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Simple and Combined Pretreatment of a Mixture of Forestry and Aromatic-Medicinal Plant Waste by Chemical, Physical and Enzymatic Methods

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ABSTRACT

Forestry waste (FW) extracted parts ofmedicinal-aromatic plant waste (EPW) and unused parts (UPW) are considered potential resources for energy recovery (their heating value of approximately 19 MJ/kg).In order to valorize lignocellulosic biomass, a pretreatment process is required to hydrolyze the recalcitrant lignocellulosic complex into fermentable simple sugars. The aim of this study is to determine the best method of pretreatment that takes into account treatment time, efficiency, and environmental friendliness. The mixture of FW, EPW, and UPW was treated by simple and combined treatment using different methods like acid sulfuric (Ac), steam explosion (SE), and enzymatic (E) (cellulase and hemicellulase).The results showed that the combined and simple Ac treatments are the mostefficient compared with SE and E treatments in the hydrolysis of polysaccharide of cellulose with a rate respectively of 90.5% and 77.6% and hemicellulose with a rate respectively of 80.63% and 87.14%. In addition, both of the preceding methods release an important rate of total phenolic compounds. Combined treatment demands high time but is friendly (approximately 1 day), and Ac treatment is less time-consuming (about 25 min) but harmful to the environment and causes the corrosion of equipment.In conclusion, combined treatment can be the best method and the high time required can be reduced with the progress of the research.

Keywords: biomass; lignocellulosic complex; pretreatment; cellulose; plant waste.

INTRODUCTION

In Africa, the demand for energy has increased for the past 10 years, as energy consumption has grown from 15.99 Ej in 2010 to 18.58 Ej in 2019 with a rate of development per annum of 2.5% (Statistical Review of World Energy, 2021). Moreover, the enhancing depletion of fossil fuels and their disadvantages on health and the environment derived from greenhouse gas emissions leads to a search for alternative sourcesof renewable energy like lignocellulosic biomass. This is a potential source to produce second-generation bioethanol and biomethane (Grippi et al., 2020).

Despite the availability of plant biomass and its richness in organic matter, mainly cellulose and hemicellulose (Bendaoud et al., 2022). Their valorization requires a pretreatment step that constitutes more than 40% of the total processing cost of the biomass conversion process (Sindhu et al., 2016). During this pretreatment step to degrade recalcitrant lignocellulosic complex, which, lignin is the major barrier to accessing the polysaccharides; cellulose, and hemicellulose and hydrolyze it into fermentable simple sugars for bioenergy production (Zabed et al., 2019)

Among the most common methods used in the pretreatment of lignocellulosic biomass are

chemical pretreatment with H_2SO_4, physical-chemical pretreatment with a steam explosion, and enzymaticpretreatment. The chemical pretreatment is a rapid reaction but is expensive due to the high chemical cost and it can produce toxic elements and disrupt microbial activities in anaerobic digestion (Mohammad Rahmani et al., 2022; Thamizhakaran Stanley et al., 2022). Steam explosion (SE) is an efficient, environmental-friendly and industrially scalable pretreatment method and makes biomass more operative to enzyme (cellulase) attack due to the disintegration of structural components of lignocellulose and could be able to enhance ethanol yield during the anaerobic digestion process into approximately 90% (Fabiano et al., 2015; Kumari and Singh, 2018; Stanley et al., 2022). Enzymatic pretreatment is also environmental-friendly and becomes a suitable way because it requires less energy, while fewer fermentation inhibitor products are generated. Among the enzymes utilized in enzymatic pretreatment are Cellulase and Hemicellulase (Brummer et al., 2014; Maitan-Alfenas et al., 2015).

In the region Fes-Meknes of Morocco, Forestry residues, and medicinal and aromatic wastes, are among the lignocellulosic biomass that has a high level of cellulose and hemicellulose, and lower content of lignin compared to others biomass such as olive pomace, olive waste and household waste (Bendaoud et al., 2022).

The objective of this study is to determine the most efficient and best pretreatment method(s) which takes into consideration treatment time, hydrolysis yield, and being less polluting. This work was carried out on a mixture of forestry waste biomass and medicinal- aromatic plants waste.

