AMELIORATING SISAL LEAF WASTES IN PURVEYING PLANT MACRONUTRIENTS

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Abstract: Sisal (*Agave sisalana*) is extensively grown in Kenya. It is a high waste generating crop. Waste disposal has remained a challenge to the sisal processing set ups. The wastes are used as landfills and or are burned to ashes despite being rich in plant macronutrients. Direct use scorches crops due to high acidity. Though composting takes too long before the biomass can be in use, it remains the recognizable approach, The study sought to establish pH trends of decomposing pretreated SLW with a view of seeking approaches to shorten the otherwise long period. Sisal leaf wastes (SLW) from young, mid old and old leaves were soaked in distilled water fresh, sun or shade dried and corresponding pH monitored. Effects of boiling SLW in lye and sodium hydroxide on deterioration were compared. It was established that natural decomposition of SLW is accompanied with a pH change from ≤ 4.8 (acidic) to > 7.5 (basic). SLW from young sisal leaves as well as shade dried ones deteriorated faster compared to those soaked fresh or after sun drying. Those (SLW) boiled in lye had evolution of the biogas earlier than either the fresh or 2M NaOH treated (boiled) samples. The SLW soaked without pretreatment on average took the longest period before biogas was detected. It is apparent from this study that boiling the SLW in an alkali shortens the decomposition period hence enables an easy access to macronutrient. Utilization of SLW in enhancing crop production would be an economical pathway of their disposal.

Key words: **Sisal, leaf, wastes, disposal, challenge, decomposition.**

1. INTRODUCTION

Sisal (*Agave sisalana*) production and yield in most countries like Kenya has been on the decline in the last four decades except Brazil (whose production) and China (yield) were on the rise. This was attributed to decreasing fiber prices and poor crop husbandry (Shamtu, 2000). The introduction of new applications for sisal fibers coupled with the push for eco-friendly fibers has however reversed the aforementioned trend. The need for value addition of agro-industrial waste products, which may become useful to society, has grown in recent years.

Sisal is a high waste generating crop. The leaf decortications residue is one of the most abundant agro-industrial residues in East African. Only 2.7-7.3% of the decortications of the sisal leaves produce the hard fiber that is used for various purposes depending on the age of the plant as well as the efficiency of the decortications process; the remaining 97.3-92.7% (Lock, 1969) consists of solid waste (mucilage) and waste liquid (juice of the sisal) that are normally discarded by sisal farms (Muthangya *et al.*, 2009). Kenyan sisal industries generate an estimated 340,000 tons waste which is well above the 27,000 tons of fibre produced. The untreated wastes are disposed of by both burning and dumping the ashes into water bodies and / or as whole in land filling. Such practices are not sustainable and contribute to environmental pollution (Ade *et al.*, 2011). In Tanzania the biomass is used to generate electricity hence, improving the profitability of the sisal industry. It is hoped that other sisal producing countries like Kenya will soon implement such eco-friendly activities. Sisal biomass is proposed in the manufacture of bio-fertilizers, since crop residues have proven potential for the manufacture of fertilizers (Taiwo, 2011).

Anaerobic microbiological decomposition is a process in which micro-organisms derive energy and grow by metabolising organic material in an oxygen-free environment resulting in the production of methane (CH_4). The anaerobic digestion processes consist of four phases, each requiring its own characteristic group of micro-organisms. These are hydrolysis, acidogenesis, acetogenesis and methanogenesis (Lettinga, Hulshoff-Pol, and Zeeman, 1999). The first steps of anaerobic digestion can occur at a wide range of pH values, while methanogenesis only proceeds when the pH is neutral (Lettinga and Haandel, 1993). For pH values outside the range 6.5 - 7.5, the rate of methane production is lower. A sufficient amount of hydrogen carbonate (frequently denoted as bicarbonate alkalinity) in the solution is important to maintain the optimal pH range required for methanogenesis. Several compounds exhibit a toxic effect at excessive concentrations such as ammonia, cations such as Na^+, K^+ and Ca^{++}, heavy metals, sulphides and xenobiotics, which adversely affect methanogenesis (Lettinga, Hulshoff-Pol, and. Zeeman, 1999).

