POLITECNICO DI TORINO

Dottorato di Ricerca di Ingegneria Chimica, XXXI ciclo

Simona Pentassuglia

Novel microbe-based technologies for bioelectricity and biofuel production

This Ph.D. thesis deals with two novel microbe-based technologies: Microbial Fuel Cells (MFCs) and Gas fermentation. These approaches are both reliant on the potential utilization of bacteria as cell factories for bioelectricity and biofuel production, respectively. Microbial Fuel Cells (MFCs) are bioelectrochemical systems (BESs) able to harvest the chemical energy stored in organic matter and directly convert it into electrical energy, through special microbes capable of transferring electrons to an insoluble electron acceptor. During the first year of my Ph.D, the research activity was chiefly focused on the application of floating MFCs (FMFCs) in real marine environment. The objective of this study was to assess the potential applicability of an innovative MFC-based floating configuration as portable power source for low energy-demanding devices. With this aim, two experiments were conducted during both summer and winter in La Spezia bay (Italy), for 58 and 45 days, respectively. The cell prototype used consisted of a small-scale single chamber MFC with an open air cathode (Figure 1.). Multiple MFCs were placed in a polymeric floating system (Figure 1.) and this set-up was employed during *in situ* measurements.

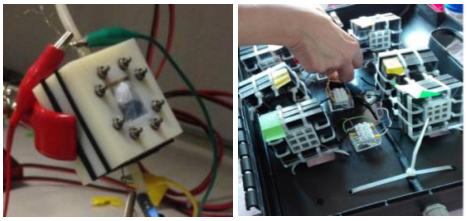

Figure 1. *Left: picture of the squared single-chamber MFC with an open air cathode, fabricated by 3D-printing technology. Right: assembly of the floating system (Adapted from Massaglia et al. 2018).*

Commercial carbon felt was used as both anode and cathode electrode. Additionally, an agar-based synthetic solid-state electrolyte (SSE) was placed within the cells, to promote microbial metabolic activity during the start-up phase, by gradually releasing nutrients. Initially, the work was focused on the development of a new strategy for *in situ* anodic biofilm formation, consisting in the colonization of the anodic electrode directly into the marine sediment. During a laboratory test, the electrochemical performance of the *in situ* enrichment anodes was examined by voltage monitoring across a 560 Ω resistor and compared to that of the anodes developed through the conventional approach. Whilst the obtained results did not reveal substantial differences, the *in situ* enrichment procedure was preferred over the standard approach, due to its advantages (cost-effective, less –time consuming and reduced effects on microbiota composition). Successively, during the on field measurement campaigns MFCs were assembled within the above-mentioned floating system, which was anchored at 2 m from the pier in a bay near La Spezia. A tailored software allowed the remote acquisition of voltage data throughout the entire duration of the experiments. During summertime experiment, four out of the six MFCs employed showed a stable power output of 6 mW/m^2 (Figure 2.). Similar results were achieved during the wintertime measurement, with no appreciable seasonal effects (Figure 2.). Based on the obtained results it can be concluded that the novel, compact and cheap FMFC set up developed in this study can be successfully employed as portable power-supply for remote area.

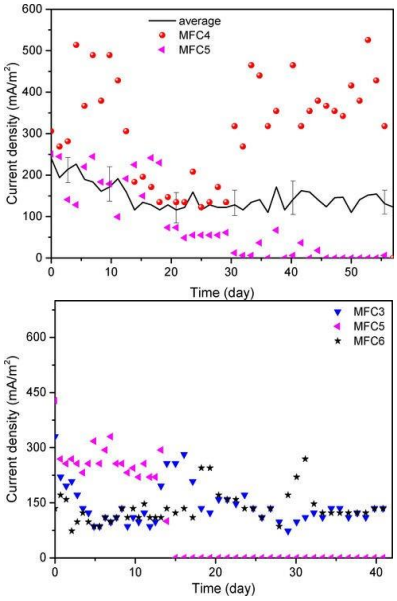

Figure 2. *Current density as a function of time monitored during the summertime in situ experiment. The continuous line shows the average value of four out of the six MFCs (Top graph). Current density as a function of time monitored during the wintertime in situ experiment (Bottom graph) (Adapted from Massaglia et al. 2018).*

During the research activity carried out at the Synthetic Biology Research Centre (SBRC) (Nottingham, UK), the focus of my study was shift from bioelectrochemical systems toward gas fermentation technology for biofuel production. Gas fermentation is an attractive microbe-based technology, which converts C1 gases (such as CO and CO_2) into a plethora of biofuels and high-value chemicals. The biocatalyst used was the industrially-relevant *Clostridium autoethanogenum*, a gram-positive, strict-anaerobic acetogen able to grow autotrophically using CO and CO_2+H_2 as carbon and energy sources. *C. autoethanogenum* natively produces ethanol, acetate, 2,3-Butandiol (2,3-BDO) and lactate. The redox-sensing transcriptional repressor Rex has been found to control the expression of genes involved in fermentation and energy metabolism. Deletion of the *rex* gene in some representative Clostridium species resulted in increase of solvent production. The main objective of this project was to investigate for the first

time the role of Rex in gas fermentation, simultaneously enhancing ethanol production. With this aim, first a mutant carrying deletion of the *rex* gene locus and referred to as Δ*rex*, was constructed by the means of CRISPR-Cas9 genome editing tool, making the current work further innovative. In order to verify whether the phenotypic profile resulting from the *rex* deletion strictly correlates to the chromosomal gene modification, the *rex* gene was over-expressed and the resulting strain indicated as *rex**. The growth profile and fermentation product spectra of the constructed strains were analyzed and compared to those of the Wild Type (WT). Phenotypic characterization was conducted in both serum-flasks and in a stirred tank reactor (STR) (Figure 3.). Tests in serum-flasks were performed for 10 days with 1 bar overpressure of pure CO. The bioreactor was operated in semi-batch mode with batch liquid and continuous CO supply (flow rate-20 ml/min) for approximately 14 days. Process parameters such as pH, redox, temperature and agitation were automatically monitored and/or controlled.

