Presented at the Research Congress 2013
De La Salle University Manila
March 7-9, 2013

FABRICATION OF BIOREACTOR UTILIZING HOLLOW FIBER MEMBRANE FOR RUMEN HYDROLYSIS OF SWEET SORGHUM

Raymond B. Barajas, George P. Qua, Kenneth A. Icaro, Florinda T. Bacani*
Chemical Engineering Department, De La Salle University, 2401 Taft Ave., 1004 Manila
*Corresponding author: florinda.bacani@dlsu.edu.ph

Abstract: This study was able to fabricate and evaluate a bioreactor that utilizes hollow fiber membrane modules. Results have shown that pH of 7.0 and 7.2 yielded the highest glucose content for sweet sorghum grains and sweet sorghum stalks, respectively. In addition, results have confirmed the presence of soluble sugar utilizers. This soluble sugar utilizing microbes were observed as competitors of fiber degrading microbes in the rumen fluid bacterial consortium. An increase and decrease in glucose content were seen as evidences for the activity of fiber degrading microbes and sugar utilizing microbes. Also, results have shown that utilizing hollow fiber membrane modules increased ethanol yield. Separation of undesired products from the hydrolase such as inactive microbes lessened the inhibition of the fermentation process. Lessening the inhibition increased the activity of yeast that resulted to an increased fermentation yields.

Key Words: bioethanol; sweet sorghum; rumen fluid; hollow fiber reactor; hydrolysis

1. INTRODUCTION

The excessive use of fossil fuels causes fuel depletion globally and along with it, the harmful effects of using fossil fuel are becoming more evident nowadays. Biofuels in the form of bioethanol from lignocellulosic agricultural waste has been a part of a solution to this problem (Huibregts and Rejinders 2009; Balat 2010). The production of cellulosic ethanol as an alternative source of fuel energy is now possible due to the various studies made to lessen our dependence on fossil fuels. This study focused on utilizing rumen fluid found in ruminants containing microorganisms that secrete enzymes that are capable of hydrolyzing lignocellulosic material and convert them to simple sugars.

Rumen fluid, the fluid responsible for the degradation of feeds in ruminants (Dijkstra et al., 2005) such as Philippine riverine-buffalo known as carabaos (Gundran and Mingala, 2007) is now one of the breakthroughs in enzyme hydrolysis due to its ability to saccharify lignocellulosic biomass. Out of many lignocellulosic materials, sweet sorghum, *Sorghum bicolor* L. Moench, is a very promising and capable feedstock for bioethanol production (Liu and Wang, 2008). As a new material for bioethanol production, sweet sorghum is favored because of its glucose content. The bark and pith cell walls of the sweet sorghum contains 49.7% and 59.2% (Billa et al., 1996) glucose, respectively, on a dry weight basis, with proper pretreatment, the expected ethanol yield is high. With newer technologies available, ruminant animals are now being considered as sources of microbes for fermenting lignocellulosic fibers, specifically, sweet sorghum. With stomachs capable of digesting fibrous materials,

microorganisms from the stomachs of these animals can be harnessed in microbial digestion of cellulose containing materials. Furthermore, the stomachs of these animals (called rumen or paunch) can be considered as a "bioreactor" capable of storing and sustaining the rumen microorganisms' growth.

This study fabricated a batch hollow fiber bioreactor that mimics the environment of rumen microbes for bioethanol production. The problem in feedstock was addressed using *Sorghum bicolor* L. Moench also known as sweet sorghum. The rumen of riverine-water buffalos was the source of naturally occurring enzymes. The hollow fiber membrane was tested for the separation of the desired products from the undesired ones. The hydrolase (the product after rumen hydrolysis) was evaluated using *Saccharomyces cerevisiae* and ethanol yield after fermentation (with and without hollow fiber membrane separation) were determined.

