ULTRASONIC ASSISTED AMARANTH STEM PRETREATMENT FOR BIO-ETHANOL PRODUCTION

Idan Chiyanzu, Amanda Mditshwa, Sanette Marx
Focus Area: Energy Systems, School of Chemical and Minerals Engineering, North-West University (Potchefstroom Campus), Potchefstroom, South Africa, Tel: +27 18 299 1988, Fax: +27 18 299 1535, Email:24043605@nwu.ac.za

ABSTRACT: An investigation was undertaken to determine the effect of ultrasound on enzymatic hydrolysis rate and enhanced bioethanol yields. Compositional analysis of the feedstock reveals that amaranth lignocellulose contains 36.35% cellulose and 22.57 % hemicellulose. Two sets of experiments were conducted each involving the use of alkaline or acid solutions and ultrasonic irradiations. The effect of energy-input, sonication time, calcium hydroxide and sulfuric acid concentrations (10 and 30 $g.kg^{-1}$) at a constant biomass loading (50 $g.kg^{-1}$) was studied. 30 FPU equivalent of cellulolytic enzyme activity was added to each pretreated biomass and incubated at 50°C for 48 hours at pH 4.8. High Performance Liquid Chromatography (HPLC) was used to quantify the total monomeric sugars and ethanol, while the solid residues were characterized by Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). Results show that ultrasonic-assisted dilute acid pretreatment was the most favorable conditions to obtain more fermentable sugars. The highest total sugar yield (350 $g.kg^{-1}$ substrate) was obtained at 270 kJ/g energy-input for 30 min in presence of 30 $g.kg^{-1}$ of H_2SO_4 solution. Pretreatment of amaranth lignocellulose in dilute alkali solution yielded modest total sugar yield (240 $g.kg^{-1}$ substrate) under similar conditions of 270 $kJ.g^{-1}$ energy-input for 30 min at 30 $g.kg^{-1}$of $Ca(OH)_2$ loading. Significant disruption of biomass structure was observable after pretreatment with dilute acid than when dilute alkaline was applied. FTIR spectra show peaks associated with carbon-carbon double bonds, acetyl group and hydroxyl decreasing which is consistent with removal of sugars and oligomers from amaranth lignocellulose. Therefore, a combination of ultrasound irradiations and dilute acid has shown to be effective and promising approach for pretreatment of amaranth stem when fully optimized.
Keywords: Ultrasound, pretreatment, dilute alkaline, dilute acid, amaranth, bioethanol.

1 INTRODUCTION

The global demand for energy is increasing rapidly because of high fuel consumption and growing industrialization. The increased energy demand has highlighted the limited supply of fossil based energy and the urgent need for renewable alternatives (Sunday, 2011). According to Sunday [1] approximately 2.4 billion people worldwide lack access to modern fuel. Fossil-based fuels are widely used for energy resources and are major contributors to environmental pollution [2]. Increasing environmental pollution has resulted in global climate change. Researchers have been driven to find alternative ways that produce a fuel that is ecological friendly, sustainable, and affordable.

Biofuels are fuels derived from biomass such as renewable organic materials from plants or animals [3]. Biomass feedstock is classified into: first, second and third generation feedstock. The first generation feedstock are those crops that are used mostly as food and feed for humans and are rich in sugar, oil, and starch [4]. However the use of first generation feedstock biofuel production has raised arguments that fuels competes with food and is responsible for increase in food prices [5]. The latter has resulted in the shift towards using second generation feedstock. Second generation feedstock are dominated by lignocellulosic biomass such agricultural residues [6] and also municipal and industrial waste [5]. Lignocellulose consists of cellulose and hemicellulose, which are converted into sugars through chemical pretreatment and biological processes and eventually fermented to bioethanol [2]. However, second generation biofuels has raised concern over the land use requirements and changes and as a result researchers have directed the focus towards third generation fuels [6]. Third generation biofuels produced from microscopic organisms are considered to be viable alternative energy resource that do not possess the major drawbacks associated with first and second generation biofuels [7]. Third generation feedstock are non-food crops such as microalgae [2] and microbes [7]. Biofuel feedstock can be converted into solid, liquid, and gaseous form of biofuels. Amongst all the liquid biofuels, biodiesel and bioethanol, are the most studied and promising as alternative fuels or used as blends with petroleum petrol [8].

