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Full Length Research Paper

Two-stage anaerobic digestion of aerobic pre-treated sisal leaf decortications residues: hydrolases activities and biogas production profile

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A two-stage system was investigated for anaerobic digestion (AD) of aerobically pre-treated sisal leaf decortication residue (SLDR) with regard to hydrolytic enzymes and biogas production. The system consisted of a solid-bed bioreactor for hydrolysis connected to methanogenic bioreactor packed with sisal fibre decortication residues (SFDR) as biofilm carriers. Some of the enzymes produced by microorganisms to hydrolyse SLDR were found to be pectinase, filter paper cellulase, amylase, - glucosidase, carboxylmethyl cellulase, xylanase and protease. Enzyme activities observed in the acidogenic bioreactor were much higher than those in the methanogenic bioreactor. The hydrolysis and the methanogenic stages were well separated, as indicated by the high carbon dioxide production, high volatile fatty acids (VFAs) concentration and low pH in the acidogenic bioreactor compared with high methane production, low VFAs concentration and above neutral pH in the effluent of the methanogenic bioreactor. Digestion of SLDR gave energy yields of 2.45 kWh/kg volatile solids added in the form of methane. The integrity of the methane filter was maintained throughout the period of operation producing biogas with 51 - 70% methane content. A stable effluent pH showed that the methanogenic bioreactor had good ability to withstand the variations in load and VFAs concentrations that occurred in the two- stage process. In conclusion, the results of this study showed that the two-stage system was suitable for effective stabilization and biomethanation of SLDR.

Key words: Anaerobic digestion, two-stage, sisal decortications residues, aerobic pre-treatment, biogas, hydrolases.

INTRODUCTION

One of the main trends of today's waste management policies worldwide is to reduce the stream of waste going to landfills and to recycle the organic material and the plant nutrients back to the soil (van Lier et al., 2001). Anaerobic digestion (AD) is one way of achieving this goal and it will, furthermore, reduce energy consumption or be net energy producing. Also AD for the production of biogas to replace oil and natural gas is in active development around the world and is focusing on the use of cheap organic matter (such as agro-industrial, municipal solid and sewage waste) as feed-stocks in AD (Nishio and Nakashimada, 2007). There has been a fast development in biogas technology and the technology moves forward quickly to high rate anaerobic digestion systems such as two-stage processes for AD of solid agro-industrial wastes (Parawira et al., 2007).

Sisal leaf decortication residue (SLDR) is one of the most abundant agro-industrial residues in Tanzania. About 900,000 tonnes and 225,000 tonnes of SLDR and short fibres residues, respectively were generated for the year 2007 alone (Mshandete et al., 2008a). These residues are disposed off untreated and in most cases

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burnt, dumped in water bodies and/or landfilled, such practices are not sustainable and contribute to environmental pollution (Yu et al., 2002). This is due to lack of suitable and feasible bioconversion technologies for production of value added products from sisal residues such as biogas, bioethanol etc. Nevertheless, recent laboratory AD studies have shown that sisal residues are potential feedstock for biogas production in Tanzania (Mshandete et al., 2004a, 2005a, 2006). This attempt is in line with the core aim for the development of AD technologies to efficiently convert organic solid wastes to methane, which is driven by the need for alternative renewable sources of energy and the need to mitigate greenhouse gases emissions and nutrients leakage from landfills (Yadvika et al., 2004; Burrell et al., 2004; Nishio and Nakashimada, 2007).

