APPLICATIONS OF ENZYMES IN POLLUTION CONTROL IN THE TEXTILE INDUSTRY: A REVIEW

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Abstract

Enzymes are versatile catalysts with a growing number of applications in biotechnology. Their properties render them also attractive fir wuste/pollu font treatment processes and their use might be advantageous over conventional treatments. This review highlights enzymes that are suitable for waste treatment, with a focus on cell-free applications or processes with extracellular and immobilized enzymes. Biological wastes are treated with hydrolases, primarily to degrade biological polymers in a pre-treatment step. Oxidoreductases and lyases are used to biotransform specific pollutants of various natures. Examples from pulp and paper, textile, foodandbeverageas well as water and chemical industries illustrate the state of the art of enzymatic pollution treatment. Research directions in enzyme technology and their importancefor future developmen t in environmental biotechnology are discussed.

***Keyword*:** *Enzymts, pollution, textile, industry.*

INTRODUCTION

Over the past century, there has been an increased awareness on the effects of pollution, and public pressure has influenced both industries and government, Environmental pollution is no longer unavoidable. There are increasing demands to replace traditional industrial processes by less or non-polluting anes. During this gradual shift which is <i major challenge for us and the coming generations,, the treatment of wastes from current human activities and as a heritage of our industrial history remains a problem to be solved. Only as these treatments gradually merge with environmentally benign industrial processes, will a truly suuwinatlc economy become a reality.

Tliw U.«P nf nmy.ymss in industrial processes is usually linked to a reduced consumption of energy ** wmll **as** gh«migals and thus beneficial for the environment. The enzyme world market grew at a double-digit rate in the last seven years and was about $5,1 billion f N 800 billion) in 2009 (Sanchez and Dcmain, 2010). Enzymes catalyze specific reactions and mostly act under moderate conditions (temperature, pH, solvents and ionic strength).

1 truer pnjymrs rrprrsrrit a promising tool for the ywlwc'tivw rymuval yf pollutants; from waste streams. Enzyme specificity also precludes undesired side rcactiona, which would otherwise increase reactant consumption and currysponclmjjly raise the cost of treatment a great advantage over conventional chemical treatment processes, The application of cncjrmca to waste treatment was already proposed in the 1930s (Aitten, 1993), however the degradation of a pollutant (parathion) by enzymeswas first illustrated in the late 1970s (Munnecke, 1976).

The purpose of this review is to highlight enzymes that are suitable for waste treatment applications, to summarize waste treatment situations in which the use of enzymes is feasible, and to define the issues/perspectives and potential bottlenecks relevant to the development of enzyme-based waste treatment processes.

TEXTILE INDUSTRY

Production of textiles can be divided into two parts; the fiber and fabric manufacturing and the dyeing process. Only a few possible applications of environmental biotechnology were found for the first, in connection with the low number and amounts of byproducts generated. Wu *et al.* (2008) described a protease-based bioprocess for the production of bioactive peptides derived from sericin found in silk industry effluent. Sericin hydrolysate produced with a commercial protease exhibited good antioxidant activity and tyrosinase inhibiting activity particularly appreciated in food, cosmetic and pharmaceutical industries. Another prospective application refers to poly (trimethylene terephthalate) (PTT), a linear aromatic polyester used in textile industry as fiber, but also in films, filaments and plastics manufacturing. A higher hydrophilicity of such a material is appreciated for

further processing (e.g. dyeing) and generally obtained through alkaline and plasma treatments. Enzymatic hydrolysis may represent an alternative for surface modifications. Polymers and oligomers

of PTT were treated using diverse polyesterases (cutinases and Upases) (Eberl etal., *2008) . The ability of polyesterases to functionalize FTT, which is poorly biodegradable, might also be of interest to increase its bioavailability during waste processing. Finally, biotechnological degumming of bast fibers of ramie and sunn hemp (found in East India) using pectinases and xylanases after a mild chemical treatment presents an option to manufacture textile materials (Kapoor* et al, *2001).*