Ethanol (ethyl alcohol), one of the liquid fuels made from biomass, has for many years been applied as oxygenate to petroleum fuels providing significant reduction in particulate and NOx emissions from their combustion [9]. Many more preference for use of bioethanol include its high-octane number, high compression ratio and a shorter burn time, and thus it has a theoretical efficiency advantage over petrol in an internal-combustion engine. Currently, the most viable route for ethanol production is by means of first-generation feedstock such as sugar and starch.

Amaranth is a potential second generation feedstock for ethanol production that is relatively cheap. Amaranth possesses characteristics such as fast growth rate, good tolerance to stress and high potential biomass yield [10]. It has a C4 photosynthetic pathway and nitrogen acquisition which makes it a fast growing plant. Amaranth is also known to absorb heavy metals from surrounding soil and can thus be used for the rehabilitation of contaminated soils [11].

Various types of pretreatment methods for production of bioethanol such as physical, physico-chemical, chemical and biological methods and combinations thereof [12] has been investigated. Application of ultrasound irradiation in combination with dilute acid or alkaline pretreatment strategies has been shown to me more effective in breaking down lignocellulose material into fermentable sugars than just the alkaline or acid pretreatment [13]. Application of ultrasound-assisted pretreatment under selected conditions can increase the overall bioethanol yield rapidly through the increased porosity of cellulose fibre [13] and the cleavage of glycosidic linkages in lignin [14]. It can also promote decrease of mass transfer limitations and improve hydrolysis [15].

The present study assesses the application of ultrasonic pretreatment in combination with dilute acid and alkali solutions to convert amaranth lignocellulose to bioethanol *via* fermentation of the liberated monomeric sugars. The effect of parameters such as dilute acid and alkaline concentration, biomass loading and energy-input on the total sugar yield will be investigated.

2 MATERIALS AND METHODS

2.1 Raw materials

Amaranth plants were obtained from a local farm belonging to the Department of Agriculture in Potchefstroom, South Africa (27°43'43 16" S - 27° 04' 47.71 °E). The plants were harvest as whole plants with roots and grains. The plants were transported to the university laboratories in plastics bags. The stems were sun dried to a moisture content of 100 $g.kg^{-1}$ stems and then milled to an approximately sieve size of 1.7 mm using a Hammer mill (Trapp-TRF 70). The milled samples were stored in airtight storage bags at ambient conditions until used in the experiments. The composition of amaranth stems was analyzed by ARC Irene laboratories and is given in Table I.

Table I: Compositional analysis of amaranth feedstock

Analysis	wt.%	Standard method
Dry matter	93.81	ASM013
Ash	6.19	ASM013
*Protein	16.38	ASM048
Fat (ether extract_	9.41	n/a
Neutral detergent fiber	0.85	ASM044
Acid detergent fiber	46.24	ASM060
Acid detergent lignin	34.07	n/a
Higher heating value (MJ/kg)	13.39	ASM053
Cellulose[a]	26.06	Calculated
Hemicellulose[b]	12.17	Calculated

*For the conversion of nitrogen to protein content a factor 5.25 was used.
[a]Cellulose content = ADF-ADL
[b]Hemicellulose content = NDF-ADF

2.2 Pretreatment

A loading of 50 $g.kg^{-1}$ milled amaranth was used in either acid (H_2SO_4) or alkali ($Ca(OH)_2$) solutions (10 or 30 $g.kg^{-1}$ in water). An ultrasonic bath was used to supply indirect ultrasonic irradiation to pretreatment samples. The biomass in acid or alkali solution was kept at a constant ultrasonic bath temperature of 40°C for up to 300 minutes at varying power settings (75, 150, 375, 600, and 750W) at a frequency of 35 kHz.

2.3 Hydrolysis

The pretreated sample was hydrolyzed with a commercial cocktail of cellulase enzyme (NS 22192) from Novozymes, (Denmark). The filter paper unit of the enzyme was determined according to the standard procedure recommended by IUPAC as described by [16]. The enzyme activity was found to be 47.4 FPU. A loading of 30 FPU per gram of substrate was used with 1.25$g.L^{-1}$ Tween 80 at a pH of 5.0. Tween 80 was added as a surfactant to limit the absorption of cellulase to lignin in the broth. The pH was adjusted to 5.0 using either $Ca(OH)_2$ or H_2SO_4 prior to the addition of the enzymes. The solution was then incubated in 250mL GL 45 laboratory glass bottles at 50°C for 48 hours at 150 rpm shaking speed in a rotatory shaker. The hydrolysate was then filtered and the liquid fraction was analyzed for sugars using high performance liquid chromatography (HPLC).

