ISERME2024 Proceedings
2nd September 2024 – Sapporo, Japan
https://doi.org/10.31705/ISERME.2024.9

Optimization of Rice Straw Hydrolysis to Convert Ligno-Cellulose to Simple Sugars

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Abstract

Development and use of renewable energy is a key option towards poverty alleviation and to mitigate global warming. Annual rice straw yield of 2.7 million metric tons in Sri Lanka is the most abundant lingo-cellulosic crop residue for bioconversion into ethanol. This research focused on the hydrolysis phase in ethanol production to optimize the rice straw digestion in the saccharification process. In this study, four anaerobic leaching columns in which the base filled with gravel and sand were used. Except for the control, the other three contained an additional soil layer of 60 mm that was obtained beneath a pile of decomposing rice straw. Then, 500g of rice straw was added to each column. In one of the soil added columns, elute was re-circulated. Rice straw to water ratio was maintained at 1:20 by adding water and straw. Samples were taken at daily basis, except for non-recycled one with analysis of initial and final day for pH, conductivity, TDS, salinity, brix, TS, VS, VSS, TSS, VSS, and BOD.

The non-recycled, soil added column gave increased values of conductivity, TDS, salinity, TSS, VSS, and well-grown white mycelia on straw. Highest values of measured parameters were reported in re-circulated column. The increment of cumulative conductivity, TDS, salinity, VS, TSS, and VSS, of non-recycled, soil added column was significant rather than the control. In applying Michaelis Menten kinetics, Vmax and Km for the column without soil are 0.33 and 26.7, with soil were 0.42 and 18.37 and with re-circulation were 7.22 and 0.15, respectively. The latter with higher rate of conversion with less inhibition, proved better affinity between substrate and enzyme. The elusions of TS for without and with soil were 58.0g and 71.5g. These converted to 40.1g and 50.8g of simple sugars and they were 8.0% and 10.1% of total straw weight, expressing in cellulose mass, varied between 22.9%-26.7% and 29%-33.8% of simple sugars. Thus, deduced energy contents are 1.03-1.40 MJ/kg and 1.30-1.77 MJ/kg. Hence hydrolysis of rice straw with soil, has a potential to recover 175 million liters of ethanol.

Keywords: Hydrolysis, Rice Straw, Sugars, leaching columns

1. Introduction

Among major barriers towards a sustainable development, Sri Lanka faces inadequacy of energy, environmental degradation and poverty among both urban and rural communities. Moreover, with the increasing rate of annual energy demand of 10%, with increasing population and investment activities, supplying energy based on fossil fuel and hydropower is no more practicable and alternatives are needed to be encouraged. To overcome the issues, conversion of agricultural crop residues and other biological by products into an energy source is an appropriate option. Manufacturing bio-fuel using rice straw is one possibility in Sri Lanka as the country producing about 2.7 million metric tons of paddy resulting almost same amounts of paddy straw annually. This research focused on the hydrolysis phase in ethanol production to optimize the rice straw digestion in the saccharification process.

2. Materials and Methods

In this study, four anaerobic leaching columns in which the base filled with gravel and sand were used. Except the control, the other three contained an additional soil layer of 60 mm that was obtained beneath a pile of decomposing rice straw. Then, 500g of rice straw was added to each column. In one of the soil added columns, elute was re-circulated. Rice straw to water ratio was maintained at 1:20 by adding water and straw. Samples were taken at daily basis, except for non-recycled one with analysis of initial and final day for pH, conductivity, TDS, salinity, brix, TS, VS, VSS, TSS, VSS, and BOD.

