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# Effect of Acid-Alkaline and Thermal Pre-treatment to Cassava Pulp Feed for Batch Reactors in Optimization of Bio-Methane Yield

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## Abstract

Cassava starch mills in Nakhon Ratchasima province operate biogas plants to generate renewable energy from surplus cassava pulp using anaerobic digestion (AD) technologies. However, the biogas yields fluctuate and digestion failure occurs due to suboptimal digester configuration and lack of understanding of the specific properties of cassava pulp substrate. This study used acid-alkaline and thermal pre-treatment to modify the cassava pulp substrate to enhance biogas yields. Concentrated 36 N sulphuric acid ($H_2SO_4$) and 20 M sodium hydroxide (NaOH) was chosen as an acid-alkaline pre-treatment to adjust to the required pH for the substrates, and 45 min at 200 ˚C for the thermal pre-treatment. Extreme pH adjusted substrates such as T1, T2, T12 and T13 required both acid and alkali in high volume, and inhibition occurred from both acid and alkali resulting in retardation of fermentation by hydrolytic bacteria, a lower volatile fatty acid to total alkalinity ratio (VFA/TA), more depletion of reducing sugars and a lower bio-methane yield. The results showed Soluble Chemical Oxygen Demand (SCOD) obtained from decomposition of lignocellulosic structure of fresh cassava pulp by combined thermal-chemical pre-treatment, was found highest in T2 which was pre-treated at pH 2 having more than 100 g $L^{-1}$. Though SCOD could be enhanced by acid-alkaline pre-treatment, it led to inhibition driven by radicals of acid and alkaline. Three different mixing ratios, i.e. 3 %, 5 %, and 10 % (w/v) were compared against without pre-treated samples, and found 5 % Total Solids (TS) was most suitable after subjected to acid-alkaline pre-treatment and produced biogas yield at 4125.2 mL $kg^{-1}$ TS in batch digestion for 21 d. Pre-treatment was found increase bio-methane by up to a factor of six.

**Keywords:** Cassava pulp; Acid-alkaline; Thermal pre-treatments; Anaerobic digestion; SCOD

## Introduction

The combined impact of massive extraction of fossil fuel and its environmental costs has driven the pursuit of renewable energy sources such as biomass, solar, wind, hydropower and geothermal energy. Thailand promotes the renewable energy sector, harnessing energy from biomass to reduce its heavy dependence on energy imports [1]. The country's agricultural sector offers tremendous potential to generate renewable energy from agro-industrial by-products and waste. With a steady increase in annual production, Thailand ranks as Southeast Asia's top cassava producer. According to the Thai Tapioca Starch Association, production surpassed 33 million tons in 2015/2016. The industry generates massive quantities of numerous by-products in its starch production chains. On average, for each ton of cassava starch produced, 1.4-1.6 tons of highly fibrous carbon source enriched cassava pulp and 19-22 $m^3$ of wastewater are also produced [2]. The low protein content of cassava pulp renders it unattractive for animal feed manufacture. Creating serious environmental burdens and a mounting industrial waste management problem for starch mills.

Anaerobic digestion (AD) technology for energy production for either bio-methane ($CH_4$) or bio-ethanol ($CH_3CH_2OH$) is a proven technology to manage agro-industrial wastes and recover energy from diverse biomass substrates [3]. However, the technology includes numerous restrictions and limitations for developers to harness dependable and stable energy supplies under the constraints of cost and benefit. Anaerobic digestion process begins with the processes of hydrolysis, acidogenesis, acetogenesis and methanogenesis in order, through symbiosis of diverse anaerobic bacteria and synthesized products of each phase [4]. Biomass containing carbohydrates, proteins and lipids in the form of complex polymers are subjected to hydrolytic decomposition into monomers such as amino acids, sugars, alcohols and fatty acids [4-6]. During acidogenesis, these hydrolysis products are transformed into intermediary products such as acetate, propionate, ethanol and lactate [7]. Acetogenesis is responsible for converting these intermediary products predominantly into acetic acid ($CH_3COOH$), hydrogen ($H_2$) and carbon dioxide ($CO_2$) [4, 7-8]. The final stage (methanogenesis) enerates $CH_4$ and $CO_2$ [8-9]. The success of biogas and biogas plants depends on many environmental variables and design parameters such as temperature, loading rate, retention time, solids concentration, C/N ratio, alkalinity, pH, inhibitors and stimulators [10]. The digestion system depends on the availability of resources and plant capacity. Despite many studies on the comparative potential of different substrates and reviews of anaerobic digestion technologies, specific research regarding cassava pulp remains lacking.