2.4 Fermentation

The hydrolysates obtained after hydrolysis were filtered using vacuum filtration with whatman no. 246 filter paper. The solid residue was analyzed using Fourier Transmittance Infrared spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) and the filtrate were used for fermentation. *S. cerevisae* was activated from the dry dormant state using small amounts of the hydrolysis filtrate. A *S. cerevisae* loading of 50 $g.kg^{-1}$ was aseptically inoculated into the hydrolysate broth. The broth was incubated at 32 °C for 48 hours at a shaking speed of 120 rpm under anaerobic conditions. The fermentation products i.e. residual sugars, ethanol and cell density were monitored by taking out aliqouts at selected time preiods.

2.5 Analytical Methods

2.5.1 High Performance Liquid Chromatography (HPLC)

The pH of sample was adjusted to 7.0. A volume of 2.0 ml of the sample was transferred to a clean polytop and the sample was diluted by adding 2.0 ml of distilled water. The sample was filtered into a clean HPLC vial using a 0.45µm and 0.2 µm syringe filter consecutively. Analysis was done using an Aligent series 1200 HPCL instrument fitted with a Shodex column for sugars and ethanol and an Aminex column for analysis of organic acids, HMF, and furfural. The instrument was fitted with a reflective indiex (RI) detector and water and 5% sulfuric acid was used as mobile phase for the Shodex column an Aminex columns respectively.

2.5.2 FTIR analysis

Untreated and pretreated amaranth stem samples were analyzed using FTIR (Shimadzu 2000)at 4 cm^{-1} and 40 scans per sample. The samples were prepared by mixing 3 mg of amaranth stem to 300 mg of potassium bromide (KBr) and the samples were then analyzed using the transmittance measure mode within the range of 400 – 4000 cm^{-1}.

2.5.3 Scanning Electron Microscopy (SEM) analysis

Samples of untreated and pretreated amaranth stem were analyzed in by Sem (FEI Quanta 250 FEG) in high vacuum mode to see morphological changes on the biomass.

2.5.4 Ultra-violet Spectrophotometer (UV)

Cell growth during fermentation was measured using a UV spectrophotometer at 600nm absorbance.

3 RESULTS AND DISCUSSION

3.1 Ultrasonic-assisted dilute acid pretreatment

3.1.1 Effect of time on the pretreatment

The effect of sonication time on the total sugar yield from amaranth stem was investigated with 10 $g.kg^{-1}$ and 30 $g.kg^{-1}$ of H_2SO_4 in water at a fixed ultrasonic power of 750 W and varying treatment times.

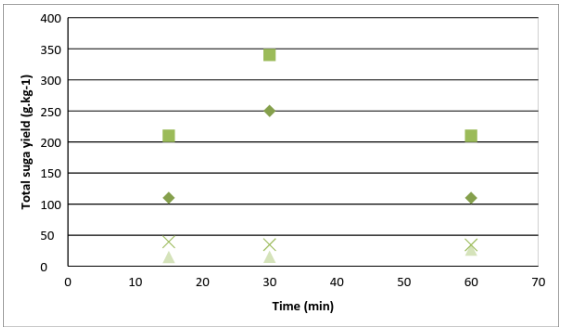

Figure 1: Effect of pretreatment with and without ultrasonication on total sugars yield at a power of 750 W (♦ - with sonication in 10g.kg of H_2SO_4, ■ – with sonication in 30 g.kg of H_2SO_4, ▲- without sonication in 10g.kg of H_2SO_4, × - without sonication in 30g.kg of H_2SO_4).

