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# ENHANCEMENT OF ANAEROBIC DIGESTION OF SISAL LEAF DECORTICATION RESIDUES BY BIOLOGICAL PRE-TREATMENT

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## ABSTRACT

In recent years, the use of agro-industrial residues as feedstocks for biogas production has gained great attention worldwide due to limited reserves of fossil fuels. The sisal industry in Tanzania generates large quantities of sisal leaf decortication residues (SLDR) with good potential for biomethane production. However, the process is limited by the lignocellulosic nature of SLDR, making it not effectively degraded under anaerobic conditions. The effects of biological pretreatment of SLDR under solid state fermentation with a ligninolytic CCHT-1 strain and *Trichoderma reseei* at different inoculation rates and incubation periods to improve methane production were investigated. The methane production potential of the pretreated substrate was determined in batch anaerobic bioreactors. SLDR was pre-treated with CCHT-1 for 4 days at an inoculation rate of 10 % (wet weight inoculant/ SLDR) gave methane yield of 0.203±0.019 $m^3$ $CH_4$/kg $VS_{added}$, while pre-treatment of SLDR with *T. reseei* for 8 days at an inoculation rate of 25 % (wet weight inoculant/SLDR) gave methane yield of 0.192±0.024 $m^3$ $CH_4$/kg $VS_{added}$.This was an increment of between 24 to 30% in methane yield, compared to 0.145±0.015 $m^3$ $CH_4$/kg $VS_{added}$ obtained for the untreated samples. In conclusion, the results demonstrated the suitability of biological pre-treatment method using fungi for enhanced anaerobic digestion of SLDR.

**Keywords**: sisal leaf decortication residue, biological pre-treatment, anaerobic digestion, enhancement, fungi.

## INTRODUCTION

Anaerobic digestion technology previously employed in waste water treatment has been widely applied in management of various solid organic wastes, such as animal manure, food wastes, agricultural residues, and municipal solid wastes, which differ from wastewaters due to their high insoluble organic matter content and chemical oxygen demand (COD) (Yanfeng, H., Yunzhi, P., Yanping, L., Xiujin, L. and Kuisheng, W., 2008). During the process, the biomass is transformed into biogas, a mixture of methane and carbon dioxide, which is a clean renewable energy and organic compost after aerobic stabilisation of the digestate (Neves, L., Ribeiro, R., Oliveira, R. and Alves M.M., 2006). The need to supply energy to drive economic growth is one of the key global development challenges. A particularly desirable option is production of biogas from renewable organic biomass for sustainable energy provision. Therefore the search for alternative energy sources from organic biomass by anaerobic digestion is an ongoing effort in developed and developing countries.

Tanzania is one of the major producers of sisal in the world. The total holding of the sisal sector is about 235,000 hectares of which 165,000 hectares are suitable for sisal development (Sheya, S.M. and Mushi, S.J.S., 2000). The industry generates 100 $m^3$ and 25 tonnes of waste water and solid residues, respectively per tonne of sisal fibres produced. Production of 45,000 tonnes sisal fibre in the year 2007 resulted in the generation of 4.5 million $m^3$ of sisal decortication wastewater and 1,125,000 tonnes of solid sisal decortication residues composed of about 900,000 tonnes of SLDR, the rest being short fibres residues (Mshandete, A.M, Björnsson, L., Kivaisi, A.K., Rubindamayugi, M.S.T., Mattiasson, B., 2008a). Currently in most cases, both sisal solid residues and wastewater are disposed of untreated resulting in serious environmental pollution problems (Mshandete, A.M, Björnsson, L., Kivaisi, A.K., Rubindamayugi, M.S.T., Mattiasson, B., 2008a). On the basis of recent investigations by Mshandete, A.M., Björnsson, L., Kivaisi, A.K., Rubindamayugi, M.S.T., Mattiasson, B., (2008b), SLDR are a suitable substrate for biogas production and already they are used as a feedstock of a large scale biogas plant at one of the sisal factories in Tanzania. However, their bioconversion efficiency appears to be limited by their lignocellulolytic nature.

