Understanding methanotroph ecology in a biofilter for efficiently mitigating methane emissions

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Abstract

In New Zealand, the majority of the greenhouse gas (GHG), methane (CH_4) emissions are from the agriculture sector (enteric fermentation, manure management) and the remainder from solid waste disposal, coal mining and natural gas leaks. A soil-based biofilter made from volcanic pumice soil (isolated from a landfill in Taupo, New Zealand) and perlite has been tested and promoted to mitigate high concentrations (3 300 ppm – 100 000 ppm) of CH_4 emissions from a dairy effluent storage pond. This soil-perlite mixture exhibited excellent physical (porosity, water holding capacity and bulk density) characteristics to support the growth and activity of an active methanotroph community. Methanotrophs comprise a diverse group of aerobic alpha and gamma proteobacteria (type I and type II methanotrophs, respectively) that are present naturally in soils where CH_4 is produced. However, there is little information on the methanotrophs community structure, population diversity and abundance in this soil-based biofilter. Understanding the activity of these diverse genera under varying soil conditions is essential for optimum use of biofiltration technology, and is the main aim of this thesis.

This thesis describes a study to use molecular techniques (PCR, quantitative PCR, T-RFLP and molecular cloning) (Chapter 3) to reveal the population dynamics of methanotrophs (type I, type II and various genera – *Methylobacter/Methylomonas/Methylosarcina*, *Methylococcus* and *Methylocapsa*), in order to build a more efficient CH_4 biofiltration system. Methanotroph population dynamics in two fundamentally different prototypes of volcanic pumice soil biofilters – a column and a floating/cover biofilter studied are presented in Chapters 4 and 5.

The column biofilter study (Chapter 4) examined the performance of a previously used acidic soil-biofilter medium that was further acidified from pH 5.20 ± 0.20 to 3.72 ± 0.02 by H_2S present in the biogas (from the dairy effluent pond).. The more acidic soil biofilter medium (volcanic pumice soil and perlite, 50:50 v/v) was reconstituted with optimal moisture content (110% gravimetric dry wt or ~ 60 % WHC) and achieved a maximum CH_4 removal rate of 30.3 g m^{-3} h^{-1}. In addition, the population of *Methylocapsa*-like methanotroph increased by 400 %, demonstrating

the ability of these soil microorganisms to adapt and grow under acidic pH conditions in the biofilter. The results from this study indicated that (i) when primed with CH_4, a soil biofilter can effectively regain efficiency if sufficient moisture levels are maintained, regardless of the soil acidity; (ii) changes in the methanotroph population did not compromise the overall capacity of the volcanic pumice soil to oxidise CH_4; and (iii) the more acidic environment (pH 3.72) tends to favour the growth and activity of acid-loving *Methylocapsa*-like methanotroph while being detrimental to the growth of the *Methylobacter* / *Methylococcus* / *Methylocystis* group of methanotroph.

In the floating biofilter (Chapter 5), original acidic soil biofilter medium (pH 5.20) as used in column study was assessed to remove CH_4 from the effluent pond surface for a period of one year (December 2013 to November 2014). Field evaluation was supported with a concurrent laboratory study to assess their CH_4-oxidising capacity, in addition to identifying and comparing the methanotroph community changes in the soil when exposed to field conditions. Results indicated that (i) irrespective of the season, the floating biofilters in the field were removing 67 ± 6% CH_4 throughout the study period with a yearly average rate of 48 ± 23 g CH_4 m^{-3} h^{-1}; however, the highest CH_4 removal rate achieved was 101.5 g m^{-3} h^{-1} CH_4, about 300 % higher than the highest CH_4 removal rate by the acidified column biofilter (Chapter 4); (ii) the acidity of the field floating biofilters increased from a pH value of 5.20 to 4.72, but didn't suppress the genera of methanotrophs (particularly *Methylobacter*/*Methylosinus*/*Methylocystis*); (iii) the laboratory-based floating biofilters experienced biological disturbances with low and high CH_4 removal phases during the study period, with an yearly average CH_4 oxidation removal of 58%; and (iv) both type I and type II methanotrophs in the field floating biofilters were more abundant, diverse and even compared with the methanotroph community in the laboratory biofilters. This study has demonstrated the ability of the floating biofilters to efficiently mitigate dairy effluent ponds emissions in the field, without requiring any addition of nutrients or water; however, during very dry conditions, occasional addition of water might be needed to keep the biofilter bed moist (≥ 23±4 % dry wt).

Earlier New Zealand studies and the current studies (Chapters 4 and 5) were based on the use of a particular volcanic pumice soil as biofilter medium. However, the limited availability of volcanic pumice soil and associated transportation costs limited the wider application of this technology within New Zealand and internationally. This necessitated the assessment of other farm soils and potentially suitable, economical, and locally available biofilter materials that could potentially be used by the farmers to mitigate CH_4 emissions (Chapter 6). The potential biofilter materials, viz. farm soil (isolated from a dairy farm effluent pond bank area), pine biochar, garden waste compost, and weathered pine bark mulch were assessed with and without inoculation with a small amount of volcanic pumice soil. All materials supported the growth and activity of methanotrophs. However, the CH_4 removal was high (> 80%) and consistent in the inoculated - farm soil and biochar, and was supported by the observed changes in the methanotroph community. The CH_4 removal was further enhanced (up to 99%) by the addition of nutrient solution. Field evaluations of these potential materials are now needed to confirm the viability of these materials for recommending them for use on farms.

