Ozonolysis: an advantageous pretreatment for lignocellulosic biomass revisited

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ABSTRACT

Ozonolysis, as a lignocellulosic biomass pretreatment, goes back to 80s; however, in the last years it is becoming widespread again owing to its efficiency and mild operation conditions. Ozone reacts preferably with lignin than carbohydrates, promoting biomass destructuration and delignification, and so the sugar release by enzymatic hydrolysis. The hydrolysate from pretreated biomass has being used as sugars source for second-generation fuels production, mainly ethanol, methane and hydrogen. Short-chain carboxylic acids are the main inhibitory compounds generated, being properly removed by water washing. The most common inhibitory compounds reported for other pretreatments, furfural and HMF (5-hydroxymethylfurfural)[†], are not found in ozone-pretreated hydrolysates. Composition of pretreated biomass and ozone consumption depends on several process parameters: reactor design, moisture content, particle size, pH, reaction time, ozone/air flow and ozone concentration. Additional studies are necessary to clarify process parameters effect and to optimize the process to achieve high yields with economic feasibility.

Keywords: ozone; pretreatment; lignocellulosic biomass; sugar release; biofuel.

1. Introduction

Fuels production from renewable sources is becoming crucial due to the growing dependence on the oil production, the unavoidable depletion of fossil fuel reservoirs, and the need to reduce greenhouse gases emissions because of its influence on global climate change. In this regard, the biorefinery concept involves the conversion of biomass in different products such as fuels, thermal energy and chemicals.

[†] Abbreviation: HMF, 5-hydroxymethylfurfural.

Lignocellulosic biomass is the main potential raw material for biorefinery, because of its sugar content, high availability and low price It is a complex structure mainly formed by cellulose (40-50%), hemicellulose (25-35%) and lignin (15-35%), and other components such as extractives and several inorganic materials. Lignin is a heterogeneous cross-linked aromatic polymer with a three-dimensional structure that protects and covers cellulose, hindering its biodegradation. Cellulose is a linear polymer formed by glucose units that can form strains or fibrils due to the formation of hydrogen bonds between polymer chains generating recalcitrant structures. Hemicellulose is an amorphous and branched polymer, which may make it less resistant than cellulose, composed by random pentose and hexose units.

The core process in a biorefinery is the production of second-generation biofuels. The main stages involved in this process are a biomass pretreatment, an enzymatic hydrolysis to convert sugar carbohydrates into monomers and finally a fermentation to generate products of interest.

The primary challenge for biomass-based refinery is the efficient, economic and sustainable design of pretreatment in order to break and to open the complex lignocellulosic structure and thus facilitating the enzymes access during hydrolysis, for ultimately enhancing the rate and yield of sugars release. This stage represents up to 20% of the total production costs.

Among all alternatives, ozonolysis is one of the most promising lignocellulosic biomass oxidative pretreatment for lignin degradation with minimal effects on the hemicellulose and cellulose contents. Table 1 resumes the main advantages and disadvantages of ozonolysis compared with other pretreatments (Travaini et al., 2014).

Ozone is one of the strongest oxidizing agents (E^0= 2.07 V, 25 °C), soluble in

water (110 mg/L, 25 ºC), and readily available for use after its production from oxygen in a strongly endothermic reaction. Due to ozone electron deficiency in the terminal oxygen, it attacks preferably lignin, an electron-rich substrate, than carbohydrates. Most research on ozone pretreatment is related to bleaching in pulp paper industry but its applications have increased principally over the last two decades, such as in ground and industrial wastewaters treatment.

Most research on ozone pretreatment is related to bleaching in pulp paper industry but its applications have principally increased over the last two decades, such as in wastewater advanced oxidation processes and for the reduction of sewage sludge production in wastewater treatment plants. In the search for new alternatives, ozone has been used as delignificant with different types of biomass, such as cereal straw, wood pulp and wood chips, cotton stalk, grass, newsprint and magazine pulps or sugarcane bagasse, among others, and to increase the digestibility of other biomass such as macroalgae or municipal solid waste. Ozonolysis was recently used in the presence of solvents to depolymerise lignin to produce compounds suitable for blending with petrochemical fuels. Hence, the application of this pretreatment could lead to a major improvement in industrial processes that use delignificated biomass as raw material, for example second-generation biofuel production (mainly ethanol and biogas, but also butanol and biohydrogen) or even enzymes production (Travaini et al., 2014).

Binder et al. (1980) delignificated wheat straw with ozone to increase its digestibility achieving a reduction of lignin content of 60%, with 75% of glucose released by enzymatic hydrolysis compared with just 20% for the untreated straw. Neely (1984) demonstrated the enhancement of enzymatic hydrolysis sensitivity for a variety of lignocellulosic biomass (poplar and pine shavings, red oak sawdust, peanut

shells, bagasse, wheat straw and green hay) with 1-2 hours ozone treatment and a consumption of 4-6 g ozone by 100 g of biomass. Vidal and Molinier (1988) reported an increase from 0% to 57% in the enzymatic hydrolysis yield of ozonized poplar sawdust and a decrease in the percentage of lignin from 29% to 8%.

Silverstein and Chen (2007) applied ozone pretreatment to cotton stalks. They did not obtain the expected effect, probably because of the operation conditions. Nevertheless, Kaur et al. (2012) achieved over 42% reduction of lignin content, 53% glucose release and 89% ethanol yield with the same material. Kojima and Yoon (2008) improved enzymatic hydrolysis from 37% to 58% ozonating newsprint and magazine pulps.

García-Cubero et al. (2010) studied the ozone effect on cereal straws, relating sugar release yield with the acid insoluble lignin fraction. They obtained enzymatic hydrolysis yields up to 88.6% (11.2% acid insoluble lignin) and 57% (12.1% acid insoluble lignin) after ozonolysis compared with 29% (17.1% acid insoluble lignin) and 16% (22.1% acid insoluble lignin) on untreated wheat and rye straws (García-Cubero et al., 2009). Schultz-Jensen et al. (2011a) obtained 52% ethanol yield by saccharification and fermentation of washed pretreated wheat straw for 3h, reducing the lignin content by 60%. Heiske et al. (2013) investigated ozone pretreatment of wheat straw to produce biogas, attaining a bioconversion to methane of 45%. Wu et al. (2013a, 2013b) investigated ozonolysis pretreatment (from 15 to 90 min of ozonation time) for enhancing biohydrogen production from wheat and barley straws. Optimal hydrogen production in the ozonated samples was obtained for 45 minutes, with 158% and 166% augmentation for wheat and barley straw respectively, compared to the untreated material.

Mamleeva et al. (2009) achieved residual lignin content as low as 1% after ozonolysis of aspen sawdust. Lee et al. (2010) significantly improved glucose release yield in coastal Bermuda grass from 24% to 53% with a consumption of ozone of 26.4 g of ozone by 100 g of biomass. Yu et al. (2011) reduced the total lignin content on loblolly pine and mixed southern hardwood pulps, with a maximum carbohydrate conversion for the latter of around 80%. Miura et al. (2012) ozonated Japanese cedar chips, finding remarkable hemicellulose removals. Panneerselvam et al. (2013a) tested enzymatic hydrolysis for four types of ozonated energy grass obtaining up to 100% glucose conversion and 60.6% xylan conversion for washed samples.

Travaini et al. (2013) pretreated sugarcane bagasse with ozone to increase lignocellulosic material digestibility. Acid insoluble and total lignin decreased whereas acid soluble lignin increased in all experiments, reaching cellulose and xylan recoveries over 92% after the pretreatment. Yields of sugar enzymatic hydrolysis release increased from 6.64% to 46.06% and from 2.05% to 52.44% for glucose and xylose, respectively, under the best experimental conditions.

Schultz-Jensen et al. (2013) applied ozonolysis to pretreat the macroalgae *Chaetomorpha linum*, increasing ethanol yield from 31 g ethanol/100 g glucan for untreated macroalgae to 41 g ethanol/100 g glucan for ozonated macroalgae. Li et al. (2015) studied the ozonolysis of maize stover. After pretreatment, both lignin and xylan content decreased while cellulose was only slightly affected. The best result provided 78% lignin removal, increasing the glucose yield after enzymatic hydrolysis from 18.5% to 80%.

