 1.5 year period, while NO_3^--N concentrations decreased considerably, probably reflecting incorporation into the soil's microbial pool. Olsen-P levels decreased marginally in the filter media during the trial (Table 3). The drop in Olsen-P does not appear to have adversely impacted methanotroph activity, and again likely reflects incorporation into the filter's microbial pool. Overall, while the field filter performed strongly for 1.5 years without intervention, the decrease in available N and P in the system suggest that biofilters need to be continuously monitored for nutrient levels, and ammendments may be necessary at some point.

Assessment of H_2S impact on field filter performance

In contrast to the other measured physicochemical parameters, which were generally optimal for CH_4 oxidation, the pH throughout the entire filter decreased over the course of the trial (Table 3). As mentioned previously, low pH is potentially inhibitory for methanotroph activity (Nikiema et al. 2007). The base of the filter was particularly acidic (3.9). This pH decrease with depth in the filter over time was likely caused by oxidation of H_2S in the biogas and subsequent generation of sulphuric acid. Measurements of the biogas composition entering the biofilter (taken in March 2010) revealed a mean H_2S concentration of 443 +/–41 ppm. By contrast, the H_2S concentration in the gas exiting the biofilter was 0 ppm, indicating that complete H_2S oxidation was occurring within the filter.

The biogas H_2S concentration observed in this study was high compared with H_2S values reported by Rasi et al. (2007). These authors documented maximum H_2S values of 427 ppm in landfill biogas and 20–170 ppm for sewage and farm biogas. The elevated H_2S values in the effluent pond biogas in this study may be a result of a high-sulphur diet for the cows. Regardless of the cause of the H_2S accumulation in the biogas, the presence of this acid-forming trace gas has the potential to affect biofilter performance. To establish whether CH_4 oxidation was negatively affected by H_2S oxidation, laboratory batch CH_4 oxidation tests were conducted on filter media collected from different depth horizons. The results are shown in Figure 10.

Results in Figure 10 indicate that significant H_2S oxidation occurred in the filter, evidenced by decreasing pH and increasing sulphate-S values down the filter profile. It initially appeared that this sulphur oxidation clearly impacted negatively on CH_4 oxidation, because the CH_4 oxidation rate at the base of the filter was only about 55% of the rate near the filter's surface (Figure 10). However, as was seen in the previous section, moisture content also plays a key role on CH_4 oxidation by the studied soil. Methane oxidation rates by the volcanic soil with a moisture content of 85% (by dry weight) are approximately half the oxidation rates by the same soil with a 40% moisture content (Figure 7). Hence, the difference in CH_4 oxidation rates between the top and base of the field filter can be accounted for by moisture content alone, as the moisture content at the filter's top was 44% by the end of the trial, compared with 85% at its base (Table 3). It is possible that with time, sulphur oxidation could become inhibitory for methanotrophs within the field filter. From a practical viewpoint, sulphur oxidation could be overcome by liming the filter to maintain near-neutral pH or by scrubbing H_2S from the biogas using iron chips. However, despite the pH being well below the optimal range (5–8.1) for CH_4 oxidation (Nikiema et al. 2007) throughout the filter profile, the data in Figure 8 clearly show that the filter's overall CH_4 oxidation rates were not severely impacted by the end of the trial. In fact, the highest CH_4 oxidation rates were observed during the last few days of monitoring (Figure 8), suggesting the effects of

sulphur oxidation and subsequent acid generation may take a long time (>1.5 years) to impact significantly on methanotroph growth and activity in engineered biofilters.

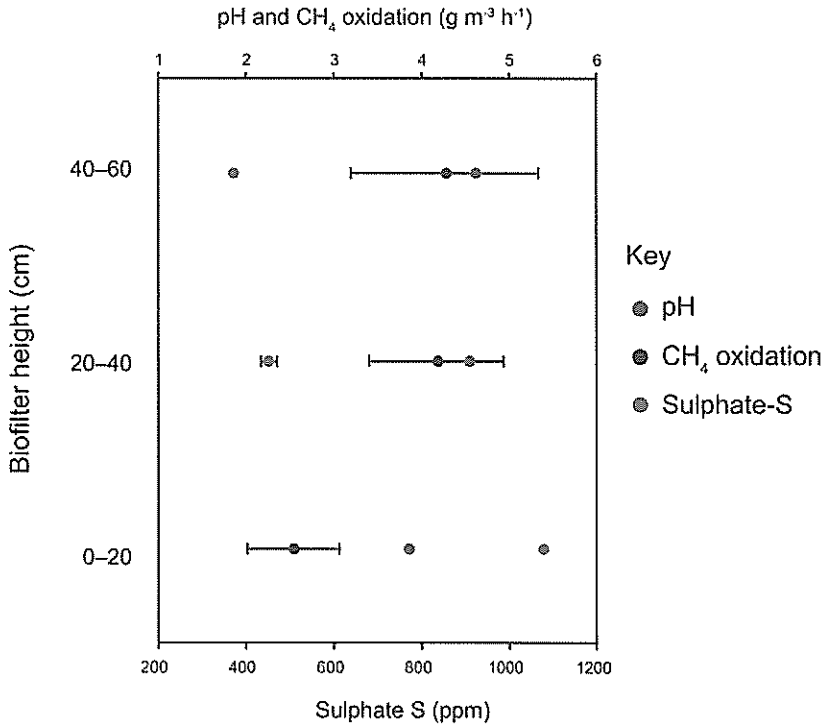


Figure 10 pH, sulphate-S and CH_4 oxidation rates in different depth horizons of field filter. Mean of duplicate values and ranges shown.

While the filter generally performed well, it is clear that CH_4 oxidation rates were variable over the trial (Figure 8). Oxidation was highest in summer whereas over winter, oxidation rates were <5 g m^{-3} h^{-1} and, occasionally, no oxidation was observed in the filter. This seasonal variation in CH_4 oxidation rates has been reported in previous studies (Melse & Van Der Werf 2005; Scheutz et al. 2009). The main factors causing this seasonally variable performance appear to be CH_4 influx rates and temperature. Methane influx rates and CH_4 oxidation rates were significantly positively correlated (r^2=0.42, p[two-tailed]≤0.001). Biofilter temperature and CH_4 oxidation rates also showed significant positive correlation (r^2=0.46, p[two-tailed]≤0.001), agreeing with the results of the laboratory temperature tests. These correlations are pertinent at a practical-scale because seasonal variations in biogas production and wide temperature fluctuations are features of many dairy effluent ponds. In the Manawatu region, where the trial was performed, summer temperatures often reach 25°C. The maximum air temperature recorded during the experiment was 30°C. By contrast, winter temperatures approaching 0°C are not uncommon: a low of –1°C was reached during the trial. Diminished methanotroph activity and growth at these cold temperatures is a significant obstacle for biofilter performance as Scheutz et al. (2009) note that oxidation rates can reach a standstill over winter. Actively heating the filter may overcome reduced CH_4 removal rates over winter, although this approach will likely be expensive, which compromises the low-cost/low-maintenance aspect of this technology. However, the reduction in biofilter performance over winter may be offset by the low CH_4 emissions produced during this period, as methanogenic activity is also reduced. Seasonal CH_4 production rates from the pond assessed in this study are shown in Figure 11.

