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ScienceDirect
Bioresource Technology 99 (2008) 4723–4731
BIORESOURCE TECHNOLOGY

The effect of biomass density on cellulose solubilisation rates

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Received 26 April 2007; received in revised form 22 September 2007; accepted 24 September 2007
Available online 19 November 2007

Abstract

The aim of this work was to compare the impact of inoculation density on the rate of cellulose hydrolysis by a rumen derived culture with that of a microbial enrichment from an organic waste anaerobic digester. The results showed a linear relationship between the mass of biomass at the start of the first order degradation phase (X_o) and the first order hydrolysis rate (r) for both rumen inoculated and leachate inoculated cellulose digestions and that the slopes of these relationships were not distinguishable. This suggested that differences in the microbial community, media and other environmental factors had a lesser impact on the hydrolysis rate compared to the effect of the number of cells in the system. This could be of great importance to industrial applications of anaerobic digestion technologies as it suggested that if cells densities in the waste treatment digesters could be boosted to match those seen in the rumen, then the rates of the cellulose hydrolysis would rise.

Keywords: Cellulose; Anaerobic digestion; Biomass; Landfill; Rumen

1. Introduction

Degradation of cellulose occurs in a wide range of anaerobic environments such as landfills, rice paddies, anaerobic sediments and the rumen of some herbivores (Leschine, 1995). It is accepted that cellulose is the rate limiting substrate in anaerobic digesters treating organic solid waste (Noike et al., 1985). Increasing the rate of cellulose degradation would allow for higher throughput and greater yield in anaerobic treatment systems which endeavour to recover energy from organic wastes in the form of methane (Chynoweth and Pullammanappallil, 1996).

The microbiology and microbial ecology of the rumen has been extensively studied for more than 50 years and numerous reviews on the topic of cellulose degradation in the rumen have been published (Hobson and Stewart, 1997; Hungate, 1966, 1975; McAllister et al., 1994; Miron et al., 2001). It is known that the microbial consortium of the rumen is highly efficient at degrading cellulosic feedstocks such as grasses and grains which form the majority of ruminant diets. It is also well known that the rumen consortium is 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).

Operationally, it is well established that rumen inocula will hydrolyse cellulose from organic waste at rates unobtainable with organisms enriched from sewage or organic solid waste. The application of rumen contents as an inoculum to treat cellulose rich organic solid waste has been explored by a number of authors (Barnes and Keller, 2003; Blasig et al., 1992; Gijzen et al., 1987; Hu et al., 2004; Nair et al., 2005; Song et al., 2005).

Reactors inoculated with rumen contents are useful as positive controls for experiments using inocula sourced from

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doi:10.1016/j.biortech.2007.09.070

landfill leachate as they provide an indication of the upper range of cellulose solubilisation rates that can be expected under given circumstances. The rumen and many engineered anaerobic digesters are anaerobic, mesophilic environments with approximately neutral pH and highly cellulosic feedstocks and the degradation process in these systems is dependant on the interaction of a range of microorganisms.

Bacteria from both the rumen and waste treatment environments have been shown to have cellulosomes, protuberances bound to their cell walls that are made up of a variety of cellulolytic enzymes along with structural proteins and proteins that are thought to facilitate binding to the cellulose surface (Lynd et al., 2002). The cellulosomes of *Clostidium thermocellum* have been isolated and well studied (Gilad et al., 2003; Guimaraes et al., 2002; Kataeva et al., 2002; Kurokawa et al., 2002; Ng et al., 1977; Zhang and Lynd, 2002). It has been shown that the different segments that make up the cellulosome work in unison to facilitate the depolymerisation of cellulose. A range of other cellulolytic organisms, including *Ruminococcus albus, Clostridium cellulosolvans*, and *Clostridium cellulolyticum* have been found to have similar protuberances on their cell surfaces that are thought to behave in a similar manner (Desvaux et al., 2000; Morrison and Miron, 2000; Rivard et al., 1991). The presence of these cell bound enzyme complexes indicates why many cellulolytic bacteria must bind to the cellulose surface to degrade cellulose effectively.

