

Green Process for Transforming Lignocellulosic Residues into Novel Bio-Based Vitriimer Composites

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One of the main research challenges in recent years has been to valorize waste and by-products coming from the agro-food industry. To this purpose, the CYCLEVIT project aims to valorize lignocellulosic residues to produce vitrimers. In particular, this work aims to present a more sustainable process for lignin and cellulose recovery compared to the traditional one which involves acid and alkaline hydrolysis. Indeed, cellulose and lignin are the basis to produce vitrimers. The key idea of the process presented was to combine autohydrolysis, to remove the hemicellulose from raw material, and organosolv to separate lignin from cellulose. The optimal condition for the recovery of lignin and cellulose was identified for the autohydrolysis process, based on both previous scientific work and a validated mathematical model. The solid obtained from autohydrolysis was subsequently subjected to the organosolv process, to evaluate the optimal condition for lignin and cellulose recovery. Different temperatures (180°C-200°C) and solid-liquid ratio (1:10 -1:20 g/ml) were tested. It is interesting to point out that the 60 % v/v of ethanol solution used for this process was obtained by mixing the ethanol with the liquor resulting from the autohydrolysis process. Based on the results obtained, a first process layout was proposed which allows a cellulose and lignin recovery of approximately 75% and 21% respectively and water savings of about 35%.

1. Introduction

Nowadays, the agricultural and food industries generate significant amounts of residues. Some of these wastes are by-products that could be reused to obtain new products with high added value, such as food additives, biofuels, biochemicals, or energy (Narisetty et al., 2023). In this regard, many studies and research have been done, but many challenges are still open, including the production of high-added value products from natural sources instead of fossil ones. In this framework, the CYCLEVIT project (funded by Fondazione Cariplo) has the aim to replace fossil-based thermosetting polymers with lignin and cellulose-based ones, the Vitrimers. The latter are a new class of materials that at room temperature behave like classic thermosetting polymers, but, when heated, gradually decrease their viscosity flowing like viscoelastic fluids (Röttger et al., 2017). Moreover, compared to traditional thermoset materials, they can be repaired, reshaped, and reprocessed by extrusion or injection significantly decreasing the environmental impact (Yue et al., 2019). One of the challenges of the CYCLEVIT project is the sustainable recovery and production of cellulose and lignin starting from lignocellulosic residues like wheat straw, rice husks, etc. As reported in scientific literature, one of the most effective ways to extract cellulose and lignin from residues is diluted acid hydrolysis followed by alkaline hydrolysis (Vadivel et al., 2017). The acid hydrolysis, usually done with sulfuric acid, aims to break down hemicellulose into sugars while the alkaline hydrolysis is carried out to treat the solid recovered from the acid hydrolysis to purify the cellulose dissolving the lignin. The latter can be recovered from the alkaline liquor through acid precipitation, using for instance sulfuric acid (Lee H.V. et al., 2014). Despite being effective, these treatments are not sustainable from an environmental point of view, mainly because they need fresh reagents for each batch and they produce a large volume of acidic and alkaline liquor that have to be properly disposed of, increasing also

the cost of the process. For these reasons, the aim of this work was to present and discuss a greener process for the recovery of cellulose and lignin from agro-food residues. The basic idea was to replace the acid and alkaline treatments with autohydrolysis and organosolv treatment, respectively. Indeed, the autohydrolysis treatment reaches the same scope as the acid hydrolysis, that is the degradation of hemicellulose, using only water as solvent, operating at mid-pressure and slightly higher temperature (Fiorentini et. al., 2022). On the other hand, the organosolv process is applied to separate lignin from the cellulosic fraction using 60% v/v ethanol as solvent, thus avoiding the use of NaOH for alkaline treatment (de la Torre et. al., 2013). Despite the several studies in the scientific literature regarding these treatment processes, to the authors' knowledge, there aren't extensive and in-depth studies that have been carried out to recover cellulose and lignin combining autohydrolysis and organosolv. Therefore, a first investigation of this novel process has been done in this work. In particular, wheat straw was selected as the raw material on which to conduct the initial trials given both its high content of cellulose and lignin, and the large amount of information that can be found in the scientific literature related to the autohydrolysis process (Bassani et. al., 2021). In this way, it was possible to focus more attention on the organosolv treatment where some additional trials were done at different temperatures from 180°C to 200°C, and different solid-to-liquid ratios, from 1:10 g/ml to 1:20 g/ml, to set the best conditions for lignin and cellulose recovery. Moreover, it's important to point out that, to further optimize the process, the liquor coming from autohydrolysis was recycled in the organosolv process to obtain the 60% v/v ethanol solution. In this way, it is possible to reduce water consumption and avoid the disposal of acid liquor while at the same time increasing the recovery of lignin. Indeed Nitsos et al. (2016) reported that acid compounds can act as catalysts during the organosolv process, promoting the de-lignification and autohydrolysis liquor contains a certain amount of acetic acid (Fiorentini et al., 2022).

