

Wheat Straw bio-refining

Part II: Optimization of Alkali Pre-treatment method with Microwave

Radiation for fermentable sugars separation.

Onome Festus , Vasiliki K. Skoulou and Sharif H. Zein*

Chemical Engineering, Faculty of Science and Engineering, School of Engineering and Computer Science, University of Hull (UoH), Kingston Upon Hull, HU6 7RX, U.K.

STRUCTURED ABSTRACT

Background: The potential of microwave-assisted heating for enhancing the alkaline pre-treatment of lignocellulosic waste is highlighted in this work.

Objective: To maximize separation of fermentable sugars from wheat straw.

Methods: Wheat straw hydrolysis assisted by microwave was carried out by setting up a statistical experimental design method and further investigating the main process parameters, namely: temperature (°C), microwave power (W), NaOH concentration (M) and wheat straw pre-treatment time (min) towards maximization of fermentable sugars extraction from wheat straw. The reducing sugars yield (response) of the alkaline pre-treated and microwaved wheat straw in lab scale provided data for building a predictive model which reflected interactions, significance and impact of the process parameters (factors) on the wheat straw hydrolysis yield.

Results: SEM and FTIR images of untreated and alkaline pre-treated wheat straw were studied for investigating the morphological changes of wheat straw surface quality and structure resulting from the microwave and/or alkaline pre-treatment. In addition reducing sugars yield of 87%wt. from wheat straw pre-treated at 180°C, 550W microwave power, 0.65M of NaOH for 25min was achieved. This result was significantly higher compared to the one from the straw which was only hydrolysed enzymatically (30 %wt).

Conclusion: It was found that shorter pre-treatment times were obtained at higher temperatures, alkali concentration and moderate microwave power levels. The results obtained were further optimized and indicated that the microwave-assisted alkaline pre-treatment of wheat straw technique is an attractive pre-treatment method which reduces the wheat straw pre-treatment time and enhances the hydrolysis yield.

Keywords: Microwave-assisted pre-treatment; experimental design; lignocellulose; fermentable sugars; enzymatic hydrolysis; wheat straw; bioethanol

Address correspondence to this author at the Chemical Engineering, Faculty of Science and Engineering, School of Engineering and Computer Science, University of Hull, Kingston Upon Hull , HU6 7RX UK; Tel: ++40 1482 466 753; E-mail: s.h.zein@hull.ac.uk.

Abbreviations:

CCD: Central Composite Design

1. INTRODUCTION

Lignocellulosic biomass is today the largest source of renewable energy in the EU and despite growth rates in the solar (photovoltaics) and wind energy, biomass-energy is expected to remain the major renewable source until 2030. Along with bioenergy production, a future bio-based economy will bring a wide range of additional opportunities to develop new biomass-based industries, open new markets for biomass-derived products and use waste sources as a valuable resource in a more sustainable way. Nowadays, there is need for a clear and long-term stability of the biomass policy framework along with support schemes to unlock the potential of biomass waste, especially lignocellulosics, and support the development of innovative bio-businesses. Towards that direction the COP21 agreement in Paris set a long-term goal for providing new opportunities, as well as indicated challenges for the bio-energy sector and as a result, the R&D of bio-businesses. There is still however the need to bridge the gap between laboratory research, large scale demonstration and industrial applications so as to properly address the investment costs and financial risks of such attempts.

For a sustainable feedstock supply, all market opportunities for biomass feedstock need to be considered and having in mind the above, wheat straw is an attractive and abundant residue with a worldwide production of 1×10^{11} tons/yr [1]. Lignin, cellulose and hemicellulose comprise the main building complexes of the plant's tissues [2]. Cellulose is made up of glucose units while hemicellulose and cellulose comprises different sugar molecules such as pentoses and hexoses as well as small amounts of uronic acid [3]. All these varying sugar molecules are fermentable and can be separated by hydrolysing the major building blocks of lignocellulosic waste, cellulose and hemicellulose referred usually in literature as holocellulose.

The increasing interest in the utilization of lignocellulosic biomass like wheat straw is that such agricultural waste sources consist of a primary source for the production of fermentable sugars, which serve as feedstock towards liquid biofuels such as bioethanol [4-8]. Wheat straw is an abundant source for UK too and especially the area of Hull and Humberside its potential to produce fermentable sugars as well as bioethanol is shown in Table 1. The commercial value of the bioethanol produced from the locally available stocks could reach an annual value of ~1.12 M£.

However, wheat straw waste like any other lignocellulosic waste, requires some kind of pre-treatment before converting it into bioethanol in order to improve this feedstock's digestibility and amenability to enzymatic hydrolysis. The necessity of pre-treatment arises from the fact that lingo-cellulose presents complex structural associations between not only hemicellulose and cellulose, but also lignin as well as some inherent biomass enzymes reducing its digestibility. Concerning the natural macromolecular component of the plant cell wall, lignin, it offers rigidity and a layer of protection to cellulose and hemicellulose against the microbial attack. The alkaline pre-treatment step also decreases crystallinity of the cellulose macromolecule, which is in fact a desirable outcome for the subsequent hydrolysis step as cellulose is more hydrolysable in its amorphous, rather than its crystalline form [9].

Table 1 Wheat straw cultivation/harvesting details and the potential of cellulose separation and bioethanol production in the area of North Humberside and Hull.

Area (ha) [10] (DEFRA, 2014)	251,000
Wheat yield (ton/ha)	8.40
Wheat Crop Productivity (ton/yr) [10]	2,108,400
Wheat Residues -to-Product ratio [11]	1.36

Wheat Residues availability for alternative uses (%) [12]	15
Wheat Straw Residues (*ton/yr)	430,114
Wheat straw cellulose content- Moderate estimation (33 wt %) (*ton/y)	141,937
Wheat Straw cellulose content-high estimation (40 wt%) (*ton/yr)	172,045
Wheat straw dry mater (~90 wt%) (ton/yr)	387,103
Bioethanol Production (m ³ /y) (~0.29 m ³ /ton dry matter) [13]	112,260
Bioethanol estimated value (~ 0.010£/L)	1,122,600 £

Wood contains as high as 40-50wt% of cellulose while in some parts of specific plants i.e. cotton and tobacco leaves reaches the levels of 95-99 wt% [14]. Cellulose (C₆H₁₀O₅)_n is a natural biopolymer, a polysaccharide structure based on glucose (C₆H₁₂O₆) monomers and polymerizes through d-glycosidic bonds [15]. It can be found in either amorphous or crystalline form and is insoluble in aquatic solutions while its hydrogen bonds (-H-) take part in the formation of macromolecular levels responsible for its strength and thermal stability [16]. Nowadays, almost 95% of the produced cellulose is related to the technical wood manufacture industry and is also consumed by the paper production industry [17].

Hemicellulose, on the other hand, is always accompanied by cellulose. It consists of the basic component of plant cells and sums up to the 25-30 wt% of woody biomass species. Hemicellulose is a linear biopolymer composed of simple sugar molecules such as xylose and arabinose, as well as glucose and monose but in lower weight percentages compared to the previous mentioned. Apart from those simple sugar molecules, hemicellulose is comprised of pentoses and exozes [16, 17]. Hemicellulose, on the contrary to cellulose, is a water soluble product and it represents a feeding source for digestion processes and bioethanol production as in fact it is the source of simple sugar molecules production [17].