Besides these studies, ozonolysis has been used in combination with other pretreatments. For Japanese cypress treated with a combination of ozone-oxidation and

dioxane-water extraction (Yokota et al., 2006), mild ozonization for 10-30 min was sufficient to remove lignin from the thin sections of cell walls. Barros et al. (2013) combined ozonolysis with wet disk milling for sugarcane bagasse and straw. The glucose yields for ozonolysis followed by wet disk milling were 89.7% for bagasse and 63.1% for straw, whereas for wet disk milling followed by ozonolysis resulted in 81.1% for bagasse and 92.4% for straw.

Gitifar et al. (2013) studied the ozonization of sugarcane bagasse previously treated with diluted sulfuric acid in autoclave. Delignification and sugar production increased by applying the acid pretreatment; no data about degradation compounds was provided. Karunanithy et al. (2014) explored a sequential extrusion-ozone pretreatment to improve sugar recoveries. When comparing with control samples, glucose, xylose, and total sugar recovery rates attained increases of 3.42, 5.01, and 3.42 times for switchgrass and of 4.5, 2.7, and 3.9 times for big bluestem. Shi et al. (2015) applied ozonolysis and planetary ball milling to promote the enzymatic hydrolysis of corn straw.

Ozonolysis has been also used for other applications, like enzymes production. The suitability of ozonolysis for pretreating wheat straw to be used as substrate for enzymes production by *Trichoderma reesei* was tested elsewhere (Rodriguez-Gomez et al., 2012).

Despite all these laboratory-scale research, full-scale biomass pretreatment with ozone has not been developed yet. Ozone generation is an important drawback considering two factors: its current production costs and the large amount of ozone that process needs. Technological advances are steadily reducing ozone production costs, decreasing a 30% in the last four years. Schultz-Jensen et al. (2011b) estimated an

electrical energy requirement of 2.38 MJ per 100 g of ozone and, nowadays, only 1.65MJ are consumed[‡]. Since combustion of 100 g ethanol releases 2.67 MJ, an economically feasible ethanol production by applying ozonolysis requires the optimization of pretreatment conditions minimizing ozone consumption while maximizing efficiency to, finally, reduce costs. Heiske et al. (2013) obtained similar conclusions for biogas production from ozonated wheat straw.

Despite the poor initial energetic balance, many promising alternatives emerge when ozonolysis is integrated in the biorefinery concept. Fermentation of released sugar to other high-value products or the integration of alcohol production with a subsequent methane production process, or even the generation of electricity and heat, from their corresponding residues would improve the economic balance (Papa et al., 2015).

Probably, the most promising alternative would be the recovery of the degradation products from lignin (the unique renewable source of aromatic compounds) and hemicellulose as value-added products, besides biofuel production. The sequential release pattern of various degradation products (e.g. phenols, benzene, fatty acids) during pretreatment suggests that ozonolysis would be an appropriate pretreatment to obtain lignin-derived products (Schultz-Jensen et al., 2011a). Ozonolysis was recently used in the presence of different solvents to depolymerize lignin and produce compounds suitable for petrochemical fuels blending. Ozonolysis using ethanol as solvent resulted in a low conversion of oxygenated aromatics for short reaction times, whereas a range of saturated esters was obtained over 24-h reaction time. Short chain oxygenates can be used as fuel additives, replacing a certain percentage of hydrocarbon

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[‡]Technical information provided by Jose Antonio del Rey Martin. Operation manager of ViAQUA - Gestión de Aguas de Galicia SAU, Agbar Group, Santiago de Compostela, Spain.

fuel while improving some of fuel properties (Chuck et al., 2013).

From this overall perspective, this review describes the main progress in the use of ozonolysis as lignocellulosic biomass pretreatment, the most usual routes of ozone-biomass chemical reactions and inhibitory compounds formation, biomass compositional and structural changes produced by ozone, and a compilation of the effects of ozonation parameters on biofuel production.

2. Chemical reactions and compositional and structural changes

2.1. Chemical reactions

The ozone, a powerful oxidant, is highly reactive toward lignin, but it shows low selectivity. It can therefore react with carbohydrates and other compounds, degrading them and generating byproducts that may act as inhibitory compounds in downstream processes. The most accepted routes of reaction are described below; however, many secondary reactions have not been studied yet and remain unclear.

2.1.1. Reactions between ozone and lignin

Studies have proved the potential of ozone pretreatment for delignification of lignocellulosic biomass, which depends on the substrate and process parameters. Different reaction mechanisms have been proposed: selective reaction with carbon−carbon double bonds, attack to aromatic centers and glycosidic bond cleavage (Bule et al., 2013). Ozone preferentially reacts with olefinic, aromatic and phenolic compounds because of their electron density. The reactivity and the route of reaction of these compounds change according to the substituents.

The reactions of ozone with aromatic compounds involve an initial electrophilic

attack followed by a hydroxylation of the aromatic ring, which increases electrophilic substitution reactivity of the ring. Hence, a probable subsequent step is a 1,3-cycloaddition. Ionic 1,3-dipolar cycloaddition occurs by Criegee mechanism (Criegee, 1975; Souza-Correa et al., 2013b), which opens olefinic double bonds, and 1,1-cycloaddition occur via π- and σ-complexes. Another reaction mode is the ozone insertion into carbon-hydrogen bonds in alcohol-, aldehyde- and ether-type structures. In the case of aryl and alkyl ethers, the reaction results in the cleavage of the ether bond (Gierer, 1982; Olkkonen et al., 2000; Ragnar et al., 1999).

The chemical analysis of samples evidences that ozone attacks partially acid insoluble lignin transforming it to acid soluble lignin and degrading a fraction of the total lignin. Experiments with bagasse sugarcane resulted in a 66.8% of acid insoluble lignin reduction, acid soluble lignin increased from 3.13% in the raw material to 7.21% and total lignin decreased 39.6% (Travaini et al., 2013).

2.1.2. Reactions between ozone and carbohydrates

Reactions between ozone and carbohydrates are in the range of 10^6 times slower than those between ozone and lignin, although carbohydrate oxidation may become relevant depending on system parameters. The ozone, in water, generates reactive hydroxyl radicals through the formation of superoxides (the primarily formed radical), which react with carbohydrates resulting in random cleavage of glycosidic bonds (Bule et al., 2013; Gierer, 1982; Staehelin and Hoigne, 1985). Direct ozone attack on carbohydrates may also occur, resulting in the formation of carbonyl and carboxyl groups. The attack of β-glucoside involves the 1,3-dipolar addition on the anomeric carbon in the C-H bond, generating hydrotrioxide hemiorthoester. Afterward, this ester-

type compound undergoes several routes of fragmentation (Olkkonen et al., 2000).

2.2. Inhibitory compounds formation

During ozonolysis pretreatment, initial ozone-oxidized products may react further with extra ozone, generating different inhibitory compounds depending on the reactivity of functional groups.

During ozonolysis, sugar degradation generates mainly oxalic acid, formic acid, acetic acid and levulinic acid. By contrast, furfural and HMF, commonly found as sugar degradation compounds, were not detected (Travaini et al., 2013).

Lignin degradation products include a wide range of aromatic and polyaromatic compounds that may be subsequently converted into carboxylic acids. Various studies found the relation between the production of inhibitory compounds and percentage of biomass delignification (Hoigné and Bader, 1983; Travaini et al., 2013). Oxidation products of p-coumaric and ferulic acids (aromatic aldehyde and acids including p-hydroxybenzaldehyde, vanillin, vanillic acid, caproic acid, azelaic acid, and p-hydroxybenzoic acid) are also found (Quesada et al., 1999; Schultz-Jensen et al., 2011a).

Kádár et al. (2015) found also fatty acids produced by wax degradation in ozonated wheat straw, as a result of pH diminution.

