PRODUCTION OF INDIGO AND ITS DERIVATIVES THROUGH GAS FERMENTATION

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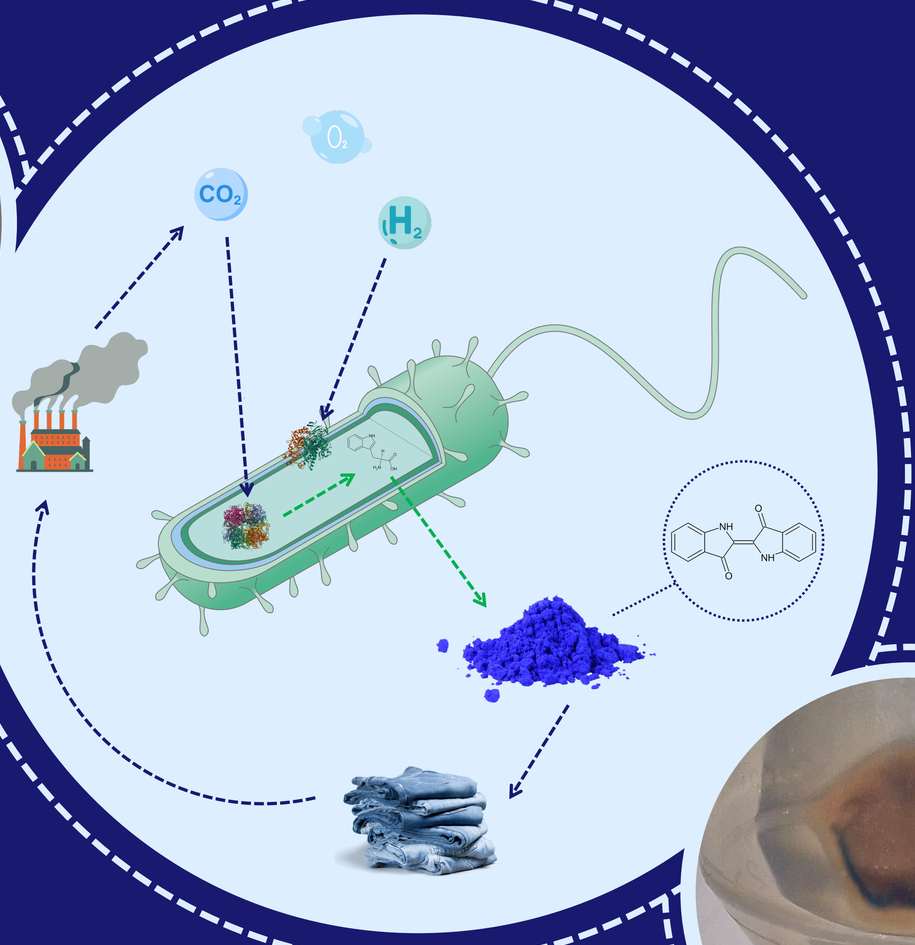
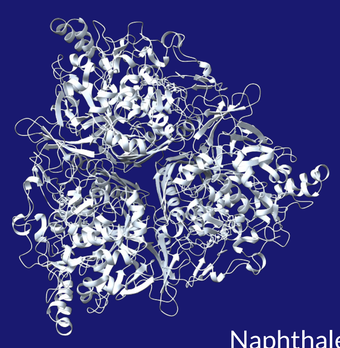
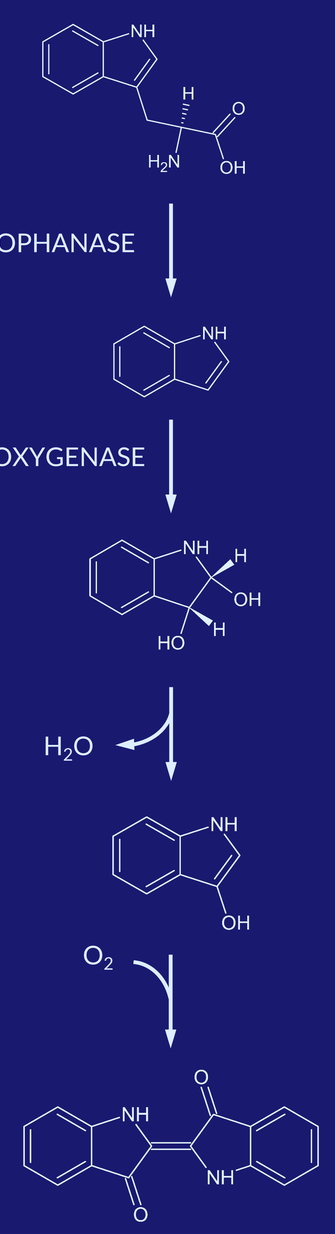
A SUSTAINABLE TEXTILE INDUSTRY

The textile industry is one of the most polluting industries on the planet, still relying on traditional chemical synthesis methods and solvents that are harmful to the environment and human health. On top of this, the industry will surely face continuous economic growth due to the demands of an increasing world population. In this context, biotechnology could be key to accelerating the transition towards a more sustainable and circular model, thanks to the industrial readiness of biotechnological processes for producing biopolymers and pigments. Among the latter, blue indigo is one of the most employed dyes in the Denim fashion industry. Bio-production of indigo was first demonstrated in *Escherichia coli* in the '90s, and since then different microbial hosts and enzymes have been tested to improve titers and yields[1]. However, no large-scale process for indigo bioproduction is available to date. Here, we propose the chemolithotroph *Cupriavidus necator* H16 as a cell factory for a proof-of-concept production of indigo dye and its derivatives through gas fermentation.