2. Material and Methods

As mentioned, wheat straw was selected as raw material for the initial trials. Wheat straw was ground using a hammer mill with a 2 mm diameter sieve and was subjected to the lignocellulosic fractionation process illustrated in Figure 1. As reported in the introduction, such lignocellulosic fractionation process can be divided into two main treatments: autohydrolysis and organosolv whose main purpose is to allow the separation and isolation of lignin and cellulose from the solid obtained from the first autohydrolysis treatment.

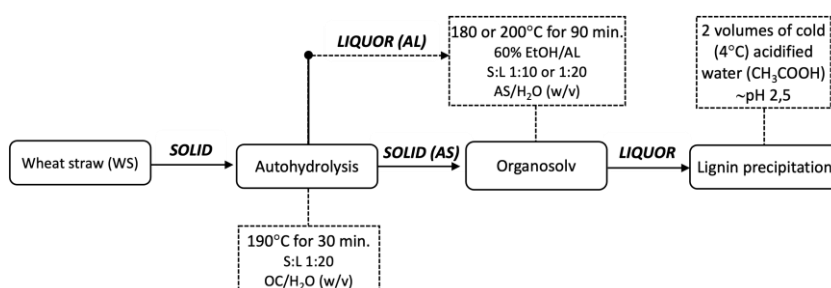


Figure 1 - Fractionation process for lignin and cellulose recovery from wheat straw - Experimental plan

2.1 Autohydrolysis Treatment

This treatment was conducted in a high-pressure reactor (Highpreactor BR-1000, 990 mL, Berghof, Germany) into which the wheat straw sample and distilled water were placed. The treatment was conducted at 190°C for 30 minutes with a solid-to-liquid ratio of 1:20. These conditions were selected with the goal of almost completely degrading hemicellulose while maximizing cellulose recovery. This was achieved through the use of a mathematical model developed in a previous work regarding wheat straw autohydrolysis (Bassani et. al., 2021). Furthermore, these conditions are also confirmed in the work of Fiorentini et. al. (2022) where similar conditions were found in high cellulose recovery. Subsequently, the hydrolyzed sample was filtered to separate the acidic liquor from the solid pulp using Whatman paper filters (N°589/2). The liquid fraction mainly contains water, sugars and acetic acid derived from the solubilization of hemicellulose and sugar degradation products while the solid fraction was dried at 60°C for 24 hours and contains mainly lignin and cellulose. To determine the proportions of lignin, cellulose, and hemicellulose in the autohydrolysis solids, a structural carbohydrate analysis was performed.

