JPIChE 50 (1) 2022: 01-20

Journal of Pakistan Institute of Chemical Engineers

Since 1969

Journal homepage: www.piche.org.pk/journal

DOI: https://doi.org/10.54693/piche.05011

Check for updates

# Strategic Lignin Removal from Lignocellulosic Matrix of Wheat Straw Using Selectivity Characteristics of Ozone.

*K.S. Baig[1*], K.S. Quraishi[2], A. Farrukh[1], N. Mehmood[1]*

*Submitted: 26/10/2021, Accepted: 30/03/2022, Online: 31/03/2022*

**Abstract**

*Current study was planned to investigate the ozone selectivity for lignin in wheat straw delignification process under controlled conditions. Operating variables such as ozone concentration, reaction time of ozone with wheat straw and flow rate of ozone were explored and the focus was to prevent depolymerization of cellulose in the lignocellulosic matrix. The novelty in the technique is the strategic use of operating parameters to get maximum lignin removal and least cellulose damage in wheat straw matrix. The removal of lignin was maximum till the first 60 minutes for the ozone flowrates of 4 L $min^{-1}$, 2 L $min^{-1}$ and 1 L $min^{-1}$ of reaction. Further increase in reaction time above 60 min made the process less selective. The experimental work performed on the delignification of agricultural waste by using ozone is very rare and in addition to this, the literature about delignification of wheat straw by ozone is almost none. Results support the idea that the lignin can both sustain and restrict the disintegration of carbohydrates. The flow rate of 2L $min^{-1}$ with a contact time of 60 min is recommended for optimum removal of 85% of lignin.*

**Keywords:** Ozone, Lignin, Lignocellulose, Ozonolysis, Wheat Straw

## 1. Introduction:

The enthusiasm in developing alternative forms of energy is burgeoning due to bilateral perquisites in the field of economy and environment. Since the effect of effluents, namely crude oil, on the eco-system, the turbulence in the prices of fuel and because of raising health concerns, the idea of further refinement in the sources of energy is further adjoined [1,2] The production of fuels from fossil-based resources is appeared to be unsustainable because of depletion of these resources [3] As a renewable, abundant non-edible raw material, lignocellulosic biomass has garnered more and more attention over the last few decades. It widely exists in woody plant, herbaceous plant, aquatic plant, forestry and agricultural residues, industrial and municipal solid waste, and so on, and numerous studies have shown that lignocellulose could be converted to biofuels and value-added chemicals and materials in biorefinery process.

Lignocellulosic biomass is mainly consisting of cellulose, hemicelluloses and lignin. The quantity of these constituents varies with plant to plant. Cellulose is the main substance in the biomass structure [4] Cellulose is made from polymeric chains which are associated with one another and form strong bonds with neighboring chains [5] Lignin is a three-dimensional polymeric material, it

[1]*Department of Chemical Engineering, Wah Engineering College, University of Wah, Quaid Avenue, Wah Cantt, Pakistan.*
[2]*Department of Chemical Engineering, Pakistan Institute of Engineering and Applied Sciences (PIEAS), Nilore, Islamabad, Pakistan.*

**Corresponding Author:** khurram.shahzad@wecuw.edu.pk

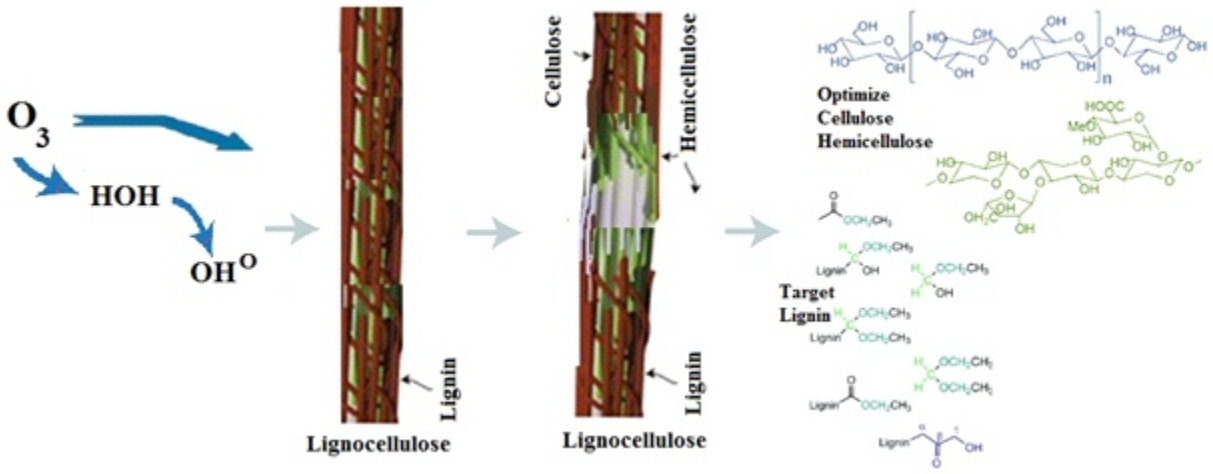



**Figure 1:** Lignocellulose Delignification

bonds the fibrous material such as cellulose and hemicellulose, and it is also distributed within the fibers [6] . Lignin is largely responsible for the protection of the cellulose [7] Hemicellulose is a polysaccharide which is a component of cell walls making biomass [8]. Several approaches have been made for the efficient lignin removal. The effectivity of an approach is mainly based on the maximum removal of lignin and less degradation of hemicellulose or cellulose [912] Lignin is an irregular macromolecule of phenylpropane units [6]. Its monomeric composition depends strongly on the plant species from where it is obtained [13]. Carrying out reactions on woody substances is like the reaction on any other solid polymer surface but the difference is that the polymers are homogeneous but woody surfaces are not [14]. The problem in these reactions is the choice of suitable reagent which can transport into the not well distributed throughout the woody matrix [15]. Therefore, non-homogenous reaction surfaces generally resulted in the products (such as 3-hydroxy-4-methoxybenzoic acid, 4-hydroxy-3-methoxybenzoic acid, 2 Butanone, etc) of varying or useless properties due to less efficient reactions.

Ozone is a strong oxidizing agent which has electrophilic and nucleophilic sites. Selectivity is the ratio of the rate of reaction with lignin to the rate of reaction with carbohydrates. Selectivity of ozone favored the attack of ozone on lignin in opposition to carbohydrates [16,17]. Under various ozone reaction conditions, the rate of reaction with carbohydrates determines the selectivity while the ozone was free to react with carbohydrates or lignin or by products or with the free radicals. Colodette *et al* (1993) investigated the ozonation as a delignification agent [18]. Southern pine Kraft pulp was delignified and they found that there was a selectivity problem associated with the ozonation reaction [Rosen et al, 2019]. It seemed that the non-selective behavior was the problem because it promoted the oxidation of all organic materials present [19]

In the study of bleaching of pulp, Gierere and Zhang (1993) pointed out that some radicals were formed during the reactions of ozone with pulp. The radicals formed during reaction, reacted with lignin and carbohydrates, rapidly[20]. Ozone reacted with organic substances by addition reactions, and it break the olefinic and activated aromatic bonds. A group of researchers considered it a main pathway chemical reaction for ozonation of lignin analogous compounds when the reaction medium is non-aqueous [2125] In aqueous reaction system, ozone might eliminate an electron from the substratum such as phenolates [23,26] and the hydroxyl radical are formed as the result of electron transfer[27]. In the other reaction path, a superoxide radical might be created with reacting ozone. During ozonolysis, the superoxide radicals are converted into hydroxyl radicals [28] or the other way around [29]. It could be argued to decide which one of the radicals were formed first.

In order to investigate the causes of the cellulose degradation during ozonation of pulp, Kang *et al*, (1995) studied three lignin-carbohydrate model systems [30] . The three model compounds used were: i) methyl β-D-glucopyranoside ($C_7H_{14}O_6$), ii) dextrin $(C_6H_{10}O_5)_n$, and iii) a bleached kraft pulps. From the ozonation of lignin analogous chemical substances, it was observed that the lignin is capable to support and confine the disintegration of the carbohydrates. The protective effect of the lignin compounds was due to the competition with the carbohydrates for the ozone [31,32] It was observed that additional hydroxyl radicals were created during the ozonation of lignin than the self-degradation of ozone [30,33] The degradation of carbohydrate is enhanced because of the easier creation of hydroxyl radicals which is only possible due to its phenolic structures. The study of radical formation during the reactions of ozone with lignin and carbohydrates have shown that the primary product of reaction turns into the secondary ones by further reaction with ozone [3335]. The disintegration of cellulose during reaction of ozone with kraft pulp was investigated by using lignin-carbohydrate model systems and it was observed that the free phenolic hydroxyl groups in the presence of lignin model compounds promote the disintegration of cellobiose [36,37]. The accelerated degradation continued until the lignin was completely used up by the available ozone. In contrast, the etherified phenolic hydroxyl groups in the presence of lignin model compounds retarded the degradation of cellobiose. Phenolic hydroxyl groups formed during the reaction of ozone and lignin, in further reactions produced hydroxyl radicals. The hydroxyl radicals have accelerated the degradation of cellobiose during the ozonation in aqueous medium. Lind *et al*. (1997) advocated that ozone in its molecular form ($O_3$) contributed to degradation of up to 50% of the polysaccharides in reaction with pulp [38]. Ragnar *et al*. 1999 claimed that molecular ozone ($O_3$) was very selective [39]. This unsettled situation became a problem for the researchers to decide which radical should be removed for an optimum delignification reaction.

The experimental work for delignification of agricultural waste by using ozone is very rare in the literature and literature about delignification of wheat straw by using is almost none. The present work elaborates on main aspects of above debate whether ozone degrade polysaccharides or not. The author hypothesized that lignin and cellulose are not uniformly distributed in wheat straw matrix. The contribution of super oxide radicals and/or hydroxyl ions in delignification with ozone was also elucidated. The major drive of this research was to study the selectivity of ozone in the procedure of lignin removal from the lignocellulosic matrix of wheat straw. As Canada is one of the leading countries among the producers of wheat, so, the availability of wheat straw cannot be a problem [40,41] The study was accomplished by i) conducting vigorous literature survey, ii) performing experiments, and ii) monitoring reaction conditions, such as reaction time, ozone concentration and flow rate. The results obtained were analyzed in the perspective of selectivity and from the kinetic point of view.