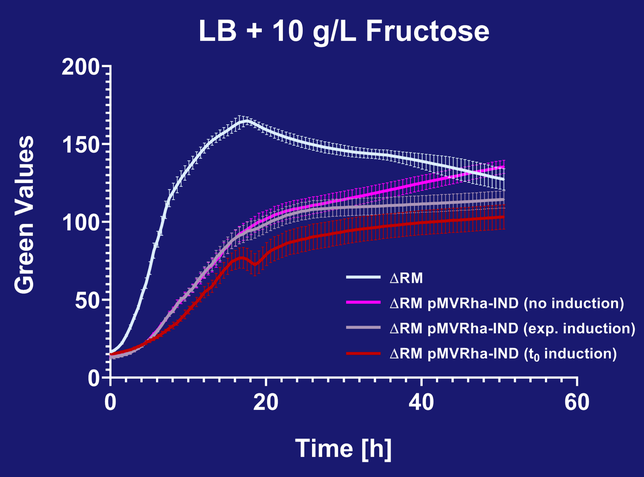


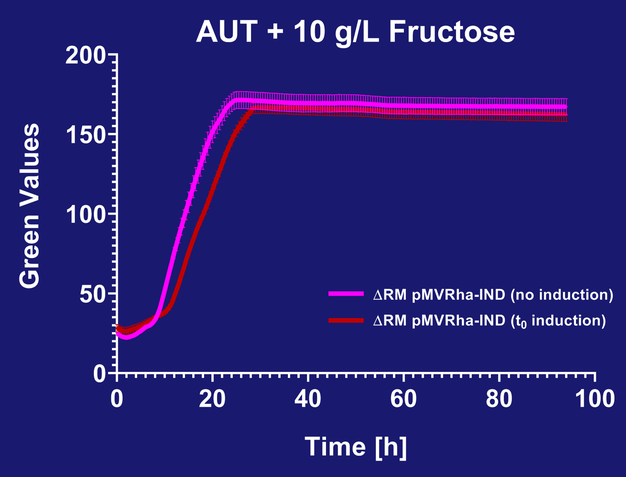
Naphthalene 1,2-dioxygenase (NDO) from *Pseudomonas* sp. NCIB 9816-4



HETEROTROPHIC GROWTH BEHAVIOUR

The heterotrophic growth of the engineered strains was monitored in a Growth Profiler (EnzyScreen, NL) in complex and defined media, and compared to the reference strain *C. necator* ΔRM[2]. Indigo production was induced with 10 mM rhamnose at t_0 and in the exponential cultivation phase to observe any growth effects. Strains were grown at 30°C, 225 rpm. A production of 3,8 mg/L was achieved in heterotrophic conditions.

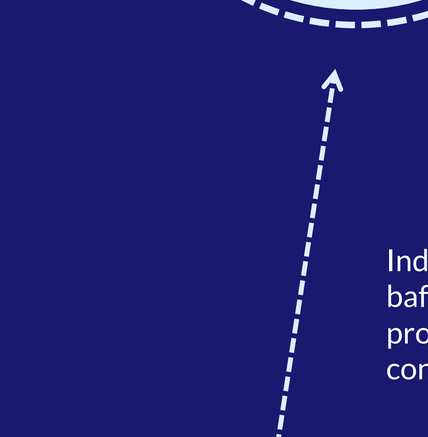


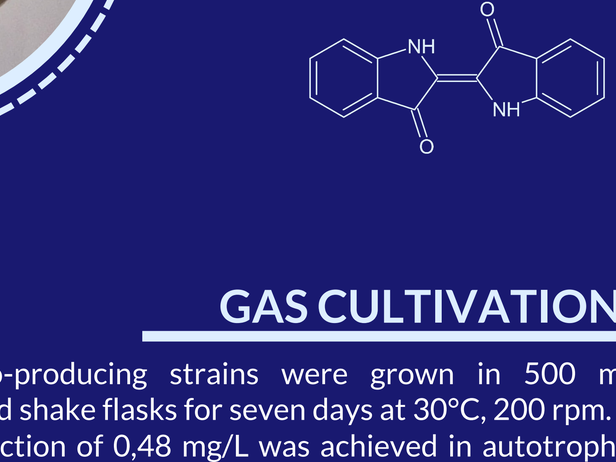


GAS CULTIVATIONS

Indigo-producing strains were grown in 500 mL baffled shake flasks for seven days at 30°C, 200 rpm. A production of 0,48 mg/L was achieved in autotrophic conditions. This gas mixture was changed every 24h:

$H_2 : CO_2 : N_2 : O_2 = 80 : 10 : 6 : 4$





WHAT'S NEXT

- Metabolic engineering strategies are planned to achieve tryptophan overproducing strains for improving precursor availability for indigo biosynthesis
- Alternative oxygenases will be tested to shift the production towards compounds with a higher added value, *e.g.* indirubin

REFERENCES

1. Linke, J.A., Rayat, A. & Ward, J.M., Bioresour. Bioprocess. 10, 20 (2023), DOI: 10.1186/s40643-023-00626-7
2. Vajente M., Clerici R., Ballerstedt H., Blank L.M., & Schmidt S., ACS Synthetic Biology Article ASAP DOI: 10.1021/acssynbio.4c00380

Funded by the European Union

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 955740. This presentation reflects only the author's view, the Agency is not responsible for any use that may be made of the information it contains.