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Full Length Research Paper

Effect of aerobic pre-treatment on production of hydrolases and volatile fatty acids during anaerobic digestion of solid sisal leaf decortications residues

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The effect of aerobic pretreatment on the production of hydrolases and volatile fatty acids during anaerobic digestion of solid sisal decortications leaf residue (SLDR) was investigated. Batch solid waste bioreactors with working volume of 2 litres were used in this study. Batch loads of aerobically treated or untreated sisal leaf residue inoculated with activated sludge mixed culture were packed into the bioreactors and operated anaerobically for 400 h. The fermentation products were mainly (mg/g total volatile fatty acids, VFAs): acetic acid (287), n-butyric acid (201), n-valeric acid (96) and caproic acid (62) as well as with low amounts of propionic acid and iso-butyric acid for aerobic pre-treated sisal leaf waste solids. Contrarily, for the untreated system, the fermentation products were chiefly (mg/g total volatile fatty acids): propionic acid (317), iso-butyric acid (276), n-butyric acid (96), acetic acid (84) and insignificant amounts of n-valeric acid, iso-valeric acid and caproic acid. Although the activities of hydrolytic enzymes found were similar for both treated and untreated SLDR, proportions of VFAs obtained with the former residues appeared to be better substrates for biomethanantion than those obtained from the latter substrates. These results indicated the potential of aerobic pre-treatment for enhanced bioconversion of SLDR. The present study, reports for the first time the types and levels of VFAs and hydrolases produced during anaerobic digestion of aerobic pre-treated SLDR and could be used as a basis for designing a pilot scale process.

Key words: Aerobic pre-treatment, hydrolases, volatile fatty acids, anaerobic digestion, sisal leaf decortications residues.

INTRODUCTION

During biodegradation pathways in anaerobic digestion, hydrolysis involve depolymerisation of the organic polymers by hydrolytic-fermentative bacteria, through the action of extracellular hydrolytic enzymes namely, cellulases, proteases and lipases, into oligomers and monomers (sugars, amino acids, long-chain fatty acids and glycerol). The intermediates formed during hydrolysis are fermented by relatively faster growing acidogenic bacteria into succinate, lactate, alcohols, volatile fatty acids (VFAs), hydrogen (H_2), carbon dioxide (CO_2) etc (Wang et al.,1999). Finally, acetate, H_2, CO_2, methylamines and formate are converted to methane by slow growing methanogens (Yu and Fang, 2001). However, the rate of hydrolysis depends on the extracellular enzymes produced by the fermentative acidogens, the source of inoculum, types and nature of substrate composition, substrate loading, and the specific surface area of the particulate substrate (Mata-Alvarez et al., 2000). This requires an understanding of the hydrolytic enzyme production and their actions in the first, acidogenic phase of anaerobic digestion (Parawira et al., 2005). Furthermore, it requires a selection of most appropriate pre-treatment method based on the composition of the substrate (solid waste, slurries) (van Lier et al., 2001). Aerobic pre-treatment is an emerging relatively new, dyna-

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mic and versatile low technological approach for production of more readily available substrate for methanogens during subsequent anaerobic process (Ugwuanyi et al., 2004a). The principle behind aeration is that the resulting mixed culture of aerobic and facultative anaerobic bacteria functions more efficiently than a monoculture, as found in strictly aerobic process. Accumulation of VFAs in aerobic system is considered evidence of fermentative metabolism (Ugwuanyi et al., 2005a, b). Nevertheless, most of previous reported studies on VFAs accumulation during aeration were carried out under thermophilic conditions using low solid substrates such as municipal sludges and potato peel waste (Hagesawa et al., 2000; Fothergill and Mavinic, 2000; Ugwuanyi et al., 2005a, b). However, aerobic pre-treatment of solid agro-industrial residues during anaerobic acidogenesis at ambient temperature, which describes the types and levels of VFAs and hydrolases produced is one the least investigated topics in this area.

The composition of organic acids produced during anaerobic acidogenesis significantly affects the performance of the subsequent methane bioreactor. Accumulation of VFAs particularly acetate, is desirable since it encourages rapid development of methanogenic populations, leading to improved methanogenesis in the subsequent anaerobic digestion stage. However, the spectrum and pattern of each individual acid varies from one acid to another depending on the nature of the substrate and physico-chemical conditions (process variables) employed (Lata et al., 2002; Parawira et al., 2004; Min et al., 2005). Although, considerable studies on the acidogenic phase has been done, it involved low-solid content substrates using in most cases continuously stirred tank bioreactors (Ahn et al., 2001; Yu and Fang, 2001; Elefsiniotis et al., 2005; Min et al., 2005). Such bioreactors are unsuitable for high solid content feedstock. Therefore, a solid-bed bioreactor has been proposed for hydrolysis and acidogenesis of high solid feeds. However, little information is available in the literature on the acidogenic phase for complex organic particulates. The lack of such knowledge is one of the major barriers for a wide introduction of the two-phase process for particulate organic fractions feed stocks (Hwang et al., 2001). Furthermore, it has been reported that initial high concentrations and accumulation of odd-chain organic acids such as propionic and valeric acids lead to the inhibition of the growth of several microbial species with negative influence on the methanogens (Schink, 1997; Wang et al., 1999). Most of the attempts reported previously on selective production of organic acids in acid bioreactor are by pH control and organic loading using waste slurries (Horiuchi et al., 2002; Ugwuanyi et al., 2005 b). Therefore, research on methods for selective production of VFAs from solid organic biomass, which is suitable both during start-up and subsequent process stability of the subsequent anaerobic stage, is imperative.