The biodegradability and hence, biogas potential, of lignocellulosic substrate depends mainly on the composition of the cellulose, hemicellulose and lignin fractions. A direct correlation between the biodegradability fraction and the lignin content has been reported by Hartman, H. and Ahring, B. K., (2005). The physical structures and chemical compositions of lignocellulosic materials could be altered through various methods of pretreatment making them more accessible and more readily biodegradable to anaerobic microorganisms, which would increase digestion efficiency and biogas production (Yanfeng, H., Yunzhi, P., Yanping, L., Xiujin, L. and Kuisheng, W., 2008).

The effect of pre-treatment of lignocellulosic material has been recognized for a long time (McMillan, J.D., 1994). Van Lier, J.B., Tilche, A., Ahring, B.K, Macarie, H., Moletta, R., Dohanyos, M., Hulshoff pol, L.W., Lens, P. and Verstaete W., (2001) postulated that, future developments of anaerobic treatment of lignocellulosic materials would be the enhancement of the

process by pre-treatment as a core technology in recycling processes. Therefore, in recent years considerable efforts have been made to further improve the performance of anaerobic digestion of different wastes, especially solid wastes, by means of pre-treatment (Ward, A..J., Hobbs, P.J., Holliman, P.J. and Jones, D.L., 2008). Pretreatment prior to anaerobic digestion has been proven to be one of simple and effective methods to improve biodegradability and biogas production of lignocellulosic materials (Yanfeng, H., Yunzhi, P., Yanping, L., Xiujin, L. and Kuisheng, W., 2008). Pre-treatment can be mechanical, physico-chemical or biological or in combinations. Biological pre-treatment methods have not been developed as extensively as physical-chemical methods for improving hydrolysis of lignocellulosic substrates such as sisal residues. However, these methods have the advantage that they are simple and do not require major capital investments (Mshandete, A.M., Björnsson, L., Kivaisi, A.K., Rubindamayugi, M.S.T., Mattiasson, B., 2008c). The ability of aerobic pre-treatment to enhance the subsequent mesophilic anaerobic digestion of SLDR using an activated sludge mixed population as a source of inoculum has been investigated in batch cultures. Nine hours of pre-treatment of SLDR prior to anaerobic digestion demonstrated a 26% higher methane yield when compared to the SLDR without pre-treatment (Mshandete, A.M., Björnsson, L., Kivaisi, A.K., Rubindamayugi, M.S.T., Mattiasson, B., 2005).There are however, no other published reports regarding biological pre-treatment of SLRD aimed at improved subsequent biogas production. Therefore this study investigated for the first time biological pre-treatment of SLDR with two fungal species Trichoderma reesei and strain CCHT-1 which could improve the subsequent anaerobic digestion for increased biogas production.

## MATERIAL AND METHODS

### Substrate and anaerobic inoculum

SLDR, a leafy biomass produced during sisal decortications was obtained from a sisal-processing factory at Hale sisal estate, Tanzania. An active anaerobic inoculum used in this study was obtained from a 10 year old pilot batch manually stirred tank bioreactor digesting SLDR at Hale Sisal Estate. Twenty-five litres plastic containers with airtight lids were used to carry the inoculum at ambient temperature to the laboratory. The SLDR was characterised and stored at-20 °C until used. The composition of the substrates is shown in Table-1.

**Table-1**. Composition of SLDR (mean ± SD).

| Determination | Fresh SLDR |
|---|---|
| Total solids (TS) % | 14.1 ±0.1 |
| Volatile solids (VS) (% of TS) | 85.5±0.6 |
| Ash (%TS) | 14.5±0.6 |
| Organic carbon[a] | 48.3±0.2 |
| Total nitrogen[a] | 1.78±0.9 |
| Carbon:Nitrogen ratio (C:N) | 24 |
| Neutral detergent fibres (NDF) [a] | 45.5±0.7 |
| Acid detergent fibres (ADF) [a] | 43.0±0.2 |
| Lignin [a] | 9.5.±2.1 |
| Cellulose[a] | 68.6±1.6 |
| Hemicellulose[a] | 5.5±0.7 |

