

**OXIDATION PROCESSES IN BIOREFINERIES, FROM PRE-TREATMENT TO FINAL PRODUCTS**

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**ABSTRACT**

The biorefinery technology aims to produce renewable fuels and chemicals from lignocellulosic non-edible biomass waste. To achieve sustainability, it must isolate and utilise all biomass fractions, i.e. cellulose, hemicellulose and lignin. In line with the refinery processes, the first and most costly stage is the fractionation of biomass. This is followed by the conversion of the biomass fractions into fuels and chemicals by chemical and biochemical methods. In recent years, oxidation processes for biomass utilisation have been developed; they are of particular interest as they can accelerate the individual steps and provide high value-added products. Oxidative delignification is an effective way to remove lignin and increase the hydrolysis of cellulose. Furthermore, the development of oxidative processes for conversion of sugars and lignin is of particular interest; it requires the development of new selective catalytic systems to ensure high yields towards desired products.

In this work, we reviewed current research progress in the following oxidative processes: a) pre-treatment of biomass, b) upgrading of sugars and c) production of oxidants with Power to X technologies. We evaluated a pretreatment based on the replacement of commonly used inorganic acids, such as sulfuric acid, with O<sub>2</sub> gas; this resulted in low degradation by-product yields, high degrees of delignification (DD) and good quality biomass fractions. We investigated several process parameters such as solvent, temperature, O<sub>2</sub> addition and the use of polyoxometallic salts (POMs) that can enhance oxidative delignification. Higher temperatures and longer processing times were beneficial in producing pulps with low lignin content (~1 % w/w) while maintaining high cellulose (100 %) and hemicellulose (40 %) recovery in the solid. The remaining hemicellulose was recovered as soluble oligosaccharides that were not degraded due to the absence of organic acids. Cellulose and hemicellulose sugars can be biochemically converted to different products such as ethanol, lactic acid, omega-3 fatty acids. Alternatively, their oxidation to high value-added chemicals such as aldonic and aldaric acids is of particular interest using photo- and electro- catalytic technologies and materials already developed in the context of hydrogen production and wastewater treatment.

**KEYWORDS:** biorefinery, delignification, sugar oxidation, photoelectrocatalysis, aldonic and aldaric acids

**INTRODUCTION**

Biorefineries aim to transform biomass into a spectrum of valuable products mimicking their petrochemical counterparts. The development of biorefineries has to deal with the additional challenge of biomass variety and complexity. Different types of biomass can and should be fed to biorefinery processes such as lignocellulosic materials, agricultural residues, and algae. The capability to valorise different types of biomass is crucial as it reduces the dependence on fossil

resources, mitigates environmental impact, and contributes to the development of a circular and bio-based economy. Biorefineries provide a platform for the production of a diverse range of chemicals, including biofuels, organic acids, and specialty chemicals. The inherent flexibility of these novel processes is a key advantage that creates opportunities for a wide array of high-value products.

There are a number of challenges that need to be overcome so that biorefineries can be commercially viable. The major challenge that needs to be addressed is the complexity of the feedstock, that is lignocellulosic biomass. Its complexity makes its efficient fractionation and conversion of each fraction into desirable products harder and more costly. Moreover, biomass does not have a steady composition and necessitates tailored approaches for effective processing, making achieving consistent product yields more difficult<sup>1</sup>. Biorefinery-based chemical production includes high capital and operational costs, hence developing economically competitive processes is critical to address challenges related to scale-up and cost-effectiveness. Therefore, it is imperative that new processes, technologies and catalytic systems are developed, specifically tailored to biomass valorisation. There are three key aspects in biorefineries that are crucial if they are to become sustainable and economically viable alternatives to petrochemical refineries: biomass pretreatment, biomass fraction conversion and integration with power-to-x technologies. Oxidative processes present interesting alternatives to the conventional ones; when coupled with power-to-x technologies and the production of “green” oxidative media they can have close to zero carbon footprint. In this work we review the state of the art and the future potential of these three areas of research and discuss some advantages of oxidative pretreatment.