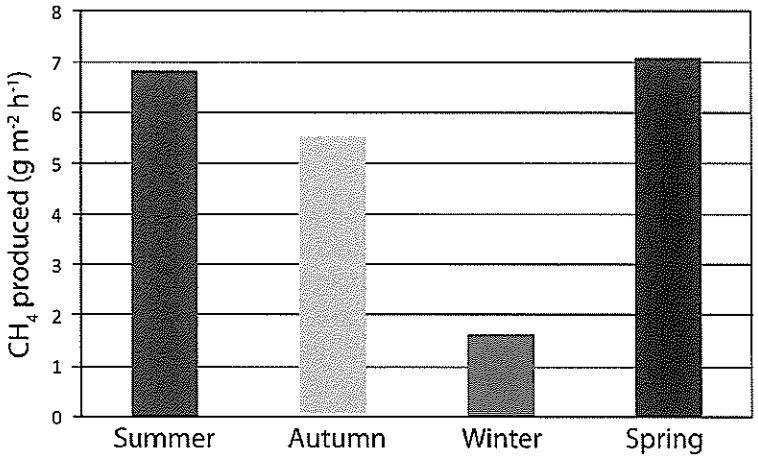


Figure 11 Average seasonal biogas production by effluent pond. Values are means of half-hourly measurements.

It can be seen that winter CH_4 production rates are only about 25% of summer, spring and autumn generation rates (Figure 11). A similar finding was observed by Craggs et al. (2008) for CH_4 production from a dairy effluent pond in northern New Zealand. These authors reported winter emission rates to be approximately 25% of production rates during the other seasons. Therefore, even if no oxidation occurs in a biofilter over winter, only about 8% of yearly emissions would escape during this time.

It is important to note that there is very little published data on CH_4 production rates from dairy effluent ponds. Furthermore, herd sizes and waste production volumes vary between farms. Our study indicates CH_4 emission rates of approximately 180 m^3 d^{-1} for a 1000-m^2 pond treating effluent from 450 cattle, yet this figure is significantly higher than the two other published CH_4 emission rates for New Zealand dairy ponds: 45 m^3 d^{-1} for a 1700-m^2 pond treating effluent from 700 cattle (Craggs et al. 2008); and 12 m^3 d^{-1} for a pond receiving waste from a herd of 450 (McGrath & Mason 2004). The emission rates recorded during our study may be high compared with typical dairy farms. This is because discussions with the manager of the farm assessed in this work revealed that, due to the research-based operation of the site, the studied effluent pond periodically receives input of high-strength organic solids (such as milk waste) in addition to manure washdown from the milking shed. The study by McGrath and Mason (2004) was based on an observational technique to measure emission rates, which may have compromised the accuracy of their findings. Hence, the figure from Craggs et al. (2008) is used as the benchmark CH_4 emission rate for typical effluent ponds through the remainder of this manuscript. However, the figure reported by Craggs et al. (2008) is normalised to a 1000-m^2 pond and becomes 26 m^3 CH_4 d^{-1}, which is considered more representative of emission rates from an average New Zealand herd size (350).

Considerations for implementation of full-scale biofilters on dairy effluent ponds

The pilot-scale field biofilter demonstrated sustained and effective CH_4 removal from a dairy effluent pond over a period of 1.5 years. The filter's removal efficiency increased at the end of the trial indicating successful acclimation of methanotrophic bacteria within the engineered structure. Based on CH_4 oxidation rates achieved by the filter towards the final stages of the experiment, a full-scale filter volume of approximately 50 m^3 could effectively

offset emissions from a typical New Zealand dairy farm waste pond (about 26 m^3 CH_4 d^{-1}). Despite negligible oxidation rates over winter, the full-scale filter would operate at >90% removal efficiency. This corresponds to an annual offset of approximately 135 tonnes CO_2-e per year, assuming a GWP for CH_4 of 23 relative to CO_2 over 100 years (Melse & Van Der Werf, 2005).

An economic incentive would be needed to stimulate the uptake of biofilters by New Zealand dairy farmers. However, with the possible inclusion of agriculture in New Zealand's Emissions Trading Scheme there could soon be an economic driver to encourage the uptake of GHG mitigation technologies. Based on a carbon pricing of US$25 (NZ$32) per tonne, this study has shown that a total of US$3.4K (NZ$4.3K) per year could be offset by a biofilter treating dairy effluent pond emissions. If biofilters were installed on the effluent ponds of just half of New Zealand's 11 000 dairy farms, 0.74 million tonnes of CO_2-e could be offset annually, which would be worth an economic value of NZ$23 million (US$18 million). However, the capital cost of biofilters is also required to make an economic assessment. As there are no examples yet of full-scale systems in operation, the capital cost is difficult to estimate. However, this should decrease as demand for this technology grows. Melse and Van Der Werf (2005) reported a cost of US$25K (NZ$32K) for a biofilter treating a CH_4 flux of 170 CO_2-e tonnes/year (similar to the emission rate of a typical dairy effluent pond). A gas collection system needs to be included in the calculations, which costs approximately US$19K (NZ$24K) (NIWA 2010). The total capital expenditure for the cover/filter system, thus, comes to about US$49K. Hence, it could take approximately 15 years to pay-off the upfront cost of the filter, making the current system not economically viable. However, the economics may become more feasible by modifying the current biofilter design. Rather than deploying separate pond cover and filter units, an alternative approach is to merge them into a single structure overlying the pond surface, thereby reducing installation costs. The cover would ideally comprise a lightweight permeable material supporting the filter media. Moreover, by spreading the filter media over the entire surface of the pond, it may be possible to reduce filter thickness to such an extent that oxygen supply to the methanotrophs can be achieved solely by diffusion, eliminating the need for expensive ventilation devices.

Pond cover filter laboratory experiment

We are currently researching the feasibility of using a cover/filter system to oxidise CH_4 emissions from a dairy effluent pond. A preliminary laboratory cover/filter experiment using compost soil (which has been the most active media tested so far) has been running for approximately 9 weeks (Figure 12). For the first 4 weeks the filter was able to oxidise nearly 100% of CH_4 fed through it at a rate commensurate with typical pond emission rates (based on data from Craggs et al. 2008). Recently, the performance of the filter has declined and it is currently removing approximately 40% of the inlet load. This decline likely reflects an inherent instability in the compost soil's methanotroph population rather than a problem with the cover filter design. This is because the same drop in performance has recently been observed for the same soil in a chamber experiment, where a CH_4/air mixture is fed upward from the soil's base. Further monitoring of the pond cover experiment is needed and other soil types (including the volcanic soils) will be tested under this set-up. In addition, the cover filter design is about to be tested under field conditions. Nonetheless, the early data yielded from the pond cover laboratory experiment have indicated that this cover/filter design is potentially an effective approach to reduce the installation costs of biofilters targeting dairy effluent pond CH_4 emissions.