Anaerobic digestion is a multi-stage process in which hydrolysis is the first step. During hydrolysis, complex insoluble substrate macromolecules such as polysaccharides are hydrolysed into smaller units by a large number of microbial species that act in concert synthesizing and secreting different hydrolysing enzymes (Cirne et al., 2007). Polysaccharides are converted to simple sugars; hydrolysis of cellulose by the enzyme complex cellulase yield glucose, hemicellulose degradation results in monosaccharides such as xylose, glucose, galactose, arabinose and mannose; while starch is converted to glucose by amylase enzymes (Lai et al., 2001). It is commonly found that during AD of solid lignocellulosic material such as agro-industrial and municipal wastes, crop residues and energy crops both hydrolysis of complex polymeric components and accessibility of hydrolytic microorganisms to the solid matter, constitute the rate limiting-step (Mata-Alvarez et al., 2000; Cirne et al., 2007). Therefore, one strategy is pre-treatment in order to break the polymer, which prevents penetration by microorganisms or extracellular enzymes. Pre-treatments can be carried out in different ways, namely physically, chemically or biologically, or in combination (Gabe and Zacchi, 2007). The first two methods have been extensively studied to improve lignocellulosic substrate properties for enhanced subsequent AD (Sawayama et al., 1997; Held et al., 2002). However, biological pre-treatment methods have not been developed as extensively as physical-chemical methods for improving hydrolysis of lignocellulosic substrates prior to AD. These methods have advantages that they are usually simple and do not require major capital investments (Lissens et al., 2003). To this effect, aerobic thermophilic pre-treatment of sludge waste prior to conventional AD has been reported to improve methane yield by 50%, which was attributed to extracellular enzymes secreted by aerobic bacteria (Hagesawa et al., 2000). On the other hand, Mshandete et al. (2005b) recently, reported enhancement in methane yield of 26% when sisal pulp waste was aerobically pre-treated under mesophillic conditions using activated sludge mixed culture as an inoculum in batch anaerobic bioreactors. However, information on two-stage AD of aerobic pre-treated SLDR with regard to hydrolytic enzymes and biogas production is totally non-existent.

Two-stage AD has been considered more effective than the conventional single-stage systems in the conversion of easily degradable solid substrates to biogas (Kalia et al., 1992; Chanakya et al., 1992). However, with concentrated soluble or high-solid feeds, volatile fatty acids production (VFAs) proceed at a much faster rate than the rate of conversion of VFAs to methane, thereby causing acid accumulation, a drop in pH below 6 and consequent inhibition of methanogenesis (Yu et al., 2002). To avoid such problems, it is desirable to use a two-stage configuration to avert the imbalance between the processes of acidogenesis and methanogenesis, by physically isolating these two major microbial phases in two separate bioreactors. Also two steps process is advantageous in the case of solid wastes, because it permits the separation of the solid and the liquid phase, allowing the operation of high rate anaerobic methanogenesis for the liquid. A two-stage approach has previously been successfully applied to fruits and market waste (Mtz-Vituria et al., 1995), municipal solid waste (Pavan et al., 2000) agro-industrial residues (Stamatelatou et al., 2003), solid potato waste (Parawira et al., 2007). The first stage includes both hydrolysis and acidification, producing leachate with soluble organic compounds, mostly VFAs, from the solid feedstock. The leachate is then converted into biogas in the second, methanogenic stage. The treated liquid is returned to the solid-phase for recirculation through the solid bed/hydrolysis bioreactor (Parawira et al., 2007). The methanogenic bioreactor can be designed as an attached growth bioreactor such as packed bed bioreactor (anaerobic filter) with a long solid retention time due to attachment of biomass on carrier (Hanaki et al., 1994). The microbial growth in a biofilm also has the advantage of protecting the sensitive methanogens from toxic shocks, variations in load and VFAs concentrations (Hanaki et al., 1994; Andersson and Björnsson, 2002; Mshandete et al., 2004b; Mshandete et al., 2008b).

The present study constitutes part of our effort to understand the separation of AD process into acidogenic and methanogenic phases, with respect to the hydrolases acting on solid biomass during anaerobic digestion. Hydrolysis of the complex biopolymers is carried out through integrated action of a variety of the hydrolytic enzymes released in the bulk liquid (free enzymes), attached to particulate matter or cell-bound (Goel et al., 1998; Parawira et al., 2005). However, enzymes released in the bulk liquid were investigated to see if they can explain some of the hydrolysis dynamics in the two-stage system. The feasibility of producing biogas from SLDR was evaluated using two-stage AD system, which consisted of a high-solids hydrolysis/acidification bioreactor and a methanogenic bioreactor with sisal fibre

Table 1. The procedure and conditions employed for enzyme assays during two stage anaerobic digestion of solid sisal decortications residues (Acetate buffer pH 6 (0.05 M)