Cellulases and hemicellulases are classified as dedicated enzymes to break down cellulose and hemicellulose to glucans and xylans, respectively. The activity however of these enzymes is limited when lignin is present in the feedstock material. This is the reason why efficient separation of holocellulose from lignin is a very important pre-treatment process for future bio-economies and biorefineries. The negative impact of the presence of lignin on the fermentable sugars separation and hydrolysis yield from the lignocellulosic biomass waste has been repeatedly reported in scientific literature [1, 3, 18-19].

Different methods of biomass pre-treatment such as utilisation of concentrated and dilute acid, alkali, steam explosion and ammonia fibre explosion have been tested in the past to improve biomass digestibility resulting in variable results and a variety of yields [6, 8, 20]. Among others, alkaline pre-treatment is one of the most common and effective methods of processing lignocellulosic waste, with alkalis such as calcium hydroxide Ca(OH)₂, sodium hydroxide (NaOH) and potassium hydroxide (KOH) to be commonly used as pre-treatment means. Alkaline pre-treatment offers several advantages over other common methods of pre-treatment such as steam explosion and acid pre-treatment. Some of the advantages of alkaline pre-treatment are that it not only requires milder operating conditions, but is also cost effective, does not have an adverse effect by deteriorating the quality of cellulose during pre-treatment and does not lead to the formation of inhibitory compounds such as furfural and 2-formaldehyde (2) [3, 21]. The latest is a desirable property for such kind of pre-treatments especially when followed by other downstream operations such as enzymatic hydrolysis and fermentation as the presence of inhibitory compounds prevents the activity of enzymes and in general, microorganisms responsible for fermentation [3, 22]. However, at low alkali concentrations and low temperatures, the conventional pre-treatment methods require longer residence time for achieving higher pre-treatment yields [23].

Today the conventional pre-treatment methods are quite energy consuming, expensive - capital intensive, require long residence times in order to be completed and have very close monitoring [1,20]. In order to balance the above mentioned drawbacks, the microwave assisted heating technique has started attracting attention as it gained a widespread range of applications in other chemical processes while it has also been lately found an application and in combination with other methods to biomass pre-treatment. Microwave assisted heating presents the advantages of lower

energy needs, uniform heating of the feedstock material without the need of conduction as in other conventional heating methods and is easy in operation [24]. These advantages lead to more efficient heating, quicker reaction times as well as limited equipment setup effort and generation of waste [25]. Microwave pre-treatment of biomass waste is an emerging area and whilst not exhaustively investigated, it holds a great potential for improving the feedstock pre-treatment processes. Among such processes, it works well for improving the digestibility of lignocellulosic waste in lower processing times especially when combined with other widely used conventional pre-treatment processes such as the conventional alkaline pre-treatment. The objective of this research is to evaluate the impact of the microwave-assisted heating in the alkaline pre-treatment of wheat straw and the impact of the main pre-treatment operating conditions of: temperature (T), concentration of alkali (C), microwave radiation power (P) and processing time (t) on the reducing sugar yield from the subsequent enzymatic hydrolysis of the lignocellulosic waste of wheat straw.

1 EXPERIMENTAL DESIGN

1.1.1 Experimental design for microwave-assisted pre-treatment

The experimental conditions (matrix) designed using the statistical design software DESIGN EXPERT which is based on the Central Composite Design (CCD) method is a form of a Surface Response Methodology (SRM), which has been described in detail in the previous study [26]. Such kind of an experimental design strategy enables embodiment of the advantages of performing a specific number of experiments which are foreseen to lead to the optimum lab experimental results and thus contribute to reduction of time, manpower and resources spent for performing unnecessary experiments. Full consideration of the multivariate nature of the process parameters (factors) implicating in the experiment, as well as consideration of the definite interactions between these parameters and the impact on the response yield (reducing sugars yield or fermentable sugars separation from the wheat straw) has been taken into account. The process conditions and their effect on the reducing sugars yield studied here were: the temperature, concentration of alkali, microwave power and microwave pre-treatment time. The calculated response factor represents the reducing sugars yield from the enzymatic hydrolysis of both untreated and alkaline pre-treated and microwaved wheat straw waste. A number of experimental runs designed to be performed by setting the upper and lower levels of the process parameters (upper and lower factors limits) is shown in Table 2.

Table 2 Process parameters (factors) and levels (upper lower limits) for an optimum experimental design outcome.

Process Parameter (Factor)	Low	High
Temperature (°C)	150	180
Residence time (min)	10	40
Microwave power (W)	250	800
Alkali (NaOH) concentration (M)	0.3	1.0

Running the DESIGN EXPERT software, a total number of 30 optimum experimental runs-best suited process conditions for an optimum result (maximization of the sugars yield from wheat straw waste) were obtained based on different combinations of the lower and upper levels of four (4) process parameters (factors) with one (1) response being the hydrolysis yield (wt%). The 30 experiments comprised of 16 factorial points, eight (8) axial points and six (6) centre points with each experimental parameter to be studied at five (5) different levels or process parameter values (Table 3).

Table 3 Levels of experimental process parameters (factors).

Process Parameter (Factor)	Level 1	Level 2	Level 3	Level 4	Level 5
Temperature (°C)	150	158	165	178	180

Process Parameter (Factor)	Level 1	Level 2	Level 3	Level 4	Level 5
Residence time (min)	10	18	25	33	45
Microwave Power (W)	300	425	550	675	800
Alkali (NaOH) concentration (M)	0.300	0.475	0.650	0.825	1.00

2 MATERIALS AND METHODS

Wheat straw was purchased from a local farm in the Yorkshire area and stored in the laboratory away from moisture and any contamination from the environment. Sodium hydroxide (NaOH), D-glucose anhydrous, sodium acetate buffer solution (pH 5.2±0.1), 3, 5-dinitrosalicylic acid (DNS, 98%), sodium bisulphite, potassium sodium tartrate tetrahydrate (99+%) and cellulase enzyme (CelluclastR 1.5L) were purchased from Sigma-Aldrich. All chemicals were of analytical grade and deionized water was used for all experimental purposes. The enzyme used each time was securely stored in a freezer below 6°C prior to its use.

2.1.1 Methods

Wheat straw was milled to smaller particle sizes with the use of a blender and passed through a mesh to obtain uniform sizes and eliminate the fine particles which make the filtration process difficult after pre-treatment. A batch of 2 g of wheat straw waste was weighted and used as feedstock for each experimental run. The wheat straw samples were placed manually into the microwave reactor vessels and 50ml of NaOH solution was added with the inclusion of magnetic stirring bars to facilitate automated stirring inside the microwave. The lignocellulosic waste- alkali mixture was stirred with a spatula to form a homogeneous mixture, ensuring that the wheat straw was fully immersed in the alkali solution. The reactor vessels were then sealed and placed in the appropriate rotating base of microwave reactor system. A standard laboratory type Ethos EX microwave was made by CEM Corporation with a maximum operating capacity of 1,000W, 1bar and 200°C. Further details concerning the microwave assisted pre-treatment rig can be found elsewhere [26].

