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A BIOCATALYTIC PROCESS FOR MILDER BLUE DENIM DYEING

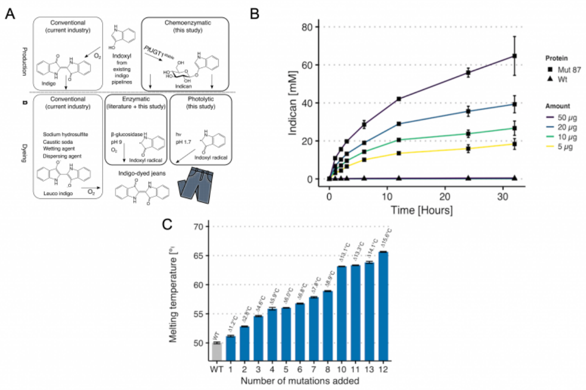
AUTHORS

Gonzalo BIDART / DTU, KEMITORVET 220, KONGENS LYNGBY
Natalia PUTKARADZE / DTU, KEMITORVET 220, KONGENS LYNGBY
David TEZE / DTU, KEMITORVET 220, KONGENS LYNGBY
Leila LO LEGGIO / KU, UNIVERSITETSPARKEN 5, COPENHAGUE
Sumesh SUKUMARA / DTU, KEMITORVET 220, KONGENS LYNGBY
Ólafur ÖGMUNDARSON / UNIVERSITY OF ICELAND, ARAGATA 14, REYKJAVÍK
Anna-Mamusu SESAY / DESIGNSKOLEN KOLDING, ÅGADE 10, KOLDING
Charlotte JANSEN / DTU, KEMITORVET 206, KONGENS LYNGBY
Katrine QVORTRUP / DTU, KEMITORVET 206, KONGENS LYNGBY
Folmer FREDSLUND / DTU, KEMITORVET 220, KONGENS LYNGBY
Ditte WELNER / DTU, KEMITORVET 220, KONGENS LYNGBY

PURPOSE OF THE ABSTRACT

Indigo is the most used dye for blue denim worldwide(1). Its synthesis and the dyeing process require chemical steps that are environmentally damaging, including the use of reducing agents and alkali for indigo solubilization. Different approaches aim at replacing the harmful processes with ecologically attractive alternatives, but the economic and social aspects of sustainability are often overlooked, resulting in poor implementation(2,3,4). The glycosyltransferase PtUGT1 adds a glucose moiety to the reactive indigo precursor indoxyl to form indican, preventing spontaneous oxidation and keeping the dye-precursor soluble(5). Forming indigo directly in the yarn through indican dyeing is a promising route that uses mild conditions(5). Indican eliminates the requirement for reducing agents while still ending as indigo, the only known molecule yielding the unique hue of blue denim. However, currently there isn't a source of bulk indican. To efficiently leverage indican in denim dyeing, two processes must be developed that have environmental benefits over the conventional method while still being affordable: a) a production process & b) a dyeing process (Fig. 1A). Here, we address these two aspects, considering the three dimensions of sustainability, i.e., 1) economic viability, 2) environmental performance, and 3) social impacts. To make biocatalytic indican synthesis economically feasible, we performed a Techno-Economic Assessment (TEA), which pinpointed that the main parameters to decrease reaction costs would be to implement a UDP-glucose recycling system, replace the buffer for water, and to increase substrate concentration above 80 mM. Unfortunately, PtUGT1 is inactive at this substrate concentration (Fig. 1B). Leveraging the structural information of PtUGT1 obtained by X-ray crystallography (PDB ID: 5nlm)(2), we therefore rationally designed 108 mutants to increase enzyme stability to tolerate the required high substrate concentration. As a result, we have developed several active PtUGT1 variants with up to 15°C increase in melting temperature (TmB) (Fig. 1C), which correlated with increased chemo-stability and a strong reduction in substrate deactivation at the required concentrations. This allowed the biocatalytic synthesis of indican from up to 100 mM indoxyl with a 65% yield (Fig. 1B). Further, TEA, social sustainability, and comparative Life Cycle Assessment (LCA) of indican dyeing processes showed that, either enzymatic dyeing or a newly discovered and developed photolytic method, would outperform the current indigo dyeing process in 15 or 14 of 18 impact categories, including global warming, terrestrial ecotoxicity, marine ecotoxicity, and ozone depletion, among others (Fig. 2). Overall, our results emphasize the power of integrating technical and sustainability aspects from early stages of biocatalytic process development.

FIGURES



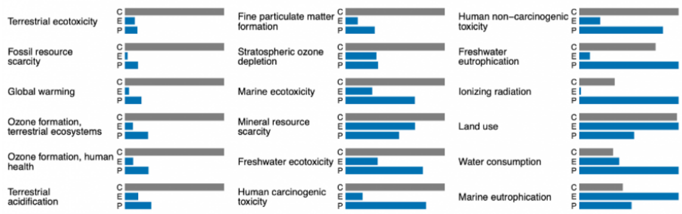


FIGURE 1

Figure 1

A) Overview of indigo and indican production and dyeing processes. B) Kinetics of indican synthesis using 100 mM indoxyl-acetate as substrate. C) DSF comparing TmB of PtUGT1 WT and designed mutants.

FIGURE 2

Figure 2

LCA midpoints (C = conventional indigo dyeing, E = enzymatic indican dyeing, P = photolytic indican dyeing)

KEYWORDS

Biocatalysis | Techno-Economic assessment | Life-cycle assessment | Rational engineering

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