The composition of cell walls, the major component of plant biomass, varies widely among species (Popper *et al.*, 2011) and may vary within an individual, depending on the cell type or in response to environmental conditions (Knox, 2008). Some cell types, such as those that make up the vascular tissues, have very thick walls that are usually rigidified with lignin. These cell types, which can constitute the majority of mass in a plant body, typically undergo a period of cell wall deposition to form "secondary cell walls" after cell expansion is complete. Other cell types, such as the mesophyll cells of leaves have relatively thin cell walls that minimize diffusive resistance to gases and the fluxes of the products from photosynthesis. The primary determinants of the quantity and composition of cell walls are developmental controls (Etchells *et al.*, 2012).

Cell walls can be considered to be composed of five main components: cellulose, hemicellulose, pectin, lignin, and minerals that are collectively referred to as lignocellulose. The bonds in lignin are very difficult to break under normal conditions. Lignin is a complex, phenolic polymer that acts as a protective barrier encasing cellulose and hemicellulose. It also provides structural support for and transport of water within the plant (Grabber, 2005).

Lignin can be removed from plant cell walls to varying degrees by extreme treatments including heat, acids, bases, oxidizing agents, and solvents. Most pretreatments cleave ester and ether linkages to varying degrees. During lignification, several types of covalent bonds are formed between the lignin monomers, including β aryl ethers, esters, and carbon–carbon bonds. The most common intermolecular linkages in lignin are β-O-aryl ethers formed by coupling the 4-hydroxyl group of the phenyl ring with the propanoid side chain of another subunit. β-O-4' aryl ethers represent 30%–50% of linkages in wood and up to 90% of linkages in grasses, and many pretreatments, including dilute acid, preferentially cleave these linkages (Villaverde et al., 2009). In contrast, ester linkages, which can be high in some grasses, are broken under alkaline conditions. Carbon–carbon linkages in lignin are the most recalcitrant, but they can be cleaved by oxidative catalysis.

Macronutrients are in part structural components that constitute the plant cells and part as salts that play specific roles. Nitrogen is a key component of proteins, pyrimidines, nucleic acid (DNA and RNA), in polyphyrine structures present in chlorophyll and cytochrome enzymes. Phosphorous on the other hand is a component of every living cell, phospholipids, nucleic acid, nucleic proteins, co- enzymes NAD/NADP/ATP. Calcium cements the walls of the cells together forming cell membranes and lipid structures while potassium exists as a soluble inorganic salt or in organic acid salt in the cells. The plant macronutrients therefore are found as part of the lignocellulose biomass whose main bonding material lignin, a polymer of three major phenolic components: p-courmaryl alcohol, conferyl alcohol and sinapyl alcohol (Rubin, 2008) are susceptible to alkali attack. The alkali dissolves lignin away and in essence making it easy for micro-organisms penetration.

The ferulic acid linkage between the lignin and hemicellulose fractions is the point of reaction during NaOH pretreatment (Buranov and Mazza, 2008). The ester bond between the ferulic acid and the carbohydrate is highly susceptible to alkali degradation, as the hydroxide ion (dissociated from NaOH) increases the rate at which the hydrolysis reaction occurs as compared to water (Bruice, 2004). The mechanism of alkaline pretreatment is such that the hydroxide ion attacks the carbon of the ester bond, whether between the lignin and carbohydrate or even between two lignin components or two carbohydrate components. A tetrahedral intermediate forms but quickly collapses when a negatively charged oxygen atom expels an alkoxide (–OCH$_3$) from the carboxylic acid. In a very fast reaction, the resulting alkoxide acts as a base, deprotonating the carboxylic acid. The result is the irreversible hydrolysis of the ester bond, weakening the structural integrity of the lignocellulose.

Pretreatment with sodium hydroxide results in several structural modifications of lignocellulose that are beneficial for enzymatic hydrolysis. Bonds linking the protective lignin barrier with hemicellulose are broken. Depending on the pretreatment conditions, lignin is partially or totally solubilized, and degradation of the hemicellulose fraction may occur. Sodium hydroxide pretreatment also swells the lignocellulose particles, leading to an increase in surface area and greater accessibility to the cellulose fraction. Additionally, a decrease in the degree of polymerization and crystallinity of the cellulose is likely, increasing the enzymatic digestibility of the polysaccharide.