## 2. Materials and Methods:

### 2.1 Plant Material:

Wheat straw (*Tritium sativum*) is a discarded lignocellulosic material. For this study wheat straw was taken from an acreage situated in the north of Toronto city. The dust and clay particles were removed from straw by gentle palpates. Then straw was milled to reduce size by Retsch Inc., Philadelphia, USA. After size reduction, the wheat straw was sieved to get the pre decided mandatory sizes. The moisture constituent of the wheat straw was calculated using LAP-001 method adopted from the National Renewable Energy laboratory (NREL)[42] and values were 1.6-1.8 %.

### 2.2 Sample Formulation:

This study is based on a technique which author has proposed as a pretreatment for effective delignification. The proposed pretreatment is mainly based on exploiting physical and chemical properties of wheat straw and water. This proposed treatment worked in a two steps procedure:

In the first step, the oven dried samples were immersed in 1% NaOH solution for a time of 24 hours. So that the natural wax and proteinic

coatings from the wheat straw surface could be removed [43] This step was necessary otherwise there would be very little reaction between solid state wheat straw and ozone because of coated protection of wheat straw surface. Then they were filtered, neutralized with deionized water. Samples were oven dried in an oven at 105 °C, made by Labline Inc., USA, till a consistency in weight was observed.

In the next step, a specific amount of water was sprayed to moist the dried sample. The amount and mode of the application of moisture has been given in detail in our other study. Then the samples were wrapped in aluminum foil, placed in covered plastic dishes, further wrapped in cellophane and placed at 4 °C to avoid moisture loss for 24 h. The moistures were allowed to be absorbed into the wheat straw which increased the surface area of wheat straw by swelling. This step increased the active sites for reaction with ozone. Then samples were subjected to ozonation for specific time after preparation

**2.3 Ozone Generation and Exposure:**

Pure oxygen was used to generate ozone using an ozone generator, PC1-WEDCO, Model GL-1, West Caldwell, New Jersey, USA. The generator was designed to produce ozone in the concentration range of 13.1 to 65.5 mg/L in ozone-oxygen stream. Linde Canada Limited supplied oxygen in cylinders to use in the generator.

Various kinds of reactors were used to provide interaction between flowing ozone streams through wheat straw. One of the reactors was having the walls of clear PVC, the particles of wheat straw turned into charged particles on reaction with ozone, attaching with the walls of PVC reactor, the reaction with ozone was not adequate, and in addition the wastage of material was unbearable. The most satisfactory reactor having a stainless-steel reaction chamber was a fluidized bed column reactor of 3.5 cm x 25 cm size. Control valves were there to control the in and out flows of ozone through the reactor. At first, passing through a diffuser, ozone entered in the reaction chamber with a homogenous distribution where it interacts with wheat straw uniformly. The amount of incoming ozone was measured with spectrophotometrically (at 257 nm).

**2.4 Potassium Iodide Method:**

Unreacted ozone in the effluents was collected in an iodide trap (gas absorber) that was containing 0.2 M potassium iodide solution, placed in ice jacket. The effluent gas was bubbled through it. The reaction between ozone and potassium iodide solution is:

$$O_3 + 2KI + H_2O \qquad O_2 + I_2 + 2KOH \qquad (1)$$

Ozone trap relying on this reaction has been used in several research works and was found effective [4448]

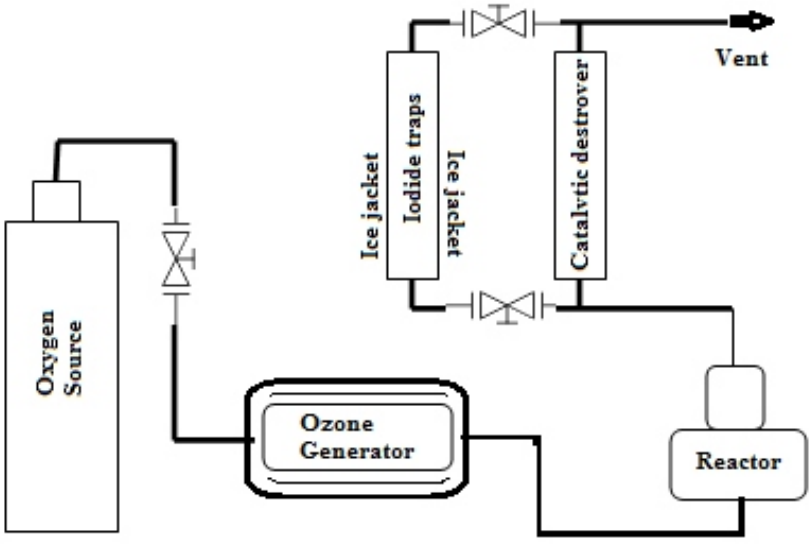



**Figure 2:** Set up for the detection of ozone consumption [49].

Then the amount of ozone was determined by titrating the liberated iodine in excess of potassium iodide solution with standard sodium thiosulphate solution, using phenolphthalein as indicator:

$$I_2 + 2S_2O_3^{2-} \rightarrow 2I^- + S_4O_6^{2-} \quad (2)$$

One mole of iodine reacts with two moles of thiosulphate and we know that one mole of iodine is produced by one mole of ozone.

**2.5 Detection of Lignin:**

The hydrolysis of the ozonated samples was conducted in 15 ml of 72% $H_2SO_4$ for the contact time of 20 min at 4 $^oC$, then 2 h at 22 $^oC$, and afterwards boiled for 4 h in 3 % $H_2SO_4$. During room temperature hydrolysis, mixing at every 15 min is important to have homogenous contact of acid with particles (wheat straw), a less attention to the detail may affect results. After cooling, the solution was filtered by using glass filter. After three-stage hydrolysis with $H_2SO_4$, the samples were dried at 105 $^oC$ overnight. The calculated weight of dried solids agrees to the amount of acid-insoluble lignin plus the ash contents in the sample and it is described as Klason lignin. The weight of the ash contents was established by heating solids to 575 $^oC$ for 3 hours. The standard biomass protocol from National Renewable Energy Laboratory (NREL) was used to determine the amount of ash[50] . The Laboratory Analytical Procedure (LAP), LAP-003 of NREL was followed to determine acid insoluble lignin [51] and acid soluble lignin in wheat straw samples was evaluated by using LAP- 004 of NREL [51]. The UV / Visible Spectrophotometer was used to determine the concentration of acid soluble lignin in the filtrates, at 205 nm.

**2.6 Detection of Glucose:**

The residues of Klason lignin were used to calculate total holocellulose (Cellulose and hemicellulose) in the wheat straw sample. 150 ml of water was heated to 75°C and 5 grams of the residues were added in it while keep stirring the solution. Then, 10 drops of CH3COOH (glacial) and 1.5 g $NaClO_2$ were added. Same number of reagents were added periodically after each hour till the total time was 4 hours. The suspended slurry was cooled, filtered and washed with distilled water, then with $C_3H_6O$ and dried at 105°C. The holocellulose is then estimated gravimetrically [52].

**3. Results and Discussion:**

The degree of delignification of wheat straw lignin was determined at various flow rates of ozone ranging from 1L/min to 4L/min at 20 °C and 1 atm. The experimental results obtained during this study are depicted in Figure 2. Each point obtained was an average of the experiments conducted at triplicate.

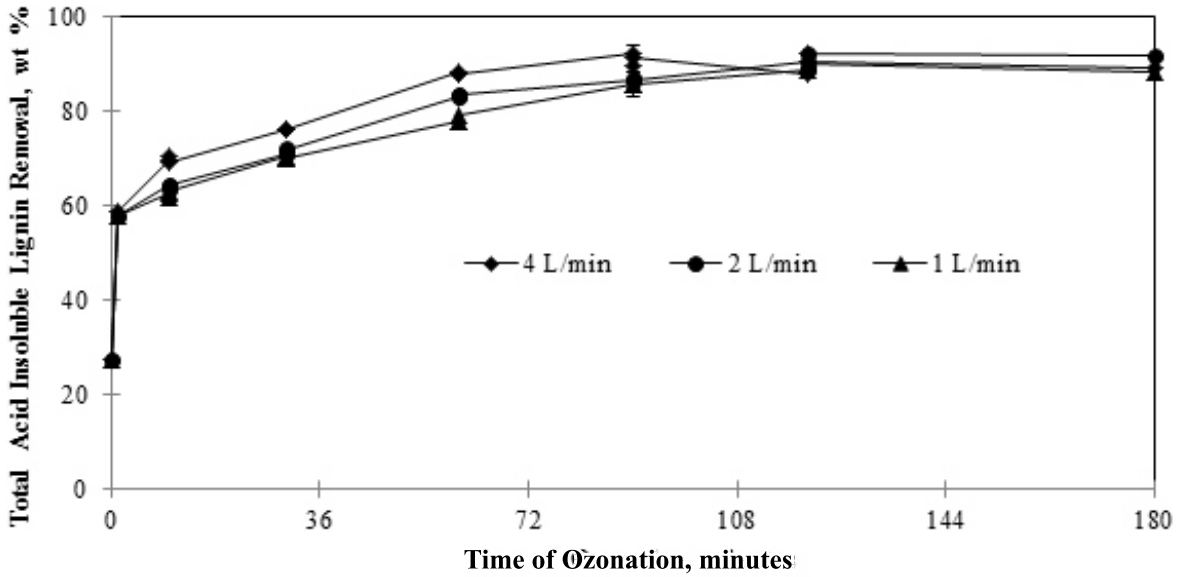



**Figure 3:** Lignin removed as a function of time of reaction [50]

The delignification was fast in the start; it increased as the reaction proceeded. For the experiments conducted at 4L/min the curve started leveling off after 60 min of the reaction, for 2L/min this stage arrived at 90 minutes and for 1L/min this stage was arrived late i.e., 120 minutes of the ozonation. Initially the reaction was very fast due to the open availability of ozone for every reaction site, with the passage of time the sites after reaction with ozone were not available for further reaction. Hence, ozone travelled through pores and move through the sites to proceed as intra-particular reaction. The occupancy of sites, hindrances to transport for ozone from used sites to the next ones and the formation of reaction products caused the reaction rates to decrease. So, there was a decrease of reaction with increase in reaction time.