After pre-treatment, the processed wheat straw firstly allowed to cool down, removed from the microwave system, filtered and repeatedly washed with deionized water. The filtrate was continuously checked with litmus paper until neutral pH was observed. The final solid residue was dried in a convection oven at a moderate temperature of 105°C overnight to prevent charring and further degradation of the material. The dried wheat straw residue was weighed, stored in airtight vials in a desiccator, maintained for further analysis and exploited as a feedstock for the enzymatic hydrolysis.

2.2 Enzymatic hydrolysis

2.2.1 Experimental variables and design

The enzymatic hydrolysis of both raw and pre-treated wheat straw samples prepared from previous experiments was performed. The main operating conditions (factors) taken into consideration during the hydrolysis step were the enzyme loading, temperature, PH, incubation time and the revolutions per minute (rpm) and these were set along optimum conditions for cellulase enzymes chosen for the hydrolysis. Cellulase enzymes usually perform better at temperatures of 45 - 50°C, PH of 4.5 – 5.0 and incubation time of about 72hrs [4, 20, 27].

The enzymatic hydrolysis took place as follows. A sample of 0.4g each from the raw and microwave/alkaline pre-treated wheat straw into separate 100ml Erlenmeyer flasks was taken. A 50ml solution of 0.1M acetate buffer pH 4.8 was added to the flasks followed by the addition of 5ml sodium azide to prevent microbial growth and deactivation of the enzymes.

The wheat straw and flask contents were pre-incubated in an INFORS HT Multitron incubating shaker for 45min to ensure elimination of any microbial contamination. Thereafter, 1ml of the cellulase enzyme of Celluclast 1.5L was added to each flask. The flasks were placed in the incubating shaker and allowed to incubate for 84hrs at 50°C and 160RPM.

After completion of the hydrolysis process, the flasks and hydrolysed samples were withdrawn from the incubator and quickly heated at 100°C for 10-15min targeting to the de-activation of the enzymes. The final content was then

centrifuged for about 10min. The supernatant was stored in a refrigerator and used for analysis and estimation of the concentration of reducing sugars released following the 3,5- dinitrosalicylic acid (DNS) method as described by Miller [28].

In addition, the FTIR spectroscopic analysis was performed in a Thermo Nicolet 1s5 FTIR for both the untreated and pre-treated wheat straw in order to study the changes in the chemical structure of the wheat straw waste and changes in functional groups as a result of the microwave assisted alkali pre-treatment. The disappearance or reduced intensity of certain functional groups, especially those related to the lignin, which separated in a previous stage [26] and hemicellulose gives an idea of how effective these pre-treatment conditions are. The specifications of the spectrometer for the analysis were measured between wavelengths $4,000\text{cm}^{-1}$ and 600cm^{-1} with the detector set at a resolution of 4cm^{-1} and 16 scans per sample.

Scanning Electron Microscope (SEM) images of untreated and pre-treated wheat straw were taken at resolutions of 1500X using a Hitachi TM 1000 microscope.

Reducing sugars released from the enzymatic hydrolysis were estimated by the 3, 5- dinitrosalicylic acid (DNS) method as described by Miller [28]. A calibration curve with known concentrations of glucose was created and the absorbance readings of each solution were taken after reacting it with DNS reagent. The supernatant from the hydrolysis was reacted with DNS reagent and the absorbance of the solutions was measured with a UV spectrometer at 540nm and 1cm path-length. From the calibration curve, the concentration of reducing sugars yield in the samples was estimated.

3 RESULTS AND DISCUSSION

3.1 Impact of pre-treatment on wheat straw physical properties.

Microwave assisted alkaline (NaOH) pre-treatment had an impact on the physical properties of the wheat straw for all the conditions and experimental runs carried out. After pre-treatment, the wheat straw indicated a substantial swelling behaviour, weight loss and increase of its surface area (Figure 1). After pre-treatment, the produced solution contained both dissolved lignin and hemicellulose.

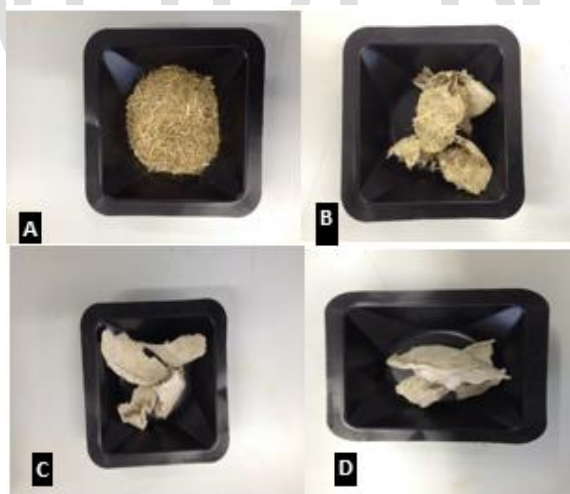


Figure 1 Images of: (A) raw wheat straw waste, and (B), (C), (D) microwave assisted, alkali pre-treated wheat straw.

The reducing sugars released from wheat straw were confirmed by the DNS method [28]. The solubilisation of lignin in the alkaline (NaOH) solution after pre-treatment is a result of the cleavage of the α -aryl ether bonds of the lignins as the poly-phenolic units [29]. Once these bonds are disrupted, the solubilisation and separation of lignin units from cellulose are enhanced.

Hemicellulose macromolecule is perhaps the easiest amongst the three components of lignocellulosic waste to hydrolyse. In the sodium hydroxide (NaOH) solution, the solubilisation of hemicellulose is achieved due to the breakage of the hydrogen bonds (-H-). According to Ternrud, Lindberg and Theandera [30], the alkali is able to break the ester-

linked groups in hemicellulose, as well as other components of the lignocellulosic biomass cell wall. As a result, there is tendency towards increasing the hydrophilic properties and subsequent solubilisation in the alkali.

On average, a 59 wt% weight loss was observed for all the different conditions of pre-treatment designed. The highest weight loss of 66 wt% was obtained at the highest temperature tested that of 180°C (**Table 1**). The alkali treatment induced a breakage of the chemical bonds and resulted in the swelling of cellulose but it proved not efficient enough to solubilise components such as cellulose, as cellulose is not soluble in typical organic solvents [31-32].

3.2 FTIR analysis

The raw wheat straw showed several characteristic peaks such as the strong, broad absorption band between 3,350 – 3,027 cm^{-1} with a peak value at 3,351 cm^{-1} . This peak corresponds to the stretching vibrations of the associated hydroxyl (-OH) groups commonly associated with alcohol and phenols [33]. For all the pre-treated samples, the -OH stretching bands were further broadened compared with untreated wheat straw, as shown in **Figure 2**. This broadening of the band describes increased hydrophilicity of the pre-treated wheat straw as hemicellulose and lignin are removed from the material and then attributed to the presence of cellulose [34].