MATERIALS AND METHODS

Materials

The type of lignocellulosic biomass selected for this work are forestry waste (FW), extracted parts of medicinal-aromatic plant waste (EPW) and unused parts (UPW) (Bendaoud et al., 2022). FW was collected from the Ain Chkef Forest located in the province of fez (33°59’07.0”N, 5°01’05.0”W). The medicinal-aromatic plant waste were selected from different cooperatives in the Fez Meknes region.

The collected samples were dried at 55 °C for 72 hours until they reached a constant weight. Then they were crushed and sieved. The size of the processed samples was optimized to 0.25 mm.

Treatment methods

The acid treatment was done by sulphuric acid (H2SO4). The concentration of the sulphuric acid and the treatment time were optimized. The optimal conditions used were 0.5% sulphuric acid for 20 minutes (Fitria et al., 2019).

One kilogram of waste mixture (FW, PPW and EPW) was washed with pure water to remove impurities and placed in an Erlenmeyer flask containing 3 liters of distilled water. The substrate was pretreated with steam in an autoclave (20 minutes at 270 °C), the time of depressurization was 1-2 seconds every 5 minutes (Eloutassi et al., 2014a; Yu et al., 2022).

Enzymatic hydrolysis is catalyzed by the enzymes during 4 days: Cellulase and Hemicellulase extracted from *Trichoderma Reesei*. The preparation of the enzymes was obtained from the supernatant of the culture medium of the *Trichoderma Reesei* strain grown on cellulose as carbon and energy source. 20 ml of the concentrate was added to 100g of substrate. The time, pH and temperature of the culture were optimized (Maitan-Alfenas et al., 2015; Wu et al., 2022).

The substrate was combined treated with (Ac) and (SE) for 10 minutes under the same optimal conditions applied for the simple treatment and then hydrolyzed enzymatically by cellulase and hemicellulasefor 2 days (Figure 1).

Cellulose and hemicellulose were measured by Van Soest’s method (Godin et al., 2010; Van Soest et al., 1991). The results are expressed in percent as follows:

- Cellulose = (ADF- ADL) /initial dry mass.
- Hemicelluloses = (NDF - ADF) /initial dry mass.

The total phenolic compounds were analyzed by the Folin–Ciocalteu method using gallic acid as a standard, as it is described by Singleton and Rossi (Singleton and Rossi, 1965).

Statistical analysis

The treatment and analysis of the results was done by the RStudio (R Core Team, 2022). The comparison between the different methods of pretreatmentwas executed by the one-factor ANOVA test followed by Tukey’s test with $p < 0.01$.

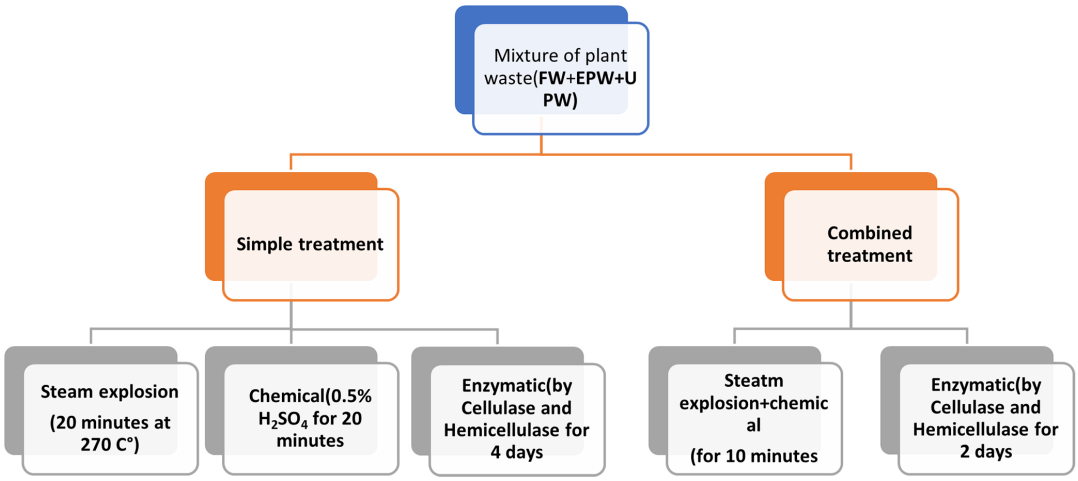

Figure 1. Treatment process by different methods of a mixture of lignocellulosic biomass waste