It has been hypothesized that disruption of this cross-linking enhances enzyme adsorption and enzyme effectiveness. Removal of lignin by NaOH often leads to the release of acetyl groups and uronic acid substitutions, which can enhance the digestibility of cellulose and hemicellulose (Wan et al., 2011). However, hydrolytic enzymes can be inhibited by some of these degradation products, such as xylooligomers (Qing et al., 2010), organic acids and phenols (Kim et al., 2011). Balance is the key to achieving optimal lignin removal while limiting the production of inhibitory compounds. Cui et al. (2012) found that delignification was influenced by NaOH loading, time, and moisture content during long-term wet storage of corn stover. As NaOH loading increased from 4 g to 40 g NaOH per 100 g biomass, lignin degradation increased moderately from ~7% to ~15%.

2. MATERIALS AND METHODS

Sisal leaf samples (young, mid old and old) were collected in duplicate from mature sisal plants on farms in Lugari, western Kenya. The leaves were stripped before extrusion of sisal fibers to obtain sisal leaf wastes (SLW). The SLW samples were used fresh, sun dried and shade dried. Drying was done for two weeks 8 hours a day until constant weight was attained. Five kilograms of dry maize cobs (DK variety) were weighed onto steel metallic sheet and burned to obtain the cobs ash. The ash was transferred into a plastic container, mixed with 1 litre of distilled water and stirred thoroughly before filtering to obtain the cobs ash lye. The procedure was repeated twice using 1 litre of water, stirring and filtering off the residue respectively. The total lye filtrate was stored in a three litre plastic container. 2 M NaOH was made by dissolving 20 g in 250 ml distilled water.

2.1 pH and decomposition of sisal leaf wastes (SLW)

The pH of decomposing sisal leaf biomass was investigated. Ten grams of freshly prepared sisal leaf biomass (constituting set I) were soaked in 100 ml of distilled water in 250 ml beakers and stirred. The procedure was repeated for sisal leaf biomass that had been directly sun dried (set II) and shade dried (set III). The contents for each of set I, II and III had initial pH taken and left standing for thirty days. This was done with a view to monitor pH changes as the sisal biomass decomposed in order to determine how sisal leaf biomass would be treated to enhance a quick access to macronutrients.

2.2 Methanogenisis in Alkali Pretreated Sisal Leaf Wastes (SLW)

Dry sisal leaf wastes (10 g) were weighed into sets of round bottomed flasks labeled SW (sisal wastes), CASD (cobs ash sisal digest) and NaD (sodium hydroxide digest). Deionized water (200 ml) was added to each of the flasks SW. This was done in triplicate. The sisal leaf wastes in set ups labeled CASD were each mixed with 200 ml lye extracted from maize cobs ash while flasks labeled NaD had 200 ml of 2 M NaOH added. All the set ups were each heated with a Bunsen burner flame for ten minutes before cooling to room temperature (25° C). Cumulative gases generated were collected over 0.5 M HCl solutions in an inverted measuring cylinder and volume monitored daily.

3. RESULTS AND DISCUSSION

3.1 pH Changes and Decomposition of Sisal Leaves

The study investigated the pH changes in decomposing sisal leaf wastes. Sisal leaf wastes from relatively young, mid old and old were soaked in distilled water when fresh, sun dried and shade dried. The pH was monitored daily. This was to determine how treatment of the biomass affected the initial pH of sisal leaves of different ages, pH changes, expected optimum period before end of decomposition. It too facilitated projection on a way of shortening the composting period. Table 1 shows analyzed results when treated sisal leaf wastes were soaked in water and left standing.

