STEAM PRE-TREATMENT OF LIGNOCELLULOSIC WASTES FOR BIOMETHANOGENESIS: A PRELIMINARY STUDY

Aslam Sk Heerah[1], Ackmez Mudhoo*[1], Romeela Mohee[1] and Sanjay K. Sharma[2]

[1]Department of Chemical & Environmental Engineering, Faculty of Engineering, University of Mauritius, Reduit, Mauritius.

[2] Computational and Green Chemistry Research Laboratory, Institute of Engineering & Technology, MIA, Alwar (India)

* Email: ackmezchem@yahoo.co.uk

ABSTRACT

Lignocellulosic biomass was subjected to steam pre-treatment at 95^{o}C and 103 KPa for four consecutive steam cycles each lasting 45 minutes followed by anaerobic digestion lasting for 38 days. Total solids, volatile solids, pH, volatile fatty acids (VFA), chemical oxygen demand (COD) and biogas, methane and carbon dioxide production rates were monitored. The first set of experiments showed that steam pre-treatment had increased the final filtrate VFA level two-fold to 30.1meq/L and that filtrate COD had increased from 10,500mg/L to 16,000mg/L. The next set of experiments for the anaerobic digestion of steam-cooked and non steam-cooked biomass gave the following results: 49.6% decrease in COD from 38,450mg/L, 90.1% decrease in VFA from 42.5meq/L and 49.4% decrease in Total solids from 8.40% for steam-cooked biomass, and 34.5% decrease in COD from 33,400mg/L, 93.0% decrease in VFA from 27.5meq/L and 47.8% decrease in total solids from 11.5% for non steam-cooked biomass. The biomethanogenesis performance after 36 days of anaerobic digestion was as follows: 12,720mL and 12,760mL biogas from non steam-cooked (50.3% CO_2 and 49.7% CH_4) and steam cooked biomass (35.3% $CO_2$and 64.7% CH_4), respectively.

Keywords*: *Lignocellulosic, Anaerobic digestion, Steam pre-treatment, Biomethanogenesis, Biogas

INTRODUCTION

Anaerobic conversion of organic wastes is a solid waste treatment technology that has gained increased interests during the last years [1-7] as an emerging organic waste treatment option which is addressing several of the environmental solid wastes pollution concerns[8]. Different bacterial populations are involved in this process whereby organic compounds are biodegraded to produce a mixture of gases (mainly methane and carbon dioxide) [9,10]. The advantages of this digestion process include a complete containment and control over the gas and leachate-odour generated and the ability to harness biogas[11,12] as a useful fuel source. The major constraint that has been reported with the anaerobic technology is the slow rate of stabilization of solid waste within the anaerobic system. Municipal solid wastes (MSW) in common with most other biomass wastes (forestry and agricultural residues) contain a high content of lignocellulosics fibre (5-25% of the total waste mass) that is not readily digestible[13]. Various pretreatment and conditioning procedures[14], employing chemical and enzymatic hydrolysis or particle size reduction (physical) of lignocellulosic materials have provided some modest improvements to digestion yields.

Steaming process is a recent technology[15] which has gained much interest worldwide. Steam acts as a physico-chemical agent to release the cellulosic materials enmeshed in the lignin, thus increasing the amount of smaller molecules available for further processing[13]. The rate of steaming is also important since the time taken for depolymerisation is less in the thermal process as compared to natural degradation within anaerobic systems[16]. Plant cell wall material is chiefly composed of three important constituents: cellulose, lignin, and hemicellulose. Lignocellulose, is a composite of cellulose fibers embedded in a cross-linked ligninhemicellulose matrix[17]. In general, processed municipal solid wastes contain 40-50 % cellulose, 12% hemicellulose, 10-15% lignin by dry weight[18]. Lignin is particularly difficult to biodegrade, and reduces the bioavailability of the other cell wall constituents. The cellulose and hemicellulose fractions are biodegradable and make up over 90% of the biochemical methane potential of municipal solid wastes. However, not all the hemicellulose and cellulose are available for anaerobic digestion[18]. The physiology of these three constituents best explains why lignocellulosics are so resilient to biological processes such as enzymatic hydrolysis and fermentation. The cellulose microfibrils are imbedded in a matrix of noncellulosic polysaccharides, mainly hemicellulose and pectic substances[19], which complicates the hydrolysis of cellulose to glucose even further. Pre-treatment of lignocellulosics materials to open up the complex structure is essential for enzymatic attack and efficient bioconversion to processes such as hydrolysis, fermentation and biomethanogenesis[20].

