2017-06-26

Ferric Chloride Assisted Plasma Pretreatment of Lignocellulose

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Recommended Citation

Ravindran, R., et al. (2017) Ferric chloride assisted plasma pretreatment of lignocellulose, *Bioresource Technology* (2017), doi: <http://dx.doi.org/10.1016/j.biortech.2017.06.123>

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Funder: Technological University Dublin (DIT), Dublin, Ireland

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Accepted Manuscript

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PII: S0960-8524(17)31027-1
DOI: <http://dx.doi.org/10.1016/j.biortech.2017.06.123>
Reference: BITE 18360

To appear in: *Bioresource Technology*

Received Date: 25 April 2017
Revised Date: 21 June 2017
Accepted Date: 22 June 2017

Please cite this article as: Ravindran, R., Sarangapani, C., Jaiswal, S., Cullen, P.J., Jaiswal, A.K., Ferric chloride assisted plasma pretreatment of lignocellulose, *Bioresource Technology* (2017), doi: <http://dx.doi.org/10.1016/j.biortech.2017.06.123>

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Ferric chloride assisted plasma pretreatment of lignocellulose

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Abstract

In this study, a novel pretreatment for spent coffee waste (SCW) has been proposed which combines two techniques viz. atmospheric air plasma and FeCl_3 to create a superior pretreatment that involves Fenton chemistry. The pretreatment was optimised employing Taguchi Design of Experiments, and five parameters were taken into consideration viz. biomass loading, FeCl_3 concentration, H_2SO_4 concentration, plasma discharge voltage and treatment time. The composition analysis of the pretreated SCW revealed substantial amounts of lignin removal, with a maximum for process conditions of 70kV for 2min in an acidic environment containing 1% H_2SO_4 . FTIR, XRD and DSC were performed to characterise the samples. The pretreated SCW after enzymatic hydrolysis yielded 0.496g of reducing sugar/g of SCW. The hydrolysate was subjected to fermentation by *S. cerevisiae* and led to the production of 18.642g/l of ethanol with a fermentation efficiency of 74%, which was a two-fold increase in yield compared to the control.

Keywords: Lignocellulose; biomass pretreatment; spent coffee waste; cold air pressure plasma; Fenton reaction; bioethanol production

1 Introduction

With petroleum resources dwindling, the world has a renewed interest in sustainable sources of energy such as bioethanol. Lignocellulose is one the most abundant renewable resources in the world. In recent years, lignocellulosic plant wastes from various industry sectors such as food and agriculture have widely been researched as potential substrates for bioethanol production. Spent coffee waste (SCW) is the solid material obtained after brewing of instant coffee and is a rich source of lignocellulose. Over the past few years' global coffee production and consumption has been on the rise. Coffee is one of the most popular beverages in the world, with global coffee production reaching 8.5 billion tonnes in 2015/2016 (ICO, 2016).

Lignocellulose is a complex polymer of polysaccharides and lignin. In nature, plant cell wall can naturally resist degradation by microbial, enzymatic and chemical attack. Consequently, the efficient utilisation of polysaccharides is challenging. This calls for suitable methods that can remove recalcitrance of lignocellulose. Pretreatment of lignocellulosic biomass are necessary to disrupt the lignocellulosic complex to expose cellulose to enzymatic hydrolysis (Ravindran & Jaiswal, 2016a). Numerous studies have reported on pretreatment methods and their efficacies over the past few decades. Some of the most effective pretreatment methods that offer commercial feasibility include steam explosion, dilute acid pretreatment and ammonia fibre expansion (Campbell et al., 2013; Rocha et al., 2015). Recently, treatment with ferric chloride was investigated as a feasible pretreatment measure for bagasse, rice straw and wood fibre by Chen et al. (2015). Studies involving plasma generated ozone for delignification of wheat straw and subsequent bioethanol production was studied by Schultz-Jensen et al., (2011).

Several pretreatment methods have been devised for lignocellulose over the past decade. However, very few studies have focused the effective pretreatment of SCW. For example, characterisation of polysaccharides extracted from alkali pretreated SCW was performed by Ballesteros et al., (2015). Conde and Musatto (2015) studied the effectiveness of hydrothermal pretreatment strategy in the isolation of polyphenols from SCW. Similarly, Scully et al. (2016), applied hydrothermal pretreatment for the industrially important sugars. However, the authors were unable to come across any study that involved mimicking Fenton reaction using plasma for lignocellulosic waste such as spent coffee waste and its potential application in bioethanol production.

