Research Paper Biotechnology

Lignocellulose Degradation of *Ocimum Sanctum* During Anaerobic Digestion: Effect of Inoculum Densityand Selenium Trace Metal

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ABSTRACT

One potential source of fuels and chemicals is lignocellulosic plant material, the world's most abundant renewable source of organic carbon. Anaerobic digestion to convert lignocellulosic biomass has been assayed; however, further advances in this technology, are required to achieve economic potential.

In the present study, the rates of anaerobic biodegradation of lignocellulosic biomass and biogas production from Holy basil (*Ocimum sanctum*) in the presence of heterogeneous cow dung as inoculums (5%, 10% and 20%) and Selenium trace metal was investigated in batch reactors for 45 days. The digester mixed with 10% inoculums and 2 mM Selenium showed the maximum performance. As the Lignocelluloses were derived from herbaceous plants, they were degraded more rapidly. Overall, these results indicate that with appropriate inoculum and the addition of trace metal can greatly enhance rates of anaerobic degradation of lignocelluloses.

KEYWORDS Lignocelluloses, Inoculum, cowdung, digester

INTRODUCTION

One potential source of fuels and chemicals is lignocellulosic plant material, the world's most abundant renewable source of organic carbon (Benner and Hodson, 1985). Plant cell wall material is composed of three important constituents: cellulose, lignin and hemicellulose. Lignin is particularly difficult to biodegrade, and reduces the bioavailability of the other cell wall constituents. Lignin is a complex polymer of phenylpropane units, which are cross linked to each other with variety of different chemical bonds (Zainol and Rahman, 2008).

Benner and his associates (1984) detected low rates (<10% in 275 days) of anaerobic biodegradation of lignin to methane and carbon dioxide in anoxic sediments (20 to 30°C) from a variety of marine and freshwater environments. Thermophilic (55°C) anaerobic digestion of several types of lignocellulose showed significant but low percentages (2 to 4%) of lignin degradation during 60-day incubations (Benner and Hudson, 1985). Anaerobic digestion to convert lignocellulosic biomass has been assayed; however, further advances in this technology, are required to achieve economic potential. The present study was aimed at assessing, by batch digestion, the potential for biogas production of locally available plant residue: Tulasi (Holy basil). The anaerobic digestion process is a complex process requiring the presence of several different microorganisms. It is, therefore, imperative that appropriate inoculum and its amount be selected for the degradation process to proceed. Trace metals are essential constituents of cofactors and enzymes and several studies have shown that their supplementation stimulates and stabilizes the biogas process performance (Feng et al., 2010; Fermoso et al., 2010; Gustavson et al., 2011).

The work was carried out with basil biomass as the substrate originating from the offerings in the temples. The rates of anaerobic biodegradation of lignocellulose and biogas production from Holy basil (*Ocimum sanctum*) were investigated in the presence of 5%, 10% and 20% inoculum density and the presence of Selenium in trace amount.

MATERIALS AND METHODS

Collection and Preparation of Material

Ocimum sanctum, commonly known as holy basil, is a herbaceous plant found throughout the south Asian region (Hannan *et al.*, 2006) was collected from the famous Simhachalam temple, Visakhapatnam, where it is otherwise discarded as waste. The leaves were washed with distilled water, air dried in shade, and used as a substrate. The substrate was then blended to make a paste.

Experimental Set Up

In each digester bottle, 1500 ml was slurry (6% TS w/v) of the substrate, and heterogenous cowdung (5%, 10%, 20% TS v/v) was added as active inoculum. The experimental set up was designed as reported by Singh and his associates (2007). Selenium in the form of elemental Selenium, was added to the digester at a concentration of 2mM and the overall performance of the digester was evaluated.

Analysis of the Parameter

Biogas produced in the digester was measured once a day by reading the level of saline water displaced by gas pressure (Singh *et al*., 2007). The contents of the digester were mixed once a day by shaking them manually for 5 minutes. The experiment was carried out for a period of 45 days. The pH was maintained in the range of 6.8 to 7.2. The total solids (TS), volatile solids (VS),), Chemical Oxygen Demand (COD) were analyzed at a regular interval of 5 days using standard methods (Eaton *et al*., 2005). Lignin and Cellulose were also analyzed at an interval of 5 days as per the method given by Zainol and Rahman (2008). The data was subjected to statistical analysis using regression.