The traditional wet sisal decortications process in Tan-Tanzania generates 100 m^3 and 25 tonnes of waste water and solid residues, respectively per tonne of sisal fibres produced. With projected production of 45,000 tonnes sisal fibre for the year 2007, it means generation of 4.5 million m^3 of sisal decortications wastewater and 1,125,000 tonnes of solid sisal decortications residues, of which about 900,000 tonnes is sisal leaf decortications residues (SLDR), the rest being short fibres residues. SLDR represents one of the large unutilised bio-energy potentials. Currently, both sisal solid residues and wastewater are disposed untreated resulting in serous environmental pollution problems. On the other hand, recently SLDR has been recently reported to be suitable feedstock for biogas production (Mshandete et al., 2004; 2005). However, the extent of bioconversion of SLDR into biogas is limited by many factors. One such factor is the spontaneous high levels production of VFAs, especially propionic acid, which has been reported to be toxic to the anaerobic process (Rubindamayugi and Salakana, 1997). Therefore, one of the strategies to avoid subsequent SLDR anaerobic digestion failure is to decrease the amount of propionic acid produced during acidogenesis. In the present study, the effect of aerobic pre-treatment on the hydrolases and VFAs profiles during acidogenic phase of anaerobic digestion of SLDR was studied using activated sludge as an inoculum at ambient temperature. To authors' knowledge such aforementioned study has never been investigated elsewhere.

MATERIAL AND METHODS

Chemicals, sources of waste and inoculum

The following reagents were used: carboxymethyl cellulose (CMC), xylan (from oat spelts), salicin, soluble starch and avicel (Sigma-Aldrich Co. Ltd, Gillingham, Dorset, UK). All other chemicals used were of analytical grade. Sisal leaf decortications residue produced during sisal processing was obtained from a sisal-processing factory at Ubena Zomozi, Tanzania. The SLDR was stored at –20°C until used. Activated sludge inoculum (ASI) was obtained from an activated sludge process of a municipal wastewater treatment plant at Eslöv, Sweden. Characteristics of the SLDR and ASI are as previously reported by Mshandete et al. (2005).

Experimental set-up

Two sets of experiments were carried out to examine the effect of aerobic pre-treatment of sisal leaf decortications residues on the subsequent performance of the acid-phase of two-stage anaerobic digestion. The first set, consisted of SLDR and ASI without aeration (conventional) and designated NP (not pre-treated aerobically). The other set designated P1 (pre-treated aerobically) had SLDR and ASI aerated for 9 h according to Mshandete et al. (2005). Cylindrical-conical made of glass anaerobic bioreactors with a 2 litres working capacity was used. Six hundred grams of SLDR were loaded into two separate bioreactors. The SLDR had a total solids (TS) content of 14% of which 82% is volatile solids (VS). To each bioreactor 1000 ml tap water and 300 ml of ASI were added. To avoid the destruction of micro-organism communities, it is preferable to take care that the organic matter is aerated without mechanical aids by an air supply or pneumatic through-flow system.

Table 1. pH and temperature optima of extracellular hydrolytic enzymes in during anaerobic acidogenesis of solid sisal leaf decortications residues (Sample supernatant of 0.5 ml in 1.0 ml acetate buffer pH 6 (0.05 M).