All values are averages of triplicates
[A] % of dry weight

### Microbial cultures

Two pure cultures were used as inocula for biological pre-treatment. *Trichoderma reesei* QM-9414 was generously supplied by the Department of Biochemistry, Uppsala University, Sweden and strain CCHT-1 was obtained from dumps of decomposing sisal residues at Hale sisal Estate in Tanzania where it was found growing under natural environmental conditions. *Trichoderma reesei* was selected for pre-treatment in light of its capacity to secrete readily and in large quantities a complete set of extracellular cellulases, for the degradation of crystalline cellulose (Penttilä M., Limon, C. and Nevalainen, H., 2004). Pure cultures of the two fungi were maintained on 2% malt extract agar slants at a temperature of 4$^{o}$C and were grown at 27±1°C on plates of the same medium for inoculum preparation. Inocula of the two microbial strains were prepared as described in the case of mushroom spawn production according to Stamets, P., (2000) using sterilized wheat grains and expanded on sisal fibre dusts which was then used in pre-treatment as an inoculum.

### Bioreactors

Pre-treatment of SLDR with the fungi was carried out in rectangular plastic containers measuring 23cm x14cm x 9cm (Cello® Domestoware (Mkate), Tanzania). A total of 136 aeration holes of 0.7 cm in diameter and 3cm apart from one another were made in all the container sides. The anaerobic digestion experiments with the treated residues and control (un-treated residue) were performed in 0.5 l bioreactors consisting of wide mouth Erlenmeyer conical flasks with a working volume of 260 ml as described by Mshandete, A.M., Björnsson, L., Kivaisi, A.K., Rubindamayugi, M.S.T. and Mattiasson, B., (2005).

**Experimental strategy**

To minimize depletion of fermentable sugars during fungus culture incubation and making them available for methane production, the optimization of microbial inoculum concentration and the incubation period was done in this study. Pre-treatment using two fungi was investigated based on the existing knowledge on microbial enzymes and the physical events in the anaerobic digestion process. It was presumed that, the methane yields would increase with inoculation rates as well as pre-treatment periods to up to optima points.

**Pre-treatment of SLDR**

Pre-treatment of SLDR with different inoculum concentration of CCHT-1 and *Trichoderma reesei* was done under solid state fermentation (SSF) prior to anaerobic digestion in batch anaerobic bioreactors. Inocula concentrations of, 1, 3, 5, 10, 15, 20, 25, 30, 40, and 50 % for CCHT-1 and *Trichoderma reesei* (inoculum/SLDR wet weight) were used to inoculate 450 gram of SDLR (moisture content approximately 50-60%. A control (un-treated) SLDR was included and all the SSF containers were incubated for a period of ten days at ambient temperature of 28±2 °C.

To determine the optima pre-treatment periods, five different periods of 2, 4, 6, 8, and 10 days were investigated for both species under solid state conditions in bioreactors described above. Optima inocula concentration of 10% and 25% for CCHT-1 and *Trichoderma reesei*, respectively were used. The unpretreated substrate was included as a control.

**Anaerobic digestion experiments**

The anaerobic digestion experimental set-up for the pre-treated substrates consisted of 35 batch bioreactors. The volume of anaerobic inoculum added to all digesters was kept constant at 200 ml (5.84 gVS). Each digester was fed with 5.84 gVS of pretreated substrate in the ratio of 1:1 ratio (substrate:inoculum). One bioreactor containing untreated SLDR was included as a control and a digester containing only the anaerobic inoculum was included for background biogas which was subtracted from that produced in the digesters containing the substrate (SLDR). The bioreactors were run in triplicates at an ambient temperature of 28±2°C and shaken manually for one minute thrice daily to provide substrate mixing. The methane content was determined after every 48 hours prior to biogas volume measurement as described in the analytical section.