Substrate (w/v)	Sample supernatant (ml)	Acatate buffer (ml)	Enzyme assayed	pH	Temperature (°C)	Incubation time (min)	Reference
Starch (0.5%)	0.5	1.0	Amylase	6.0	50	30	Giraud et.al. (1991)
Filter paper (50mg)	0.5	1.5	FPase	6.0	50	60	Ghose (1987)
CMC (0.5%)	0.5	1.0	CMCase	6.0	60	60	Ghose (1987)
Salicin (0.5%)	0.5	1.0	-glucosidase	6.0	60	60	Wood and Bhat (1988)
Xylan (0.5%)	0.5	1.0	Xylanase	6.0	50	60	Bailey et. al. (1992)
Pectin (0.5%)	100 l	400 l	Pectinase	6.0	50	30	Gupta et. al. (1993)

decortication residues (SFDR) biofilm carrier in a packed bed.

MATERIAL AND METHODS

Chemicals, substrate and inoculum

The following reagents were used: filter paper, (Whatman grade 41, ash less filter), carboxymethyl cellulose (CMC), xylan (from oat spelts), pectin (from citrus fruits) and salicin (Sigma-Aldrich Co. Ltd, Gillingham-Dorset, UK), soluble starch and azocasein (Sigma Chemical Co. St. Louis, MO, USA). All other chemicals used were of analytical grade. SLDR and SFDR produced during sisal decortications were obtained from a sisal-processing factory at Ubena Zomozi, Tanzania. Activated sludge inoculum (ASI) and anaerobic sludge (ANS) from a municipal wastewater treatment plant at Eslöv, Sweden, were used as inoculum. This plant receives sewage sludge and industrial effluent mainly from potato-processing plant. The SLDR was stored at –20°C until used. Characteristics of SLDR, SFDR and ASI are as previously reported by Mshandete et al. (2005a, b; 2006).

Experimental design

The solid-bed bioreactor (SBB) was fed in batch mode with SLDR and operated with recirculation of the leachate through the bed. The leachate from this bioreactor was fed to the methanogenic bioreactor (MEB) and the overflow was *r*ecirculated through the SBB. The laboratory two-stage system consisted of solid hydrolysis/acidification bioreactor (HAB) and MEB with SFR biofilm carrier in packed bed. HAB consisted of a cylindrical -conical glass with a capacity of 3 litres, had a length of 550 mm and an outer diameter of 94 mm. A wire sieve (3 mm gauge) was installed 50 mm above the bottom of the cone to support the solid waste substrate while still allowing the liquid to pass through it. One thousands two hundred grams of SLDR were loaded into the bioreactor. Tap water 1000 and 400 ml of ASI were added to the bioreactor. Afterwards, the mixture was aerated at a flow rate of 1 litre/min for 9 h (Mshandete et al., 2005b). The airflow rate was determined with an air flow meter (Rota Wehr-2, Sigurdholm AD, Olshammargatan, Stockholm, Sweden). The concentration of dissolved oxygen (DO) during aerobic period was determined with an oximeter (OXi 320, WTW, Germany). After aeration pre-treatment time elapsed, the bioreactor was closed at the top with butyl rubber to maintain anaerobic conditions. The bioreactor was operated at an ambient temperature of 26 ± 0.3°C. The leachate from the HAB containing the microbes from ASI was continuously recirculated at 20 ml/min. and sprinkled over the packed bed of solid SLDR.

The MEB had a volume of 1 litre, with height of 420 mm and an internal diameter of 60 mm. The bioreactor was filled with pre-digested SFDR packing media to facilitate the microbial attachment and growth. The packing had a density of 145 g/l and the SFDR was inoculated with 500 ml of ANS and was operated at mesophilic conditions (35 - 37°C). The MEB had a constant recirculation of 10 ml/min. from top to the bottom and was equipped with gas-tight bag at the top to collect biogas. The MEB was designed to operate as up-flow anaerobic filter (UAF).

System operation

To start the experiment, initially the hydrolysis/acidification stage was operated with internal recirculation of the leachate. The leachate collected at the bottom was sprayed on the top of the bed. Decreasing culture pH, increasing VFAs concentrations and rising carbon dioxide of the bioreactor headspace indicated acidogenic culture development. When the leachate, after 2 - 4 days, reached a level approximately of 18 - 20 g chemical oxygen demand (COD)/l, total VFAs (TVFAs) about 9 g/l and pH 5.1, circulation over the MEB was initiated. The organic loading rate (OLR), 2 - 10 g COD/l/d, varied according to the fluctuations in the COD in the liquid. The average OLR was 5.4 (± 2.8) g COD/l/d. The overflow from the MEB was recycled to the HAB for replenishing and provision buffering capacity to prevent excessive acidification. The purpose of this mode of operation was to alleviate inhibition of the fermentative, acetogenic and methanogenic microorganisms thereby promoting the overall efficiency. The experiment was continued for up to 40 days.