Figure 1 shows a slight increase in total sugar yield as pretreatment time increases for runs without ultrasound. As for ultrasonic-assisted pretreated samples, an increase in total sugar yields was observed from 0 to 30 min and a decrease in total sugars was observed after 30 min. The total sugar yields without ultrasound assistance were comparatively lower to those obtained from ultrasonic-assisted pretreatment. The significant increase in total sugar yields with time when ultrasound was used indicates the process's effectiveness in enhancing dilute acid pretreatment of amaranth stems. The disruption of amaranth structure is associated with acoustic cavitation of ultrasound which is known to disrupt the bonds within polymers [17] and the breaking down of heterocyclic ether bonds between monomers found in the polymeric chain caused by dilute acid pretreatment [18]. The highest total sugar yield (340 $g.kg^{-1}$) was obtained after 30 min of sonication pretreatment in 30 $kg.g^{-1}$ sulphuric acid solution in water. This demonstrates that amaranth stem requires short sonication times for liberation of high amounts of monomeric sugars. However, prolonged sonication treatment induces the degradation of monomeric sugars as was observed in this study at sonication times longer than 30 minutes.

3.1.2 Effect of sulphuric acid concentration on total sugar yields

The influence of sulphuric acid loading on total sugar yield at different power densities (different wattages in the same treatment time of 30 minute) was studied and results are shown in Figure 2.

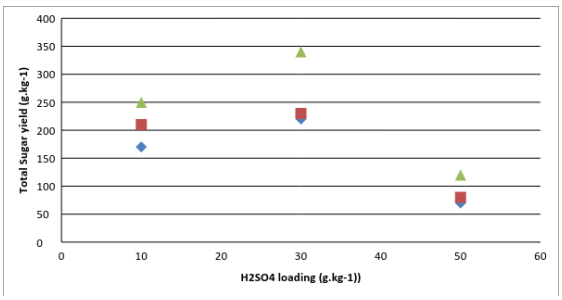

Figure 2: The effect of H_2SO_4 loading on the total sugars yield at a fixed treatment time of 30 minutes and different power setting, (♦ - energy input of 54 $kJ.g^{-1}$, ■ – energy input of 216 $kJ.g^{-1}$, ▲ – energy input of 270 $kJ.g^{-1}$).

From Figure 2 it can be seen that total sugar yield increased with an increase in sulphuric acid concentration up to a maximum at an acid loading of 30 $g.kg^{-1}$ H_2SO_4 in water. Maximum total sugar yield of 340 $g.kg^{-1}$ was obtained with 30 $g.kg^{-1}$ H_2SO_4 in water at 270 $kJ.g^{-1}$ energy input. At the same power density, 50 $g.kg^{-1}$ H_2SO_4 in water gave significantly lower total sugar yields than 10 and 30 $g.kg^{-1}$. The increase in total sugars yield may be attributed to the fact that dilute sulphuric acid hydrolyses and removes most of the hemicellulose as dissolved sugars (xylose) and glucose yield from cellulose increase with hemicellulose removal [19]. Also, the hydrolysis of hemicellulose increases with an increase in H_2SO_4 concentration, resulting in an increase in sugar yield with an increase in sulphuric acid concentration. The decrease observed in total sugars at 50 $g.kg^{-1}$ H_2SO_4 in water concentrations is associated with degradation of sugars at increased concentrations.

3.1.3 Effect of biomass loading on total sugar yields

The effect of biomass loading on total sugar yields was investigated (10, 50, and 100 $g.kg^{-1}$) at a sulphuric acid loading of 30 $g.kg^{-1}$ in water and an energy input of 270 $kJ.g^{-1}$. The results for total sugar yield and by-product (acetic acid) yield are presented in Figure 3.

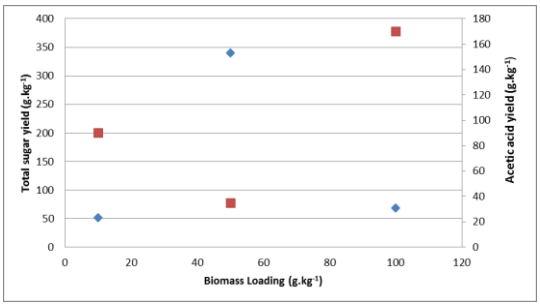

Figure 3: Effect of biomass loading on total sugar yield (♦ - Total sugar yield, ■ - Acetic acid yield.