Plasma is the fourth state of matter. It contains atoms, ions and molecules in a metastable state with a net electric charge of zero (Devi et al., 2017). Atmospheric plasma discharges may be obtained artificially by many means of electromagnetic wave disturbances resulting from the application of current or ionization radiation in way such that a measurable charge is created in the gas without the generation of any heat. This gives rise to positively and negatively charged electrons, intermediate highly reactive species ($H^{\bullet}$, $O^{\bullet}$, $OH^{\bullet}$ etc.) atoms, molecules and UV photons with neutral charge (Sarangapani et al., 2015, Sarangapani et al., 2016). Ozone, one of the reactive species generated because of atmospheric air plasma specifically degrades lignin when lignocellulosic materials are subjected to plasma treatment. This leaves the solid fraction rich in polysaccharides which can be utilised. Studies involving plasma as a pretreatment measure have reported high dry matter concentration, relatively low pretreatment time, low temperature of operation and absence of any inhibitory compounds as the major advantages. The phenolic compounds that arise from the lignin degradation can be removed by simply washing the solid material with water (Souza-Corrêa et al., 2014).

Fenton chemistry is an oxidation reaction which gives rise to highly reactive free radicals. In the presence of a Fe^{3+} ion and H_2O_2 the ferric ion is oxidised to an Fe^{2+} ion resulting in the formation of H_2O and O_2 along with two reactive species viz. $\text{HO}^\bullet$ and $\text{HOO}^\bullet$ (Fenton, 1894; Pignatello et al., 2006). The Fenton reaction does not require high temperatures, pressure or any other chemical in the form of a catalyst for initiation. However, it does require an acidic environment (Jung et al., 2009). The generation of reactive species in the presence of Fe^{3+} can lead to the occurrence of Fenton chemistry which can be used for the depolymerisation of lignin in plant biomass. Several studies have reported where the Fenton reaction has been exploited as a pretreatment measure (Jung et al., 2015; Kato et al., 2014).

Pretreatments can involve several parameters to achieve for effective delignification of lignocellulose. In most common pretreatments such as dilute acid hydrolysis or organosolv pretreatments, these parameters include acid concentration, temperature, pressure, ethanol concentration etc. (Ravindran et al., 2017a). The abundance of parameters makes it difficult to determine the optimal levels of each factor to attain the target objective. Taguchi design of experiments describes an effective method to evaluate the effects of different parameters statistically in a process that has multiple factors. Furthermore, it minimises the errors caused due to variations in the experimental process. Taguchi design of experiments works by testing the effects of varying parameters simultaneously. This allows the accurate estimation of individual factors thus selecting the best combination for factors that will result in a robust process (Azad et al., 2016).

In the present study, we investigate a new pretreatment strategy for lignocellulose by combining a nonthermal plasma discharge and Fe^{3+} ions to create an environment for the Fenton reaction

along with ozone to delignify lignocellulose, and spent coffee waste was chosen as a model. The pretreatment process was optimised by the Taguchi Design of Experiments that involved five parameters viz. biomass loading, acid concentration, ferric chloride concentration, voltage and time. Furthermore, the pretreated SCW was enzymatically hydrolysed using the optimised parameters and the hydrolysate obtained was employed for bioethanol production.

2 Materials and methods

2.1 Feedstock and other chemicals

Spent Coffee Waste (SCW) was collected from a local coffee outlet located in Dublin city. The waste material was dried in a hot air oven at 80°C for 48h (Kwon et al., 2013). The dried SCW was then stored at room temperature in a cool and dry place for further experiments. All the chemicals such as FeCl₃, sulphuric acid and saccharifying enzymes such cellulase from *Trichoderma reesei* and hemicellulase from *Aspergillus niger* were purchased from Sigma Aldrich, Ireland. Cellulase was purchased in liquid form. Following protocols devised by National Renewable Energy Laboratory (Adney & Baker, 1996), the cellulase activity was assayed. The hemicellulase, on the other hand, was obtained as powder form and dissolved in sodium acetate buffer (pH 4.8, 50mM) to make up a concentration of 10 g/l. The subsequent activity of the enzyme was assayed followed protocols described by Rickard and Laughlin (1980). Cellulase enzyme registered an enzyme activity of 77 FPU/ml while hemicellulase showed 72 U/ml enzyme activity.

2.2 Component analysis

The compositional variations in SCW before and after pretreatment was analysed by using protocols devised by the National Renewable Energy Laboratory, USA. As a control measure the composition of native SCW was also determined. Biomass was hydrolysed by mixing it with 72% H_2SO_4 for 60 min at 30°C. The acid concentration was then diluted to 4% with deionised water and autoclaved at 121°C for 1h. The solids were then separated from the liquids and dried at 80°C for 48h following which the acid insoluble lignin was determined by drying at 595°C for 24h. The liquid was assessed for the acid soluble lignin by measuring the absorbance at 205nm and the sugar concentrations were determined by HPLC (Rezex ROA H^+ , Waters e2695 Separation module, Refractive Index detector).