In this study, ozonolysis was carried out at ambient temperature and pressure to prove that it was a milder reaction. A separate experiment was performed for prolonged contact time (approximately 5 hours) to evaluate selectivity of ozone and it was observed that this oxidation process could be a risk of significant loss of organic matter such as hemicellulose and cellulose. Because 40 to 50 % material loss was measured. It occurred because the aromatic rings in lignocellulosic structure were broken down (primary products) and the primary products formed were further oxidized and gives secondary products. It was deduced that a part of the ozone started reacting with cellulose and hemicellulose constituents of wheat straw aside from lignin. Similar results were also reported by other groups of researchers [5355] [55,56,57]. Let's conceive the chemical reactions taken place during ozonation of wheat straw while supposing that ozone was not decomposed, and wheat straw was mainly composed of lignin and polysaccharides. Hence, Equation 3 is,

Ozone + Lignin Products (3)

Binder *et al*., 1980, reported that the products formed during ozonolysis were acetic acid, formic acid, formaldehyde and methanol. The dissolved organic carbons (DOC) were containing 20% of the products formed [53]. The most part of the products left behind were other types of carboxylic acids for example muconic acid ($C_6H_6O_4$) and oxalic acid ($C_2H_2O_4$) [55,57]. For simplicity suppose that all lignin is of phenyl propane type, therefore, Equation 3 can be modified as Equation 4: A

$$4O_3 + C_9H_{12} \quad C_2H_2O_4 + C_6H_6O_4 + 2H_2O + CO_2 \qquad (4)$$

As the delignification reaction of ozone proceeds, the exposed cellulose fibers may be broken down into many short cellulose chains [5860]. Let us suppose the reaction is as given in Equation 5:

Ozone + Polysaccharides Products (5)

The ozonation influence the degradation of polysaccharides in two (2) opposite pathways: i) the creation of small chains of cellulose, which enhanced the delignification, ii) the generation of lactones which showed the adverse effect [61]. Suppose all polysaccharides are in the form of glucose, then the ozonation reaction would convert it to Gluconic acid and Glucuronic acid like Equation 6:

$$30_3 + 4C_6H_{12}C_6 \quad C_6H_{12}O_7 + C_6H_{10}O_6 + 3H_2O + C_6H_{10}O_7 + C_6H_{10}O_7 + 30_2 \qquad (6)$$

Water formed during the reactions and the water present as moisture reacted with ozone and produce hydroxyl, peroxide and superoxide radicals as in Equation 7:

$$0_3 + H_20 \quad O_2^{0-} + 20H \qquad (7)$$

The reaction between hydroxide ions and ozone lead to the formation of one superoxide anion radical and one hydroperoxyl radical [62,63] as given in Equation 8:

$$0_3 + 0H^- \quad O_2^{0-} + Ho_2^0 \qquad (8)$$

The $HO_2^{\circ}$ radical formed could react with ozone and generate Oh°radicals, the oxidizing power of $OH^{\circ}$ radical is 2.72 V in acidic vehicles and 2.32 V in neutral vehicles [64], Equation 9.

$$0_3 + H_20 \quad 20_2 + 0H^0 \qquad (9)$$

A group of researchers [65] agreed with the results of this study that $OH^{\circ}$ radicals were produced during: (i) decomposition of ozone which transform $OH^{-}$ ions [66], (ii) reaction of ozone molecule with lignin [67]

### 3.1 Kinetics of the reaction of ozone with wheat straw:

Kinetics of the reaction was studied, and it was observed that it follows pseudo second order model as given in the Figure 3. The ozonation process assumed to proceed in three steps, surface and diffusion reactions followed by intra-particular diffusion reactions, discussed in another publication by the author. In the present study, all the three reactions are represented by a single, pseudo kinetics second order rate constant k.

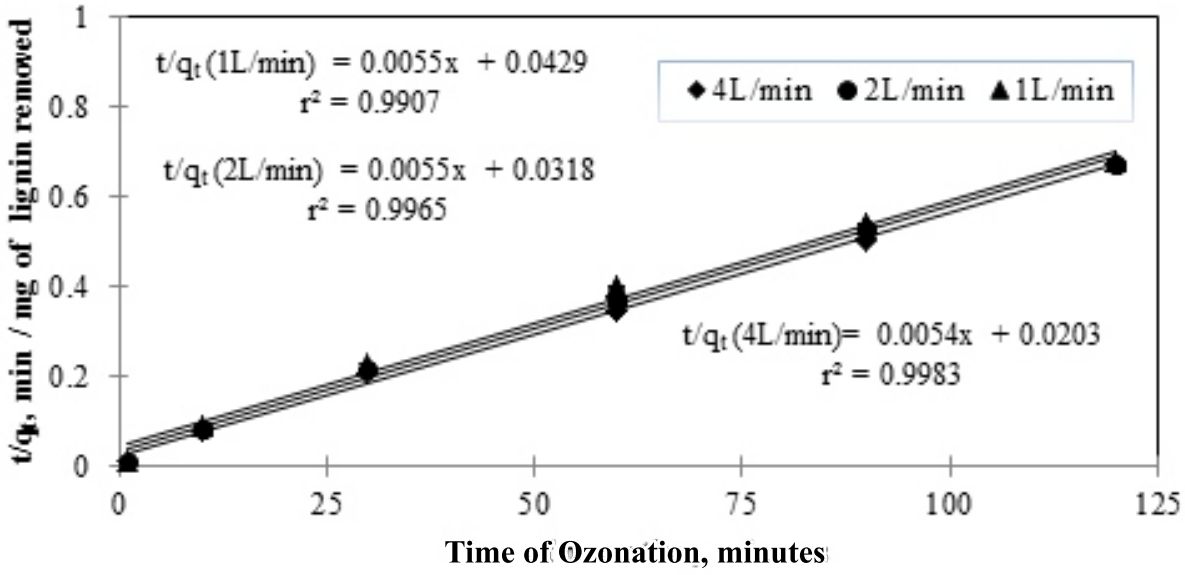



**Figure 4:** Lignin removal kinetics follow pseudo second order reaction.

The plot $t/q_t$ vs t for the ozonation kinetics of wheat straw for different flow rates of ozone, where '$q_t$' was the amount of lignin removed after time 't' of ozonation. The experimental data points obtained for all the flow rates ranging from 1 L/min to 4L/min at ozone concentration of 2%. The trend lines obtained through the plotted data points showed that the coefficient of determination was over 99%. It means that 99% variation in $t/q_t$ is due to variation in time of ozonation. The kinetic study conducted for ozonation of glucose present in the wheat straw also showed that it followed the pseudo second order model. In fact, the excessive supply of ozone during this reaction leads to chemical reaction, which follow pseudo second order reaction model. A graph of parameters '$t/q_t$' against 't' for the kinetics of ozonation of glucose present in wheat straw for different flow rates of ozone is given in the Figure 4. The degradation of glucose by reaction with ozone (between 2L/min to 4L/min) follow very close pattern. These higher flow rates of ozone support side reactions such as coupling of delignification radicals and further depolymerizing of degraded glucose fragments. The coefficient of determination for the glucose exposed as the result of ozonation of wheat straw were more than 99% which was a good representation of the reaction kinetics.

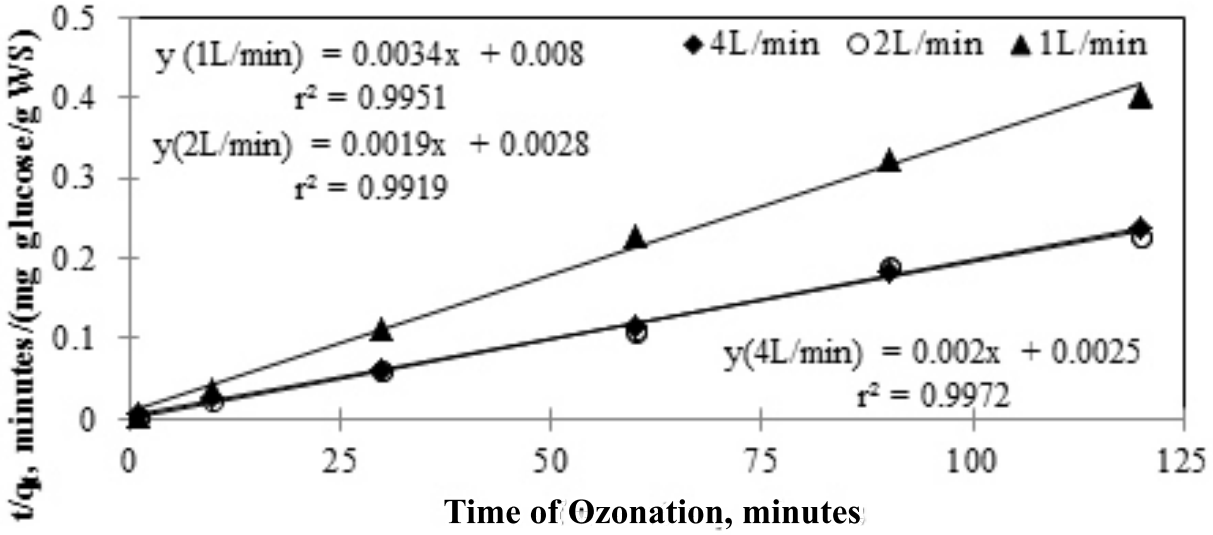



**Figure 5:** Glucose disintegration by ozone follow pseudo second order kinetics.

The higher $r^2$ values clearly suggested that the interaction of $O_3$ with lignin and glucose in the matrix of wheat straw was successfully represented by the proposed pseudo second order kinetic reaction model.