Weak carboxylic acids (mostly acetic, formic and oxalic acid) are reported to be the major inhibitors generated from biomass ozonolysis. Various carboxylic acids, besides formaldehyde and methanol, were found in ozonated wheat straw samples (Binder et al., 1980); for ozonated wood chips, the acids acetic, formic, oxalic, glyoxalic and tartaric were identified (Ben'ko et al., 2013) while in ozonated macroalgae less than

0.5g/100g of formic and acetic acids were detected (Schultz-Jensen et al., 2013). The inhibitory effect of carboxylic acids is pH dependent, since they have to be in a undissociate form to penetrate cell membrane. As intracellular pH is higher, they dissociate intracellularly decreasing internal pH affecting cell functions. Therefore, the fermentability of ozone-pretreated hydrolysates can be improved by raising pH. High oxalic and acetic concentrations (6.5 and 1.5 g/L, respectively) strongly inhibited fermentation of ozonated wheat straw hydrolysates to ethanol by *Pichia stipitis* consuming just a 10% of sugar content (Bellido et al., 2013).

In most publications, water washing of ozonated samples resulted to be an effective detoxification step, removing inhibitory compounds while enhancing glucose release after enzymatic hydrolysis. Simple carboxylic acids and phenolic compounds, as well as nearly 30 lignin degradation products, were found in the washing water of ozonated wheat straw (Schultz-Jensen et al., 2011a)

Nevertheless, it is important to remark that the washing step also leaches xylan, since it becomes partially soluble after ozonolysis oxidation. Water washing of ozonated sugarcane bagasse removed 100% of formic and lactic acids generated during ozonolysis, remaining small concentrations of xylitol and acetic acid. The washing step solubilized up to 2.13% of glucose and 8.16% (w/w) of xylan. For the test where the washing step provided the maximum effect, glucose release increased from 35.22% to 45.39% and xylose release decreased from 52.44% to 26.40% (Travaini et al., 2013). For maize stover, the washing step only increased glucose yield around to ten percentage points, but decreased xylose yield a 42% (Li et al., 2015). Schultz-Jensen et al. (2011a), working with ozonated wheat straw, found that this washing step reduced the hydrolysis duration. In this work, similar xylose yields were obtained for both

washed and unwashed material. Ethanol yields for one-hour ozonated wheat straw did not result affected by detoxification step, 45% yield for both, washed and unwashed material. Three-hour ozonated unwashed samples could not be fermented, whereas a 52% ethanol yield was reached for washed ones.

2.3. Structural and morphological changes on ozone pretreated samples

Further understanding and identification of structural and morphological changes of pretreated biomass are crucial for improving the ozonolysis process. Several techniques have been used, besides chemical composition analysis, in order to acquire a broader comprehension of these changes, which are shown in the following subsections.

2.3.1. Structural changes

Bule et al. (2013) investigated the selective modification/degradation of lignin subunits of wheat straw after ozonolysis, finding significant changes on the lignin structure. NMR analysis suggested the degradation of β-O-4 moieties and aromatic ring opening in different lignin subunits. The spectrum showed differences between aromatic structures of the control and ozone pretreated samples, concurrent with a decrease in concentration of the aromatic carbon signal. They observed changes attributed to the methoxy groups, suggesting the possible breakdown of ester-linked structures.

Mamleeva et al. (2009) investigated the conversion of lignin during aspen sawdust ozonation by UV and Fourier transform-infrared (FT-IR) spectroscopy. The study showed the destruction of aromatic rings, and the formation of new ring-conjugated structures (aldehydes, ketones), relatively resistant toward ozone. Aliphatic

carbon acids and carbonyl compounds were formed as a result of both lignin and hemicellulose partial oxidative destruction. In addition, the spectra indicated that a part of the softwood lignin is not subjected to ozone action. Souza-Correa et al. (2013b) studied lignin oxidation of sugarcane bagasse, using mass spectrometry and FTIR. Their results supported Criegee's mechanism of ozone attack towards lignin double bonds.

Kádár et al. (2015) applied Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR) to study the chemical changes on the surface of ozonated wheat straw. They observed a significant decrease in aliphatic compounds, related with the degradation of the cuticular and epicuticular waxes and a decrease of intensity of the peaks assigned to aromatic skeletal vibrations, attributed to lignin degradation. UV spectrometry of ozone treated thermomechanical paper pulp wastes showed that ozone attacks first the surface of the pulp fiber, namely the cell wall, and then reaction spread into the cell wall (Kojima and Yoon, 2008).

Yu et al. (2011) evaluated changes in crystallinity index for loblolly pine and mixed southern hardwood chips pretreated in alkaline conditions followed by ozonation. They found a general augmentation after pretreatment, attributed to a decrease on the amount of amorphous components (lignin and hemicellulose). In contrast, Ben'ko et al. (2013b) found that crystallinity index varied with the pretreatment intensity: while short times of ozonolysis resulted in no significant changes, longer pretreatment times caused firstly a decrease and ultimately an increase (38% for non-pretreated samples and 37, 28 and 44% for 15-min, 60-min and 150-min ozonated samples, respectively). These variations were attributed to a cellulose amorphization and a subsequent recrystallization caused by sample processing.

2.3.2. Morphological changes

Scanning Electron Microscopy (SEM) analysis showed a disturbed structure and nanoscopic fibrous morphology in ozone-pretreated biomass. These changes were clear for materials sensitive to the ozone attack, as sugarcane bagasse. Lignin removal allowed the visualization of biomass microfibers only in some areas, probably from the primary cell wall, where lignin content is lower and delignification could easily release cellulose microfibrills. However, this effect only increases the specific surface area by generating a porous structure, whereas the delignification of the second cell wall allows better saccharification yields (Barros et al., 2013). SEM microscopy of ozonated bagasse after the washing step clearly showed the destructive effect of ozone pretreatment on lignin. The washing step leached acid soluble lignin and hemicellulose, and more grooves were appreciated (Travaini et al., 2013). SEM was also used to observe the degradation of waxes, which appear as flakes at the surface of wheat straw. In can be observed that the stronger the ozone pretreatment, the lower the amount of wax flakes found (Kádár et al., 2015).

The delignification of Japanese cypress by ozonization followed by dioxane-water (9:1 v/v) extraction was studied by microspectrometry, using the Wiesner color reaction. Ozone delignification process started from lumen to middle lamella, with the secondary wall ozonized faster than middle lamella. Therefore, ozone reacted topochemically depending on the tissue lignin content (Yokota et al., 2006).

Accessible pore volume (APV) was measured for alkaline-ozone pretreated wood chips by differential scanning calorimetry (DSC), and the results were interpreted by the accumulation of freezing bond water (Yu et al., 2011). The APV decreased for pretreated samples up to 40% delignification. For higher lignin removal values, the

APV gradually increased, although it remained beneath values of the non-pretreated samples. The observed reduction might be an indication of structural collapse of fibers caused by the ozone delignification of surface structures, with fibers becoming twisted and severely shrunken. The ozone penetration into the pulp of highly delignificated samples does not counterbalance the APV reduction by external fibers collapse.

3. Effects of process parameters

As described by different authors, many process factors have influence on the ozonolysis pretreatment efficiency (Table 2), such as: reactor design, moisture, particle size, pH, ozone concentration, ozone/air flow rate and time of ozonolysis. The only study about the temperature influence was conducted by Sugimoto et al. (2009) mentioning experiments at 0 ºC and 40 ºC, providing only results of ozone consumption and no pretreatment efficiency data. The knowledge about the effect of the most remarkable process parameters on the ozonolysis pretreatment is summarized in this section.

3.1. Effect of reactor design

Reactor design is one of the fundamental parameters in ozonolysis pretreatment as it determines the effective ozone concentration profile, which has a considerable effect on ozone consumption, reactions kinetics, sugar release and interacting with other process parameters. The reactor configuration should provide a contact between ozone and substrate as effective as possible, in order to favor the oxidation reaction. Moreover, it is important to ensure the highest efficiency and lowest residual ozone mass due to the high costs associated to ozone generation.

Theoretically, two different model reactors can be defined: perfect mixed and plug flow. For ideal mixing model, ozone concentration in the reaction medium is homogenous and the same as in the reactor outlet flow; whereas for plug flow model, ozone concentration decreases with the reactor length, from the inlet to the outlet. Different research works have used a variety of reactor design options, from mixed to plug flow models: batch reactor (Vidal and Molinier, 1988), rotatory reactor (Miura et al., 2012), Drechsel trap reactor (Cesaro and Belgiorno, 2013), cylindrical reactor (Neely, 1984), bubble column reactor (Cesaro and Belgiorno, 2013), multi-layer fixed bed reactor (Heiske et al., 2013) or fixed-bed reactor (García-Cubero et al., 2012; Panneerselvam et al., 2013b; Travaini et al., 2013) as showed schematically in Fig. 1.