Two (2) unique peaks were observed at 2,920 and 2,850 cm^{-1} , which represent the C-H stretching vibrations in the untreated wheat straw and =CH₂ stretching vibrations ascribed to the double bonds in some of the components of lignin, respectively. Both peaks disappeared, leaving a weakened and broad absorption band around that wavenumber in the spectra for pre-treated wheat straw. The strong peak at 1,734 cm^{-1} observed in the untreated wheat straw describes C=O stretching vibrations characteristic of aldehydes and carboxylic acids [33]. Alemdar and Sain and Janoobi et al. [35,36] also noted that peaks at 1,735 cm^{-1} are characteristic of α , β unsaturated esters and are ascribed to the ester linkages present in the carboxylic group of ferulic and p-coumaric acids components of lignin as well as groups within hemicellulose. In the samples subjected to the microwave alkali pre-treatment, this peak was observed to have significantly diminished or disappeared, indicating the removal of lignin and hemicellulose.

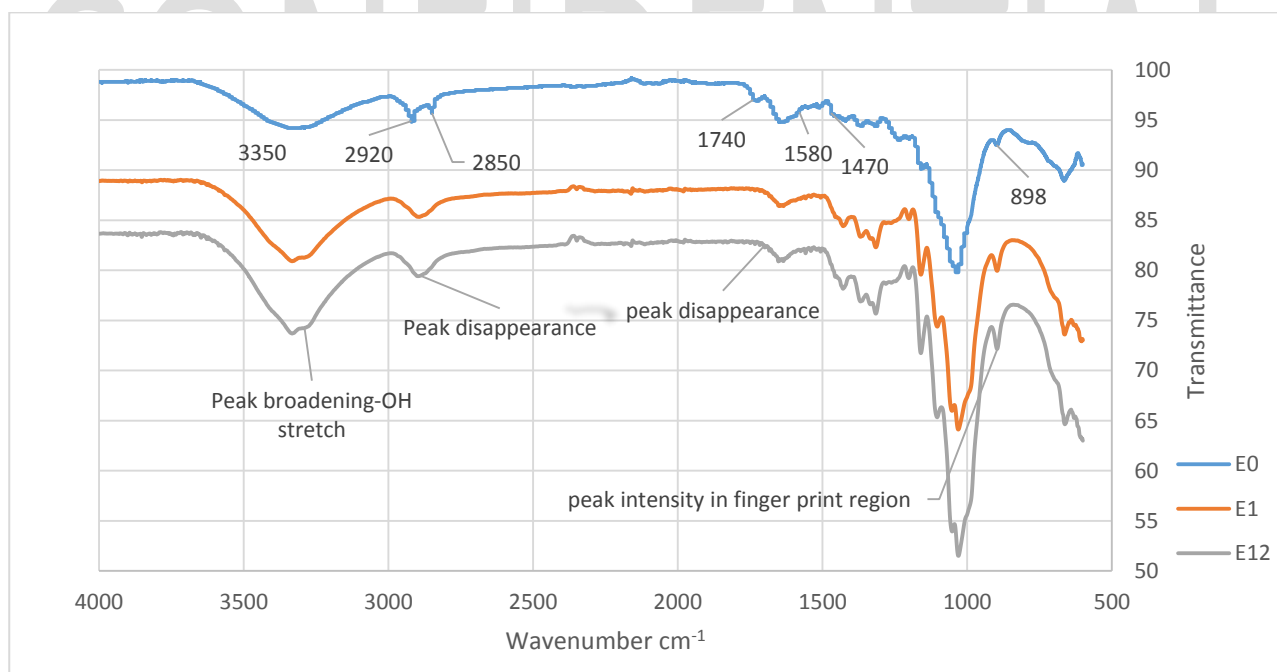


Figure 2 Comparison of the FT-IR spectra of: (E0) untreated wheat straw, and (E1) (E12): alkaline and microwaved pre-treated wheat straw.

Other peaks noticed in untreated wheat straw within the finger print region 1,500 to 400 cm^{-1} of the spectra showed improved peak intensities and clarity in the pre-treated material. Peaks within the finger print region though complicated, are usually characteristic of a particular compound. The peak at 897 cm^{-1} was steady and prominent in all pre-treated samples which further indicates the increase in the amount of cellulose after pre-treatment.

3.3 SEM analysis

SEM images of untreated and pre-treated wheat straw (**Figure 3**) indicated surface morphological changes in the physical appearance and surface morphology of the wheat straw **micro-fibres**. Pre-treated wheat straw fibres appeared to be more **of 'porous' nature** compared to the untreated **sample**. As the basic structural components of plant cell wall, lignin and hemicellulose form both the primary and secondary cell wall structure around cellulose. **The** impact of the alkali pre-treatment, concentration and temperature aids in disrupting the structural associations between lignin, cellulose and hemicellulose, which leads to swelling of the material and an increased surface area. The longer wheat straw fibres were also broken down to form smaller fibrils of about 50µm **of characteristic length**.

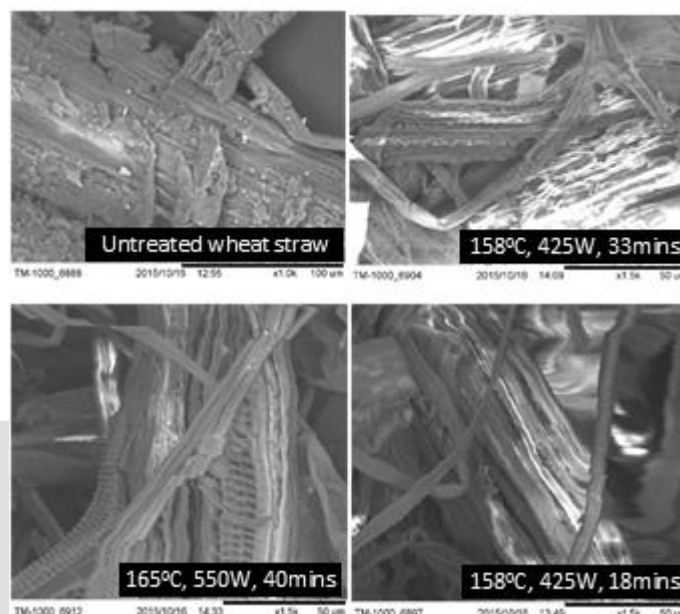


Figure 3 Effect of microwave assisted alkali pre-treatment on the surface morphology (SEM images) of the untreated wheat straw.

3.4 Reducing sugars yield

Attractive yields of reducing sugars were obtained from the enzymatic hydrolysis of **the** pre-treated wheat straw, being also an indication of the effectiveness of the microwave assisted pre-treatment **method** of **the** straw. The yields were **entered** in the design resulting in the model with a quadratic equation shown below Equation (1) which had an R² value of 0.74.