**3.2 Ozone concentration:**

In order to examine the influence of the [$O_3$] on the lignin removal process, ozone was introduced at various concentration of ozone in ozone-oxygen streams ranging from 1 wt% to 5 wt %. The amount of ozone was maintained for 60 minutes at a fixed flow rate of 2L/min and during this part of study. The results of this study are given in the Figure 6

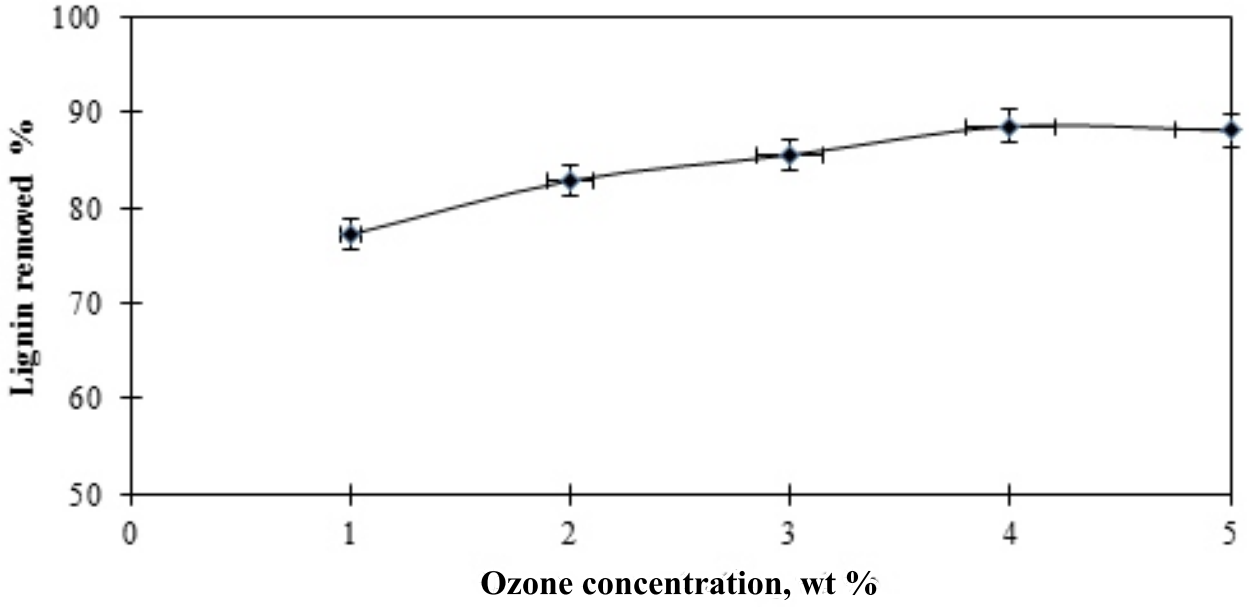



**Figure 6:** Percent of lignin removed as a function of the $O_3$ concentration in the $O_3$ - $O_2$ gas stream.

Increase in the ozone concentration [$O_3$] increases the rate of interaction according to the Le Chatelier's principle more of the reactant molecules '$O_3$' are present to generate the reaction products. The increase in ozone concentration provided more access to the substrate's reaction sites. In general, chemistry reactions as concentrations of reactants reached a high limit then further increase in the concentration has little effect on the rate of reaction. Same was the case here, increase in the [$O_3$] has a pronounced influence on the rate of lignin removal up to 4 wt % and further increase in ozone concentration has decreased delignification. The decrease in delignification can be attributed to the coupling of lignin phenoxy radicals (2,6-di4-butyl-4-methyl and 2,6-di-i-butylphenoxy, 2,4,6-tri-í-butylphenoxy) and formation of bigger lignin molecules [68]. The effect of reaction of ozone at various concentrations on the cellulose of wheat straw is given the Figure 6. The amount of lignin exposed by the reaction was almost the same for 1-2 wt % concentration of ozone. It started decreasing with increase in ozone concentration.

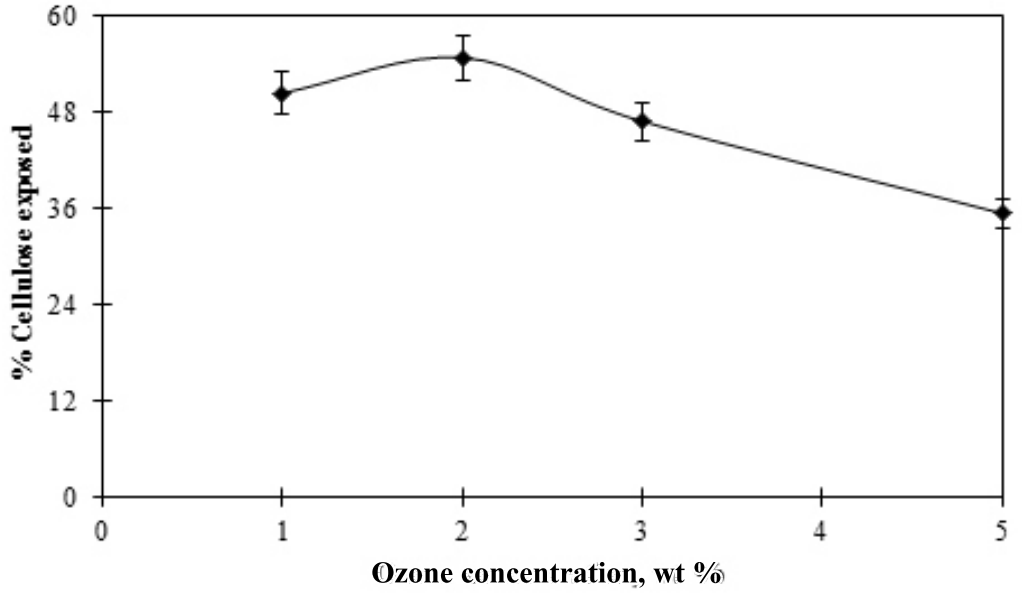



**Figure 7: Percent of cellulose exposed as a function of the [$O_3$] in the $O_3$ - $O_2$ gas stream.**

The Figure 6 and Figure 7 have shown considerable dissimilarities in the concentration profiles for lignin and cellulose degradation. The dissimilar concentration profiles lead to diverse reaction results. It is clear from the results that delignification was increased by increase in concentration and then it started decreasing. The same thing happened to cellulose degradation i.e., it increased initially then it started decreasing. The delignification was maximum between the ozone concentration of 4 wt % to 5 wt % while the degradation of glucose started with a higher value reached to its maximum at 2 wt % ozone concentration and further increase in concentration in the ozone decreased the degradation of cellulose. The increase in ozone concentration in ozone supply start contributing to secondary reactions because there was excess of ozone, limited reaction sites, and towards the prolonged reaction times the site were not accessible. Ozone is an oxidizing agent; its molecules reacted with other electron rich molecules for example double bond or aromatic groups present in wheat straw lignin. It has been observed in this study that ozone was not selective in its reactions because molecular ozone act as a dipole, as an electrophilic agent, and as a nucleophilic agent. However, during ozonolysis, the lignin was degraded, and the carbohydrates present in wheat straw were found degraded, as well. The ozone is not selective in oxidation reactions. The

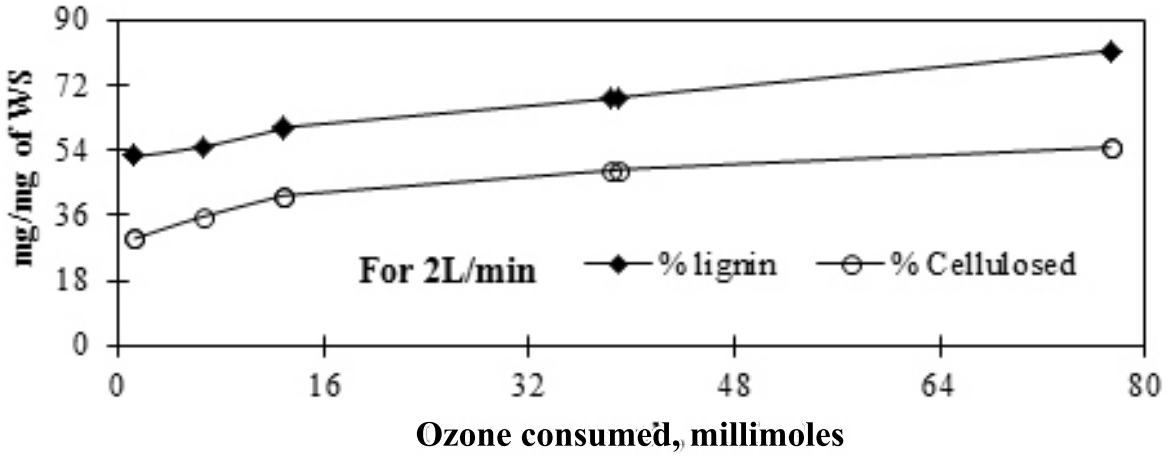
ozone ($O_3$) has a high oxidation potential value of 2.07 V which further increases with the presence of a catalyst $OH^{\circ}$ species to 2.8 V. This oxidation potential characteristic makes ozone more demanding candidate for reactions. The hydroxyl radicals ($OH^{\circ}$) produced by the reaction of ozone with water. The degradation of carbohydrates was due to the existing of hydroxyl radicals ($OH^{\circ}$) [6871]. In the presence of oxygen, the organic radical formed is generally further oxidized to yield a carbonyl group

$$R^{\circ} + O_2 \quad R_2 \qquad (10)$$

$$RO_2 \quad R_{ox} \quad {}_2 \qquad (11)$$

Hydroxyl and superoxide radicals are short-lived intermediate species formed during the delignification processes. Although they are look-alikes, the characteristics of these two radicals are quite different. The hydroxyl radical ($HO^{\circ}$) is denoted hydridooxygenyl according to modern IUPAC nomenclature, while the superoxide radical ($O_2^{\circ-}$) is named dioxideyl(1). Reitberger *et al* (1999) studied radical formation during ozonation in pulping process and reported that $O_2^{\circ-}$ (superoxide) has mainly reducing behavior, selective, diffusible, delignifying while $OH^{\circ-}$ (Hydroxyl) has strongly oxidizing nature, non-selective, non-diffusible, and partly delignifying [72] . The term *diffusible* reflects the fact that superoxide has a lifetime and can thereby move in a solution from its site of formation, whereas this is not the case for the extremely short-lived and reactive hydroxyl. Superoxide is to be considered delignifying due to its capability of ring-opening of aromatic rings, while hydroxyl is partly delignifying since it can hydroxylate aromatic rings. Superoxide can be protonated to hydroperoxyl (hydridodioxygenyl), $HO_2^{\circ}$ with a p*K*a-value of 4.88. Hydroxyl behaves as an acid with the corresponding base, the oxyl (oxide-$y_1$(1)), $O^{\circ-}$ The p*K*a-value of hydroxyl is 11.9. The half-life of ozone is about 30 minutes at standard temperature and pressure and $OH^{\circ-}$ has a half-life of $10^{-9}$ second. Hence, the $OH^{\circ}$ radicals are species that cannot move in the solution or diffuse in the fibers (unable to diffuse). Therefore, the $OH^{\circ}$ radicals react only in the place where they are generated. On the other way, the $O_2^{\circ-}$ radical penetrate the fiber [73]. Ragner (2000) stated his experience that the $O_2^{\circ-}$ radical was found selective, since it did not react with carbohydrates, or its reaction was negligibly small. It indicated that its existence was not a problem for the degradation of carbohydrates. On the other hand, the $O_2^{\circ-}$ radical diffuses inside the fiber, generate $OH^{\circ}$ radicals and disintegrate cellulose [34]. Therefore, the $O_2^{\circ-}$ radical increased the formation of $OH^{\circ}$ radicals and served as carrier of the $OH^{\circ}$ [73] into the cellulose fibers.