Enzyme assay

Enzyme activities were determined to monitor the progression of acidogenic and methanogenic fermentations. Ten ml liquid samples from HAB and MEB were centrifuged at 13,000 x g for 10 min using a BIOFUGE 13 (Heraeus Instruments, Germany). The supernatant was collected and used for enzyme assays. Reducing sugars produced in the enzyme-treated samples were assayed using the dinitrosalicylic acid (DNS) method of Miller (1959). Acetate buffer (pH 6.0, 0.05 M) was used in all enzyme assays. The reducing sugar produced after incubation was determined at 540 nm using an Ultrospec 1000 UV/visible spectrophotometer. The conditions and procedures employed for analysis of amylase, filter paper cellulose (FPase), Carboxymethyl cellulase (CMCase) -glucosidase, xylanase and pectinase are summarized in Table 1. For amylase, FPase, CMCase, xylanase and -glucosidase one

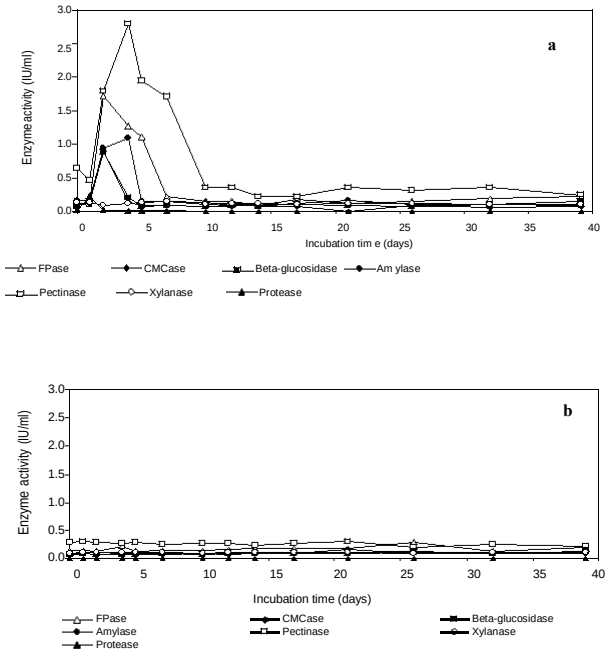

Figure 1. The extracellular enzyme profiles from effluent of (a) the hydrolysis/acidification bioreactor and (b) the methanogenic bioreactor of the two-stage anaerobic digestion of aerobically pre-treated SLDR.

enzyme unit in each case was defined as the amount of enzyme, which releases 1 µmol of reducing sugars under assay conditions per minute. Pectinase activity was assayed by a slight modification of the method by Gupta et al. (1993). Supernatant of 100 µl was mixed with pre-warmed 400 µl of 0.5% (w/v) pectin (from citrus fruits), in acetate buffer (pH 6.0, 0.05 M) and was incubated at 50°C for 30 min. One unit of pectinase activity was defined as the amount of enzyme, which releases 1 µmol of monogalacturonic acid under assay conditions per minute. Protease activity was assayed using the azocasein method. Supernatant of 1 ml was incubated with pre-warmed 1 ml of 0.5% (w/v) azocasein in 200 mM Tris-HCl buffer (pH 7.4) and was incubated for 60 min at 50°C. The reaction was stopped by adding 2.0 ml of 10% (w/v) trichloroacetic acid. The mixture was then centrifuged at 3,000 x g for 10 min. The supernatant was removed and the absorbance read at 380 nm (Kole et al., 1988). The activity of the protease was expressed in arbitrary units, where IU was equivalent to an optic density (OD_{380}) change of 0.1 per min. from the absorbance of the enzyme blank (Mawadza, 1998). The control for all enzymes was Millipore water which replaced the volume of the supernatant added for each particular enzyme investigated.

