Fluorescent Microscopy of Fecal Samples From Five Orders of Mammals to Investigate Methanogen Presence

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Abstract

Animal wastes have been studied as potential fuel sources via bio-digestion. Feces have also been used to inoculate biodigesters. Biodigesters are a waste solution which converts organic matter into a fuel source such as methane. They utilize microbes to breakdown organic matter into substrates, which are then converted into methane as fuel. The final step's productivity depends on the methanogen content of the biodigester. This study serves to examine the feces of captive animals for use as a digester inoculum. The aim was to assess the potential of different feces for methanogen contribution through literature and sample analysis via fluorescent microscopy looking for F420 autofluorescence. Coenzyme F420 is a fluorescent coenzyme involved in redox reactions in methanogens and has been used in their identification and observation. The samples were from herbivores in the orders Rodentia, Lagomorpha (rabbits), Perissodactyla (odd toed ungulates), Artiodactyla (even toed ungulates) and Diprotodontia (some marsupials). The project thus far has been impeded by two dilemmas: the scarcity of methanogens and obscuring from foliage. Due to these contrasting problems, the aim has been minimizing foliage obscurity while retaining enough methanogen presence. It was observed with a series of dilutions that a 1:10 dilution reduced foliage impact.

Introduction

This review explores the available literature in search of ways to begin looking for feces that would be well suited to improve the inoculation of anaerobic digesters.

Biodigesters are a waste solution used in a variety of countries and that show promise worldwide. Their benefits extend to both developed and less-developed countries as not only a waste solution, but also as a fuel source that in the US could produce $77 million (Cornejo & Wilkie, 2010; Wilkie, 2008).

Methanogen presence is one of the limiting factors in many starting anaerobic digesters (Wilkie, 2005). To fix this deficiency, often an anaerobic digester is inoculated with feces with the intent of adding anaerobes and bacteria such as Firmicutes and Bacteroides to breakdown and digest waste and process it much like they would in a gut microbiome (Sun et al., 2015).

With the methanogen content being a pivotal step, one would desire an inoculum with high methanogen content to use as a starter for a new digester.

Methanogen presence in the vertebrate gut microflora of a variety of animal species is around 0.5% to 3% of the overall microflora (Lamendella et al., 2011; Sorlini et al., 1988; St-Pierre & Wright, 2012a, 2012b).

Methanogens historically have been visualized via Coenzyme F420 autofluorescence when observed under fluorescent microscopy (Doddema & Vogels, 1978). This has been used reliably in testing for fecal contamination among other uses.

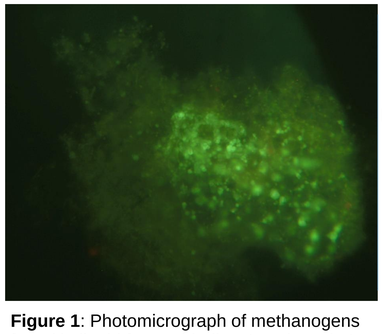

Figure 1: Photomicrograph of methanogens found in liquid from a biodigester, viewed at 400x magnification.

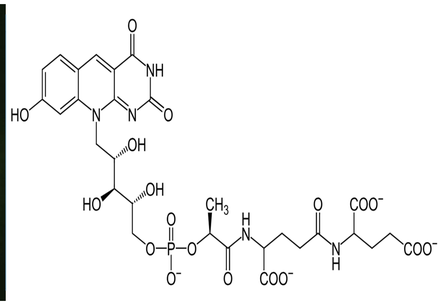

Figure 2: Coenzyme F420

Objective

The primary objective of this study was to analyze a variety of feces to examine their potential for inoculating biodigesters.

Methods

The methods included analysis of relevant figures and data in the study along with use of critical reasoning in order to draw similarities between animals in the study and those obtainable in the local area.

Fecal samples were collected from the local area from five mammalian orders including, Perissodactyls, Artiodactyls, Rodents, Lagomorphs, and Diprotodonts.

The samples were viewed using fluorescent microscopy at 420 nm to observe autofluorescence of Coenzyme F420.

Photomicrographs were taken using a SPOT Insight 2 Mp CCD Scientific Color Digital Camera System.

F420 autofluorescence was used as a way to scan the feces for the presence of methanogens.

Fecal Samples were viewed both as simple wet mounts and as dilutions.

Dilutions were also viewed as a whole and divided into supernatant and pellet after centrifuging.

Results

Table 1: Study Animals Methanogenicity Subset from Hackstein 1996

Animal	Relative Animal in Hackstein study	Methane Average nmol/g/h	Methane Max nmol/g/h	M/N
Sheep	*Capra hireus*	4230	10000	M
Goat	*Capra hireus*	4230	10000	M
Domestic Donkey	*Equus przewalskii caballus*	118	286	M
Wallaby	*Wallabia rufogrisea*	185	283	M
Rabbit	*Oryctolagus cuniculus*	42	227	M
Llama	*Lama g. guanicoe*	73	202	M
Patagonian Cavy	*Dolichotis patagonum*	25	145	M
Hamster	*Mesocricetus auratus*	9	29	M
Grey Squirrel	*Sciurus carolinensis*	0.01	4	N

The microscopy showed that in most species there was significant fecal foliage that could be reduced by a 0.1ml of feces to 1.4ml of water dilution.