Hydrolysis yield (YH)

$$\begin{aligned}
 &= 31.2328 - (0.36009 * T) + (0.14175 * C) - (5.1403e - 003 * P) - (0.026170 * T) \\
 &- (2.7455e - 003 * T * C) + (9.20559e - 006 * T * P) - (3.11141e - 007 * T * t) \\
 &+ (4.12946e - 004 * C * P) - (0.023504 * C * T) + (5.14309e - 006 * P * T) \\
 &+ (1.10531E - 003 * T) + (0.60945 * C) + (2.67577e - 006 * P * P) \\
 &+ (8.26233e - 004 * T * T) \qquad \qquad \qquad \text{Equation (1)}
 \end{aligned}$$

Where:

YH= Hydrolysis yield (wt%)
T = Temperature (°C)
C= Concentration of alkali (M)
P = Microwave power (W)
t = Time (min)

The normal plot of residuals (**Figure 4**) indicates that the errors in the model are normally distributed.

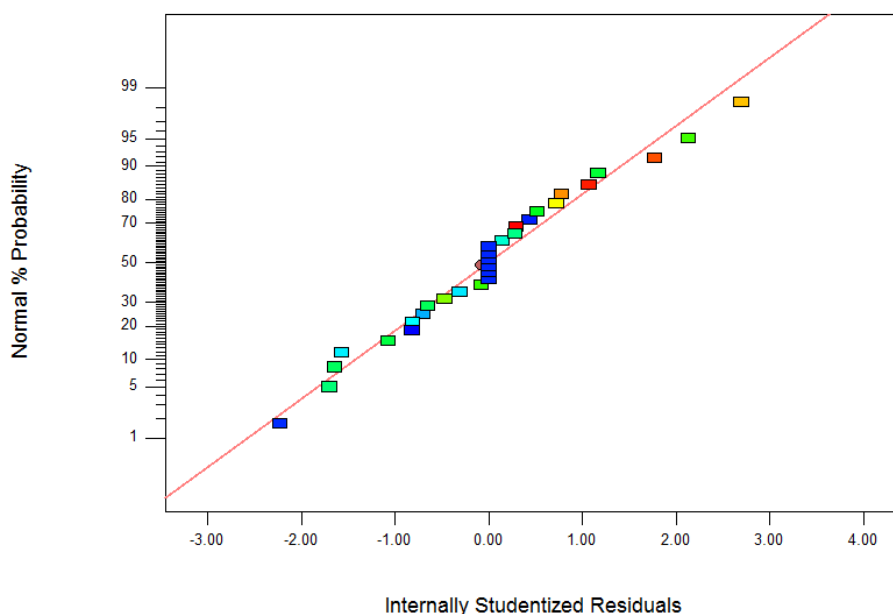


Figure 4 Normal plot of residuals.

The hydrolysis yields (YH in wt%) obtained are tabulated in **Table 4**. The highest yields of fermentable sugars of 87 wt%, 85 wt% and 81 wt% were obtained at pre-treatment conditions of (180°C, 550W, 0.65M for 25min), (173°C, 425W, 0.475M for 33mins) and (173°C, 425W, 0.475M for 18min), respectively.

Overall, the concentration of the alkali (up to 1M NaOH) was adequate for pretreatment, however it was shown that key process parameters (factors) with significant p-values less than 0.05 were the temperature (T) and microwave power (W). Time had a p-value of 0.2 hence it was an insignificant factor for the model. The pre-treatment time (t) was also dependent on the severity of other factors like temperature and microwave power.

A comparison of the wheat straw pre-treatment was carried out in the same conditions of alkali concentration, temperature, and same or different residence times. It was shown that the pre-treatment with the higher microwave power gave a better hydrolysis yield at lower temperature. Also, at higher power, the pre-treatment time was reduced, as expected.

Table 4 Weight loss (wt%), hydrolysis yield (wt%) and process conditions of microwave-alkaline pre-treatment of wheat straw.

Run Code	Temperature (°C)	Time (min)	Power (W)	NaOH (M)	Weight loss during pre-treatment (wt%)	Hydrolysis yield (wt%)
E1	158	33	675	0.825	59	58
E2	158	33	675	0.475	59	55
E3	173	18	425	0.475	60	68
E4	165	25	550	0.650	56	48
E5	173	33	425	0.825	60	71
E6	173	18	675	0.825	59	76
E7	165	25	550	0.650	56	48
E8	165	25	800	0.650	63	64
E9	165	25	550	0.300	52	46
E10	165	25	550	0.650	57	48
E11	158	18	675	0.475	52	47
E12	173	33	425	0.475	61	85
E13	158	33	425	0.825	63	78
E14	173	18	675	0.475	56	48
E15	173	33	675	0.475	64	83
E16	158	33	425	0.475	62	63
E17	165	25	550	0.650	57	48
E18	165	40	550	0.650	60	64
E19	165	25	550	0.650	57	48
E20	158	18	425	0.475	53	69
E21	165	10	550	0.650	62	65

Run Code	Temperature (°C)	Time (min)	Power (W)	NaOH (M)	Weight loss during pre-treatment (wt%)	Hydrolysis yield (wt%)
E22	158	18	675	0.825	56	53
E23	173	18	425	0.825	60	81
E24	158	18	425	0.825	54	56
E25	165	25	550	1.000	61	61
E26	150	25	550	0.650	59	57
E27	173	33	675	0.825	61	62
E28	165	25	550	0.650	57	48
E29	165	25	300	0.650	60	61
E30	180	25	550	0.650	66	87

When however high temperature and mid-level microwave power conditions ($\leq 425\text{W}$) applied, those favoured pre-treatment and the hydrolysis yield as the impact of microwave power was generally found to be limited to mid-level conditions ($\leq 425\text{W}$). At higher microwave power levels, the effect on the pre-treatment was no longer significant especially at high temperatures and alkali concentration (Figures 5-6). The driving force in these sets of experiments is that a mid to low level microwave power can be utilised effectively, provided that the pre-treatment is carried out at higher temperatures ($\leq 180^\circ\text{C}$).