Substrate	Substrate (ml)	Enzyme	pH	Temperature (°C)	Incubation time (min)	Reference
Avicel 0.5% (wv)	0.5	Avicelase	6.0	50	60	Wood and Bhat (1988)
CMC 0.5% (wv)	0.5	CMCase	6.0	60	60	Ghose (1987)
Salicin 0.5% (wv)	0.5	ß-Glucosidase	6.0	60	60	Wood and Bhat (1988)
Xylan 0.5% (wv)	0.5	Xylanase	6.0	50	60	Bailey et. al. (1992).
Starch 0.5% (wv)	0.5	Amylase	6.0,8.0	50	30	Giraud et. al. (1991)

Therefore, for the P1 set, air was introduced in an up flow mode at a flow rate of 1 litre/min for 9 h using air supply flow system. It was established in our previous batch studies that, 9 h of aeration promoted hydrolysis and solubilisation of SLDR and subsequently improved the anaerobic digestion (Mshandete et al. 2005). The airflow rate was determined with an air flow meter (Rota Wehr-2, Sigurdholm AD, Olshammargatan, Stockholm, Sweden). The concentration of dissolved oxygen during aerobic period was determined with an oximeter (OXi 320, WTW, Germany). The relative concentration of dissolved oxygen (DO) recorded was 8.0 mg/l. In order to maintain anaerobic conditions both P1 and NP were closed with butyl rubber stopper at the top. P1 set was closed after aeration while NP set was previously closed from the beginning. It has previously been reported that VFAs production increases in the range of 22-30°C and decreases at 35°C (Banerjee et al. 1998). Therefore in this study bioreactors were operated at an ambient temperature of 26 ± 0.3°C, while the temperature recorded inside both bioreactors during operation were 28 ± 2°C in P1 and 27 ± 2°C in NP. A wire mesh sieve (1 x 1 mm) was installed above the bottom of the cone in each bioreactor to support the solid waste substrate while still allowing the liquid to pass through it. The leachate from the bioreactors was recirculated continuously at 20 ml/min and sprinkled over the packed bed of SLDR. The leachate percolated down through the bed and out of the bioreactor through the bottom part. Samples were collected at the first hour, thereafter every 6 h for the first 24 h, afterwards every 12 h until the experiment was stopped after 400 h.

Analytical methods

The samples from the bioreactors were centrifuged at 3000-x g for 3 min (WIFUG Lab Centrifuges STUDIE-M, UK) and the supernatants were used for pH, chemical oxygen demand (COD) and VFAs analysis. The pH was measured using, a TIM titration manager with an ABU 901 Autoburette (Radiometer, Copenhagen, Denmark) and the VFAs were monitored with high performance liquid chromatography, Varian 9000 HPLC (Varian, Wulnut Creek, CA, USA), using a BioRad column 125–0115 column for fermentation monitoring (Biorad, Hercules, CA, USA). The COD and total nitrogen were determined according to standard methods (Apha, 1998).

Separation of enzymes and their assays

Enzyme activities were determined for each bioreactor by centrifuging 8 ml of well-mixed leachate 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 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 procedure employed for enzyme assays are summarized in (Table 1). For amylase, carboxymethyl cellulase (CMCase), β-glucosidase avicelase, one enzyme unit in each case was defined as the amount of enzyme, which releases 1 µmol of reducing sugars under assay conditions per min while for xynalase one unit of enzyme activity was defined as the amount of enzyme, that releases 1 µmol of xylose under assay conditions per min. Studies on temperature optimisation were performed in acetate buffer (pH 6.0, 0.05M) by incubating the reaction mixtures at 20-90°C for 1 h. Studies of pH optimisation were carried out by the appropriate temperature in buffers with pH ranging from 4-10. In this study, protease was also assayed; however, it showed insignificant activity thus not reported. On the other hand; although, filter paper cellulose and pectinase showed significant activity, their results were very erratic thus not reported.

RESULTS

Extracellular hydrolytic enzyme profiles

Bacteria excrete enzymes that hydrolyse particulate substrates to small molecules, which can pass through the cell membrane. Once inside the cell, these simple molecules are oxidized to provide energy and to synthesize cellular components. The enzymes that hydrolyse the lignocellulosic components of SLDR with and without aeration were investigated using ASI in acidogenic bioreactors. The production with time of a wide range of extracellular hydrolytic enzymes measured in the leachate is given in Figure 1. The enzyme production from NP was more or less similar with that from P1. The leachate was continuously recirculated through the bed and the production of extracellular enzymes for both P1 and NP generally increased rapidly and reached peak values within the first 50 h of incubation. After 50 h of incubation, the activities of the enzymes remained at relatively constant levels until 400 h of incubation, although there were variations in the levels of various enzymes.

Enzyme activities during hydrolysis/acidification

Comparisons of enzyme activities in the hydrolysis/acidification bioreactors (P1 and NP) show some similarities in the levels and times of peak activity. The total avicelase activity varied between 0.06 and 0.67 IU/ml and 0.08 to 0.67 IU/ml in P1 and NP, respectively. The total CMCase activity ranged between 0.10 and 0.59