The dyeing process conferring colour to textile materials is a major activity in textile industries which requires large amounts of water for dyeing, rinsing and cleaning. The removal of dyes from generated effluents is a matter of concern considering the progressively stringent environmental legislations. Indeed, textile effluents are not only unaesthetic (coloured) but toxic and partly carcinogenic. Dyes used in tannery and textile manufacturing are mainly synthetic and , categorized according to physical and chemical properties (C.g, Solubility and charge) as acidic, basic, direct, disperse, reactive, sulfur (Hao et aL, *?nnn) As these properties arc diverse, and because ayes were originally desired to be resistant to chemicals, water, light and microbial degradation, OfflUCntS Containing dyw an* Inherently difficult to clean Consequently, existing chemical processes, c.g. coagulation, advanced oxidation processes (AOPy), adsorption, rmorination, arc partially ineffective and nOt economical for the treatment of dye mixtures (Hiounl* tl al.r*2011). Furthermore, tevHle effluents exhibit bacterial toxicity and low biochemical OKygen demand (BOD)/ehem«-al Wy^On demand (COD) ratio (N 0.1), i.e. lOW moaceradabilirv, As a result, a combination ot* ***chemiral rnagulfttion and OXldatiOn followed by •UTvbiV biulvHKill oxidation is an often employed*** *process (Hao* d aL *ZUUO), which may be fused in an* ***lltVHWtwl biochemical treatment.*** **So** ***far, white TOt fungi fWRP) With their liBnin modifying system are*** *the most dficicnl aerobic orgaruomo to break down coloured pollutants Wesenberg* ct aL *(2003) reviewed the dewlwrlzation and detoxification capacity of WRF. HOWOVer, drawbacks associated with whole rrll rcoctora like Uncontrolled blOmaSS Woducrton are difficult to overcome. I lere again, the application of suitable enzymes might be beneficinl-*

and wntliraqulnnnic structures account for more than 80% Of all textile dyestuff produced (3OPHIED European FP6 project, 2011). Azo are dyes arai'tHrivfld by nitrogcnnitrogcn double (plwnyla iob«nienr) and anthraquinOniC dyes are based on anthracene-9,10-dione (Hao* et al., *2000). Laccases were shown to act on a variety of synthetic dyes; Acid Blue 62 (Vanhulle* et al., *2008), Acid Blue 81, Reactive Blue 19 (Vanhulle* et al., *2007), Malachite Green (Maalej-Kammoun* et al, *2009), Reactive Black* 5, *Reactive Yellow 15, Reactive Red 239 (Crist6vao* et al, *2009), Reactive Red 120 (Arica* et al, *2009), methyl orange (Zille* et al, *2005), crystal violet (Dai* etal, *2010) are some examples. Peroxidases were also assayed for dye degradation, e.g. methylene blue (Alam* et al, *2009), bromophenol blue and methyl orange (Liu* et al, *2006). Akhtar* et al. *(2005) evaluated the potential of a cheap peroxidase from bitter gourd for dye decolourization over a broad range of 21 industrial dyes with various chemical structures. The tested peroxidase was able to degrade nine of the dyes without mediators, and all of them when catalysiswasmediatedwith 1-hydroxybenzotriazole (HBT). Beside peroxidases, CDHs participate synergistically with laccase in the decolourization of the anthraquinonic dye Acid Blue 62 (Vanhulle* et al., *2007,2008). In a study of Enayatzamir* et al. *(2009), culture broth containing laccase and CDH was used to degrade synthetic dyes. They showed that CDH could be used instead of HBT to enhance decolourization of the dyes Direct Violet 51 and Ponceau xylidine due to hydroxyl radicals generated.*

Coupling reactions lead to less soluble polymers of azo and anthraquinonic dyes and often occur upon application of LMEs (Enaud et al, *2010). The predominance of those chromophores in industry, together with a reaction mechanism involving free radical cascades often leading to polymers, is a clear justification why membrane technologies associated with an enzyme treatment are extensively studied, and considered as a promising textile effluent treatment process. For instance, Katuri* et al. *(2009) combined filtration and enzymatic treatment by efficiently immobilizing and stabilizing laccases on a chitosan membrane. More than 95% of an azo dye (Acid Black 10 BX, 20 ppm) could be degraded on 51 cycles of operation. Enzyme immobilization is an effective way to decrease the treatment cost since it allows several reactor configurations together with catalyst reuse. Osma* et al *(2010) used laccases immobilized on silanized alumina pellets activated with glutaraldehyde and achieved the complete degradation of the resistant diazo dye Reactive Black 5 (500 ppm) in a fluidized bed reactor (FBR) and different CSTRs as well as in a continuously operated tubular reactor with this biocatalyst. Matto and Husain (2009) exploited*