3. Results and Discussio

3.1 Variation of pH value of Columns

The pH value of column with and without soil varied in between 4.5 and 6.5, indicating acidogenic conditions within the column. The pH value of elute for the column with recirculation varied within the range of 6-7, which is an indication of the optimum pH value for hydrolysis

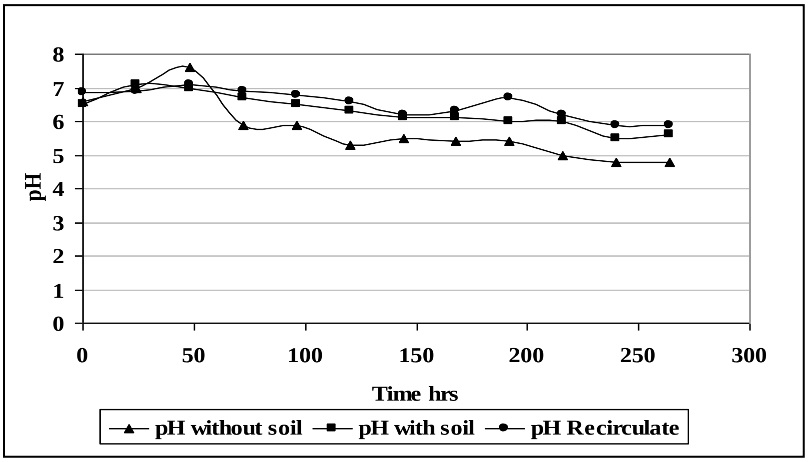


Figure 1 Comparison of pH variations in anaerobic leaching columns

3.2 Conductivity variation of elute with time

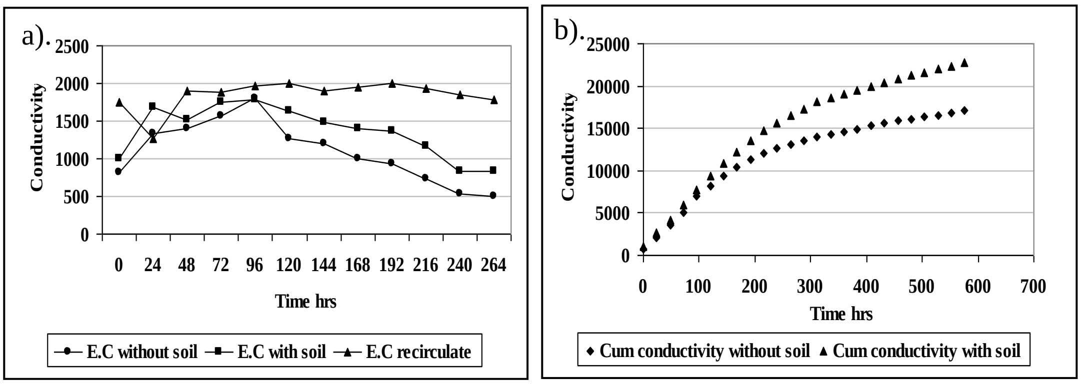


Figure 2 a). Conductivity variations of columns b). Cumulative conductivity variation

The cumulative conductivity of column with soil and without soil, have similar pattern of increasing rates. But a distinct increment of conductivity is illustrated by the column with soil at the latter part of the graph. Among the anaerobic leaching columns the column which recalculated, illustrates the higher conductivity values, (Figure 2) indicating the maximum release of inorganic ions due to rapid microbial activity by intense contact of soil microbes with the substrate.

3.3 Total Dissolved Solid (TDS) variation of elute with time

Among the anaerobic leaching columns, the column with recirculation (Figure 3), show higher TDS. In the primary phase, TDS of column with and without soil remained slightly above 400g/l and 200g/l, respectively, although the TDS in recirculation column remained above 800g/l indicating highest availability of substrate for microbes due to intense mixing. The mixing action has increased the TDS concentration [1], [2], [3], thus, increase in TDS implies a high concentration of volatile fatty acid (VFA) and ethanol [3].

a). b).

