Thermophilic anaerobic digestion of cattle manure reduces seed viability for four weed species

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Abstract: Anaerobic digestion of manure and other biowaste has been gaining public attention for producing biogas as a renewable energy. More digestate materials after harvesting biogas available will be used as biofertilizers, soil conditioners and amendments for land application. However, digestate is required to be free of weed seeds. The effect of anaerobic digestion on the survival of weed seeds has not been studied extensively. This study examined four weed seeds, wild oats (*Avena fatua* L.), wild buckwheat (*Polygonum convolvulus* L.), wild mustard (*Sinapis arvensis* (DC.) L.C.Wheeler) and volunteer canola (*Brassica napus* L.) that were placed in batch cultures with feedlot cattle manure at 55℃ for 7 and 24 hours. The results showed that after being subjected to anaerobic digestion for 7 hours, wild oats, volunteer canola and wild mustard had zero viability. Wild buckwheat had remaining viable seed after the 7 and 24 hour anaerobic digestion treatment as shown by the tetrazolium test. However, the remaining viable wild buckwheat seeds were considered to be incapable of normal growth and development.

Keywords: weed seeds, anaerobic digestion, *Avena fatua*, *Polygonum convolvulus*, *Brassica napus*, *Brassica kaber*
DOI: 10.3965/j.ijabe.20120501.008

Citation: Eckford R E, Newman J C, Li X, Watson P R. Thermophilic anaerobic digestion of cattle manure reduces seed viability for four weed species. Int J Agric & Biol Eng, 2012; 5(1): 71－75.

1 Introduction

Anaerobic digestion transforms biowastes, including manure into biogas for green energy production. The digestate, which is the material after harvesting biogas, can be utilized to produce biofertilizers, soil conditioners and amendments, and other value-added products. Utilizing biowastes to produce value-added products has many environmental benefits, including substantial reduction of CO_2 and other greenhouse gas emissions while protecting the land and water supplies from pathogens and excessive nutrients. If the anaerobic digestate is free of pathogens and weeds and balanced with plant nutrients, it can become a source of nutrient-dense, slow-release biofertilizers for agricultural industry including both conventional and organic food growers, golf courses and horticulturalists.

The studies on effects of rumen digestion and composting on weed seed viability in manure indicated that the duration of seed in the rumen and compost, temperatures during composting, and seed coat hardness are key factors in reducing weed seed viability in manure[1-3]. In a multi-year study, Bilek[4] gathered information on the environmental impacts and economic viability of various manure handling systems. As part of the study, seed viability reduction in anaerobic digesters was studied. Dormant seeds, common to the area, were subjected to *in vitro* rumen fermentation and various storage conditions. They were then placed in anaerobic digesters for 20 days at temperatures ranging from 35℃ to 41℃. *Panicum miliaceum* (L.), *Setaria faberi* (Herrm.) *and Polygonum persicaria* (L.) did not germinate and were assumed to have been killed during rumen treatment. Abutilon theophrasti (Medicus),

Received date: 2010-11-09 **Accepted date:** 2011-12-01
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Chenopodium album (L.), Amaranthus retroflexus (L.) had no cumulative difference in weed seed germination among the various treatments. Therefore, the effects of anaerobic digestion on weed seed viability for this study were unclear.

Anaerobic digesters rely on moderate to high temperatures, and a highly active microbial community to process manure. Substantial reduction of weed seed viability using anaerobic digestion has been reported. *Sorghum halepense* (L.) and *Panicum dichotomoflorum* (Michx.) seeds took 20 days at 35℃ using batch and daily-fed digesters to eliminate seed viability[5]. *Rumex obtusifolius* (L.) and *Lycopersicon lycopersicum* (L.) seeds were destroyed using a batch digester in 14 days at 55℃[6]. However, the use of Completely Stirred Tank Reactors (CSTRs), has become increasingly common to process the large volumes of manure from high-intensity livestock operations. Retention time is one of the key factors for CSTR operations with shorter retention time reducing capital cost and improving economic return. To date a minimum time for substantive reductions in weed seeds viability has not been established. The purpose of this study was to determine if viability of weed seeds is affected after exposure to anaerobic digestion at 55℃ for 7 and 24 hours, which is the minimum retention time that the fresh materials stay in a CSTR digester.

2 Materials and methods

2.1 Experimental set-up

Anaerobic digestion was conducted with triplicate batch cultures with 5% total solids substrate. The substrate was bed pack manure from a local cattle feedlot or digestate from anaerobic digestion systems. The digestion vessels were Wheaton™ culture bottles with butyl rubber septa lids. After the cultures were prepared, the Wheaton™ bottle headspace was sparged with ultra-high pure nitrogen (UHP-N_2) to create anaerobic conditions. The cultures were incubated at 55℃ in a C24 incubator shaker (New Brunswick Scientific Co., Edison, NJ) for 24 hours before weed seeds were added to determine if anaerobic digestion was occurring. Light was excluded from the incubators in order to simulate conditions in anaerobic digestion system tanks. The digesters were monitored for biogas production and quality. Pressure reading was used for calculating the biogas production. A PT420 transducer (SRP Control Systems, Mississauga, Ont.) fitted with a readout pressure meter was used for pressure reading. Headspace biogases were analyzed by GC with a Varian CP 2003 Micro-GC and Molsieve 5Å PLOT and Poraplot U (10 m) columns for biogas quality. Hydrogen, CH_4, CO_2, and H_2S were measured.