2.3 Surface modification using atmospheric air pressure plasma

Various parameters affecting the metal chloride assisted plasma pretreatment were optimised by adopting a Taguchi design. Five parameters *viz.* biomass loading, ferric chloride concentration, dilute acid concentration, time and voltage were taken in to consideration for experimental design. Three levels were considered for each parameter. Based on these parameters and levels an L_{27} orthogonal array of standard configuration of five 3 level factors was set up (Table 1) and was generated by using Minitab 17[®]. A prototype high voltage but low power (~150W) dielectric barrier discharge plasma source was used for this study. The system consisted of a high voltage transformer (230 V, 50 Hz), a ground electrode and voltage variac (output voltage can be controlled from 0 to 120 kV). The optimisation trials were operated at 60, 70 and 80 kV as per the experiment design. The input voltage and current was monitored by employing an oscilloscope (InfiniVision 2000 X-Series Oscilloscope, Agilent Technologies Inc., USA) (Fig 1).

The samples were held in the centre of a polypropylene container by means of a petri dish. On completion of each trial, the containers were sealed in polypropylene bags (B2630; Cryovac Sealed Air Ltd, Dunkan, SC, USA) and stored in a cool and dry place for the reaction to take place (Lu et al., 2014). After pretreatment the solids were filtered out. Any residual FeCl_3 was removed by washing the solids five times with deionised water. They were then freeze dried and stored for further experiments.

2.4 Enzymatic hydrolysis

The parameters for maximum reducing sugar production was derived from a study performed previously in our laboratory (Ravindran et al., 2017b). Briefly, the pretreated SCW was hydrolysed by employing a biomass loading of 1%, cellulase and hemicellulase loading of 1.5 ml (23.1 FPU/g of dry biomass) and 0.37 ml (5.33 U/g of dry biomass), respectively while maintaining a pH of 6.7. The ambient temperature for the reaction was maintained at 50°C while the reaction volume was set to 50 ml. On completion of the experiments the suspensions were transferred to 30 ml polypropylene tubes, centrifuged at 7000 rpm for 10 minutes. The supernatant was collected and were checked for reducing sugar content using dinitrosalicylic acid and the values obtained were input into the design software to decipher optimised hydrolysis conditions.

2.5 Individual sugar, inhibitor and organic acid analysis

The presence and quantification of monosaccharides and organic acids was performed using a Allaince HPLC (Waters, e2695 Separation module) with a Rezex ROA-Organic acid H+ (8%) column, (350 x 7.8 mm; Phenomenex, UK) and 0.005 M H_2SO_4 as the mobile phase at 65°C maintaining a flow rate of 0.6 ml/min (Jaiswal et al., 2012). The same type of guard column was

used with the regular column and was kept outside the compartment to avoid overheating beyond the manufacturers recommended limit (60°C). An isocratic mobile phase of 0.01M sulphuric acid was used to detect and estimate the number of inhibitors such as furfural and hydroxymethyl furfural in the hydrolysate. The HPLC system was equipped with an autosampler, degasser and a UV detector (210 nm) and a refractive index (RI) detector.

2.6 Bioethanol production

The sugar rich hydrolysate, free from undissolved solids, obtained after saccharification was subjected to fermentation to produce bioethanol. *Saccharomyces cerevisiae* obtained from the DIT microbiology repository was employed as the fermentative microorganism. For inoculation, the microbe was grown in a medium containing glucose (1g/100ml), and yeast extract (0.1g/100ml) supplemented by (NH₄)₂SO₄ (0.5g/100ml), MgSO₄·7H₂O (0.05g/100ml) and KH₂PO₄ (0.1g/100ml) and was incubated at 30°C and 120 rpm for 18h. For bioethanol production, 0.5ml of actively growing culture of *S. cerevisiae* was added into the reaction mixture. The reaction mixture consisted of the hydrolysate which was supplemented with yeast extract (0.1%) and peptone (0.1%) was autoclaved at 121°C for 15 minutes. The pH was maintained by the addition of 1% (v/v) acetate buffer (pH 5.4, 20mM). Fermentation was carried out in 100 ml conical flasks with a total reaction volume of 50 ml at 30°C for 72h. After fermentation, the reaction mixture was centrifuged and filtered using 0.4 µ filters and the ethanol produced was quantified by using Gas Chromatography [Bruker Scion 456-GC coupled with flame ionisation detector (FID)]. The injector temperature was set at 220°C while the column oven was set at an initial temperature of 80°C. which was ramped up to 160°C at a rate of 40°C

per min and held for 7 minutes. A flame ionisation detector was used to analyse the ethanol content and set at a temperature of 200°C.