The Figure 8 explained that the ozonation reaction at a flow rate of 2L $min^{-1}$ delignification and cellulose degradation increased was dependent on the ozone consumption. At low flow rate (1L$min^{-1}$) the ozone was completely used up by delignification reaction while the reaction of cellulose degradation was almost the same throughout the reaction.



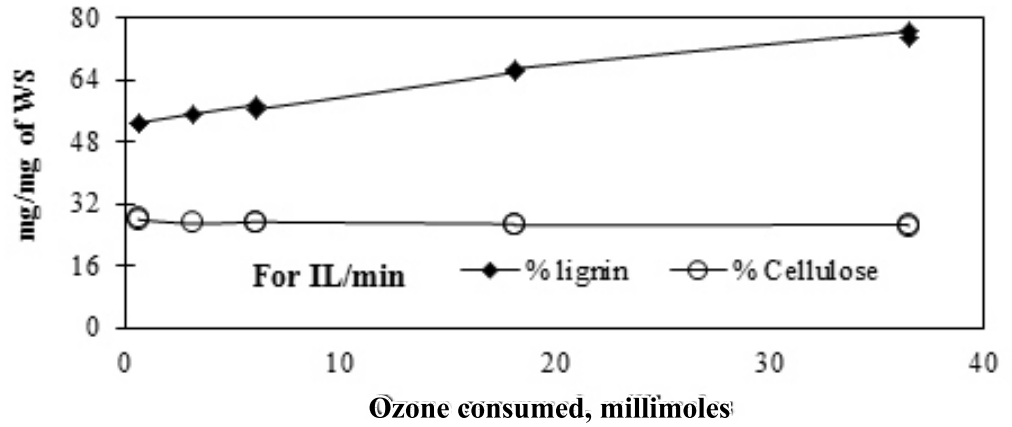



**Figure 8: Percent of lignin delignified and cellulose degraded as a function of ozone consumed.**

It proved author's hypotheses (mentioned in introduction para 6) that lignin and cellulose are not homogenously distributed in the wheat straw matrix. If lignin and cellulose are homogenously distributed in wheat straw the amount of lignin removed should be the same as the amount of cellulose exposed. Or at least in the proportion as they were constituted in wheat straw but the results obtained were different. It means that ozone came in reaction with lignin first. Ozone reacted at the available reaction sites of lignin and started moving towards cellulose where it is present in a sponge like structure. While at the same time ozone passed through the pores of wheat straw and started reacting with cellulose in the interior of the straw. As the rate of delignification decreased with time, it may be expected that at some point the rate of ozone consumption will decrease but it did not happen. This observation told author that cellulose is on the hit now. Mbachu and Manley, 1981, came across the similar situation when they were studying ozonation of spruce lignins in 45 % acetic acid solution [75]. The molecular weights of the dissolved products were measured and concluded that the weight-average molecular weights increased initially with time of ozonation but started decreasing after 15 minutes. It led to add another piece of knowledge that the soluble products were defragmented and further the defragmented products were using ozone as well. Another reason could be the formation of oxalic acid, which was formed as a result of delignification, and decreased the degradation of depolymerized glucose fragments [76,77. Figure 9 (below) is a comparison of amount of ozone supplied and the amount of ozone consumed during the ozonation process as the function of reaction time.

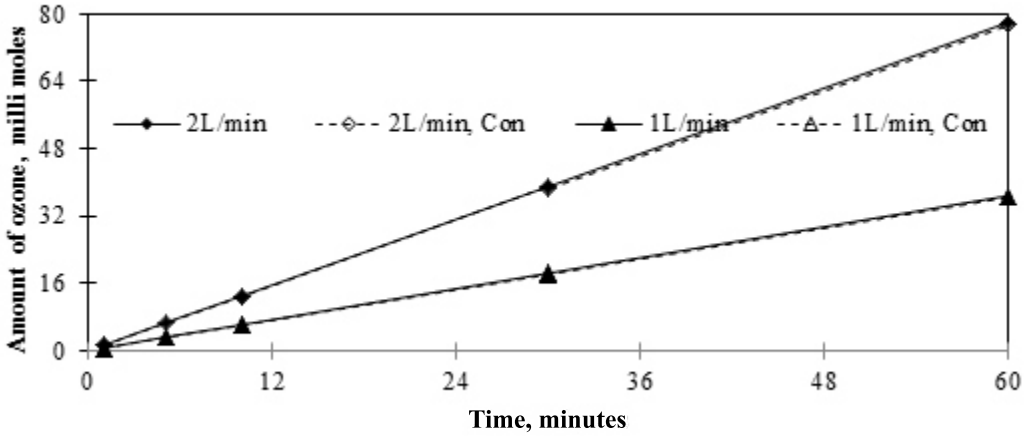



**Figure 9:** Ozone supplied and consumed as a function of reaction time.

It shows that the ozone consumption increases as the flow rate increases. At the large flow rate of ozone, large quantity of ozone was going into the reactor despite the fact higher amount of ozone gets involved in the secondary reactions with biomass.

Consider Figure 10 where the percent of delignification of the lignin present is plotted against the percent of cellulose exposed due to the ozonation reaction.

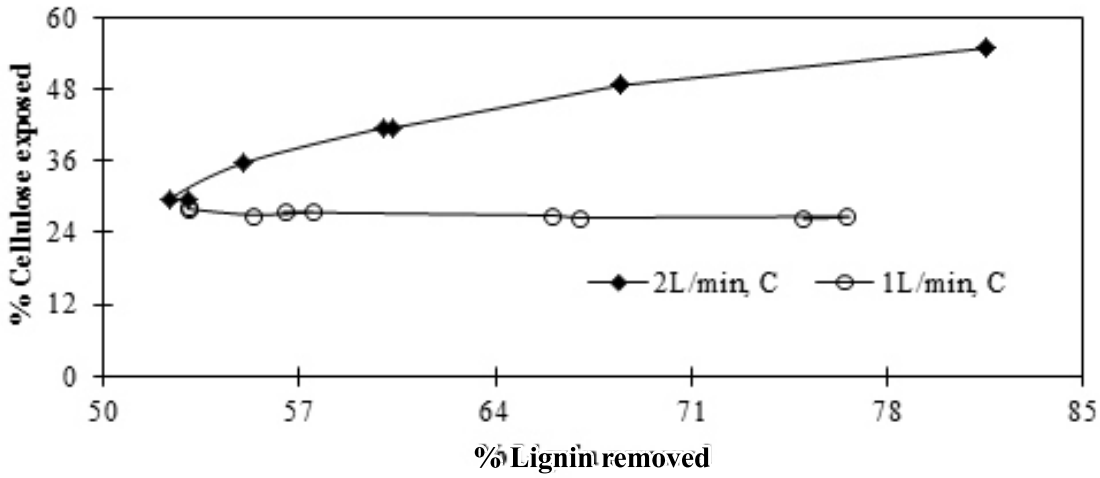



**Figure 10:** Cellulose exposed as a function of lignin removal.

According to the Figure 10 (above) the rate of delignification increased accompanied by same sharp increase in cellulose exposed for the first 5 minutes. After this quick reaction the amount of lignin removed is elevated further and the degree of delignification decreased and at the same time the cellulose degradation also decreased. The slope at the first two (2) points is 2.44 while it is decreased to 0.47 for the last two (2) point in the curve for the ozone flow rate of 2L/min, which means that there was a significant decrease in the degradation of cellulose. In other words, the degradation of cellulose was decreased to over one fifth of the delignification rate at the time of reaction. It can be implied that the reaction of ozone towards cellulose was hindered. The difference of the extent of reaction became very much clear at ozone flow rate of 1L/min when increase in ozonation is not serving any increase in degradation of cellulose which means reaction mechanism was not supporting reaction of ozone with cellulose. At low flow rates of ozone supply, there is no forced diffusion to the wheat straw metrics. Therefore, ozone reacts with the surface and lignin is being degraded while cellulose is not. This is another proof of hypothesis that lignin is mainly located at the surface of straw and it is not evenly distributed. The less degradation of cellulose can be attributed to: i) low direct accessibility of ozone to the cellulose sites, ii) protection of cellulose by the formation of reaction products such as oxalic acid, iii) the lignin can act both ways i.e., it can both support and restrain the degradation of the carbohydrate. Comparing with the results described by Ragnar *et al* 1999 for the produced radicals, it could be deduced that increase in delignification was due to the generation of hydroxyl radicals and slowing down due to oxalic acid.