Analytical methods

VFAs concentrations, pH, soluble COD, alkalinity, gas content and methane production were also determined to monitor bioreactor system performance. The volume of biogas was measured using a wet-type precision gas meter (Schlumberger, Karlsruhe, Germany). Biogas composition was measured using a Varian 3350 gas chromatograph (Walnut Creek, CA, USA) fitted with a Hay sep Q 80/100 mesh column, a molecular sieve column and a thermal conductivity detector. Helium was used as a carrier gas at a flow rate of 12 ml/min. The column temperature was 70°C and the injector and detector temperatures were 110 and 150°C, respectively. The compounds detected were methane, carbon dioxide, nitrogen and oxygen. The partial alkalinity (PA), total alkalinity (TA) and pH were determined according to Andersson and Björnsson (2002). VFAs were monitored with a high performance liquid chromatograph (Varian 9000 HPLC, Wulnut Creek, CA, USA), using a BioRad column 125 – 0115 (Biorad, Hercules, CA, USA). Samples for the analysis of VFAs were centrifuged at 3000 x g for 3 min. and the supernatant was acidified with concentrated sulphuric acid, stored at –20°C awaiting further analysis. The frozen samples were allowed to thaw and then filtered through 0.45 µm filters before analysis (Minisart, Satorius AG, Göttingen, Germany). The COD equivalents of the five VFAs (delivered from complete oxidation reactions of the individual VFAs to CO_2 and H_2O), were used to calculate percentages COD in the form of VFA (Ince, 1998). COD and total nitrogen were determined according to standard methods (APHA, 1998). Analysis of ash mineral (K, P, Ca, Mg, Mn and Na) constituents of anaerobic digested SLDR were analysed using an atomic absorption spectrophotometer (A- Analyst 300 model, Perkin- Elmer Corporation, Norwalk, CT, USA) according to AOAC (2002).

RESULTS AND DISCUSSION

Profiles of hydrolytic enzymes in HAB and MEB

The extracellular enzymes found in HAB (Figure 1a) generally increased rapidly and reached peak values within the first 12 days of AD, although the extent and the time of peak values differed for each enzyme. After 12 days of AD, the activities of the enzymes remained at relatively constant levels throughout the study period. The activity profile of the hydrolases decreased in the following order: pectinase > FPase > amylase > -glucosidase > CMCase > xylanase > protease. Although CMCase -glucosidase, xylanase and protease had peak values, they showed no major change in the activity from the initial levels to the end of the study period. The observed increase in the hydrolases activity for HAB in the first 12 days may be a result of the availability of easily biodegradable material (Figure 3a). The decline in the activity of hydrolases after 12 days of AD showed that substrate become a limiting factor, since the substrates available were utilized by methanogens in the MEB for biogas production. The observed patterns of enzyme activities give an insight into the multi-complex dynamics of extracellular enzyme synthesised and secreted during hydrolysis of SLDR into reducing sugars, which can be fermented to biogas (Mshandete et al., 2005b). This implies that determination of enzyme activities could be a potential tool for anaerobic digestion monitoring, since the hydrolytic enzyme activity will, to some extent, be controlled by the composition of the substrate mixture (Parawira et al., 2005) which could be a step towards the development of strategies to enhance hydrolysis and ultimately increasing the methane production rates and yields from bioreactor-based digestion of lignocelluloses substrates in two-stage AD (Cirne et al., 2007).

The extracellular enzymes activity profiles in MEB which ranged from 0.0012 - 0.30 IU/ml (Figure 1b) were comparable with a range between 0.0002 - 0.35 IU/ml found in HAB after 12 day of AD. However, the enzymes