All types of biomass are comprised of cellulose, hemicellulose, and lignin, which in combination are known as lignocellulose [11]. Each individual biomass substrate possesses a distinct structural configuration and composition, making them resistant to breakdown during AD processes [12]. Although cellulose of common substrates is soluble in water, and hemi-cellulose with lignin chains are sensitive to thermal and chemical decomposition, upon subjected to suitable pre-treatment, they start to dissolve, enriching the nutrient content of the feedstock substrate. Therefore, pre-treatments may be adjusted, depending on substrate type, to enhance solubility and enzymatic degradation. Of several pre-treatment options available, thermal, chemical, and ultrasonic methods have proved effective, time saving, and economically feasible, whereas biological and enzymatic methods are less attractive because of their complexity and slow conversion rates [13]. Selection of pre-treatment methods relies on the properties of individual biomass and applicability. In recent years, researchers have been focusing on diverse substrates, ranging from forest by-products, agricultural residues and herbaceous grasses to municipal waste [14-17]. However, cassava crop residues remain to be studied as only Thailand produces cassava as a commercial cash crop and uses cassava pulp as a feedstock for biogas plants.

Several authors have reported the use of pre-treatment methods for cassava waste to produce diverse ranges of product from bio-ethanol to hydrogen. Phowan and Danvirutai reported a hydrogen yield of 432 ml $H_2$ $g^{-1}$ $COD_{reduced}$ and hydrogen production rate of 2,281 ml $H^2$ $L^{-1}$ $d^{-1}$ achieved using dilute sulphuric acid pre-treatment up to 5 % with a reaction time of 30 min at a temperature of 121 ˚C [18]. Similarly, for ethanol fuel, a study conducted by Srinorakurata and co-workers using combination of dilute sulphuric acid of 0.2-5.0 M and α-amylase enzyme at a temperature of 60-120 ˚C for 30 min, maximum ethanol production was found 3.62 % (w/v) in a 10 L fermenter at 24 h [19]. Also, Kosugi and co-workers conducted pre-treatment of cassava pulp to produce ethanol using hydrothermal and enzymatic hydrolysis. In these studies, the researchers used $H_2SO_4$ at a concentration of 0.1 or 2.0 %, K7 strain, and heated at 120 to 180 ˚C for 1 h, proving ethanol could be produced up to 40 mL $kg^{-1}$ cassava pulp within 7 d of fermentation [20]. With regard to NaOH addition, Penaud et al. proved that upon hydrolysing biomass COD for 30 min at 140 ˚C and pH 12, COD could be increased to 71 % of any substrate [21]. Although there are plenty of researches relating to pre-treatment methods to enhance liquid biofuels, in the field of biogas the novel research remains to be explored.

This study fills this gap by highlighting optimization of biogas yield from cassava pulp feed batch reactors by concentrated acid-alkaline and thermal pre-treatment. Cassava pulp was subjected to strong sulphuric acid ($H_2SO_4$), sodium hydroxide (NaOH) and thermal hydro-lysis prior to anaerobic digestion in batch reactors at different pH ranges and 3 different solid contents (3 % TS, 5 % TS and 10 % TS). The influence of acid-alkali and thermal pre-treatment results and digesters performance was compared against control sets. This study aims to enhance biogas yields in existing cassava pulp feed biogas plants by applying the findings and to encourage a change from the current practice of directly feeding cassava pulp as a common feedstock by mixing cassava pulp with mill effluent.