Table 1. Chemical composition of mixture of plants waste: forest waste (FW), extracted parts wastes (EPW), and unused parts wastes (UPW) of medicinal-aromatic plants

Concentration (g/l)	Total polysaccharides (TPS)	Cellulose (Ce)	Hemicellulose (He)	Lignin (L)	Glucose	Xylose	Mannose	Galactose	Total phenolic compounds (TPC)
Mixture of FW, PPW and EPW	25.21	9.51	5.68	4.75	5.75	3.35	1.61	0.69	2.05

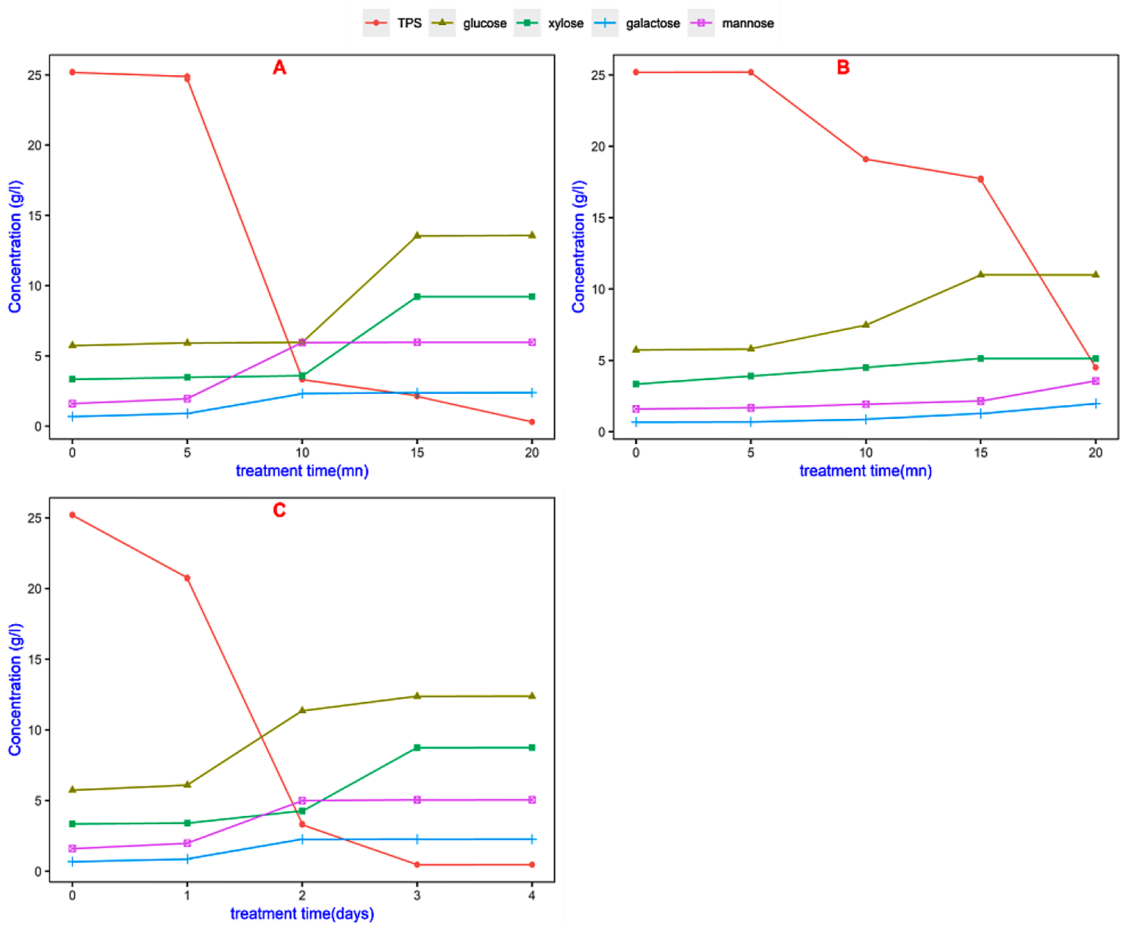

Figure 2. Treatment of biomasses by different methods: (A) acid treatment H_2SO_4, (B) steam explosion, (C) enzymatic treatment

RESULTS

The results of an analysis of a mixture of biomass waste's chemical components (FW, EPW et UPW) are represented in Table 1. These results showed that the mixture contains cellulose polysaccharide in substantial amounts as well as glucose, the most prevalent simple sugar.