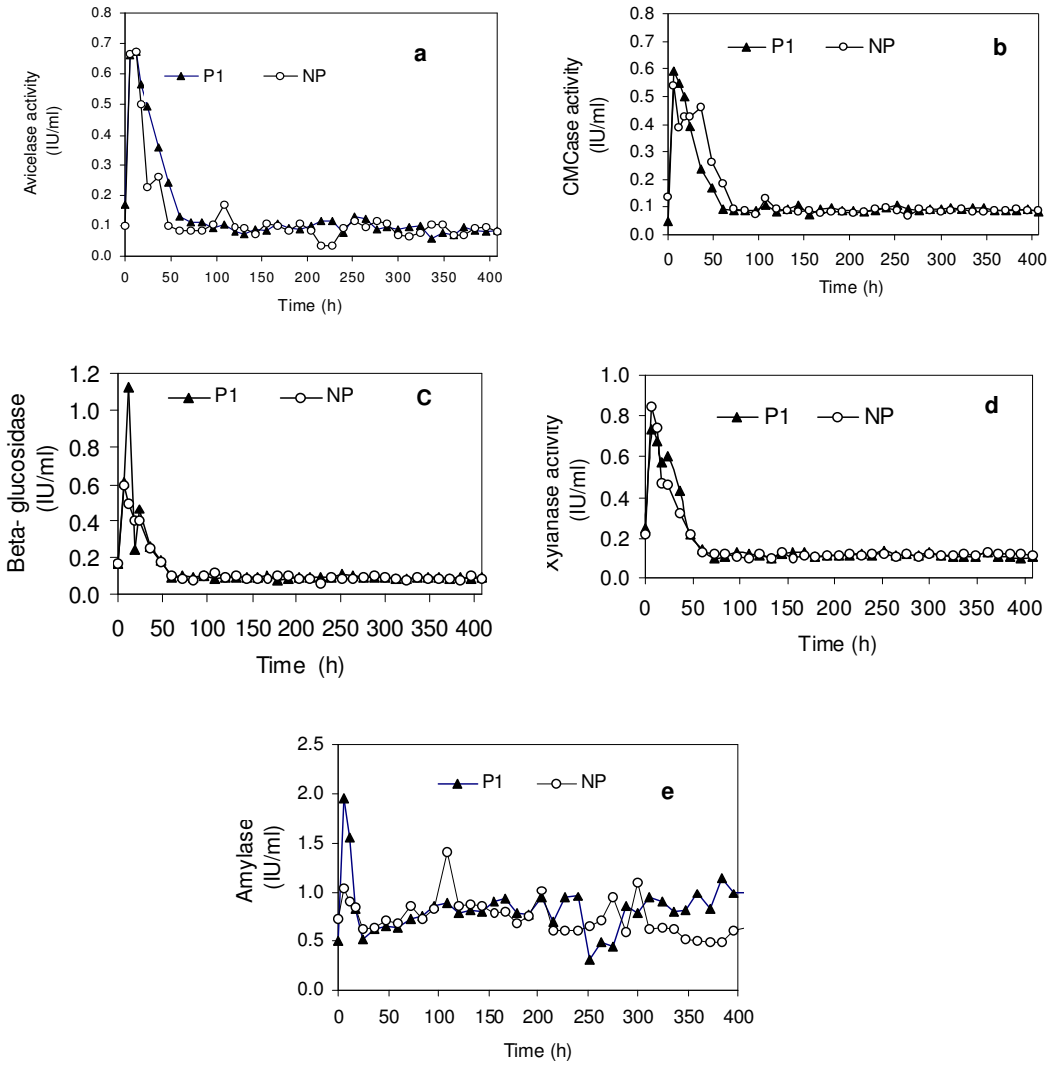

Figure 1. The production with time of extracellular enzymes (a) Avicelase, (b) CMCase, (c) βglucosidase, (d) Xynalase, (e) Amylase during hydrolysis/acidification of solid sisal leaf decortications residue; (P1) with aerobic pre-treatment, (NP) without pre-treatment.

IU/ml in P1 and 0.10 and 0.54 IU/ml in NP bioreactor. The range of total β-glucosidase activity in bioreactor P1 was between 0.10 and 1.12 IU/ml and 0.10 and 0.61 IU/ml in NP. The total xylanase activity ranging between 0.11 and 0.74 IU/ml and 0.11 and 0.84 IU/ml were recorded in P1 and PN bioreactors, respectively. The variation of total amylase activity ranged between 0.30 and 1.95 IU/ml in P1 and 0.50 and 1.41 IU/ml in NP bioreactor.

pH and temperature optima of hydrolytic enzyme

It was necessary to standardize the pH and temperature optima activity for the enzymes, since unknown enzymes were being assayed. The optimal values are presented in Table 1. Amylase showed optimum pH at 6.0 and 8.0; however, the enzymes were only assayed at pH 6.0. Avicelase, CMCase, β-glucosidase and xynalase activeties were optimal at pH 6.0. The temperature optima, at which enzymes were assayed, were 50°C for avicelase, xylanase and amylase while CMCase and β-glucosidase were at 60°C.