turnip peroxidase immobilized on wood shavings for the decolourization of a direct dye, Direct Red 23. Their continuously operated packed bed reactor, in series with an activated silica filter, was operated over 4 months with satisfactory results (64% removal efficiency retained). Hematin a hydroxylated form of heme has been considered mas an economical alternative for some peroxidase-based catalysis. Pirillo *et al* (2010) studied the catalytic degradation of model dyes Eriochrome Blue Black R and Fluorescein with HRP/HjO, in comparison with a mimetic system consisting in hematin/H_sOj. More than 85% elimination of both dyes was achieved with HRP in contrast to 30% of Eriochrome Blue Black R with onlyhematin. Recently, Khouni *et al.* (2010) compared the efficiency of nan of i 11ration, coagulation/flocculation and commercial laccases (DeniliteQIIS, Novozyme) for the treatment of a model effluent Blue Bezaktiv S-GLD150 and Black Novacron R plus typical salts. Beside the very effective imnofiltration *(99%* colour removal), enzymatic catalysis was shown to be technically competitive since better results (98% colour removal) were obtained compared to the most efficient coagulants and flocculants (93% colour removal).

Enzyme Inhibition by high salt concentrations and metal ions may potentially hamper the feasibility of an enzymatic treatment for textile effluents. Marine fungi and their laccases were considered for their ability to decolourize black liquor and textile dyes in salty effluents containing carbonates, sulfides, aulfatcs, chlorides, etc. (P'Souza *et al*,, 2006). **MurgHun *et al* (5009) studied the influence of Ca^,** to^{81}, an"^{1}, Cr^{u}, Cu" and Fe*^{1} on the laccase redox-**rrMdialHid decolour iza lion of Remazol Black«B and** Remaaol Brilliant Blue R (50 ppm), Except for Fe" that highly inhibited enzyme activity, their presence d i d not *f* xert much effect.in textile effluents, a bacterial reductive cleavage ofthe *ana* dye bonds may occur and release colourless nmiiipA(Rtnlr$_r$ 5001). Aromatic amines which are highly toxic pollutants are also released from other anthropogenic activities like rubber manufacturing, chemical, plastic and paper processing. Karim & HW«iln (5009) have Investigated the potential of bitter gourd peroxidase for the degradation of aniline, m chloroanilinc, N,N-dimethylaniline, diphenylflmine, m-toluidinc and p-aminobenzoic acid. All tcatcd aromatic amincQ were recalcitrant to enzymatic treatment but when pcroxidaSC activity was mediated by u-diamsidine, satisfactoiy results were obtained with most of them (50 to removal).

Alongside oxidoreductases, a hydrolase was evaluated for the remediation x>f tannery effluenfc. Tannase from Aspergillus candidus entrapped in alginate beads efficiently removed effluent colont and reduced tannin concentrations below the discharge limit (Murugan & Al-Sohaibani, 20KJX Unlike whole cell treatments with A. candidus, enzymatic treatment did not affect other physicochemical properties of raw effluents, nor did it decrease the BOD. Cell-free tannase may thus be applied in a pretreatment step for effluent detoxification and decolourization before a conventional biological treatment.

RESEARCH DIRECTIONS IN ENZYME TECHNOLOGY

Enzyme catalysis has been exploited in food and beverage processing for centuries. Nowadays scientists and engineers are aware of the enormous potential and versatility of enzymatic reactions and are facing the challenge of their exploitation. This challenge is significant as the cheap supply of highly active and stable biocatalysts has yet to become a reality. Very recently, tools to improve existing biocatalysts, whole cells or enzymes, used in established processes have been partially reviewed (be Carvalho, 2011). Here, we present examples for the use of these tools in connection with above mentioned enzymes which also have potential environmental applications.