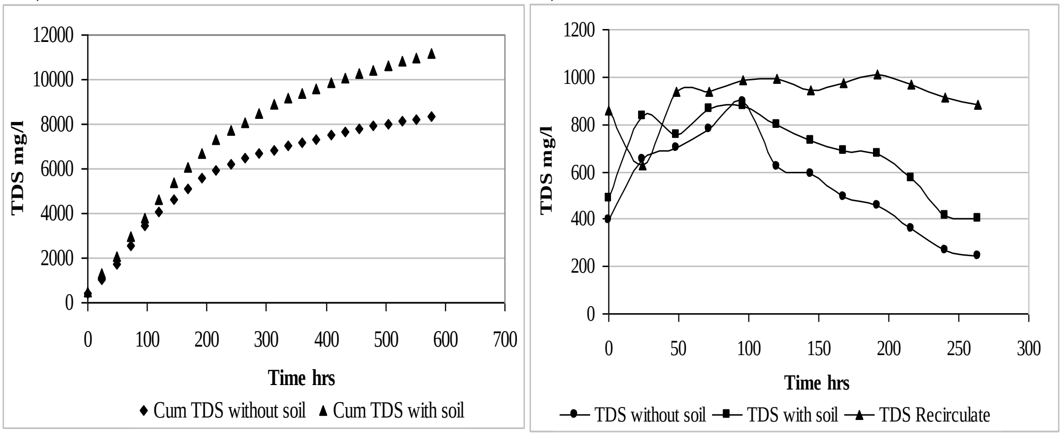


Figure 3 a). Total Dissolved Solid Variation in all columns b) Variation of Cumulative Total Dissolved Solid in all columns

3.4 Total solid (TS) variation of elute with time

a). b).

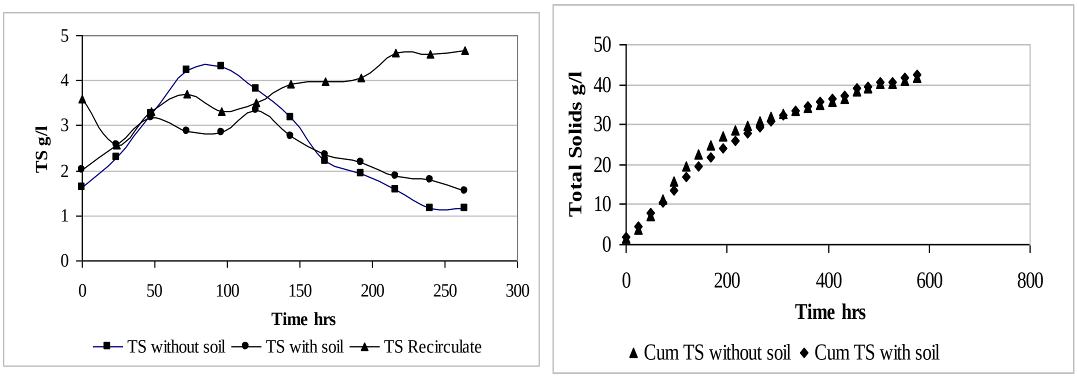


Figure 4 (a.) Total Dissolved Solid Variation in all columns b). Variation of Cumulative Total Dissolved Solid

Total Solids of column with and without soil remained slightly above 1.5g/l and 1g/l respectively, although the TS of the column with recirculation remained above 3g/l indicating highest availability of substrate for the growth of microbes due to more affinity of soil microbial enzymes with rice straw.

3.5 Total Suspended Solid (TSS) variation of elute with time

Cumulative TSS content increased in column with and without soil in similar pattern. With the progress of time, the difference in cumulative TSS content increased in columns with and without soil. TSS of column with and without soil remained slightly above 0.1g/l, although the TSS of column with recirculation remained above 0.5g/l indicating higher microbial population within leaching column.