Cesaro and Belgiorno (2013) tested two different reactors to ozonate the organic fraction of municipal solid waste diluted with distilled water (ratio 1:3 w/w). The first reactor was a Drechsel trap and the second reactor consisted of a bubble column: this last one was more effective, a higher amount of ozone reacted with the substrate and lower residual ozone leaved the reactor.

Neely (1984) evaluated the influence of the reactor design (rotating horizontal cylinder, stirred-bed reactor and fixed- bed reactor) with different types of biomass (pine, oak, poplar, wheat straw, peanut shells, corn stover and bagasse). Its studies concluded rotating cylinder reactor was the most satisfactory for general use. Nevertheless, scarce information about the reactor design was provided and the comparison was not systematic.

Vidal and Molinier (1988) studied the poplar sawdust ozonolysis in both fixed bed column (30 and 75% (w/w) of water in a dry matter basis) and stirred semibatch reactor (50 g/L initial concentration). The number of moles of ozone consumed to

oxidize one C9 unit was three in the solid and seven in the slurry. The stirred reactor provided higher ozone concentration at the outlet of the reactor.

In experiments with 5% (w/w) suspension of wheat straw in a tank reactor, 5 hours of reaction time were necessary to achieve about 50% delignification with an ozone consumption of 0.2 g O_3 by g of dry wheat straw (Binder et al., 1980). García-Cubero et al. (2012) achieved the same 50% delignification of wheat straw in a fixed bed column with 40% (w/w) moisture and 2 hours reaction time. The reaction consumed 100% of fed ozone the first 60 minutes and 97% of fed ozone in 120 minutes once the breakthrough time was attained, which corresponded to 0.09 g O_3 by g of dry wheat straw.

These studies showed that mixed reactors work with constant ozone flow and concentration, but the ozone starts to escape from time zero. It occurs due to the mixing in the reactors, the mass transfer barrier between solid and gas phases, and the slight solubility of ozone in experiment with a liquid phase. The fixed bed reactor seemed to be more efficient than the stirred reactor, comparing the ratio ozone consumption/solubilized lignin. In any case, the study of parameters effect should take into account that reactor model and moisture are directly related: low concentration of substrate provides ideal mixing and low moisture provides plug flow reactor.

Nowadays, most of the researchers use plug flow reactors with theirs modifications (Li et al., 2015; Shi et al., 2015). García-Cubero et al. (2012) studied the profile of ozonolysis effect on rye and wheat straw in a fixed bed reactor (50 cm high and 2.7 cm diameter). The delignification and sugars release yields decreased along the column in all the experiments, proving the plug flow model.

Heiske et al. (2013) compared single layer and multiple layer bed reactor

configurations to enhance the bioconversion of wheat straw to methane. Reaction goes multi-longitudinally through thin layers from ozone inlet to outlet; the layers became saturated until ozone arrives at the outlet zone, escaping without react. The single layer reactor provided treated straw with 16.2% lignin concentration and 14.52% sugars available. The lignin content decreased in the three layers of the multiple-layer reactor from top (17.5%) over mid (13.0%) to bottom (7.2%), increasing the sugars availability (top 8.8%, mid 16.9%, and bottom 28.4%). Nevertheless, methane production yields indicated little but no substantial difference between the layers. The inhibitory effects of lignin degradation products found in the analysis of the pretreated wheat straw washing water balanced out the beneficiary effects of a stronger treatment.

Panneerselvam et al. (2013b) experimented with four varieties of energy grasses in a fixed bed reactor using two different feed flow configurations: firstly, direct ozone flow and secondly, reverse ozone flow by inverting the reactor. Reverse flow configuration showed the highest biomass delignification, the highest sugar recovery, the lowest retention time and the strongest reaction between the bedsides. .

3.2. Effect of moisture content

Most authors agree that sample moisture is the most important ozonolysis process parameter, because of its function as mass transport medium, and its effect on ozone and generated radicals' effective concentrations. As described previously, moisture content interacts closely with the reactor model: high water content is related with mixed reactors and, thus, with homogenous ozone concentration, whereas low water content promotes plug flow reactor models.

The reaction starts with ozone transference from gas phase to free water, then

from free water to bounded water, and finally the reaction between ozone and lignocellulosic biomass occurs. (Choi et al., 2002; Li et al., 2015). Therefore, at the beginning of pretreatment, virtually no ozone exists in the water reaction face, being all the ozone in the free water. When ozone passes to the bounded water, reaction starts. During pretreatment, reaction rate slows down, being ozone mass transfer the limiting step.

Each type of biomass has its optimal moisture content. According to Neely (1984) this optimal moisture content corresponds to the saturation point of the fibers. At low water concentrations, the ozone mass transfer is limited, and it cannot react properly with all biomass, resulting in shorter breakthrough times. If the water concentration is excessive, a thick film of water blocks biomass pores favoring large residence time of ozone and promoting its decomposition in another hydroxyl radicals. Reactions then follow another non-selective pathways, and an excessive ozone consumption is observed (Mamleeva et al., 2009; Neely, 1984; Puri, 1983; Vidal and Molinier, 1988). Li et al. 2015 studied the interactions between moisture content and particle size in maize stover ozonolysis, finding that water activity (the ratio between free and bounded water) regulates optimal moisture content. For each particle size, different optimal moisture content for delignification were found, but the water activity measured for them was always around 0.775 (Fig. 2).

Usually, the optimal water content for agricultural residues is higher than that observed for woods, due to their capacity to bond water. Souza-Correa et al. (2013a) studied 10, 25, 50 and 75% (w/w) moisture content for ozonization of sugarcane bagasse, and found the optimum result at 50% moisture content. Travaini et al. (2013), working with sugarcane bagasse in a fixed bed reactor, achieved a 46% of glucose yield

at 80% (w/w) moisture content, six percentage points more than at 40% (w/w) moisture content. Low water contents favored inhibitory compounds formation. Bule et al. (2013), working with wheat straw in a 1 cm high fixed bed reactor and moisture contents between 30-90% (w/w), found the optimal glucose release at 90% (w/w). This extra high value can be attributed to the small size of the bed, which may mitigate the thick water layer formation.

Ben'ko et al. (2013a), working in a fixed layer flow unit reactor with aspen chips, studied water contents from 7% (w/w, air-dry wood) to 90% (w/w, water suspension), applying ozone absorption as efficiency criterion. They found that extreme values do not absorb ozone, with the maximum absorption at 40% (w/w) moisture content. This value is higher than that of wood fiber saturation, 20-23% (w/w), at which just cell walls have water, and the cavities are empty. This reinforces the need of free water for great ozonization. For Japanese cedar the optimal moisture content is around 40% (w/w), but after this value free water overload results excessive (Miura et al., 2012; Sugimoto et al., 2009). For red oak treated in a stirred bed reactor, the optimal water content was between 25-35% (w/w), with an augmentation of ozone consumption when increasing the water content (Neely, 1984). Ueda et al. (2014) studied the influence of moisture content of shiitake spent culture media (wood meal, *Quercus serrata*). They obtained the best moisture content value between 20-40%; whereas working with water content higher than 60%, lignin was not properly degraded.

The air-ozone flow and the increase of temperature caused by the exothermic oxidation reactions can have a remarkable drying effect, decreasing considerably the moisture during the ozonolysis (Mamleeva et al., 2009). Feeding to the reactor an air-ozone flow saturated of water can minimize this moisture reduction, but the increase of

temperature in the reactor displaces the equilibrium of water saturation and this drying effect is, in fact, difficult to avoid.