2.7 Characterisation of pretreated SCW

2.7.1 X-ray diffraction

X-ray diffraction studies were employed to study any changes imparted in the crystallinity of the lignocellulose substrate by the pretreatment strategy (Ravindran et al., 2017c) XRD of the pretreated and native SCW was performed in a Siemens D-500 X-ray diffractometer. The voltage and current for the radiation were set at 40 kV and 30 mA using Cu K α as the radiation source ($\lambda=0.154$ nm). The diffraction angles were spanned at $2\theta=5^{\circ}$ - 50° .

2.7.2 FTIR analysis

FTIR spectroscopy was performed on raw and pretreated SCW to identify possible structural changes as a reflection of the variations in the functional groups in the SCW before and after pretreatment. A Perkin Elmer Spectrum GX FT-IR (UATR) Microscope (USA) was employed in this study as per the method described in Ravindran et al. (2017c). In brief, the FTIR spectra for SCW samples were recorded from 4000 to 400 cm^{-1} with 16 scans at a resolution of 0.3 cm^{-1} in transmission mode.

2.7.3 Thermal Behaviour study using DSC

Differences in the thermal behaviour of SCW after pretreatment was studied using differential scanning calorimetry (DSC), using 55 mg of the pretreated SCW in an aluminium pan with an empty pan used as a reference. The thermograms were obtained by increasing the temperatures from 20°C to 500°C at a rate of 10°C per min at a constant nitrogen atmosphere. All

measurements were carried out between 25°C and 500°C with a linear increase of 10°C in a Shimadzu DSC-60 installed with TA-60WS software.

2.8 Estimation of total phenolic content

The total phenolic content in the pretreatment liquor was determined by following protocol described by (Jaiswal et al., 2013). Briefly, 100 µl of sample was mixed with 2 ml of 2 mM Na₂CO₃ solution and incubated for 2 min at room temperature. 100 µl of 1N Folin-Ciocalteu's phenol reagent was added followed by incubation for 30 min in darkness at room temperature. After the reaction was complete, the absorbance was measured at 720 nm. The results were expressed as microgram per ml of the sample through calibration curve of gallic acid.

2.9 Determination of hydrogen peroxide concentration

Hydrogen peroxide formed because of plasma treatment was quantified by oxidising potassium iodide measuring the amount of iodine formed using spectrophotometry at 390 nm. In brief, 100 µl of 1M KI solution and 50 µl of phosphate buffer were mixed with 50 µl of plasma treatment liquor. The mixture was incubated for 30 minutes after which the absorbance was measured at 390 nm (Boehm et al., 2016).

2.10 Optical emission spectroscopy

Optical emission spectroscopy was performed to determine the active chemical species formed during the non-thermal plasma discharge. This was carried out using a Stellarnet EPP 2000C-25 spectrometer at a resolution of 1.5 nm. The light from the plasma was coupled via an optical fibre. The diffraction grating in the spectrometer had a radius of curvature of 40 mm with 590 grooves per mm and an entrance slit width of 25 µm. The fibre (numerical aperture=0.22) was

optimised for performance in the ultraviolet and visible region of electromagnetic spectrum. The spectrometer operated within the wavelength range of 190 nm to 850 nm. The integration time was set at 5000 ms and 5 samples were averaged for the collection of spectra. The emission spectra were qualitatively analysed to assign chemical species to the peaks. The noise in the spectra was cancelled, averaged and analysed using National Institute of Standards and Technology (2012) atomic spectra database and literature published elsewhere (Meiners., 2010).

2.10.1 Statistical Analysis

Quantitative experimental tests were carried out in triplicate and the results are reported as the mean $\pm$ the standard deviation. The Taguchi statistical analysis was conducted by employing MINITAB 17 software. To evaluate the sensitivity of each parameter, determine the optimum combination of operating parameters a variance analysis (ANOVA) was performed at confident level of 95%.