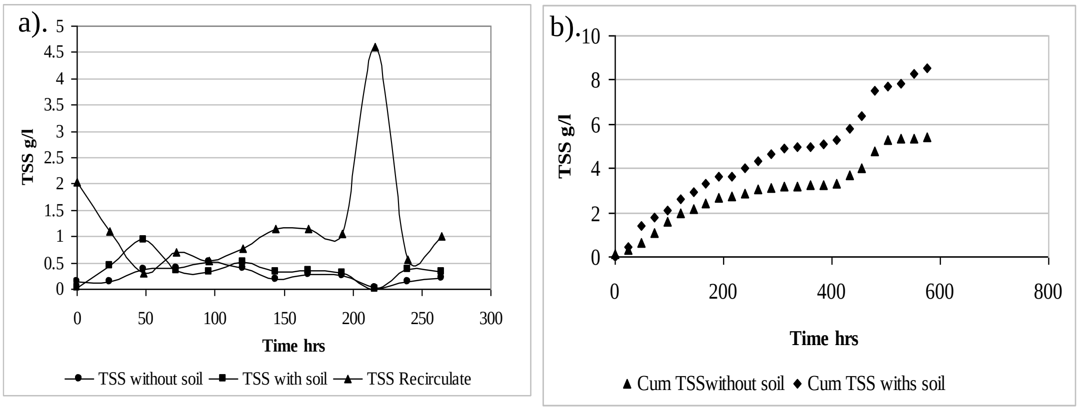


Figure 5 a). Total Suspended Solid Variations in all columns b) Variation of Cumulative Total Suspended Solid Variations

3.6 Volatile Solids (VS) variations of elute with time

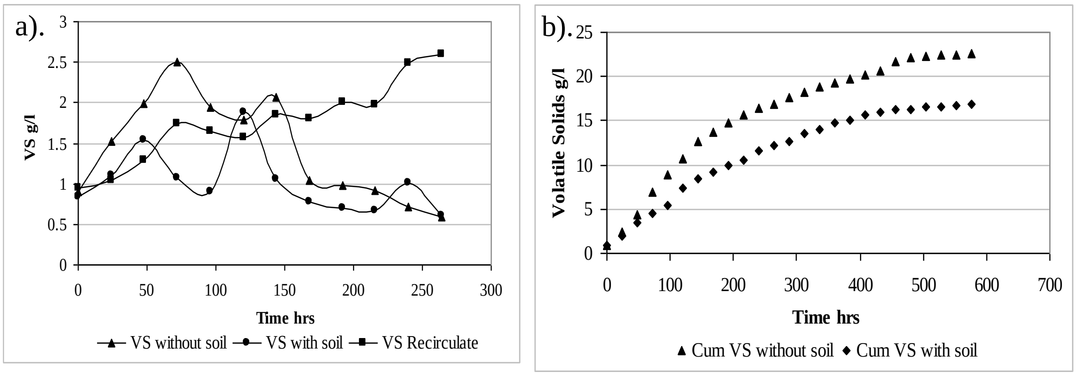


Figure 6 a). Total Volatile Solids Variations Figure 6 in all columns b). Variation of Cumulative Volatile Solids

After a lapse of time, the difference in cumulative VS values increased because of microbial population within columns utilized volatile solids at a higher rate for their reproduction. Column with soil had higher cumulative VS values than the column without soil, indicating higher utilization of volatile solids by soil microbes. Volatile Solids of column with and without soil remained slightly above zero level at 0.5g/l and 1.0g/l, respectively, although the VS of the column with recirculation remained above 1.5g/l indicating higher soil microbial utilization of volatile solids.

3.7 Volatile Suspended Solid (VSS) variation of elute with time

Like most of the parameters, the cumulative VSS contents increased in column with and without. With time, the difference in cumulative VSS values increased because the rate of biomass production increased. The column with soil has higher cumulative VSS values than the column without soil due to presence of higher amount of active microbial cells. VSS of column with and without soil did not exceed 0.6g/l and 0.4g/l, respectively, although the VSS of the column with recirculation did not exceed 0.8g/l indicating higher production of biomass.