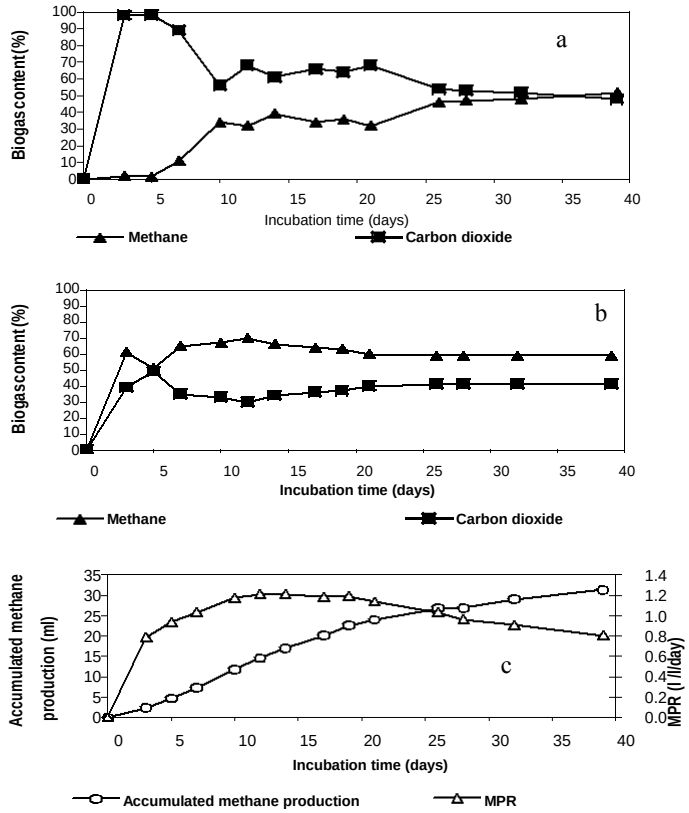

Figure 2. (a) Biogas composition in hydrolysis/acidification stage, (b) biogas composition in methanogenesis stage, (c) methane production rate and accumulated methane production in methanogenesis stage during anaerobic digestion of aerobic pre-treated SLDR.

occurred in lesser but stable amounts throughout the experiment. The presence and activity of extracellular hydrolases in the MEB is possibly not much a result of the activity of methanogens but possibly represents more the carry-over of the enzymes from the HAB. The influent in MEB include the raw substrates, enzymes, end products and microorganisms of the HAB. Microbial population shift between HAB and MEB during AD of organic matter to form biogas are theoretically known to exist but technically are rudimentarily understood and have been reported recently by Cirne et al. (2007). In this study, the acidogens were possibly destroyed in the MEB leading to the release of cellular contents, which includes extracellular hydrolases. Kaseng et al. (1992) and Parawira et al. (2005) made similar observations for extracellular profiles in laboratory scale two-stage AD of raw settled sewage sludge and solid potato wastes, respectively. The present data reports on the pattern and appearance of the different extracellular enzymes in the liquid. The integrated actions of the variety of extracellular enzymes in the liquid and cell-bound enzymes/attached to particulates are required for complete hydrolysis of the complex biopolymers (Goel et al., 1998; Burrell et al., 2004; Parawira et al., 2005).

However, other extracellular hydrolytic enzymes, which can be biofilm (cell associated/cell bound) or tightly bound to the surface of the organic particles after being released, were not investigated in this study.

Biogas composition and methane productivity

The carbon dioxide and methane content of biogas and methane production rate (MPR) during the two- stage digestion of the solid SLDR is shown in Figures 2a, b, c). The productions of carbon dioxide without accompanying methane production and decrease in pH in the hydrolytic/acidogenic stage are signs of well functioning hydrolysis and acidification (Hai-Lou et al., 2002). This was observed during the first 7 days of SLDR anaerobic digestion, carbon dioxide production was high; methane production was inhibited due to high VFAs production and the low pH. From day 11 to day 39 the methane content increased gradually to a peak value of 52%. The MPR in hydrolytic/acidogenic stage ranged from 0 - 0.077 l/l/d. The methane content of biogas in the methanogenic stage was 51 - 70%, which is beneficial for energy recovery. This is in agreement with methane content of 71% reported by Yu et al. (2002) in UAF of the two-stage, anaerobic digestion of grass. The decrease in daily methane production after 39 day of anaerobic digestion indicated that digestion was completed. The methane yield was 0.25 m^3 CH_4 kg VS^{-1}. The total methane yield, expressed, as the energy content of the total 35 l of methane produced, was 2.45 kWh kg/VS added (1 m^3 CH_4 = 9.8 kWh) . This is comparable to 2.44 kWh kg/VS calculated from the data reported from two-stage AD laboratory scale using ground to 25 mm and thermal treated at 110°C rice straw as a substrate at 35°C (Zhang and Zhang, 1999).

