FINAL REPORT

In the frame of the project in the **first year** pre-experiments were carried out with our old ED equipment having small surface area in one hand, and manufacturing possibilities of organic acids were studied on the other hand.

Multi-channel data collecting system was built for the ED and spectrometric analytical method was adjusted to follow the processes. The results of the tests help us for start and the effective operation of the new ED equipment.

The production of organic acids by enzymes pre-experiments were carried out to determine the effects of operational parameters. The analytical method (set earlier for the fermentation) was used here, as well. We worked together with foreign partners (University of Zagreb) in the preparation and conduction of the experiments.

Among the organic acids produced by fermentation we have selected itaconic acid. During the investigation of the fermentation – having studied the literature critically – a reliable analytical method (HPLC) was set firstly, then the most effective strain (according to literature), *Aspergillus terreus* was purchased, which was used for successful fermentations.

In the **second year** of the project the planned new electrodialysis equipment was managed to purchase, thus the research work was continued according to the original research plan. Itaconic acid fermentations were carried out to identify the key parameters influencing the operation: (i) aeration, (ii) pH and (iii) substrate concentration. Optimization of these parameters were started immediately by integrating the membrane process to the system, and measurement series – applying experimental design – were conducted, where a control, "membrane free" fermentation was set, as well. Results:

(i) For the aeration a silicon tube membrane – allowing oxygen through the wall – was inserted into the fermenter, which provided the high oxygen level evenly under suitable operation.

(ii) Studying the pH adjustment, in front of the ED equipment (where the multi-channel data collecting system was built in) a microfiltration module (its material is PVDF) had to be introduced which was able to reject the microbial mass. In this way the ED equipment could be operated, and the itaconic acid was possible to recover from the fermentation broth applying bipolar membranes.

(iii) The effect of substrate concentration was studied in the range of 80-250 g/l, and the concentration of inocula (1.5-15 g/l) was varied, as well. It seems that the extremely high inocula concentration is not preferred from the product formation points of view.

Optimization was carried out for all the three parameters, and it turned out that aeration rate had the most significant effect. Finally, the special productivity was managed to increase from 3.3 to 6.2 g/l.day.

In the **third year** of the project further experiments for itaconic acid production were carried out to determine the optimal range of the operational parameters using the *Aspergillus terreus* NRRL 1961 strain. The fermentation was conducted in the Lambda Minifor bioreactor (Figure 1), its volume was 1.8 l, in batch mode of operation, using 120 g/l initial glucose concentration, with 5 % inocula, applying the suitable aeration described earlier. The concentration of acid formed was determined by HPLC. The fermentation process was evaluated from kinetical points of view, as well, the length of lag phase and the maximal yield were determined as 1.52 day and 3.83 g/l.day, respectively.

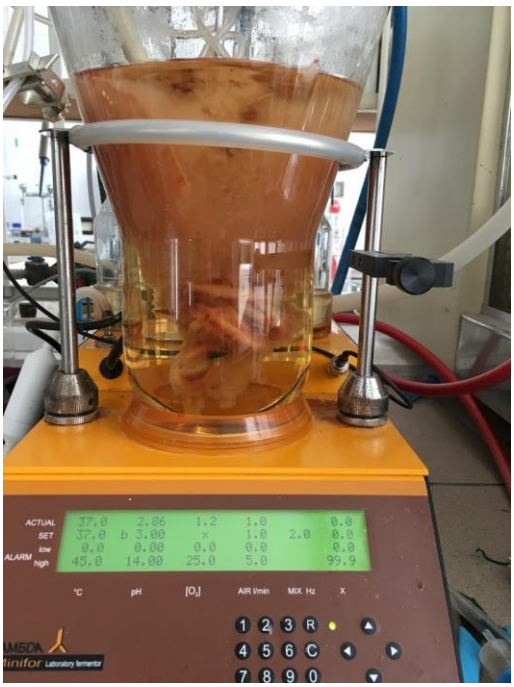

Figure 1: Itaconic acid fermentation

The fermentation – as a novelty in this field – was followed on-line by measuring the CO_2 formed using gas phase analysis, we have proven that there was a linear connection between the gas content and the quantity of the microbial biomass formed.