**Analytical Methods**

The generation of biogas was measured every two days. The composition of 5 ml samples of the biogas was estimated by the absorption of carbon dioxide and hydrogen sulphide in concentrated alkaline solution using serum bottles as described by Ergüder, T.H., Tezel, U, Güven, E, and Demirer, G.N., (2001). The volume of biogas produced during the experiment was measured using a graduated 100 ml gas-tight plastic syringe with a sample lock according to Mshandete, A.M., Björnsson, L., Kivaisi, A.K., Rubindamayugi, M.S.T., Mattiasson, B., (2005). Methane yield from the biologically pretreated SLDR was compared with untreated SLDR. The pH before and after anaerobic digestion of the biomass and effluents was determined using a pH 209, meter (Hanna instruments® USA). Total solids, volatile solids (TS, VS) and the ash content of the substrate and inoculum were determined by the oven-drying and ignition method, respectively according to standard methods, American Public Health Association., (1995). Total carbon was determined by the dry combustion method previously described by Allen, S.E., (1989). The organic matter content of the SLDR was done by the dry combustion method previously described by Lyimo, T.J., Pol, A. and Op den Camp, H.J.M., (2002). The total fibres were determined by the permanganate method as Neutral detergent fibre (NDF) and Acid detergent fibre (ADF) according to the method of Goering, H.K. and Van Soest, P.J., (1970). Total nitrogen was determined by the Kjeldahl method according to standard methods standard methods American Public Health Association., (1995).

**RESULTS**

**Effect of pre-treatment of SLDR with different fungal inocula concentrations on methane yield**

Pre-treatment with strain CCHT-1 at different inoculation rates (Figure-1) enhanced the AD process with increasing concentration to an optimum methane yield of 0.203±0.019 $m^3$ $CH_4$/kg $VS_{added}$ at 10% inoculum concentration. Further increase in inoculum concentration during pre-treatment resulted in a decrease in methane yield. The observed increment in methane yield was 30% (Figure-2) in comparison to the untreated SLDR where 0.145 $m^3$ $CH_4$/kg $VS_{added}$ yield was obtained. The Neutral detergent fibre (NDF) content decreased from 45.5±0.71 to 37.5±1.4 during pre-treatment with strain CCHT-1, with a 13.2% decrease corresponding to the highest methane yield after anaerobic digestion. On the other hand, inoculation of the substrate with different inoculum concentrations of *Trichoderma reesei* enhanced the AD process from 0.145±0.015 $m^3$ to 0.192±0.024 $m^3$ $CH_4$/kg $VS_{added}$ (Figure-3) corresponding to methane yield increment of 25 % (Figure-4). Further increase in *T. reesei* inoculum concentration led to a decrease in methane yield with 0.086 $m^3$ $CH_4$/kg $VS_{added}$ being recorded where 50% inoculum concentration was used (38 % decrease in methane yield). The NDF content (%) decreased from 45.5±1.8 to 38.2±1.1 at 50% inoculum concentration. Methane content of the biogas produced varied from 50 to 66% (data not shown) of which the highest was obtained for the pre-treated substrate.

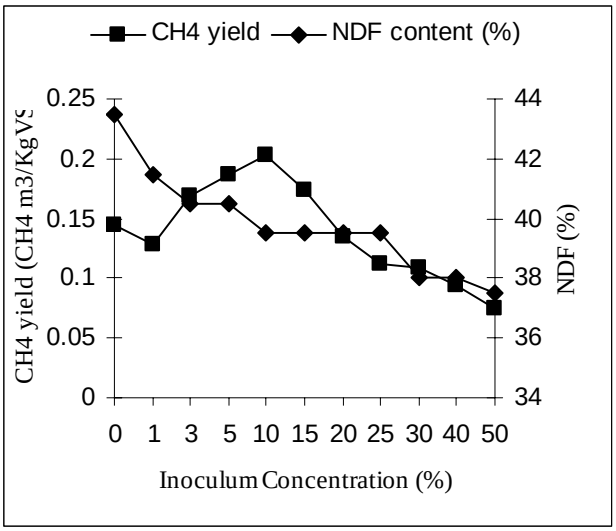


**Figure-1**. Methane yields and NDF content (%) of SDLR pre-treated with strain CCHT-1 at different inoculum concentrations.