## Experiment

### 1) Materials and methods

Fresh cassava pulp was collected from Korat Starch Factory located in Nakhon Ratchasima province in north-eastern Thailand. Prior to collection, a qualitative farm survey was conducted to identify the cassava variety. Four cassava cultivars predominated, namely CMC 76, KU 50, Rayong 60, and Rayong 90 in commercial operations in this province. Upon proximate analysis for each individual sample collection, fresh cassava pulp of all variety was found to contain 55-70 % moisture on a wet basis, 60-65 % of starch and 15-18 % of fiber [22]. Wastewater from the starch mill was sampled and stored at 4 ˚C until use. APHA standard methods (1995) were used to determine Soluble Chemical Oxygen Demand (SCOD), Carbon to Nitrogen ratio (C/N), Volatile Fatty Acids (VFAs), Total Alkalinity (TA), and reducing sugars [23]. Inoculum was taken from parent biogas plant, maintained at 35˚C and utilized within 12 h of collection time. Inoculum properties having pH value about 7.5-8.0. Laboratory samplings were done prior to start anaerobic digestion in batch reactors.

### 2) Acid-alkaline and thermal pre-treatment

Cassava substrates were made in 3 % TS, 5 % TS and 10 % TS (w/v) by mixing solid cassava pulp with 1,000 mL of effluent wastewater from the starch mill. Concentrated sulphuric acid ($H_2SO_4$) of 36 N and sodium hydroxide (NaOH) of 20 M were used to adjust to the desired pH ranging from 1-13, denoting pre-treatments from T1 to T13. The boiling point of the substrates (comprising mixtures of fibrous cassava pulp and mill effluent starts) at 150 ˚C. Thus for practical purposes, the temperature and reaction time for acid-alkaline and thermal pre-treatment were set at 200 ˚C for 45 min on a hot plate. The reaction vessels were then left to cool and undergo acid-alkaline attack for a further 6 hrs. Finally, the hydrolyzed samples were neutralized back to pH 7.5 for digestion. Therefore, the whole pre-treatment mechanism includes forward and backward neutralization to reach the desired pH set before and after thermal pre-treatment. Two control samples, one in natural wastewater with neither acid-alkaline nor thermal pre-treated (C1) and one with only pH 7 adjusted (C2) but was not subjected to thermal pre-treatment, were compared against all acid-alkaline and thermal pre-treated sample sets (T1-T13). For trial investigation on the effect of acid-alkaline and thermal pre-treatments, sampling was run on 3 % TS, and then integrated the results for remaining 5 % TS and 10 % TS.

### 3) Biogas potential assay and statistical analysis

To obtain optimum gas yield from the batch reactors of each set, batch reactors were set up in polypropylene bottles of 650 ml content. The head space was largely eliminated by adding 300 ml of prepared substrate and 300 ml of inoculum. Batch reactors in three different solid contents, i.e. 3 % TS, 5 % TS and 10 % TS were set up separately and compared. The Feed to Microorganism ratio (F/M) was set at 0.5 and anaerobic digestion was maintained under 33 ˚C (+/-2) in thermo-control room. The digestion period (HRT = SRT) was set at 21 d. Biogas was collected via a water displacement system and total biogas yield was measured at the end of the digestion period. To avoid possible interference, variances between each sample during laboratory investigation of control parameters were eliminated by triplicating each individual sample set.

## Results and discussion

### 1) Effect of chemical pre-treatment on pre-treatments and substrate properties

The effect of acid-alkaline pre-treatment contributed not only to accelerated solubility of the carbon source attached to the lingocellulosic structure of the substrate, but also in adjusting to the desired pH upon addition of the relevant acid or base. The stronger the acid or base, the more rapid the changes in pH during hydrolysis. Thermal treatment at 200 ˚C did not significantly change pH of the substrate (a mixture of cassava pulp and mill effluent) from its original value of pH 4.3. The addition of concentrated $H_2SO_4$ and NaOH catalysed decomposition of lignocellulosic structure of cassava pulp. The amount of acid and alkali required is reported in Figure 1. While substrates with higher solid content required a higher volume of chemicals, the resulting high solubilisation of COD led to excessive loading of the batch digester, causing inhibition of microbial activity to initiate the AD process. The overall w/v of substrate was ultimately maintained by using strong acid and base in hydrolysis since uniform substrate volume is necessary to control prior to start digestion within the specified solid to liquid ratio and F/M ratio of 0.5.

