Microbial CO_2 conversion to value-added products.

Global warming due to the increase of atmospheric CO_2 concentration is the driving force for the development of strategies that aim to exploit CO_2 as raw material to produce interesting compounds for industry. According to the Carbon Capture and Utilization (CCU) concept, the development of new microbial factories, that exploit the CO_2 derived from industrial anaerobic digestion platforms to produce value-added chemicals, can contribute to mitigate climate change.

Acetone is a raw material used in the chemical industry both as a solvent and as a precursor for the synthesis of deeply used polymers and its worldwide demand is increasing.

Acetogenic bacteria can produce acetate using CO_2 and/or CO as carbon source with H_2 as energy source. Among them, *Acetobaterium woodii* can grow both heterotrophically on organic compounds and autotrophically on CO_2 and H_2 to naturally produce acetate. By a metabolic engineering approach, *A.woodii* was modified with the genes coding for the enzymes of the acetone pathway derived from *Clostridium acetobulylicum*, to make it able to produce acetone. In the autotrophic condition, microbial fixation depends on the mass transfer of the gaseous substrates – which is particularly low for H_2 - to the cells suspended in the liquid phase. By increasing substrates availability, performing fermentation processes at high pressure, increased compounds' productivities can be achieved.

The aim of this work is to investigate, at a laboratory scale, different autotrophic process conditions in pressurized bioreactors for acetone production, by employing an *A.woodii* modified strain. In order to perform fermentations at high pressure, modified *A.woodii* resistance up to 11 bar was first assessed. The bacterium was grown in heterotrophic condition and different pressurization-depressurization protocols were tested. Results obtained confirmed the ability of *A.woodii* to survive and grow up to 11 bars.

Important operative parameters, such as inoculum strategy, optimal pH range, and definition of the feeding gas mix were tackled to optimize the pivotal pre-seeding step for autotrophic fermentations at high pressure.

A gas mix composed of 70% H_2 and 30% CO_2, pH control at 7, and inoculum in the reactor at $OD_{600} > 0.2$ using heterotrophic cultures of the bacterium in exponential phase were the preliminary conditions chosen.

A.woodii was then grown in an autotrophic gas and liquid batch fermentation at 10 bar. 1 L of culture was grown in a 2 L stirred reactor. The pressure was increased by providing the H_2:CO_2 mix. Results showed that the biocatalyst grew and carried on its main autotrophic metabolism at high pressure in this operating condition.

Next, tests in liquid batch and continuous gas supply were set up. First, a reference experiment at atmospheric pressure and then, fermentations at 10 bar, using different combinations of biomass and in-flow gas rates, were performed. Results showed the ability of *A.woodii* to survive and fix carbon. Nevertheless, in this operating condition, besides acetate and acetone, the carbon flow was redirected towards the production of formic acid while biomass production is avoided.

Interestingly, if, before inoculation, the fresh medium is pressurized with the gas feeding mix, biocatalyst behavior is similar to what was recorded when it was grown at high pressure. According to this result, it was suggested that this phenomenon could depend on an increased

concentration of HCO_3^- in the medium. Tests were performed to verify this hypothesis, both at atmospheric and at high pressure, by testing different carbon dioxide partial pressures and maintaining constant the partial pressure of the hydrogen and the total pressure of the process. Results demonstrated that formic acid synthesis is fostered by higher CO_2 partial pressure.
Moreover, independently of the operating condition applied, experiments in the reactor and in serum bottles suggested an influence of acetate concentration in the medium on acetone production by modified *A.woodii* strain. At a concentration of acetate higher than 100-120 mM, a higher part of the carbon flow is directed towards acetone production.
An experiment in gas and liquid continuous allowed investigating the behavior of the biocatalyst in a range of pressure between 1.7 bar and 10 bar. At low pressures, two different in-flow gas rates were screened. Results confirmed that, at the highest pressure tested, bacteria growth was impaired and formic acid production was fostered, while the highest acetate and acetone productivities were recorded at 2.25 bar applying 20 ml/min of in-flow gas mix.