Along with these similarities, there are several important differences between these systems which must also be considered. It has been shown that the microbial community in the rumen is dominated by different organisms than those found in landfills and anaerobic digesters. Culturing studies have stated that the *Fibrobacter succinogenes*, *R. albus* and *Ruminococcus flavifaciens* species are among the most important cellulose degrading bacteria in the rumen (Hungate, 1975; Weimer and Odt, 1995) , while in landfills and anaerobic digesters other species of *Firmicutes*, primarily *Clostridia*, tend to be dominant (Burrell et al., 2004; Van Dyke and McCarthy, 2002).

The rumen environment has yet to be duplicated *in vitro*. The synergy between the ruminant and the microbial community is an important aspect in the function of the rumen. The rumen microbes provide a means of degrading fibrous feed into volatile fatty acids which the animal can absorb across its intestinal lining and use for growth. In turn, the animal provides a regulated, temperature and pH controlled environment with gentle stirring and mastication of feeds which is ideal for the growth of the microorganisms.

Although *in vivo* rates of rumen cellulose solubilisation are yet to be achieved in the laboratory the rates of cellulose solubilisation in reactors inoculated with rumen contents are unobtainable using inocula from engineered systems such as anaerobic digesters or landfills (Song et al., 2005). Song et al. (2005) conducted parallel experiments to compare rates of cellulose colonization and degradation with rumen and leachate inocula reactors under identical reaction and incubation conditions. They concluded that the rate of solubilisation in the rumen inoculated system was faster even though less inoculum (on a volumetric basis) was used in the rumen cultures.

Experiments in previous studies have been inoculated with a wide range of starting inoculum, ranging from 3% to 10% (v/v) of cell culture (Desvaux et al., 2000; Miller and Wolin, 1995; Mourino et al., 2001; Schofield et al., 1994; Shi et al., 1997; Song et al., 2005). Mourino et al. (2001) demonstrated that the first order hydrolysis rate constant in rumen inoculated systems improved with increases in inoculum volumes up to 20% (v/v), with no further improvement beyond this point. However, in none of these studies has the actual biomass concentration been measured during the digestion. Any comparison to assess the effect of inoculum volume on ultimate hydrolysis rates would rely on the assumption that biomass density (g/L) was similar in inocula across studies. In addition to this, no similar studies using inocula from engineer waste treatment systems have been conducted.

The aim of the experiments presented in this paper was, therefore, to investigate the effect of inoculum intensity (i.e., the initial cell density) on the rate of cellulose solubilisation by a mixed culture taken from the rumen of a cow compared to a microbial consortium from a mixed waste anaerobic digester.

2. Methods

2.1. Inoculum

Rumen contents were collected from a fistulated steer which was fed a forage diet at the time of sampling. The collected material was filtered through four layers of nylon mesh (1mm × 1mm) to remove coarse solids prior to being placed into a nitrogen purged, insulated flask which provided an anaerobic, temperature controlled environment for the duration of transportation (approximately 45 min).

The leachate inoculum was collected from a 5 L lab-scale anaerobic digester that was treating a simulated waste mix designed to mimic the organic fraction of MSW (Kayhanian and Hardy, 1994). The base medium in the anaerobic digester was 3 L of 11.2 g/L sodium bicarbonate buffer solution. The reactor had been operating for approximately 1 month at the time of leachate extraction and was producing methane and VFAs as a result of the degradation of the solid waste.

2.2. Reactor set-up

Cellulose digestions were carried out in 2 L reactors with 1 L working volumes. The same reactor was used for gas and slurry sampling. All reactors were incubated at 38 °C (±0.5 °C). Digestions were carried out in basal mineral salts solution (artificial saliva) (Coleman, 1987) supplemented with either clarified, sterilised rumen fluid in the rumen inoculated experiments or sterile leachate in the leachate inoculated experiments. The composition of this