3.3. Effect of particle size

Since ozonolysis pretreatment is a surface reaction, particle size has an important role as process parameter. Nevertheless, any mechanical step will increase the process cost, and an optimal particle size should be pursued, as very small particles promote agglomeration and sugar degradation. A ball mill was used for particle size reduction of corn straw before ozonolysis in a fixed bed reactor with particles from 160 µm to 21 µm, obtaining the highest glucose conversion with 64 µm particles (Shi et al., 2015). The particle size reduction favored delignification and glucose release, although it decreased xylose yield, probably because of xylose degradation. Schultz-Jensen et al. (2011b), working in a fixed bed reactor with wheat straw, tested the effect of three particle sizes: 0.5, 1 and 2 mm. Glucan and xylan conversion for each particle size were 23, 50 and 19.4%, and 57.5, 75 and 45%, respectively. Ozone losses were higher for 2 mm particles, probably due to a decrease on reaction kinetic caused by a lower surface area, promoting the unreacted ozone going out from the reactor. For 0.5 mm particles, the decrease of sugars conversion was attributed to agglomeration effect.

For grinded sieved maize stover pretreated in a fixed bed reactor (particle sizes fractions between 20/40 and 80/150 mesh, and less than 300 mesh, the smaller the particle size, the greater delignification was achieved, with the optimal moisture depending on particle size (Fig. 2). Around 75% delignification and 80% glucose conversion were achieved for particle size lower than 300 mesh (Li et al., 2015).

The combination of wet disk milling and ozonolysis allowed a less expensive

process from an energetic point of view, when compared to individual pretreatments, obtaining glucose release yields of 81.1% for bagasse and 92.4% for straw (Barros et al., 2013). Working with red oak in a stirred bed reactor with 50% (w/w) moisture content to obtain 80% digestibility, the reaction time decreased four times when reducing the particle size from 1000 to 106 μm. However, a reduction below 0.5 mm provided small advantage (Neely, 1984).

García-Cubero et al. (2009), studying the particle size effect (<1 and 3-5 mm) for wheat and rye straw in a fixed bed reactor, found that particle size does not influence significantly on delignification and sugars yields. Vidal and Molinier (1988) had the same conclusion with 1 and 2 mm particle of poplar sawdust. Souza-Correa et al. (2014) working with sugarcane bagasse, found small improvements on delignification efficiency (from 75% to 80%) reducing particle size (from 2 to 0.08 mm). In contrast, cellulose conversion ratio increased (from 61% to 79%) as the particle size diminished, observing the most significant change for particles below 0.5 mm. The way in which particle size affects ozonolysis depends on the type of biomass studied, the range of particle sizes and their relation to moisture content.

3.4. Effect of pH

The pH of water used for adjust biomass moisture content can influence the pretreatment. Additionally, pH decreases during ozonolysis process by formation of organic acids from degradation reactions (Binder et al., 1980; García-Cubero et al., 2009; Yu et al., 2011). Alkaline media favors the delignification of biomass, because it removes lignin bonded to carbohydrates. However, at pH greater than 4 ozone destruction is favored, and another reactive species are generated that can attack

carbohydrates (Pan et al., 1984).

Aspen wood chips ozonization, neutral and with 2% and 12% NaOH, showed a decrease of sugar release with the increase of NaOH concentration, attributed to cellulose decomposition (Ben'ko et al., 2013a). García-Cubero et al. (2009) studied the ozonolysis of wheat and rye straw in a fixed bed reactor moisturized with water and 20% NaOH solution. The alkaline media resulted in carbohydrate degradation and lower delignification. Binder et al. (1980), working in 5% liquid media suspension of wheat straw, found a higher ozone consumption in alkaline media. No ozone was found in outlet gas at initial times, indicating ozone degradation. A higher delignification was achieved for green liquor of mixed wood chips when ozonated in pH 2 than compared to neutral medium (Yu et al., 2011). In conclusion, the effect of pH should be counterbalanced between ozone destruction and delignification.

3.4. Ozone consumption: effect of reaction time, ozone concentration and ozone flow

The most important variable of ozonolysis pretreatment, from an economic point of view, is the ozone consumed by gram of dry biomass pretreated. Ozone consumption is expected to be closely related to delignification and hence, to sugar hydrolysis yield (Fig. 3) (García-Cubero et al., 2009; Shi et al., 2015; Travaini et al., 2013). Nevertheless, excessive ozone consumption promotes the production of inhibitory compounds by sugar degradation and by reaction with low molecular lignin compounds (Binder et al., 1980; Neely, 1984).

Ozone consumption is the most complex variable to compare, since it is directly dependent on many process parameters as reaction time, ozone concentration and inlet gas flow; and influenced by the other process parameters discussed before. Li et al.

(2015) pretreated maize stover in a fixed bed reactor, varying the ozone consumption from 100 to 250 mg of ozone by g of biomass. They did not find a relation between ozone consumption and delignification and sugar yield because of the effect of moisture, as water content could influence the ozone decomposition rate. Travaini et al. (2013), ozonated sugarcane bagasse in a fixed bed reactor. An experiment with 3.07% (v/v) ozone concentration, 40% (w/w) moisture content and 180 minutes of reaction time with a consumption of 0.32 g of ozone by g of dry bagasse resulted in 55% delignification and 23% enzymatic hydrolysis glucose release. Another experiment using 3.44% (v/v) ozone concentration and 80% (w/w) moisture, 45 minutes of reaction time and a consumption of 0.12 g of ozone by g of dry bagasse, provided 33% of delignification but enzymatic hydrolysis glucose release yield of 45%.

The ozone consumption varies with the reaction time in a similar way for all types of biomass. Initially, the preferential reaction is between ozone and lignin: ozone consumption is very fast, and the highest delignification and enzymatic hydrolysis sugar rates are achieved. After this time, a stabilization period begins, when ozone consumption quickly decreases, and delignification and sugar release yields increase very slowly. In a last step, sugar polymers start to be degraded and the hydrolysis sugar release diminishes (Fig. 4). Partial and global reaction times are specific for each combination of process parameters and type of biomass (Binder et al., 1980; García-Cubero et al., 2009, 2012; Neely, 1984; Schultz-Jensen et al., 2011b).

The relation between ozone concentration and sugar release yield is not clear. In several studies, when using a fixed bed reactor, the increase of ozone concentration does not result in a proportional sugar yields increment. For red oak, an increase of ozone from 3 to 3.37% (v/v) augmented digestibility from 64 to 89%, whereas just 91%

digestibility was found with 6.08% ozone concentration (Neely, 1984). For sugarcane bagasse, the sugar release increased with ozone concentration but not proportionally, from 16% working with 1.37% (v/v) ozone concentration, 105 minutes reaction and 0.09 g of ozone by g of biomass to 46% with a 3.44% (v/v), 60 minutes reaction and 0.12 g of ozone by g of biomass (Travaini et al., 2013). For wheat and rye straw, ozone concentrations of 2.7 and 3% (v/v) provided similar results for delignification and sugar release (García-Cubero et al., 2009).With energy grasses, ozone concentrations of 40, 50 and 58 g/L provided similar results of delignification and sugar release too (Panneerselvam et al., 2013b).

The increase in ozone/air flow reduces the reaction time, but increases ozone consumption. Inlet overflow provides an excess of ozone, which is going to be available for reaction, obtaining a virtually constant ozone concentration in the reactor. Working with pine shavings, ozonization with 3% (mol/mol) ozone concentration, 17 L/min flow and 10 minutes of reaction time provided around 60% digestibility, and the experiment with the same ozone concentration, 0.5 L/min flow and 90 minutes reaction provided 65% of digestibility. In the first case, 0.066 g ozone by g of biomass was consumed, while the consumption was just 0.024 g ozone by g of biomass for the second case. Large flows promote faster reactions with higher ozone consumption, what could make process more expensive and generate inhibitors, as discussed before (Neely, 1984). When reaction time was fixed, larger flows resulted in lower sugar yields for wheat and rye straw in a fixed bed reactor (García-Cubero et al., 2009), but the optimal reaction time for glucose yield (2 hours) decreased two times working with wheat straw in a fixed layer reactor (Schultz-Jensen et al., 2011b).

4. Conclusions

Ozonolysis has proved its efficiency as pretreatment for diverse lignocellulosic biomass providing high delignification (≈80%) and total sugar release yields (≈75%) with very low carbohydrate losses. The low generation of inhibitory compounds enables subsequent enzymatic hydrolysis and fermentation steps for biofuels production. The best results, in terms of ozone consumption and efficiency, were obtained working with fixed bed reactors. Moisture is the most important parameter and passes through a maximum depending on the material. Key factor in global process economy is biorefinery conceptualization, by valorizing the whole biomass residues to produce other energy forms, hemicellulose-derived compounds and lignin ozone-oxidized products.