2.2 Anaerobic digestion treatments

Seeds from four weed species were subjected to digestate treatments at 55℃ for 7 and 24 hours after the initial 24 hour incubation period. The selected weed seeds were wild oats (*Avena fatua* L.), wild buckwheat (*Polygonum convolvulus* L.), wild mustard (*Sinapis arvensis* (DC.) L.C.Wheeler) and volunteer canola (*Brassica napus* L.). These are common weeds in western Canada[7] and represent a range of hardness of seed coat and expected dormancy. After the first 24 hour incubation when anaerobic digestion had become well established in the cultures, the bottles were opened under a UHP-N_2 flow to maintain anaerobic conditions. Nylon bags containing 100 weed seeds were added to selected bottles and the bottles were returned to the incubator with mixing at 120 r/min. Two hours after the addition of weed seeds, pressure readings were taken to ensure that microbiological processes had resumed. Cultures were removed from the incubator after the designated 7 and 24 hour incubations; the seed bags were rinsed with distilled water and sent for seed germination and viability testing.

For anaerobic digestion, triplicate control cultures with bed pack feedlot manure or digestate were incubated with no addition of seeds. These cultures were monitored for pressure increase from biogas production and biogas quality to show that anaerobic digestion was proceeding as expected for the substrate. Each weed species represented an individual trial as differences among them were expected *a priori*. Therefore, each germination and viability trial was coupled with water-imbibed weed seed control of 100 seeds that were not subjected to anaerobic digestion.

2.3 Germination and viability testing

Petri dishes were lined with two layers of filter paper and moistened with 5 mL distilled water. Weed seeds were added to the petri dishes and the petri dishes were placed in a seed germinator programmed for day/night temperatures of 25℃/15℃ and light of 16/8 hour duration, respectively. Distilled water was added as needed to maintain moist filter paper. Germination assessments were conducted at 7 and 16 days after the weed seeds adding to the petri dishes. Germination was considered to have occurred when the radical was 2 – 3 mm in length.

Non-germinated seeds were tested for viability according to the procedures as outlined in the "Handbook on Tetrazolium Testing"[8]. The Tetrazolium test provides a quick estimate of seed viability. With results within short period this test can be very helpful when time is a factor or for species which have very long germination requirements. This is to ensure that the assessment, particularly for buckwheat seeds that may require longer time to germinate, reflects their true viability. Depending on weed species, 5 – 10 mL of a 1% w/v solution of 2, 3, 5-triphenyl tetrazolium chloride (TTC) and distilled water was added to each petri-dish containing water-imbibed weed seeds that had failed to germinate. Where required, testa of each seed was incised in order to allow the TTC solution to permeate. Petri dishes were left at room temperature and covered to eliminate light. After 24 hours, viability assessments were conducted evaluating seed colour change from off-white to pink after exposure to TTC. Total viability was determined by adding geminated seeds to seeds deemed viable from the TTC test.

2.4 Statistical analysis

All data were subjected to an arcsin-square root transformation prior to analysis as is appropriate when proportional data fall outside the linear range of 30 to 70[9]. Data were analyzed using PROC MIXED in SAS. Transformed and back-transformed means are presented.

3 Results and discussion

For all anaerobic digestion (AD) treatment cultures, either methane or hydrogen content in the biogas generated at the culture containers indicated that anaerobic processes were occurring and healthy. Table 1 summarizes the experimental results. For germination and viability of the controls, total viable seed (percentage), as measured by the germination and tetrazolium tests, was greater than 90% for all four weed species. Wild oat, volunteer canola and wild mustard all had a seed germination rate in excess of 92% with minimal or no additional viability in non-germinating seed. Wild buckwheat germinated at a rate of less than 20%, and non-germinating seed viability accounted for an additional 77% – 79% of the total viable seed. These results indicated that the quality of seeds used in this experiment is very good (Figure 1).

Under AD treatments, the germination rate was zero to less than 0.2% for all four species. The wild buckwheat controls did differ from the other weed seed. This is due to the nature of buckwheat, it requires longer time to germinate or break its shell, as Tetrazolium test, with 77% viability. Under AD treatment viability decreased to 13.7% with 7-hour treatment, 13.7% and 4.7% with 24-hour treatment.

Results indicate that concerns over weed seed spread through manure are significantly reduced or eliminated depending on weed species[10], when manure is subjected to a short duration of anaerobic digestion. Most seeds of the species investigated were destroyed by a 7-hour anaerobic digestion treatment in batch cultures at 55℃. It was shown that a 7-hour retention time would be sufficient to destroy most weed species entered into a digester. Among the weed seeds used in this study, only wild buckwheat posed a problem with regard to weed seed survival and subsequent dispersal. Wild buckwheat has a hard pericarp and scarification which can be required to break dormancy[11]. It is likely that wild buckwheat seed would be destroyed if it remained in a digester for the full retention time. In large-scale anaerobic digestion systems, there is a specified retention time, usually days, with substrate movement in and out of the system. The minimum amount of time materials are in a digester is 7 hours. For systems with a 14-day retention time, less than 7.5% of the material would be removed on a daily basis from the digester. Considering

the possibility that new materials being moved out of a digester is less than 50%, if wild buckwheat seeds were present, the percentage of the seed that would germinate would be minimal and seed viability would be less than 0.5%. In addition, if these seeds went through rumen digestion first, the risk of their survival through this anaerobic digestion is extremely low.