A variety of biological, physical and chemical methods has been assessed for their technical and economical feasibility at pre-treating lignocellulosic residues. These include acid or alkali treatment, ammonia and urea physical grinding and milling, fungal degradation and steam explosion and combined alkali and heat treatment. The best pre-treatment options are those which combine elements of both physical and chemical methods[21]. High-pressure steaming, with or without rapid decompression, has been claimed as one of the most successful option for fractionating wood into its three major components and enhancing the susceptibility of cellulose to enzymatic attack[22]. Steam explosion is the most commonly used method for pretreatment of lignocellulosic materials. Steam explosion is initiated at a temperature of 160 to 260ºC with a corresponding pressure 0.69 to 4.83 MPa for several seconds to a several minutes before the material is exposed to atmospheric pressure[19] for cooling. High-pressure steam radically modifies the plant cell wall structure, yielding a dark brown material from which partially hydrolysed hemicelluloses are easily recovered by water-washing, leaving a water-insoluble fraction composed of cellulose, residual hemicelluloses and a chemically modified lignin that can be further extracted by mild alkali, dioxane, ethanol, or oxidative agents such as alkaline hydrogen peroxide and sodium chlorite[23]. Temperature is also an important factor that has been considered for pretreatment whereby saturated steam temperatures ranging from 140 to 240°C have been used over a wide range of residence times into the steam gun[23]. It is known that during a thermal treatment of wood, carbonic acids, mainly acetic acid[24], will be formed as a result of cleavage of the acetyl groups of particular hemicelluloses[25]. The study presented in this paper has been designed to assess the effects of steam pre-treatment on the anaerobic digestion of lignocellulosics fractions obtained from municipal solid wastes as a preliminary research prior to the pilot-scale design of a steam pretreatment unit.

Biomethanogenesis:

Biomethanogenesis is the generation of methane gas from the biological degradation of organics substrates under certain controlled conditions of pH, temperature and microbial species dominance. It is actually the process by which bacteria decompose organic matter using carbon dioxide as an electron acceptor in absence of dioxygen or other electron acceptors. Biomethanogenesis may be regarded as a process, which is used to reduce levels of atmospheric carbon dioxide by the conversion of renewable resources to significant quantities of substitute natural gas[26]. An overall scheme of biomethanogenesis, depicted in Figure 1, indicates that the principal substrates of methanogenic bacteria are acetate and hydrogen /carbon dioxide (or formate)[27].

Methane is a greenhouse gas that is increasing in the atmosphere; its concentrations from numerous sources are of serious concern. It is a dangerous gas, with 21 times more global warming potential than carbon dioxide[28]. Over a time span of 100 years, methane has a global warming potential of 23 relative to $\mathbf{CO_2}$. Therefore, during this time, one ton of methane produces the same greenhouse gas effect as 23 tons of $\mathbf{CO_2}$. When methane burns the reaction is-

$$\mathbf{CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O}$$

By harvesting and burning landfill gas and other prospective sources of methane, its global warming potential is reduced by a factor of 23, in addition to providing energy for heat and power. Methane is a colourless and odourless gaseous hydrocarbon at ambient temperatures and has a critical temperature of -82.2^0C and a critical pressure of 45.8 atm. Some essential properties of methane are given in Table 1. It is combustible when the O_2:CH_4 ratio exceeds 2.0 producing CO_2 and water. Its heating value is 2350KJ/kg. Because of its high availability and high energy content it is widely used as a fuel representing about 20% of the US energy supply in 1983[29,30]. The concentration of methane increased from 0.70ppm to 1.68ppm from 1787 to 1987 and is increasing by about 1.0% per year[31].

EXPERIMENTAL

Substrates preparation

The substrates chosen were of lignocellulosic nature (grass clippings and acacia branches). According to their respective C/N (carbon to nitrogen) ratios as shown in Table 2, co-digestion with vegetable waste and chicken waste, was found to be necessary to achieve the desired level of moisture content (%MC) and C/N ratio. The proper mix of substrates was determined after adjusting the C/N ratios and moisture contents. For this purpose the desired bulk C/N ratio was set to be within 16-19 as recommended by Cecchi *et al.*[32]. All the substrates were first mechanically chopped to a uniform size using a shredder and a grinder. Preliminary analyses indicated that the ratio of water, bulk substrate and inoculums to be added should be in a 1:1:1 (v/v) ratio in order to achieve a total solids content of 6-12%. The solids content of the mix studied have varied from 8.2 to 8.7%.