3 Results and discussion

3.1 Effect of pretreatment on the composition of spent coffee waste

The Fenton reaction involves the generation of hydroxyl radicals or similarly powerful oxidising agents in the presence of a metal ion such as iron or hydrogen peroxide. One of the several chemical reactions involved in Fenton chemistry includes the depolymerisation of lignin by dealkylation which then facilitates the enzymatic digestion of cellulose and hemicellulose components (Arantes et al., 2012). This novel pretreatment strategy attempts to delignify SCW by means of providing a suitable environment for the Fenton reaction to take place by providing ferric ions and hydrogen peroxide while maintaining a low pH. Composition analysis of native and plasma pretreated SCW was performed to determine the changes in individual components

brought about by the pretreatment strategy. Native SCW was rich in glucomannan and galactomannan content which was evident from the galactose (13.7 ± 0.3 g/100g of SCW) and mannose (21.2 ± 0.5 g/100g of SCW) content with respect to glucose (8.6 ± 0.1 g/100g of SCW). Trace amounts of arabinose was also found in SCW (1.7 ± 0.2 g/100g of SCW). Similar findings were reported by (Ballesteros et al., 2015; Ballesteros et al., 2014). On the other hand, the plasma pretreated SCW consisted of 18.76 ± 0.2 g of galactose, 24.51 ± 1.3 g of mannose, 10.74 ± 0.9 g of glucose and 2.24 ± 1.2 g of arabinose per 100g of SCW. From the results, it is evident that there was an increase in the total polysaccharide content in SCW after the pretreatment. As with lignin content, the acid insoluble lignin reduced from 31.12 ± 0.2 g in native SCW to 18.6 ± 0.45 g in pretreated SCW (per 100g SCW).

3.2 Effect of different process parameters on metal chloride assisted plasma pretreatment

Table 1 represents the Taguchi experimental design and associated reducing sugar results obtained in the study. Results showed a maximum reducing sugar yield was obtained from trial no. 26 (0.493 g/g of SCW) (refer Table 1). The pretreatment parameters for this trial was found to be 12.5% biomass loading, 3% FeCl_3 concentration, 1% H_2SO_4 concentration, exposure time of 2 min and a voltage of 70 kV. Fig 2 comprises of contour plots that depict the interactions of the process parameters considered in this study that contribute to the release of reducing sugar after enzymatic hydrolysis of the pretreated SCW. A general trend observed was that a higher biomass content resulted in higher reducing sugar concentration after enzymatic hydrolysis. From Fig 2a, it is evident that a high biomass content (% w/v) exposed to plasma for lower time periods (min) was favourable in enhancing the reducing sugar yield. At a biomass concentration

of 12.5% (w/v) and a plasma exposure time of around 2 min the reducing sugar release was found to be in the range of 0.40 g/g of pretreated SCW. Increasing the exposure time detrimentally effected the reducing sugar yield. This was possibly because of the extensive damage incurred by the polysaccharide fraction along with lignin which left less cellulose and hemicellulose for enzymatic degradation.

Fig 2b represents the effects of voltage and biomass on the eventual release of the reducing sugar from SCW in the form on a contour plot. From the plot, it is evident that low voltages and low biomass loading rates (8-10%) was largely ineffective in breaking down the complex lignocellulose structure as the reducing sugar yield was low. As the voltage increases (70-80 kV) the effectiveness of the pretreatment increases. At higher voltages, higher concentrations of reactive species are generated which in turn accelerates the delignifying degradation process (Moiseev et al., 2014). Fig 2c shows the relationship between H_2SO_4 concentration and biomass. A lower concentration of acid (1% acid conc.) was found to favour higher reducing sugar formation. This could be because the higher concentrations of acid tend to produce inhibitory compounds which negatively affect enzymatic hydrolysis. Fig 2d shows the interaction between FeCl_3 concentration and biomass loading. A higher concentration of FeCl_3 resulted in higher reducing sugar yields. This may be because of the accelerated generation of reactive radicals are a result of the Fenton chemistry due to higher concentrations of Fe^{3+} ions.

3.3 Statistical analysis and regression model equation

The ANOVA outputs performed on the L_{25} Taguchi Orthogonal Array is provided in table 2. ANOVA studies gave insights on the role of each parameter based on Fischer's test (F-value), probability and sum of squares to check the significance of each parameter in the model. A large

F-value is suggestive of the importance of a parameter applied in the process. Accordingly, three parameters viz. biomass loading, FeCl₃ conc. and H₂SO₄ conc. were found to have higher F-values compared to voltage and time. Acid concentration had the most prominent effect on the reducing sugar yield with an F-value of 244.84 followed by FeCl₃ conc. (F-value=151.2) and lastly biomass loading (F-value=113.04). Furthermore, only three parameters (biomass loading, FeCl₃ conc. and H₂SO₄ conc.) were found to be significant ($p < 0.05$) with respect to influencing reducing sugar yield. Voltage did not have a significant impact ($p > 0.05$) on the effectiveness of the pretreatment. A similar observation was reported by Misra et al. (2015), where atmospheric air pressure plasma was used to study the variations in anthocyanin content in strawberries. The variability in observations of this study can be attributed to the change in plasma chemistry due to variations in the nature of the lignocellulosic substrate which have not been included in this study.

