Chemical pretreatment of *Hippophae rhamnoeides* prunings towards their biotechnological exploitation through anaerobic digestion

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Lignocellulosic biomass is one of the richest and most abundant sources of valuable chemical compounds on earth, exhibiting a huge exploitation potential towards the production of renewable energy. Due to the severe environmental and subsequently, economic impacts caused by the immense and inconsiderate use of fossil fuels, lignocellulosic residues pose an exceptional alternative energy resource, the use of which does not contribute to the greenhouse effect and promotes energy independence, following the principles of circular economy, while being cost-effective at the same time (Bello et al., 2018). Over the past decades, the application of several advanced biological and thermophysical processes has demonstrated the conversion of lignocellulosic biomass into various types of solid, liquid or gas fuels, rendering them able to produce directly or indirectly electrical and thermal energy. However, given the extremely diverse sources of lignocellulosic biomass on nature, as well as its complex structure, many barriers have yet to be overcome to fully unlock its potential and accelerate the transition towards a greener energy at a global scale (Tursi, 2019).

Among the several types of lignocellulosic materials, wood-related residues, originating mainly from the forestry industry (shavings, sawdust etc.) and from other agro-industrial sources (leaves, twigs, prunings etc.) consist an altogether unexploited stream of wastes, thus, pointing out the urgency for the application of more sustainable techniques for their proper treatment (Singhvi and Gokhale, 2019). Among the various potential treatment methods, focusing on the biological processes, anaerobic digestion, poses an interesting, reliable and sustainable option for the treatment of organic residues, by converting them into a methane-rich gas mixture (biogas), which can serve as an energy source, both thermal and electrical, similarly with natural gas. However, the complex composition of lignocellulosic biomass, consisting of large natural polymers, namely cellulose, hemicellulose and lignin, creates a serious impediment towards their depolymerization and thereafter their conversion into methane, through the multiple metabolic pathways of anaerobic digestion. Thereby, pretreatment of these materials is essential, in order to increase the accessibility of the sugar-based macromolecules, like cellulose and hemicellulose, from the anaerobic digestion microbial population, and consequently render the process more efficient, with higher methane productivity (Antonopoulou and Lyberatos, 2013).

Based on literature, there are several chemical, enzymatical, thermal and mechanical pretreatment techniques, each affecting the structure of lignocellulose differently. Among these techniques, chemical pretreatment with dilute acid (e.g. H_2SO_4, HCl, H_3PO_4) at an elevated temperature holds several advantages, including reliability, speed, effectiveness in hemicellulose depolymerization and saccharification and generally, reduced production of inhibiting substances such as furfural, HMF, acetic acid etc. (Saha et al., 2005).

The present study is a first attempt to explore the feasibility of the exploitation of *Hippophae rhamnoeides* (sea buckthorn) prunings (HRP), an emerging type of agro-industrial residue, through the process of anaerobic digestion, towards the production of valuable biogas and thereby energy. The focus of this work was oriented towards the study of the first step of the process, i.e. the chemical pretreatment (hydrolysis) of this feedstock, utilizing dilute H_3PO_4, aiming to solubilize effectively the hemicellulose fraction of the material and render easier the depolymerization of cellulose from the anaerobic microorganisms at the second stage of the process. The effect of four parameters, namely feedstock loading, temperature, acid concentration and duration of pretreatment was evaluated, based on maximizing the saccharification yield from the initial feedstock, to discover the optimum pretreatment conditions.

Initially, HRPs were subjected to mechanical pretreatment (milling) and freeze-drying, followed by their physicochemical characterization (Table 1). The parametric analysis was conducted for each parameter independently at the following ranges: feedstock loading 4 – 40% (w/v), temperature 100 – 131 ^{o}C, acid concentration 1 – 4 % (v/v), duration of pretreatment 15 – 120 min, based on similar works for other lignocellulosic materials. Also, SEM images from both the initial and the treated materials were taken, to further assist comparison and evaluation of the effect of hydrolysis.