### 3.4 Model of Selectivity Reactions:

Through literature survey and based on our experimentation it can be stated that ozone reacts with lignin and polysaccharides without any discrimination. The rate selectivity parameter for two parallel reactions can be defined as following [78] :

$$Selectivity = \frac{Rate\ constant\ for\ delignification}{rate\ constant\ for\ the\ degradation\ of\ cellulose} \quad (12)$$

The selectivity of ozonation reaction to the components of wheat straw can be defined by Equation 13:

$$S = \frac{rl}{rc} \quad (13)$$

Where $r_L$ is the rate of delignification and $r_C$ is the rate of degradation of cellulose. The rate of reactions can be illustrated as Equation 14 15:

$$r_L = \frac{dc_L}{dt} = k_L C_L \quad (14)$$

$$r_c = \frac{dc_c}{dt} = kc c_C^{\beta} \quad (15)$$

$dC_L$ / dt is the rate of change in lignin concentration with time. $dC_C$/dt is the rate of change in concentration of cellulose with time. $k_L$ is the rate constant for lignin and $k_C$ rate constant for cellulose. $C_L$is concentration of lignin and $C_C$ is the concentration of cellulose. Where and are concentration exponent in the corresponding rate laws. Therefore, from Equation 13 and 14 we can write:

$$S = \frac{dC_L}{dt} \Big/ \frac{dC_C}{dt} \rightarrow \quad (16)$$

The Equation 16 (model of selectivity) would be used in the discussion of selectivity of reactions of ozone in later part of the section results and discussion. In the Figure11, upper part shows the rate of delignification, and the lower part is about the rate of degradation of cellulose as a function of the time of Ozonation. The first observation was that $dC_L$/dt and $dC_G$/dt present almost the same pattern along the reaction with the reaction.

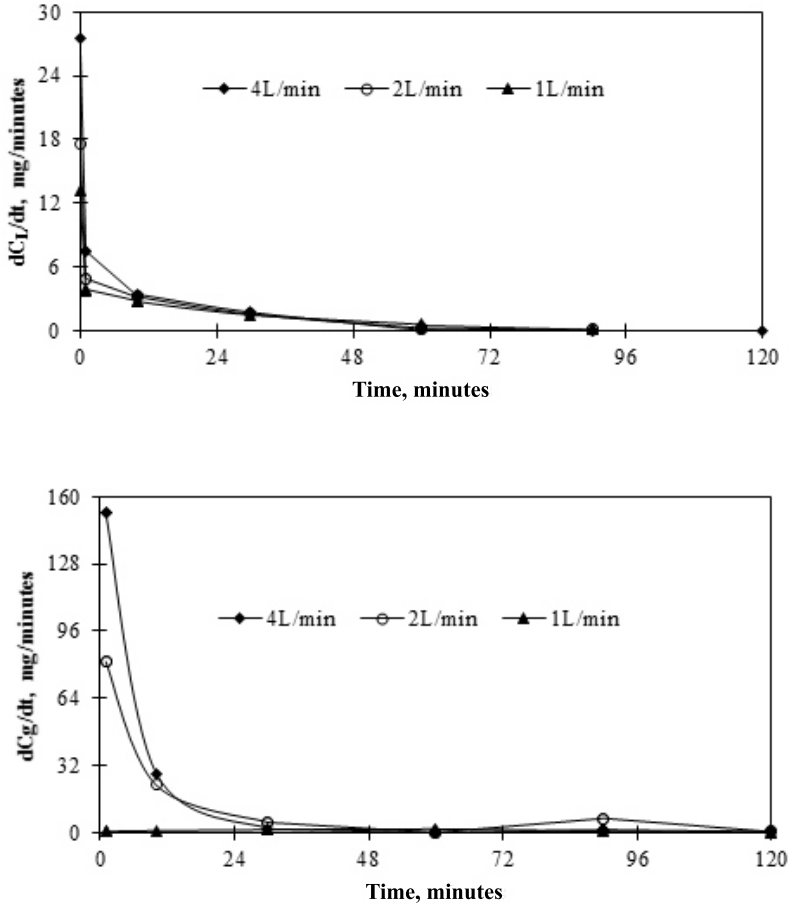



**Figure 11:** Rate of change of reaction.

The values of $dC_L/dt$ after 30 minute of reaction was in the range of 6- 9% for the flow rates of 1 L/min to 4 L/min of ozone. After 60 min, rate of delignification further decreased to 3 5% for the given flow rates. The decrease in $dC_L/dt$ was correlated with the reduction of lignin contents in the wheat straw fibers. The values of $dC_G/dt$ after 30 minute of reaction was in the range of 2- 13% for the flow rates of ozone between 1 L/min to 4 L/min. After 60 min, rate of degradation of cellulose further decreased 1 6% for the applied flow rates. The decrease in $dC_G/dt$ could not be attributed to the number of polysaccharides that remain almost unchanged during the reaction with ozone. The Table 1 was intended to show the selectivity of the reaction of ozone with the lignin and cellulose present in wheat straw based on their reaction rate constants

**Table 1:** Selectivity of ozonation in wheat straw

| **Parameters** | **4 L min$^{-1}$** | **2 L min$^{-1}$** | **1 L min$^{-1}$** |
|---|---|---|---|
| Rate constant of delignification, $k_L$ | 1.44E-03 | 9.51E-04 | 7.05E-04 |
| Rate constant of cellulose degradation, $k_C$ | 1.59E-03 | 1.29E-03 | 1.45E-03 |
| Selectivity | 9.06E-01 | 7.37E-01 | 4.86E-01 |

It can be observed that change in flow rate has not significant effect on the selectivity. Comparing [ozone] in the $O_3$-$O_2$ reaction stream it can be observed that increased concentration makes reaction more selective due to the formation of oxalic acid. The Table 2 was designed to show the selectivity of ozone based on their reaction rate constants. It can be observed that the selectivity of ozone is $10^4$ $10^5$.

**Table 2:** Interaction of ozone with model compounds

| **Species Name** | **Species type** | **Rate constants** | **Reference** |
|---|---|---|---|
| styrene | lignin | $1.00 \times 10^5$ | [79] |
| p-cresol | lignin | $3.30 \times 10^4$ | [80] |
| Cellobiose | carbohydrate | 0.21 | [81] |
| d-Glucose | carbohydrate | 0.17 | [79] |

The differences between the results given in Table 2 and in our investigations could be due to several reasons. 1) The reaction performed by Hoigne and Bader (1981) are homogenous type because these reactions were performed in liquid phase while in our study it was a heterogeneous type i.e, ozone in gas phase and it reacted with the components of wheat straw in solid phases. 2) Hoigne and Bader reacted pure solutes (model compounds) with ozone while in our study lignin, cellulose, hemicellulose, proteins, waxes they all were in natural state in the matrix of wheat straw. 3) Reactants in Hoigne and Bader's system are in free to move state while in our study lignin and cellulose are embedded in a matrix in wheat straw and ozone has to find way to reach to the far placed reaction sites. 4) Moisture contents in wheat straw. Moisture on ozonation generated $OH^{\circ}$ radicals. OH radicals are very much less selective than ozone. Consider Table 3 where the selectivity of $OH^{\circ}$ radical was in the range of 5-6 times.

**Table 3:** Selectivity of hydroxyl radicals for model compounds

| **Species Name** | **Species type** | **Rate constants,** | **Reference** |
|---|---|---|---|
| Veratrolglycol | lignin | $1.5 \times 10^{10}$ | [82] |
| Veratrolglycol β-glycol ether | lignin | $1.7 \times 10^{10}$ | |
| Me-β-D-glucopyranoside | carbohydrate | $3.2 \times 10^9$ | |
| Me-β-D-xylopyranoside | carbohydrate | $2.6 \times 10^9$ | |

This observation led to think that the $OH^{\circ}$ radical formed, reacted more readily with carbohydrates than ozone molecule. The moisture contents were used to make wheat straw swell and give accessibility to ozone because of increased surface area, worked very well we were able to reduce lignin contents up to 92 % in the wheat straw sample of the amount of lignin present naturally. Consider Table 4 where sensitivity of the rate of reaction is given as a function of time and flow rate.

**Table 4:** Selectivity of ozonation, $(dC_L/dt) / (dC_G/dt)$

| Time | 4L $min^{-1}$ | 2L $min^{-1}$ | 1L $min^{-1}$ |
|---|---|---|---|
| 10 min | 4.49E-02 | 6.03E-02 | 8.83E-02 |
| 30 min | 1.17E-01 | 1.39E-01 | 1.05E+00 |
| 60 min | 1.29E+01 | 6.48E+00 | 3.33E-01 |

It is to be noted that with a decrease in flow rate from 4L/min to 1Lmin the selectivity increased. The increase in selectivity was almost 50 %. Increase in time of reaction from 10 minute to 60 minutes it decreased from a value of 4.49E-02 to 1.29E+01 for ozone supply of 4L/min. For the case of ozone flow rate at 1L/min the selectivity is decreased initially up to 30 minutes and then it started increasing till 60 minutes. The behavior of ozone at 1L/min was quite natural i.e., after 30 minutes of ozonation the amount of oxalic acid was increasing the protection barrier remained increasing till 60 minutes of ozonation reaction time. In our studies lignin was more accessible to degrade because of the action of ozone and $OH^{\circ}$ radicals. A compromise is required between the time of ozonation and the characteristics of the desired product. For example, Table 5 indicated the 69 % of lignin removed was associated with the removal of around 27 % of cellulose degraded when ozone was supplied for 30 minutes at a flow rate of 1L/min at the concentration of ozone 2% wt in ozone oxygen stream. Increase in reaction time to 60 minutes lead to percent removal of 77 % of lignin with almost no change in glucose degradation. This can be attributed to the formation of a protection layer by oxalic acid.

**Table 5:** Comparison of lignin removed and cellulose degraded

| Time1 | L $min^{-1}$ | | 2L $min^{-1}$ | | 4L $min^{-1}$ | |
|---|---|---|---|---|---|---|
| Minutes | % AIL Removed | % Glucose degraded | % AIL Removed | % Glucose degraded | % AIL removed | % Glucose degraded |
| 30 | 69 | 26.93 | 71 | 48 | 75 | 48.7 |
| 60 | 77 | 26.54 | 85 | 54 | 88 | 54.82 |

- AIL: Acid insoluble lignin

For a system where ozone was supplied at a flow rate of 2L/min, the amount of lignin removed was 71 % and the amount of cellulose degraded was 48 %. Increase in reaction time made the lignin removed to 85 % with an associated increase in glucose degradation by 54%. This increase in glucose degradation could be due to the convective action caused by the supply of ozone which increases the diffusion power of ozone and breaks the protective layers of oxalic acid.