Traditional anaerobic digestion (TAD)

An acclimatization period of 40 days of the substrates to the inoculum was important. After 40 days of adaptation, a reactor representing the conventional method of anaerobic digestion was setup using the acclimatized sludge at the 1:1:1 (v/v) working ratio.

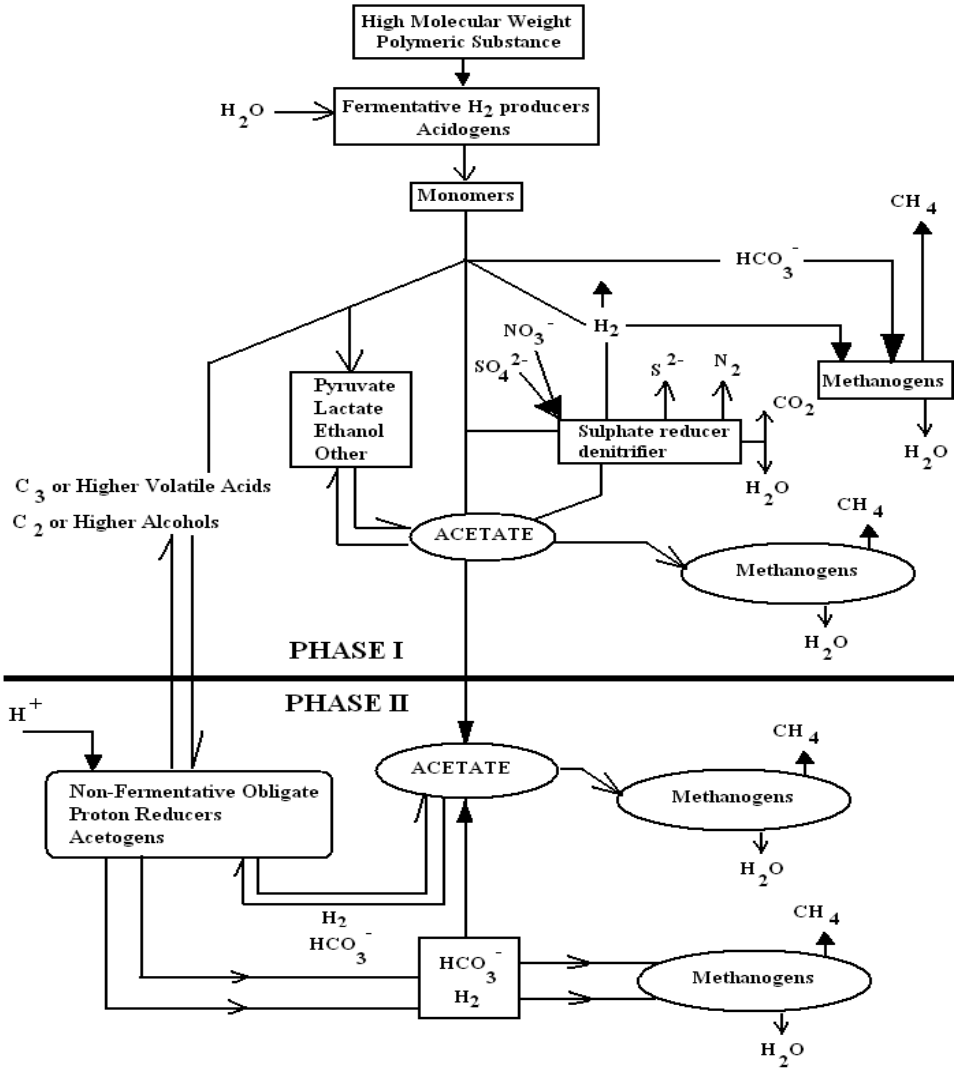

Figure-1: Methanogenic decomposition of compounds in nature

Table-1: Properties of Methane

Molecular Formula	CH_4
Heating Value	2350 KJ/kg
Ratio of O_2:CH_4 required for combustion	2.0
Boiling Point	-162^0C
Critical Temperature	-82.25^0C
Critical Pressure	45.8atm.
Solubility in water at 35^0C	17mg/L

Table-2: Characteristics and quantity of substrates

Substrate	Mass (kg)	C:N ratio	%MC
Shredded acacia branches	2.0	29.14	58.3
Green grass clippings	1.0	49.10	76.8
Vegetable wastes	1.0	30.00	90.0
Chicken wastes	1.0	15.20	30.0
Effective value in mix		*18.75*	*55.4*