A regression model equation was generated as a part of the ANOVA study which comprised all the factors considered in this study. This equation can predict the reducing sugar yield according to any set of parametric settings within the range of the process parameters. The regression equation is as follows:

$$\text{Reducing sugar (g/g)} = 0.1864 + 0.01827 * \text{biomass loading} + 0.05798 * \text{FeCl}_3 \text{ conc.} - 0.1473 * \text{H}_2\text{SO}_4 \text{ conc.} - 0.00609 * \text{time} + 0.001188 * \text{voltage}$$

From a high R² value of 96.42% and the adjusted R² of 95.56% illustrated that the model adequately fit the data.

3.4 Incidence of inhibitors in pretreatment liquor

The pretreatment liquor was subjected to HPLC analysis to determine any incidence of inhibitory or monosaccharide components. As was expected trace amounts of individual sugars such as glucose, galactose and mannose and, a small fraction of cellobiose was found in the liquor. An interesting finding was the presence of hydroxymethyl furfural (HMF) in the treatment liquors which were high in sulphuric acid concentration. The concentration ranges of HMF in pretreatment liquors ranged from 0.5g to 1.2g/100g of SCW. This may have been detrimental in the enzymatic hydrolysis of the pretreated SCW. It has been well documented that glucose in acidified solutions can give rise to hydroxymethyl furfural (Van Dam et al., 1986). The inhibitory compounds may have been formed by the degradation of glucose by acid. The increasing intensity of plasma coupled with the higher exposure times may have accelerated the process of HMF formation.

3.5 FTIR, XRD and DSC profiles of untreated and pre-treated spent coffee waste

Fourier Transform Infrared Spectroscopy is used to identify or analyse the changes induced by a pretreatment process on lignocellulosic residues. Each component in lignocellulose (cellulose, hemicellulose and lignin) will have bonds and functional groups that are specific to each of them. Any changes in structure such as the breakage of bonds or removal of fractions can result in changes that can identified in the FTIR spectra. The peaks at 897 cm^{-1} represents glycosidic linkage between cellulose and hemicellose components. The peak intensity was considerably lower in the spectrum of the pretreated SCW. The absorbance of band at 1035 cm^{-1} , which indicates C-O, C=C and C-C-O stretching between polysaccharides and lignin decreased after pretreatment (Tamaki and Mazza, 2011). Furthermore, the decrease in band 1200 cm^{-1} indicated

the breakage of hydrogen bonds between cellulose and hemicellulose. The peak at 1290 cm^{-1} is associated with the crystallinity of SCW (Binod et al., 2012). This peak was found to be diminished in the pretreated SCW spectrum. The band at 1400 cm^{-1} is representative of the lignin present in the sample. This band was seen to be considerably reduced which indicates that the plasma pretreatment was effective in lignin removal. Band 1750 cm^{-1} is an indication of the ketone/aldehyde bonds in hemicellulose (Bodirlau and Teaca, 2009). A peak for this region was found in the pretreated sample spectrum, albeit in lower intensity, probably due to the stretching of these bonds. The band 2920 cm^{-1} represents methyl and methylene groups in lignocellulose (Haripriya et al., 2014). These bands were not as prominent in the pretreated sample spectra, which is an indication of possible demethylation of SCW by reactive radicals generated during the plasma treatment. The absence of peaks in the band range $3000\text{--}3500\text{ cm}^{-1}$ is indicative of stretching of --OH groups for the pretreated SCW (Jahan et al., 2011).

Lignocellulose is generally crystalline as well as amorphous in nature due to its chemical composition. Cellulose is the component in plant biomass that provides its crystallinity. Crystalline cellulose is more resistant to degradation due to chemical, enzymatic or microbial attack compared to its amorphous counter-part. Conversely, hemicellulose is amorphous in nature and is susceptible to degradation by chemical agents or microorganisms. The efficient utilisation of cellulose involves a shift from its crystalline nature to amorphous form (Ravindran & Jaiswal, 2016b). X-ray diffraction studies help to understand the crystallinity of the lignocellulose. Peaks at $2\theta=22^\circ$ are indicative of the crystallinity of the material. Meanwhile, peaks at $2\theta=18^\circ$ represents the amorphous regions in a lignocellulosic substrate. Native SCW was found to be a mixture of equally crystalline and amorphous with broad peaks ranging from $2\theta=15^\circ$ to $2\theta=25^\circ$. However, in the plasma pretreated SCW there was an absence of such peaks

in the crystalline region. Coupled with the development of peaks in the amorphous region it is clear that the plasma treatment leads to decrease in crystallinity of lignocellulose. Although not many studies are available on the changes brought about by air plasma on lignocellulosic fibres, (Baltazar-Y-Jimenez & Bismarck, 2007) reported that the crystallinity of cellulose fibres is affected by the plasma treatment.