2.2 Organosolv Treatment

To separate lignin from cellulose in the solid recovered from the autohydrolysis the organosolv process was performed. The organosolv treatment was carried out in the same reactor used for the autohydrolysis (Highpreactor BR-1000, 990 mL, Berghof, Germany). As reported in the introduction, organosolv was tested at two different temperatures (180°C and 200°C) and two different solid-to-liquid ratios (1:10 and 1:20 g/ml), keeping the treatment time (90 minutes) and solvent concentration (60% v/v of ethanol) constant in all cases. Moreover, the solvent is prepared using ethanol and, instead of water, the acid liquor, with a pH of about 4, comes from autohydrolysis. This acidity is related to the presence of acetic acid (Bassani et al., (2020) which can act as a catalyst for the organosolv process to promote lignin recovery, as also confirmed by Baruah et. al. (2018). It is important to emphasize that the choice of operating conditions was made based on the scientific literature (Sidiras et al. (2022)) and that it was decided to vary only two parameters (i.e., temperature and solid-liquid ratio) as this is an initial study to verify the potentiality of the process. After the treatment, the mixture was filtered and the solid fraction was washed with distilled water and dried in an oven at 50°C for one night (Huijgen et al., 2010), while the liquid fraction was first acidified with two volumes of cold (4°C) acidified water (with acetic acid, final solution pH 2.5) and then centrifuged and filtered to separate the precipitate containing lignin. The composition of the recovered solids was evaluated also in this case using the structural carbohydrates analysis.

2.3 Fat and Protein Content

To determine the fat and protein content, the procedure described by Didaoui et. al. (2023) was followed. Petroleum ether was used as the solvent for fat extraction using the Soxhlet method, while the Kjeldahl method was employed to assess protein content.

2.4 Structural Carbohydrates

The analysis was carried out using the methodology described by Sluiter et al. (2013). In brief, the solid material underwent two consecutive acid hydrolysis steps: the first using 72% sulfuric acid at 30°C for one hour, and the second using 4% (v/v) sulfuric acid at 121°C for one hour. Following the hydrolysis procedures, the lignin content was determined by weight difference relative to the initial sample. The hemicellulose and cellulose contents were estimated from the glucose and xylose concentrations in the resulting liquor, using factors of 0.88 and 0.9, respectively, to account for the non-sugar components associated with these carbohydrate fractions.

3. Results and Discussion

Initially, the composition of wheat straw was evaluated to properly assess the potential recovery of both cellulose and lignin at the end of the fractionation process. The results are reported in Table 1 and are in line with what has been reported by Fiorentini et al., (2022), where, for instance, the cellulose and total lignin content are about 31.5% and 21.3% respectively.

Table 1 - Wheat straw characterization

	Composition		Composition
Moisture content (%)	7.58 ± 0.16	Acid insoluble lignin (% on dw)	17.44 ± 0.61
Fat (% on dw)	1.45 ± 0.04	Acid soluble lignin (% on dw)	1.74 ± 0.03
Proteins (% on dw)	4.03 ± 0.13	Hemicellulose (% on dw)	15.21 ± 0.08
Ashes (% on dw)	5.35 ± 0.14	Cellulose (% on dw)	35.85 ± 0.50

3.1 Autohydrolysis treatment

As indicated in Figure 1, the autohydrolysis process is the first fractionation phase carried out on the raw material. Table 2 reports the composition of the lignocellulosic fraction in the solid recovered after the autohydrolysis and the recovery yield of the lignocellulosic compounds evaluated considering that the solid yield of autohydrolysis treatment is 57.59% ± 0.24 on dm. This latter value is in agreement with what was reported by Fiorentini et al. (2022), in which a solid recovery of 60.9% ± 2.7 was found. Table 2 highlights that cellulose is the main component in the solid (54.33%), the second one is lignin (36.49%) while hemicellulose has been almost completely removed (1.96%). This aspect is in agreement with the goals of the treatment as autohydrolysis aims to degrade hemicellulose while maximizing cellulose recovery (82.50%). The latter recovery is in accordance compared with what was reported by Fiorentini et al. (2022). Moreover, the solid recovered has a composition that is almost all cellulose and lignin (more than 90%), making it suitable for the following organosolv treatment. The last comment concerns the recovery of lignin which is higher than 100%. This could be due to the formation of pseudo-lignin structures (Cheng et al., 2018) resulting from polymerization or

condensation reactions involving substances derived from furfural and hydroxymethylfurfural. The latter are byproducts of the degradation of xylose and glucose generated from the breakdown of hemicellulose and cellulose respectively. Consequently, it can be inferred that the effective recovery of lignin is approximately equal to or slightly below 100%.