Table 1: Mean ± SD of pH of pretreated sisal leaf of different relative age

Treatment	Relative age (Leaves)	Mean ± SD per day of soaking								p-value (days)
Fresh	Old	4.19 ± 0.19^{ab}	3.66 ± 0.21^{bc}	3.30 ± 0.15^{c}	3.30 ± 0.16^{c}	3.31 ± 0.16^{c}	3.71 ± 0.52^{b}	4.03 ± 0.44^{ab}	4.60 ± 0.98^{a}	0.012
	Mid	4.23 ± 0.24^{b}	3.62 ± 0.10^{bc}	3.33 ± 0.13^{c}	3.38 ± 0.09^{c}	3.43 ± 0.13^{c}	3.80 ± 0.29^{bc}	4.63 ± 0.84^{a}	5.22 ± 0.68^{a}	0.000
	Young	4.26 ± 0.28^{b}	3.77 ± 0.25^{b}	3.71 ± 0.38^{b}	3.65 ± 0.43^{b}	3.67 ± 0.48^{b}	4.20 ± 0.61^{b}	5.27 ± 0.82^{a}	5.40 ± 0.69^{a}	
	Mean (pH)	4.22 ± 0.22	3.67 ± 0.21	3.41 ± 0.28	3.44 ± 0.32	3.46 ± 0.34	3.91 ± 0.54	4.61 ± 1.04	5.03 ± 0.87	0.003
	P-value	**0.848**	**0.451**	**0.131**	**0.248**	**0.119**	**0.210**	**0.194**	**0.185**	
Sun Drying	Old	4.38 ± 0.29^{xb}	4.15 ± 0.13^{b}	3.90 ± 0.21^{b}	4.18 ± 0.51^{b}	4.50 ± 0.73^{b}	5.63 ± 1.02^{a}	6.18 ± 0.87^{a}	6.60 ± 0.98^{a}	0.001
	Mid	4.43 ± 0.38^{x}	4.23 ± 0.22^{c}	3.95 ± 0.10^{c}	3.93 ± 0.25^{c}	4.35 ± 0.57^{c}	5.95 ± 0.54^{b}	6.65 ± 0.34^{ab}	6.90 ± 0.46^{a}	0.000
	Young	5.30 ± 0.56^{ybc}	4.38 ± 0.23^{cd}	4.13 ± 0.32^{d}	4.43 ± 0.55^{cd}	4.77 ± 0.80^{c}	5.63 ± 0.40^{bc}	6.17 ± 0.40^{ab}	6.47 ± 0.50^{a}	0.001
	Mean (pH)	4.65 ± 0.56	4.23 ± 0.19	3.98 ± 0.22	4.15 ± 0.44	4.52 ± 0.65	5.75 ± 0.67	6.31 ± 0.58	6.67 ± 0.66	-
	P-value	**0.042**	**0.033**	**0.025**	**0.122**	**0.445**	**0.448**	**0.519**	**0.754**	
Shade drying	Old	4.36 ± 0.34^{c}	4.13 ± 0.10^{bc}	3.86 ± 0.19^{c}	4.11 ± 0.47^{c}	4.44 ± 0.63^{bc}	5.43 ± 1.03^{b}	6.08 ± 0.91^{a}	6.46 ± 0.95^{a}	0.001
	Mid	4.43 ± 0.38^{c}	4.23 ± 0.22^{c}	3.95 ± 0.10^{c}	3.93 ± 0.25^{c}	4.35 ± 0.57^{c}	5.95 ± 0.54^{b}	6.55 ± 0.34^{ab}	6.90 ± 0.45^{a}	0.000
	Young	5.33 ± 0.45^{b}	4.66 ± 0.36^{b}	4.40 ± 0.65^{b}	4.81 ± 0.69^{b}	5.07 ± 0.68^{b}	5.91 ± 0.67^{a}	6.46 ± 0.53^{a}	6.86 ± 0.61^{a}	0.001
	Mean (pH)	4.73 ± 0.59	4.33 ± 0.40	4.08 ± 0.47	4.33 ± 0.64	4.65 ± 0.69	5.72 ± 0.82	6.32 ± 0.69	6.70 ± 0.74	-
	P-value	**0.035**	**0.499**	**0.391**	**0.366**	**0.742**	**0.790**	**0.629**	**0.713**	
Overall	**P- value**	**0.359**	**0.009**	**0.028**	**0.036**	**0.004**	**0.000**	**0.003**	**0.001**	

Same small letters in rows (a, b, c) and columns (x, y) showed no significant differences

The initial mean pH values of fresh, sun dried and shade dried sisal leaf biomass when soaked in distilled water were 4.22±0.22, 4.65±0.56 and 4.73±0.59 respectively (Table 1). Other studies report sisal leaf pH value of 4.0 (Kategile, 1986) and 4.8 (Purseglove, 1992). The values found in this study fall within this range. Sisal leaf wastes are acidic. Presence of organic acids including oxalic acids is responsible for the initial acidity of sisal leaves biomass.