Discovery of new enzymes

Better enzymes are needed to meet the requirements of process engineering and to enable sustainable bioprocesses to compete equitably with "older* processes. The search for new enzyme activities among known or previously unknown organisms is called bioprospecting. The classic culture dependent way of screening organisms lor interesting metabolic capabilities or finding them "accidentally" and subsequently identifying, isolating and characterizing responsible enzymes is time consuming, laborious and seldom systematic, yet it represents an established and proven methodology. Even in the era of "omics", this methodology has its value in prospecting enzymes and remains important in combination with molecular biological methods, like cloning, heterologous expression, etc. Examples of its persisting relevance include the discovery of novel secreted fungal peroxidases, like aromatic peroxygenases and dye decolourizing peroxidajWr exhibiting surprising catalytic properties M reviewed by Hofrichter *et al.* (2010).

For several decades, extremophiles have more than excited the interest of researchers. The first commercialized enzyme from such organisms was the DNA polymerase of Thermus aquaticus (Chien *ei al.,* 1976). Nevertheless, key drawbacks associated with the culture of extremophiles are the harsh conditions imposed on the equipment during fermentations and downstream processing, e.g. high salt concentrations (halophiles), high temperatures (thermophiles) or pressures (piezophilea) (De Carvalho, 2011) .

Advances in molecular biology and analytical techniques coupled with automation and computerized data processing enables a modern lab to have a high throughput approach in enzyme prospection, Genome sequence databases, or even more powerful metagenome databases, allow an in Silico screening of the whole diversity of enzymes, not limited by their expression or the culturing of the respective microorganisms (Steele *et al,* 2009). Whereas genome mining is suitable for prokaryotic enrymcaj introns in eukaryotic genea currently hamper a broad exploitation of fungal, animal and plant genomes, tml3 Calling for the application of (meta) tranBWiptonuc approaches. The next major **VreaXumiuyh can be expected, as proteomics is starting to pfoVidO Comprehensive libraries. ThUS.** in combination with the above mentioned methods, proteomlc* offers the possibility to directly derive specific enzymes based on metabolic capabilities of mivropial communities. Furthermore, it is able to overcome limitations of nucleic acid based approaches like post translational modifications or alternative spUcinR which can alter catalytic propwtW significantly (Dhingraef *al,* 2005).

JiiiH enzymes

industrial applications of enzymco often require conditions CpH, iOTUO strength, organic solvents) dut an. tiol congruent With Optimal activities and Stability ae suggested by an enzyme's physiological context. TO fulfill rtw riJL|mifrnent3 of industry, protein engineers Can U80 directed evolution and rational design to tailor Catalytic efficiency *(Km,* Kgat[1]), <mpml stability (pH, T, solvents) and even mOfilfY the reaction mechanisms (BplTiSCheuer & !'ohL 2001). The impact of these two methodologies on unxyine technology and blOpIOCQBB engineering IS Slowing In Importance. Rational design mttOflUCeS deflnud changes in the aminO acid wtpcncc Uaing SltO directed mutagenesis based on the knowledge of three dimensional structures of erurrmeS, functions: and mechanisms. Molecular modeling predicts how mutations would affect enr.ymc properties BUCh as vwlectivity, activity and stability, using databases and structures of homologous enzymes as a model if the structure of the studied protein is unknown (Bornscheuer & Pohl, 2001). Cedrone *et al.* (2000), for example, were able to increase nitrile hydratase activity of papain by 4 orders of magnitude. Lutz (2010) highlighted new computational tools for protein design dedicated to small libraries built on knowledge of protein sequence, structure and function. Rational design might still be in its infancy, but an increasing understanding of structure function relationships and post translational processing of proteins alongside with steadily increasing computational power for simulations and modeling, will soon make this methodology one of the most valuable tools for protein engineers.

In contrast, a popular method mimicking natural evolution called directed evolution requires much less information. Indeed using random mutagenesis, it is possible to improve catalytic properties without information about protein spatial structure. However, for directed evolution one still needs to have the gene coding for the enzyme of interest cloned and ready for expression. In earlier approaches mutations were introduced in whole bacterial genomes. In addition, suitable methods for the screening of the obtained mutants for desired properties must be at hand. By its nature, this approach does not have a high success rate (i.e. percentage of mutants with desired properties) since it introduces random point mutations while substantial changes in enzyme functions may require changes of not only single amino acids but modifications of larger portions of the amino acid sequence (insertion, deletion, inversion, etc.), occurring naturally during recombination events. Nevertheless, directed evolution represents an approach yielding impressive results, especially when mutations affect the active site (Arnold, 2006). This was shown for one variant of OPH which exerted a 25 fold higher activity against methyl parathion compared to the native enzyme (Cho *et al,* 2006). In a review, Singh *et al* (2008) also reported that directed evolution using DNA shuffling had produced bacterial mutants expressing OPH with a 725 fold higher hydrolytk activity against the pesticide chlorpyrifos. Stabilizing mutations often affect the protein surface by adding electrostatic and hydrogen bonds (Eijsink *et al,* 2004). Zumarraga *et al* (2007) stated that mutations at the surface rather than in the active site of laccase improved its stability in solvents. The laccase mutant they obtained after five rounds of directed evolution exhibited 19.2 fold higher stability than