Steam pre-treated anaerobic digestion (SPAD)

The steam pre-treated anaerobic digestion (SPAD) involved an additional unit operation prior to anaerobic digestion. This step required pre-treatment of the substrate under a steamed batch system in a conventional pressure cooker. The same substrates mix conditions as 55.4TAD were employed. After the steaming process was completed, the resulting mixture was mixed with the required amount of sludge such that the overall working ratio of 1:1:1 (v/v) was still maintained. The resultant slurry was digested anaerobically. A conventional pressure cooker was used and operated at a maximum of 95-100ºC and 103 KPa. The retention time (number of steam cycles or steam digestion, SD) within the system was a determining factor for the steaming process. In this study, the steam pre-treatment process consisted of four consecutive steam cycles. Every steam cycle consisted of 10 to 15 minutes of heating at 95-100ºC and 103KPa followed by 30 minutes of cooling to room conditions. An acclimatization phase of the sludge with the steam-cooked substrates was run. Acclimatized sludge derived from TAD acclimatization was actually mixed with the pre-treated substrate and allowed to adapt for a period of 40 days. Once the four cycles and analyses were over, the resulting mixture was mixed with the acclimatized sludge so that the ratio of substrate, water and sludge was maintained at 1:1:1 (v/v). The whole mixture was then digested anaerobically under mesophilic conditions. The frequency of monitoring and parameters analysis were similar to those performed for TAD.

Experimental set-up and analytical methods

Two anaerobic reactors were set up for comparison. The first setup was a reference reactor representing the typical anaerobic digestion (TAD) of solids and the second one was similar to TAD but involved an additional unit operation for steam pre-treatment (SPAD). The mesophilic anaerobic digester (bioreactor) was a glass-made airtight system accommodating a total volume of 2L. It was equipped with a mechanical stirrer having an operation speed range 0-1000 revolutions per minute and was mounted vertically on the central axis of the bioreactor. The system also consisted of probes for gas outlet, sampling of reactor contents and sensors for temperature and pH. To provide for mesophilic conditions, a thermostatically controlled water bath constantly operating at 35oC was used. The digester was submerged to 75% of its volume in the water bath. In order to simulate a pressurized steam chamber, a conventional 6L aluminium pressure cooker was used. The cover of the vessel comprised a synthetic rubber gasket in-between, a locking looping system to make it leak proof, a safety valve and a vent weight for providing the working pressure only. A gas cooker (Liquid Petroleum Gas type) was used to heat the pressure vessel for the steam cycle. For every steam cycle, the same flame intensity (medium flame intensity) was used. For the collection of carbon dioxide and methane, a carbon dioxide scrubber and a methane collection system were used, respectively. The scrubber had a capacity of 0.5 L and the methane collector had a volume of 5L. The scrubber solution was analysed every week and replaced with freshly prepared standardised KOH. For the methane collection, as

the gas volume exceeded 4 L, a plastic syringe, was used to remove the gas so as to bring back the volume to zero. Thus, no methane was lost. *Every week, grab samples of reacting substrates were withdrawn from the bioreactor at the sampling point* for volatile fatty acid (VFA), chemical oxygen demand (COD), average moisture content, total solids content and volatile solids analysis. Biogas was analysed for methane and carbon dioxide contents. pH was sensed by the pH probe and was read off directly from the pH display screen. Moisture content and total solids were determined by heating the 3-4g of sample at at 105^{o}C in an oven for 24 hours until constant mass was reached. The moisture content (%MC) was calculated as the loss in weight after drying divided by sample the wet weight times 100%. The total solids content was equal to 100% - %MC. Volatile solids content was determined according to the BS1377 standard method. VFA levels were determined using a combination of volumetric (titration) analyses and the use of the TITGRAM software. For biogas analysis, 25 mL of the well mixed scrubber solution was taken and titrated with using two indicators successively (phenolphthalein followed by methyl orange). Calculations performed thereafter using the titration values gave the volume of CO_2 scrubbed. The volume of methane that had collected by upward water displacement was easily readable from the calibrated gas collector. COD was determined following the standard method detailed in APHA (1998)[33].