When pH values of samples soaked fresh, sun or shade dried were considered, it was observed that pH values in the entire period of composting significantly varied ($p<0.05$) regardless of the relative age of the sisal leaf. pH generally rose in all samples towards an optimum value that was well above 7. pH values of samples soaked fresh (separated by relative ages) per day (down the columns) were compared. There was no significant difference ($p>0.05$) between pH values of samples (separated by relative age of the leaves). Samples sun dried before soaking exhibited significant difference ($p<0.05$) for the initial successive pH values measured. Sun drying greatly affected young leaves causing an overall difference in pH, Young sisal leaves have tender tissues and the extend of effect by the sun drying was more compared to the relatively older leaves. On the other hand shade dried samples showed significant difference ($p=0.035$) only between the initial pH values of samples as separated by relative age of the leaves. Shade drying does not crystalize as much of the organic acids in the young leaves

as does sun drying. Moreover levels of oxalic acid in sisal leaves differ with the maturity of the plant (Rahman and Kawamura, 2011).

The overall effect of different treatments on the pH of soaked sisal leaf wastes per day, analysis of variance showed no significant difference ($p = 0.359$) in the initial pH of all samples separated by treatments. However there was significant difference ($p < 0.05$) in successive daily pH measurements. The overall daily averages (pH) per treatment over the period of composting were compared (Fig 1).
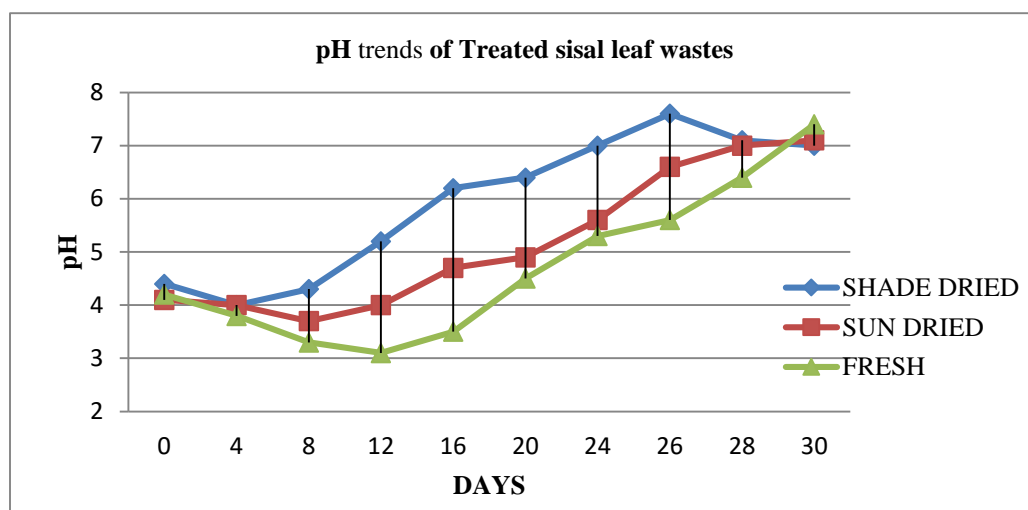
Figure 1: Comparison of effect of treatments on pH changes of soaked sisal leaf wastes

Different treatments affected the rates of change in pH values rather than the pattern or trend. In figure 1 shade dried samples had the fastest change in pH. Sun dried samples fell between the shade dried and those soaked fresh. Those soaked fresh took longer to attain a given pH. Fresh sisal pulp contain at least 10% soluble sugars (DM) which fall to less than 1% after few days (Herrera *et al*., 1981) due to rapid fermentation producing lactic acid. The fermentation starts immediately after fiber extraction (Preston and Leng, 1987). The acid produced lowers the pH.

Presence of oxalic and other organic acids in sisal plant tissues are responsible for the low pH of the mixtures when soaked. Sun drying significantly reduces acid value of plant materials partly due to crystallization or oxidation (Hassan *et al*., 2007). Sun drying only crystallizes the acid with the greatest effect being on younger leaf wastes. Gebremariam and Machin, (2008) observed that sun drying does not affect pH but only promotes crystallization of the acids in the sisal wastes. Sun drying lowers the amount of water and so destructive enzymes are inactivated (Hassain *et al*., 2010) unlike shade drying, a slow process that allows destruction to start off. The extent of crystallization of the acid in the sisal waste differs. Relatively old leaves with fully developed structural matrix do obscure the effect of sun drying. The crystallization of oxalic acid in young sisal leaves was presumably more and had initially less acid crystals to dissolve when wastes were soaked. It is noticeable that whether use of sun dried or fresh sisal leave wastes, trends in pH changes are independent of the age of the leaves. If sisal leaf wastes were to be used straight then shade drying before composting would be recommended for it creates an environment for rapid change in pH compared to other treatments.

