

**Project title: Innovative technologies for valorizing lignocellulosic waste to bioplastics, LIGNOBIOPLAST Objectives stage 2. Experimentation and optimization of the technologies for obtaining bioplastic from lignocellulosic waste -initial:**

► Development of technologies for the production of bioplastics (PLA and PHA) from lignocellulosic waste, using fermentation processes - Final; ► Technology experimentation; ► Optimization of laboratory technology for the production of bioplastics from lignocellulosic waste; ► Physico-chemical characterization of bioplastics obtained by LIGNOBIOPLAST technology; ► Dissemination of results (1 ISI article, 2 participations at international conferences).

**Summary stage 2**

Stage 2 held for 12 months (01.01.2023 -31.12.2023) comprised **4 activities**: ► *Act 2.1. Development of fermentation processes for the production of bioplastics (PLA and PHA) - Final.* A report on the development of bioplastics production technologies (PLA and PHA) from lignocellulosic biomass was carried out using fermentation processes specific to each type of bioplastic. ► *Act 2.2. Method experimentation. Optimization of the laboratory method for the production of bioplastic from lignocellulosic waste.* The experimentation report for the technology of obtaining polylactic acid (PLA) from lignocellulosic biomass was elaborated. It includes the following technological steps: pretreatment with carbon dioxide in supercritical conditions, the process of saccharification and simultaneous fermentation (SSF) with *L. rhamnosus* to lactic acid, purification of lactic acid, polymerization of lactic acid by irradiation in the microwave field in the presence of tin chloride to PLA and purification of the obtained PLA bioplastic. The experiment report for the technology of obtaining polyhydroxyalkanoate acid (PHA) from lignocellulosic biomass was elaborated. It includes the optimal solution selection criteria, a summary of experimental results and the mass balance of PLA and PHA technologies. Based on the experimental data, it can be concluded that the PLA production technology from lignocellulosic biomass must be resolved as follows: pretreatment with CO<sub>2</sub> in supercritical conditions carried out at 180°C for a reaction time of 45 minutes and a pressure of 100 bars, the SSF process carried out at 37°C for a fermentation time of 72 h using the strain *L. rhamnosus* ATCC 7469 at a pH of 5.5, lactic acid polymerization by microwave treatment at 140°C for 30 min using 0.4% SnCl<sub>2</sub>. The proposed PHA production technology from lignocellulosic biomass involves pretreatment with CO<sub>2</sub> in supercritical conditions carried out at 180°C for a reaction time of 30 minutes and a pressure of 100 bars, delignification with ammonia carried out at 80°C for 12 h, enzymatic hydrolysis performed at 50°C for 72 h and fermentation with the *Bacillus megaterium* strain at 35°C for 48 h. ► *Act 2.3. ► Physico-chemical characterization of bioplastic obtained by LIGNOBIOPLAST technology.* The physico-chemical characterization report of the two types of bioplastics (PLA and PHA) was elaborated through: electron impact ionization mass spectroscopy, nuclear magnetic resonance spectroscopy, Fourier transform infrared spectroscopy (FT-IR), analysis thermogravimetric (TGA), X-ray diffraction (XRD) and PLA analysis by scanning electron microscopy (SEM). The chemical structures of the obtained bioplastics were established and the molecular mass was calculated. ► *Act 2.4. Results dissemination.* The results obtained during the stage were disseminated through: the publication of 2 ISI articles and 3 communications by participating at 3 international conferences. The following scientific articles were published: „*L-Poly(Lactic Acid) Production by Microwave Irradiation of Lactic Acid Obtained from Lignocellulosic Wastes*”, *International Journal of Molecular Sciences*, **2023**, 24, 9817. <https://doi.org/10.3390/ijms24129817> and „*Poly(3-hydroxybutyrate) production from lignocellulosic wastes using Bacillus megaterium ATCC 14581*”, *Polymers*, **2023**, 15, 4488. At the 4<sup>th</sup> International Conference on Materials Sciences and Engineering, 11-12 aug 2023, the oral presentation „*Production of bioplastics (L-poly(lactic acid) and polyhydroxybutyrate acids) from agricultural biomass wastes*” was presented and participated with two communications (poster) at the 6<sup>th</sup> World Conference and Exhibition (WCCE-2023), 11-12 Sept, Barcelona, Spainia „*Production of polyhydroxyalkanoates from lignocellulosic biomass*” and „*L-poly(lactic acid) production from lignocellulosic biomass waste via microwave irradiation*”.

## Results stage 2

### ► Articles

- Published ISI articles: 2

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1. Lacrimioara Senila, Oana Cadar, Eniko Kovacs, Emese Gal, Monica Dan, Zamfira Stupar, Dorina Simedru, Marin Senila, Cecilia Roman, *L-Poly(Lactic Acid) Production by Microwave Irradiation of Lactic Acid Obtained from Lignocellulosic Wastes*, **International Journal of Molecular Sciences**, 2023, 24, 9817. <https://doi.org/10.3390/ijms24129817> (Impact factor – 5.6, Relative influence score -2.264, Q2).
2. Lacrimioara Senila, Emese Gal, Eniko Kovacs, Oana Cadar, Monica Dan, Marin Senila, Cecilia Roman, *Poly(3-hydroxybutyrate) production from lignocellulosic wastes using Bacillus megaterium ATCC 14581*, **Polymers**, 2023, 15,4488, <https://doi.org/10.3390/polym15234488>. (Impact factor – 5.0, Relative influence score -1.787, Q1).

### ► Participation at conferences: 3

- Communications: 3

### Participation at conferences: 3 (3 comunications)

1. Eniko Kovacs, Oana Cadar, Daniela Alexandra Scurtu, Anca Becze, Dalma Kovacs, Lacrimioara Senila, Diana Elena Dumitras, Production of bioplastics (L-poly(lactic acid and polyhydroxybutyrate acids) from agricultural biomass wastes, 4<sup>th</sup> International Conference on Material Sciences and Engineering, 11-12 aug 2023, online conference (**oral presentation**).
2. Senila Lacrimioara, Kovacs Eniko, Scurtu Daniela Alexandra, Becze Anca, Kovacs Dalma, Cadar Oana, Production of polyhydroxyalkanoates from lignocellulosic biomass, 6<sup>th</sup> World Conference and Exhibition (WCCE-2023), 11-12 Sept, Barcelona, Spania (**poster**).
3. Kovacs Eniko, Senila Lacrimioara, Scurtu Daniela Alexandra, Becze Anca, Kovacs Dalma, Cadar Oana, L-poly(lactic acid production from lignocellulosic biomass waste via microwave irradiation, 6<sup>th</sup> World Conference and Exhibition (WCCE-2023), 11-12 Sept, Barcelona, Spania (**poster**).

### ► Experiment reports: 4

- Report on fermentation processes for the production of bioplastics (PLA and PHA) - final;
- Experiment report on the bioplastic production technology (PLA and PHA);
- Experimental report on the optimization of bioplastic production technologies (PLA and PHA);
- Report on the physico-chemical characterization of the obtained bioplastics (PLA and PHA).