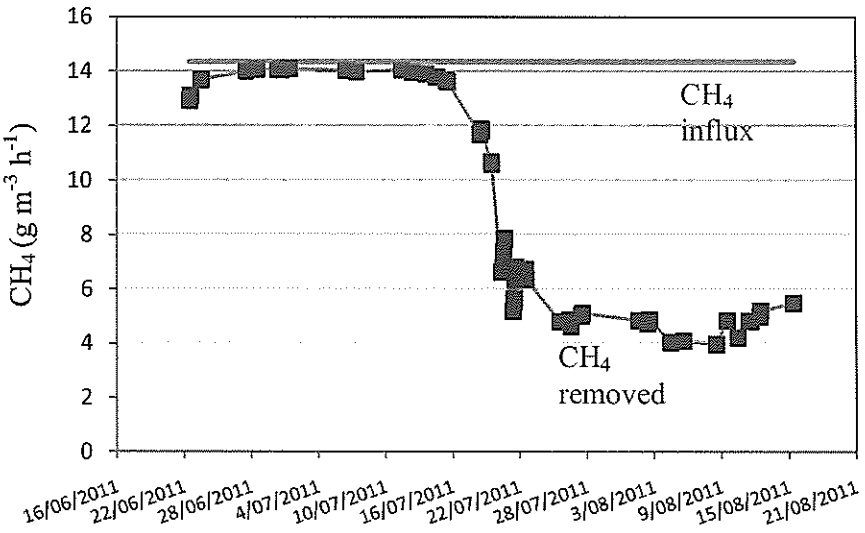


Figure 12 CH_4 oxidation rates in the laboratory pond cover filter.

2.5 Conclusions

- In a long-term laboratory experiment, volcanic pumice soils showed effective and sustained oxidation of high CH_4 fluxes (24 g CH_4 m^{-3} h^{-1}) with no intervention, which is promising for the development of biofilters as a low-cost and low-maintenance GHG mitigation technology.
- Preliminary molecular analyses have shown Type II methanotrophs (mainly *Methylocystis-sp.*-related) are the dominant organisms associated with the oxidation of high CH_4 fluxes.
- Laboratory experiments and modelling of data produced revealed that biofilters are unfeasible for treating CH_4 emissions from housed animals, as the quantity of CH_4 removed is too small in relation to the size of the filter required. However, the results showed that biofilters are a feasible approach to mitigate CH_4 emissions form typical dairy effleunt ponds.
- Our prototype field biofilter treating CH_4 emissions from a dairy effluent pond performed strongly (removed up to 16 g CH_4 m^{-3} h^{-1}) over a 1.5-year period.
- These results suggest that a 50-m^3 biofilter would effectively oxidise CH_4 emissions from a typical dairy effluent pond.
- The economics of the filter design can be improved by merging the filter and gas capture cover into a single unit (i.e. cover/filter).
- Preliminary results from a laboratory experiment have shown that this design is effective, with a soil cover/filter able to remove up to 100% of a CH_4 influx commensurate with emissions from a typical effluent pond.

2.6 Recommendations

- Longer term testing of the cover/filter design is needed to assess the stability of methanotroph populations within this biofilter design.

- Testing of various soils (including volcanic pumice and compost) is recommended to identify the most suitable substrate for use in a field cover/filter system (which will be tested in the coming year).
- Methods for deploying the cover/filter system on pond surfaces will be tested using various materials such as rubber-matting, straw and floating polystyrene cells.
- Understanding of key methanotroph strains in the studied soils will be undertaken to optimise the conditions favourable for their growth and activity, which ultimately controls filter performance.

3 Inhibition of rumen methanogenesis using clays

3.1 Background

Despite the high priority given to finding an effective, safe technology to reduce CH_4 emissions from ruminants, none has yet been found. Much research has focused on decreasing CH_4 emissions per animal through feed quality management, and the use of feed additives including oils and other chemicals (e.g., condensed tannins, monensin). One report from the UK (Bayaru et al. 2001) suggested that use of fumaric acid-based feed additives could result in marked reductions in CH_4 productions from cows, but this has not been confirmed. Another approach from Obihiro University, Japan, reported in the Japanese media, added a mixture of nitrate and cysteine to cows' diet, and demonstrated that this effectively reduced CH_4 emissions without affecting milk yield or flavour. Other studies, including some in New Zealand, are focussing on better understanding rumen microbial ecology as a route to designing targeted strategies; including developing vaccines for reduced rumen CH_4 emissions.

We have adopted a different approach by using natural and/or modified clays to attempt to reduce enteric CH_4 emissions. This idea may offer an effective way to suppress methanogen activity in the rumen based on geophagy (soil ingestion), emerging science and our accumulated knowledge of how soil–microbial interactions can influence microbial activity.

What is geophagy?

Geophagy is the deliberate consumption of soil and clay minerals by animals, including humans (Wilson 2003). For example, animals instinctively use clay minerals to cure wounds and soothe irritations (Carretero 2002); whereas for centuries humans have used clay minerals as gastrointestinal protectors, osmotic oral laxatives, and antidiarrhoeaics. Various hypotheses have been offered to explain the beneficial (and side) effects of geophagy, including: adsorption by clay minerals of toxins, bacteria and even viruses, detoxification of noxious or unpalatable compounds in the diet, alleviation of gastrointestinal upsets, the supplementation of mineral nutrients, and the reduction of excess acidity in the digestive tract (Wilson 2003).

What properties of soil or clay deposits are important?

The high specific surface area and sorption capacity of soils and clay minerals can partly explain why they can be effective in improving animal health. For example, clay minerals

and charcoal have been mixed with animal feed to reduce or eliminate animal disease caused by the presence of mycotoxins in feed (Huwig et al. 2001). Humans have also commonly consumed soil materials to control digestive problems (e.g., diarrhoea; Aufreiter et al. 1997).

Other properties are apparently also important. For example, in a study of New Guinean birds, Diamond et al. (1999) found that consumption of soil by frugivores appeared to provide protection against plant toxins in their diet. The effectiveness of the soil was attributed mainly to its high cation-exchange capacity, high content of cation-binding minerals and binding of large quantities of tannic acid and quinine. They hypothesised that these properties were effective in binding poisonous and/or bitter-tasting secondary compounds in ingested fruits and seeds.

The mineral content of soils is the principal property behind consumption of soil as famine food by humans (Aufreiter et al. 1997). It is clear from these examples that soil and clay materials can be effective in influencing the health and survival of animals and humans, through their influence on physiological and biochemical processes in the gastrointestinal tract. We contend that clays might also influence the microbial ecology of the rumen, thereby altering methanogen activity and enteric CH_4 emissions. This was our working hypothesis.