The trends in pH values across the entire period were not affected by the treatments as in all cases they followed the same pattern. Similar patterns were observed in the deterioration of samples separated by relative age of the leaf wastes (Fig 2).
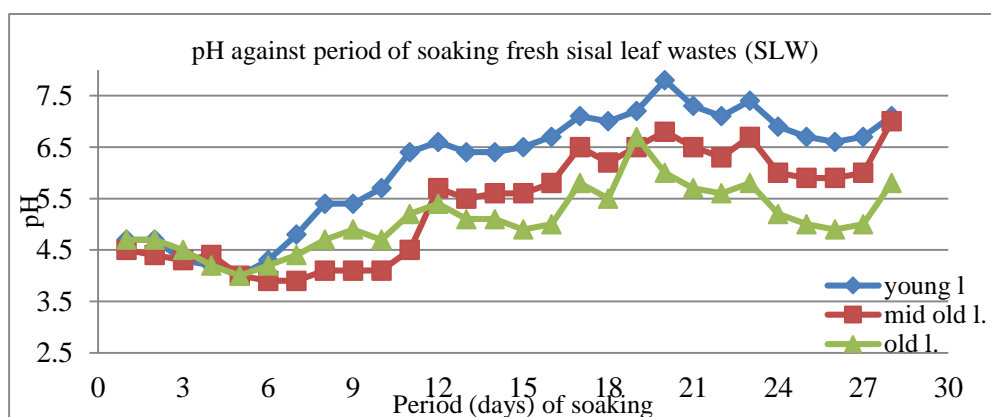

Figure 2: pH trend curves for old, mid old and young sisal leaves

The pattern followed started with a pH drop after soaking until a minimum value was attained. This was followed by a subsequent rise before slight drop to a second minimum after which there was a rise in pH. When sisal leave wastes were soaked, hydrolysis involving liquefaction of the bio degradable organic carbon (DOC) producing soluble sugars, amino acids

and long chain fatty acids on the whole lowered the pH (Sleat and Mah, 2006)   The pH values in all the cases then increased with time before again showing a drop. This rise in pH was due to bacterial breakdown of the amino acids as well as the long fatty acids responsible for lowering pH (Sleat and Mah, 2006). The rise in pH that ensues is however immediately followed by drop that can be explained as acidogenesis in which acidogenic (acid forming) bacteria form hydrogen, short chain volatile fatty acids including acetic acid and alcohols from the soluble compounds earlier formed (Sleat and Mah, 2006). The rise in pH that followed was due to methanogenesis, a process that leads to production of methane and carbon (IV) oxide (Salum and Hodes, 2007).

Though decomposition of the plant biomass makes macronutrients readily available, the rate is significantly affected by pH. The composting period allows pH change from acidic to basic as microorganisms break down the lignocellulosic material. When sisal leaves' biomass was soaked there was a significant rise in pH range from 4.5 to 7.9. Mechanisms to fasten composting or digestion of sisal leaf wastes would target not only breaking down the lignocellulosic material but also seek raising pH of the samples. It was suggestive that soaking in a base or distilled water be considered as a pre-treatment of sisal leaf biomass for the formulation. The rate of decomposition is usually dependent on temperature, moisture, aeration and pH (Lines-Kelly, 1993; Walls-thumma, 2000).

