Bioresource Technology 185 (2015) 79–88

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

# Bioaugmentation with an anaerobic fungus in a two-stage process for biohydrogen and biogas production using corn silage and cattail

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## HIGHLIGHTS

- Bioaugmentation with a rumen fungus was performed in an anaerobic two-stage system.
- Comparable $H_2$ and $CH_4$ yields were obtained after 60 days of digestion.
- Bioaugmentation improved $H_2$ and $CH_4$ production rates and VFA degradation rate.

## ARTICLE INFO

*Article history:*
Received 19 December 2014
Received in revised form 12 February 2015
Accepted 24 February 2015
Available online 28 February 2015

*Keywords:*
Anaerobic digestion
Dark fermentation
*Typha latifolia*
Leach bed reactor
UASB reactor

## ABSTRACT

Bioaugmentation with an anaerobic fungus, *Piromyces rhizinflata* YM600, was evaluated in an anaerobic two-stage system digesting corn silage and cattail. Comparable methane yields of 328.8 ± 16.8 mL $g^{-1}$ VS and 295.4 ± 14.5 mL $g^{-1}$ VS and hydrogen yields of 59.4 ± 4.1 mL $g^{-1}$ VS and 55.6 ± 6.7 mL $g^{-1}$ VS were obtained for unaugmented and bioaugmented corn silage, respectively. Similar $CH_4$ yields of 101.0 ± 4.8 mL $g^{-1}$ VS and 104 ± 19.1 mL $g^{-1}$ VS and a low $H_2$ yield (<1 mL $g^{-1}$ VS) were obtained for unaugmented and bioaugmented cattail, respectively. However, bioaugmentation resulted in an initial increase in $CH_4$ and $H_2$ production rates and also increased volatile fatty acid degradation rate for both substrates. Our study demonstrates the potential of bioaugmentation with anaerobic fungus for improving the digestibility of lignocellulose substrates for biogas and biohydrogen production.



## 1. Introduction

In 2010, the share of bioenergy was 50 EJ or 9% of global energy and it is projected to increase sustainably to 60–120 EJ of primary energy in 2050 (Searle and Malins, 2014). Lignocellulose feedstock is the most abundant and available biomass in nature and its utilisation in the bioenergy sector is vital to attain national and global objectives. In this study, corn silage and cattail, which are lignocellulose biomass, were used as feedstocks for biogas and biohydrogen production. Maize is a widely used energy crop due to its high biomass yield (21–30 kg TS $ha^{-1}$) and its short growing season of 3 month (Amon et al., 2007). However, its growth requires nutrients, water and pesticides, which increases the production cost. Also, there are ethical concerns with the use of maize (food) for biofuel production (Kazamia and Smith, 2014). As a consequence, maize for biofuel production is increasingly being cultivated in a crop rotation system on marginal land (Gelfand et al., 2013). Cattail, on the other hand, is a wetland plant, pest resistant and can grow on poor soil, reaching a high biomass yield of 40 kg TS $ha^{-1}$ (Zhang et al., 2011). The horizontal growth of their rhizomes enables fast re-establishment. Besides the use of cattail for bioenergy production, the harvested biomass also recovers plant nutrients (N and P) from polluted water bodies (Zhang et al., 2011). One disadvantage of the use of cattail for biofuel production might be the need for non-conventional equipment for harvest.

The full potential of lignocellulose feedstock is however, difficult to unlock due to the complex associations between cellulose, hemicellulose, and lignin. Lignocellulose composition is diverse depending on the source, and its slow degradability necessitates expensive pretreatments, both of which are barriers for the expansion of biofuel production (Chang et al., 2010). Pretreatment of lignocellulose feedstock is often energy

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http://dx.doi.org/10.1016/j.biortech.2015.02.100
0960-8524/

demanding, requiring high temperatures and also use of chemicals with potential environmental hazards (Singh et al., 2014). Biological pretreatment is an attractive alternative, which uses enzymes and microorganisms that naturally degrade lignocellulosic material in their natural habitat and under physiological conditions (Fotidis et al., 2014; Peng et al., 2014). However, biological pretreatment is slower and less efficient than thermochemical methods.

Herbivores rely on symbiotic anaerobic fungi, bacteria and other microbes in the rumen for the secretion of a spectrum of cellulase degrading enzymes, which hydrolyse complex lignocellulose feedstocks into soluble sugars and volatile organic compounds. These microorganisms are well adapted to the rumen environment and have been reported to significantly degrade lignocellulose material (Chang et al., 2010; Haitjema et al., 2014). As such, anaerobic fungi have many biotechnological applications which include probiotic improvement of the nutritive value of poor forage, a source of potent polysaccharide enzymes, and simultaneous scarification and production of ethanol in livestock and biofuel production (Gruninger et al., 2014; Haitjema et al., 2014) Enrichment of specific microorganisms (bioaugmentation) is a technique that is also used in anaerobic digestion processes for the improvement of lignocellulose hydrolysis, nutrient recovery, biogas process start-up and process inhibition recovery from ammonia and volatile fatty acids (VFA) accumulation (Goud et al., 2014; Westerholm et al., 2012). Previous studies have evaluated bioaugmentation of anaerobic digestion processes with rumen anaerobic bacteria, but only a few studies have investigated the use of anaerobic fungus, which plays a major role in the degradation of lignocellulose feedstock in the rumen (Procházka et al., 2012).