Clay additives are commercially available (e.g., Rumenite©) for dusting on pastures or as a feed additive, and are used to reduce rumen acidosis and scours, as well as improving animal weight gain. The primary ingredient, bentonite (a montmorillonite swelling clay), is thought to affect the role of protozoa in the rumen. Some evidence for reduced CH_4 emissions from using natural dolomites has recently been reported *in vitro* using meadow hay and barley grain as substrates (Váradyová et al. 2007). However, there are no reports of experiments using clays and with substrates in common use in New Zealand like grass or lucerne chaffage. If our hypothesis proves correct, the use of clay as a feed additive has the potential to be rapidly developed, tested, and introduced on the farm with no animal health issues.

Apart from their effects on the microbial ecology of the rumen (e.g., Rumenite©), clays could have a similar role to condensed tannins (CT). When fed to ruminants, feeds containing CTs, such as some *Lotus sp.,* sulla, sainfoin and the flowers of white clover, can reduce CH_4 emissions from lactating cows by up to 13% (Woodward et al. 2004). This is thought to be due to the CTs binding to plant proteins in the rumen, reducing protein degradation to ammonia, and increasing absorption of essential amino acids from the small intestine. This in turn increases milk and milk protein production and feed conversion efficiency. Other benefits include a reduction in bloat and amelioration of parasitism (Waghorn et al. 1987, Woodward et al. 2004). Therefore, reducing CH_4 emissions may not only divert energy into increased production by more efficient use of forage, but also benefit animal health. Reduction in methanogenesis may also arise from reduced hydrogen production, alternative hydrogen sinks or through a direct effect on methanogens (Woodward et al. 2004). For example, the apparent effectiveness of fumaric acid and derivatives in reducing CH_4 emissions (Bayaru et al. 2001) could be due to this organic acid acting as a hydrogen sink. Smectites can also reduce the amount of hydrogen produced during colonic fermentation (Frexinos et al. 1986, Arbeille et al. 1991).

Among the inorganic constituents of soil, it is the clay fraction (<2 μm equivalent spherical diameter) that is the most reactive. The large surface area and peculiar surface charge characteristics of clays predispose them to retain water and nutrients, and explains their large propensity for binding organic substances, including microbial cells and metabolites, in soil

(Theng & Orchard 1995). Clay and mineral surfaces are a preferred habitat for soil microbiota as a result of their relative enrichment by ions, water and organic matter. The nature of this interaction with microorganisms is complex, and conceptually challenging because both microbial cells and clay surfaces are negatively-charged. However, metal oxides in soil (e.g., Fe and Al oxides) have positively charged surfaces at pHs commonly found for most soils (i.e. 4.5–7), and these minerals can therefore attract and bind bacteria.

Clays and clay-size minerals can also promote the activity of microorganisms in their vicinity by keeping the pH of micro-habitats within the optimum range and by absorbing microbial metabolites that would otherwise be detrimental to growth. On the other hand, clay and mineral surfaces may accumulate toxic substances and immobilize extracellular enzymes, and therefore exert a depressive effect on microbial activity (Saggar *et al.* 1994). Perhaps of greater importance in terms of microbial survival is that clays can alter the architecture of soil aggregates by creating pores with necks of less than 6 μm in diameter. These pores are freely accessible to bacteria but not to their predators, notably soil protozoa. We can also modify clays to produce so-called "smart clays" with characteristics tailor-made for specific purposes (Hedley *et al.* 2007).

If clays can be shown to reduce rumen methanogenesis, and the mechanism(s) understood, it should then be possible to modify the surface of a clay that shows promise to optimise its efficacy in reducing enteric CH_4 emissions.

3.2 Objectives and hypothesis

- Test the efficacy of a range of clays for reducing CH_4 emissions *in vitro*.
- Examine key variables (clay type, loading (clay:forage dry weight ratio), surface properties, rumen pH, individual cow) to understand the mechanism(s) of clay effects on rumen methanogenesis and to optimise the efficacy of clays in reducing methane production.

Hypothesis

Addition of clays to the rumen will reduce the production of methane without adversely affecting animal metabolism.

3.3 Methods

Our project was primarily designed as an *in vitro* rumen digestion episode, where a known quantity and quality of herbage was added to artificial saliva and an sample of fresh cow rumen content, and incubated anaerobically at blood temperature (38°C) over a time period representing (ca. 7 h) one digestion cycle

McDougall's buffer (artificial saliva)

McDougall's buffer ($NaHCO_3$ 9.8g/L, $Na_2HPO_4 \cdot 12H_2O$ 9.3g/L, NaCl 0.47g/L, KCl 0.57 g/L, $MgCl_2$ 0.06g/L) was prepared the day before incubations to ensure components were completely dissolved. On the day of incubations CO_2 was bubbled through the prepared buffer to remove any dissolved oxygen.

Reducing agent

A solution of cysteine sulphide was prepared immediately before each incubation by mixing together 0.315 g cysteine hydrochloride , 48 ml water, 2 ml 1M NaOH , and 0.315 g sodium sulphide.

Clays tested

Table 4 lists the clays tested, and their sources.

Clay	Source
Bentonite (Calben)	New Zealand
Zeolite 1	New Zealand
Zeolite 2	New Zealand
Na-montmorillonite (Kunipia-F)	Japan
Acidified zeolite 1	Modified in house
Zeolite 3	New Zealand
Allophane	New Zealand
Kaolinite (PC1057R) (hydrothermally altered)	New Zealand
Halloysite	New Zealand
Allophane TK14	New Zealand
NPM4 (porous silica)	Japan
Na-montmorillonite (ZJNAM)	China
Na-montmorillonite (FHMNA)	China
Montmorillonite (Hudson)	Australia
Montmorillonite (P)	Australia
Layered double hydroxide (LDH)	Synthesised in house
Tonsill FF (210)	Germany
Halloysite-ODTMA	Modified in house
Acidified montmorillonite	Modified in house
Talc	China
SBEN400 (organoclay)	Japan
Kaolinite 10	New Zealand
Kaolinite 11	New Zealand
Kaolinite 12	New Zealand
Kaolinite 13	New Zealand
Kaolinite 14	New Zealand

As we had very little of the clay that, in preliminary experiments, showed some promise (Kaolinite PC1057R) and the original deposit could not be located, we were assisted by Dr Colin Harvey (GNS, retired) in locating and sampling other hydrothermally altered kaolinites (10–14) from sites in the central North Island.

Collection of rumen liquor

For all but two experiments where more than one cow was used, rumen liquor was collected fresh from the same fistulated cow (AgResearch), 2–3 hours after the onset of eating in the morning. Rumen liquor, strained through cheesecloth, was collected into a warmed thermos flask, and immediately taken back to the laboratory, where the pH of the liquor was measured. A 6-ml subsample was taken and immediately frozen for volatile fatty acid (VFA) analysis, A fresh sample of herbage taken from the paddock used by the cow was also analysed by feedTECH at AgResearch.