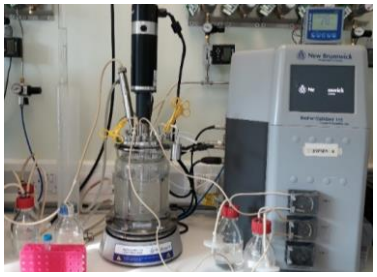

Figure 3. *2L stirred tank reactor (STR) used during this study. The bioreactor was operated in semi-batch mode with batch liquid and continuous CO supply.*

The results achieved from the serum-bottle tests demonstrated that the deletion of the *rex* open reading frame did not substantially affect growth on CO (Figure 4.). Conversely, the complemented strain displayed a slightly slower growth rate, resulting in a prolonged lag phase. Changes in headspace pressure were measured throughout the entire experiment. The three strains were all able to reduce almost the same amount of headspace pressure (WT= -0.49 ± 0.01 bar; Δ*rex*= -0.51 ± 0.03 bar; rex*= -0.46 ± 0.01 bar) (Figure 4.).

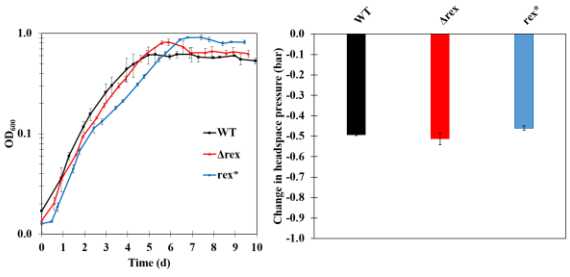

Figure 4. *Growth profile (left) and change in headspace pressure (right) from the start to the end of cultivation of C. autoethanogenum WT, Δrex and rex* strains, conducted in serum-flasks with CO (1 bar overpressure). Data is reported as the average of each triplicate and error bars are represented as standard error of the mean (s.e.m.).*

Regarding metabolite profile, no substantial discrepancies were detected in terms of ethanol and acetate production. Conversely, as shown in Figure 5. Δ*rex* outperformed the control strain by exhibiting 83% increase in 2,3-BDO production. With respect to lactate, a 17-fold higher concentration was observed in Δ*rex*, compared to the WT and *rex** strains.

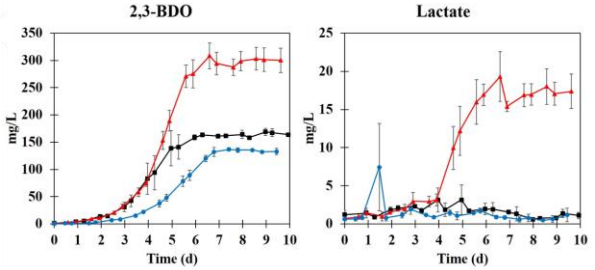

Figure 5. *2,3-BDO and lactate profile during batch fermentation of C. autoethanogenum WT (black), Δrex (red) and rex* (blue) strains, conducted in serum-flasks with CO (1 bar overpressure). Metabolites were quantified by HPLC. Data is reported as the average of each triplicate and error bars are represented as standard error of the mean (s.e.m.).*

These findings corroborates with the *in silico* analysis according to which Rex putatively modulates genes coding enzymes involved in lactate and 2,3 –BDO synthesis. When cultures were continuously fed with CO, no appreciable metabolic changes were displayed instead. However, the WT reached a maximum ethanol concentration of ~20 g/L, far higher than that reported in previous studies with similar working conditions. The acetate productivities reached at the end of the acidogenesis phase were 23.4 ± 3.6 mg/L·h, 21.6 ± 1.8 mg/L·h and 23.3 ± 2.3 mg/L·h for WT, Δ*rex* and *rex**, respectively. Moreover, an ethanol productivity of 56.7 ± 6.7 mg/L·h was calculated for Δ*rex*, similar to that of the WT (59.7 ± 1.4 mg/L·h) (Figure 6.).

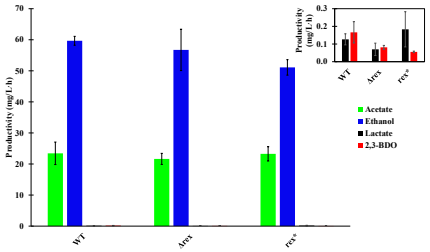

Figure 6. *Productivity (mg/L·h) of ethanol and acetate. The inset shows the zoom of 2,3-BDO and lactate productivities. Data is reported as the average of each triplicate and error bars are represented as standard error of the mean (s.e.m.). Acetate productivities are referred to the peak time point whereas all the other values were calculated at ~300 h.*

The experimental evidence of the Rex role in 2,3-BDO synthesis is an attractive finding that requires further investigations. However, the results obtained during bioreactor fermentations suggest that *rex* is not a valuable target for metabolic engineering studies seeking to increase ethanol production in *C. autoethanogenum*.