2. METHODOLOGY

This study investigated the effects of pH on fibre degradation of rumen fluid hydrolysis. Preliminary experiments were conducted to confirm if 39±1°C and Mcdougall's buffer were to sustain the viability of rumen microbes. The preliminary experiments were conducted in 600 mL flasks filled with 200 mL of Mcdougall's buffer and feed of sweet sorghum grains and stalks. A feed load of 20g/L and 3% (v/v) rumen fluid was used as constant parameters. Experiments were conducted to determine the pH that gives the highest glucose content. 600 mL flasks were used to incubate the rumen microbes at pH of 6.8, 7.0, and 7.2 based from the study of Hu et al. (2004). The pHs were adjusted accordingly using HCl. The conditions that yielded the highest glucose content were then used for the scale-up experiment. A 5 L bioreactor with a 3 L working volume was used. The hydrolase resulting from the rumen fluid hydrolysis was then passed through a G6 hollow fiber membrane glucose separation. The resulting solution was fermented at 250 mL flasks with 135 mL working volume at pH of 5.5 and room temperature. Both hydrolysis and fermentation experiments were conducted at anaerobic conditions. Figure 1 summarizes the overall process of the experiments conducted. The glucose content for the experiments were analysed using the DNS method. On the other hand, ethanol concentration was determined using the refractometry. The resulting refractive index was confirmed by comparing the data obtained with existing literatures.

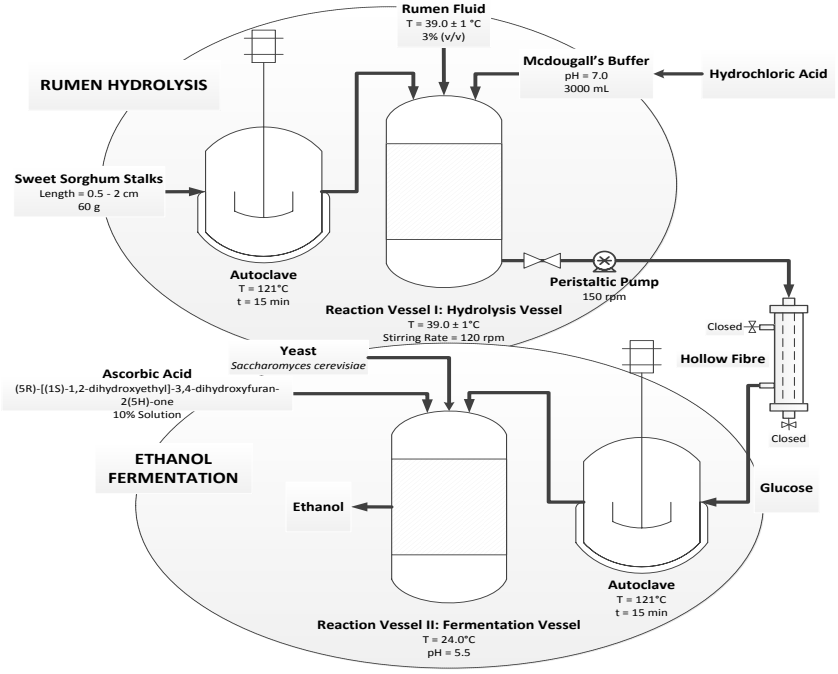

Figure 1. Process Flow Diagram

3. RESULTS AND DISCUSSION

Results of this study are summarized in Figure 2 and Figure 3. These results have confirmed the presence of fibre degrading microbes as well as the presence of soluble sugar utilizing microbes. Theodorou and France (2005) have identified these soluble sugar utilizing microbes as *Megasphaera elsdenii*. The sweet sorghum grains yielded more glucose content compared to the sweet sorghum stalks. Sweet sorghum stalks initially contains glucose while sweet sorghum grains do not contain glucose. Results presented that the initial glucose content of sweet sorghum stalks favoured the growth of the soluble sugar utilizers. The significant decrease in glucose content of the sweet sorghum stalk hydrolase indicated the dominance of *Megasphaera elsdenii* while the absence of glucose in sweet sorghum grains favoured the growth of fibre degrading microbes. As an emphasis, only a few studies have confirmed the presence of soluble sugar utilizers. The results of this study may serve as basis for further researches confirming the presence of other soluble sugar utilizing microbes.

Fermentation results presented the significance of using hollow fiber membrane modules. The hollow fiber membrane was used to separate the inactive rumen microbes from the glucose rich hydrolysate solution. It was observed the glucose rich solution that was passed through the hollow fiber membrane yielded more ethanol compared to the glucose rich solution which was not passed through the membrane. The separation of the inactive rumen microbes from the glucose rich solution lessened the inhibition in the fermentation process. Results have shown that use of hollow fiber membrane separation modules increased the activity of *Saccharomyces cerevisiae*.