Experimental results for 3 % TS hydrolysis revealed that substrates having extreme pH, i.e. T1, T2, T12 and T13 required higher volumes of both 36N $H_2SO_4$ and 20M NaOH. Alkaline demand reached up to 15.9 mL and 17.5 mL, respectively, in T1 and T13, and acid consumed up to 7.2 mL in T1 and 6.5 mL in T13. Overall, all sets of substrates required NaOH addition to adjust to the designated pH range during hydrolysis and to neutralize back to pH 7.5, the optimum pH to trigger anaerobic digestion [24-25]. NaOH in which $Na^+$ radical evolved as an inorganic salt during chemical reaction with carbohydrate from cassava pulp, they serve as a nutrient source for microorganisms in the AD process [26-27]. While $Na^+$ content in the range of 100-200 mg $L^{-1}$ had stimulatory effects, an inhibitory effect began at 3,500 mg $L^{-1}$, and was toxic above 5,500 mg $L^{-1}$ [27].

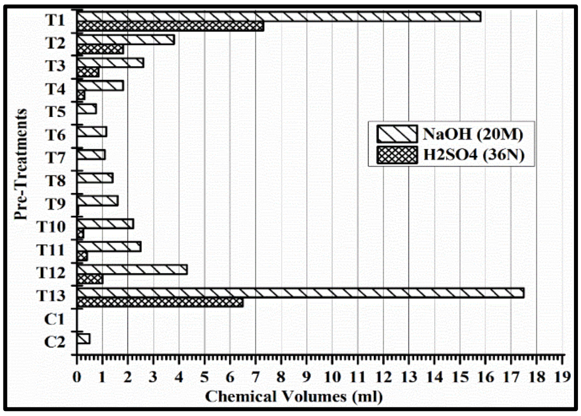

**Figure 1** $H_2SO_4$ and NaOH consumption for hydrolysis of 3 % (w/v) of cassava pulp.

The experimental results revealed that the extreme pH treated substrates that demanded substantial volumes of both strong NaOH and $H_2SO_4$ (up to 4,000 mg $L^{-1}$ of sodium and 2,500 mg $L^{-1}$ of sulphate, Figure 1), were found to have inferior digestion performance in comparison to those substrates treated with neutral ranges. This may be due to inhibition of both sodium ($Na^+$) and sulphate ($SO_4^{2-}$) ions occurs during anaerobic digestion. Even though strong acid and alkaline conditions can enhance solubility of the degradable organic fraction, the increase in soluble COD was also related to the effect of the combination of both thermal and chemical pre-treatment. While thermal pre-treatment at above 150 ˚C for more than 30 min can exterminate the effects on enzymes, fungus and microorganisms in the substrates, lower concentrations of acids and alkalines significantly can significantly change the final solid to liquid ratio (w/v), then subsequently affects F/M ratio and modifies into new solid content of the digester. Therefore, despite thermal pre-treatment could enhance solubilisation of carbon source in the substrates, it required higher F/M ratio for AD process to be initiated.

### 2) The influence of thermal-chemical pre-treatments on SCOD

Experiment results indicated that substrates subjected to pH 2 (T2) were found to generate the highest soluble chemical oxygen demand (SCOD) of more than 100,000 mg $L^{-1}$ (Figure 2). With the exception of substrates treated to pH 13 (T13), the remaining sample sets produced SCOD ranging between 70,000 to 90,000 mg $L^{-1}$. It can be concluded that substrates subjected to highly acid conditions had higher SCOD formation than alkaline treated samples.