RESULTS AND DISCUSSION

Effects of steam pre-treatment

Results for pH, VFA and COD variations obtained from the experiments carried out for the two batches of substrates (steaming and non-steaming) are shown in Figure 2. The pH for the nonsteamed batch attained a minimum steady level of 8.1, as compared to the steamed batch for which pH was still decreasing even after the fourth SD. The pH after 225 minutes was 7.81. The decrease in pH was mainly due to the release of organic acids during the steaming process. Besides the fact that the non-steamed batch was a closed system, the decrease in pH could mainly be attributed to the easily and quickly hydrolysable particulates within the bulk substrates by the microbes. VFA started to rise from 6.70 meq/L to reach 10.00 meq/L for the 1st SD as compared to the non–steamed batch for which VFA levels showed no appreciable increases from the original VFA concentration. As reported by Castro and Machado[34], the compositional changes occurring during steam pre-treatment, release sugars from the hemicellulosic matrix which in turn affect VFA proportion. As from the first SD to the fourth SD, the steamed batch experienced an important increase in VFA production and release before attaining a value of 30.1meq/L after the fourth SD. On the other hand, the non-steamed substrates showed an increase from 6.70 to 14.20meq/L VFA. This increase in VFA concentration can be attributed to the partial degradation of the more biodegradable substrates by the microorganisms, but was not as high as that observed from the steam cooked biomass.

From preliminary experiments it was found that for a substrate of around 29,000-33,000 mg/L bulk COD the value would decrease to 21,000-24,000 mg/L after the four steaming cycles. It was observed that the COD value for the filtrate derived from each steaming process, showed a general increase. This was expected since an increase in filtrate COD would mean an increase in solids disintegrated from the bulk substrate due to steam disruption. This process can be characterized as an interphase mass transfer since there is movement of particles from bulk solid (feed matrix) to bulk liquid (filtrate). It was observed that, as the steam cycles were increased,

the bulk substrate mixture gradually became a dark brown slurry and which was similar to the observations of Ramos[23]. It was seen that the increase in COD was much more pronounced in the steamed batch (84% increase) than in the non-steamed batch (7.1%). It was also found that for the steamed and non-steamed substrates no significant changes were observed in filtrate COD during the first 50 minutes, this being equivalent to a lag phase before the disintegration and interphase transport of substrates particles into the bulk filtrate. A 35% increase in COD from an initial value of 9,800mg/L was recorded at the end of the second steam cycle, a cumulative 45% increase after the third cycle, and finally a net 84 % increase after the fourth cycle.

Biomethanogenesis performance

The focus of this study was to analyze the effect of steam pre-treatment on solid organics and the subsequent effects on anaerobic digestion. A conventional reactor (TAD) was set up for comparison with the SPAD reactor.