Dosage of total polysaccharides and simple sugars

After only 10 minutes of treatment, the Ac treatment (Figure 2A) quickly hydrolyzed the polysaccharides at a rate of over 88%, and after 20 minutes of treatment, the polysaccharides were virtually totally destroyed.

These results also revealed a significant release of monosaccharides, primarily glucose and xylose, which, after 15 minutes of treatment, reach maximum concentrations of 15 g/l and 10 g/l, respectively.

In terms of time and yield, the simple physical treatment of the substrates by SE (Figure 2B) was less effective than the Ac treatment because it takes more time to produce the same effects as the Ac hydrolysis. With regard to the release of simple sugars, the yield of this method is relatively poor; glucose and xylose concentrations are both limited to less than 4 g/l and roughly 10 g/l, respectively, while the yield for the other monosaccharides is nearly zero.

According to the data in Figure 2C, enzymatic treatment E appears to be successful at converting polysaccharides into simple sugars. Although it takes a long time -nearly two and a half days- to decompose almost all of the polysaccharides, this treatment methods was able to do so.

Figure 3 illustrates the results of combined treatment of the previous simple techniques of chemical, physical, and enzymatic treatment (Ac, SE, and E). The hydrolysis yield of polysaccharides is extremely significant and mimics the results of Ac treatment, but, onthe one hand, it is a process that requires a lot longer (nearly 1 day) than Ac and SE treatments, and, on the other hand, it may be regarded as reasonably quick compared to enzymatic hydrolysis, which takes approximately 2.5 days.

Dosage of cellulose, hemicellulose and total phenolic compounds

The result of hydrolysis of polysaccharides (cellulose and hemicellulose) and Total Phenolic Compounds released by different pretreatment methods are represented in Figure 4. Ac and combined method of treatment are efficiency in hydrolysis of Ceand He. After 20 minutes, Ac treatment can degrade Ce and He at a rate of more than 94%, and the combined method is able to digest approximately 91% of polysaccharides in only 10 minutes.

But both methods release a significant amount of TFCwhich increased from 2.5 to more than 30

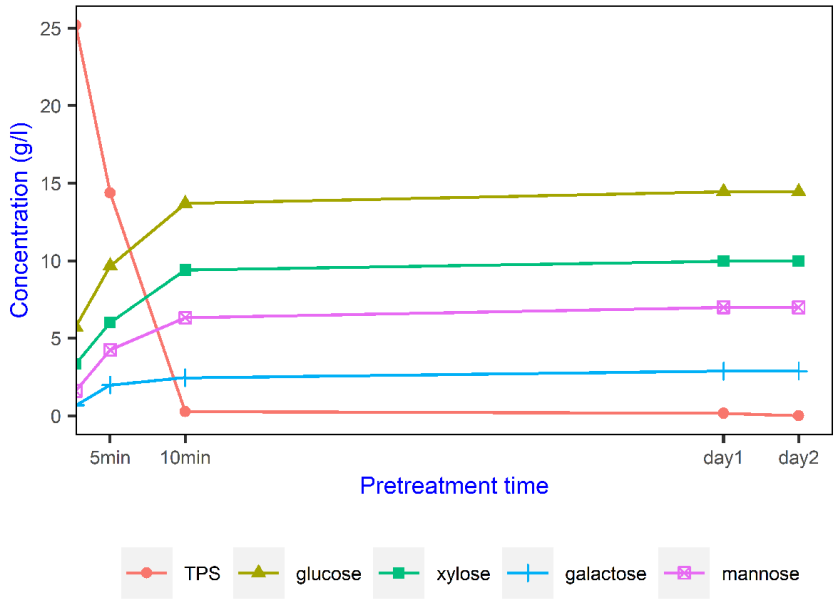

Figure 3. Combined treatment of biomass (SE + Ac + E)

g/l compared to the E and SE methods which released less rate of TFC that represents respectively 20.76 and 22.32 g/l.