Most of the samples from Petting Zoo Ocala showed negligible methanogen levels.

Squirrels from Gainesville showed considerably higher methanogen levels under the microscope than did the other animals.

The literature favored medium to large ruminants from Perissodactyla.

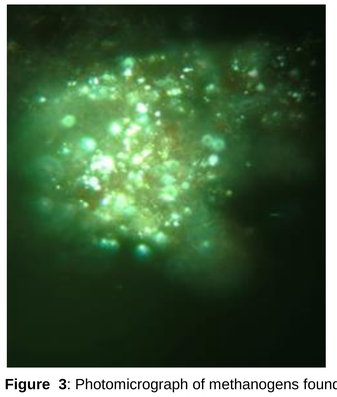

Figure 3: Photomicrograph of methanogens found in undiluted squirrel feces at 400x magnification.

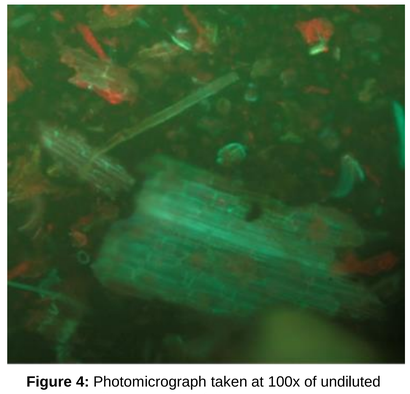

Figure 4: Photomicrograph taken at 100x of undiluted goat feces illustrating high foliage presence.

Conclusions

Despite the microscopy results showing a greater density of methanogens in the squirrel feces than the others, the meaning of the results is unclear. This lack of clarity is exacerbated by the low amounts of methanogen levels in animals with high expected methanogen content. Due to this discrepancy, it is likely that these results are confounded instead by some other variable, some of which may be freshness of the samples or handling time or oxygen exposure. Some of these are a result of temporal and spatial constraints on access to the animals and their feces, e.g. transport time was required for the feces from most of the animals as they were located further away than the squirrels. Despite the squirrels showing high methanogen content comparatively, it may still not be viable to use preferentially due to the inefficiencies of harvesting and using such small feces.

Figure 5: Biodigester at the BioEnergy and Sustainable Technology Laboratory.

References

Cornejo, C. & Wilkie, A. C. (2010). Greenhouse gas emissions and biogas potential from livestock in Ecuador. *Energy for Sustainable Development*, 14(4), 256–266. https://doi.org/10.1016/j.esd.2010.09.008

Doddema, H. J. & Vogels, G. D. (1978). Improved identification of methanogenic bacteria by fluorescence microscopy. *Applied and Environmental Microbiology*, 36(5), 752-4.

Hackstein, J. H. P. & van Alen, T. A. (1996). Fecal methanogens and vertebrate evolution. *Evolution*, 50(2), 559–572. https://doi.org/10.1111/j.1558-5646.1996.tb03868.x

Lamendella, R., Santo Domingo, J. W., Ghosh, S., Martinson, J., & Oerther, D. B. (2011). Comparative fecal metagenomics unveils unique functional capacity of the swine gut. *BMC Microbiology*, 11(1), 103. https://doi.org/10.1186/1471-2180-11-103

Sorlini, C., Brusa, T., Ranalli, G., & Ferrari, A. (1988). Quantitative determination of methanogenic bacteria in the feces of different mammals. *Current Microbiology*, 17(1), 33–36. https://doi.org/10.1007/bf01568816

St-Pierre, B. & Wright, A.-D. G. (2012a). Diversity of gut methanogens in herbivorous animals. *Animal*, 7(s1), 49–56. https://doi.org/10.1017/s1751731112000912

St-Pierre, B. & Wright, A.-D. G. (2012b). Molecular analysis of methanogenic archaea in the forestomach of the alpaca (Vicugna pacos). *BMC Microbiology*, 12(1), 1. https://doi.org/10.1186/1471-2180-12-1

Sun, L., Pope, P. B., Eijsink, V. G. H., & Schnürer, A. (2015). Characterization of microbial community structure during continuous anaerobic digestion of straw and cow manure. *Microbial Biotechnology*, 8(5), 815–827. https://doi.org/10.1111/1751-7915.12298

Wilkie, A.C. (2005). Anaerobic digestion: biology and benefits. In: Dairy Manure Management: Treatment, Handling and Community Relations. NRAES-176, p.63-72. Natural Resource, Agriculture, and Engineering Service, Cornell University, Ithaca, NY. http://biogas.ifas.ufl.edu/Publs/NRAES176-p63-72-Mar2005.pdf

Wilkie, A.C. (2008). Biomethane from biomass, biowaste and biofuels. In: Bioenergy, p.195-205. J.D. Wall, C.S. Harwood and A. Demain (Eds.), ASM Press, Washington, DC. http://dx.doi.org/10.1128/9781555815547.ch16

Acknowledgements

This research was conducted as part of the *2018-19 CALS University Scholars Program* at the BioEnergy and Sustainable Technology Laboratory, Soil and Water Sciences Department, UF/IFAS.