Further increase in ozone supply to 4L/min, did not make any change in situation may be because the available sites for the reaction were almost consumed. Similar results are shown by Rosen et al (2019) that vigorous ozonation and complete lignin removal should not be an aim of ozonation, rather making the process economical [83]. The present study also recommends an optimum lignin removal

with low inlet ozone flow rates.

**4. Conclusions:**

Ozone reacts with all the components present in the wheat straw matrix. The reactivity of ozone is dependent on its accessibility to the reaction sites available. During ozonation reactions, initially all the reactions sites are available to ozone to react, hence delignification and degradation of lignin proceeded. As the reaction proceeded reaction products started forming then ozone was also consumed in the reactions with secondary reactions. Reactions of ozone were hindered by the formation of oxalic acid during delignification reactions and during degradation of cellulose.

Lignin and cellulose are not uniformly distributed in wheat straw matrix. It can be assumed that it is true for all lignocellulosic materials. Presence of lignin protect cellulose from degradation and vice versa.

Kinetics of delignification and degradation of cellulose studies simultaneously, followed pseudo second order reaction for lignin and cellulose. The removal of lignin should be optimized with a control on the experimental conditions to avoid damage of cellulose contents.

**Availability of data and materials:**

The data that support the findings of this study are available from the corresponding author, [K. S. Baig], upon reasonable request.

**Competing interests:**

The authors declare that they have no conflict of interest.

**Funding**

No funding was provided for this project

**Authors' contributions:**

Substantial contributions to the conception or design of the work; analysis, interpretation of data for this work by K. S. Baig and finally approved for publication. Therefore, the author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work.

**References:**

1. K. Narula, Global energy system and sustainable energy security, in: The Maritime Dimension of Sustainable Energy Security, Springer, 2019: pp. 2349.
2. F. Martins, C. Felgueiras, M. Smitkova, N. Caetano, Analysis of fossil fuel energy consumption and environmental impacts in european countries, Energies. 12 (2019). https://doi.org/10.3390/en12060964.
3. N.M. Holden, E.P. White, Matthew.C. Lange, T.L. Oldfield, Review of the sustainability of food systems and transition using the Internet of Food, Npj Science of Food. 2 (2018). https://doi.org/10.1038/s41538-018-0027-3.
4. R. Ruan, Y. Zhang, P. Chen, S. Liu, L. Fan, N. Zhou, K. Ding, P. Peng, M. Addy, Y. Cheng, E. Anderson, Y. Wang, Y. Liu, H. Lei, B. Li, Biofuels: Introduction, in: Biomass, Biofuels, Biochemicals: Biofuels: Alternative Feedstocks and Conversion Processes for the Production of Liquid and Gaseous Biofuels, Elsevier, 2019: pp. 343. https://doi.org/10.1016/B978-0-12-816856-1.00001-4.
5. C. Brigham, Biopolymers: Biodegradable Alternatives to Traditional Plastics, in: Green Chemistry: An Inclusive Approach, Elsevier Inc., 2017: pp. 753770. https://doi.org/10.1016/B978-0-12-809270-5.00027-3.
6. W.G. Glasser, About Making Lignin Great AgainSome Lessons From the Past, Frontiers in Chemistry. 7 (2019). https://doi.org/10.3389/fchem.2019.00565.
7. Y. Lu, Y.C. Lu, H.Q. Hu, F.J. Xie, X.Y. Wei, X. Fan, Structural characterization of lignin and its degradation products with spectroscopic methods, Journal of Spectroscopy. 2017 (2017). https://doi.org/10.1155/2017/8951658.
8. W. Farhat, Investigation of hemicellulose biomaterial approaches?: the extraction and modification of hemicellulose and its use in value-added applications, n.d. https://tel.archives-ouvertes.fr/tel-02305657.

[9] D. Tian, R.P. Chandra, J.S. Lee, C. Lu, J.N. Saddler, A comparison of various lignin-extraction methods to enhance the accessibility and ease of enzymatic hydrolysis of the cellulosic component of steam-pretreated poplar, Biotechnology for Biofuels. 10 (2017). https://doi.org/10.1186/s13068-017-0846-5.

10. I. Bernal-Lugo, C. Jacinto-Hernandez, M. Gimeno, C.C. Montiel, F. Rivero-Cruz, O. Velasco, 1-step pretreatment, 2019.
11. B. Wang, Y.-C. Sun, R.-C. Sun, Fractionational and structural characterization of lignin and its modification as biosorbents for efficient removal of chromium from wastewater: a review, Journal of Leather Science and Engineering. 1 (2019). https://doi.org/10.1186/s42825-019-0003-y.
12. K.S. Baig, J. Wu, G. Turcotte, Future prospects of delignification pretreatments for the lignocellulosic materials to produce second generation bioethanol, International Journal of Energy Research. (2019). https://doi.org/10.1002/er.4292.
13. C. Xu, R.A.D. Arancon, J. Labidi, R. Luque, Lignin depolymerisation strategies: Towards valuable chemicals and fuels, Chemical Society Reviews. 43 (2014) 74857500. https://doi.org/10.1039/c4cs00235k.
14. Adhesives for Wood and Lignocellulosic Materials, n.d.
15. C. Nicosia, J. Huskens, Reactive self-assembled monolayers: From surface functionalization to gradient formation, Materials Horizons. 1 (2014) 3245. https://doi.org/10.1039/c3mh00046j.
16. W. Den, V.K. Sharma, M. Lee, G. Nadadur, R.S. Varma, Lignocellulosic biomass transformations via greener oxidative pretreatment processes: Access to energy and value added chemicals, Frontiers in Chemistry. 6 (2018). https://doi.org/10.3389/fchem.2018.00141.
17. W. Schutyser, T. Renders, S. van den Bosch, S.F. Koelewijn, G.T. Beckham, B.F. Sels, Chemicals from lignin: An interplay of lignocellulose fractionation, depolymerisation, and upgrading, Chemical Society Reviews. 47 (2018) 852908. https://doi.org/10.1039/c7cs00566k.
18. J.L. Colodette, U.P. Singh, A.K. Ghosh, R.P. Singh, Ozone bleaching research focuses on reducing high cost, poor selectivity., Pulp and Paper. (1993).
19. Y. Rosen, H. Mamane, Y. Gerchman, Short ozonation of lignocellulosic waste as energetically favorable pretreatment. Bioenergy Res 12: 292301, (2019).
20. J. Gierer, Y. Zhang, The role of hydroxyl radicals in ozone bleaching process, in: INTERNATIONAL SYMPOSIUM ON WOOD AND PULPING CHEMISTRY, 1993: pp. 951960.
21. T. Eriksson, Relative rate of ozonation of lignin model compounds, in: Proceedings of Third International Symposium on Wood and Pulping Chemistry, Vancouver, Canada, 1985, 1985.
22. H. Kaneko, S. Hosoya, J. Nakano, Degradation of lignin with ozon: Reactions of biphenyl and alpha-carbonyl type model compounds with ozone., Journal of the Japan Wood Research Society. (1981).
23. T. Eriksson, T. Reitberger, Formation of hydroxyl radicals from direct ozone reactions with pulp constituents, in: INTERNATIONAL SYMPOSIUM ON WOOD AND PULPING CHEMISTRY, 1995: pp. 349354.
24. S. Hendessi, Low temperature ozonation of Canadian Athabasca bitumen, (2015).
25. M.B. Smith, M.B. Smith, Functional Group Exchange Reactions: Aliphatic and Aromatic Substitution and Elimination Reactions, Org. Synth. (2017) 97160.
26. P.R. Tentscher, M. Bourgin, U. von Gunten, Ozonation of para-substituted phenolic compounds yields p-benzoquinones, other cyclic á, â-unsaturated ketones, and substituted catechols, Environmental Science & Technology. 52 (2018) 47634773.