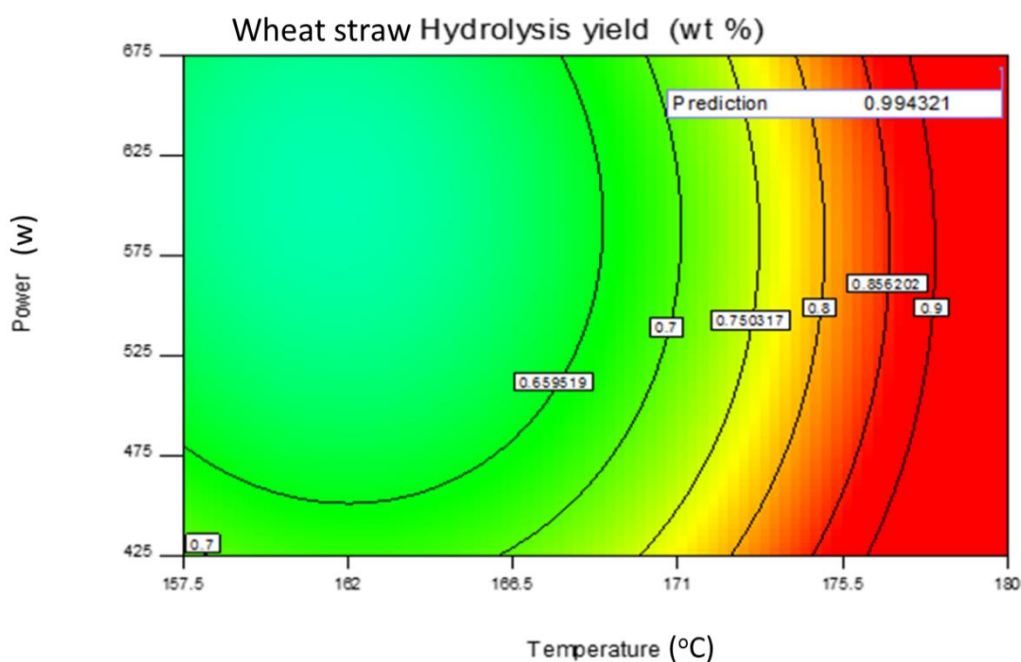


Figure 5 Effect of temperature and microwave power on the potential of maximization of the wheat straw hydrolysis yield (wt%).

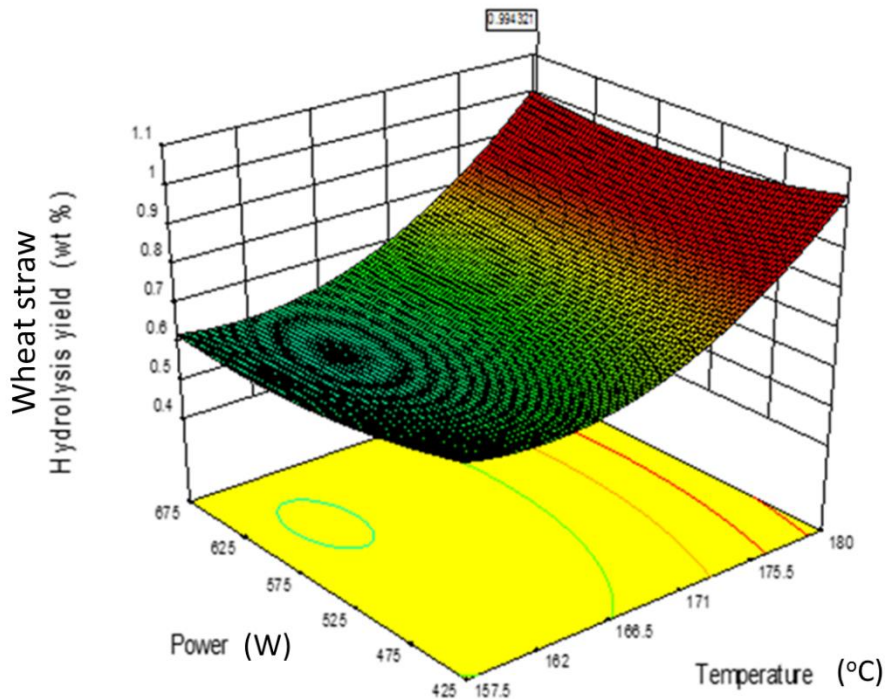


Figure 6 The 3-D representation of the effect of temperature and microwave power on the potential of maximization of wheat straw hydrolysis yield (wt%).

The impact of these trends on time showed that pre-treatment time is relatively shorter for experiments where higher temperature, higher concentration or higher microwave power were selected as optimum process conditions (**Figures 7-8**). This is a key area of interest especially when conventional alkali methods of pre-treatment utilise heating by conduction which takes several hours to achieve the pre-treatment of lignocellulosic biomass. Intanakul, Krairiksh and Kitchaiya [37] reported attractive yields from microwave assisted pre-treatment of wheat straw using glycerine at microwave power of 240W and temperatures of 200°C for 10min. Zhu et al. [20] also reported higher separation levels of lignin and improved hydrolysis process of wheat straw at 700W and 25min compared to conventional alkali pre-treatment carried out at 60min.

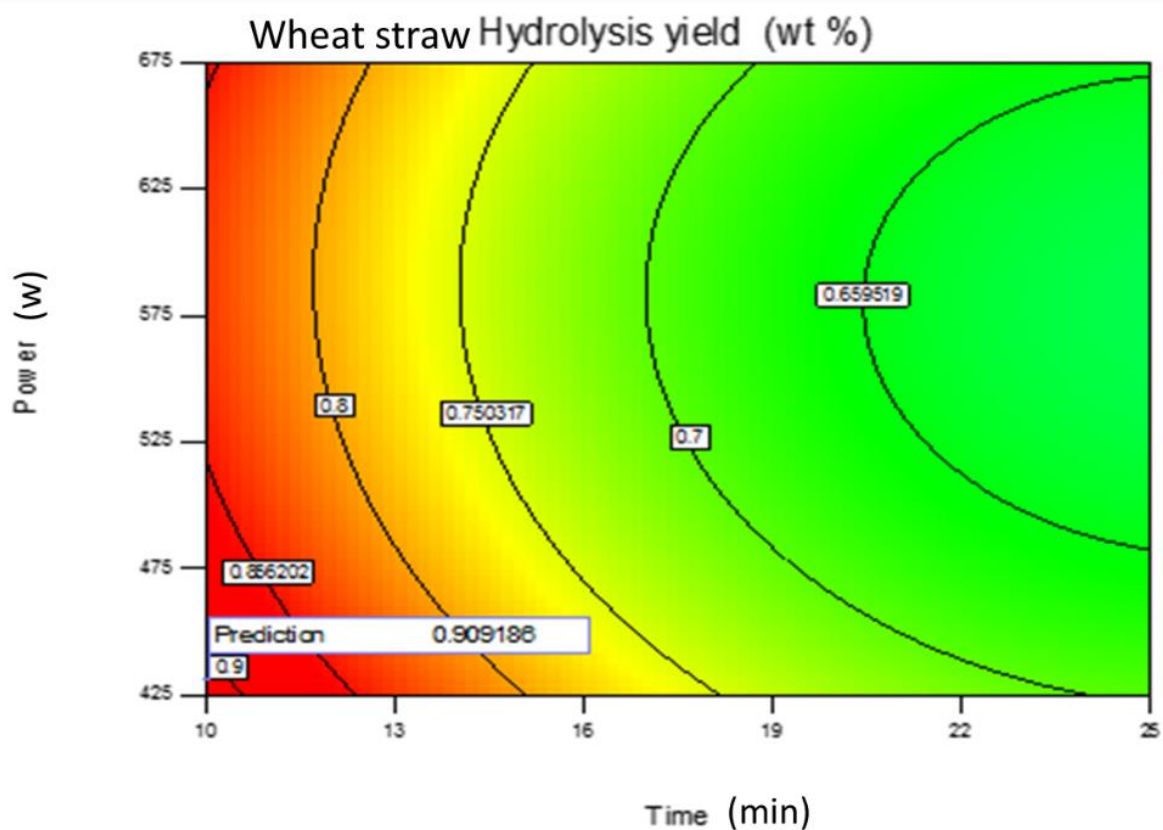


Figure 7 Effect of the microwave power (W) and the irradiation time (min) on the potential of maximization of wheat straw hydrolysis yield (wt%).