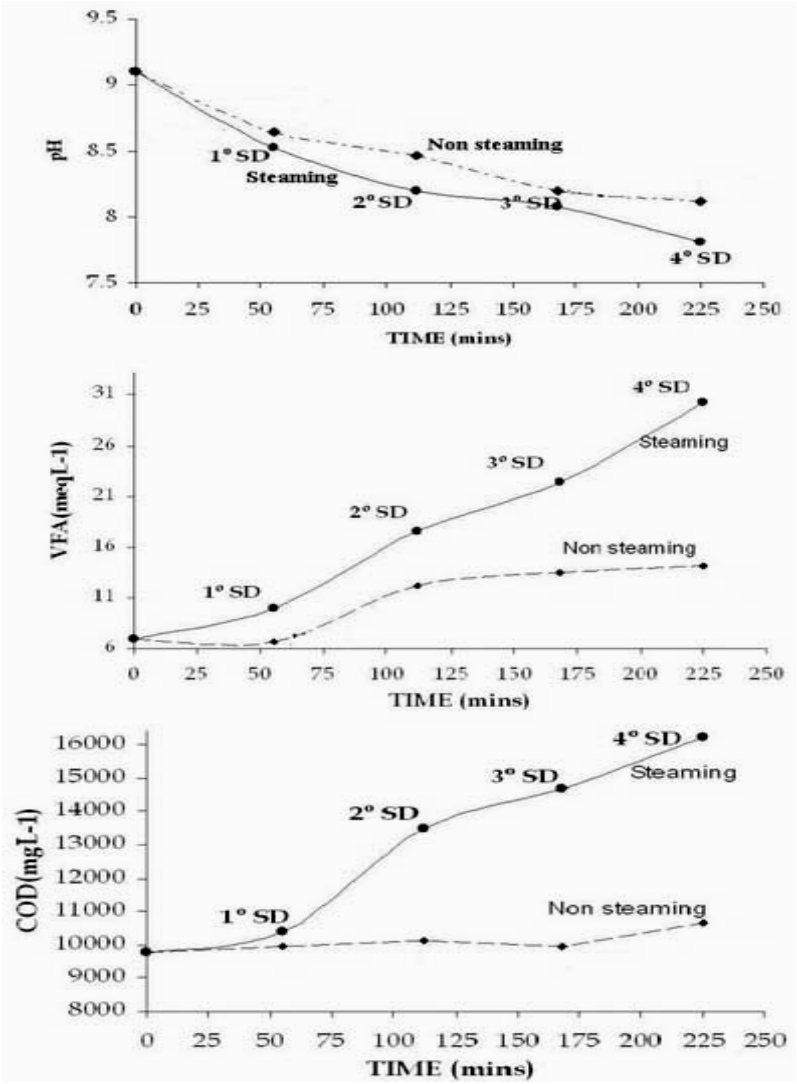

Figure-2:Comparison of pH, VFA and COD variations under steaming and non-steaming conditions

In addition to the parameters monitored earlier, the biogas composition, the rate and extent of biodegradation together with the stability of both systems were considered. Table 3 summarizes the initial and final values for the parameters monitored during the additional experiments carried out in order to assess the biomethanogenesis performance.

The results for pH, VFA, total solids content, volatile solids content and COD are presented in Figure 2. During the first 3 days, acidogenesis prevailed within the system most probably due to the easily hydrolysable vegetable wastes in the feedstock mixture by the microorganisms and this accounted for the sharp rises in VFA concentration for both TAD (from 12.9meq/L to 27.5meq/L) and SPAD (from 21.0meq/L to 41.3meq/L).

The two-fold increase in VFA occurring on day 3 accompanied by a drop in pH to 6.95 showed the quick adaptability of the acidogenic microbial population to the processed substrates that were readily available for acidogenesis. At the end of 36 days retention time, 39.5% and 44.1% total solids reduction was recorded in the non-steamed and steamed anaerobic digestion process, respectively. The corresponding decreases in volatile solids were 15.7 % and 18.8 % for non-steamed and steamed anaerobic digestion, respectively.

Table-3: Initial and final values of physical parameters from experimental runs for biuomethanogenesis analysis

	TAD		SPAD	
	Initial	**Final**	**Initial**	**Final**
Bulk density (kg/m^3)	920	1024	968	10.43
pH	8.18	8.20	7.80	7.45
VFA (med/L)	12.9	1.7	7.80	7.45
COD(mg/L)	33400	24800	38450	25700
%total solids	11.500	6.00	8.40	4.25
%volatile solids	82.00	67.60	80.00	63.40
Final cumulative volume of methane (mL)	*6730*		*8300*	

From day 1 to day 7, a 9.2% rise in COD was noted. Analyses done on day 36 showed that a 23.2% reduction in COD had been achieved while the COD removal efficiency on day 40 was 26.3%. The acidogenesis phase of SPAD prevailed for the first 2 days to form many solubilized organic compounds in the filtrate of the slurry thus accounting for an increase in COD from 38,450mg/L to 42,500mg/L. The rise could also be attributed to an increase in biomass content due to growth of micro-organisms. Conversely to TAD, as from day 3, the COD for SPAD started to decrease steadily to attain a removal efficiency of 16.2 % on day 22. At the end of a 36-days retention time, 39.3% reduction of COD was obtained for the steam-cooked biomass while only 23.3% COD removal was recorded with the non steamed-cooked biomass.

Biogas production

The amount of biogas that is produced depends entirely on the amount of volatile solids degraded under anaerobic conditions. It was interesting to note that, both systems attained comparable volumes of biogas after a period of 36 days. TAD and SPAD reached 12,720 mL and

12,760 mL biogas respectively showing that both systems were effective in producing biogas. However, there was a major difference in the composition of the biogas in terms of methane and carbon dioxide contents as shown in Figure 3 and summarized in Table 4.