VFAs and lactic acid (LA) production

The time course for the production of VFAs and LA is shown in Figure 2. The fermentation products after 400 h digestion of SLDR solids were predominantly (mg/g total VFAs): acetic acid (HAc),287, lactic acid (LA), 287, butyric acid (n-BA), (201), valeric acid (n-VA), (96), caproic acid (CA),62 as well as low amounts of propionic acid (HPr), iso-butyric acid (i-BA) and no iso-valeric acid (i-VA) for aerobic pre-treated SLDR solids. With untreated SLDR solids, the fermentation products were chiefly (mg

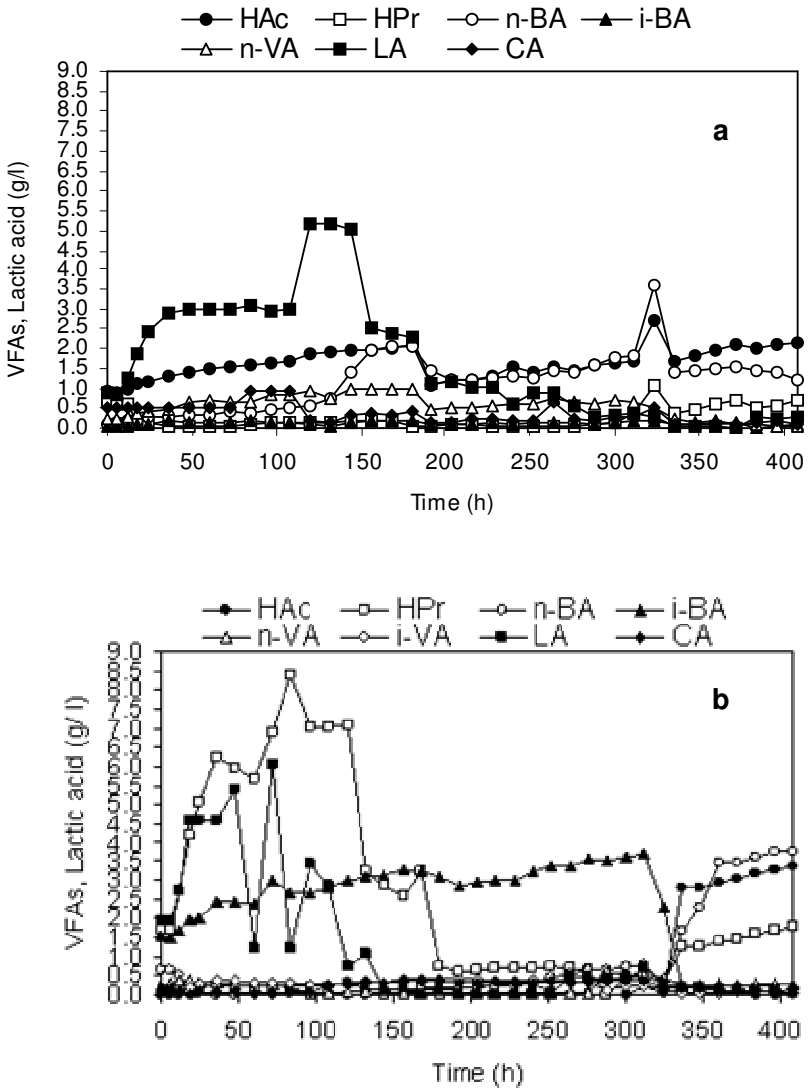

Figure 2. Time course for the production of VFAs and lactic acid during hydrolysis/acidification of solid sisal leaf decortications residues; with (a) with aerobic pre-treatment and (b) without pre-treatment.

g^{-1} total VFAs): HPr (317), i-BA (276), LA (148), n-BA (96), HAc (84) low amounts of n-VA, i-VA and CA. The production of HAc, n-VA and n-BA increased fairly continuously with P1 during the period of the study, while the production of HPr decreased rapidly during 300 h of digestion, and then increased continuously to the end of the study period. On the other hand CA production was almost constant after 180 h of digestion. A different production pattern was observed with NP. HPr production increased rapidly to the highest peak during 84 h of digestion, remained almost constant during the rest of the study period. n-VA production increased rapidly during 80 h of digestion while that of i-BA and i-VA increased continuously during 320 h of digestion, after that period their production dropped sharply. It was interesting to note that the sharp decrease of i-BA and i-VA after 348 h coincided with increased production of HAc and n-BA.