RESULTS AND DISCUSSION

The advantage of using a mixture of micro-organisms as is the case of cow dung is that almost all products, like pentoses, hexoses, volatile products and even some inhibiting compounds like furfural and soluble lignin compounds (in not too high concentrations) can be degraded after a period of adaptation (Hendricks and Zeeman, 2009). Temperature used throughout this study is ambient temperature. pH influences the activity of the hydrolytic enzymes and the microorganisms which are active within certain, usually narrow pH ranges (Angelidaki and Ahring, 1994; Angelidaki and Sanders, 2004). The pH tested was maintained between 6.5 -7.5 range which is optimum for biogas production. A cumulative biogas of 0.258dm^3/kg, 0.448 dm^3/kg and 0.301 dm^3/kg with 5%, 10% and 20% inoculum density respectively was produced. Among

the three experimental set ups, 10% inoculum resulted in maximum biogas potential. When selenium was added to the digester, cumulative biogas of 0.613 dm3/kg was observed.

It was observed that solids reduction to be highest at 10%, followed by 5%, then by 20%. There was an escalated increase in the reduction of Total and Volatile Solids in the digesters with Selenium concentrations (80.26%, 70.71%). A decline in COD throughout the incubation period was observed with all the inoculum concentrations. It was observed that highest reduction of COD (66.8%) was past 45 days for inoculum concentration of 10% v/v. The low concentration of Selenium has creeped up the digestion rates (75.19%). Process performance and process stability cab be judged by lower COD values indicating better degradation (Patel and Madamwar, 1998).

There was a continuous degradation of Lignin in the digesters but the Cellulose content increased with increase of the retention time. The Lignin degradation enhanced after 25 days of retention time. The presence of 2mM Selenium has reduced the recalcitrance of Lignin for biodegradation and initiation of degradation was observed after 10 days of interval. At 10% innoculum density, the Cellulose accumulated upto 40.50% after 40 days and then decreased by 21% past 45 days. The degradation of Lignin started after 20 days and attained a maximum degradation of 32.02%.The digesters with 5% and 20% inoculum concentration showed comparatively lower level of performance than 10%.The Cellulose content initially accumulated up to a maximumof 55.29% but the content decreased after 30 days and the degradation rate was 31.73%, with 2mM and 4mM metal concentration.

The initial works performed by other researchers (Benson et al., 1984; Benner and Hudson, 1985) required a higher retention time for Lignin degradation. But the recalcitrance of Lignin was only a maximum at 10 days in the present work which may be attributed to the blending of the substrate before loading the digester and the herbaceous nature of the basil plant. The initial Cellulose content increased with time as the Lignin biodegradation help to reveal more Cellulose because Lignin and Cellulose are cross linked to each other (Zainol and Abdul-Rahman, 2008. The statistical results corroborated with the experimental data (Figure 1).

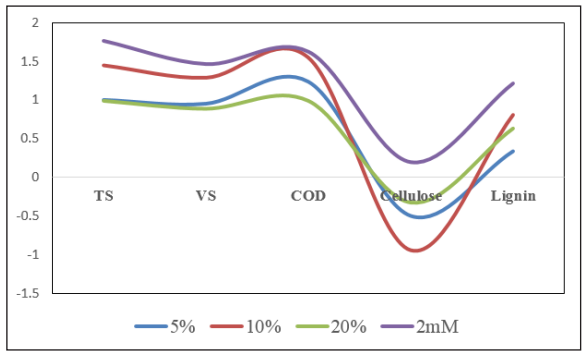


Figure 1: Statistical analysis - trends of degradation of various parameters with 5%, 10%, 20% inoculum density and 2mM Selenium trace metal concentration.

In the present study, Selenium at the concentrations of 2mM had a positive effect on the biogas generation and the overall performance of the digester when compared with the 10% inoculum density. There was a 1.38 fold increase in the biogas produced with Selenium. According to Shamberger (1985), Selenium is an essential trace element at low concentration having beneficial effects.

Conclusion

- The digester mixed with 10% inoculum showed a good performance.
- Selenium has further activated the process of anaerobic digestionand also improved the percentage of biogas formation when compared with 10% inoculum density alone.
- The increasing order of performance of the digester is 20% inoculum< 5% Inoculum< 10% Inoculum< 2mM Se.

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