The adjustment and control of pH is extremely important in this fermentation, according to our experimental results the pH-shifting strategy lead to the most effective acid production. In this way the initially pH = 3 value was decreased to 2.5 and maintained in this level for 48 hours, thus the product yield was managed to reach 0.35 g/g glucose.

The bipolar electrodialysis system (Figure 2) was tested firstly with model solutions, containing itaconic acid, then model solutions containing itaconic acid and other relevant compounds (e.g. saccharose), finally real fermentation broths were used and the optimal parameters of the separation were determined.

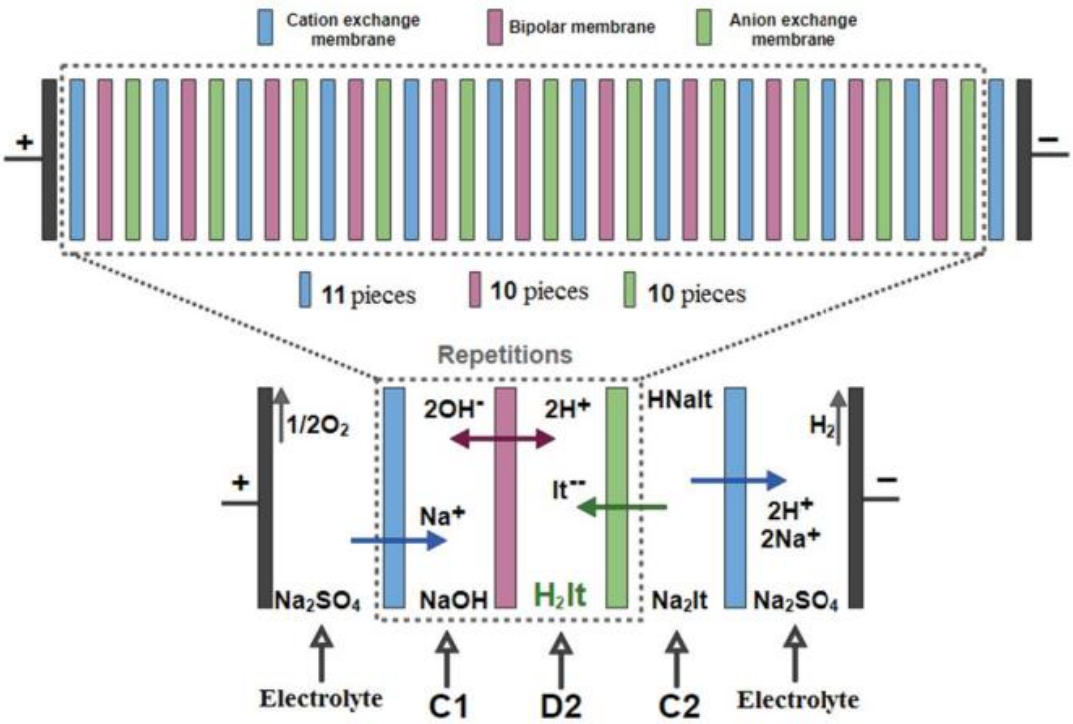

Figure 2: The scheme of the bipolar electrodialysis system

In the **fourth year** of the project the integration of the fermentation and membrane separation was realised and long-term measurements were carried out to study the itaconic acid production.

After thoughtful preparations 2-week long fed-batch experiments were managed to conduct with the *Aspergillus terreus* NRRL 1961 strain by setting the optimal conditions (earlier determined), and the itaconic acid formed was recovered by the electrodialysis equipment (tested and operated under the optimal conditions). Then semi-continuous, long-term measurements were carried out, where product removal and separation was realised by ED, thus an integrated system was operated for itaconic acid production.

The project was extended due to the COVID epidemic and in the **fifth year** of the project we continued the investigations on the integration of fermentation and ED, moreover on the comparative evaluation of the experimental results.