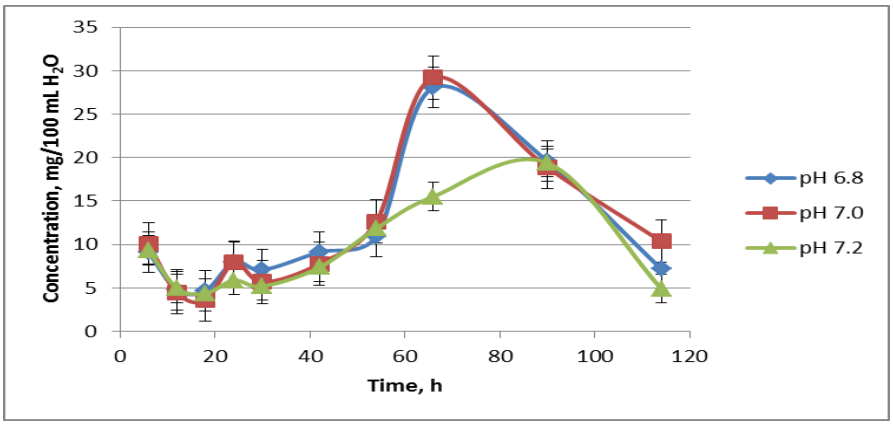

Figure 2 Summary of Result for Sweet Sorghum Grains

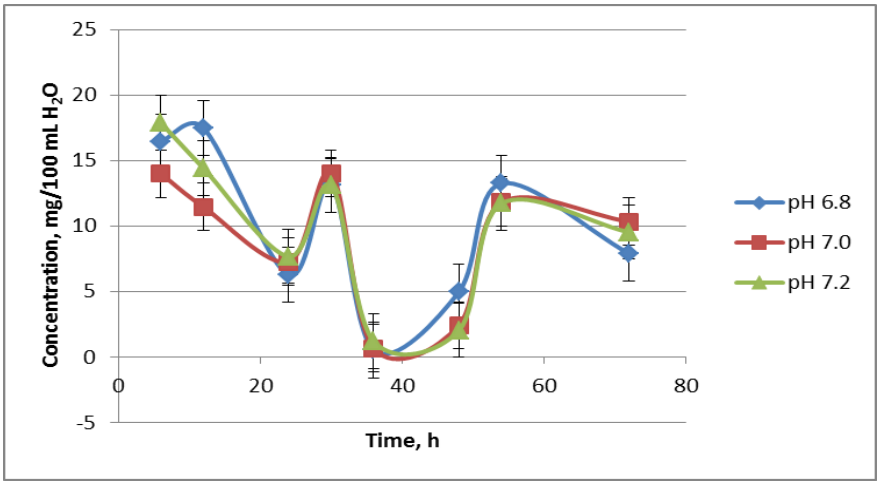

Figure 3 Summary of Result for Sweet Sorghum Stalks

4. CONCLUSIONS

This study was able to address the gaps in bioethanol production. Proper feedstock selection was conducted based from literature. Rumen fluid was identified as natural sources of fibre degrading microbes and a hollow fiber membrane module was used to increase the ethanol yield. All of these were incorporated in a bioreactor. Based from the results, the fabricated bioreactor in this study is a potential solution in addressing the major problems in bioethanol production. In addition, results of this study are seen as opportunities for further researches and these researches will further address problems concerning bioethanol production.

5. ACKNOWLEDGEMENTS

We would like to acknowledge Dr. Perla Florendo and her staffs from Philippine Carabao Center - Central Luzon State University (PCC-CLSU), Nueva Ecija.

6. REFERENCES

Balat, M (2010). Production of bioethanol from lignocellulosic materials via the biochemical pathway: A review. Energy Conversion and Management.pp 1-15.

Billa, E, Koukios E, Koullas, D, and Monties, B, (1996). Structure and composition of sweet sorghum stalk components. Industrial Crops and Products 6. pp. 297-302.

Dijkstra J., Forbes J. M., and France J. (2005), Quantitative Aspects of Ruminant Digestion and Metabolism.

Gundran, R. and Mingala, C. (2007). Assessment of water buffalo health and productivity in a communal management system in the Philippines. Trop Anim Health Prod 40.pp 61-68.

Hu Z. H., Gang W., and Yu H.Q. (2004). Anaerobic Degradation of Cellulose by Rumen Microorganisms at Various pH Values. Biochemical Engineering Journal; 21: pp. 59-62.

Huibregts, M.A.J., and Reijnders, L. (2009). Frequently Asked Questions in the Transport Biofuel debate.Springer.pp 149-162.

Liu, C., and Wang, F. (2008). Sweet sorghum: A promising crop for bioethanol. Journal of Biotechnology 1365.p S456.

Theodorou M. K., and France (2005). Quantitative Aspects of Ruminant Digestion and Metabolism (2nd Edition). Pp. 207-210.