Substrate solubilisation

The relationship between TVFAs concentration and soluble COD for both treated and untreated SLDR is shown in Figure 3. The amount of TVFAs for NP and P1 varied from 2 to 12 g/l. Initially the concentrations increased rapidly to maximum value of 12 g/l during 84 h for NP and 6 g/l for P1 after 144 h of digestion, although there was another high peak of 9 g/l at 324 h of digestion. In general, the TVFAs production for both substrates decreased progressively attaining almost nearly constant production for the rest of the operating period. Solubilisation of organic matter in terms of soluble COD increased fairly rapidly within the first 100 h and then in-

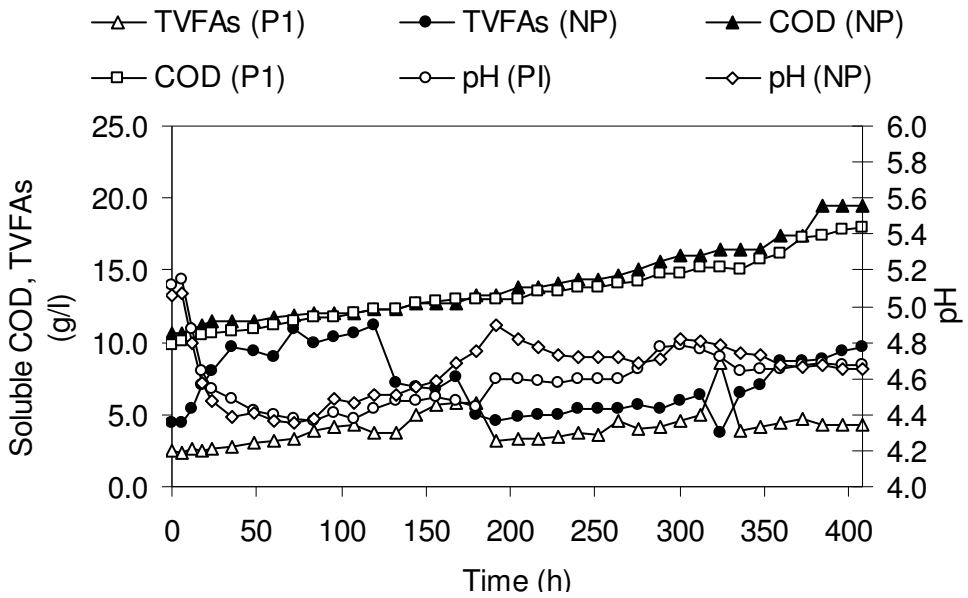

Figure 3. Temporary variation of organic matter solubilisation in terms of TVFAs and soluble COD, and changes in pH during hydrolysis and acidification of solid sisal leaf decortications residues.

increased slowly to reach a maximum of about 18 and 19 g COD/l for P1 and NP, respectively, after 400 h of digestion.

The pH in both bioreactors was quickly reduced from 5.0 to around 4.4 within 120 h after start-up as the concentration of TVFAs rapidly increased. The pH remained around 4.6 for the rest of the study for both treated and untreated SLDR Figure 3.

DISCUSSION

The enzyme production profile from NP was more or less similar to that from P1 (Figure 1). This trend shows that aeration rate and waste load of SLDR employed in this study exerted insignificant influence on the enzyme production by mixed microbial community in ASI. However, Ugwuanyi et al. (2004 a, b) reported the effect of different aeration rates, waste load and retention time on the evolution of hydrolytic enzymes for agricultural waste slurry. Since this study used one fixed waste load and retention time, the effects of varying aeration rates, waste load and retention time on the profile and extent of production of hydrolytic enzymes with the residue needs to be determined. The production of the enzymes for both P1 and NP generally increased rapidly and reached peak values within the first 50 h of incubation and remained at relatively constant levels until after 400 h of incubation, although there were variations in the levels of the various enzymes.

The rapid increase in enzyme production could be attributed to the source of inoculum used and the availability of easily biodegradable materials. This is because the mixed microbial population in ASI was adapted for degrading organic matter by secreting extracellular enzymes under aerobic conditions in activated sludge wastewater treatment systems. It has been reported that in an activated sludge the major activity of the hydrolytic enzymes is associated with the cell or lies within the extracellular polymers of the floc i.e. entrapped in the floc. This immobilization of the enzyme activity on the flocs seems to be advantageous as the micro-organisms need not waste energy in replenishing the enzyme pool continuously and have easy access to the substrate (Goel et al., 1998). The possible explanation for the levelling off of enzyme activity after 50 h of incubation is inhibition by reaction products (end products) on the interaction of the enzyme and the initial SLDR substrate. During 50 h of digestion, pH decreased from 5.0 to around 4.6 which levelled off in the remaining period of the study. Undisasociated VFAs inhibit growth of several microbial species. The organisms at pH below 5, take up undisasociated VFAs within the cell causing a decrease in the intracellular pH. As a result, proton gradient across the membrane cannot be maintained and transport of various nutrients is impaired, which adversely affects the enzyme activity (Srivivasan et al., 2001). Other assumptions include glucose and cellobiose inhibition of the various steps in hydrolysis of the cellulose molecule to form glucose. The observed rise and fall in the levels of enzymes cannot be explained precisely with the present data. It may be due to various factors, which include changing chemical composition of the substrate, stability of enzymes (how long the enzyme remains active once produced), the location of the enzymes after their biosyn-

thesis (free or cell bound), and attachment of the released enzyme to the substrate (Parawira et al., 2005).