Thermal behaviour studies of lignocellulose before and after pretreatment were conducted to analyse the changes in the biomass in terms of its properties as a polymer. This was performed using differential scanning calorimetry (DSC). DSC provides insights into the physical and chemical characteristics of lignocellulose. Several properties such as crystallisation, heat capacity, melting, crystalline orientation and glass transition can be recorded using DSC. The changes induced by the pretreatment in the thermal behaviour of lignocellulose has been widely studied by several researchers (Ballesteros et al., 2014; Nguyen et al., 1981). Therefore, DSC was performed to study the changes induced in the physical properties of SCW after plasma pretreatment. The DSC thermograms are provided as Fig. 3. The thermogram for native SCW was marked by an exothermic event between 20°C and 102.9°C with an associated enthalpy of 114.99 J/g. This may have occurred because of vaporisation of water and the crystalline nature of the sample. The exothermic event was followed by a phase transformation inducing a change in the heat capacity. The glass transition temperature was recorded at 259.2°C. The effect of plasma on the physical properties of SCW was evident as there were marked differences between the thermograms of the pretreated and native SCW. The DSC thermogram for plasma treated SCW showed distinct peaks that suggested an event for glass transition, crystallisation, melting and ending transient. The glass transition temperature was recorded at 283°C.

3.6 Estimation of total phenolic content and hydrogen peroxide in pretreatment liquor

Exposing aqueous solutions to plasma discharge results in the generation of large amounts of hydrogen peroxide. This results in the initiation of Fenton reaction that further degrades individual components in lignocellulose such as lignin. The hydrogen peroxide in the pretreatment liquor formed was quantified. It was found that the liquid obtained after pretreatment contained 647.28 μM of hydrogen peroxide after 24h of plasma treatment. A majority of the H_2O_2 formed is due to its solubilisation from gaseous phase into liquid phase. Therefore, by retaining the reactive species in the gas phase in contact with the liquid phase for longer periods time higher amounts of H_2O_2 could diffuse into the liquid. Furthermore, earlier studies confirmed that concentrations of H_2O_2 in solutions subjected to plasma treatment were stable over several weeks when stored in closed containers at 4°C (Boehm et al., 2016).

Spent coffee waste is high in phenolic compounds. The effect of plasma on the phenolic content of pretreatment liquor was analysed by Folin-Ciocalteu's phenol reagent. The control contained 908.35 μg GAE/g of SCW while the pretreated liquor contained 672.86 μg GAE/g of SCW. Exposing SCW to dielectric barrier discharge plasma resulted in the total phenolic content by 26% for an experimental setting of 70 kV and 2 min. The reduction in total phenolic content can be attributed to the formation of reactive species and the availability of oxygen in the atmosphere which actively degraded phenolic compounds in SCW (Ramazzina et al., 2016). Earlier studied conducted by [Grzegorzewski et al. \(2011\)](#) reported a reduced phenolic content in lamb lettuce leaves when treated with atmospheric air pressure plasma.

3.7 Identification of radical species formed during dielectric barrier plasma

The chemical reactive species in the gas phase formed during the dielectric barrier discharge was analysed by optical emission spectroscopy (OES). The spectrum of the radiation emitted by the plasma was grating and the intensity was measured as function of the wavelength (Fig 4). The non-thermal plasma was operated at voltage of 70 kV the emission spectra was obtained for a wavelength range of 180-900 nm. The emission spectrum revealed that the emission was near the UV region (300-400nm). Emissions from N_2 and N_2^+ species exhibited distinct peaks. The small peaks observed at the region corresponding to 250-300nm were a resultant of the presence of OH. Meanwhile the very low intensity peaks observed at 750 nm and 780 nm corresponded to singlet oxygen. The formation of singlet O may be attributed to the particle collisions as well as the quenching of $O(^3P)$ and $O(^5P)$ energy. Similar observations were reported by Pearse et al. (1976) and Laux et al. (2003) in different studies involving identification of molecular spectra and atmospheric air pressure plasma. From these inferences, it was quite clear that a certain amount of lignin degradation was achieved by reactive species formed during plasma treatment.

3.8 Bioethanol production using plasma treated SCW

SCW was found to be a potential candidate for bioethanol production. The hydrolysate obtained after enzymatic digestion of the plasma pretreated SCW was used for bioethanol production using *S. cerevisiae*. A maximum ethanol content of 18.642 g/l was obtained after fermentation for 72h. The bioethanol production using the control sample yielded 9.231 g/l of ethanol. A 2-fold increase in bioethanol yield using the optimized process parameters was found with a fermentation efficiency of 74%. This result is inline with previous studies carried out by Kwon et al. (2013), which reported a high ethanol fermentation efficiency of 80% using lipid extracted

SCW. Similar findings were reported by Sindhu et al. (2011) where they optimised dilute acid pretreatment for the processing of sugar cane trash and eventual bioethanol production to obtain a titre of 11.365 g/l.