Results show a sharp increase in the total sugar yield from when the biomass loading was increased from 10 to 50 $g.kg^{-1}$ after which a significant decrease was observed in total sugar yield. The maximum total sugar yield of 340 $g.kg^{-1}$ was obtained with 50 $g.kg^{-1}$ biomass loading. At a biomass loading of 10 $g.kg^{-1}$ the total sugar yield is comparatively low while the acetic acid yield is high. The same is observed at a high biomass loading of 100 $g.kg^{-1}$. As the increase of substrate loading, the acetic acid and inhibitors concentrations increased and that might have led to the low total sugar yields. On the other hand, the total sugar yield was low at a substrate loading of 10 $g.kg^{-1}$, probably due to insufficient lignocellulose matter that was meant to be converted.

3.1.4 Effect of energy input on pentose and hexose sugar yields

The effect of energy-input on C-5 and C-6 sugar yields was determined at a biomass loading of 50 $g.kg^{-1}$ of amaranth stem was pretreated in 30 $g.kg^{-1}$ sulphuric acid (H_2SO_4) at a constant treatment time of 30 minutes and varying power settings for the ultrasonic bath.

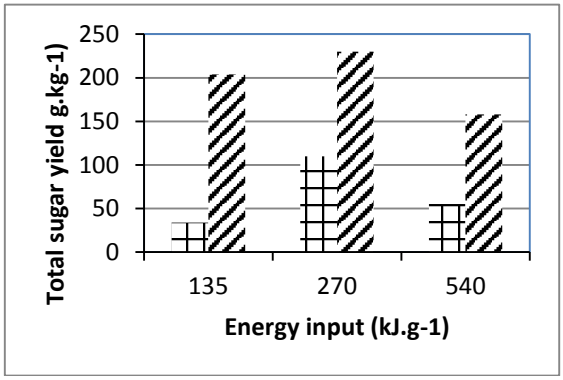

Figure 4: Effect of sulphuric acid pretreatment on yield (g.kg^{-1}) of C-5 and C-6 sugars liberated (▦ - hexose sugars, ◪ – pentose sugars).

Figure 4 shows that the amount of pentose and hexose sugars liberated increased when an increase in energy-input. The maximum amounts of pentose (110 g.kg^{-1}) and hexose sugars (230 g.kg^{-1}) were liberated at 270 kJ.g^{-1} in 30 minutes. The theoretical conversions were 90% and 88.5% for pentose and hexose sugars, respectively. The high conversions confirm that ultrasonic-assisted acid treatment was effective in breaking down cellulose crystallinity for improved enzymatic hydrolysis as well as liberate xylose from the hemicellulose structure of amaranth stems.

3.1.5 Morphological analysis of acid pretreated samples

Scanning electron microscopy (SEM) analysis was used to assess the morphological changes to the plant material during pretreatment. Figure V shows the SEM micrographs of milled amaranth stem, a control samples pretreatment with only H_2SO_4 and stems pretreated with ultrasound in dilute H_2SO_4.

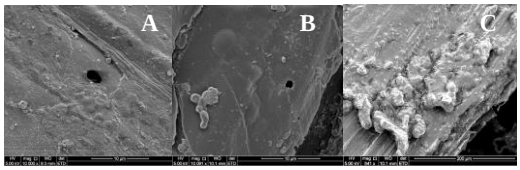

Figure 5: Scanning electron microscope images of raw amaranth stem (A), H_2SO_4 pretreated amaranth stem (B) and ultrasound-assisted H_2SO_4 pretreated amaranth stem (C).

3.2 Ultrasonic-assisted dilute alkaline pretreatment

3.2.1 Effect of time on the pretreatment

The effect of pretreatment time on the total sugar yield from amaranth stem were investigated at a constant power setting of 750 W in 10 g.kg^{-1} or 30g.kg^{-1} $Ca(OH)_2$ in water solution. The results are presented in Figure 6**.**

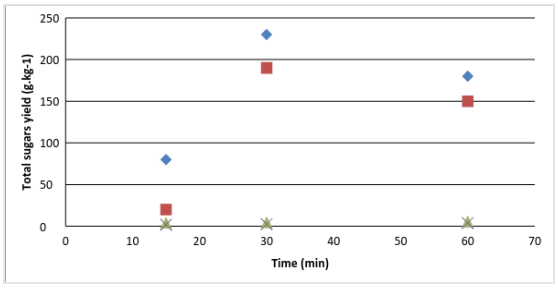

Figure 6: Effect of sonication time on total sugars yield at a sonication power of 750W (♦ - with sonication in 30 g.kg^{-1} $Ca(OH)_2$, ■ – with sonication in 10 g.kg^{-1} $Ca(OH)_2$, ▲ - without sonication in 30 g.kg^{-1} $Ca(OH)_2$, × - without sonication in 10 g.kg^{-1} $Ca(OH)_2$).