3.2 Effect of Alkali Pre-treatment on Decomposition of SLW

The final phase in decomposition of plant biomass is methanogenisis that involves conversion of acetate, carbon (IV) oxide and hydrogen into biogas (methane and carbon (IV) oxide). The study sought to establish effect of lye, 2 M NaOH pre-treatments of sisal leaf wastes in attainment of methanogenisis phase compared to a control with no treatment. Dry sisal leaf wastes weighed into round bottomed flasks (labeled SW, CASD, NaD) were mixed with deionized water, lye extract and 2 M sodium hydroxide respectively. The mixtures were boiled, cooled before connecting to gas collection set up. The biogas generated was collected over 0.5 M HCl into a measuring cylinder. Total volume (ml) collected per day in each set was used to plot a graph (Fig 3).
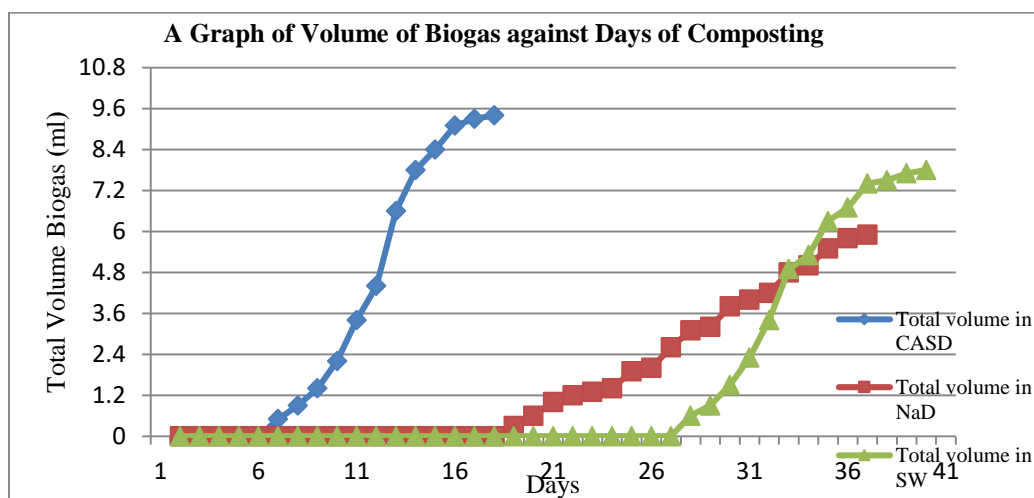

Figure 3: Effect of sisal leaves pretreatment on biogas production

Significant volume of biogas was first seen in set ups in which sisal leaf wastes were pre-treated with lye (CASD- cobs ash sisal digest). This was six days after composting. The biogas evolution in the set ups in which sisal leaf wastes had been pre-treated with 2 M NaOH (labeled NaD-sodium sisal digest) were observed from the 18th day after setting up. This was ten days after CASD set ups. Generation of biogas in the control set ups SW (sisal wastes) was only after 26 days of composting. The pre-treatment affected the start of the methanogenic phase. The lye not only breaks down the lignocellulosic materials but also provides a favourable pH range 6.5 -7.8 required for the methanogenesis phase (Lettinga and Haandel, 1993). Jay et al., 1997 reports a pH of 6.8 as the optimum condition that favours this phase. In addition concentration of Na^+, K^+ and Ca^{2+} from lye is not as high to have a toxic effect on the phase too (Lettinga and Haandel, 1993).

Maize cobs ash extract has potassium and sodium carbonates that hydrolyze in water to give hydroxides. The hydroxides in low concentrations do the hydrolysis. High concentrations of strong alkalis such as 2 M NaOH not only break down the lignocellulosic material but also provide pH range well above 12 that does not favour immediate conversion of acetate, carbon (IV) oxide and hydrogen to methane. Biogas production however started off earlier than when sisal leaf wastes were soaked without pretreatment. It was conclusive that lye extract be used in pre-treatment of sisal wastes or a lower concentration of alkali solutions to shorten composting period. In a comparative evaluation of different pre-treatment methods on biogas production from paddy straw, Vishwas et al., (2017) reported an increase of 49.7% when a 2% concentration NaOH was used. In a separate study Demirel and Schever, (2008) reported a significant enhancement in the production biogas when potassium hydrogen carbonate was used for pretreatment of sugar beet silage.

4. CONCLUSION

 Resistance of plant cells to deconstruction (recalcitrance) is a characteristic of the lignocellulosic biomass material. Sisal leaf wastes (SLW) as plant material are no exception. Utilization of SLW as fertilizers has over time been limited partly

because of its high level of acids as well recalcitrance. The resistance is due to the highly crystalline structure of cellulose embedded in a matrix of polymers-lignin, hemicellulose, acetyl groups. The well-knit structure hinders a quick access to macronutrients for in biomass rich like those from sisal leaves. Though acids, alkaline solutions and oxidative catalysts can help reduce the resistance, lye extracts from plant ashes not only breaks down the lignin bonding material but also provides ideal environment that facilitates a quick decomposition of the remaining matter. SLW boiled in a lye extract of maize cobs, decomposes faster (evidenced by biogas production) availing plant macronutrients that are useful in enhancement of plant growth and yield.

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