BIOCATALYTIC SYNTHESIS OF INDIGO AND INDICAN FOR BLUE DENIM DYEING

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Indigo is the well-known dye that gives rise to the iconic blue denim look. It has been used since ancient times when it was extracted from plants. When the demand exploded with the industrial revolution, the industry turned to the more convenient chemical synthesis of the dye pioneered by Adolf von Baeyer.[1] The synthesis is based on aniline, a petroleum-derived chemical that is suspected carcinogenic and has high acute toxicity.[2] The synthesis also includes several other problematic chemicals, such as formaldehyde, hydrogen cyanide, and sodamide. The markets for denim, and thus indigo, are expected to grow, and so is the associated pollution, posing a threat to the health of people and the planet. We search for alternative routes and processes to overcome this challenge. There are numerous examples of *in vivo* approaches to indigo biosynthesis, but the biocatalytic alternative is less explored.[3] Our enzymatic approach involves a 7-step cascade to indigo from basic building blocks, as well as 3 enzymatic recycling systems for co-factors. We adopted a modular approach as a convenient method for analysis, as well as for the identification of bottleneck enzymes. Following the optimization of process conditions, we proceed with a life cycle assessment and techno-economic analysis to identify the environmental and economic drawbacks of this approach by comparing it to the conventional synthesis method. However, the source of the dye is only part of the issue related to indigo dyeing. Due to the intrinsic properties of indigo, the dye is reduced to its soluble counterpart *leuco*-indigo to dye the denim (Fig. 2A). This chemical process involves a strong reducing agent at high pH to retain the soluble dye in the dyeing vat, in a process with large environmental footprint. Fortunately, an enzymatic alternative has been established where the water-soluble glycoside-protected indoxyl can be used as the dyeing agent through enzymatic deprotection by glucosidase activity or photolytic cleavage (Fig. 2B).[4] The main drawback to this method is the availability of indican and therefore we also explored the enzymatic synthesis of this dyeing agent. We work continuously with sustainability and economic assessments (LCA and TEA) to improve our processes, and will present the projects' current stage, including technical maturity, economic, and environmental performance.

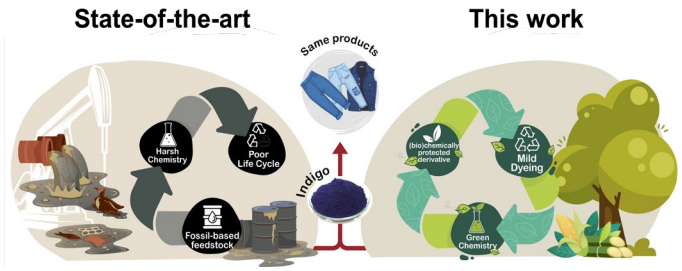

Figure 1. Employing biocatalysis as an alternative for indigo blue synthesis

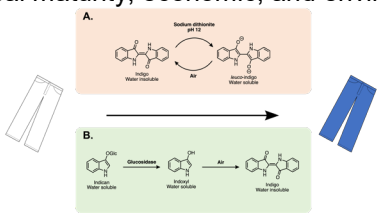

Figure 2. A) Indigo based dyeing. B) Indican based dyeing

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