Executive Summary

EFFECT OF EXTRACTIVES AND CRUDE PROTEINS ON THE KINETICS OF HYDROLYSIS IN A SOLID STATE BIO-REACTOR

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Potential towards using ligno-cellulosic material as an anaerobic process substrate for biogas production is quite high in a country like India having large resource of fermentables such as agro-residues, leaf litter and urban garbage. The ligno-cellulosic herbaceous materials are composed of cellulose, hemicellulose and lignin held together by cementing material (pectins). The cells also have structural proteins known as the crude proteins. Cellulose and lignin together constitute a major fraction of the biomass. Cellulose and hemicellulose are easily degradable. However, lignin cementing these components limits the rate and extent of hydrolysis of the substrate.

The process of anaerobic digestion involves four biochemical steps namely hydrolysis, acidogenesis, acetogenesis and methanogenesis. The first step of hydrolysis involves breaking of complex polymers to monomers by the action of fermentative bacteria. The monomers are used by the acid utilizing bacteria to produce volatile fatty acids (VFAs) in the process and are known as acidogenesis. The VFAs produced are converted to acetate, hydrogen and carbon dioxide. In the final step the acetate is converted to methane by aceticlastic methanogen and hydrogen and carbon dioxide are reduced to methane by hydrogenophilic methanogens.

Various designs of reactors have evolved over the years for anaerobic digestion of potential fermentables. Biomethanation of non-homogeneous biomass was found to be difficult in a conventional biogas plant (cow dung based plants). Hence new designs were developed which could use the physico-chemical properties of the leafy biomass to ferment the non-homogenous substrate. One such design is the solid state bioreactor (Chanakya et al., 1993, 1997, 1999), which works on the principle of dry fermentation (>10% solids) thereby avoiding usage of large amounts of water.

In the anaerobic digestion process where feed rates, temperature fluctuations and VFA production play an important role in the stable functioning of the system mathematical models have been developed over the years for better understanding of the process and efficient designing of robust systems. Hydrolysis being one of the important steps and considered to be rate limiting step in ligno-cellulosic biomass anaerobic fermentation (Tong et al., 1990) has also been modelled. Though various models have evolved over years starting with the Haldane kinetics (Andrews and Graef, 1971) to the surface based kinetics (Sanders, 2000) to describe the process of hydrolysis, most of these models were developed for degradation in a homogenous reactor (CSTR). Heterogeneous systems such as the SSB reactor used for the anaerobic digestion of non-homogeneous leafy biomass; modeling studies on these have been limited.

In this study, kinetics of hydrolysis process is studied. Rate parameters have been evaluated for a variety of feedstocks, the effect of the subcomponents (extractives and crude proteins) on the process of hydrolysis is addressed and a mathematical expression was arrived at relating the rates of initial hydrolysis as a function of extractives and crude proteins. Also the effect of lignin on the kinetics of hydrolysis has been studied. Finally the results obtained have been validated and compared with values from the literature.

The data from earlier experiments using various (dry and fresh) feedstocks conducted at the Centre for Sustainable Technologies, (CST; Chanakya et al., 1997, 1999) were used for the evaluation of the kinetic parameters in order to understand the process of hydrolysis in the SSB reactor. First order hydrolysis rate was used for the kinetic analysis for particulate hydrolysis (Tong et al., 1990; Vavilin et al., 1996) has been considered. Rate expression is arrived at by correlating the fraction of extractives and crude proteins to the rates of initial hydrolysis. The rate parameters obtained are used for analyzing the effect of concentration of lignin on the hydrolysis process. Values from literature are further used to validate and compare the rates of hydrolysis.

The results obtained showed that the hydrolysis in the case of pectin bound substrate was higher upto 70% in case of paper mulberry and least hydrolysis was observed in case of *Acacia auriculiformis*upto 40%. This provides the upper and lower bound limits on the extent of hydrolysis. Urban garbage a third substrate was also used for a comparison of the extent of hydrolysis since it is a heterogeneous substrate having a high concentration of lignin.

In the case of paper mulberry the rates of hydrolysis was found to increase till 25^{th} day and fall down rapidly thereon. This phenomenon could be attributed to the attachment of the bacteria causing an increase in the rate and subsequent decrease in the rate is due to the binding of lignin to cellulose and hemi-cellulose limiting their access to the bacteria. Similar results were observed in the case of all the fresh feedstocks except for *Acacia.* Among the dry feedstocks only sugarcane bagasse seems to follow a similar trend, this could be attributed to the limited accessibility of the subcomponents to the degradability of the feedstock.

Study by Lehtomaki et al (2007) had shown that the crude proteins and extractives play a major role in determining the rates of hydrolysis in a ligno-cellulosic substrate. In this study the effect of fraction of extractives and crude proteins was used to explain the process of

initial hydrolysis and a correlation is obtained using the data by a a linear correlation with the experimental data analyzed. The expression obtained is validated using the data available from the literature. The data seems to fit well except in case of papaya pulp.

The overall rates of hydrolysis were obtained and it was found that the fresh feedstocks have higher rates of hydrolysis (average of 0.154 /d). Acacia although being a fresh feedstock showed low rates of hydrolysis. The reason attributed for such low rates of hydrolysis were physical encrustation of cellulose and hemi-cellulose by lignin. Paddy straw too showed a lower rate due to presence of high concentrations of silica, which lowered the rate of hydrolysis because of decrease in the available surface area.

The effect of lignin on the rates of hydrolysis though prominent, with dry lignin bound feedstocks showing decreased rates, the rate of decrease could not be generalised owing to limited number of data points. Finally a comparison of rates with pure substrate and values from literature showed comparable results in some cases and higher rates in some (fresh feedstock) when fed in fed batch mode.

In this study the hydrolysis of pectin bound and lignin bound substrate was addressed and it has been observed that the pectin bound fresh feed stock has a high extent of hydrolysis. The initial rates of hydrolysis were found to be affected by the concentrations of extractives and crude proteins and a linear correlation for the same was obtained. Lignin concentration did not influence the rates of initial hydrolysis but plays a significant role in the overall decrease in the rates of hydrolysis.

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