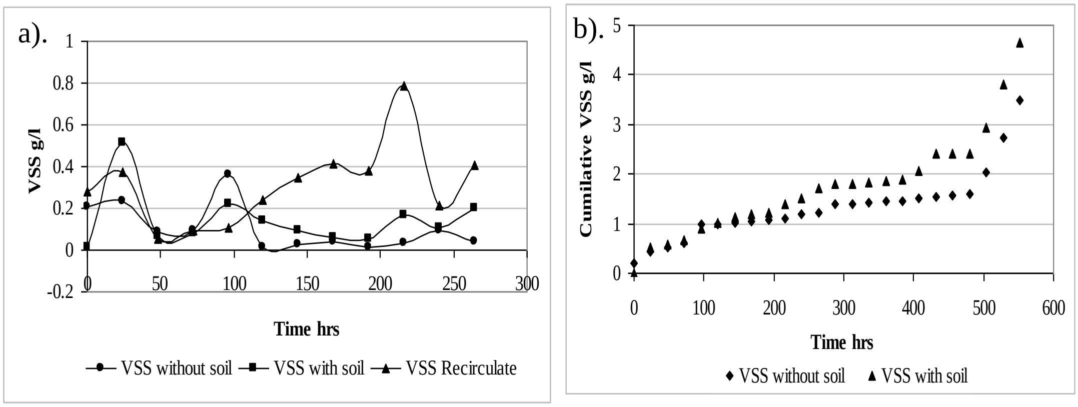


Figure 7 a) Volatile Suspended Solid Variation b). Variation of Cumulative in all columns Volatile Suspended Solids

3.8 Biological Oxygen Demand (BOD) variation of elute with time

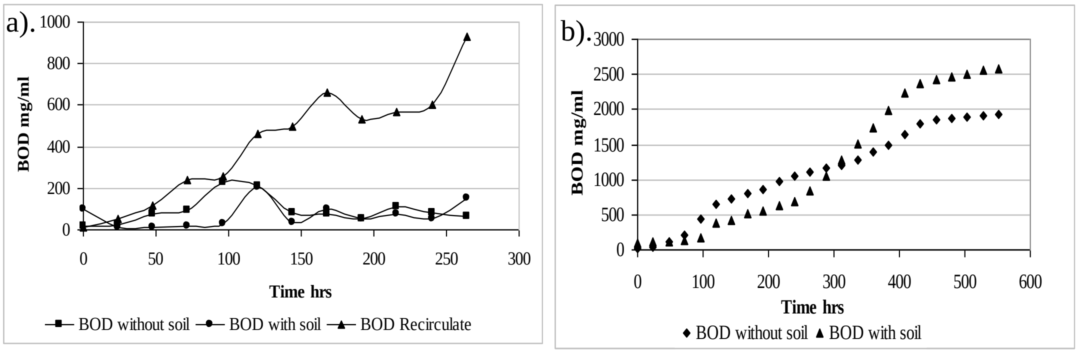
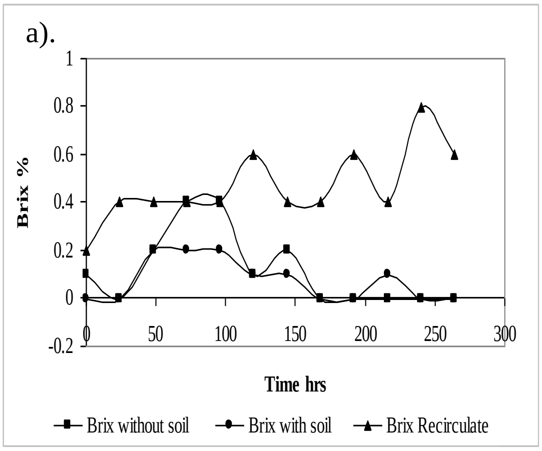


Figure 8 a). Biological Oxygen Demand in all columns b). Variation of Cumulative Variation Biological Oxygen Demand

The cumulative values interpreted higher cumulative BOD_5 values initially for column without soil and in the latter phase an opposite effect. In fact, it was the reverse order for the column with soil. BOD_5 of column with and without soil did not exceed 250 mg/l and 200 mg/l, respectively, although the BOD_5 of the column with recirculation reached 930 mg/l, indicating marked increment due to biodegradation process, which was enhanced by the elute recirculation and reduction of the substrate particle size [4].

3.9 Brix (Sugar content) variation of elute with time

The column without soil had more Brix values than the column with soil in the primary phase due to more conversion of cellulose and less utilization of simple sugars by microbes within the system. In the next phase, more brix values for column with soil due to rapid activity of cellulolytic bacteria in soil. The column with recirculation, sugar content increased, reached to a plateau and once again increased distinctly till the maximum, maintaining more Brix values than other columns, indicating the highest conversion rate to simple sugars due to intense activity of cellulolytic soil microbes such as *Trichoderma spp*.



