**Bioprocess development using municipal solid waste for the production of crude enzymes and fumaric acid in electrochemical bioreactor**

Chrysanthi Pateraki1,\*, Eleni Derveni1, Melissanthi Gkatzogia1, Korneel Rabaey2, Apostolis Koutinas1

*1* Agricultural University of Athens, Iera Odos 75, Athens, Greece

*2* Ghent University, Coupure Links 653, B-9000 Ghent, Belgium

*\*Corresponding author: paterakichr@aua.gr*

**Highlights**

* Municipal solid waste
* Biorefinery
* Fumaric acid production
* *Candida blankii*

**1. Introduction**

Valorisation of renewable resources is nowadays crucial for the production of bio-based products (chemicals, polymers and fuels) through fermentation. The biodegradable fraction of municipal solid waste (MSW) represents a potential low-cost feedstock for the production of bio-based products. Waste composition varies greatly across the world, depending on the level of socio-economic development. Bio-based fumaric acid production is mainly conducted with fungal fermentation on solid or liquid cultures. There are many challenges on fungal fermentations (like oxygen transfer, pellet generation etc) that could be faced by utilising yeasts. The aim of this work is the valorisation of biowaste fraction of MSW, for the production of fumaric acid. The sugar fraction was hydrolysed with crude enzymes that were previously produced by solid state fermentation using a fungus, *Aspergillus awamori*. The resulting hydrolysate was used as a nutrient supplement that contained assimilable sugars, free amino nitrogen (FAN) and inorganic phosphorus (IP). Bioconversion of sugars to fumaric acid was carried out using *Candida blankii*, a natural fumaric acid yeast strain. Fed-batch fermentations were conducted with the MSW hydrolysates in order to optimise the final fumaric acid production, yield and productivity. Separation of fumaric acid and electrocatalytic conversion of fumaric acid to succinic acid was achieved with membrane electrolysis, a novel electrochemical extraction technique in which electrodes are present in the fermentation broth to drive (bio)electrochemical reactions while utilising the electro-motive force to transport charged acid salts from a cathode across an anion exchange membrane (AEM or CEM) into a low volume, acidic anode solution.

**2. Methods**

The organic fraction of municipal solid waste (OFMSW) contained food waste and yard waste from selected households. Solid state fermentations were carried out with the fungal strain *Aspergillus awamori* at different initial moisture contents (50, 55, 60, 65 and 70%)*.* OFMSW hydrolysates were produced by mixing varying quantities of OFMSW with the enzyme-rich suspension. OFMSW hydrolysis was conducted at 55 oC with 100 g/L final solid concentration. Value added products were extracted from the raw material using hexane for lipid fraction, ultrafiltration with 3 kDa membrane for protein fraction and treatment with acidified ethanol for pectins. The remaining solids were hydrolysed utilising extracted crude enzyme consortia that were previously produced by solid state fermentation (SSF) using *A. awamori*. The hydrolysate was finally used as a substrate for fumaric acid production through microbial fermentation by *Candida blankii*. Fumaric acid was further converted to succinic acid in the fermented broth using an electrochemical bioreactor.

**3. Results and discussion**

OFMSW has been used as the sole substrate for the production of crude enzyme consortia via solid state fermentation of *Aspergillus awamori*. Glucoamylase, cellulase, cellobiase, invertase, phytase and protease enzyme activities were determined in order to select the optimal initial moisture content of 55%. Extraction of lipids, proteins and pectins was evaluated using untreated or enzymatically treated OFMSW and material balances were estimated in order to identify the optimal refining scheme. The OFMSW carbohydrates were enzymatically hydrolysed and the sugar-rich hydrolysate was used for fumaric acid production via fermentation with the yeast strain *Candida blankii*. Fumaric acid production exceeded 40 g/L and electrocatalysis was applied for its conversion and separation of succinic acid with a conversion yield of 80 %.



**Figure 1.** Biorefinery development of MSW

**4. Conclusions**

OFMSW was used for the production of crude enzyme consortia and fumaric acid. Value added co-products were recovered from OFMSW hydrolysate. Electrocatalytic conversion of fumaric acid to succinic acid was achieved via an electrochemical bioreactor.

**Acknowledgments**

 Received funding by the Bio Based Industries Joint Undertaking under the European Union’s Horizon 2020 research and innovation programme, grant agreement No 745828, entitled “Chemical building blocks from versatile MSW biorefinery” (Acronym: PERCAL).