### **Traditional biomass pretreatment**

The main objective of the pretreatment is to remove lignin, a polyphenolic polymer that surrounds the cellulose and hemicellulose, protecting them from pathogens, microorganisms, and enzymes<sup>2</sup>. Hence, pretreatment disrupts the complex carbohydrate–lignin complex, facilitating the subsequent extraction of sugars and other valuable components<sup>3</sup>. Various pretreatment methods have been extensively studied, ranging from physical methods, such as milling and extrusion, to chemical methods, including acid and alkali pretreatments<sup>4</sup>. Organosolv pretreatment is an interesting option as it is very efficient in delignifying lignocellulosic biomass and producing a high quality and purity cellulose pulp. However, organosolv pretreatment has some critical limitations regarding the equipment and process costs, as well as its ability to efficiently delignify softwood species that are more recalcitrant to this process. Addition of inorganic acids increases the severity of the pretreatment, and hence the delignification efficiency, but necessitates the use of more expensive corrosion resistant equipment and the post-treatment of the acidic liquid streams<sup>5</sup>.

### **Oxidative biomass pretreatment**

There are several different oxidative processes that have been developed for biomass pretreatment.

#### Wet Oxidation

Wet oxidation is a promising option; its main advantage is its ability to efficiently delignify lignocellulosic biomass; it has a minimal impact on cellulose, particularly when conducted under moderate conditions. The solubilized lignin fragments can be further valorized for high-value bioproducts<sup>6</sup>. Wet oxidation benefits the lower production of furfural and HMF, strong inhibitors in the fermentation step. However, the hemicellulose fraction is partly lost; this leads to a reduction in sugars yield. Wet oxidation requires elevated temperatures and pressures, it's an energy-

intensive process. Moreover, it creates a corrosive environment that requires corrosive resistant materials for the reactor construction. All of the above make its commercialization difficult. So far wet oxidation has been mostly studied for the treatment of aqueous wastewaters as it has low operating costs and minimal air pollution discharges, while the main limitations are the high capital costs and safety implications associated with a system operating at severe operating conditions<sup>7</sup>.

Hydrogen Peroxide: Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) has emerged as a versatile and environmentally friendly reagent for biomass pretreatment. Its key advantages are that it is an environmentally benign oxidant, avoiding the use of harsh chemicals and generating water and oxygen as byproducts, minimizing environmental impact and reducing the need for additional waste management<sup>8</sup>. It is easy to degrade with minimal toxicity to the environment. It occurs naturally, one of its uses in organisms is the decomposition of lignin, hence it is highly selective in lignin degradation. On the other hand, hydrogen peroxide pretreatment often requires longer reaction times, it is still quite expensive and because it degrades easily and cannot be recycled it is more difficult to apply it in commercial scale.

Ozon: Ozonolysis involves the use of ozone (O<sub>3</sub>) to cleave lignin and cellulose linkages within biomass, facilitating subsequent enzymatic hydrolysis and increasing fermentable sugar yields. Recent advancements in ozonolysis technology have focused on improving reactor design and optimizing process parameters to enhance efficiency. Its main advantages are that it is highly selective towards lignin degradation and that it maintains the formation of inhibitory by-products at low levels. However, ozone is still expensive and reactor scale-up and integration into existing biorefinery processes are challenging and more research is needed on cost-related challenges<sup>9</sup>.

Oxidative organosolv: Recent advancements of the more established organosolv pretreatment involves the use of organic solvents in the presence of oxygen or air to selectively extract lignin from biomass. Our group has developed an oxidative organosolv process, named OxiOrganosolv<sup>10</sup> that combines the advantages of organosolv and wet air oxidation. Its main advantages are that it is very efficient and selective in lignin removal while preserving cellulose and hemicellulose intact, it is highly compatible with different types of feedstock, it minimizes sugar degradation and the production of inhibitory by-products. The main challenges that need to be overcome are associated with solvent recovery and recycling that can have a significant effect on the process cost. Moreover, reducing the required temperature and pressure for biomass pretreatment is a critical aspect. In our latest work we have utilized different types of poly-oxo-metalate (POM) catalysts in an effort to reduce process severity while maintaining process efficiency and selectivity.