As shown in Figure 6, there is a sharp increase observed on the total sugar yield with increase in pretreatment time until a maximum yield was reached at 30 minutes. The highest sugar yield of 230 g.kg^{-1} biomass was obtained after 30 minutes of pretreating with 30 g.kg^{-1} $Ca(OH)_2$ in water at a conversion efficiency of 61% total sugar. Generally, ultrasound improves the liberation of monomeric sugars through hydrodynamic shear forces produced by ultrasound promoting the breakage of chain polymers in presence of an alkaline solution. The aryl ether linkages found in lignin are disrupted, increasing enzyme accessibility, resulting in increased total sugar yields. Ultrasonic-assisted alkaline treatment increased efficiency conversion of total sugar from 5.3 to 61% as compared to non-sonicated alkaline treatment of amaranth stem. As was observed with ultrasonic-assisted acid pretreatment, prolonged sonication damage the liberated monomeric sugars.

3.2.2 Effect of $Ca(OH)_2$ concentration on total sugar yield

The effect of $Ca(OH)_2$ concentration on total sugar yield, from amaranth stem was investigated at varying ultrasonic energy-input at a fixed treatment time of 30 minutes.

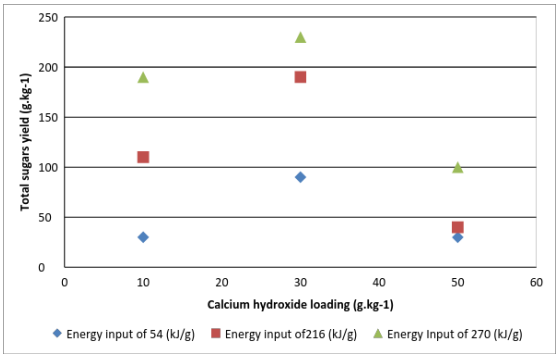

Figure 7: Effect of $Ca(OH)_2$ loading on the total sugar yield at a fixed treatment time of 30 minutes and various power setting, (♦ - energy input of 54 kJ.g^{-1}, ■ – energy input of 216 kJ.g^{-1}, ▲ – energy input of 270 kJ.g^{-1}).

As seen from Figure 7 when $Ca(OH)_2$ concentrations was increased from 10g.kg^{-1} to 30 g.kg^{-1}, total sugar yield also increase until a alkaline loading of 50 g.kg^{-1} $Ca(OH)_2$ in water. The highest total sugar yield of 230 g.kg^{-1} was observed with 30 g.kg^{-1} $Ca(OH)_2$ in water at

270 kJ.g^{-1} energy-input. The increase of total sugar yield is attributed to the cavitation effect caused by ultrasound and alkaline reaction. Alkaline solutions saponifies the intermolecular ester bonds crosslinking xylan and hemicelluloses and lignin, thereby causing swelling of the lignocellulose structure, and separation of the linkages between lignin and carbohydrates resulting in disruption of the lignocellulose structure [20]. Lower total sugar yield is observed at higher concentrations of $Ca(OH)_2$. Normally, high amounts alkaline solution in lignocellulose pretreatment forms irrecoverable salts or is incorporated as salt in the biomass, thereby inhibiting the degradation and subsequently liberation of oligomers and monomeric sugars.

3.2.3 Effect of biomass loading on total sugar yield

The effect of biomass loading on total sugar yields was evaluated at different biomass loadings (1, 5, 10 g.kg^{-1}) using 750 W power for 30 min in the presence of 30 g.kg^{-1} $Ca(OH)_2$ solution in water. Figure 9 shows the relationship between biomass loading and total sugar yield.