Table-4: Final volumes (mL) of biogas, CO_2 and CH_4

	TAD		SPAD	
	t=0	t=36 days	t=0	t=36 days
Biogas	0	12720	0	17760
CO_2	0	6400	0	4500
CH_4	0	6330	0	8300

It can be observed that after two days of retention in the bioreactor, the carbon dioxide volume scrubbed was 1100 mL and the corresponding volume of methane collected was 700ml. The variations of biogas production for TAD indicated that the rate of carbon dioxide formation was higher than the rate of methane formation as from the start of the reaction[35]. The production of carbon dioxide and methane reached a common rate as from day 36, and both curves showed a stabilizing trend after that day. This indicated that the biogas production potential of the reactor had been exhausted and was fading. The total amount of carbon dioxide and methane collected for TAD after 36 days was 6400 mL and 6330 mL, respectively and corresponded to 50.3% carbon dioxide and 49.7 % methane by volume. A value of 0.098 L biogas / g volatile solids added was calculated for TAD after the 36 days retention time. After a retention time of 36 days, a cumulative production of 8300 mL methane and 4500 ml carbon dioxide was recorded for SPAD corresponding to 64.6% methane and 35.3% carbon dioxide by volume. A 0.13 L biogas /g volatile solid was deduced for SPAD. The increase in biogas yield can be attributed to the effect of the 4-cycle steam pre-treatment. The recalcitrant volatile solids fraction of the substrate (especially lignin) was disrupted and broken, to an extent so as to liberate partly the embedded biodegradable fractions (cellulose or hemicellulose) to the biological degradation system. The higher total solids reduction (49.4%) obtained in SPAD can also support the observation that more solids had been degraded within the system. Zinder and Koch[36] have shown that interspecies hydrogen transfer may be important in acetate metabolism in the thermophilic digesters where the conversion of acetate may involve oxidation to hydrogen and carbon dioxide as an intermediate step, instead of the more common direct aceticlastic conversion of methyl group to methane (usual mode). Thus it is most probable that steam pre-treatment favoured the anaerobic digestion system to maximize the intermediate step instead of the aceticlastic conversion. Thus, in general, SPAD was much more successful in hydrogen-utilized formation of methane, leading to its higher methane content as compared to TAD.

CONCLUSION

Results from the study demonstrated that within 4 SD (45 minutes of supplied heating and cooling), significant physico-chemical changes occurred with an increase in VFA from 6.7 meq/L to 27.5 meq/L and a corresponding decrease in pH to 9.1 from 7.8. Physical appearance of the steamed slurry confirmed the effect of steaming as a process of physico-chemical transformation of lignocellulosics wastes into mainly organic acids. The higher evolution rates and volumes of methane and carbon dioxide from the anaerobic digestion of steamcooked

biomass advocated the merit of steam pre-treatment over the non-steam biomass anaerobic digestion process. A higher yield in biogas production was obtained from SPAD. A 0.13 L biogas /g volatile solid added was deduced for SPAD whilst only 0.098 L biogas/g volatile solids added was computed for TAD. Also, the improvement in methane content was more with SPAD than with TAD. Biogas production recorded for TAD consisted of 49.7 % methane as compared to SPAD where the volumetric methane content was 64.6%. The essence of these findings is therefore the enhanced calorific value of the biogas produced from the anaearobically digested steam pre-treated biomass.