The increased yields at elevated temperatures can be attributed to the effective solubilisation of lignin and hemicellulose, thus increasing the degree of delignification and improved digestibility for enzymatic hydrolysis. Compared to conventional heating, perhaps the effect of the microwave is to quickly excite the polar components of the reaction mixture, which in turn transfers this thermal energy to the biomass/alkali mixture and overcoming the energy barrier required for the disruption and severing of bonds.

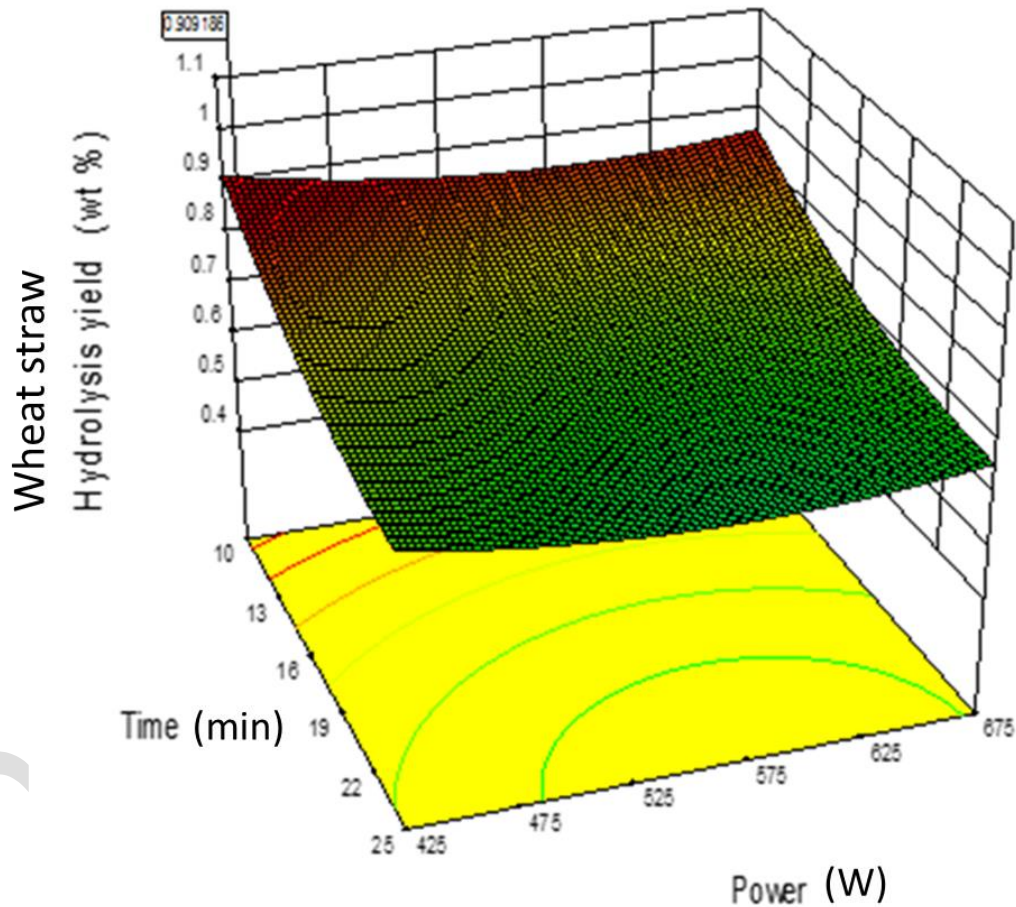


Figure 8 The 3-D representation of the effect of time and microwave power on the potential of the maximization of wheat straw hydrolysis yield (wt%).

The results obtained indicate that microwave assisted heating is better than conventional alkaline methods as well being able to impact positively on the hydrolysis yield, however the mechanism of the impact of the microwave towards reducing the pre-treatment time is considered an area for further study to be able to make the most of the method.

Whilst the efficiency of the enzymatic hydrolysis is dependent on the degree of pre-treatment, it is worth mentioning that other key factors pertaining to the hydrolysis like enzyme loading, pH, incubation temperature and time as well as microbial attack which can render the enzymes inactive, all playing a part in determining the yield from hydrolysis. Also, the mechanism of enzyme reactions are complex and not easily explained by simple reaction pathways which can further complicate the process of building a direct correlation between the variables chosen for designing the pre-treatment experiments and the hydrolysis yield.

Optimization of the response based on the model obtained indicated that a 94 wt% hydrolysis yield can be obtained in less than 15min if the pre-treatment conditions are 177°C, 396W and 0.85M of NaOH.

4 Conclusion

Experimental results obtained indicated that the microwave-assisted pre-treatment of wheat straw led to better results compared to the conventional pre-treatment of wheat straw as increased hydrolysis yield by removing lignin and hemicellulose, thereby increasing the digestibility of the straw, reducing the cellulose crystallinity and increasing the accessibility for more efficient enzymatic hydrolysis. The key process parameters were the temperature, alkali concentration and microwave power while the pre-treatment time depended on the combined effect and severity of these process parameters. Higher temperatures were required at moderate microwave power and dilute alkali concentrations to effectively pre-treat straw in shorter time than the conventional method in order to maximise the cellulose extraction, a valuable precursor towards fermentable sugars.

The fermentable sugars yield from enzymatic hydrolysis of pre-treated wheat straw reflected the impact of the pre-treatment conditions and how much sugars were acquired from wheat straw. Although there are other factors such as enzyme loading, incubation temperature and pH which also influence the enzymatic hydrolysis, microwave-assisted alkaline pre-treatment has the potential to achieve the goals of obtaining high yields of fermentable sugars from lignocellulosic biomass like wheat straw, which gave a maximum yield of 87% wt. of reducing sugars from wheat straw pre-treated at 180°C, 550W of microwave power, with 0.65M of NaOH for 25min.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