E and SE treatment methods seem to be less efficient than the previous methods in terms of time, as they require a fairly long period of time to ensure significant degradation of the lignocellulosic complex. The SE treatment requires 20 minwhich represent double time of Ac treatment to degrade most of the polysaccharides and the E treatment needs almost 2.5 days.

A statistical comparison using the anova test revealed a significant difference between the applied treatment methods after 10 minutes

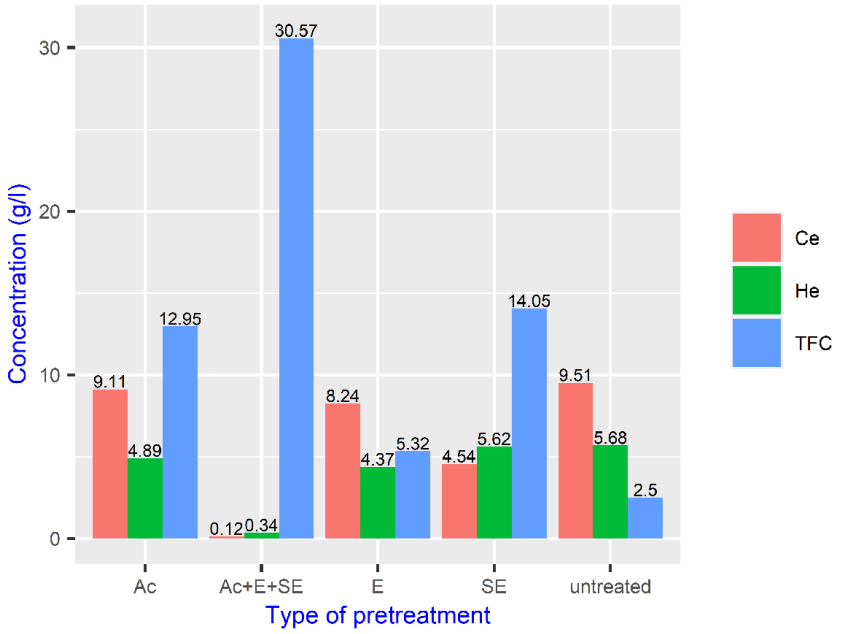

Figure 4. Comparison of treatment methods in hydrolysis of Ce, He and releasing of TFC after 10 Mn of treatment. Untreated represents initial values

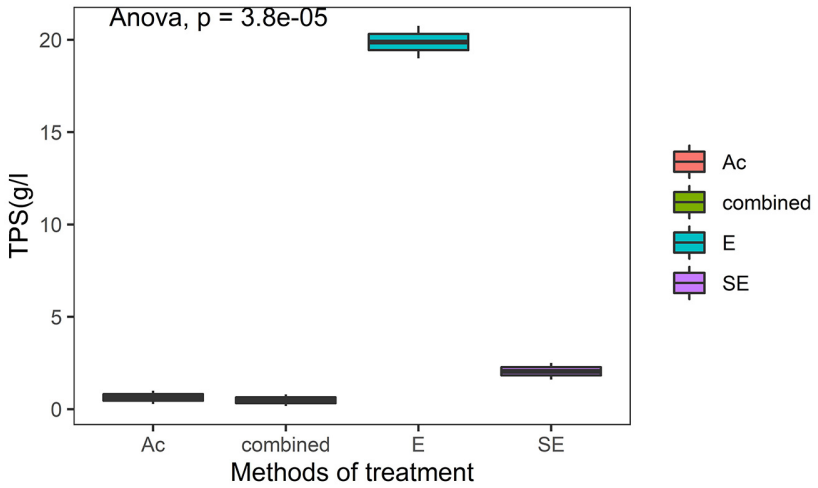

Figure 5. Comparison of TPS concentration mean by different methods

Table 2. Comparison of treatment methods by Tukey test

Tukey test						
Type of treatment	Combined-Ac	E-Ac	E-combined	SE-combined	SE-E	SE-Ac
P value	0.9967286	0.0000780***	0.0000759***	0.3171155	0.0000989***	0.3837972

*** significant with $p < 0.01$.

of treatment ($p=3.8\cdot10^{-5}$) (Figure 5). A Tukey test showed that this distinction is between the "enzymatic" treatment method and the other treatments (Ac, SE, and combined) (Table 2). Therefore, the enzymatic treatment method is inefficient in comparison to other treatments due to the lengthy amount of time needed.

