Bioresource Technology 124 (2012) 371–378

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Methyl syringate: An efficient phenolic mediator for bacterial and fungal laccases

Tânia Rosado [a], Pedro Bernardo [a], Kamila Koci [a], Ana V. Coelho [a], M. Paula Robalo [b,c,*], Lígia O. Martins [a,*]

[a] Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal
[b] Área Departamental de Engenharia Química, Instituto Superior de Engenharia de Lisboa, Rua Cons. Emídio Navarro, 1, 1959-007 Lisboa, Portugal
[c] Centro de Química Estrutural, Complexo I, Instituto Superior Técnico, Av Rovisco Pais, 1049-001 Lisboa, Portugal

HIGHLIGHTS

- The mechanism for phenolics oxidation by bacterial laccases is proposed for the first time.
- The efficiency of laccase-mediator system using phenolics is independent of the enzymes properties.
- Reactivity vs. stability of phenoxy radicals is a key issue in the laccase-mediator systems.
- C–O coupling products were identified from a newly putative radical coupling route.
- A catalytic cycle of laccase-mediator systems with an interplay of competitive routes is proposed.

ARTICLE INFO

Article history:
Received 6 June 2012
Received in revised form 3 August 2012
Accepted 4 August 2012
Available online 11 August 2012

Keywords:
Laccases
Phenolics
Natural mediators
Non-phenolic
Lignin units
Radical coupling

ABSTRACT

The aim of the present work is to provide insight into the mechanism of laccase reactions using syringyl-type mediators. We studied the pH dependence and the kinetics of oxidation of syringyl-type phenolics using the low CotA and the high redox potential TvL laccases. Additionally, the efficiency of these compounds as redox mediators for the oxidation of non-phenolic lignin units was tested at different pH values and increasing mediator/non-phenolic ratios. Finally, the intermediates and products of reactions were identified by LC–MS and ^{1}H NMR. These approaches allow concluding on the (1) mechanism involved in the oxidation of phenolics by bacterial laccases, (2) importance of the chemical nature and properties of phenolic mediators, (3) apparent independence of the enzyme's properties on the yields of non-phenolics conversion, (4) competitive routes involved in the catalytic cycle of the laccase-mediator system with several new C–O coupling type structures being proposed.

1. Introduction

Lignocellulose has been receiving considerable attention as a source of renewable chemicals, materials, and fuels for future sustainable development (Martínez et al., 2009). Owing to the structure of the plant cell wall, the presence of the lignin matrix covering the polysaccharide components is the main obstacle in the cell wall decomposition. Enzymatic delignification is of special interest for production of high-quality pulps, as well as for dissolving (sulphite) pulps for regeneration of cellulose. Moreover, the increasing interest on lignocelluloses as raw material opens a broad field of research for the enzymatic processing of plant biomass. Lignin itself represents a potential source of aromatic added-value chemicals including polymers and simple monoaromatic compounds, and also an alternative to non-renewable surfactants (Stewart, 2010).

Laccases are both involved in the biosynthesis and biodegradation of lignin. Therefore, they have the highest potential for modification of lignocellulosic materials and isolated lignins. Laccases are oxidoreductases that have been found useful for diverse biotechnological applications including the detoxification of industrial effluents, from the paper and pulp, textile and petrochemical industries, as well as a bioremediation agent to clean up herbicides, pesticides and certain explosives in soil (Couto and Herrera, 2006; Riva, 2006). Apart from its action in depolymerising and polymerising processes, laccases have also been shown to be powerful enzymes for coupling molecules and create low-molecular-weight products in high yield (Wells et al., 2006; Mikolasch and Schauer, 2009).

The oxidation of lignin-related substrates by laccases is restricted to the oxidation of phenolic lignin moiety that comprises

* Corresponding authors Address: Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal (L.O. Martins). Address: Área Departamental de Engenharia Química, Instituto Superior de Engenharia de Lisboa, Rua Cons. Emídio Navarro, 1, 1959-007 Lisboa, Portugal. (M.P. Robalo).
E-mail addresses: mprobalo@deq.isel.ipl.pt (M.P. Robalo), lmartins@itqb.unl.pt (L.O. Martins).

0960-8524/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2012.08.023