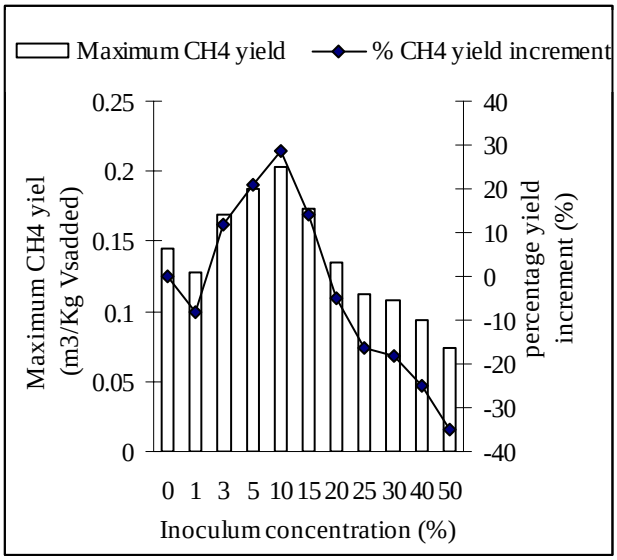


**Figure-2**. Methane yield and increment over the control for SLDR treated with CCHT-1 at different inoculum concentrations.

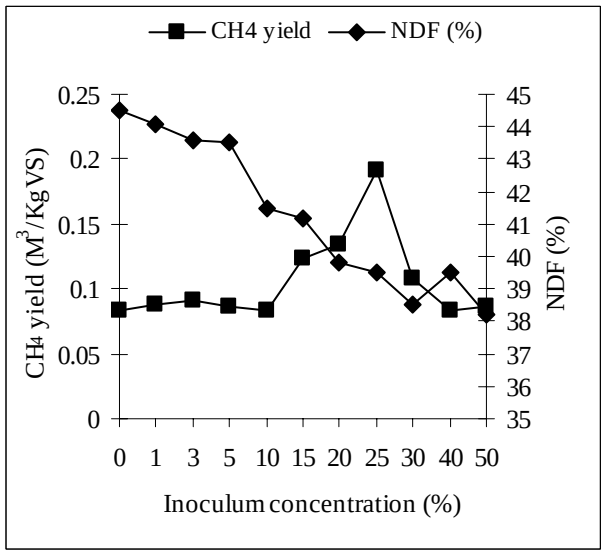


**Figure-3**. Methane yields and NDF content (%) of SDLR pre-treated with *Trichoderma reesei* at different inoculum concentrations.

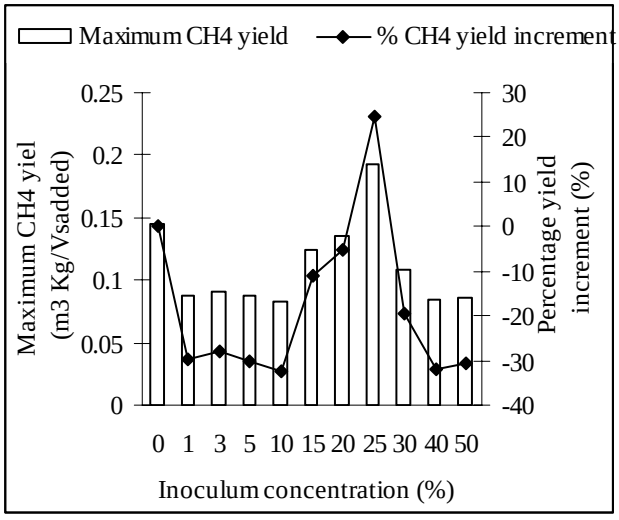


**Figure-4**. Methane yield and increment over the control for SLDR treated with *T. reesei* at different inoculum concentration.