Waste treatment, renewable energy production, and nutrient recycling together with the mitigation of eutrophication and greenhouse gas effects are the main benefits of anaerobic digestion. Anaerobic digestion is a biological process whereby organic compounds are digested by mixed consortia of synergistic microorganisms in the absence of oxygen and in a series of metabolic steps (hydrolysis, acidogenesis, acetogenesis and methanogenesis) to produce $CH_4$ and $CO_2$ as final products (Jha et al., 2011). Anaerobic digestion can be performed in a one-stage process in which all four metabolic steps occur in a single vessel. Alternatively, hydrolysis and acidogenesis can be separated from acetogenesis and methanogenesis into two or more vessels in a two-stage process. Hydrolytic microbes have a higher growth rate and require a pH of 5.5–6.5 for optimum growth, while methanogens are slow growing and require an optimum pH of 6.8–7.6 (Jha et al., 2011). Hence, bioaugmentation of the hydrolytic reactor with anaerobic fungi, which grows at an optimum pH of 6.5, could be more applicable in a two-stage anaerobic digestion process than a one-stage process. In addition $H_2$, which is an intermediary product in anaerobic digestion, is a valuable renewable fuel that can be produced in the first stage of the two-stage anaerobic process.

The objectives of the current research were to investigate bioaugmentation with the anaerobic fungus, *Piromyces rhizinflata* YM600, in a bio-methane potential (BMP) batch test and in a two-stage anaerobic digestion for biohydrogen and biogas production using corn silage and cattail.

## 2. Methods

### 2.1. Feedstocks

Cattail (*Typha latifolia*) from the previous year's growth was harvested in April 2014 near Lethbridge, Alberta. Corn silage was supplied from a feedlot facility in the County of Lethbridge in April 2014.

### 2.2. Culturing of anaerobic fungus: P. rhizinflata YM600

The anaerobic fungus *P. rhizinflata* YM600 was originally isolated from a *Bison bison* rumen sample, and for this trial, it was revived from stock cultures stored over liquid nitrogen in the LRC microbial collection at the Agriculture and Agri-Food Canada Research Centre, Lethbridge. Fungal biomass was produced in Lowe's semi-defined medium B with 5% (v/v) clarified rumen fluid added and barley straw (0.01 g $mL^{-1}$) as the carbon source (Lowe et al., 1985). Anaerobic fungal cultures were grown under anaerobic conditions using previously reported anaerobic technique (Hungate, 1950) and after incubation for 3 days, the fungus and spent medium were utilised for bioaugmentation.

### 2.3. Bio-methane potential batch test

Methane production from corn silage and cattail, with and without the bioaugmentation (10% v/v of *P. rhizinflata* YM600 and spent medium) were performed in batch assays. The inoculum was used as a control and the background methane production was subtracted from the substrates. Another run for the digestion of anaerobic fungus only was performed to subtract the contribution of methane production from the anaerobic fungus. Anaerobic sludge used as inoculum was collected from a 2.8 MW full-scale plant, Lethbridge Biogas LP, which treats agro-industrial wastes under mesophilic condition (40 °C). The cellulose degrading ability of the inoculum was assessed by the digestion of Avicel cellulose. The tests were performed in triplicate in 0.5 L Erlenmeyer flasks at 37 °C for 60 days. Addition of the inoculum and substrate was in the ratio (ISR) of 1:1 based on g VS. The rest of the experimental procedure and set-up was as previously described (Nkemka and Murto, 2013).

### 2.4. Two-stage anaerobic digestion process

Corn silage and cattail were digested in duplicate anaerobic two-stage system, comprised of a leach bed reactor connected to an upflow anaerobic sludge blanket (UASB) reactor, each with a 1.3 L volume (Fig. 1). Granular sludge used as inoculum in the UASB reactor was collected from a mesophilic pilot UASB plant (National Research Council, Montreal, QC, Canada) treating apple juice wastewater. The rest of the experimental set-up and procedure was as described earlier (Nkemka and Murto, 2013).

At the startup, 87 g of cattail and 300 g water or 160 g of corn silage and 200 g of water were placed into the leach bed reactor. The leach bed reactors were flushed with nitrogen for 3 min and the anaerobic fungus with spent medium (10% v/v) was then added into the duplicate leach bed reactors, while 40 mL of the anaerobic medium was added in the control two-stage system. Duplicates for the treated (bioaugmenting with the anaerobic fungus) and the untreated sets were performed for both the corn silage and cattail. The liquid content in the leach bed reactors was internally recirculated at 5 mL $min^{-1}$ for one day using a multichannel peristaltic pump. Transfer of leachate between the leach bed reactor and the UASB reactor commenced on the second day using a peristaltic pump and a timer switch.

In the digestion of cattail, the peristaltic pump operated 5 times per day for 30 min, at a rate of 5 mL $min^{-1}$. For each transfer, the timer switch operated for 30 min. The total volume exchanged between the reactors was 750 mL $d^{-1}$, corresponding to a hydraulic retention time (HRT) of 1.73 days and an average organic loading rate (OLR) of 2.7 g chemical oxygen demand (COD) $L^{-1}$ $d^{-1}$. The exchange of leachate lasted for 10 days, and on Day 11 internal