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Photofuel - Biocatalytic solar fuels for sustainable mobility in Europe

Deliverable D2.3

Report on designed strains and optimized processes for production of short chain-length alcohols (n-butanol; iso-butanol) reaching at least TRL4

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Editorial		
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Publishable Summary

In Photofuel Workpackage 2, one of the aims is to generate strains of cyanobacteria capable of photosynthetic production of butanol (C_4H_9OH), a potential drop-in biofuel. Compared to ethanol, butanol has lower vapor pressure, which means that using butanol instead of ethanol will not induce a change of the base fuel due to boiling point/vapor pressure problems like E5 and E10 gasoline. Furthermore, its energy content is 30% higher, and it has lower O_2 content than ethanol, which means that more butanol can be blended into gasoline while maintaining the oxygen content according to EN 228 in the final product. Due to these properties, butanol can be used as a fuel without requiring changes in the already existing infrastructure for storage, distribution and utilization in vehicles.

Cyanobacteria are photosynthetic microorganisms, able to grow by fixing CO_2 from the atmosphere with sunlight as their energy source. By employing cyanobacteria as host organisms for production of butanol, the carbon in the resulting fuel will come from CO_2 taken up by the cells. Thus, the fuel will be carbon-neutral (Fig 1).

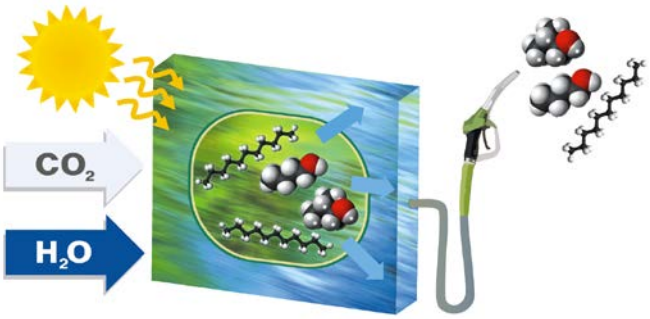

Fig 1. The Photofuel concept: Production of carbon neutral fuels by photosynthetic microorganisms.

In WP2, we are working on production strains for two forms of butanol, which differ in the carbon backbone structure: n-butanol (linear molecule with terminal -OH), and iso-butanol (branched carbon structure with terminal –OH). For both molecules, the goal is to bring the processes for their production in cyanobacteria to TRL 4 to 5, from a starting point at TRL 3. This requires improvements in the photosynthetic cells, mainly in terms of more efficient product formation. To reach this goal, our strategy involves pathway optimization and screening of enzymes for high efficiency production of the target compounds, as well as testing the effect of varying growth conditions for the cells.

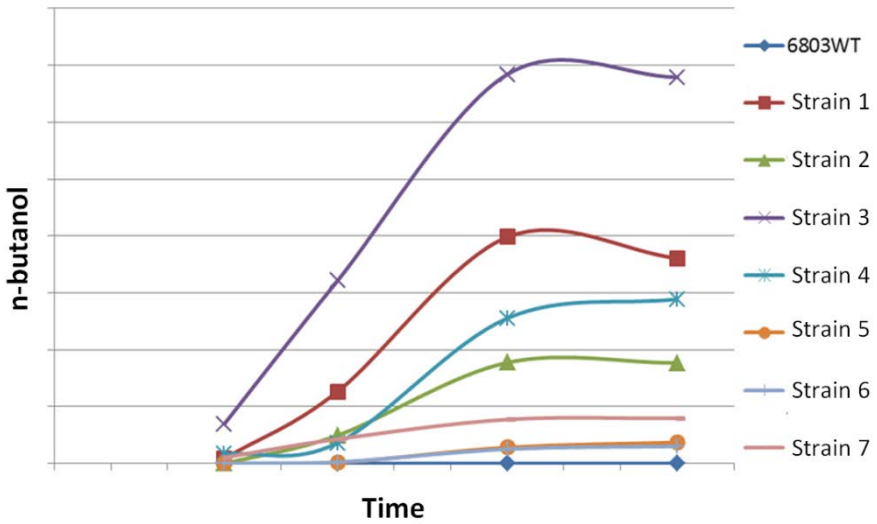

Fig 2. Butanol production in a set of modified strains, compared to the parent strain, wild type *Synechocystis* PCC 6803.

Pathways leading to the production of both n-butanol and iso-butanol have been engineered in the selected host organism, the cyanobacterium *Synechocystis*. Different combinations of native as well as modified and optimized enzymes and pathways have been screened for the capacity to produce n-butanol and iso-butanol under different environmental conditions. Rates and efficiencies of butanol production are compared and evaluated systematically between pathways and genetic constructs (see Fig 2). We use molecular biology and biochemical techniques to evaluate gene and protein expression along with growth characteristics and productivity of the cells (see Fig 3).

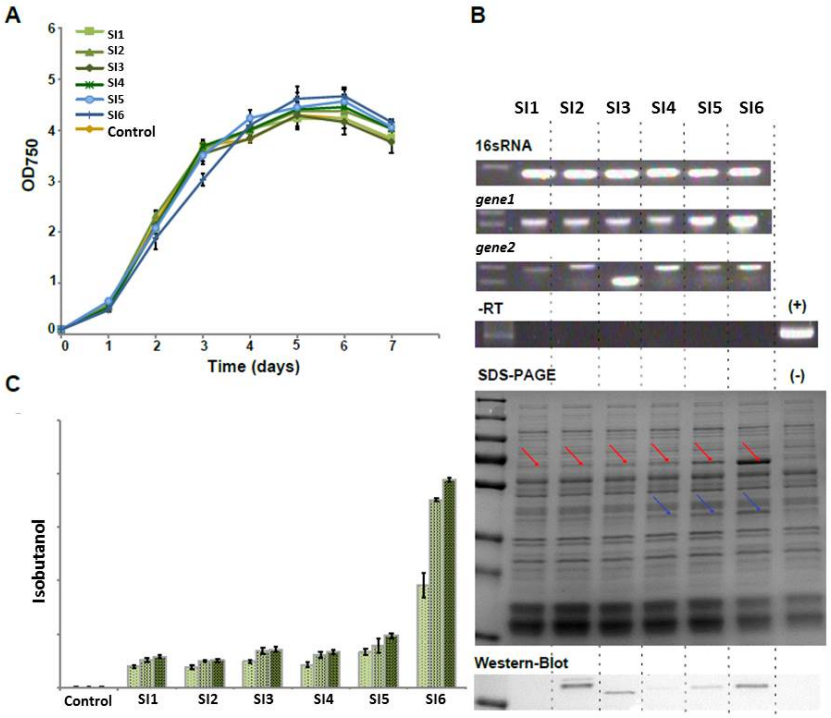

Fig. 3. Analysis of several isobutanol-producing strains.

Since the start of the project, butanol productivity from our strains has increased 7 times comparing our best first butanol producing strain with our current best producers, reaching TRL4.