3.3.1 In vitro incubation procedure

In general, incubation experiments were conducted with three treatments (no clay, clay A, clay B) and five replicates of each treatment. Anaerobic incubations were conducted in 15 x 50 ml Schott bottles adapted for gas sampling by the insertion of a gas-tight septum, sealed into the lids with silicone. One set of five bottles was used as controls and the remaining bottles were used for five replicates of each of two treatments (added clay). Approximately 2.5 g (fresh weight) of frozen chopped and minced lucerne forage was weighed into each bottle. Approximately 15 mg of clay was added to each replicate of the clay treatments.

Incubations were carried out under anaerobic conditions at 38–39°C in a water bath with a shaker. The bottles containing forage +/- clay additive were placed in the water bath up to 90 minutes before addition of rumen liquor. The buffer solution was immersed in a 40°C water bath and CO_2 gas bubbled through it for 45 minutes. Each bottle was purged with CO_2 gas for 30 seconds before, and throughout, addition of 12 ml of buffer and 0.5 ml of the reducing agent. Bottles were quickly sealed and returned to the water bath as soon as possible. The reducing agent was allowed to work for about 15 minutes, then 3 ml of rumen liquor was pipetted into each bottle, with further purging with CO_2. Care was taken to ensure no entry of air into the bottles.

Gas from each bottle was sampled with a 25-ml syringe after incubation for 1, 3, 5 and 7 hours. On insertion of the syringe, gas production, causing a pressure rise in the bottles, forced the syringe plunger to a level where the pressure equalised with atmospheric pressure. The volume of gas produced was noted. Two equal aliquots (5 ml if possible) of sampled gas were taken and injected into evacuated 12-ml exetainers. Nitrogen gas was added to each exetainer to bring the total volume of gas in the exetainer to 25 ml. The exetainers were then stored (usually 1–3 days) for later CH_4 and hydrogen (H_2) analysis by gas chromatograph. At the end of each incubation, the pH of the rumen liquor plus forage, with or without clay, was measured. Then each bottle was sub-sampled for volatile fatty acid (VFA) analysis. Subsamples were frozen immediately and stored frozen until analysis.

Incubation with tannin

In one incubation, 15 mg of a condensed tannin extracted from *Lotus corniculatus* (Sivakumaran *et al.* 2006) was tested with and without clay (kaolinite PC1057R).

3.3.2 Supplementary analyses

Volatile fatty acids

Volatile fatty acid (VFA) content of the initial rumen liquor, and the 15 incubated liquor samples was determined at Massey University (Biotechnology) after first centrifuging the subsamples at 13000 rpm for 5 minutes, then filtering through a 0.45-µm membrane filter, before the injection of 25 µl onto a Dionex Ion Chromatograph column.

Gas analysis

Methane concentration was analysed using an automated Shimadzu GC2010 gas chromatograph with a flame ionisation detector, at 250°C, with a detection limit of 0.06 ppm (Hedley et al. 2005). Hydrogen gas concentration was analysed by a Reduction Gas Detector at NIWA, Greta Point, with a detection limit of 1 ppm.

3.3.3 *In vivo* experiment

An *in vivo* experiment was conducted (November–December 2008) using sheep in AgResearch's calorimeter rooms. The experiment was conducted after receiving approval from the AgResearch Animal Ethics Committee, and with guidance from Dr Stefan Meurtzel, AgResearch. After delivery of the clay "cocktail" (60g clay/kg lucerne chaffage DM, comprising 40g acidified kaolinite (pH 5.8) + 20g Zeolite 1) a palatability trial was first conducted to ensure the sheep ate all the clay, with no refusals. No problems were encountered, with 100% success rate. The main experiment in the calorimeter rooms was subsequently conducted over 9 days with 8 ewes —4 controls in which ewes were fed lucerne chaffage only; and 4 test animals which were fed lucerne chaffage +clay.

Sheep were used in the *in vivo* experiment because cattle were not available, but also because the greater expense that would have been incurred by using cattle was considered prohibitive at this stage of the work.

Our choice of clay was based on our phase one work, which indicated a kaolinite with acid surface properties would be most likely to be effective. Insufficient amounts of the successful clay (kaolinite PC1057R) forced us to seek an alternative, and we chose a kaolinite from China with similar properties to kaolinite PC1057R. Time constraints to meet the allocated date for use of the calorimeter prevented us from testing this clay *in vitro* beforehand.

3.4 Results

There were three main phases to the project. First, a range of clays was tested for ability to reduce methane production under standard conditions as described. Second, attempts were made to optimise effects of clays on methanogenesis using the most promising clays, including "new" clays sampled as potential replacements for the very promising clay (kaolinite PC1057R) that was in short supply. Third, pure methanogen cultures were developed for a future investigation of the mode of interaction of methanogens and clays.

3.4.1 Phase one

All experiments conducted in phase one used rumen liquor from the same cow, fed a grass diet.

Gas production

Total gas (mainly CO_2) and CH_4 production during the 7-hour incubations were used to assess the efficacy of different clay treatments.

Total gas production showed a characteristic pattern of rapid accumulation during the first 3 hours followed by a slow decline in the rate of gas accumulation. This pattern likely reflects the sequence of substrate depletion by methanogen; initially labile constituents are consumed while later more recalcitrant fractions were digested. The total gas production and patterns of production varied, but in general maximum gas production occurred after 5h incubation. The major constituent of the gas was carbon dioxide.

The zeolite treatment (clay dose at 3% of lucerne dry weight) showed a significantly lower CH_4 production than the (Ca, Mg) montmorillonite-treated and control samples in one early incubation, but this could not be reproduced in subsequent incubations. In contrast, a large and significant decrease in CH_4 production (54%) occurred with the kaolinite-treated samples (Figure 13). The strong effect of kaolinite in reducing CH_4 production was confirmed in subsequent incubations. It appeared that when the initial pH was in the range 5.9–6.2, a reduction in CH_4 production was more likely.

In a further experiment with the same clay, but including a condensed tannin for comparison, strong reductions in CH_4 production were evident for all treatments (Figure 14).