**Effect of pre-treatment of SLDR at different incubation periods on methane yield**

The methane yield and NDF content (%) obtained for SLDR pre-treated with strain CCHT-1 and *Trichoderma* at different incubation periods are given in Figures 5 and 6. The percentage NDF content of the pre-treated SLDR before loading in the batch bioreactors revealed a decrease in the range of 14-23% with increased incubation period. Inoculum concentrations of 10% and 25% for CCHT-1 and *Trichoderma reesei*, respectively which previously gave the highest methane yields were used. The methane yields produced varied from 0.145-0.192 $CH_4$ $m^3$/kg $VS_{added.}$ The highest methane yields (24 % increment) (Figure-7) were obtained for SLDR pre-treated with CCHT-1 after 4 days of incubation. Pre-

treatment with *T. reesei* resulted in low methane yields in comparison to the untreated substrate (Figure-8). The pH values in all the bioreactors were in the range of 7.1-7.8 (results not shown); hence no external buffer was required.

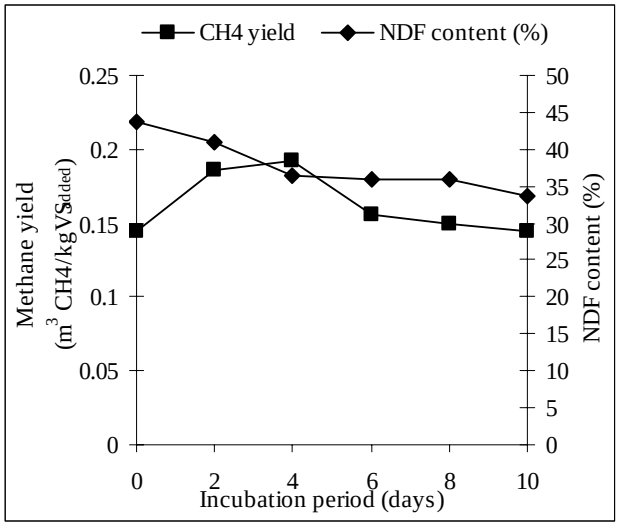


**Figure-5**. Comparison of methane yield with NDF content after pretreatment of SLDR with CCHT-1.

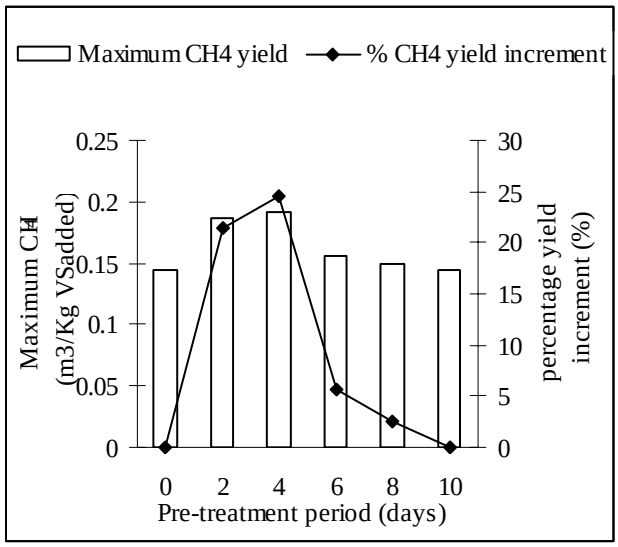


**Figure-6**. Methane yield and increment over the control for SLDR treated with CCHT-1 at different incubation periods.

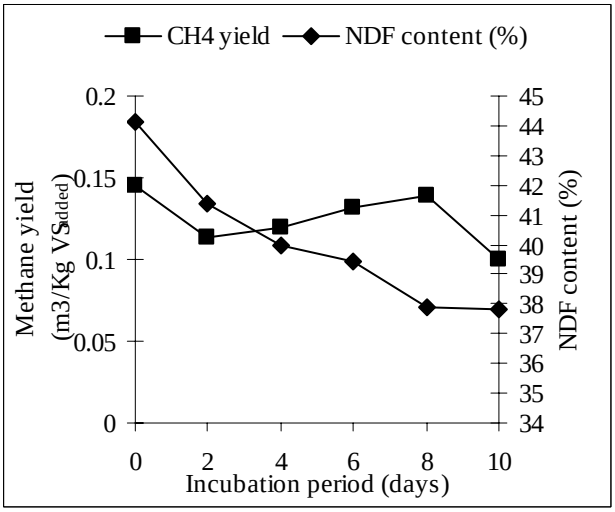


**Figure-7**. Comparison of methane yield with NDF content after pretreatment of SLDR with *T. reesei*.

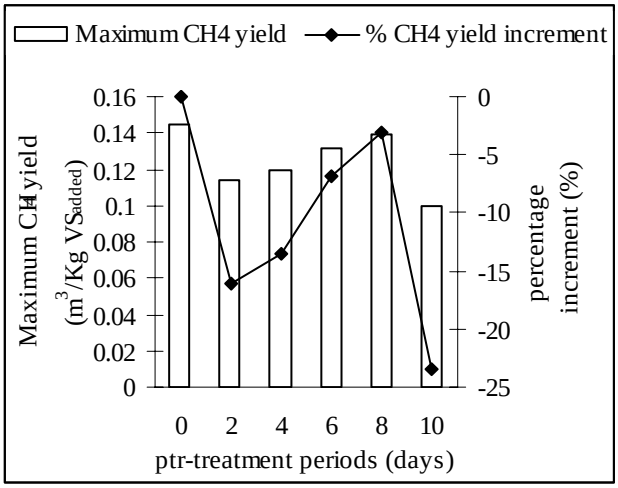


**Figure-8**. Methane yield and increment over the control for SLDR treated with *T. reesei* at different incubation periods.

## DISCUSSIONS

### Effect of pre-treatment inoculum concentration on methane yield

The results in Figure-1 show that there was an increase in methane production with an increase in inocula concentration to an optimum point. The highest methane yields from SLDR pretreated with CCHT-1 and *T. reesei* were obtained at an inoculum concentration of 10% and 25%, respectively. Further increase in inoculum concentration resulted in a decrease in methane yield after anaerobic digestion of the pretreated SLDR. SLDR being plant residues contains lignin which is linked to both hemicelluloses and cellulose forming a physical seal around them, which is an impenetrable barrier preventing penetration of enzymes. This makes it hard for microbial degradation like any other natural polymers (Howard, R.L., Abotsi, E., Jensen, V., Rensburg, E.L. and Howard,