Table 2 – Solid composition and recovery after autohydrolysis treatment

Compound	Content	Recovery (%)
Acid insoluble lignin (% on dw)	35.43 ± 0.67	117.82 ± 0.02
Acid soluble lignin (% on dw)	1.06 ± 0.02	35.18 ± 0.01
Hemicellulose (% on dw)	1.96 ± 0.20	18.11 ± 0.02
Cellulose (% on dw)	54.33 ± 1.09	82.50 ± 0.02

3.2 Organosolv treatment

The solid residue from autohydrolysis underwent an organosolv process to separate the lignin fraction that was tested in four different conditions (Figure 1). Table 3 reports the solid yield of each tested condition complying with what was expected. In fact, increasing the severity of treatment by raising the temperature and the amount of solvent promotes both lignin dissolution and cellulose degradation resulting in a lower solid yield (83.92% for the case of organosolv at 200°C and 1:20 solid-to-liquid ratio).

Table 3 - Solid yields of the organosolv treatment

	Organosolv 180°C		Organosolv 200°C	
	1:10	1:20	1:10	1:20
Solid yield (% on dw)	92.20 %	95.72 %	87.81 %	83.92 %

Since the main goal of organosolv treatment is to separate lignin from cellulose, preventing the degradation of the latter, it is necessary to verify in-deep what is the reason for this increase in degradation obtained for more severe treatments. In Table 4 are reported the compositions of the solid fraction coming from the organosolv process. It is possible to observe that the lignin content in the solid decreases at constant temperature increasing the solid-to-liquid ratio in both cases at different temperatures and, in the same way, decreases at constant solid-to-liquid ratio increasing the temperature. This confirms that more severe organosolv treatment leads to a higher de-lignification due to a higher hydrolytic cleavage of alpha aryl-ether links into fragments that are soluble in the solvent system, reducing the lignin content itself up to about 12%. Regarding cellulose, it is possible to observe similar trends that result in higher cellulose degradation at the most severe conditions. However, it is important to notice that cellulose content slightly changes between cases considering, for example, that the highest value found (i.e., about 68%) has a larger standard deviation than the other values. For this reason, it is possible to affirm that this value may not differ much from the other values obtained. Moreover, if the mean is considered, it is possible to highlight that the cellulose content in the solid residue slightly decreases, from about 68% to 59%, compared to the higher decrease of lignin content from about 36% to 12%. Therefore, more severe treatment allowed lignin to be better separated from cellulose, limiting its degradation for hydrolysis reaction.

Table 4 - Solid composition after organosolv treatments

Compound	Organosolv 180°C (%)		Organosolv 200°C (%)	
	1:10	1:20	1:10	1:20
Acid insoluble lignin (% on dw)	35.69 ± 1.13	20.68 ± 0.40	35.20 ± 2.41	12.15 ± 0.15
Acid soluble lignin (% on dw)	0.75 ± 0.03	0.92 ± 0.03	0.77 ± 0.02	1.79 ± 0.17
Hemicellulose (% on dw)	2.05 ± 0.07	1.35 ± 0.05	1.74 ± 0.03	1.15 ± 0.11
Cellulose (% on dw)	61.41 ± 1.07	66.59 ± 3.73	68.75 ± 4.25	59.16 ± 0.87

These results and considerations are also validated by the evaluation of the lignin recovery reported in Table 5. As mentioned, the lignin solubilized during the organosolv process, was recovered through an acid precipitation using a solution of cold water and acetic acid. In Table 5 it can be observed that the recovery yield of the treatment performed with a solid-to-liquid ratio of 1:20 is significantly higher than the treatment with a solid-to-liquid ratio of 1:10. In particular the organosolv performed at 200°C allowed to obtain a recovery of 20.52%, which is the highest yield obtained. Therefore, it is possible to affirm that the best conditions are those with a solid-to-liquid ratio of 1:20 at 200°C, which allows to obtain the higher lignin recovery (20.52%) with just a slight

cellulose degradation (recovery of 91.38%) and a final solid residue with a lignin content of about 12%. Finally, applying the operating conditions that maximize lignin recovery, an initial estimate of the actual savings concerning water consumption due to the reuse of the acid liquor generated during the autohydrolysis process.

