

Abstract

Anaerobic digestion features among various conceivable solutions for addressing the global issues of waste remediation and soil and water sustainability. This technology offers an environmentally friendly way to handle human and organic wastes, control pollution, mitigate greenhouse gas emissions, and recover energy and elemental nutrients. Inoculation is the most critical step in startup of an anaerobic digester. This study was undertaken to investigate alternative sources of inocula in case digester effluents are not available or if manures are inappropriate. Sediment samples were collected from wetlands in Gainesville and Florida springs. These samples were subjected to microscopic examination and methanogenic activity tests in order to assess the presence of methanogenic bacteria. Samples that demonstrated a higher methanogenic activity were selected as an inoculum source for the digestion of sugarcane bagasse. Results obtained confirmed the presence of methanogenic bacteria in different water-saturated environments. Soil samples from wetlands appear to be a better source of inoculum than spring sediments. The total methane production ranged from 132.8 to 310.6 ml per gram of chemical oxygen demand (COD) added. Biochemical methane production of the bagasse using different inocula reveals that wetland soils can be used for inoculating anaerobic digesters. The conclusion of this study is very promising for the future of the anaerobic digestion technology specifically in developing countries where this technology is at an emergent stage.

Introduction

One of the most important elements involved in the anaerobic digestion process is the variety of methanogenic bacteria for which favorable conditions have to be maintained for the efficiency of the system. In most cases, inoculation is a mandatory step in commissioning or restarting a digester. Digested effluent and animal manure are the most frequent sources used for this purpose. Although utilization of animal manure does not pose any threat to public health because most of the pathogens that can be present in the manures are killed or deactivated by the anaerobic digestion process, some people can still have a perception of risk associated with their use. This study was undertaken to investigate alternative sources of inocula in the event digester effluents are not available or if manures are inappropriate.

Objectives

The purposes of this study are to investigate methanogenic bacteria that can be used as inocula for anaerobic digestion. Specific objectives of this study are:

- Find an alternative source of anaerobic methanogenic bacteria among different samples collected from different water saturated environments such as wetland soils and spring sediments.
- Study the biochemical methane potential of anaerobic methanogenic bacteria by using sugarcane bagasse as substrate.

Investigation of Alternative Sources of Inocula for Anaerobic Digestion **Reginald Toussaint¹ and Ann C. Wilkie²**

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Methodology

- Collect inoculum sources : Soil samples were collected from wetlands in Gainesville and Florida springs.
- Microscopic examination: The ability of methanogenic bacteria to auto-fluoresce when exposed to ultraviolet light was used to assess their presence (420nm excitation/470nm emission).
- Biochemical Methane Production (BMP) test: Samples incubated at 35°C for 15days in airtight 150mL serum bottles. Gas production measured every third day by bubbling gas through 5M KOH solution to dissolve CO_2 and give displacement reading for only CH_4 .
- **Incubation and validation**: BMP tests were performed using glucose and sodium acetate as substrates. Methanogenic activity was evaluated by the amount of methane production over time.
- Anaerobic digestion of sugarcane bagasse : BMP tests using sugarcane bagasse as a substrate, were used to evaluate the inoculum potential of the microbial population of the collected wetland samples.







Figure 2. Methanogenic activity of the wetland sample with glucose and acetate as substrates . W1-W4: different sample sites of the wetland.



Dairy manure was used as a comparative control.



Figure 4. Biochemical methane production (BMP) measurement apparatus. A: Incubated sample, **B**: KOH saturated solution, **C**: Graduated cylinder

Discussion Results obtained from the microscopic examination and methanogenic activity test confirm the presence of methanogenic bacteria in different water-saturated environments. Soil samples from wetlands appear to be a better source of inocula than spring sediments. Biochemical methane production of the sugarcane bagasse using different inoculum sources reveals that wetland soils can be used for inoculating anaerobic digestion. Results obtained from different treatments are not enough to conclude whether dairy manure or wetland soils are better inocula, as no significant difference was observed between the amount of methane produced.

Conclusion Sediments of different water-saturated environment contain methanogenic bacteria suitable for inoculating anaerobic digesters. Microscopic examination and incubation of different wetland soils supports the possibility of using them as inocula. Enrichment of the samples with different substrates does not translate into a better source of inoculum but only indicates appreciating the presence of methanogenic bacteria.

References

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Figure 3. Methane production of sugarcane bagasse with wetland soils as inoculum.



Figure 5. Methanogen colonies in wetland sample, cells fluoresce blue under epi-fluorescent illumination (red arrow), 500x