4 Conclusion

Ferric chloride assisted plasma processing of SCW was found to be an effective pretreatment strategy with high lignin removal capacity. This resulted in an enhanced enzymatic digestion and high fermentation efficiency for ethanol production. A Taguchi design of experiments was successful in optimising the parameters for the plasma treatment. A maximum effectiveness of the pretreatment was achieved after a short treatment duration of 2 min at a voltage of 70 kV and in the presence of 1% H_2SO_4 . To our knowledge there are limited experiments employing atmospheric air plasma as a lignocellulose pretreatment measure let alone trying to mimic Fenton chemistry using plasma for delignification of plant biomass and subsequent bioethanol production.

Appendix A. Supplementary data Supplementary data associated with this article can be found, in the online version, at xxxxx

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Figure Captions

Fig. 1. Instrument set up for the generation of cold air pressure plasma

Fig 2. Contour plots exhibiting the interactions of various process parameters on the reducing sugar yield (g/g); (a) interactions between biomass (% w/v) and time (min); (b) interactions between biomass (% w/v) and voltage (kV); (c) interactions between biomass and H₂SO₄ concentration (%); (d) interactions between FeCl₃ concentration (%) and biomass (% w/v).

Fig 3. DSC thermogram of native and plasma pretreated SCW

Fig 4. Typical Optical Emission Spectrum (OES) of the dielectric barrier discharge in air (Operating voltage = 70 kV)

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Fig 4. Typical Optical Emission Spectrum (OES) of the dielectric barrier discharge in air (Operating voltage = 70 kV)

Table 1 Taguchi design for optimisation of process parameters involved in plasma pretreatment

Trial no.	Biomass loading (%w/v)	FeCl₃ conc. %	H₂SO₄ conc. %	Time (min)	Voltage (kV)	Reducing sugar (g/g)
1	7.5	1	1	2	60	0.299
2	7.5	1	1	2	70	0.311
3	7.5	1	1	2	80	0.317
4	7.5	2	1.4	4	60	0.296
5	7.5	2	1.4	4	70	0.302
6	7.5	2	1.4	4	80	0.311
7	7.5	3	1.6	6	60	0.300
8	7.5	3	1.6	6	70	0.313
9	7.5	3	1.6	6	80	0.318
10	10	1	1.4	6	60	0.253
11	10	1	1.4	6	70	0.264
12	10	1	1.4	6	80	0.272
13	10	2	1.6	2	60	0.309
14	10	2	1.6	2	70	0.309
15	10	2	1.6	2	80	0.321
16	10	3	1	4	60	0.413
17	10	3	1	4	70	0.428
18	10	3	1	4	80	0.455
19	12.5	1	1.6	4	60	0.269
20	12.5	1	1.6	4	70	0.296
21	12.5	1	1.6	4	80	0.300
22	12.5	2	1	6	60	0.428
23	12.5	2	1	6	70	0.452
24	12.5	2	1	6	80	0.446
25	12.5	3	1.4	2	60	0.432
26	12.5	3	1.4	2	70	0.493
27	12.5	3	1.4	2	80	0.474

Table 2 Analysis of variance data for reducing sugar yield after plasma pretreatment

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Biomass loading	1	0.037545	0.003833	113.04	0.019
FeCl ₃	1	0.060517	0.0061759	151.2	0.006
H ₂ SO ₄	1	0.036434	0.0037195	244.84	0.014
Time	1	0.002668	0.002723	147.41	0.007
Voltage	1	0.00254	0.002596	89.95	0.06
Error	1	0.005191	0.000687	54.83	0.065
Total	6	0.144895			
S=0.0157216		R-sq=96.42%	R-sq(adj)=95.56%		

S-square of root mean square; *R*²-coefficient of determination; Adj SS-adjusted sum of squares; Adj MS-adjusted mean square; *F*-*F* test; *P*-probability

Highlights

- A novel FeCl_3 assisted plasma pretreatment strategy was proposed for lignocellulose.
- Extensive delignification in spent coffee waste was achieved upon pretreatment.
- The polysaccharide fraction of spent coffee grounds was left unaffected.
- High reducing sugar yield was obtained upon hydrolysis of pretreated spent coffee waste.
- Pretreated SCW was found to be a suitable substrate for bioethanol production.