Table 5 - Lignin recovery yield from Organosolv liquor

	Organosolv 180°C (%)		Organosolv 200°C (%)	
	1:10	1:20	1:10	1:20
Lignin recovery (% on dw)	5.87 ± 0.08	13.23 ± 0.18	5.90 ± 0.09	20.52 ± 0.28

Tables 6 and 7 show the inputs and outputs of the cellulose and lignin production process calculated for the production of 1 kg of vitrimers for both the processes that consider liquor recycling and the case without considering the recycling. It can be pointed out that the recycling of acid liquor allows for a significant reduction in water consumption (about 36%) while also eliminating the need to dispose of acid liquor further reducing the impact on the environment. It is worth noting that the estimation of the process without recycling was carried out considering the same operating conditions and yields as the process with acid liquor recycling. However, to obtain the same yields in both cellulose and lignin production, the organosolv treatment of the process without recycling should operate under more severe conditions (e.g., at a higher temperature) since it does not benefit from the catalytic effect given by the presence of acetic acid. Thus, the process of recycling benefits not only from a reduction in water consumption but also from a reduction in energy consumption associated with less severe treatments. A precise estimate of this reduction can be made in future studies. It is important to point out that, this was a first study in which the possibility of reducing waste was highlighted with the goal, in the future, of having a "zero waste" process. Clearly, it will be necessary to further optimize the process. For example, while obtaining a ready-to-use lignin, it is useful to further increase the ligning yield obtaining purer cellulose as a consequence, avoiding or reducing the downstream cellulose purification treatments (e.g., basic hydrolysis).

Table 6 – Input of the process with or without the recycling of the autohydrolysis acid liquor, evaluated from the production of 1 kg of vitrimers

	<i>With liquor recycling</i>	<i>Without liquor recycling</i>
Wheat straw (kg)	5.72	5.72
Water (l)	235.22	151.86
Ethanol (l)	44.35	44.35
Acetic acid (l)	6.85	6.85

Table 7- Output of the process with or without the recycling of the autohydrolysis acid liquor, evaluated from the production of 1 kg of vitrimers

	<i>With liquor recycling</i>	<i>Without liquor recycling</i>
Residual liquid after lignin precipitation (l)	148.65	148.65
Evaporated ethanol (l)	14.56	14.56
Evaporated water (l)	39.42	39.42
Sold residue from organosolv (kg)	2.98	2.98
Lost solids (kg)	2.17	2.17
Autohydrolysis liquor (l)	83.36	-

4. Conclusions

This research focused on the discussion and investigation of the combined treatments of autohydrolysis and organosolv for the recovery of cellulose and lignin from agri-food waste in a more sustainable way concerning the traditional process which involves acid and alkaline hydrolysis. Starting with wheat straw as the selected lignocellulosic residue, the two treatments were analyzed sequentially. As regards autohydrolysis, whose operating conditions were obtained using a previously validated mathematical model, an almost complete degradation of the hemicellulose has been evaluated and at the same time a high recovery of cellulose and lignin was evidenced. Then, the subsequent organosolv treatment allowed the separation of cellulose from lignin, obtaining a lignin recovery up to about 20% and a final solid whose lignin content was reduced up to 12% with a high recovery of cellulose. The optimal operating conditions of the latter treatment were investigated and selected by varying the operating temperature and the solid-to-liquid ratio. It is useful to highlight how this combined process resulted in an overall cellulose and lignin recovery from wheat straw of about 75.4% and 20% respectively, avoiding the use of acid and alkaline solvents that have a high environmental impact. Moreover,

the presented process included the recycling of the acid liquor produced during the autohydrolysis treatment for production with ethanol of solvent to be used in organosolv. This resulted in a savings of about 36% in water consumption based on a production of 1 kg of vitrimers, thus reducing the potential environmental impact. As mentioned, this work aimed to present the potentiality of this new process, which, however, can be further investigated and analyzed to increase its sustainability both from an economic and environmental point of view (e.g. LCA analysis). For example, the residual solid still contains a significant fraction of lignin (about 12%), and therefore the organosolv process can be further optimized to fully recover all the lignin present. A further example can be related to ethanol recovery. In fact, the residual liquor after lignin precipitation will have to be analyzed to verify its actual ethanol content and estimate its possible recovery.

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