Power-to-X: Power-to-X technologies are a rapidly evolving field with significant potential for various applications, including the production of oxidative media such as ozone, hydrogen peroxide, and oxygen<sup>11</sup>.

**Ozone Production:** Power-to-X technologies for ozone production typically involve electrolysis of water followed by the conversion of oxygen into ozone through various methods, such as corona discharge or ultraviolet radiation. Electrolysis of water to produce oxygen is a well-established process with efficiencies typically above 70-80%<sup>12</sup>. However, the efficiency of converting oxygen into ozone depends on the method used (e.g., corona discharge, UV radiation), with reported efficiencies ranging from 10% to 30%<sup>13</sup>. The on-site generation of ozone, that eliminates the need for transportation and storage of ozone gas and the fact it can be produced by renewable electricity are its clear advantages.

**Hydrogen Peroxide Production:** Hydrogen peroxide production involves the electrochemical

reduction of oxygen or direct synthesis from water and oxygen. The electrochemical reduction of oxygen can achieve high Faradaic efficiencies, often exceeding 70-80%<sup>14</sup>. The direct synthesis of hydrogen peroxide from water and oxygen is still under development, with reported efficiencies varying<sup>15</sup>. Achieving high selectivity for hydrogen peroxide production over competing reactions and developing stable catalysts over extended operation periods are the main challenges of these approaches. A key process advantage of these systems is that it is easy to scale them up via a modular design. The main challenge that needs to be overcome is cost related; current hydrogen peroxide production methods, cannot compete with traditional manufacturing processes based on anthraquinone oxidation.

Oxygen Production: Oxygen (O<sub>2</sub>) is widely used in industrial processes, medical applications, and aerospace. Power-to-X technologies for oxygen production typically involve electrolysis of water or air separation processes. This is a mature technology with high efficiencies, typically exceeding 70-80%, the need for high H<sub>2</sub> production will also ensure that O<sub>2</sub> supply is high.

In conclusion, power-to-X technologies show promise for the production of oxidative media such as ozone, hydrogen peroxide, and oxygen, offering potential advantages in terms of renewable energy integration, on-site generation, and purity. However, significant challenges remain in improving energy efficiency, reducing costs, and scaling up these technologies for commercial deployment. Further research and development efforts are needed to address these challenges and unlock the full potential of power-to-X for oxidative media production. Combining the production of oxidative media with biorefinery processes to develop new oxidation conversion pathways can provide alternative routes to achieving commercially and environmentally sustainable biorefineries.

## METHODOLOGY

Beechwood (Lignocel<sup>®</sup> HBS 150–500, JRS GmbH and Co KG, Germany) was used as feedstock. The composition of the raw material was 40.1% cellulose, 19.1% hemicellulose and 23.6% lignin (Kalogiannis et al., 2020). OxiOrganosolv pretreatment process took place in a 975 mL Hastelloy C-276 Parr reactor, with solutions of 50:50 (w/w) H<sub>2</sub>O: organic solvent as liquid phase and O<sub>2</sub> as a catalyst. Acetone (ACO), ethanol (EtOH), isobutanol (iBuOH) and tetrahydrofuran (THF) were used as organic solvents. The weight ratio of dry solid biomass to solvent was 1:10. The reactor was pressurized with high-purity O<sub>2</sub> gas (99.5%), at a pressure of 16 bar at ambient temperature and then heated to a reaction temperature of 150 °C. The overall duration of the experiment was 2 h, where t<sub>0</sub> was the time the system first reached 150 °C. After cooling and depressurizing the reactor, the solid biomass was separated from the solution by vacuum filtration and washed with 500 and 750 mL of organic solvent and distilled water, respectively. It was then allowed to air dry until the weight of the sample was kept constant (typical moisture ~ 4%). The determination of cellulose, hemicellulose and lignin in the final pulp was done using the NREL method<sup>16</sup>.