- [1] Zhang, Z.; Zhao, Z. Microwave-assisted conversion of lignocellulosic biomass into furans in ionic liquid. *Bioresource Technology*, 2010, 101(3), 1111-1114.
- [2] Basu, P. *Biomass Gasification, Pyrolysis and Torrefaction: Practical Design and Theory*, Elsevier, 2013.
- [3] Kumar, P.; Barrett, D.; Delwiche, M.; Stroeve, P. Methods for Pre-treatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production. *Industrial & Engineering Chemistry Research*, 2009, 48(8), 3713-3729.
- [4] Sun, Y.; Cheng, J. Hydrolysis of lignocellulosic materials for ethanol production: A review. *Bioresource Technology*, 2002, 83(1), 1-11.
- [5] Hu, Z. and Wen, Z. Enhancing enzymatic digestibility of switchgrass by microwave-assisted alkali pre-treatment. *Biochemical Engineering Journal*, 2008, 38(3), 369-378.
- [6] Park, N.; Kim, H.; Koo, B.; Yeo, H. ; Choi, I. Organosolv pre-treatment with various catalysts for enhancing enzymatic hydrolysis of pitch pine (*Pinus rigida*). *Bioresource Technology*, 2010, 101(18), 7046-7053.
- [7] Hendriks, A.; and Zeeman, G. Pre-treatment to enhance the digestibility of lignocellulosic biomass. *Bioresource Technology*, 2009, 100(1), 10-18.
- [8] Saha, B.; Iten, L.; Cotta, M.; Wu, Y. Dilute acid pre-treatment, enzymatic saccharification and fermentation of wheat straw to ethanol. *Process Biochemistry*, 2005, 40(12), 3693-3700.
- [9] Beguin, P. The biological degradation of cellulose. *FEMS Microbiology Reviews*, 1994, 13(1), 25-58.
- [10] DEFRA-Department of Environment Food and Rural Affairs, 2014. Available online at: <https://www.gov.uk/government/organisations/department-for-environment-food-rural-affairs> [Last accessed on: 11.07.2017].
- [11] Unal, H.; Alibas, K. Agricultural Residues as Biomass Energy. *Energy Sources, Part B: Economics, Planning, and Policy*, 2007, 2(2), 123-140.
- [12] European Bioenergy Networks (2003) EUBionet Biomass survey in Europe Country report of Greece. Available online at: http://www.afbnet.vtt.fi/greece_biosurvey.pdf [Accessed on October 2017]
- [13] Kim, S., Dale, B.E Global potential for bioethanol production from wasted crops and crop residues. *Biomass and Bioenergy* , 2004, 66, 361-375
- [14] DataBase Phyllis2, Database for biomass and waste, Available online at: <https://www.ecn.nl/phyllis2/> [Accessed on October 2017]
- [15] Bidlack, J, Malone, M., Benson, R. Molecular structure and component integration of secondary cell walls in plants *Proc. Okla. Acad. Sci.*, 1992, 72, 51-56
- [16] Mohan, D.; C.U.P.; Steele P.H. Pyrolysis of Wood/Biomass for Bio-oil: A Critical Review. *Energy Fuels*, 2006, 20, 848-889.
- [17] Clark, D.; Deswarte, F., (2008). *Introduction to chemicals from biomass*, John Wiley & Sons, Ltd. ISBN: 978-0-470-05805-3
- [18] Alonso, D.; Bond, J.; Dumesic, J. Catalytic conversion of biomass to biofuels. *Green Chemistry*, 2010, 12(9), 1493 – 1513.
- [19] Hamelinck, C.; Hooijdonk, G; Faaij, A. Ethanol from lignocellulosic biomass: techno-economic performance in short-, middle- and long-term. *Biomass and Bioenergy*, 2005, 28(4), 384-410.
- [20] Zhu, S.; Wu, Y.; Yu, Z.; Chen, Q.; Wu, G.; Yu, F.; Wang, C.; Jin, S. Microwave-assisted Alkali Pre-treatment of Wheat Straw and its Enzymatic Hydrolysis. *Biosystems Engineering*, 2006, 94(3), 437-442.

- [21] Merrill, M.; Baker, A.; Satter, L. (1976). Physical and chemical pre-treatment for enhancing cellulose saccharification. *Biotechnol. Bioeng. Symp.*; (United States), Vol 6 Dept. of Agriculture, Madison, WI.
- [22] Li, J.; Henriksson, G.; Gellerstedt, G. Lignin de-polymerization/re-polymerization and its critical role for delignification of aspen wood by steam explosion. *Bioresource Technology*, 2007, 98(16), 3061-3068.
- [23] Binod, P.; Sindhu, R.; Singhanian, R.; Vikram, S.; Devi, L.; Nagalakshmi, S.; Kurien, N.; Sukumaran, R.; Pandey, A. Bioethanol production from rice straw: An overview. *Bioresource Technology*, 2010, 101(13), 4767-4774.
- [24] Kashaninejad, M.; Tabil, L. Effect of microwave–chemical pre-treatment on compression characteristics of biomass grinds. *Biosystems Engineering*, 2011, 108(1), 36-45.
- [25] Bian, J., Peng, P., Peng, F., Xiao, X.; Xu, F.; Sun, R. Microwave-assisted acid hydrolysis to produce xylooligosaccharides from sugarcane bagasse hemicelluloses. *Food Chemistry*, 2014, 156, 7-13.
- [26] Demirhan, H.; Ahmad Zul Izzi Fauzi; Skoulou, V.; Haywood, S.K.; Sharif H. Zein. Wheat Straw bio-refining. Part I: Optimization of the Microwave Radiation **Process** with Sulphuric Acid Pre-treatment. *Current Microwave Chemistry*, 2017, 4(3), 205-218.
- [27] Maitan-Alfenas, G.; Visser, E.; Guimarães, V. Enzymatic hydrolysis of lignocellulosic biomass: Converting food waste in valuable products. *Current Opinion in Food Science*, 2015, 1, 44-49.
- [28] Miller, G. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Analytical Chemistry*, 1956, 31(3), 426-428.
- [29] Gierer, J. (1985). Chemistry of delignification, part 1. General concept and reactions during pulping. *Wood Sci. Technol.*, 19(4), pp.289-312.
- [30] Ternrud, I.; Lindberg, J.; Theandera, O. Continuous changes in straw carbohydrate digestion and composition along the gastrointestinal tract of ruminants. *Journal of the Science of Food and Agriculture*, 1987, 41(4), 315-324.
- [31] Jackson, M. The alkali treatment of straws. *Animal Feed Science and Technology*, 1977, 2(2), 105-130.
- [32] Sun, R.; Lawther, J.; Banks, W. Influence of alkaline pre-treatment on the cell wall components of wheat straw. *Industrial Crops and Products*, 1995, 4(2), 127-145.
- [33] Kellner, R. (1998). *Analytical chemistry*. Weinheim [Federal Republic of Germany]: Wiley-VCH.
- [34] Binod, P.; Satyanagalakshmi, K.; Sindhu, R.; Janu, K., Sukumaran, R.; Pandey, A. Short duration microwave assisted pretreatment enhances the enzymatic saccharification and fermentable sugar yield from sugarcane bagasse. *Renewable Energy*, 2012, 37(1), 109-116
- [35] Alemdar, A., Sain, M., Isolation and characterization of nanofibers from agricultural residues – Wheat straw and soy hulls. *Bioresource Technology*. 2008, 99 (6) 1664-1671
- [36] Jonoobi, M.; Harun, J.; Shakeri, A.; Misra, M.; Oksman, K. Chemical composition, crystallinity and thermal degradation of bleached and unbleached Kenaf Bast (*Hibiscus Cannabinus*) pulp and nanofibers. *Bioresources*, 2009, 4(2), 626-639.
- [37] Intanakul, P.; Krairiksh, M.; Kitchaiya, P. Enhancement of Enzymatic Hydrolysis of Lignocellulosic Wastes by Microwave Pre-treatment under Atmospheric Pressure. *Journal of Wood Chemistry and Technology*, 2003, 23(2), 217-225.