Influence of acetate concentration on acetone production by a modified *Acetobacterium woodii.*

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Exploiting CO_2 as a raw material to produce industrial compounds and avoiding its release in the atmosphere in the gaseous form is a strategy to face global warming. According to the circular economy concept, developing new microbial factories (MFs) can allow the exploitation of the CO_2 derived from anaerobic digestion platforms to produce value-added chemicals.

Acetone is a raw material used in the chemical industry both as a solvent and as a precursor for the synthesis of polycarbonate plastics, and its worldwide demand is increasing (Hoffmeister et al., 2016).

Acetogenic bacteria can produce acetate using CO_2 and/or CO with H_2 as carbon and energy sources via the Wood-Ljungdahl pathway (Bengelsdorf et al., 2018). In particular, the acetogenic bacterium *Acetobaterium woodii* can grow 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 (Hoffmeister et al., 2016).

This work aimed to investigate how acetate concentration in the medium can influence the acetone synthesis by the modified *A.woodii* strain.

Preliminary tests were performed in serum bottles and the reactor in different operating modes. The batch mode was analyzed in serum bottles using a gas-fed-batch strategy to prevent bacteria growth from ceasing due to feedstock depletion. Results pointed out that if the concentration of acetate in the medium overcame the threshold of 100-120 mM, acetone synthesis was fostered.

In autotrophic growth, the concentration of the gaseous substrates in the liquid medium depends on the gas-liquid mass transfer- which is particularly low for H_2. To debottleneck the gas mass transfer rate for these gases and increase acetate and acetone production, the strategy proposed in this work was to perform fermentations at high CO_2-H_2 pressure. Experiments in the reactor were performed at atmospheric pressure and higher pressure, up to 10 bar. Moreover, the tests were conducted in liquid and gas batch mode and liquid batch and continuous gassing mode. Results indicated that independently from the pressure and the operative mode applied, in the experiments in which acetate production was higher, acetone reached a higher concentration.

To confirm this observation, an experiment in serum bottles was set up in which 100 mM of exogenous acetate was added to the autotrophic medium immediately before the inoculum or when cells were in the exponential phase. Moreover, serum bottles without exogenous acetate addiction in the medium were set up as a control.

Results indicated that when acetate was added before inoculation, the final acetone concentration was close to the concentration found when exogenous acetate was not added. Nevertheless, in the control case acetone synthesis started 20 hours after the inoculation, while adding acetate to the medium, acetone synthesis started immediately after the inoculation.

Then, exogenous acetate was added to the medium in the serum bottles during the exponential phase of the growth curve. In this case, acetone concentration was two-fold the value recorded in the control condition. Nevertheless, interestingly, acetone synthesis did not start immediately after the inoculation.

In the literature, another work using a modified *A.woodii* strain for acetone production showed a similar influence of acetate concentration on acetone production (Hoffmeister et al., 2016). Nevertheless, in that study, the acetate concentration in the medium that promoted the acetone synthesis was 11-fold higher (1330 mM).

Phosphate limitation was found to trigger solvent production in *C. acetobutylicum*. It was suggested that, in this condition, WLP is limited in the final step by the shortage of Acetyl-P, and thus the Acetyl-CoA pool inside the cell increases (Bahl et al., 1982; Gottwald and Gottschalk, 1985). Other authors describe a similar behavior in wild-type *A.woodii* grown on glucose in a medium with phosphate limitation. They described ethanol production as a scavenger mechanism to manage the increasing pool of intracellular Acetyl-CoA (Buschhorn et al., 1989).

This key metabolite is the branching point between the WLP and the acetone pathway in the *A.woodii* strain used in this work. Phosphate depletion from the medium was not recorded in the experiments described. Nevertheless, it could be possible that the increasing concentration of acetate in the medium led to a metabolic rearrangement inside the cell. So, once the acetate threshold was overcome, other metabolic pathways were enhanced.

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