Based on the annual SLDR production of 900,000 tons in Tanzania, two-stage anaerobic digestion of the aerobically pre-treated residue (TS% 14%, VS 82%, 0.25 m^3 CH_4 kg VS^{-1} SLDR added) could mean production of 26 million m^3 CH_4, corresponding to 25,000 m^3 of petroleum (1000 m^3 CH_4 = 0.96875 m^3 petroleum oil). Conversion of methane to electricity with small scale turbines (< 0.1 MW) of 30% electricity efficiency and large scale turbines (>1 MW) of 40% electricity efficiency would give 76 - 102 GWh of electricity, respectively. SFDR being inexpensive and abundant in the vicinity of sisal factories could be an attractive microbial carrier for large-scale methanogenic applications. Anaerobic digestion seems to be an interesting alternative for energy supply in sisal processing, also opening up for a possibility of electricity contribution to national grid and distribution to other local clients.

The overall process performance of the two-stage AD in this study was good and process failure due to acid accumulation leading to drop in pH and consequent inhibition of methanogenesis was avoided, which is one of the advantages of the two-stage system with high

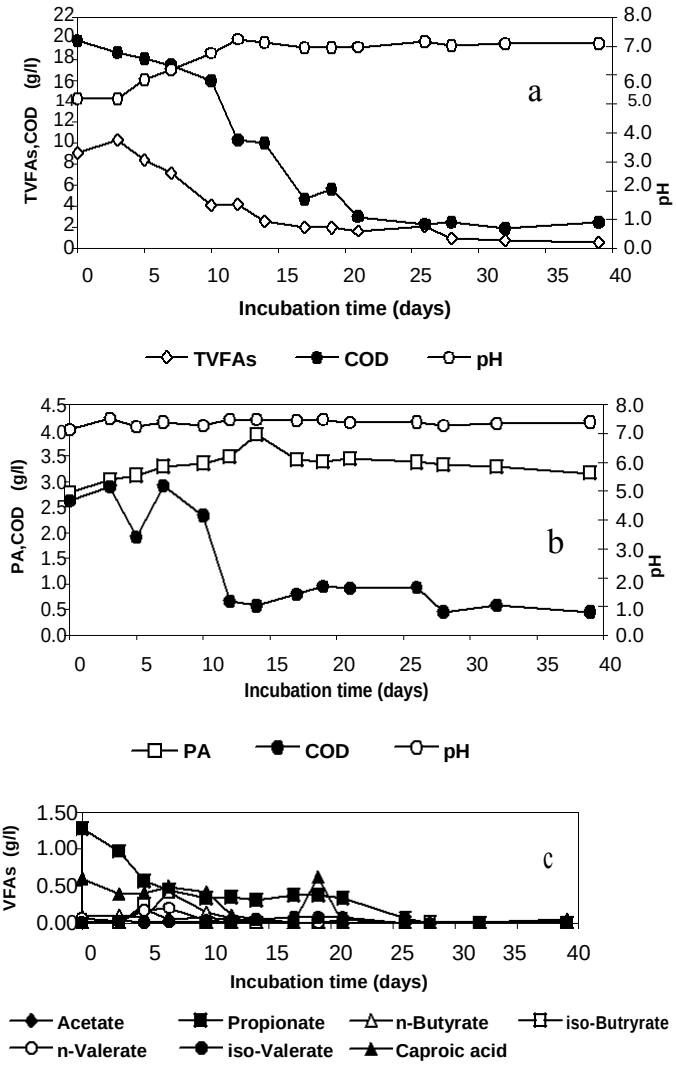

Figure 3. (a) pH, TVFAs and COD profiles in the hydrolysis/acidification stage, (b) profiles of pH, PA and COD, (c) profiles of individual VFAs in the methanogenesis stage during anaerobic digestion of aerobically pre-treated SLDR in a two-stage system.

Table 2. Physical-chemical composition of biogas manure after two-stage anaerobic digestion for 40 days.