Acknowledgements

References

1. Barros, R.D.R.O. De, Paredes, R.D.S., Endo, T., Bon, E.P.D.S., Lee, S.H., 2013. Association of wet disk milling and ozonolysis as pretreatment for enzymatic saccharification of sugarcane bagasse and straw. Bioresour. Technol. 136, 288–294.

2. Bellido, C., González-Benito, G., Coca, M., Lucas, S., García-Cubero, M.T., 2013. Influence of aeration on bioethanol production from ozonized wheat straw hydrolysates using Pichia stipitis. Bioresour. Technol. 133, 51–58.

3. Ben'ko, E.M., Manisova, O.R., Lunin, V.V., 2013a. Effect of ozonation on the reactivity of lignocellulose substrates in enzymatic hydrolyses to sugars. Russ. J. Phys. Chem. A 87, 1108–1113.

4. Ben'ko, E.M., Manisova, O.R., Murav'eva, G.P., Lunin, V.V., 2013b. Structural changes in wood during ozonation. Russ. J. Phys. Chem. A 87, 1097–1101.

5. Binder, A., Pelloni, L., Fiechter, A., 1980. Delignification of straw with ozone to enhance biodegradability. Eur. J. Appl. Microbiol. Biotechnol. 11, 1–5.

6. Bule, M.V, Gao, A.H., Hiscox, B., Chen, S., 2013. Structural modification of lignin and characterization of pretreated wheat straw by ozonation. J. Agric. Food Chem. 61, 3916-3925.

7. Cesaro, A., Belgiorno, V., 2013. Sonolysis and ozonation as pretreatment for anaerobic digestion of solid organic waste. Ultrason. Sonochem. 20, 931–936.

8. Choi, H., Lim, H.N., Kim, J., Hwang, T.M., Kang, J.W., 2002. Transport characteristics of gas phase ozone in unsaturated porous media for in-situ chemical oxidation. J. Contam. Hydrol. 57, 81–98.

9. Chuck, C.J., Parker, H.J., Jenkins, R.W., Donnelly, J., 2013. Renewable biofuel additives from the ozonolysis of lignin. Bioresour. Technol. 143, 549–554.

10. Criegee, R., 1975. Mechanism of ozonolysis. Angew. Chemie Int. Ed. English 14, 745–752.

11. García-Cubero, M.T., González-Benito, G., Indacoechea, I., Coca, M., Bolado, S., 2009. Effect of ozonolysis pretreatment on enzymatic digestibility of wheat and rye

straw. Bioresour. Technol. 100, 1608–1613.

12. García-Cubero, M.T., Coca, M., Bolado, S., González-Benito, G., 2010. Chemical oxidation with ozone as pre-treatment of lignocellulosic materials for bioethanol production. Chem. Eng. Trans. 21, 1273–1278.

13. García-Cubero, M.T., Palacín, L.G., González-Benito, G., Bolado, S., Lucas, S., Coca, M., 2012. An analysis of lignin removal in a fixed bed reactor by reaction of cereal straws with ozone. Bioresour. Technol. 107, 229–234.

14. Gierer, J., 1982. The chemistry of delignification – A general concept - Part II. Holzforschung 36, 55–64.

15. Gitifar, V., Eslamloueyan, R., Sarshar, M., 2013. Experimental study and neural network modeling of sugarcane bagasse pretreatment with H_2SO_4 and O_3 for cellulosic material conversion to sugar. Bioresour. Technol. 148, 47–52.

16. Heiske, S., Schultz-Jensen, N., Leipold, F., Schmidt, J.E., 2013. Improving anaerobic digestion of wheat straw by plasma-assisted pretreatment. J. At. Mol. Phys. 2013, 1–7.

17. Hoigné, J., Bader, H., 1983. Rate constants of reactions of ozone with organic and inorganic compounds in water—II. Water Res. 17, 185–194.

18. Kádár, Z., Schultz-Jensen, N., Jensen, J.S., Hansen, M. a. T., Leipold, F., Bjerre, A.-B., 2015. Enhanced ethanol production by removal of cutin and epicuticular waxes of wheat straw by plasma assisted pretreatment. Biomass and Bioenergy 81, 26–30.

19. Karunanithy, C., Muthukumarappan, K., Gibbons, W.R., 2014. Sequential extrusion-ozone pretreatment of switchgrass and big bluestem. Appl. Biochem. Biotechnol. 172, 3656–3669.

20. Kaur, U., Oberoi, H.S., Bhargav, V.K., Sharma-Shivappa, R., Dhaliwal, S.S., 2012.

Ethanol production from alkali- and ozone-treated cotton stalks using thermotolerant *Pichia kudriavzevii* HOP-1. Ind. Crops Prod. 37, 219–226.

21. Kojima, Y., Yoon, S.L., 2008. Improved enzymatic hydrolysis of waste paper by ozone pretreatment. J. Mater. Cycles Waste Manag. 10, 134–139.

22. Lee, J., Jameel, H., Venditti, R., 2010. Effect of ozone and autohydrolysis pretreatments on enzymatic digestibility of coastal Bermuda grass. BioResources 5, 1084–1101.

23. Li, C., Wang, L., Chen, Z., Li, Y.Y., Wang, R., Luo, X., Cai, G., Yu, Q., Lu, J., 2015. Ozonolysis pretreatment of maize stover: the interactive effect of sample particle size and moisture on ozonolysis process. Bioresour. Technol. 183, 240–247.

24. Mamleeva, N.A., Autlov, S.A., Bazarnova, N.G., Lunin, V.V., 2009. Delignification of softwood by ozonation. Pure Appl. Chem. 81, 2081–2091.

25. Miura, T., Lee, S.H., Inoue, S., Endo, T., 2012. Combined pretreatment using ozonolysis and wet-disk milling to improve enzymatic saccharification of Japanese cedar. Bioresour. Technol. 126, 182–186.

26. Neely, W.C., 1984. Factors affecting the pretreatment of biomass with gaseous ozone. Biotechnol. Bioeng. 26, 59–65.

27. Olkkonen, C., Tylli, H., Forsskåhl, I., Fuhrmann, A., Hausalo, T., Tamminen, T., Hortling, B., Janson, J., 2000. Degradation of model compounds for cellulose and lignocellulosic pulp during ozonation in aqueous solution. Holzforschung 54, 397-406.

28. Pan, G.Y., Chen, C.L., Chang, H.M., Gratzl, J.S., 1984. Studies on ozone bleaching. I. The effect of pH, temperature, buffer systems and heavy metal-ions on stability of ozone in aqueous solution. J. Wood Chem. Technol. 4, 367–387.

29. Panneerselvam, A., Sharma-Shivappa, R.R., Kolar, P., Clare, D.A., Ranney, T.,

2013a. Hydrolysis of ozone pretreated energy grasses for optimal fermentable sugar production. Bioresour. Technol. 148, 97–104.

30. Panneerselvam, A., Sharma-Shivappa, R.R., Kolar, P., Ranney, T., Peretti, S., 2013b. Potential of ozonolysis as a pretreatment for energy grasses. Bioresour. Technol. 148, 242–248.

31. Papa, G., Rodriguez, S., George, A., Schievano, A., Orzi, V., Sale, K.L., Singh, S., Adani, F., Simmons, B.A., 2015. Comparison of different pretreatments for the production of bioethanol and biomethane from corn stover and switchgrass. Bioresour. Technol. 183, 101–110.

32. Puri, V., 1983. Ozone pretreatment to increase digestibility of lignocellulose. Biotechnol. Lett. 5, 773–776.

33. Quesada, J., Rubio, M., Gómez, D., 1999. Ozonation of lignin rich solid fractions from corn stalks. J. Wood Chem. Technol. 19, 115–137.

34. Ragnar, M., Eriksson, T., Reitberger, T., 1999. Radical formation in ozone reactions with lignin and carbohydrate model compounds. Holzforschung 53.