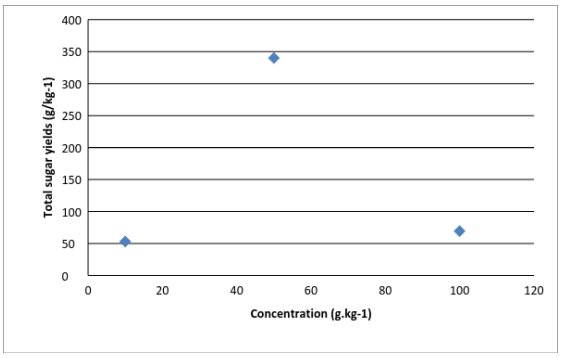

Figure 8: Effect of biomass loading on total sugar yield during ultrasonication of amaranth stems in a dilute $Ca(OH)_2$ solution

From Figure 8 it could be seen that an increase in biomass loading (10g.kg^{-1} to 50g.kg^{-1}) also resulted in increased total sugar yields of 53 g.kg^{-1} and 340 g.kg^{-1}, respectively. A high biomass loading (100 g.kg^{-1}) resulted in low total sugar yields. The maximum total sugar yield (340 g.kg^{-1} biomass) was obtained with 50 g.kg^{-1} biomass loading. The best biomass loading and pretreatment conditions is dependent on the type of lignocellulose biomass and the recalcitrance of the plant material [21]. The lower sugar yield obtained at the high biomass loading can be attributed to the increased concentration of solids that impeded the physical effect of ultrasound. Additionally, increased biomass loadings usually boosts the viscosity within the sample, thus reducing mixing and transfer rates of the alkaline solution in the sample.

3.2.4 Effect of energy input on pentose and hexose sugar yields

Amaranth stems was pretreated with ultrasound at different concentrations of $Ca(OH)_2$ (10g.kg^{-1} and 30g.kg^{-1} $Ca(OH)_2$ in water), and different power settings to investigate the effect of energy-input on the total sugar yield. The distribution of pentose and hexose sugars under ultrasonic-assisted alkaline pretreatment is shown in Figure 9.

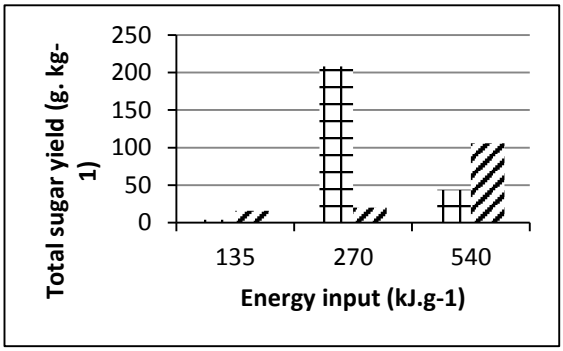

Figure 9: Effect of energy input during $Ca(OH)_2$ ultrasonic-assisted pretreatment on C-5 and C-6 sugar yield (g.kg^{-1}) (– pentose sugar, – hexose sugar)

It can be seen from Figure 9, that pentose sugars were preferentially released as compared to hexose sugars at an energy input of 270 kJ.g^{-1}. The highest amount of hexone sugars (208 g.kg^{-1}) was obtained at the highest energy input. Alkaline solutions is known to preferentially solubilise lignin and hemicellulose, allowing increased exposure of the cellulose for enzymatic hydrolysis resulting in high hexose sugars.

3.2.5 Morphological analysis of alkline pretreated samples

SEM analysis was used to study the morphological features and surface characteristics of the material before and after pretreatment with ultrasonic-assisted dilute alkaline solutions.

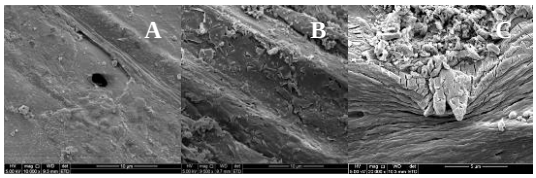

Figure 10: Scanning electron microscope images of raw amaranth stem (A), $Ca(OH)_2$ pretreated amaranth stem (B) and ultrasound-assisted $Ca(OH)_2$ pretreated amaranth stem.

4 CONCLUSION

In the present work we have shown that ultrasonic-assisted sulphuric acid pretreatment can improve enzymatic hydrolysis as compared to only dilute acid pretreatment, and mild acidic conditions (270 kJ.g^{-1} in 30 minutes) mostly release significant amounts of hexose sugars. The catalyst loading (both 30 g.kg^{-1} H_2SO_4 and $Ca(OH)_2$) in the presence of ultrasonic-assisted pretreatment also had a significant interaction effect with hemicelluloses and subsequent enzymatic release of pentose sugars. In general, higher biomass loading of milled amaranths biomass made it less responsive to ultrasonic radiations and subsequently enzymatic attack. It is certain from all results in this study that the pretreatment of amaranths lignocellulose will have to be optimised to enable its efficient utilisation in bioethanol production.