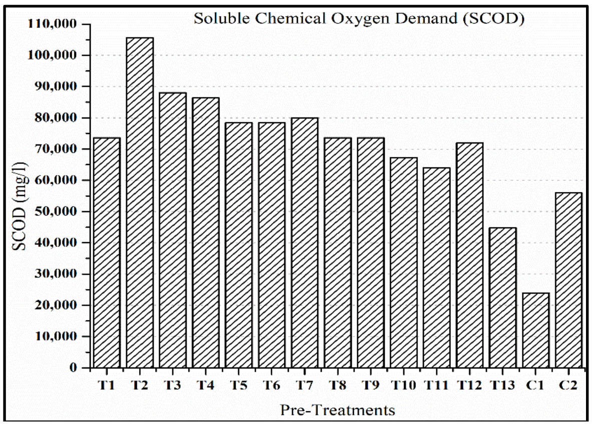

**Figure 2** SCOD produced by acid-alkaline and thermal pre-treatment of cassava pulp in 60 min of 3 % (w/v).

In addition, formation of SCOD was inversely proportional to the TSS and VSS since complex polymer compounds of cassava pulp were dissolved during hydrolysis. In contrast, SCOD production increased with total solid content in the raw substrate. Since cassava pulp is rich in carbohydrate, total solid content of more than 5 % was found inhibition effects on micro-organisms to initiate biogas fermentation processes. The control set, C1 was found to produce no noticeable amount of SCOD to its original SCOD values of 25,000 mg $L^{-1}$ and C2 being only acid-alkaline pre-treated produced 58,000 mg $L^{-1}$. Thus, the experiment revealed that SCOD originated from cassava pulp which is as major source for biogas production in this study could be enhanced either by applying either acid-alkaline or thermal pre-treatment.

### 3) The impact of pH changes on C/N ratio for each pre-treatment

Due to the strong chemical attack, samples at lower pH had a lower C/N ratio. T1 and T2 which were subjected to pH 1 and pH 2, respectively, were found to have a minimum C/N ratio of 36.6 and 36.9, respectively. The ratio became normalized between 40-44. The observed C/N ratio and the effect of pH in each treatment is summarized in Figure 3. While the C/N ratio in C1 was highest at 45, upon hydrolyzed, the C/N ratio for cassava pulp ranged between 40-44, moderately above the optimal range (20-30:1) for anaerobic digestion [28-29]. It can be assumed that solubilization of COD and C/N ratio are interconnected since higher SCOD production results in a lower C/N ratio in the substrate. The results therefore highlight the need for nitrogen supplementation. Since physicochemical composition of individual cassava cultivars is unique, sampling results can be representative of the industry's four most widely-grown cultivars [30].

### 4) The effect of hydrolysis on VFA/TA ratio and reducing sugars

This study revealed that the ratio of Volatile Fatty Acids (VFA) to Total Alkalinity (TA) values of all hydrolysed samples ranged between 0.15-0.31 with the exception of C1, having a VFA:TA ratio of 2.69. Although the syntrophic action of hydrolytic, acidogenic or fermentative bacteria of anaerobic groups generate volatile fatty acids as their synthesized products [31] [32], this group of bacteria was not active in the pre-treatment stage. Therefore, addition of acid and alkaline is the only factor that modifies the total alkalinity of the substrate. The VFA found in this stage was associated with the residual VFA present in fresh cassava pulp. Therefore, addition of acid and alkaline is critical in maintaining the VFA/TA ratio.