35. Rodriguez-Gomez, D., Lehmann, L., Schultz-Jensen, N., Bjerre, A.B., Hobley, T.J., 2012. Examining the potential of plasma-assisted pretreated wheat straw for enzyme production by *Trichoderma reesei*. Appl. Biochem. Biotechnol. 166, 2051–63.

36. Schultz-Jensen, N., Kádár, Z., Thomsen, A., Bindslev, H., Leipold, F., 2011a. Plasma-assisted pretreatment of wheat straw for ethanol production. Appl. Biochem. Biotechnol. 165, 1010–1023.

37. Schultz-Jensen, N., Leipold, F., Bindslev, H., Thomsen, A., 2011b. Plasma-assisted pretreatment of wheat straw. Appl. Biochem. Biotechnol. 163, 558–572.

38. Schultz-Jensen, N., Thygesen, A., Leipold, F., Thomsen, S.T., Roslander, C.,

Lilholt, H., Bjerre, A.B., 2013. Pretreatment of the macroalgae *Chaetomorpha linum* for the production of bioethanol-comparison of five pretreatment technologies. Bioresour. Technol. 140, 36–42.

39. Shi, F., Xiang, H., Li, Y., 2015. Combined pretreatment using ozonolysis and ball milling to improve enzymatic saccharification of corn straw. Bioresour. Technol. 179, 444–451.

40. Silverstein, R., Chen, Y., 2007. A comparison of chemical pretreatment methods for improving saccharification of cotton stalks. Bioresour. Technol. 98, 3000–3011.

41. Souza-Correa, J.A., Oliveira, C., Nascimento, V.M., Wolf, L.D., Gómez, E.O., Rocha, G.J.M., Amorim, J., 2014. Atmospheric pressure plasma pretreatment of sugarcane bagasse: the influence of biomass particle size in the ozonation process. Appl. Biochem. Biotechnol. 172, 1663–1672.

42. Souza-Correa, J.A., Oliveira, C., Wolf, L.D., Nascimento, V.M., Rocha, J.G.M., Amorim, J., 2013a. Atmospheric pressure plasma pretreatment of sugarcane bagasse: the influence of moisture in the ozonation process. Appl. Biochem. Biotechnol. 171, 104–116.

43. Souza-Correa, J.A., Ridenti, M.A., Oliveira, C., Araújo, S.R., Amorim, J., 2013b. Decomposition of lignin from sugar cane bagasse during ozonation process monitored by optical and mass spectrometries. J. Phys. Chem. B 117, 3110–3119.

44. Staehelin, J., Hoigne, J., 1985. Decomposition of ozone in water in the presence of organic solutes acting as promoters and inhibitors of radical chain reactions. Environ. Sci. Technol. 19, 1206–1213.

45. Sugimoto, T., Magara, K., Hosoya, S., Oosawa, S., Shimoda, T., Nishibori, K., 2009. Ozone pretreatment of lignocellulosic materials for ethanol production:

Improvement of enzymatic susceptibility of softwood. Holzforschung 63, 537–543.

46. Travaini, R., Marangon-Jardim, C., Colodette, J.L., Morales-Otero, M.D., Bolado-Rodríguez, S., 2014. Chapter 7 - Ozonolysis, in: Pandey, A., Negi, S., Binod, P., Larroche, C. (Eds.), Pretreatment of Biomass - Processes and Technologies. Academic Press, USA, pp 105-135.

47. Travaini, R., Otero, M.D.M., Coca, M., Da-Silva, R., Bolado, S., 2013. Sugarcane bagasse ozonolysis pretreatment: Effect on enzymatic digestibility and inhibitory compound formation. Bioresour. Technol. 133, 332–339.

48. Ueda, C., Takashima, Y., Ishiguri, F., Iizuka, K., Yoshizawa, N., Yokota, S., 2014. Ozone oxidation pretreatment for enzymatic saccharification of spent culture media after *Lentinula edodes* cultivation. J. Wood Sci. 61, 65–69.

49. Vidal, P.F., Molinier, J., 1988. Ozonolysis of lignin — Improvement of in vitro digestibility of poplar sawdust. Biomass 16, 1–17.

50. Wu, J., Ein-Mozaffari, F., Upreti, S., 2013a. Effect of ozone pretreatment on hydrogen production from barley straw. Bioresour. Technol. 144, 344–349.

51. Wu, J., Upreti, S., Ein-Mozaffari, F., 2013b. Ozone pretreatment of wheat straw for enhanced biohydrogen production. Int. J. Hydrogen Energy 38, 10270–10276.

52. Yokota, S., Iizuka, K., Ishiguri, F., Abe, Z., Yoshizawa, N., 2006. Ozone–dioxane delignification from the cell walls of Japanese cypress (*Chamaecyparis obtusa* Endl.). J. Mater. Cycles Waste Manag. 8, 140–144.

53. Yu, Z., Jameel, H., Chang, H.-M., Park, S., 2011. The effect of delignification of forest biomass on enzymatic hydrolysis. Bioresour. Technol. 102, 9083–9089.

Figure captions

Figure 1. Different reactor designs used in ozonolysis experiments: (a) batch reactor, (b) rotatory reactor, (c) Drechsel trap reactor, (d) cylindrical reactor, (e) bubble column reactor, (f) multi-layer fixed bed reactor and (g) fixed-bed reactor.

Figure 2. Adapted from Li et al. (2015). Ozone consumption on maize stover ozonolysis for three particle size ranges (20/40, 80/150 and <300 mesh): (a) as a function of moisture content and (b) as a function of water activity.

Figure 3. Adapted from Shi et al. (2015). Ozonolysis of corn straw: (a) effect of ozone consumption on delignification and water-soluble fraction release and (b) correlation between delignification and enzymatic digestibility.

Figure 4. Adapted from Souza-Correa et al. (2013a). Delignification efficiency as a function of ozone concentration absorbed on the sugarcane bagasse surface, working with 50% moisture and average size of 0.5 mm. The standard deviations on both axes were equal to or less than 5%.

Table 1

Advantages and disadvantages of ozonolysis pretreatment for lignocellulosic biomass compared with other pretreatments.

Advantages	Disadvantages
Low generation of inhibitory compounds, and specially the no generation of furfural and HMF (which might hinder following downstream stages)	Highly reactive, flammable, corrosive and toxic characteristics of ozone, leading to potentially dangerous processes
Selective lignin degradation with minimal effects on cellulose and hemicellulose	Exothermic characteristics of process may require cooling systems
Operation at ambient temperature and pressure	Special - construction materials, capable of resisting ozone high-oxidative condition: austenitic stainless steel, glass and other ceramics, Teflon, Hypalon and concrete
On-site ozone generation and direct utilization (avoiding problems related to chemical supply and storage)	
Ability of microorganisms and animals to metabolize most ozonolysis subproducts	High generation costs due to large energy demand (oxygen and electric energy costs around 0.135 € per kg of generated ozone; ozone generator equipment maintenance costs not included)[a]
Reduction of environmental pollution with a suitable process design, decomposing residual ozone at the end of the process	
Absence of liquid phase, avoiding problems related to product dilution	
No requirement of chemical additives during all the pretreatment process	

[a]. Information provided by Jose Antonio del Rey Martin. Operation manager of ViAQUA - Gestión de Aguas de Galicia SAU, Agbar group, Santiago de Compostela, Spain.

Table 2
Published ozonolysis process parameters providing the highest delignification and enzymatic hydrolysis yields.

