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Effect of biomass concentration and inoculum source on the rate of anaerobic cellulose solubilization

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ABSTRACT

This study determines cellulose solubilization kinetics from controlled batch digestions and shows the effect of inoculum biomass concentrations. Separate measurements and analyzes were performed for sessile biomass (biofilms) and planktonic biomass (free suspensions). Experiments were conducted using either leachate enriched on cellulose or rumen fluid as inoculum to assess if the effect of biomass concentration was consistent for microbial populations from different source environments. All batch digestions were fitted to a first-order kinetic model (R^2 ranging from 0.94 to 0.99). Regression analysis used to compare the first-order hydrolysis rate showed that the first-order hydrolysis rate was most strongly correlated with the concentration of sessile biomass rather than with the concentration of total or planktonic biomass. The correlation between solubilization rate and sessile biomass was statistically the same for the rumen and leachate inoculated reactors indicating that at low concentration ratios of inoculum to cellulose, the rate of cellulose solubilization is dependant primarily on sessile biomass concentration rather than the species profile of the cellulolytic community.

1. Introduction

Cellulose solubilization is the rate determining step in the anaerobic digestion of organic solid waste (Boone et al., 1993; Chynoweth and Pullammanappallil, 1996; Noike et al., 1985). An increase in the rate of solubilization should lead to an increase in the overall efficiency of the anaerobic digestion process. Reactor studies on cellulose solubilization rates are typically conducted in anaerobic, mesophilic environments with approximately neutral pH and may be inoculated from a range of source environments including landfill leachate, manure, sewage sludge, anaerobic digesters or the rumen (Anderson et al., 1994; Barnes and Keller, 2003; Burrell et al., 2004; Fields et al., 2000; Gijzen et al., 1988; Hu et al., 2004; Kostyukovsky et al., 1995; Miller et al., 2000; Murray, 1986; Tammali et al., 2003). Despite similarities in the operating conditions of reactors inoculated from different source environments, the respective microbial communities may differ in many respects, including their species profile, biofilm architecture, nutritional requirements, particle colonization and hydrolysis rates.

Microbial communities in cellulolytic environments are highly complex with interactions among numerous trophic groups required to carry out the digestion process (Chen and Weimer, 2001; Coleman, 1987; Gijzen et al., 1988; Miller et al., 2000; Miller and Wolin, 1995). *Fibrobacter succinogenes, Ruminococcus albus and Ruminococcus flavifaciens* are dominant cellulose degrading bacteria in the rumen (Weimer and Odt, 1995), while *Clostridium* species tend to be dominant in landfills and anaerobic digesters (Burrell et al., 2004; Van Dyke and McCarthy, 2002).

Anaerobic cellulose solubilization is widely regarded as first-order with respect to cellulose concentration, implying that cellulose availability is a rate limiting factor. However, solubilization rates vary among reported studies, despite the use of a common substrate and similar incubation conditions (Mourino et al., 2001; O'Sullivan et al., 2006; Song et al., 2005; Weimer et al., 1990). Among these studies, the rates of cellulose solubilization by rumen microorganisms are significantly faster than those reached by microbial communities from landfills or anaerobic digesters. Thus it is hypothesized that additional factors such as biomass concentration or inoculum source will also influence the rate of cellulose solubilization. This is further supported by Mourino et al. (2001) who reported hydrolysis rates were slower in rumen experiments in which the rumen microbial inoculum was less than 15% of the reactor volume, concluding that the biomass concentration was a factor limiting at low inoculum concentrations *in vitro*.

The aim of the experiments reported in this study was to investigate the effect of biomass concentration on the rate of cellulose solubilization. Hydrolysis under anaerobic conditions is predominantly a surface process (Fields et al., 2000; Lynd et al., 2002;

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McAllister et al., 1994; Song et al., 2005) and planktonic biomass is expected to have little direct impact on solubilization rate (Latham, 1980). To test this observation the concentration of sessile and planktonic biomass were analyzed separately. Experiments were conducted using either leachate or rumen fluid as inoculum to assess if the effect of biomass concentration was consistent for microbial populations from different source environments.

2. Methods

2.1. Inoculum

Leachate was collected from a 200 L leach bed reactor degrading Municipal Solid Waste (MSW) and transferred to a 5 L semi-continuous reactor fed cellulose. The 5 L reactor was operated in a temperature controlled incubator at 38 °C (±2 °C) and the pH was buffered at 7. Stable operation of the semi-continuous reactor was achieved by withdrawing reactor contents then feeding an equal volume of digestion medium containing 1% (wt) cellulose as a single pulse. The feeding and withdrawing procedure was performed once every two days, each time within a 15 min interval using peristaltic pumps and gas tight connecting lines (Masterflex, norprene tubing). The reactor and feed reservoir were stirred during the period of feeding and withdrawing to prevent the settling of cellulose particles and ensure that the solid retention time (SRT) and hydraulic retention time (HRT) were equal. The reactor was operated at a residence time of 10 days for approximately 6 months. Leachate inoculums were collected from the enrichment reactor after 4 months (Experiment 1) and 6 months (Experiment 2) operation.