the native enzyme in 50% (v/v) waterethanol mixtures.The choice between rational design and directed evolution depends on the level of knowledge including mechanistic'understanding and on the existence of a selection scheme for a given enzyme (Chen, 2001). For instance, directed evolution applied to cellulase is hampered by the development of an efficient selection or screening method for mutant cellulases due to the insoluble and heterogeneous nature of the cellulose substrate (Zhang *et al.*$_f$ 2006). On the other hand, Guengerieh (1995) proposed that directed evolution would be particularly attractive for the improvement of cytochromes P450, as one step detoxifying enzymes. Mutants producing active, detoxifying P450 enzymes would be capable of growing with high concentrations of substrates toxic to the host cells and may be easily selected. This is especially interesting within the scope of the present review, as the selection of enzymes for environmental applications *is* often baaed on their detaxi?eation abilities and single enzyme systems ratKer than degradation pathways are preferred, thus allowing for the construction of such selection systems.

Both approaches have already successfully produced stable and highly active biocatalysts. Following a directed evolution methodology, Bulter *ft Al.* (2003) reported the heterologous expression of laecase which exhibited a 22-fold higher **Kcat** for ABTS, a common substrate for lamases, Harford-CroBS *et al,* (2000) obtained a mutant of CVP450 from Pseudomonas putida with »n enhanced activity against PAHs (phenan-thfftnc, fluomnthene, pyrene and benzo[a]pyrene) after two directed mutations in the active site,
Dirpct»d Hvolution and rational design appear to be Complementary and their combination Increases the chance of obtaining advanced biocalalysts (Holon *el aL,* 2U02). In a two step optimisation, computer modeling is used to identify tipy Ainino acids responsible for enzyme activity and directed evolution which is vul'ty^uwiuly appligd for mutagwwsdi; of tha DNA srqnrnrr of the "hnf spot" residues and selection of mutants, The process may be iterative and after win linn rif pxmlvfd mnt^nls in *n* first round, another rational design step may be carried out.
r"h("iiy r/ *al* (1999) followed *a* combined approach in an attempt to enhance stability and activity of a fungal pcroxidaBc for its use ao dye transfer inhibitor in laundry detergents. They obtained a mutant enzyme with 174 times the stability and 100 times the oxidative activity of the wild type enzyme. And recently, the total laccase activity (including secretion by Saccharomyces cerevisiae and kinetics) was enhanced 34,000 times after eight rounds of molecular evolution (Mat6 *et al.,* 2010). This is one tangible demonstration of the possibilities offered by the above tools and concepts of protein engineering toward tailoring laccases (and, by extension, other industrially relevant enzymes) to different applications (Rodgersef«/„2010).

The drop in stability caused by the accumulation of mutations was identified and redressed by rational design. Pavlova *et al.* (2009) followed an unusual path to improve a haloalkane dehalogenase from Rhodococcus rhodochrous. They have redesigned residues in the channel connecting the bulk and the buried active site instead of concentrating on amino acids from the active site or its vicinity. After directed evolution, they obtained a mutant enzyme with a 32-fold enhanced activity toward 1,2,3-trichloropropane.

Heterologous enzyme expression has several advantages over cultivation of wild-type strains for enzyme production. Microbial fermentations performed with improved strains are usually short, economical and allow the overproduction of tightly regulated enzymes. Conversely, exploitation of e.g. wild-type white rot fungi for laccase production in bioreactors is still limited for two main reasons: first, an advanced downstream processing is required to recover the target enzymes; second, fermentations of wild-type strains are inherently linked to secondary metabolism and associated process instability, due to protease activity, polysaccharide production; uncontrolled growth, etc. (Couto & Toca-Herrera, 2007). Kunamneni *et al.* (2008) have listed 49 laccases heterologously expressed with varying degrees of success.