Enzyme activities during hydrolysis and in acidification bioreactors showed some similarities for both PI and NP (Figure 1). These results suggest that the cellulose-degrading micro-organisms synthesized and secreted several enzymes and the mixture of polymers in SLDR substrate exhibited several enzyme activities. Since solid SLDR is a complex lignocellulosic polymer the production of extracellular lignocellulose-degrading enzymes is desirable for its hydrolysis into simple soluble molecules and thus provides a good substrate for anaerobic micro-organisms (Mshandete et al., 2005). The hydrolysing activities of most of the enzymes were reasonably good and their combined multi-activity complementary interacttions resulted in hydrolysis of the solid SLDR. Synergism between separate endo and exo glucanases to achieve cellulose hydrolysis is well known. It is influenced by individual enzyme concentrations and their relative concentrations (Bhat and Bhat, 1997). A considerable degree of synergy also exists between xylanases in the degradation of xylan, which is a more complex group of polysaccharides than cellulose (Saha, 2002). Furthermore, complete hydrolysis of a complex structure of starch into glucose involves a co-ordinate action of a number of amylases (Kuriki and Imanaka, 1999). The observed patterns of enzyme activities give an insight into the multi-complex dynamics of extracellular enzyme synthesised and secreted during degradation of SLDR. This implies that it is necessary to monitor enzyme activities over time since the hydrolytic enzyme activity will to some extent, be controlled by the composition of the substrate mixture (Ugwuanyi et al., 2004b). Enzymatic depolymerisation of structural polysaccharides of the plant wall is an important process in the hydrolysis of plant materials into fermentable sugars. The products of such enzymatic hydrolysis could be biologically converted into biofuels (for example biogas and ethanol). The CMCase (endo-glucanase), Avicelase (exo-glucanases), β-glucosidase (glucosidase) and xynalase exhibited significant activity towards lignocellulose constituents of the SLDR, which demonstrates good hydrolytic activity. Cellulases and xylanases include a collection of enzymes whose primary function is to hydrolyse β-1, 4-glycosidic linkages in the plant structural polysaccharides, cellulose and xylan into their constituent sugars (Mshandete et al., 2005; Parawira et al., 2005). The presence of high amylase activity despite little starch in SLDR was probably due to the contribution from the inoculum used in the study, which routinely converts readily biodegradable organics such as potato pulp waste.

Since unknown enzymes were being assayed, pH and temperature optima activity for the enzymes were determined (Table 1). Amylases are generally known to be stable over a wide range of pH from 4 to 11. Amylase showed optimum pH at 6.0 and 8.0. However, the enzymes were assayed only at 6.0 for amylase. Avicelase, CMCase, β-glucosidase and xylanase activities were optimal at pH 6.0. The temperature optima, at which enzymes were assayed, were 50°C for avicelase, xylanase and amylase while for CMCase and β-glucosidase it was 60°C. In the literature, little can be found on the use of mixed microbes from activated sludge as an inoculum for aerobic pre-treatment aimed at investigating evolution of extracellular hydrolytic enzymes during anaerobic digestion of solid agro-industrial residues at ambient temperature. Nevertheless, some of temperature and pH values ranges observed in this study fall within what have been reported on production of extracellular hydrolyltic enzymes during digestion of agricultural slurries under thermophilic and mesophilic conditions (Ugwuanyi et al., 2004a, b; Parawira et al., 2005).

The time course for the production of VFAs and LA (Figure 2) revealed that identification of the individual VFAs formed during anaerobic acidogenesis is important, since it may provide useful information on the microbial metabolic pathways involved in the process. HAc, HPr, n-BA and i-BA are formed directly from the fermentation of carbohydrates and proteins, as well as during the anaerobic oxidation of lipids. These results are similar to those found by Horiuchi et al. (2002) when they studied the production of organic acids from complex organic medium in an anaerobic reactor with pH control. HPr is believed to be the most toxic VFA intermediate in anae--robic digestion leading to low methane productivity and process instability. Moreover, HPr is harder to degrade because its decomposition is only possible in small thermodynamic range as well as it cannot split into two acetic acid molecules. This results in longest time of digestion for all VFAs (Schink, 1997). HPr inhibits anaerobic digestion process and hence as little as possible of this acid should be produced in the acidogenic reactor (Pullammanappallil et al., 2001). The considerably higher production of HPr from NP as compared to the insignificant low quantities produced from P1 in this study could mainly be attributed to the availability of easily biodegradable carbohydrates present in NP compared to the P1 of which some were initially used by aerobic organisms and facultative anaerobic bacteria. Methanogenic conversion of HAc and n-BA is higher than that of HPr, n-VA and i-VA. The straight-chain fatty acids (normal form) were previously found to have higher decomposition rates than their branched-chain acids (iso form) (Wang et al., 1999). Therefore, the overall performance of a two-phase system could be enhanced if fractions of degradable components in the acid forming bioreactor were mainly converted to acetate and butyrate, which are known to be the best precursors for methane production, while at the same time formation of propionate which is utilized only with difficulty by acetogenic bacteria is kept low (Hwang et al., 2001). For this reason, aerobic pre-treatment of SLDR worked very well since the major products were acetate and butyrate while the formation of HPr was negligible. This could be of interest in pilot and full-scale