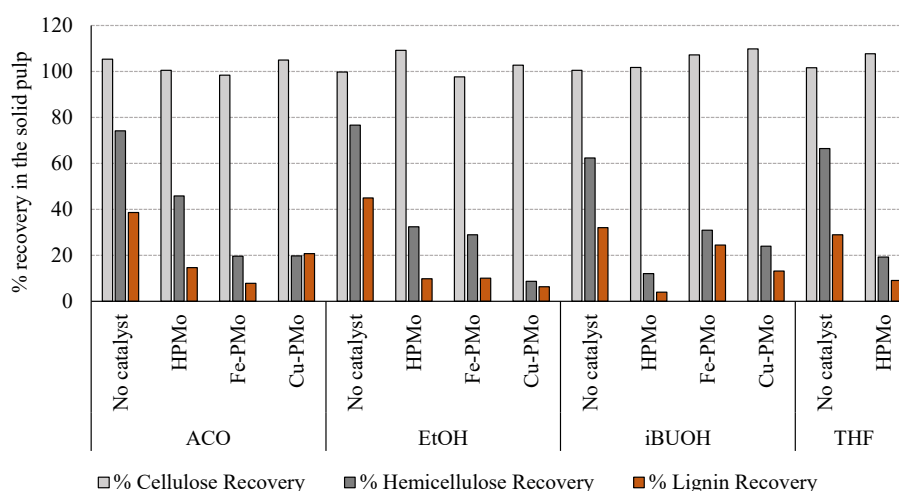
Commercial phosphomolybdic acid hydrate (H<sub>3</sub>PMo<sub>12</sub>O<sub>40</sub> x H<sub>2</sub>O or HPMo) was supplied by Sigma-Aldrich (US). Copper and iron-exchanged phosphomolybdic acid salts (M<sub>3</sub>PMo<sub>12</sub>O<sub>40</sub>, with M=Cu or Fe) were prepared as follows: 5 mmol H<sub>3</sub>PMo<sub>12</sub>O<sub>40</sub> x H<sub>2</sub>O were dissolved in 50 mL of distilled water. The above solution was added dropwise to a 50 mL aqueous solution of 1 M CuCl<sub>2</sub> or FeCl<sub>2</sub> x 4 H<sub>2</sub>O under vigorous stirring at room temperature. The mixture was stirred for about 30 min and then the temperature was raised to 50 °C and stirring was continued for 60 min, followed by drying at 80 °C. The final dried solid salts (Cu<sub>3</sub>PMo<sub>12</sub>O<sub>40</sub> or Cu-PMo and Fe<sub>3</sub>PMo<sub>12</sub>O<sub>40</sub> or Fe-PMo) were burned at 200 °C for 4 h with a temperature increase rate of 5 °C/min using air. Chemical composition was determined by Inductive Coupled Plasma - Atomic Emission Spectroscopy (ICP-AES).

## RESULTS AND DISCUSSION

Preliminary results with ACO and HPMo were conducted on a catalyst loading of 2%, 5% and 10% of the initial biomass; this corresponds to 0.2, 0.5 and 1 wt% in the aqueous liquid mixture that consisted of 50% (w/w) organic solvent. An increase in catalyst concentration resulted in pulps with higher cellulose and lower hemicellulose and lignin content, reaching the maximum delignification rate (DD) of 94.5% when 10% of catalyst was used. However, the enzymatic hydrolysis showed that saccharification of the pulp was higher with that of catalyst loading of 5%. Therefore, the above condition was selected to evaluate different organic solvents.

The results of POM-assisted OxiOrganosolv process showed that upon addition of the HPMo catalyst to all four water-organic solvent mixtures, a significant increase in the DD is achieved, especially in the case of EtOH (55% and 90.2% in the absence and presence of HPMo, respectively) and iBuOH (67.9% and 96% in the absence and presence of HPMo, respectively). The high delignification efficiency of HPMo can be attributed to its size that is similar to that of phenylpropanoid units in lignin (~1.1 nm)<sup>17</sup>. Regarding the composition of the solid pulp, in all cases, the addition of HPMo led to a decrease in lignin and hemicellulose content, clearly verifying the removal of these fractions. Significant solubilization of hemicellulose was observed, which was more profound in the case of iBuOH (hemicellulose removal 37.7% and 87.9% without and with catalyst) and THF (hemicellulose removal 33.6% and 80.7% without and with catalyst), as shown in Fig. 1. Apart from its robust oxidative potential, HPMo has distinct acidity properties, thus it is likely assisting the removal of hemicelluloses via hydrolysis, leaving the cellulosic fraction almost untouched.