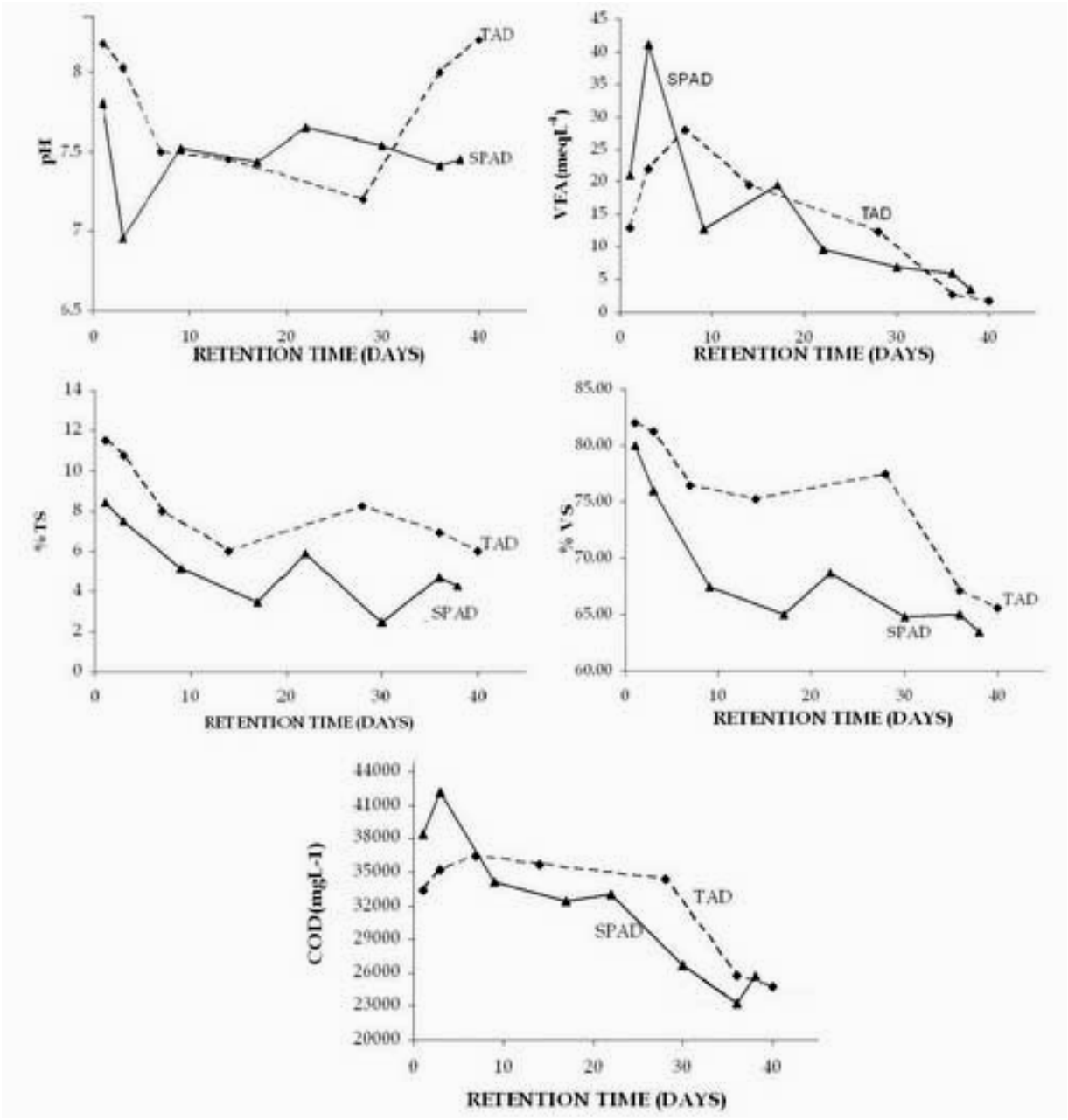

Figure-3:Variations in pH, VFA, total solids content, volatile solids content and COD for TAD and SPAD

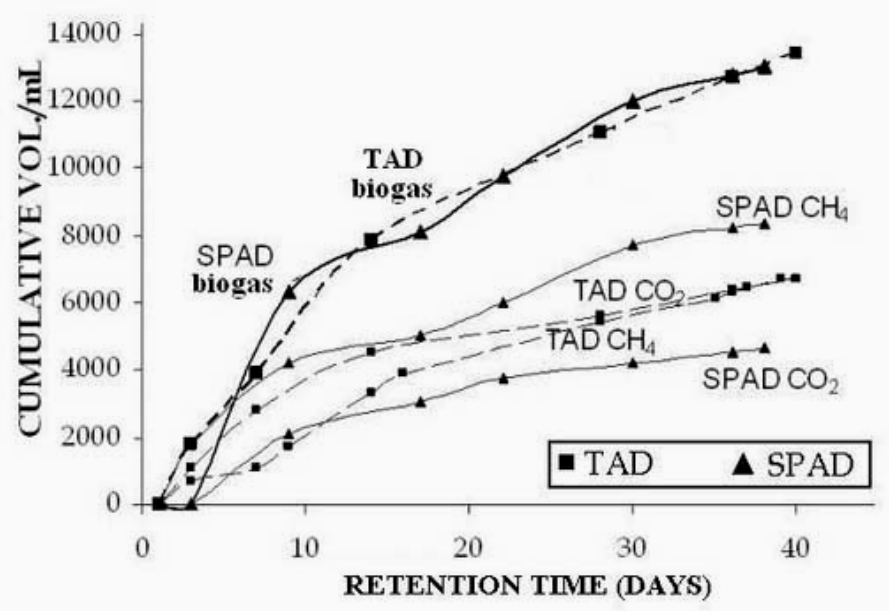

Figure-4: Comparison of biogas, carbon dioxide and methane generation rates for TAD and SPAD

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Nature teaches more than she preaches. There are no sermons in stones. It is easier to get a spark out of a stone than a moral.

-John Burroughs