Raw material	Reactor design	Moisture content (%)	Particle Size (mm)	Ozone concentration (%)	Flow (L/h)	Time (h)	Ozone consumption[a]	Delignification (%)[b]	Enzymatic hydrolysis (%)[c] NW	W	Reference
Wheat straw	Batch	95	<0.2	0.44	60	5	30	50	75 G	--	Binder el al. (1980)
	Fixed bed	40	3-5	2.7	60	2.5	--	34	88.6 G	--	García-Cubero et al. (2009)
		50	1	0.6-1	36	3	33	60	--	78 G	Schultz-Jensen et al. (2013b)
		50	1	0.6-1	36	1	--	--	49 G[d]	--	Kádar et al. (2015)
Rye straw	Fixed bed	60	3-5	2.7	60	2.5	--	45	57 G	--	García-Cubero et al. (2009)
Sugarcane bagasse	Fixed bed	80	3-5	3.44	60	1	12	66.8	42 G / 33 X	46 G / 24 X	Travaini el al. (2013)
Cotton stalks	Fixed bed	35	2	2.1	22.2	2.5		42.38	53 G / 74 X	--	Kaur et al. (2012)
Maize stover	Fixed bed	60	<0.053	2.8	60	1	25.6	78	80 G / 54 X	80 G / 54 X	Li et al. (2015)
Coastal Bermuda grass	Rotatory	30	<2	25	60	1	26.4	31 TL*	53 G / 23 X	--	Lee et al. (2010)
Energy grass	Fixed bed	30	<2	1.9	15	2	--	>51	-- / --	100 G / 60.6 X	Panneerselvam et al. (2013a)
Spent culture media	Rotatory	40	<5	6	3	1	--	~47% KL	21.8 G[e] / 6.5 X[e] / 0.3 GL[e]	--	Ueda et al. (2015)
Red oak	Cylindrical	50	0.106	3.37	30	2	--	--	89 G	--	Neely (1984)
Poplar sawdust	Fixed bed	30	2	1.63	60	3	--	72*	57 RS	--	Vidal and Molinier (1988)
Aspen wood chips	Fixed bed	40	0.6-1	2.8	10	1.67	--	--	49 RS	--	Ben'ko et al. (2013a)

[a] g of consumed ozone by 100g of biomass

[b] acid insoluble lignin decreases in regard to the initial biomass content. Abbreviations: KL: Klason lignin, TL: total lignin.

[c] percentage of original polymer converted into its respective monomers. Abbreviations: NW: non-washed, W: washed, G: glucose, X: Xylose, GL: galactose, W: washing step before enzymatic hydrolysis.
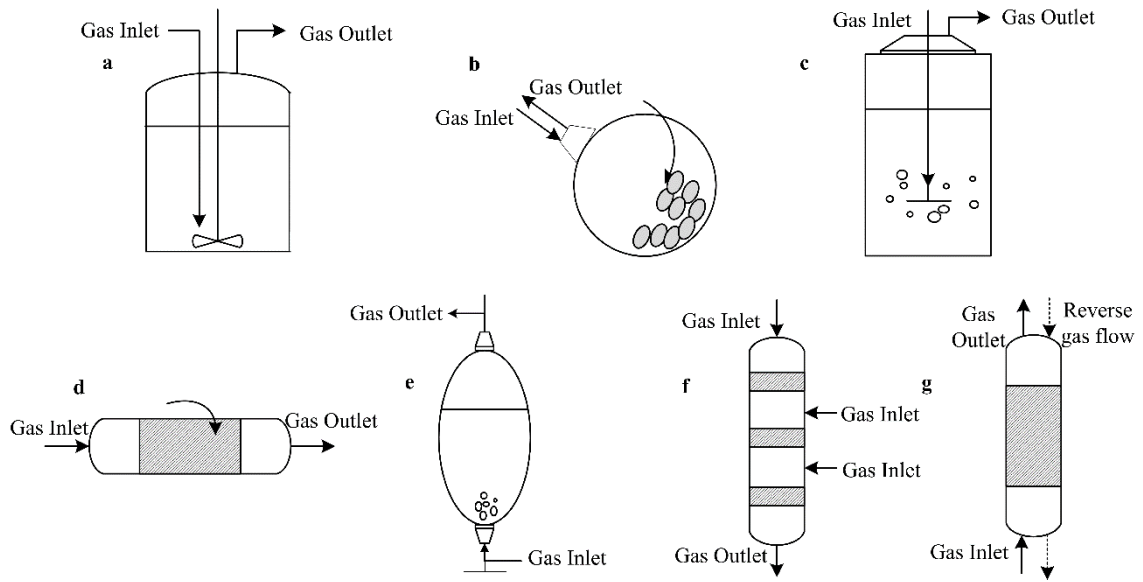
[d] only the lower third of the reactor height was used.
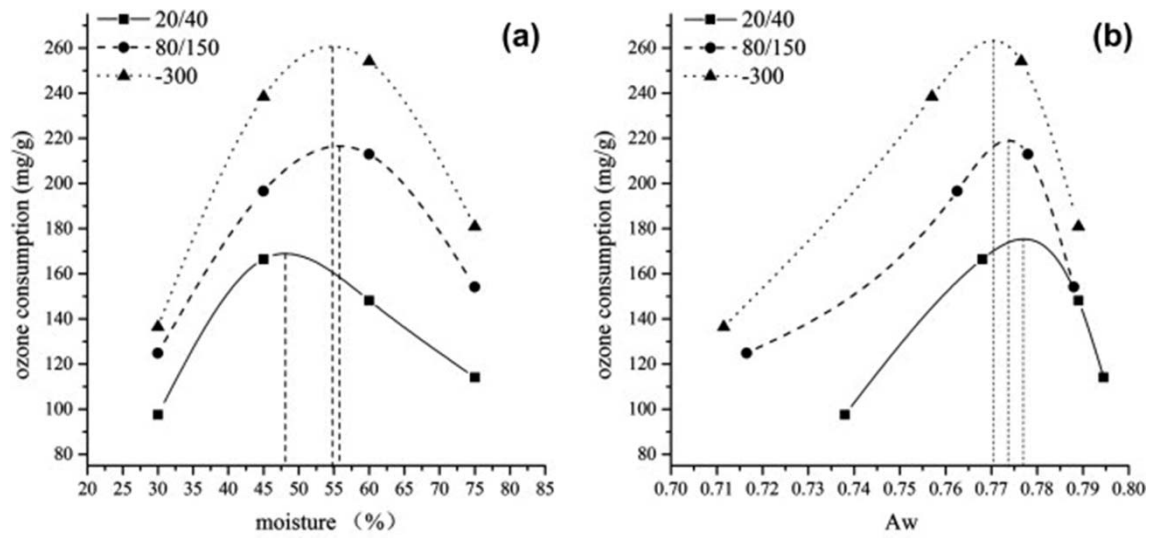
[e] g per 100 g of raw material
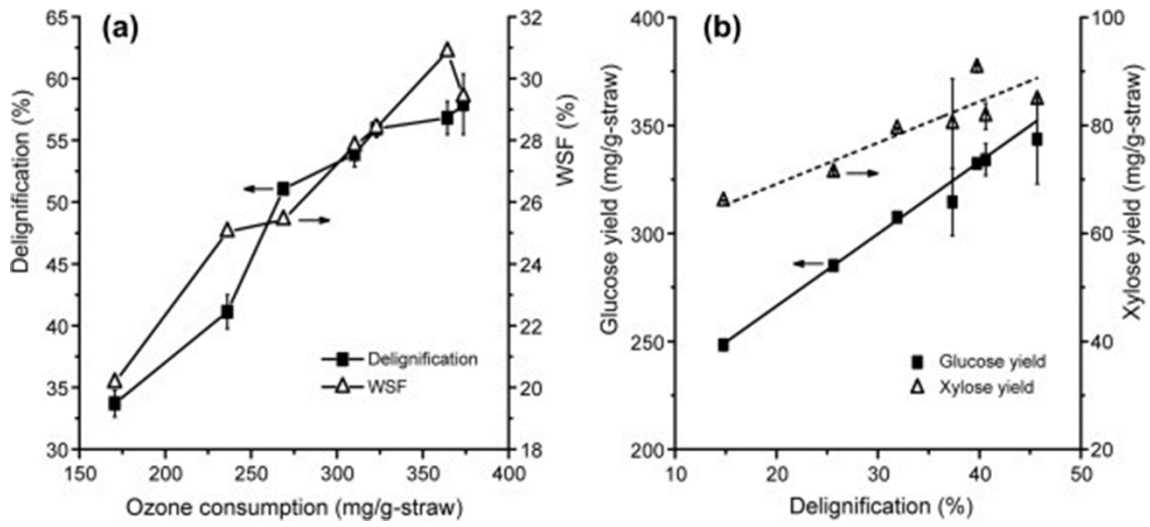
Figure 1.

Figure 2.

Figure 3.

Figure 4.