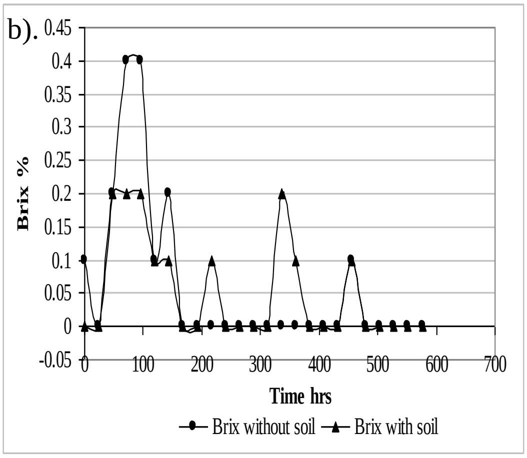


Figure 9 a). Simple Sugar content (Brix) Variation in all columns b). Variation of Cumulative Simple Sugar content (Brix)

3.10 Mycelia growth on rice straw

Well grown mycelia on rice straw were observed from the column with soil while removing the straw samples from the columns at the end of the experimentation. Mycellia is a characteristic of fungi. Cellulose degradation is a common trait among fungi within both Ascomycota and Basidiomycota (Rayner and Boddy, 1988; Cooke and Rayner 1984; Lynd *et at.*, 2002). Therefore, the observed mycelia may be a cellulolytic soil fungi. Fungi such as *Tricoderma*, *Penicillium, Fusarium, Humicola,* and *Schizophillum spp.*, can produce cellulases and hemicellulases. *Trichoderma* spp. are fungi that are present in nearly all soils and other diverse habitats and most prevalent culturable fungi

Figure 10. Well grown mycelia on rice straw obtained from column with soil

3.11 Enzyme kinetic analysis for anaerobic leaching columns

The plot of Lineweaver-Burke gives a straight line relationship for increasing cells as the substrate was utilized. It was found that Vmax and Km for the column without soil are 0.041 and 13.94 respectively, with soil Vmax = 0.33 and Km = 30.69 and with recirculation of elute, Vmax = 7.22 and Km = 0.18. Therefore, the lower Km value of elute of recirculation column indicates that there is a higher affinity between the substrate and enzyme (http://en.wikipedia.org/wiki/Michaelis-Menten). According to the Michalis Menten kinetic models, the column with soil, see Figure 11 a), it could be interpreted as an inhibition effect, but the inhibition effect is less in the column with recirculation.