DISCUSSION

The results of the simple acid treatment by H2SO4(Ac) and the combined treatment show a strong degradation of polysaccharides. during a first 10 min, combined treatmentcan behydrolyze 90.5% of cellulose and 80.63% of hemicellulose. Ac treatment can be also degraded 77.6% of cellulose and 87.14 of hemicellulose during 20 min of treatment time.Furthermore, we see a significant release of TFC for these two types of treatments.

In spite of the fact that the combined treatment requires a longer time, its effectiveness remains profitable because it releases an important quantity of simple sugars, especially glucose and xylose. SE pretreatment followed by Ac in the coupling treatment has an important influence on enzymatic hydrolysis because SE treatment increases the accessibility of enzymes to the constituents of the lignocellulosic complex, cellulose and lignin (Kumar et al., 2012; Stanley et al., 2022; Yu et al., 2022). Similar studies on "Willow" biomass have demonstrated that pretreatment with SE and enzymatic hydrolysis treatment with the cellulase enzyme led to the conversion of 80% of cellulose to glucose (Ziegler-Devin et al., 2019). According to numerous studies on various biomasses, like wheat straw, the Ac treatment has a notable impact on the hydrolysis of hemicellulose polysaccharide in particular (Solarte-Toro et al., 2019; Tian et al., 2018). Despite the efficiency of Ac treatment in the hydrolysis of hemicellulose polysaccharide, the release of toxic substances inhibits the growth of fermenting microorganisms like acetic acid and furfural (Bułkowska and Klimiuk, 2016) represents a limitation in the energetic bioconversion of biomass. So, for this reason, the idea of applying Ac treatment combined with SE and E treatment for a short time (10 min) in this study was to minimize the toxic effect of Ac treatment.

The simple Ac treatment is also very profitable, on the one hand, the treatment time is short and on the other hand, it is effective in the hydrolysis of cellulose and hemicellulose. However, Actreatment is preferable for biomasses containing less lignin in order not to release large quantities of phenolic compounds which are considered as fermentation inhibitors (Stanley et al., 2022). But on the other hand, our substrate contains a non-negligible quantity – almost 23% of the lignocellulosic complex – which explains the important quantity of phenolic compounds released.Although this method is effective at decomposing the polysaccharides in the lignocellulosic complex, it has some disadvantages that prevent it from being used on a large scale in industry. These disadvantages include the high cost of the reagents, equipment corrosion caused on by the acidity, and the difficulty of removing the acid from the hydrolysate produced after pretreatment (Banu Jamaldheen et al., 2022; Barisik et al., 2016; Tian et al., 2018).

Both simple SE and simple E treatments are effective and efficient.However, SE treatment is expensive and consumes a lot of energy (Tang et al., 2018) and E treatment requires a long processing time and can only convert a small amount of biomass (about 11%) into bioenergy (Dharma Patria et al., 2022) making it unusable on a large scale and unprofitable for investors.

The combined method of treatment, despite the lengthy treatment period, may be the best compared to the other methods examined in this study, which is to select an appropriate treatment method that takes into account the time of treatment, effectiveness, and need not pollute.

CONCLUSIONS

One of the most important steps in the bioconversion of lignocellulosic biomass is the selection of the best pretreatment technique. The results of this investigation demonstrated that the combined treatmentcould be both efficient and cost-effective, and also enabled the hydrolysis of nearly all polysaccharides in the lignocellulosic complex (more than 80%) within the first 10 minutes. In addition, compared to the other treatments investigated, such as acid, steam explosion, and enzymatic, the incorporation of biological material (enzymes) in this treatment method will have a generally non-polluting effect on the environment.

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