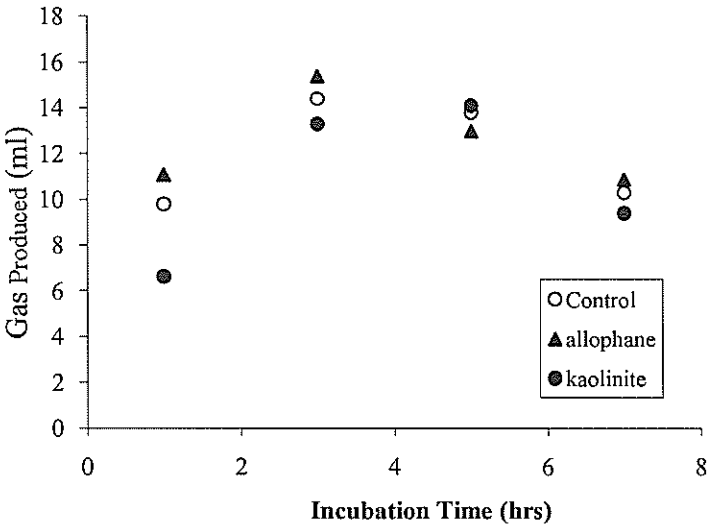
This surprising result indicated that a reduction in CH_4 production of about 65% was common to all treatments. This suggested a common mechanism may have been operating that was dependent more on surface charge characteristics than on surface area, as doubling the clay content made no difference. This is consistent with the incubation shown in Figure 13, where the clay with the much larger surface area (i.e., allophane) was ineffective in reducing CH_4 production. It also suggests that the effectiveness of the condensed tannin, which Woodward et al. (2004) found reduced rumen CH_4 emissions from lactating dairy cows by 13 %, was not primarily due to its structural chemistry.

Furthermore, considering all 15 incubations in phase one, only four incubations using kaolinite PC1057R and zeolite 1 (see Table 4) showed moderate to strong reductions in CH_4 emissions. Of these, three of the "successful" incubations were in spring or early summer when the cow was feeding on vigorously growing pasture with high or very high herbage soluble sugars and starch. Furthermore, the initial rumen liquor pHs were all between 6.0 and 6.1. One incubation, with a higher initial rumen pH of 6.38, also gave a significant but smaller reduction (the effective clay was zeolite 1, Table 4). Despite this initially positive result, subsequent incubations did not produce repeatable results.

Hydrogen analyses were made on gases collected when a significant reduction in CH_4 production was recorded (results not shown). Hydrogen concentrations over 7 hours rose from zero at T=0 to about 4000 ppmv for clay treatments, whereas for controls concentrations rarely exceeded 2000 ppmv. These results suggest that the effective clay is in some way inhibiting methanogen activity rather than microbial production of hydrogen.

Volatile fatty acids were analysed in incubations that demonstrated reduced CH_4 production. Acetate/proprionate ratios indicated that fermentation was proceeding normally (results not shown). The ratios generally varied between 2.0 and 2.6.

a)



b)

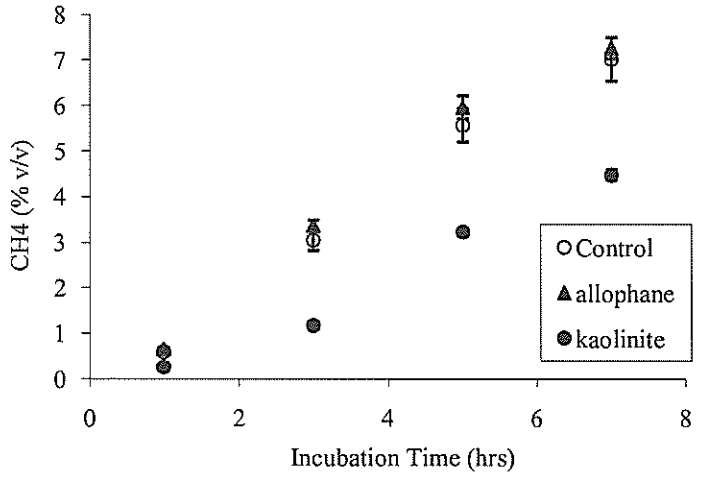
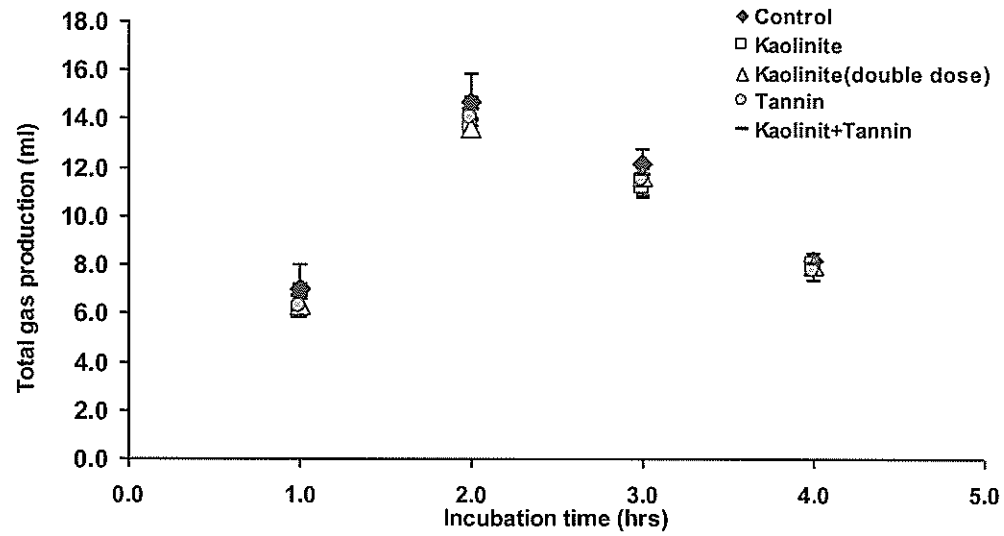


Figure 13. A comparison of the effect of allophane and kaolinite (PC1057R) additives to an in vitro rumen-lucerne incubation on (a) total gas production and (b) CH_4 production. Initial rumen pH, 6.03.



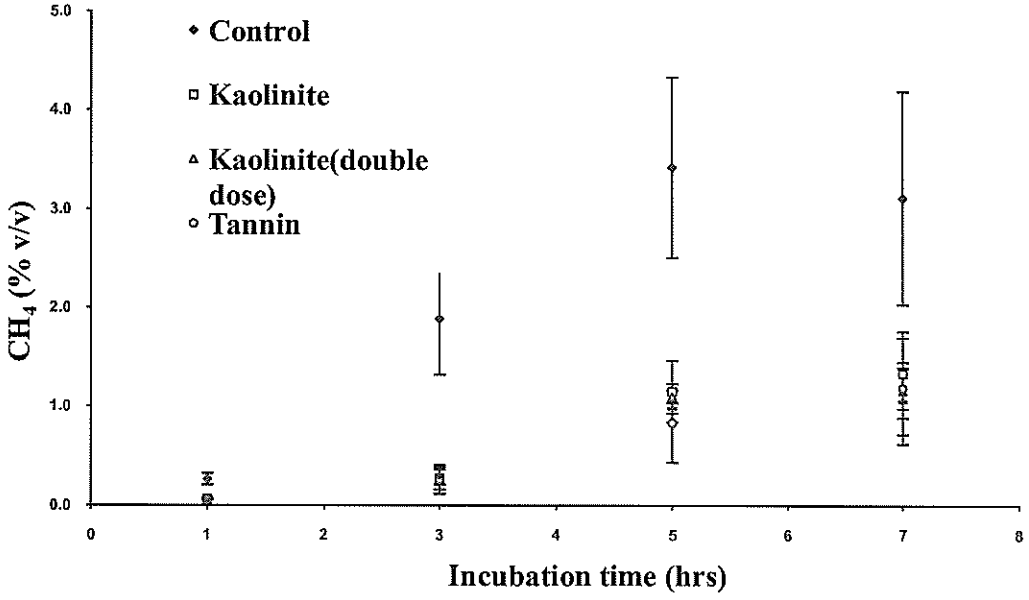


Figure 14. Effect on total gas and CH_4 production of kaolinite PC1057R (15 and 30 mg) with and without condensed tannin, and tannin alone; initial rumen pH, 6.09.