anaerobic processes digesting SLDR and other sisal residues. However, studies on the effect of aeration rates, waste load and retention time on the production VFAs remains to be investigated.

In the present study, the maximum amount of soluble COD and total volatile fatty acids (TVFAs) produced from un-aerated SLDR (NP) were not much greater than that produced from aerated SLDR (P1) under the experimental conditions used (Figure 3). In general, the TVFAs production for both substrates initially increased gradually with some fluctuations and later attained almost constant production for the rest of the operating period. Similar variations in total VFAs production patterns were observed by Lata et al. (2000) and Parawira et al. (2004) when they studied the production of organic acids from vegetable, tea and solid potato wastes. The low pH of around 4.6 was recorded for both treated and untreated SLDR during most part of the study period as a result of organic acids produced (Figure 3). Due to low pH, gas production was negligible throughout the study, indicating that methanogenic activity was effectively suppressed while acidogenic activity was enriched. Similar phenomena of methanogenic activity inhibition due acidic conditions of VFAs produced from solid organic waste in anaerobic bioreactors have been reported by other researchers (Parawira et al., 2004). Soluble COD is a parameter that represents the extent of hydrolysis and solubilisation carried out by acidogenic bacteria. Solubilisation of organic matter in terms of soluble COD in-creased fairly rapidly within the first 100 h and then increased slowly to reach a maximum of about 18 and 19 g COD/l for P1 and NP, respectively, after 400 h of digestion (Figure 3). The recirculation of the perolating culture through the solid bed promoted hydrolysis and acidification by an advantage of repeated inoculation of the bed. The COD of the leachate increased slowly after 100 h and this could be interpreted as inhibition of hydrolysis perhaps by the end-products and low pH values around 4.6 as opposed to 6.0-6.5 which is optimal for many bacteria during hydrolysis in the acid bioreactor (Veeken et al., 2000). The remaining soluble COD can be attributed to the metabolic intermediates of the fermentation process. Some of the soluble compounds contributing to COD consisted of LA, which was produced in large quantities as shown in Figure 2a. The highest total production of lactic acid was 60 g/kg in this study. The formation and subsequent conversion of lactic acid occurs as normal processes in anaerobic digesters. In the acid-phase digestion, a variety of other soluble C_1 to C_4 end products besides VFAs are formed, such as alcohols, ketones and organic acids (formic). The alcohols, which include methanol, ethanol, propanol and butanol, were not investigated in this study. All the above compounds are fermentation end products of carbohydrates metabolism.

Conclusion

In conclusion, we can say that the present study, reports for the first time the types and levels of VFAs and hydrolases produced during anaerobic digestion of aerobic pre-treated SLDR. Studies on production and secretion of microbial extracellular hydrolytic enzymes are important for designing of an efficient first acidogenic step of two-stage anaerobic digestion with improved solubilisation and bioconversion rate of polymeric compounds for enhanced methane production. Acidogenesis was feasible for both pre-treated and untreated SLDR as indicated by the low pH values, high VFA production and insignificant gas production. Negligible gas production throughout the study period implied that the methanogenic activity was effectively inhibited mainly due to acidic conditions in bioreactors. The aerobic pre-treatment of SLDR studied could be seen as potential method for the production VFAs suitable for methane production particularly acetate and butyrate with negligible HPr, which affect negatively anaerobic digestion process. Although forced (active) aeration used in this study could be seen as disadvantageous in view of energy consumption. An important factor to be taken into consideration is that, the depletion of biodegradable material in the biomass should be efficient, to avoid post- biomethanation (methane emission) after the bioreactor process is over as well as to avert the need for aerobic pre-treatment which usually applied for stabilizing anaerobic digester effluents. In an attempt to gain more understand-ing on improvement of the extent of bioconversion of the solid sisal decortications residue into biogas, studies on the effect of aeration rates, waste load and retention time on the production of hydrolases and VFAs are underway.

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