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## From lignocellulosic biomass to value-added chemicals: A one-pot, whole-cell biocatalysis approach for vanillin production using lignin oil

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### PURPOSE OF THE ABSTRACT

Vanillin is among the most 'tasteful' and universally appreciated aromatic chemicals worldwide. It is extensively used as a flavour for food and beverages and a fragrance ingredient in perfumes and cosmetics. However, almost total global vanillin demand is satisfied by petroleum-derived guaiacol. [1] The process is hazardous to the environment and unsustainable with the growing depletion of fossil fuels. Therefore, developing environmentally friendly, efficient, and sustainable routes to biobased vanillin is essential.

Here, we report on vanillin production from 4-n-propyl guaiacol, one of the main components in lignin oil obtained through reductive catalytic fractionation (RCF) [2,3] by employing recombinant *Escherichia coli* cells. Conversion is based on the expression of two engineered oxidative enzymes: a 4-n-propyl guaiacol oxidase that can convert 4-n-propyl guaiacol to isoeugenol [4] and an isoeugenol oxygenase that transforms isoeugenol into the desired product, vanillin [5] (Figure 1). To identify the suitable conditions of the whole-cell cascade, we fine-tuned different reaction conditions, such as temperature, buffer systems, pH values and timing of biocatalyst addition, using 4-n-propyl guaiacol as a model compound. Finally, a high vanillin yield (64%) was obtained (Figure 2), employing optimized conditions on a complex starting material, e.g. RCF lignin oil.

This high-performance strategy was readily scaled up to produce vanillin in more than 10% yield based on lignin in spruce, without any by-products. The whole-cell bioconversion process shows good tolerance even at high loadings of starting material and in the presence of compounds that can potentially poison or compete for the catalyst, showcasing the robustness and applicability of the employed biocatalysts. Furthermore, we show that cells in a growth medium could be used as ready-to-use biocatalysts, making this one-pot lignin oil conversion into vanillin a rather facile process. This work paves the way for the efficient production of high-titer vanillin using depolymerized lignin as the feedstock.

FIGURES

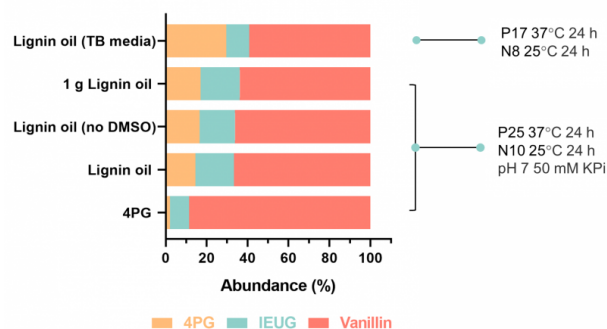
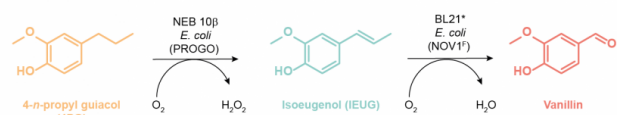


FIGURE 1

The whole-cell cascade for vanillin production from 4-*n*-propyl guaiacol

The first step of the one-pot cascade, e.g. the conversion of 4-*n*-propyl guaiacol to isoeugenol is catalyzed by 4-*n*-propyl guaiacol oxidase expressing cells, followed by the transformation of isoeugenol to vanillin by isoeugenol oxygenase expressing cells

FIGURE 2

One-pot conversions of commercial 4-*n*-propyl guaiacol and lignin-derived oils by PROGO and NOV1-S283F expressing cells

Experimental conditions (from bottom to top) for conversions of : pure 4-*n*-propyl guaiacol; lignin oil in the presence of DMSO; lignin oil in the absence of DMSO, 1g of lignin oil, and lignin oil in presence of cells in growth media.

KEYWORDS

Whole-cell catalysis | Lignin valorization | Enzymatic cascade | Vanillin production

BIBLIOGRAPHY