

# Immobilized imidazole and pyridine as coordinating ligands for ligninolytic peroxidase-like metalloporphines

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Ligninolytic peroxidases (LPs) are enzymes involved in the biological oxidation of lignin. Due to its crucial importance in various industrial processes (detoxification of wastes, bioethanol production, pulp and paper bleaching i.e.), potential of LPs in this process has been investigated, showing some notable drawbacks (such as low stability and high production costs). Thence, several biomimetic alternatives have been suggested. Since LPs active site hosts Fe(III)-heme proximally coordinated by a His residue, synthetic metalloporphines are the most promising candidates for a real structural emulation, needing a proper coordinating ligand that can be also conceivably used for catalyst immobilization onto solid supports. Imidazole is obviously the most bioinspired ligand, although the more electron-deficient pyridine could be similarly useful for metalloporphines immobilization, being able to increase catalyst redox potential, and therefore its catalytic efficiency.

Accordingly, both imidazole and pyridine-functionalized supports (silica- and polyvinyl alcohol-based) were synthesized and tested for the immobilization of metalloporphines. A preliminary screening showed that imidazole-containing supports better coordinate Mn(III)-porphines, while Fe(III)-porphines were most active when immobilized onto pyridine-bearing supports. Therefore, two principal catalysts were prepared, one imidazole-activated (IPS/MnTSPP), [1] and another pyridine-activated (PP-PVA/FeTFPP), [2]. They both shared lignin-peroxidase-like activity towards several lignin model compounds (both phenolic and non-phenolic), with the prominent difference that IPS/MnTSPP activity was centred at neutrality, while PP-PVA/FeTFPP at pH 3. In both cases catalysis took place under very mild operational conditions, making feasible an industrial scaling up. In this perspective the heterogeneous catalysts would preferably work on solid substrates. This makes compulsory the presence of a redox mediator in order to avoid any mass transfer issue. Several common redox mediators (1-hydroxybenzotriazole, *N*-hydroxysuccinimide i.e.) were tested, showing quite poor performances. However, the ideal redox mediator for an eco-compatible process is Mn<sup>2+</sup> ion, emulating ligninolytic manganese peroxidase (MnP) activity. IPS/MnTSPP catalysis was not affected by Mn<sup>2+</sup> presence under any operative condition. Quite different was the behavior of PP-PVA/FeTFPP, since it was similarly not affected by Mn<sup>2+</sup> at

acidic pH, but (as the pH was close to neutrality) the presence of this mediator was able to increase catalytic activity to values similar to those obtained at pH 3. These results were quite promising, since this can be referred as the first report of an immobilized metalloporphine showing versatile peroxidase-like activity.

Both catalysts were also able to bleach several recalcitrant textile dyes under the same mild operational conditions. In this context their activity was compared with ligninolytic enzymes (laccase, lignin, manganese, and versatile peroxidase) activity: while the latter approach suffered from significant drawbacks (low substrate specificity, high substrate inhibition in particular), both IPS/MnTSPP and PP-PVA/FeTFPP were able to bleach high amounts of each dye under conditions compatible with industrial scaling up. In all cases, mechanistic insights showed that immobilized metalloporphines were both capable of monoelectron oxidations and oxygen insertion reactions (conversely, LPs are only able to perform the first type of reaction). This led to a deeper oxidation of the organic matter: a quite desirable occurrence in the perspective of the development of an industrial process based on immobilized metalloporphines aimed to the oxidative delignification or detoxification of industrial wastewaters.

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## References

- [1] Zucca P., Mocci G., Rescigno A., Sanjust E. 2007. 5,10,15,20-Tetrakis(4-sulfonato-phenyl)porphine-Mn(III) immobilized on imidazole-activated silica as a novel lignin-peroxidase-like biomimetic catalyst. *Journal of Molecular Catalysis A: Chemical*. 278: 220-227.
- [2] Zucca P., Sollai F., Garau A., Rescigno A., Sanjust E. 2009. Fe(III)-5,10,15,20-tetrakis(pentafluorophenyl)porphine supported on pyridyl-functionalized, crosslinked poly(vinyl alcohol) as a biomimetic versatile-peroxidase-like catalyst. *Journal of Molecular Catalysis A: Chemical*. 306: 89-96.