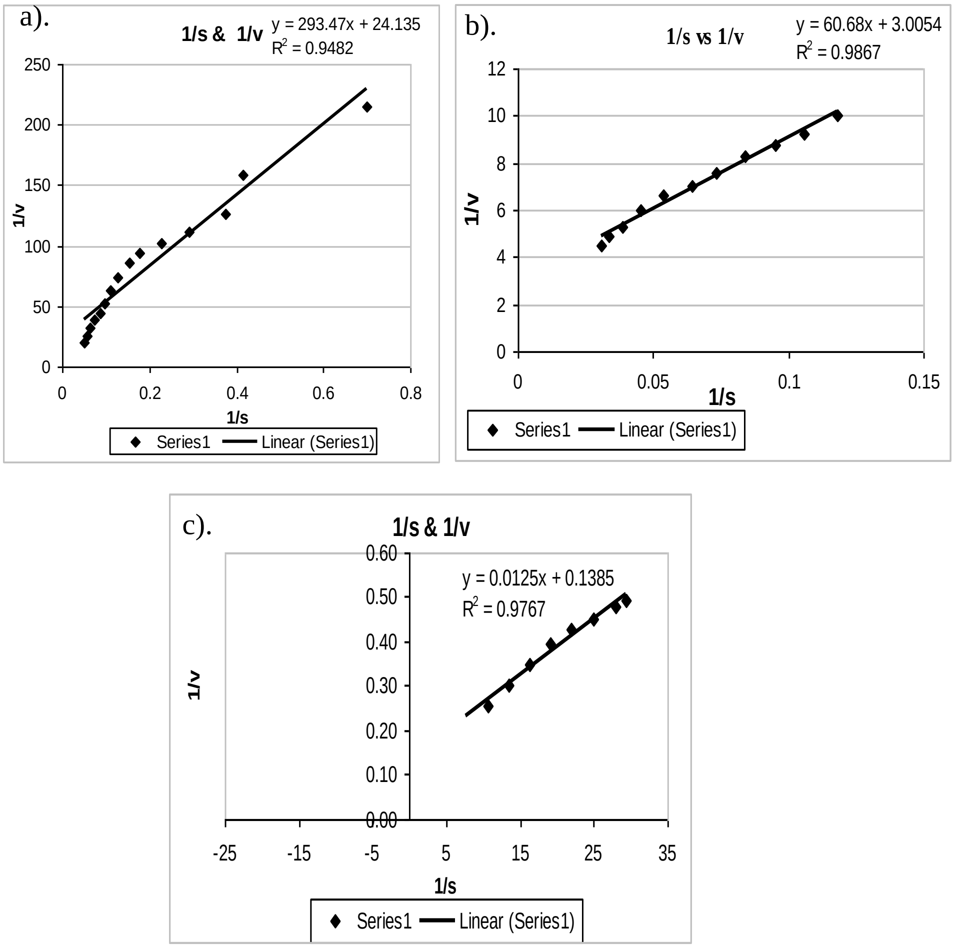


Figure 11 a.). Kinetic model for column without soil b). Kinetic model for column with soil c). Kinetic model for column with recirculation

Furthermore, in applying Michaelis Menten kinetics, it was found that Vmax was lower and Km higher in comparison to the straw column with soil, thus, resulting a very slow reaction in the column with only straw. In both of these columns, there seems to be inhibitions, since the column with recirculation obtained values of Vmax very much higher and the Michaelis constant, Km was lower than the other two conditions. Therefore, the lower Km value with a high rate of conversion shown by re-circulated column proved better affinity between substrate and enzyme. The kinetic study revealed that there are three cycles and hydrolysis reactions take place up to 28days before reaching acidogenic reactions.

4. Conclusion

The leaching columns with and without soil did elute considerably and can be substantiated in terms of sugar formations of just above 10% and 8% of total straw weight, respectively. It can be concluded that the energy conversion rate of rice straw was 16.2%. In could be deduced that hydrolysis of straw with soil, have a potential to recover 133 million liters of ethanol. However, filtration of elute through a water filter element gave only 3% of sugar and it is not sufficient for the fermentation process. It requires a filtration process that concentrates sugar content in the mash solution. It needs the development of a membrane to concentrate the sugar.

References

[1] R. Weerasekera, "Development of first stage Reactor of two-phase Anaerobic Digestion for treating MSW", B.Sc. dissertation, Department of Agricultural Engineering, University of Peradeniya, Peradeniya, (2004).

[2] S.N.M. Manikpura, "Evaluation of the Hydrolytic/Acidogenic First Stage Anaerobic Reactor for Maximum Elute Extraction". B.Sc.dissertation, Department of Agricultural Engineering,University of Peradeniya, Peradeniya, 2005.

[3] K. P. K. Jayakody, S. N. M. Menikpura, B.F.A.Basnayake, and R.Weerasekara, "Development and Evaluation of Hydrolytic/Acidogenic first stage anaerobic reactor for treating municipal solid waste in developing countries," in international Conference on Sustainable Solid Waste Management, Chennai, India, 2007, pp363-369

[4] C.H. Benson, M.A.Barlaz, D.T.Lane, J.M.Rawe, (2007). "Practice review of five bioReactor/recirculation landfills". Waste Management.vol. 27, issue 1 pp. 13–29.

[5] R. C. Loehr, Pollution control for agriculture. 2nd ed., Orlando: Academic Press, UISA, 1984.