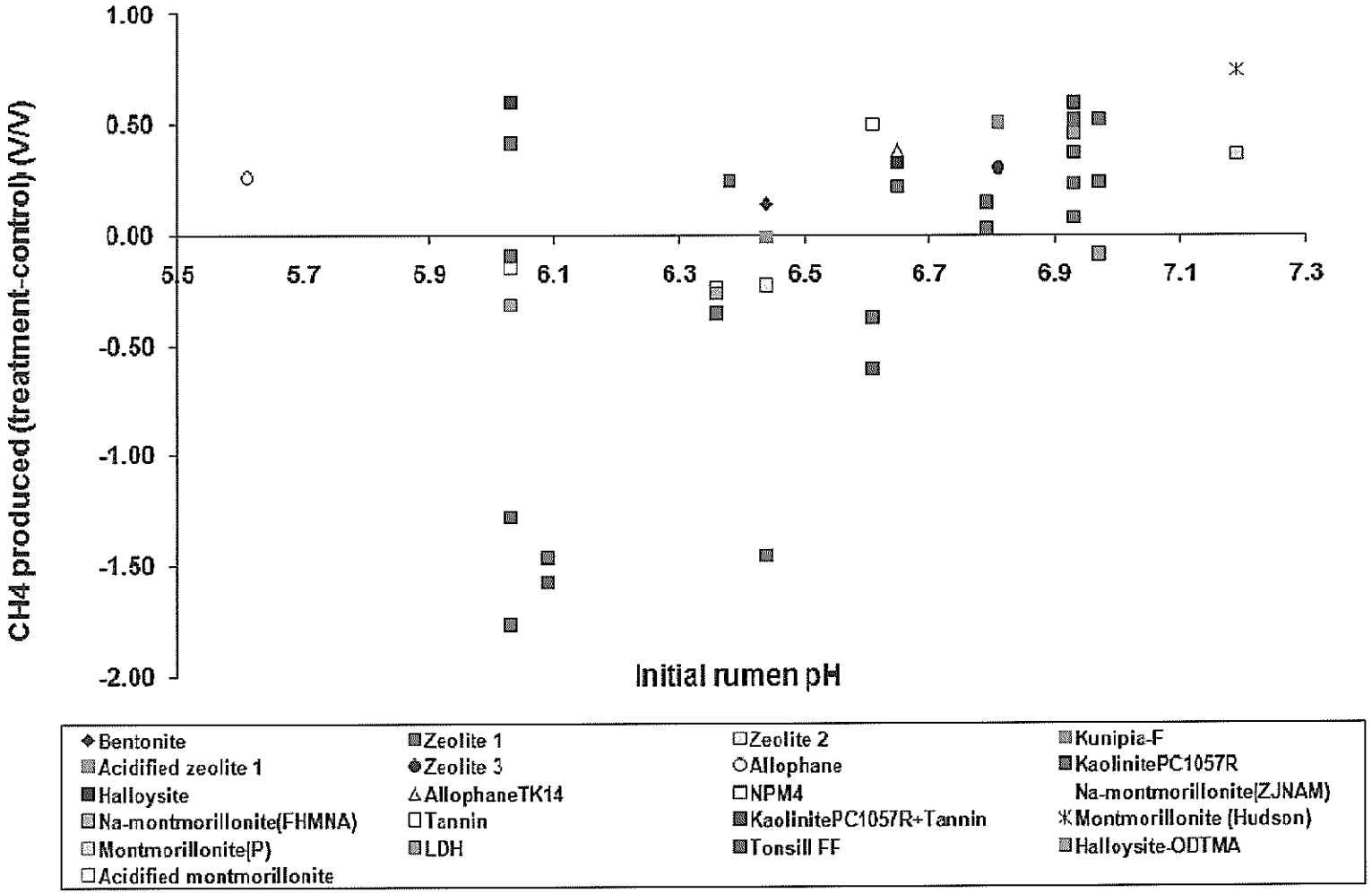


Figure 15. Summary of reduction on CH_4 production compared to controls for tannin and 19 different clays tested in relation to initial rumen pH.

3.4.2 Phase two

In phase two, a series of *in vitro* experiments were designed to probe mechanisms of the action of clays and to optimise effects of the most promising clays identified in phase one. During this phase, an animal experiment using sheep was also undertaken at AgResearch.

We undertook *in vitro* incubations and, while the same cow was used in all but two experiments, we included experiments when the original cow was being fed hay. Despite the fact that our most successful incubations occurred when the cow was fed lush grass, introducing clay into the diet would most easily be achieved when feeding supplementary feed such as hay or silage.

Do clays in combination amplify methane reduction?

Two of the most promising clays (kaolinite PC1057R and zeolite 1) were tested in combination, with the cow fed a hay diet. A moderate effect (30 % reduction in CH_4 production over 7 hours) was observed (Figure 15), whereas no effect was observed with only zeolite. The initial rumen pH was 6.03.

Effects of feed quality and rumen pH

Three replicate experiments were undertaken to investigate how feed quantity and rumen pH affected enteric CH_4 production. The first experiment used the original kaolinite, and involved sampling the rumen contents three times (T1, T2, T3) during the day. The cow had not eaten since the previous day. The initial pH of the rumen liquor was: T1, 7.07; T2, 7.09; T3, 6.84. Incubations were carried out for 6h, 4h and 4.4 h for T1, T2 and T3, respectively. The total gas produced was measured twice during the incubation periods. In all incubations, gas was sampled after one hour (no measurable total gas production), and at the end of each incubation when between 17.0 and 23.0 ml had been produced. Analyses showed a significant reduction in CH_4 production in all three incubations: T1, ca. 60%; T2, ca. 50%; T3, ca.30%.

In a replicate experiment, which took place three weeks after the first, each of the three incubations was 4 h duration. As in the previous experiment, initial rumen pHs were higher than thought optimal (pH 6.0–6.4): at T1 (10.05am) 6.90; at T2 (1.05pm) 6.99, and at T3 (3.20pm) 6.78. With the exception of T3 which showed a 38% reduction in CH_4, the observed reduction was much smaller than in the previous experiment: T1, 42%, and T2, 17%. .