Rumen contents were collected from cannulated steers maintained on a forage diet. Rumen contents were squeezed through four layers of cheesecloth to remove coarse solids and stored under a CO_2 headspace in an insulated flask to provide an anaerobic temperature controlled environment during transport. The rumen contents were used to inoculate experiments within two hours of collection from the steers.

2.2. Reactor set up

Duplicate batch digestions were carried out in 1 L glass reactors (0.6 L working volume) with sample ports to allow gas and slurry samples to be collected during each experiment. The digestion medium was a basal mineral salts solution (Coleman, 1987). The composition of this mixture, before addition of the inoculum was (in g l^{-1} unless otherwise stated) $NaHCO_3$ 5.9; K_2HPO_4 5.0; KH_2PO_4 4.0; NaCl 0.52; $CaCl_2$ (anhydrous) 0.035; $MgSO_4 \cdot 7H_2O$ 0.07; NH_4Cl 1.5; and clarified, sterile rumen fluid or sterile digester leachate at a concentration of 200 ml l^{-1}. Cellulose was added to the medium as the sole carbon source in the digestion experiments (10 g l^{-1}, 50 μm particle size, Sigmacell, Sigma, USA). The pH of the combined media and inoculum at the start of the experiment was between 7.0 and 7.3 for the leachate inoculated reactors and 6.5 and 6.8 for the rumen inoculated reactors. Reactors were stored in a temperature controlled incubator at 38 °C during the batch experiments.

The biomass concentration in the leachate experiments was varied by concentrating the amount of biomass in the leachate inocula. A volume of leachate was treated in a centrifuge at 15,000*g* for 15 min. Supernatant was discarded to reduce the volume of the sample to 10% of the reactor working volume (60 ml) and the cell pellet was re-suspended. The concentration of biomass in the inoculum was varied by changing the volume of the leachate sample treated in the centrifuge. Volumes equivalent to 10%, 20% 50% and 100% (e.g. 60 ml, 120 ml, 300 ml and 600 ml) were used in the leachate experiments. Rumen experiments were conducted at four active inoculum concentration levels: 1%, 5%, 10% and 20% inoculum by volume. In experiments where less than 20% rumen inoculum was added to the reactor, the inoculum volume was made up to 20% of the reactor volume with sterilized rumen fluid clarified at 10,000*g* for 60 min (e.g. the 5% rumen inoculum contained 30 ml active rumen fluid and 90 ml sterilized rumen fluid). This was done to provide a consistent concentration of rumen nutrients in the medium.

2.3. Analytical procedures

Biogas volume was measured by manometer at the start of each sampling event. The biogas quality (CH_4, CO_2, H_2) was determined using a Perkin Elmer autosystem gas chromatograph (GC) (APHA, 1998).

Slurry samples were filtered through 0.22 μm membrane filters to remove solids, the supernatant was then analyzed for soluble chemical oxygen demand (sCOD). Chemical oxygen demand was measured according to Standard Methods (APHA, 1998) using a Thermoreactor TR 300 (Merck, Germany) and an SQ 118 Photometer (Merck, Germany).

The concentrations of planktonic and sessile biomass in the slurry samples were estimated using the method of Jensen et al. (2008). Slurry samples collected from the reactor were separated into the cellulose fraction containing sessile biomass and the supernatant fraction containing planktonic biomass by centrifuging at low speed (150*g* for 10 min) and gently decanting (Jensen et al., 2008). The biomass concentration in the slurry sample and in each fraction was estimated using a modified Bicinchoninic Acid (BCA) protein assay (Smith et al., 1985). Cells were collected by centrifuging an aliquot of sample (2 ml) at 15,000*g* for 15 min at 4 °C. The cell pellet was resuspended in 2 ml of 5% SDS in 0.1 N NaOH and placed in a boiling water bath (100 °C for 10 min) to disrupt the cells and solubilize the proteins. Once prepared, 0.1 ml sample was added to 2 ml BCA working reagent and placed in a water bath at 60 °C for 15 min, samples were equilibrated at room temperature and the absorbance at 562 nm compared to bovine serum albumin (BSA) protein standards.

2.4. Hydrolysis and rate calculations

The extent of cellulose solubilization and volumetric rates of cellulose solubilization were calculated using a mass balance over the COD (Eq. 1). The extent is the fraction of cellulose solubilized at each sampling point and was calculated as the sum of the cumulative CH_4 and H_2 production, the instantaneous soluble COD concentrations in the solution and the cumulative biomass generation up to that point. All data were converted to units of grams of COD equivalents for application to the mass balance (Song et al., 2005).

$$\text{Extent} = \left(\frac{\text{COD}_{\text{CH}_4} + \text{COD}_{\text{H}_2} + \text{COD}_{\text{soluble}} + \text{COD}_{\text{biomass}}}{\text{COD}_{\text{initial}}}\right) \quad (1)$$

Residual cellulose data were then fitted to discontinuous first-order models (Eq. (2)) to determine the first-order hydrolysis rate (k) and the lag time. The linear model used to calculate k was only applied to data collected during the fermentation period (t); data collected from the lag period was excluded from the analysis (Weimer et al., 1990).

$$Ln(1 - \text{Extent}) = kt \quad (2)$$

3. Results

Each batch digestion in this study resulted in the solubilization of cellulose and the concurrent production of methane, biomass