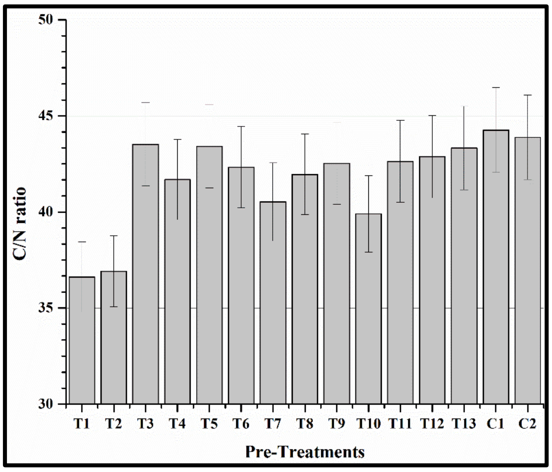

**Figure 3** Impact of pH on C/N ratio of 3 % (w/v) cassava pulp by different pre-treatments.

The experimental results indicated extreme pH treated samples, i.e. T1 and T2 required higher levels of alkali, NaOH which caused higher TA and VFA disintegration resulting in a slightly lower VFA/TA ratio than those treated at neutral pH. T12 and T13 treatments had the highest total alkalinity at 7,300 and 7,500 mg $L^{-1}$ $CaCO_3$ respectively, and with lower VFA/TA at 0.15 (Figure 4). Treatments between T2 and T10 (with pH ranging from pH 2-10 had higher residual VFA than the remaining samples. Since all samples were neutralized back to pH 7.5, total alkalinity of all samples was similar, ranging from 6,500-7,500 mg $L^{-1}$ as $CaCO_3$. Although the optimum alkalinity for anaerobic digestion was 2,500-5,000 mg $L^{-1}$ as $CaCO_3$, the observed alkalinity of pre-treated substrates was slightly higher than this optimum range [33]. However, this contra amount helps in buffering and reserving for pH drop when fermentation progresses during acidogenesis stage. Taken as a whole, the VFA/TA ratio of all samples ranged between 0.1-0.36: an optimal range for healthy biogas fermentation; all pre-treated substrates were found suitable to proceed to the AD process [34]. Likewise, VFA, more reducing sugar depletion were found in extreme pH treated substrates. Except for T1, T11, T12 and T13, the reducing sugar contents of samples ranged from 10-14 mg per 100g (Figure 5). Highly alkaline and acid treated samples yielded levels of reducing sugars as low as 5g per 100 mg on a dry pulp basis.

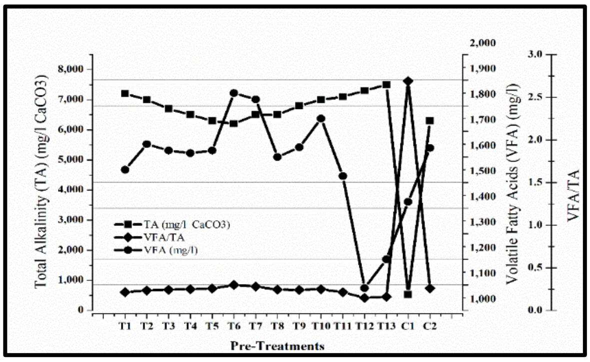

**Figure 4** Effect of different pre-treatment on VFA/TA of 3 % (w/v) of cassava pulp.

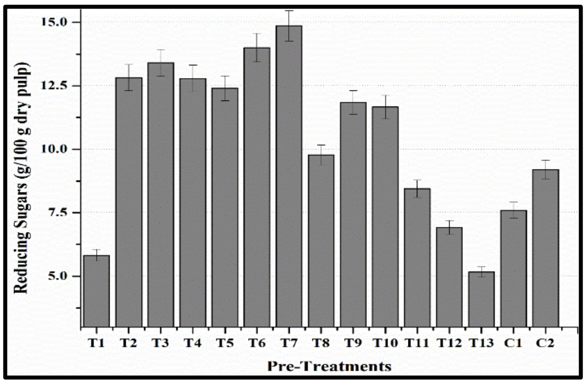

**Figure 5** Effect of different pre-treatment on reducing sugars of 3 % (w/v) of cassava pulp.

### 5) Anaerobic digestion of different solid contents and bio-methane yields

Total biogas/bio-methane yields were calculated at the end of the digestion period of 21 d. Three different total solid (TS) ratios (3 % 5 % and 10 %) were investigated (Figure 6). This was due to the fact that they encountered excessive inhibition of microorganism activity triggered by sodium and sulphate from alkaline and acid addition. At the end of the digestion period, their biogas yields fell below 1,000 mL kg$^{-1}$ TS. This yield effect occurred at all TS levels.