S., 2003). Strain CCHT-1, grows naturally on sisal residues and this could imply that it is lignocellulolytic. To degrade the substrate it most probably secretes extracellular enzymes which degraded the lignin coat in the residues prior to anaerobic digestion process as shown by the decrease in NDF content (Figure-1). The removal the NDF could have enhanced biodegradation of SLDR hence higher methane yields. This observation is in agreement with the early observations that, during pre-treatment, the structural polysaccharides contained in plant material can be partially degraded into intermediates which are suitable for methanogenic fermentation (Egg, R., Coble, C., Engler, C. and Lewis, D., 1993). On the other hand, the increase in methane yield (Figure-3) with pre-treatment with *T. reesei* may be attributed to the disruption of the cellulose structure in SLDR. This resulted in the release of more monomers which could be utilized for methane production. *Trichoderma reesei* has been reported by Mtui, G., and Nakamura, Y., (2005) as a good producer of extracellular cellulases, which degrade the crystalline cellulose this, supports the increased methane yield. Mtui, G., and Nakamura, Y., (2005) observed that aerobic pre-treatment with pure culture of *T. reesei* resulted in improved fibre digestibility which translates to higher methane production. Production of extracellular degrading enzymes is desirable in hydrolysis of sisal residues, which is highly lignocellulosic (Mshandete, A.M., Björnsson, L., Kivaisi, A.K., Rubindamayugi, M.S.T., Mattiasson, B., 2005). The results obtained in this study clearly illustrate that increase in inoculum concentration increases SLDR utilization, and hence gives enhanced methane yields. However, increase in inoculum concentration beyond the optimum resulted in a decrease in methane yield. This can be attributed to removal of more polysaccharide than lignin and also substrate starch, a similar observation reported by Jung, H.G., Valdez, F.R., Hatfield, R.D. and Blanchette, R.A. (1992).

