Project Title: **Innovative technologies for valorizing lignocellulosic waste to bioplastics, LIGNOBIOPLAST**

Objectives stage 1. Development of experimental technologies for bioplastic production from lignocellulosic waste ► Documentary study on the existing technologies for the production of bioplastics from lignocellulosic waste and a review of existing technical documentation for the development of the LIGNOBIOPLAST technology; ► Lignocellulosic biomass pretreatment; ► Development of technologies for the production of bioplastics (PLA and PHA) from lignocellulosic waste using fermentation processes - Initial; ► Dissemination of results (1 ISI article, 1 international conference participation).

Stage 1 summary.

Stage 1 of the project was carried out over a period of 7.5 months (15.05.2022 -31.12.2022), being oriented towards the fulfilment of the partial objectives set out in the project activities. ***4 activities*** were planned that contained: ►*Act 1.1. Selection of lignocellulosic waste used in the production of bioplastics and review of existing technical documentation for the LIGNOBIOPLAST technology development.* A documentary study was conducted on the types of lignocellulosic biomass waste that can be used for bioplastics production. These largely include agricultural residues (maize cobs, maize stalks, sugar beet molasses, sugar cane, cassava, cellulose, pressed carrots, hydrolysed cereal fibres, wheat straw, etc.), forest residues, etc. A review of the existing technologies for the production of bioplastics (PLA and PHA) was conducted. The conversion of biomass into bioplastics is a multistage process. Initially, lignocellulosic biomass is subjected to pretreatment, which involves the separation of the cellulosic components. This is followed by the hydrolysis of cellulose, which results in the production of monomeric sugars. Finally, microbial fermentation with specific bacteria is employed to obtain lactic acid and polyhydroxyalkanoic acid, respectively. In order to obtain PLA acid, it is necessary to undertake an additional step of polycondensation of lactic acid, which has been obtained fermentatively. ►*Act 1.2. Pretreatment of lignocellulosic biomass.* Experiments have been conducted to investigate the efficacy of various pretreatment methods for lignocellulosic biomass. A pretreatment with CO_2 under supercritical conditions at three temperatures (160, 180 and 200°C) for a reaction time of 15, 30 and 45 minutes for each temperature was proposed. The pressure was held constant at 100 bar for each experiment. A report was prepared on the solid fraction analysis (cellulose, hemicellulose and lignin content, pretreatment yield) and on the liquid fraction analysis (xylose, arabinose, glucose, mannose and galactose content, furfural and HMF). A new method for the analysis of sugars by high performance liquid chromatography (UHPLC) coupled with evaporative light scattering detector (ELSD) was developed and validated. A response methodology model (RSM) was developed to predict the optimal conditions for obtaining pulp from lignocellulosic waste under varying pretreatment parameters. The experimental data indicated that the optimal pretreatment for the maximum recovery of cellulose and monomer sugars from lignocellulosic biomass should be carried out at a temperature of 180°C for a reaction time of 45 minutes and a pressure of 100 bar. ► *Act 1.3.* ***Development of a fermentation process for the production of bioplastics (PHA and PLA) -initial****.* Two technologies have been proposed, namely (1) Technology to obtain polylactic acid (PLA) from lignocellulosic biomass, and (2) Technology to obtain polyhydroxyalkanoate (PHA) from lignocellulosic biomass. The technological schemes for each technology were proposed, and a work plan was developed. A technology for obtaining PLA from lignocellulosic biomass was designed, comprising the following main steps: pretreatment of lignocellulosic biomass with CO_2 under supercritical conditions, simultaneous hydrolysis and fermentation, purification and separation of lactic acid from the fermentation medium, polycondensation of lactic acid with production of PLA and separation and characterization of the bioplastic obtained. The *technology for obtaining PHA from lignocellulosic biomass* was designed, comprising the following steps: pretreatment of lignocellulosic biomass with CO_2 under supercritical conditions, enzymatic hydrolysis of the pretreated biomass, microbial fermentation, purification and separation of PHA from the fermentation medium and characterization of the PHA

bioplastic obtained. ► *Act 1.4. Dissemination of results.* The results obtained in this phase were disseminated through: 1 ISI article published and 1 participation in an international conference (1 communication). The ISI article entitled „*High pressure supercritical CO_2 pretreatment of apple orchard waste for carbohydrates production using response surface methodology and method uncertainty evaluation*, *Molecules*, 2022, 27, 7783. https://doi.org/ 10.3390/molecules27227783 was published. The poster with the title "*Production of bioplastics from lignocellulosic biomass*" was presented at the international conference "21st International Conference Life Science for Sustainable Development".

Stage 1 results

►Articles

- Published ISI articles: 1

► Published ISI article: 1

1. Lacrimioara Senila, Daniela Alexandra Scurtu, Eniko Kovacs, Erika Andreea Levei, Oana Cadar, Anca Becze, Cerasel Varaticeanu, *High-pressure supercritical CO_2 pretreatment of apple orchard waste for carbohydrates production using response surface methodology and method uncertainty evaluation*, *Molecules*, 2022, 27, 7783. https://doi.org/ 10.3390/molecules27227783 (Impact factor - 4.927, Relative influence score - 1.314, Q2).

► Conference participations: 1

- Communications: 1

► Conference participations: 1 (communication)

1. Lacrimioara Senila, Eniko Kovacs, Daniela Alexandra Scurtu, Anca Becze, *Production of bioplastics from lignocellulosic biomass*, 21st International Conference Life Science for Sustainable Development, 15-17 sept 2022, Cluj-Napoca, Romania (poster presentation).

►Study: 1

- Report on the existing methods for the production of bioplastics from lignocellulosic waste.

► Experimental report:

- Report on the pretreatment of lignocellulosic biomass
- Report on the fermentation processes for the production of bioplastics (PLA and PHA) - initial