In the integrated system the real fermentation broth was the feed in the bipolar ED measurements. In these experiments the ED system was in the focus and the current effectiveness values regarding itaconic acid were determined. In the adjustment of the operational parameter values we have taken into account the results of the model experiments, and the parameters appropriate for itaconic acid fermentation. During the experiments we have recognised, that

- the current efficiency regarding itaconic acid reduced from 56.1 % to 37.8 % when the initial pH of the diluate was increased from 3.04 to 7.4, but the product recovery was increased from 67.3 % to 74.7 %. Thus the separation became more complete

and fasted due to the higher pH, though the current efficiency decreased, since beyond itaconate other ionic compounds (e.g. NaOH) were present in the solution, and part of the electric current covered their transfer.

- the current efficiency regarding itaconic acid was possible to increase from 37.8 % to 76.2 %, when the number of membrane triplets in the bipolar ED was reduced to its half. In this way the voltage regarding to one triplet has doubled using similar equipment voltage level thus the electric current passing through the membrane unit was doubled, as well. In this situation the current efficiency has increased since its higher level was managed not by growing the conductivity, further ionic compounds were not added into the solution.

Figure 3 presents a complete, overall picture on the process for itaconic acid production obtained in the research project. It can be seen that itaconic acid in crystal form was managed to produce, its purity was 99.5 %, which seems convincing from the utilization points of view.

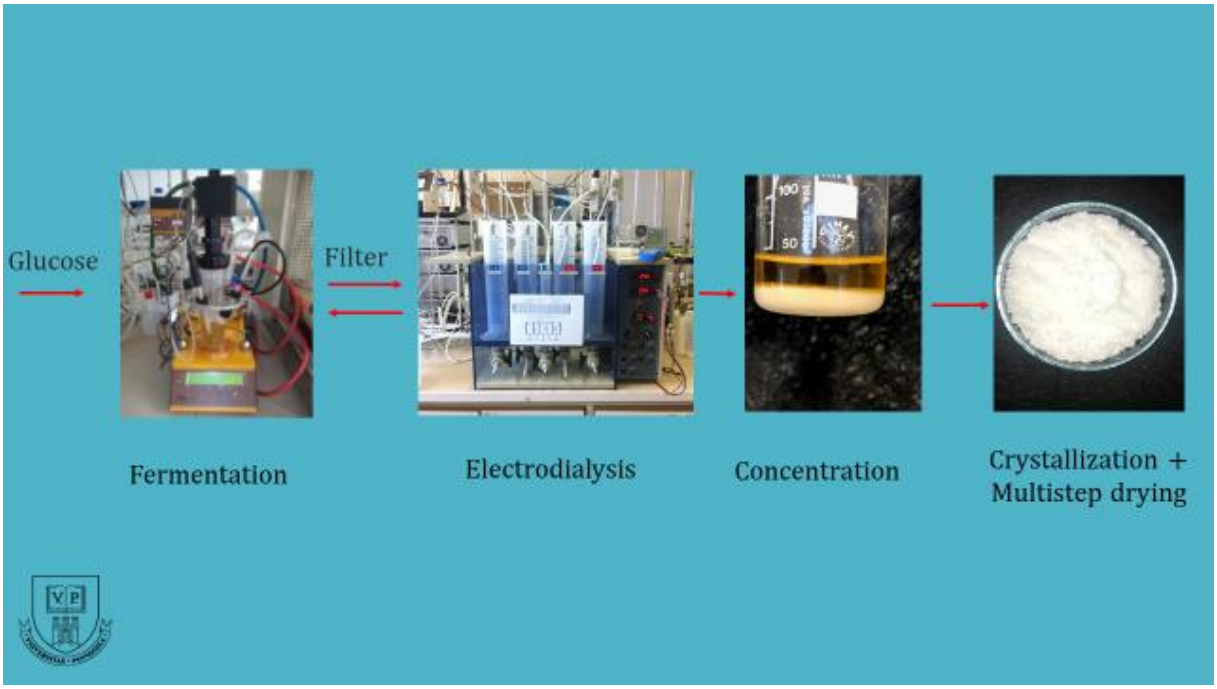

Figure 3: The complete process for itaconic acid production

Our results were presented in several conferences, 3 MSc diplomaworks and 4 BSc diplomaworks were prepared, 2 PhD dissertations are being compiled and 13scientific papers were published.