Table 1 Mean germination and viability for wild oats, volunteer canola, wild mustard and wild buckwheat

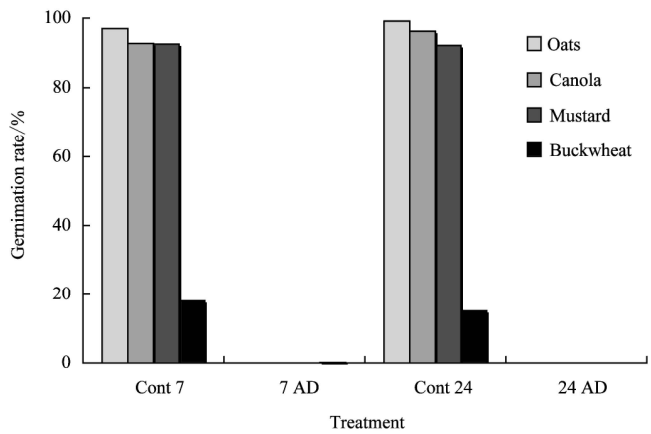
Treatment	Germination			Viability[a]		
	Transformed[b]	Diff	Back-transformed %[c]	Transformed	Diff	Back-transformed %
	Wild oats					Wild oats
7-h control	1.398	a	97.0	-	a	-
7-h digestate	-	b	-	-	a	-
24-h control	1.478	a	99.1	-	a	-
24-h digestate	-	b	-	-	a	-
	Volunteer canola			Volunteer canola		
7-h control	1.297	a	92.7	0.059	a	0.3
7-h digestate	-	b	-	-	a	-
24-h control	1.376	a	96.2	0.059	a	0.4
24-h digestate	-	b	-	-	a	-
	Wild mustard			Wild mustard		
7-h control	1.293	a	92.5	-	a	-
7-h digestate	-	b	-	-	a	-
24-h control	1.285	a	92.1	0.033	a	0.1
24-h digestate	-	b	-	-	a	-
	Wild buckwheat			Wild buckwheat		
7-h control	0.440	a	18.1	1.070	a	77.0
7-h digestate	0.033	b	0.1	0.379	b	13.7
24-h control	0.400	a	15.2	1.094	a	78.9
24-h digestate	-	b	-	0.218	b	4.7

Note: [a] Non-germinating viable seed.

[b] Percentage data transformed using arcsin-square root transformation.

[c] Back-transformed to percentage using inverse arcsin-square root transformation.

Note: Arcsin-square root of the original means and back-transformed means are presented. Treatment differences (Diff) were determined by pairwise comparisons within PROC MIXED and are indicated by differing letters, analogous to an LSD.



Note: Cont 7=water control for 7 hours; Cont 24=water control for 24 hours; 7 AD=7-hour anaerobic treatment; 24 AD= 24-hour treatment

Figure 1 Germination rate of the four tested species under 7- and 24-hour anaerobic treatments

In addition, results from this study are based on viability using the tetrazolium stain. A tetrazolium test does not measure the capacity for normal cell division, potential developmental rate or dormancy[12]. The test alone will often significantly overestimate total viability[1]. It was observed that the condition of the seed deemed viable from the tetrazolium test results suggesting that few or none of these seeds would be capable of normal development. Ruptured testae was observed with varied embryo color ranging from off-white to pale-yellow to light-gray and embryonic tissue of various consistencies ranging from flaccid, milky to grainy. Therefore, the actual fraction of germinable seed is substantially less than indicated by the tetrazolium test and may be zero for

all weeds tested in the 24-hour digestate treatment. Furthermore, Blackshaw and Rode[13] showed that rumen digestion reduces weed seed viability. They found that rumen digestion reduced germination and viability for wild buckwheat by 73.2% and 38.7%, respectively. The weed seeds in this study were not pre-digested with rumen.

4 Conclusions

The results from this study suggest that, under conditions reported here, the risk of spreading viable weed seeds that have passed through an anaerobic digester is minimal provided there is sufficient retention time, seven hours, and a suitable temperature for anaerobic digestion, 55℃. Wild oats, volunteer canola and wild mustard had zero total viability after digestate treatments. Wild buckwheat had remaining viable seed after the anaerobic digestion treatment as shown by the tetrazolium test. However, as we discussed before this test does not measure the capacity for normal cell division, potential developmental rate or dormancy. Based on our experience the remaining viable wild buckwheat seeds from this study were incapable of normal growth and development.

Acknowledgements

The authors would like to thank the technical assistance provided by Tammy Currie and Uliana Kanevets.

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