A third experiment attempted to repeat these results, but no reduction in CH_4 production was observed. This result appears likely to have been influenced by two factors that differed from the first two experiments. First a different cow was inadvertently sampled, while all previous experiments had used the same cow. Second, the timing of the rumen sample collection and feed schedule was different from that used in all previous experiments. These results suggest that both animal-specific and management (feed) factors may influence the efficacy of clays in reducing methane production.

The first two experiments were particularly interesting because the initial rumen pHs were much higher than those we previously noted as most favourable for significant effects to be observed. This series of experiments did, however, suggest we undertake experiments

specifically aimed at determining how variable our results might be if more than one rumen was used as a source of liquor.

Performance of "new" clays

Five hydrothermally altered clays collected from the central North Island (because only gram quantities of the original clay remain) were tested. Overall, methane reduction was smaller than those reported for kaolinite PC0157R.

Four experiments were carried out at Landcare Research, and one at AgResearch using their automated analysis system. In all cases, the original kaolinite was included as a control, but to date no clear evidence has emerged that the new clays are effective in reducing CH_4 production. In the AgResearch experiment, which showed no effect from any of the clays tested, including the original kaolinite, no data were available on initial rumen pH from which the liquor was taken. Failure to demonstrate a strongly positive result for any of the "new" clays has limited progress towards understanding how clay limits CH_4 production when a positive result is obtained.

Can surface hydrophobicity influence methanogen activity?

A chance observation that the surface of archaea cells is hydrophobic prompted us to include an experiment using talc (a natural hydrophobic clay). Despite the cow being grass-fed and rumen samples being taken mid-morning (conditions under which methane reduction had been demonstrated in previous experiments), the initial rumen pH was 7.38, the highest pH yet recorded in our experiments. This was well outside the optimal range (ca.6.0–6.4) for methanogens and not surprisingly, no evidence of methane reduction was obtained, despite good replication.

Is rumen variability a major controlling factor?

Following our earlier experiments that showed feed quality, quantity and initial rumen pH were important variables, we undertook an experiment using two "new" cows (jersey-crosses). Their rumens were sampled at the same time (at about 11am), and one clay was used (the original kaolinite) for each incubation, with accompanying controls with no clay added.

The initial rumen pHs were 5.82 and 5.88, and the rumen liquor was thick, green and frothy indicating high microbial activity. Total gas production was very high and consistent among replicates, even after one hour. Apart from the rumen pHs, these conditions are consistent with those encountered previously when strong effects were recorded with this clay. Despite this, no reduction in methane concentration was observed.

3.4.3 Phase three

In phase three we attempted an *in vivo* (animal) experiment. We also set up a laboratory to enable anaerobic culture of methanogens to prepare for future experiments using pure cultures of methanogens to probe clay-methanogen interactions, and how these might influence the efficacy of clays in reducing CH_4 production.

The animal experiment was conducted in the calorimeters at AgResearch, and involved 8 ewes; four ewes were fed chaffage plus clay, while the controls were fed chaffage only. Gas

measurements began 6 days into the trial, after an initial palatability trial to ensure all feed offered was consumed. Rumen contents were collected for the analysis of NH_4 and short-chain fatty acids.

Results of the animal experiment are shown below (means ± standard deviation).

Table 5. *In vivo* methane and carbon dioxide production in the presence and absence of clay

Treatment	Methane produced (g/kgDMI)	CO_2/CH_4 ratio
Clay	20.5 ± 0.92	15.3 ± 0.71
Control	19.7 ± 0.60	15.9 ± 0.33

Several possible reasons may explain the above results:

- Our choice of clay was based on our phase one work that indicated a kaolinite with acid surface properties would be most likely to be effective. Insufficient amounts of the successful clay (kaolinite PC1057R) forced us to seek an alternative, and we chose a kaolinite from China with similar properties to kaolinite PC1057R. Time constraints to meet the allocated date for the animal experiment prevented us from testing this clay *in vitro* beforehand.
- We had not previously undertaken *in vitro* experiments using chaffage.
- The clay loading chosen was based on our experience *in vitro*, and may not necessarily be optimal for the rumen.
- What may be effective in reducing methane emissions in cows might not also work for sheep.
- There could have been adaptation early in the trial to the clay additive; this could not be determined from our results because the gas measurements started about 6 days into the trial (and after the palatability phase). In hindsight, measurement of gas emissions should have started from day one.

The two positive outcomes were, first, that the clays presented no perceptible animal health or palatability problems. Second, the low standard deviations indicate that we could expect to detect even a small effect if it had occurred.

The main conclusion from this experiment was that we need to understand more about mechanisms from further *in vitro* experiments before attempting further expensive *in vivo* experiments.

In the second part of phase three, we engaged the help of an experienced rumen microbial ecologist/microbiologist (Dr Keith Joblin) to set up a methanogen laboratory. Our intention

was to culture and grow rumen methanogens for *in vitro* experiments with pure clays to better understand the methanogen-clay interaction. The laboratory was set up and rumen methanogens were cultured, grown and stored at -80°C. Though time constraints to date have prevented any pure culture-clay experiments being undertaken, cultures of pure methanogens are available if needed for further experimentation.

3.5 Conclusions

- Our hypothesis was partially confirmed, as two of the 26 clays tested did cause significant reductions in *in vitro* CH_4 emissions in some anaerobic incubations. However, our understanding of the conditions required for the clays to work is still far from complete. The clay giving the most consistent result had been exposed to hydrothermal acid washing. This removes aluminium and therefore increases holes (surface area) substantially, and creates positive charge (protons). These two changes could enhance the attachment of microbial cells to the clay surface (as microorganisms are negatively charged). Hydrogen analyses showed that methanogens rather than hydrogen-producers were affected by the clay.
- Attempts to demonstrate the efficacy of the "new" clays with similar properties to the one showing most promise have so far been elusive. It appears that the original kaolinite has a unique property that causes it to inhibit methanogen activity. If this property could be identified, we might better understand the mechanism involved and therefore make progress towards a viable mitigation strategy using clay.
- Initial rumen pH appeared to influence the efficacy of clay in reducing CH_4 emissions (Figure 15), but the mechanism is not yet clear. If clay is effective only over a narrow pH range, then this suggests adding clay to cows' feed may not be effective *in vivo* over the full digestion cycle.
- One *in vivo* experiment with sheep appeared to indicate no effect of clay, but because the experiment was performed with variables that were different from previous *in vitro* experiments, and the lack of an *in vitro* control using these variables, no conclusive results could be produced.
- Further research is needed to indicate why results are so variable. Conclusive results are likely to be demonstrated most readily in experiments using clays and cultures of pure methanogens.

3.6 Recommendations

Further research is needed to determine the mechanism of inhibition of methanogen activity by some clays, for example, by investigating clay-methanogen interactions using pure cultures and the clay showing most promise.

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