**Effect of pre-treatment periods on the extent *of* methane production from SLDR**

The results on the effects of pre-treatment periods of CCHT-1 and *Trichoderma reesei* on SLDR (Figure-5 and 8) revealed the best incubation periods of 4 days and 8 days, respectively. The highest methane yield from SLDR pre-treated with 10% inoculum concentration was recorded for 4 days of pre-treatment. This represents an increase of 24% in methane yield (Figure-6) compared to the un treated. On the other hand, the best incubation period obtained while pre-treating SDLR with 25% inoculum concentration of *T. reesei* was 8 days, where high methane yield was observed. The NDF% content (Figure-7) indicated that, there was a reduction in the total fibre with an increase in incubation period. Probably, the increase in methane yield with incubation period to an optima, indicate that the biodegradable components of SLDR were released for subsequent methane production. The results obtained for CCHT-1 are in agreement with the observation reported by Hadar, Y., Kerem, Z. and Gorodecki, B., (1993) working on cotton straw who observed, biodelignification by the edible "oyster mushroom", *Pleurotus ostreatus*, followed by 36 h of in vivo ruminal digestion removed 2.2 times more organic material than non-fungal pre-treated controls. On the other hand, Lehtomäki, A., Viinikainen, T.A., Ronkainen, O.M., Alen, R. and Rintala, J.A., (2004) reported recently that white rot fungi treatment of lignocellulosic substrates (21 days at 21°C) and short-term composting (7 days) prior to anaerobic digestion resulted in high losses of organic matter due to biological activity. In this study, prolonged pre-treatment periods resulted in lower methane yields of up to 25%. A similar major drawback was reported by Mshandete, A.M., Björnsson, L., Kivaisi, A.K., Rubindamayugi, M.S.T., Mattiasson, B., 2005, where they observed a loss of 26% and 37% in methane yield following pre-treatment of sisal pulp waste for 48 and 72 h with activated sludge mixed culture. The decrease in methane yields can be possibly due to aerobic degradation of organic material leaving less carbon for methane production.

On the whole, the results of this study are in agreement with the growth pattern of the fungi with a short growth cycle whereby, after 4 and 8 days, respectively, their degradation was highest and decreased with extended periods (maturity). The fungus short life cycle and the optimum incubation periods determined concurs with this. Tripathi, M.K., Mishra, A.S., Misra, A.K., Vaithiyanathan, S., Prasad, R., Jakhmola, R.C., (2008) reported that, white rotted material does not contain much nutrient because white-rot fungi metabolize sugar and starch in preference to lignin and cellulose in cultures. Possibly, decrease in methane yield with increase in inoculum concentration led to a decrease in readily available nutrient for biogas production. However, due to scanty published information on biological pre-treatment of sisal residues, the results of this study could not be adequately compared. The study on the effect of two-steps biological pre-treatment first by CCHT-1 followed by Trichoderma reesei and vise versa on methane yield from SLDR is underway, prior to pilot scale for industrial adoption of this technology.

## CONCLUSIONS

- The results of this study reports for the first time the potential of biological pretreatment of SLDR with CCHT-1 and *Trichoderma reesei* to enhanced biogas production.
- An enhancement of biogas generation by 30-40% was observed by anaerobically digesting pre-treated SLDR. Therefore, it was concluded that anaerobic digestion of SLDR could be enhanced (in terms of biogas yield) by biological pretreatment with optimum inoculation rates and incubation periods for the two fungi.

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