Chapter 7 summarises the molecular results from all the above studies, and describes the future studies. Molecular techniques indicated that a very diverse (Shannon's diversity, $H' = 3.9$ to 4.4) group of type I and type II methanotrophs were present in the volcanic pumice soil, which assisted the biofilter materials to perform under varying abiotic conditions. Many novel species and strains of type I and type II methanotrophs were also identified in these soils. For long-term, low cost and efficient and stable CH_4 removal, the presence of an even and abundant population (of type I and type II methanotrophs) is however essential. Nevertheless, biofilters offer much promise for mitigating CH_4 emissions from dairy ponds, piggeries, and landfills, thereby contributing to the lowering of emissions of this potent greenhouse gas to mitigate the effects of climate change.

Dedication

This work is dedicated to my parents (Farzana, Mazheruddin), siblings (Imad, Fuad, Hafsa and Dr Iqra), wife (Abeer) and my mentors (Drs Surinder, Kevin and Bernd) for their encouragement and unending support throughout my PhD.

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Publications and Presentations

Peer reviewed International scientific journals

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2. **Syed R**, Saggar S, Tate KR and Rehm BHA (2016). *Assessing farm soil, biochar, compost and weathered mulch to mitigate methane emissions*. Applied Microbiology & Biotechnology 100:9365-9379.
3. **Syed R**, Saggar S, Tate KR, Rehm BHA and Berben P (2017): *Assessing the performance of floating biofilters for oxidation of methane from dairy effluent ponds*. Journal of Environmental Quality 46:272-280.

Conference/Workshops/meetings

4. **Syed R**, Saggar S, Tate K and Rehm B (2014). *Characterizing a field methane oxidation biofilter – treating farm CH_4 emissions from Massey No. 4 dairy pond*. (Poster presentation) In: Nutrient Management for the Farm, Catchment and Community, (Eds LD Currie & CL Christensen). http://firc.massey.as.nz/publications.html Occasional Report No. 27, Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand, 11p.
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List of Abbreviations

µ	Micro
AN	Avogadro's number
ANOVA	Analysis of variance
B	Boron
bp	base pair
C	Carbon
$CaCl_2$	Calcium chloride
$CaCl_2.2H_2O$	Calcium chloride dihydrate
CH_3OH	Methanol
CH_4	Methane
CMM	Coal mine methane
CO_2–e	Carbon dioxide equivalent
$CoCl_2.6H_2O$	Cobalt chloride hexahydrate
COD	Chemical oxygen demand
Cp	Crossing point
$CuCl_2.2H_2O$	Copper Chloride dihydrate
DGGE	Denaturing gradient gel electrophoresis
EBRT	Empty bed residence time
EC	Elimination Capacity
EDTA	Ethylene diamine tetra acetic acid
EEA	European Environment Agency
E_H	Evenness
EPA	Environmental protection agency
EPS	Exopolymeric Substances
FADH	Formaldehyde dehydrogenase
FAM	6-carboxy fluorescein
FDH	Formate dehydrogenase
$FeSO_4.7H_20$	Iron sulphate heptahydrate
FID	Flame ionisation detector
FR	Francop
GC	Gas Chromatography
Gg	Giga gram
GHG	Greenhouse gas
GWP	Global warming potential
H`	Shannon's diversity
H_2S	Hydrogen sulphide
H_2SO_4	Sulphuric acid
H_3BO_3	Boric acid
HCHO	Formaldehyde
HDPE	High density poly ethylene
IPCC	Intergovernmental panel for climate change
K_2SO_4	Potassium sulphate
KCl	Potassium chloride
KH_2PO_4	Potassium mono phosphate
KJ	Kilo Joule
KNO_3	Potassium nitrate
Kt	Kilo tonne
LFG	Landfill gas

MBC	Microbial biomass C
MBN	Microbial biomass N
MDH	Methanol dehydrogenase
MfE	Ministry for Environment
$MgSO_4.7H_2O$	Magnesium sulphate heptahydrate
MMO	Methane monooxygenase
MMT	Million metric tonne
$MnCl_2.4H_2O$	Manganese chloride tetrahydrate
MOB	Methane oxidising bacteria
MSW	Municipal sewage waste
MVA	Mine ventilation air
Na_2HPO_4	Disodium phosphate
Na_2Mo_4, $2H_2O$	Sodium molybdate dihydrate
NCBI	National Centre for Biotechnology Information
$NiCl_2.6H_2O$	Nickel chloride hexahydrate
NMVOC	Non-methane volatile organic compounds
NPK	Nitrogen phosphorous potassium
PADS	Passive air diffusion system
PBS	Phosphate buffer saline
PCA	Principal Component Analysis
PCR	Polymerase Chain reaction
PHA	Polyhydroxy alkanoates
PLFA	Phospholipid-derived fatty acids
pmoA	Particulate methane monooxygenase subunit A
PMOB	Passive methane oxidation biocover
PVC	Poly vinyl chloride
qPCR	Quantitative Polymerase Chain reaction
Rpm	Revolutions per minute
RSKB	Rashad Surinder Kevin Bernd
RuMP	Ribulose monophosphate
Taq	*Thermus aquaticus*
TBE	Tris Borate EDTA
TCD	Thermal conductivity detector
TCE	Trichloro ethylene
T-RFLP	Terminal-restriction fragment length polymorphism
T-RFs	Terminal-restriction fragments
UV	Ultra violet
VOC	Volatile organic compounds
VS	Volatile solids
WHC	Water holding capacity
$ZnSO_4.7H_2O$	Zinc sulphate heptahydrate