From the obtained results (Figure 1), the significance of feedstock loading was highlighted, as a parameter that impacts greatly volume loss, which is owed to the highly porous structures of lignocellulosic materials. An almost proportional increase of volume loss was observed with feedstock loading, yet the maximum amount of sugars was achieved at 30% w/v loading. Furthermore, sugars amount was not significantly affected by the duration of pretreatment, in contrast with ammonium nitrogen that was maximized at 120 min. To conclude, the optimum pretreatment conditions were achieved at 121^{o}C, 2 % H_3PO_4, (v/v), 120 min and 30%, w/v loading. This stands in agreement with similar findings regarding other lignocellulosic materials, and therefore leads to the conclusion that chemical pretreatment with dilute phosphoric acid is an appropriate pretreatment method for our feedstock, *Hippophae r.* prunings, thus enabling us to further explore the potential for its exploitation through the process of anaerobic digestion.

Table 1. Physicochemical properties of *Hippophae rhamnoeides* (sea buckthorn) prunings

Physicochemical property*	Mean value /± S.D.
Cellulose (%)	36.51 ± 1.10
Hemicellulose (%)	17.04 ± 0.34
Lignin (%)	39.45 ± 2.12
Ash (%)	0.67 ± 0.09

* dry weight basis

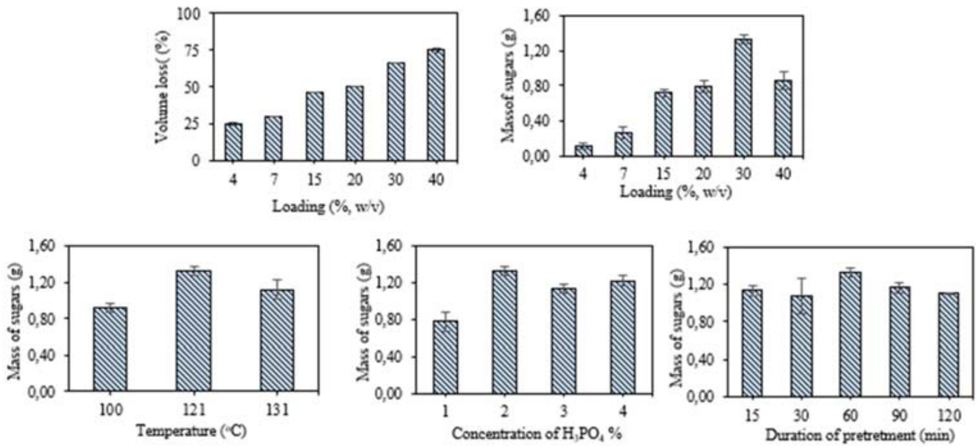

Figure 1. Impact of loading (%, w/v), temperature, concentration of H_3PO_4 (%, v/v), and duration of hydrolysis on hydrolysate content. Volume loss and mass of sugars in relationship with the studied parameters. Data are means ± SD (n=2).

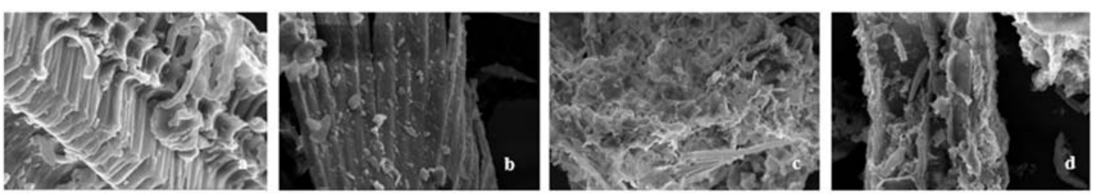

Figure 2. SEM images of *Hippophae rhamnoeides* prunings (various magnifications). Images (a) and (b) are from the untreated material, whereas (c) and (d) of the treated material at optimum pretreatment conditions.

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