The batch reactors in which substrates were treated to neutral pH ranges (pH 7-10) which are T7-T10 proved efficient in biogas fermentation, with yields more than 3 times those treated with extreme pH. Substrates treated to pH 9 (T9) and pH 10 (T10) had the highest biogas yield of 4,125.2 and 3,539.7 mL kg$^{-1}$ TS for 5 % TS and 3 % TS, respectively. Maximum biogas yield for 10 % TS was found in T9 which is pH 9 treated samples at 524.7 mL kg$^{-1}$ TS. The results provide evidence that solid content of 5 % provided the best performance under acid-alkaline ($H_2SO_4$ and NaOH) treatment, and thermal pre-treated cassava pulp with 4,125.5 mL kg$^{-1}$ TS biogas yield under a digestion period of 21 d in batch reactors.

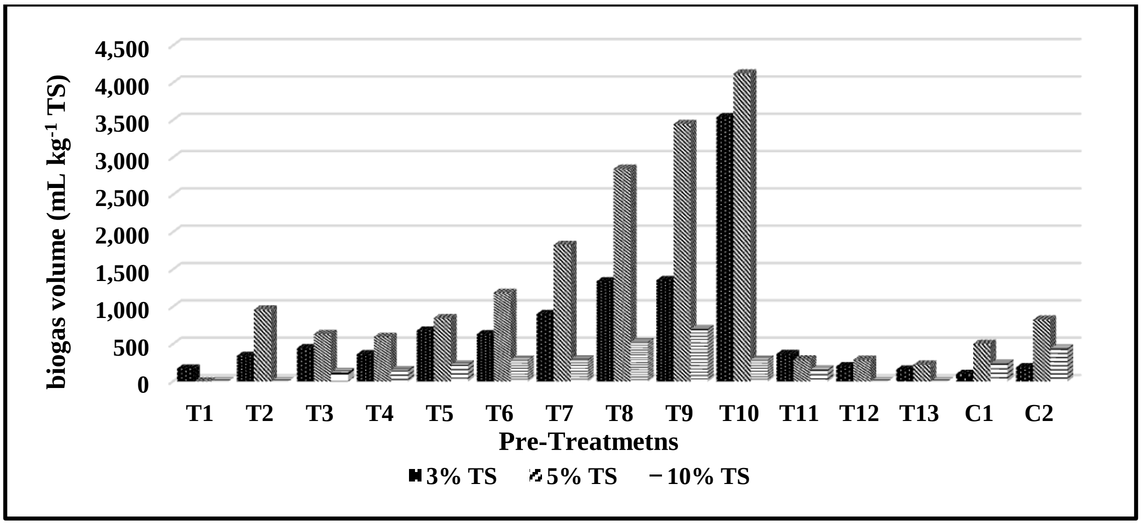

**Figure 6** Performance of batch reactors in different solid contents to each pre-treatment.

## Conclusion

Although anaerobic digestion is an effective means to manage increasing volumes of agro-industrial waste from factories, lack of research and development on the characteristics of specific substrates as feedstocks hampers development of environmentally friendly and reliable bioenergy from crop residues. This study demonstrated the high potential to boost biogas yields using either/both acid-alkaline or thermal pre-treatments prior to biogas fermentation. The high C/N ratio of cassava pulp even after hydrolysis suggests that nitrogen source supplementation or co-digestion with a second nitrogen-rich substrate should be recommended to maintain good digestion performance. In addition, even though highly acid pre-treated substrates produced higher SCOD, $SO_4^{2-}$ and $Na^+$ toxicity was observed upon undergoing AD process, resulting in lower biogas yields compared with those substrates pre-treated between pH 9 (T9) and pH 10 (T10). Therefore, this study brought compromised volume of acid-alkaline addition while selecting of $H_2SO_4$ and NaOH as common chemical pre-treatment to cassava pulp in different solid contents versus range of pH to extract optimum biogas volume. Although it is widely reported in the literature that acid and alkaline improve disintegration of lignocellulose in biomass, in the case of cassava pulp in this study, combination of both moderately higher than neutral pH by alkaline-acid pre-treatment and thermal pre-treatments for 45 min at 200 ˚C in 5 % TS produced the best outcome from batch-mode AD fermentation.

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