5 REFERENCES

[1] A.M. Sunday, Energy poverty and the leadership question in Nigeria: An overview and implication for the future, (2010), pag.48.

[2] R. T. Gahukar, Review: New source of feed stock for biofuels production: Indian perspectives, (2012), pag.24.

[3] E. Uyigue, E.O. Archibong, Scaling-up renewable technologies in Africa, (2010), pag.130.

[4] A. Gasparatos, P. Stromberg, K. Takeuchi, Biofuels, ecosystem services and human wellbeing: Putting biofuels in the ecosystem narrative, (2011), pag.111.

[5] B. Joshi, M.R. Bhatt, D. Sharma, J. Joshi, R. Malla L. Sreerama, Review: Lignocellulose ethanol production: Current practices and recent developments, (2011), pag.172.

[6] S.N. Naik, V.V. Goud, K.P. Rout, A.K. Dalai, Production of first and second generation biofuels: A comprehensive review, (2010), pag.578.

[7] S.P. Nigam, A. Singh, Production of liquid biofuels from renewable resources, (2011), pag.52.

[8] W.A. Payne, Are biofuels antithetic to long-term sustainability of soil and water resources, (2010), pag.1.

[9] M. Balat, H. Balat, and C. Oz, Progress in bioethanol processing, (2008), 2004): 551 – 573.

[10] B.P.A. De la Rosa, S.I. Fomsgaard, B. Laursen, G.A. Mortensen, L. Olvera-Martinez, C. Silva-Sånchez, A. Mendoza-Herrera, J. Gonzålez-Castañeda, A. León-Rodriguez, Amaranth (*Amaranthus hypoochondriacus*) as an alternative crop for sustainable food production: Phenolic acids and flvonoids with potential impact on ots nutraceutical quality, (2009), pag.117.

[11] M. Akond, S. Islam, and X. Wang, Characterization of biomass traits and cell wall components among diverse accessions of *amaranthceae*, (2013), Pag.37.

[12] K. Ninomiya, K. Kamade, K. Takahashi, N. Shimizu, Enhanced enzymatic saccharification of kenaf powder after ultrasonic pretreatment in ionic liquids at room temperature, (2011), pag.259.

[13] S. Nikolic, L. Mojovic, M. Rakin, , D. Pejin, J. Pejin, Utilization of microwave and ultrasound pretreatment in the production of bioethanol from corn, (2011), pag.587.

[14] P. Sakthiselvan, B. Naveena, N. Partha, Effect of medium composition and ultrasonication on xylanase production by *Trichoderma harzianum* MTCC 4358 on novel substrate, (2012), pag.12067.

[15] L.B. Werle, J.C.Garcia, R.C.Kuhn, M.Schwaab, E.L.Foletto, A. Canceler, S.L. Jahn, M.A. Mazutti, Ultrasound-assisted hydrolysis of palm leaves (Roystonea oleracea) for production of fermentable sugars, (2013), pag.128.

[16] T.K. Ghose, Measurement of cellulase activities, (1987), pag.257.

[17] M.S.U. Rehman, I. Kim, Y. Chisti, and J. Han, Use of ultrasound in the production of bioethanol from lignocellulosic biomass, (2013), pag.1391.

[18] R. Velmurugan, K. Muthukumar, Utilization of sugarcane bagasse for bioethanol production: sono-assisted acid hydrolysis approach, (2011), pag.7119.

[19] P.Kumar, D.M. Barrett, M.J. Delwiche, P. Stroeve, Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production, (2009), pag.3713.

[20] S. Singh, S. Khanna, V.S. Moholkar, A. Goyal, Screening and optimization of pretreatment for Parthenium hysterophorus as feedstock for alcoholic biofuels, (2014), pag.195.

[21] P.B. Subhedar, P.R. Gogate, Alkaline and ultrasound pretreatment for intesification of deliginification process from sustainable raw-material, (2013), pag.216.

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7 LOGO SPACE