Process limitations of a whole-cell P450 catalyzed reaction using a CYP153A-CPR fusion construct expressed in Escherichia coli - DTU Orbit (08/11/2017)

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Cytochrome P450s are interesting biocatalysts due to their ability to hydroxylate non-activated hydrocarbons in a selective manner. However, to date only a few P450-catalyzed processes have been implemented in industry due to the difficulty of developing economically feasible processes. In this study, we have used the CYP153A heme domain from Marinobacter aquaeolei fused to the reductase domain of CYP102A1 from Bacillus megaterium (BM3) expressed in Escherichia coli. This self-sufficient protein chimera CYP153A-CPRBM3 G307A mutant is able to selectively hydroxylate medium and long chain length fatty acids at the terminal position. ω-Hydroxylated fatty acids can be used in the field of high-end polymers and in the cosmetic and fragrance industry. Here, we have identified the limitations for implementation of a whole-cell P450-catalyzed reaction by characterizing the chosen biocatalyst as well as the reaction system. Despite a well-studied whole-cell P450 catalyst, low activity and poor stability of the artificial fusion construct are the main identified limitations to reach sufficient biocatalyst yield (mass of product/mass of biocatalyst) and space-time yield (volumetric productivity) essential for an economically feasible process. Substrate and product inhibition are also challenges that need to be addressed, and the application of solid substrate is shown to be a promising option to improve the process.

General information

State: Published
Organisations: Department of Chemical and Biochemical Engineering, CAPEC-PROCESS, University of Stuttgart, University of Natural Resources and Life Sciences
Authors: Lundemo, M. T. (Intern), Notonier, S. (Ekstern), Striedner, G. (Ekstern), Hauer, B. (Ekstern), Woodley, J. M. (Intern)
Pages: 1197-1208
Publication date: 2016
Main Research Area: Technical/natural sciences

Publication information

Journal: Applied Microbiology and Biotechnology
ISSN (Print): 0175-7598
Ratings:
BFI (2017): BFI-level 1
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.57 SJR 1.177 SNIP 1.173
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.254 SNIP 1.217 CiteScore 3.43
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.327 SNIP 1.458 CiteScore 3.71
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.533 SNIP 1.432 CiteScore 4.3
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.507 SNIP 1.286 CiteScore 4
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.437 SNIP 1.232 CiteScore 3.72
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.381 SNIP 1.239
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1

Scopus rating (2009): SJR 1.353 SNIP 1.062
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.224 SNIP 0.979
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 1.036 SNIP 1.021
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 1.131 SNIP 1.062
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 1.118 SNIP 1.201
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 1.169 SNIP 1.162
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 0.969 SNIP 1.24
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 0.941 SNIP 1.027
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 0.876 SNIP 1.038
Web of Science (2001): Indexed yes
Scopus rating (2000): SJR 0.834 SNIP 1.065
Web of Science (2000): Indexed yes
Scopus rating (1999): SJR 1.13 SNIP 1.253
Original language: English
Bottleneck identification, Cytochrome P450 monooxygenases, Inhibition, Process development, Whole-cell catalysis
DOIs:
10.1007/s00253-015-6999-x
Source: FindIt
Source-ID: 2283439842
Publication: Research - peer-review › Journal article – Annual report year: 2015