The cellulose-rich pulps obtained after POM-assisted OxiOrganosolv process were subjected to enzymatic hydrolysis towards the production of fermentable monosaccharides. When ACO and EtOH were used as organic solvents, % cellulose conversion to glucose looks almost similar either in the presence or in absence of HPMo. However, due to the fact that the solid pulps obtained with HPMo have a higher cellulose content, the final glucose yield is higher upon addition of the catalyst, namely 46.3 and 51 g/L with HPMo compared to 38.1 and 35.2 g/L without catalyst, for ACO and EtOH, respectively.



**Figure 1.** Lignin, cellulose and hemicellulose recovery yields in the solid pulp on a catalyst loading of 5% of the initial biomass.

## CONCLUSIONS

In the present work, an oxidative organosolv process was developed for the fractionation of beechwood by employing polyoxometalate complexes as homogeneous catalysts alongside with O<sub>2</sub> as an oxidant. The results showed that not only the commercially available HPMo, but also the in-house produced Fe- and Cu-exchanged salts promoted oxidation of lignin, which was further solubilized into the organic phase and removed, thereby yielding solid pulps with much higher cellulose content compared to those pretreated in the absence of catalyst. Evaluation of the enzymatic saccharification efficiency of the solid fractions indicated that HPMo was the most favorable catalyst to increase cellulose digestibility and glucose yields.

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## REFERENCES

1. Serrano-Ruiz, J.C. & Dumesic, J.A. *Energy Environ. Sci.* **4**, 83–99 (2010).
2. Studer, M.H. et al. *Proc. Natl. Acad. Sci.* **108**, 6300–6305 (2011).
3. Wyman, C.E. et al. *Bioresour. Technol.* **96**, 1959–1966 (2005).
4. Mosier, N. et al. *Bioresour. Technol.* **96**, 673–686 (2005).
5. Kalogiannis, K.G. et al. *Mol. Basel Switz.* **23**, 1647 (2018).
6. Sun, S., Sun, S., Cao, X. & Sun, R. *Bioresour. Technol.* **199**, 49–58 (2016).
7. Kolaczowski, S.T., Plucinski, P., Beltran, F.J., Rivas, F.J. & McLurgh, D.B. *Chem. Eng. J.* **73**, 143–160 (1999).
8. Dutra, E.D. et al. *Biomass Convers. Biorefinery* **8**, 225–234 (2018).
9. Coca, M., González-Benito, G. & García-Cubero, M.T. *Biomass Fractionation Technol. Lignocellul. Feedstock Based Biorefinery* 409–429 (2016).doi:10.1016/B978-0-12-802323-5.00018-9
10. Kalogiannis, K.G. et al. *Bioresour. Technol.* **313**, 123599 (2020).
11. Bockris, J.O. & Otagawa, T. *J. Electrochem. Soc.* **131**, 290 (1984).
12. Chisholm, G., Zhao, T. & Cronin, L. *Storing Energy Second Ed.* 559–591 (2022).doi:10.1016/B978-0-12-824510-1.00015-5
13. Rodríguez-Peña, M. et al. *Curr. Opin. Electrochem.* **27**, 100697 (2021).
14. Wang, N., Ma, S., Zuo, P., Duan, J. & Hou, B. *Adv. Sci.* **8**, 2100076 (2021).
15. Flaherty, D.W. *ACS Catal.* **8**, 1520–1527 (2018).
16. A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, D. Crocker, NREL, 2012
17. Bujanovic, B., Ralph, S., Reiner, R., Hirth, K. & Atalla, R. *Materials* **3**, 1888–1903 (2010).