Analysis	(mg/ kg, except COD and TVFAs g/l
pH	7.0
Soluble COD	< 0.5
TVFAs	0.06
Total nitrogen	9.0
Phosphorous	12
Potassium	3780
Sodium	1440
Manganese	28
Magnesium	6740
Calcium	59000

solid waste (Yu et al., 2002). The organic loading rate (OLR) in the MEB varied depending on the liquid exchange and organic concentration in the liquid. It ranged from 2 - 10 g COD/l/d due to fluctuation in the COD concentration in
the liquid. Despite the large fluctuations in the overall OLR in the MEB, the system maintained stability.

pH, total VFAs, COD and alkalinity in two stage AD of SLDR

The COD, TVFAs and pH in the hydrolytic/acidogenic stage are shown in (Figure 3a). The soluble COD of 20 g/l in the HAB at day 0 decreased to around 2 g/l at the end of the 40 days operation period. The percentage of acidified COD in the form of VFAs was 83% during greater part of the operation period and then decreased to 69% at the end of the study period. This means that hydrolysis products were quickly converted to VFAs by fermentative acidogens. During hydrolysis, rapid acidification was observed in terms of pH change in the leachate. Without pH control and addition of nutrients, the pH of the leachate decreased from 5.3 to 5.1 on zero day. This is consistent with studies showing that internal leachate recirculation promotes efficient acidification (Griffin et al., 1998; Hai-Lou et al., 2002). Increase in pH accompanied a decrease in concentration of VFAs; consequently the initiation of methane production in HAB coincided with a pH increase.

During the study period no problems regarding the performance of the MEB was encountered. The COD, pH, PA and individual VFAs profiles are given in Figure 3b, c. The COD in the effluent at the end of the study period was 0.45 g/l. The COD removal efficiency ranged between 50 - 88% (results not shown). The pH range was 7.1 - 7.5 without pH control. Thus, the MEB had good degradation capacity and generated sufficient biocarbonate alkalinity, PA (3.3 ± 0.25 g $CaCO_3$/l) to neutralise high peak loads of VFAs from HAB. The maximum effluent TVFAs concentration was 2 g/l, with propionic acid accounting for 64% (Figure 3c). This is in agreement with others study, which has reported propionate degradation to be limited in the methanogenic bioreactors (Mtz-Viturtia et al., 1995; Andersson and Björnsson, 2002; Mshandete et al., 2008b). This is because propionate-assimilating microorganisms are among the most slow-growing due to low free-energy gain from conversion of propionate to acetate and the complicated syntrophic relation to hydrogen-utilising methanogens (Schink, 1997).

Physical-chemical composition of biogas manure

As can be seen in Table 2, using two-stage anaerobic digestion, aerobically pre-treated solid SLDR can be

stabilized in a short time of 40 days into rich organic manure. The digested residue is rich in all the three major nutrients namely nitrogen, phosphorus and potassium, with insignificant content of volatile inhibitors such as VFAs and significantly reduced soluble COD and neutral pH. Lower volatiles compounds in the digested solids produce much lower odour and more stable soil conditioners. The nitrogen, released from organic matter during anaerobic digestion, becomes ammonium (NH_4^+), which is water soluble and thus readily available for plant uptake (Zhang and Zhang, 1999) . Aerobic composting post-treatment (polishing) "curing" step to stabilize the incompletely digested residues is traditionally employed to ensure maturation of anaerobically digested material and reduction of volatile inhibitors such as ammonia and organic acids (deBaere, 1999). This process, however, can also result in a significant loss of nitrogen through the volatilisation of ammonia (Salminen et al., 2001). Aerobic pre-treatment (9 h) of SLDR prior to two-stage anaerobic digestion is a new strategy with a potential for fast stabilization (40 days) of the solid SLDR, at the same time recovering energy. This means that the time taken for organic material to undergo conversion and stabilization is well below the time limit allowed for temporary storage of solid organic waste in landfill adopted developed countries such that in European Union. Yu et al. (2002) using pilot bi-phasic digester, reported AD of grass (without pre- treatment) of (190 days) which was shorter relative to the time for stabilization of the grass in a landfill.

Conclusion

Hydrolases and biochemical characterization in this study has clearly shown that it is possible to separate the AD process into acidogenic and methanogenic stages in a SFDR packed bed bioreactor. When performing two-stage AD, it is important to employ an efficient first hydrolytic step. Furthermore, the results of this study showed that the two-stage system was effective and efficient in the conversion of solid SLDR to biogas.

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