27. E.M. Cuerda-Correa, M.F. Alexandre-Franco, C. Fernández-González, Advanced oxidation processes for the removal of antibiotics from water. An overview, Water. 12 (2020) 102.
28. S. Gligorovski, R. Strekowski, S. Barbati, D. Vione, Environmental implications of hydroxyl radicals ( OH), Chemical Reviews. 115 (2015) 1305113092.
29. A.N. Onyango, Endogenous generation of singlet oxygen and ozone in human and animal tissues: mechanisms, biological significance, and influence of dietary components, Oxidative Medicine and Cellular Longevity. 2016 (2016).
30. G. Kang, Y. Zhang, Y. Ni, A.R.P. van Heiningen, Influence of lignins on the degradation of cellulose during ozone treatment, Journal of Wood Chemistry and Technology. 15 (1995) 413430.
31 M. Frei, Lignin: characterization of a multifaceted crop component, The Scientific World Journal. 2013 (2013).
32. G.N. Rivière, A. Korpi, M.H. Sipponen, T. Zou, M.A. Kostiainen, M. O¨sterberg, Agglomeration of viruses by cationic lignin particles for facilitated water purification, ACS Sustainable Chemistry & Engineering. 8 (2020) 41674177.
33. B. Wang, H. Zhang, F. Wang, X. Xiong, K. Tian, Y. Sun, T. Yu, Application of heterogeneous catalytic ozonation for refractory organics in wastewater, Catalysts. 9 (2019) 241.
34. M. Ragnar, On the importance of the structural composition of pulp for the selectivity of ozone and chlorine dioxide bleaching, Nordic Pulp & Paper Research Journal. 16(2001) 7279.
35. M.M. Sein, M. Zedda, J. Tuerk, T.C. Schmidt, A. Golloch, C. von Sonntag, Oxidation of diclofenac with ozone in aqueous solution, Environmental Science & Technology. 42 (2008) 66566662.
36. K. Magara, T. Ikeda, Y. Tomimura, S. Hosoya, Accelerated degradation of cellulose in the presence of lignin during ozone bleaching, Journal of Pulp and Paper Science. 24 (1998) 264268.
37. A. Oliva-Taravilla, E. Tomás-Pejó, M. Demuez, C. González-Fernández, M. Ballesteros, Phenols and lignin: key players in reducing enzymatic hydrolysis yields of steam-pretreated biomass in presence of laccase, Journal of Biotechnology. 218 (2016) 94101.
38. J. Lind, G. Merényi, K. Wegner, The chemistry of ozone during pulp bleaching, Journal of Wood Chemistry and Technology. 17 (1997) 297326.
39. M. Ragnar, C.T. Lindgren, N.O. Nilvebrant, On the dissociation constants of phenolic groups in lignin structures. 10th Int. Symp. on Wood and Pulping Chem, (1999).
40 D. Montane, X. Farriol, J. Salvado, P. Jollez, E. Chornet, Fractionation of wheat straw by steam-explosion pretreatment and alkali delignification. Cellulose pulp and byproducts from hemicellulose and lignin, Journal of Wood Chemistry and Technology. 18 (1998) 171191.
41. J.K. Bond, O. Liefert, Wheat Outlook, n.d.
42. B. Hames, R. Ruiz, C. Scarlata, A. Sluiter, J. Sluiter, D. Templeton, Preparation of Samples for Compositional Analysis: Laboratory Analytical Procedure (LAP); Issue Date: 9/28/2005, 2008. www.nrel.gov.
43. O. Milstein, Y. Vered, A. Sharma, J. Gressel, H.M. Flowers, Heat and microbial treatments for nutritional upgrading of wheat straw, Biotechnology and Bioengineering. 28 (1986) 381386.
44. R.R. Arnts, S.B. Tejada, 2, 4-Dinitrophenylhydrazine-coated silica gel cartridge method for determination of formaldehyde in air: Identification of an ozone interference, Environmental Science & Technology. 23 (1989) 14281430.
45. X. Zhou, K. Mopper, Measurement of sub-parts-per-billion levels of carbonyl compounds in marine air by a simple cartridge trapping procedure followed by liquid chromatography, Environmental Science & Technology. 24 (1990) 14821485.
46. J. Slemr, Determination of volatile carbonyl

compounds in clean air, Fresenius' Journal of Analytical Chemistry. 340 (1991) 672677.

47 T. Biesenthal, Q. Wu, P.B. Shepson, Relationships between Isoprene, MVK, methacrolein, and ozone in the lower Fraser Valley, BC, in: ABSTRACTS OF PAPERS OF THE AMERICAN CHEMICAL SOCIETY, AMER CHEMICAL SOC 1155 16TH ST, NW, WASHINGTON, DC 20036, 1994: pp. 246-ENVR.

48. D. Helmig, J. Greenberg, Artifact formation from the use of potassium-iodide-based ozone traps during atmospheric sampling of trace organic gases, Journal of High Resolution Chromatography. 18 (1995) 1518.

49. K.S. Baig, J. Wu, G. Turcotte, H.D. Doan, Novel ozonation technique to delignify wheat straw for biofuel production, Energy & Environment. 26 (2015) 303318.

50. A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, Determination of Ash in Biomass: Laboratory Analytical Procedure (LAP); Issue Date: 7/17/2005, 2008. www.nrel.gov.

51. A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, D. Crocker, Determination of Structural Carbohydrates and Lignin in Biomass: Laboratory Analytical Procedure (LAP) (Revised July 2011), 2008. http://www.nrel.gov/biomass/analytical_procedures.html.

52. L.E. Wise, Chlorite holocellulose, its fractionation and bearing on summative wood analysis and on studies on the hemicelluloses, Paper Trade. 122 (1946) 3543.

53. A. Binder, L. Pelloni, A. Fiechter, Delignification of straw with ozone to enhance biodegradability, European Journal of Applied Microbiology and Biotechnology. 11 (1980) 15.

54. A. Cesaro, V. Belgiorno, Sonolysis and ozonation as pretreatment for anaerobic digestion of solid organic waste, Ultrasonics Sonochemistry. 20 (2013) 931936.

55. W. Den, V.K. Sharma, M. Lee, G. Nadadur, R.S. Varma, Lignocellulosic biomass transformations via greener oxidative pretreatment processes: access to energy and value-added chemicals, Frontiers in Chemistry. 6 (2018) 141.

56. A. Cesaro, V. Belgiorno, Sonolysis and ozonation as pretreatment for anaerobic digestion of solid organic waste, Ultrasonics Sonochemistry. 20 (2013) 931936. https://doi.org/10.1016/j.ultsonch.2012.10.017.

[57] K. Kucharska, P. Rybarczyk, I. Ho³owacz, R. £ukajtis, M. Glinka, M. Kamiñski, Pretreatment of lignocellulosic materials as substrates for fermentation processes, Molecules. 23 (2018) 2937.

58. A.A. Katai, C. Schuerch, Mechanism of ozone attack on á?methyl glucoside and cellulosic materials, Journal of Polymer Science Part A?1: Polymer Chemistry. 4 (1966) 26832703.

59. R.B. Secrist, R.P. Singh, Kraft pulp bleaching. II. Studies on the ozonation of chemical pulps, Tappi Tech Ass Pulp Pap Indus. (1971).

60. N. Richet, D. Afif, F. Huber, B. Pollet, J. Banvoy, R. el Zein, C. Lapierre, P. Dizengremel, P. Perre, M. Cabane, Cellulose and lignin biosynthesis is altered by ozone in wood of hybrid poplar (Populus tremula× alba), Journal of Experimental Botany. 62 (2011) 35753586.

61. S. Wohlhauser, G. Delepierre, M. Labet, G. Morandi, W. Thielemans, C. Weder, J.O. Zoppe, Grafting polymers from cellulose nanocrystals: Synthesis, properties, and applications, Macromolecules. 51 (2018) 61576189.

62. T. Eriksson, M. Ragnar, T. Reitberger, Studies on the radical formation in ozone reactions with lignin and carbohydrate model compounds, in: International Pulp Bleaching Conference, SPCI Finlândia, 1998: pp. 119126.

63. P. Pospíšil, A. Prasad, M. Rác, Mechanism of the formation of electronically excited species by oxidative metabolic processes: role of reactive oxygen species, Biomolecules. 9 (2019) 258.

64. J. Gierer, Y. Zhang, The role of hydroxyl radicals in ozone bleaching process, in: INTERNATIONAL SYMPOSIUM ON WOOD AND PULPING CHEMISTRY, 1993: pp. 951960.

65. M.B. Roncero, J.F. Colom, T. Vidal, Cellulose protection during ozone treatments of oxygen delignified Eucalyptus kraft pulp, Carbohydrate Polymers. 51 (2003) 243254.

[66] B. Wang, H. Zhang, F. Wang, X. Xiong, K. Tian, Y. Sun, T. Yu, Application of heterogeneous catalytic ozonation for refractory organics in wastewater, Catalysts. 9 (2019) 241.

67. M. Liu, X. Chen, X. Tian, Ozone oxidation of kraft bamboo pulp for preparation of nanofibrillated cellulose, International Journal of Polymer Science. 2018 (2018).

68. D. S. Zijlstra. J. De Korte, E. P. C. de varies, L., Hameleers, E. Wilbers, E. Junk, P.J. Deuss. 2021. Highly Efficient Semi-Continuous Extraction and In-Line Purification of High â-O-4 Butanosolv Lignin. Front. Chem., 10 May 2021 | https://doi.org/10.3389/fchem.2021.655983

69. T. Kishimoto, F. Nakatsubo, Non-chlorine bleaching of kraft pulp. V. Participation of radical species in ozonation of methyl 4-O-ethyl-â-D-glucopyranoside, (1998).

70. E.E. Johansson, J. Lind, S. Ljunggren, Aspects of the chemistry of cellulose degradation and the effect of ethylene glycol during ozone delignification of kraft pulps, Journal of Pulp and Paper Science (JPPS). 26 (2000) 239244.

71. C.M. Jardim, Ozonolysis review for eucalyptus pretreatment, (n.d.).

72. T. Reitberger, T. Eriksson, M. Ragnar, P. Brandt, Radical formation in ozone bleaching, in: 10th International Symposium on Wood and Pulping Chemistry (ISWPC), Yokohama, Japan, 1999: pp. 302307.

73. J. Gierer, Formation and involvement of superoxide (O2-/HO2·) and hydroxyl (OH·) radicals in TCF bleaching processes: A review, (1997).

74. J. Du, S.H. Kim, M.A. Hassan, S. Irshad, J. Bao, Application of biochar in advanced oxidation processes: supportive, adsorptive, and catalytic role, Environmental Science and Pollution Research. (2020) 127.

75. R.A.D. Mbachu, R.S.J. Manley, Degradation of lignin by ozone. I. The kinetics of lignin degradation of ozone, Journal of Polymer Science: Polymer Chemistry Edition. 19 (1981) 20532063.

76. S.I. Mussatto, Biomass fractionation technologies for a lignocellulosic feedstock based biorefinery, Elsevier, 2016.

77. S.K. Tripathi, N.K. Bhardwaj, H. Roy Ghatak, Developments in ozone-based bleaching of pulps, Ozone: Science & Engineering. 42 (2020) 194210.

78. M. Jasiñska, Test reactions to study efficiency of mixing, Chemical and Process Engineering. 36 (2015).

79. J. Hoigné, H. Bader, Rate constants of reactions of ozone with organic and inorganic compounds in waterI: non-dissociating organic compounds, Water Research. 17 (1983) 173183.

80. J. Hoigné, H. Bader, Rate constants of reactions of ozone with organic and inorganic compounds in waterII: dissociating organic compounds, Water Research. 17 (1983) 185194.

81. P. Nompex, M. Dore, J. de Laat, M. Bawa, B. Legube, Ozonation of selected molecules constituting cellular matter, (1991).

82. M. Ek, J. Gierer, K. Jansbo, T. Reitberger, Study on the selectivity of bleaching with oxygen-containing species, (1989).

83. Y. Rosen, H. Mamane, Y. Gerchman, Short ozonation of lignocellulosic waste as energetically favorable pretreatment. Bioenergy Res 12: 292301, (2019