CONCLUSIONS

The need for environmental sustainability is the key driving force for the development of enzyme technology for environmental stewardship. This review shows that equivalent outcomes have been obtained with enzyme technology and with existing technologies, and that better results can be achieved when enzyme-based and classical technologies are combined. However, the limited number of field applications indicates a gap between academia and industry. As the use of enzymes in environmental applications often yields only limited added value, this collaboration is crucial. An integrated approach

is needed to efficiently transfer scientific findings to the market and make it a reality within the emerging bkteconomy. The development of a biocatalytic process and the path to its application need input from diverse disciplines. A comprehensive identification of the target environmental application involves not only physicochemical data but current and expected legislation along with evaluation nf potential markets for the technology to be developed. Together with life cycle assessment and prufliability atudies/ this should lead to a holiatic decision making process to pursue the development of a given technology.

REFERENCES

Aitken, M. D. (1993). Waste treatment applications of enzymes: opportunities and obstacles. Chem.Eng.J.,52;B4958.

Akhtar, S., Khan, A. A. & Husain, Q. (2005). Partially purified bitter gourd (Momordica charantia) peroxidase catalyzed decolouflaation of textile and other industrially important dyes. Bioresour Technol,, 9$; 180411

•Mnm, M. Z,, Mansw, M. F & JnlaL K. C. A. (2009). Optimization. Of decolonization of methylene blue by lignin peroxidase enryme produced from sewage sludge With Phanerochaete chrysosporium. J. Mntcr., 162! 70815.

Arieii,. M. Y., Altintas, B. & BayramoglU, G. (2009). luuiwrnlization of lacCaSC onto spacer-arm n 1 1 a c h e d nan. porous poly (CMA/T7r,HMA) beads: application for rrxtlle dye degraclaiion. BlorCSOUI Technol,, loo

Arnold, F, H, (2006) Fancy footWOrk in the space ShUf?6, Nat. Bioterhnol.,

24:32830. , n N (211)2). voigt CA, Mayo

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BorfiflChCUW, U. T & PohL M. (2001), Improved biocntnlyotS by diryclpd evolution and l protein dfiaign. Curr. Opin. Chem.

Bulter, T., Alcalde, M., Sieber, V., Meinhold, P., Schlachtbauer, C. & Arnold, F. (2003). Functional expression of a fungal laccase in Saccharomyces cerevisiae by directed evolution. Appl Environ Microbiol., 69: 98795.

Cedrone, F., Menez, A. & Quemeneur, E. (2000). Tailoring new enzyme functions by rational redesign. Curr. Opin. Struct. Biol., 10:40510.

Chen, R. D. (2001). Enzyme engineering: rational redesign versus directed evolution. Trends Biotechnol.,19:134.

Cherry, J. R., Lamsa, M. H., Schneider, P., Vind, J., Svendsen, A. & Jones, A. (1999). Directed evolution of a fungal peroxidase. Nat. Biotechnol., 17:37984.

Chien, A., Edgar, D. B. & Trela, J. M. (1976). Deoxyribonucleic acid polymerase from the extreme thermophile Thermus aquaticus. J. Bacteriol., 127:15507.

Cho, C. M. H., Mulchandani, A. & Chen, W. (2006). Functional analysis of organophosphorus hydrolase variants with high degradation activity towards organophosphate pesticides. Protein Eng. Des. Sel., 19: 99-105.

Couto, S. & Toca-Herrera, J. (2007). Laccase production at reactor scale by ?lamentous fungi. Biotechnol. Adv., 25:558569.

Cristfivao, R. O., Tavares, A. P. M., Ferreira, L. A., Loureiro, J. M., Boaventura, R. A. R. & Macedo, E. A. (2009). Modeling the discolouration of a mixture of reactive textile dyes by commercial laccase. Bioresour Technol., 100:10941099.

Dai, Y., Niu, J., Liu, J., Yin, L. & Xu, J. (2010). In situ encapsulation of laccase in micro?bers by emulsion electrospinning: preparation, characterization, and application. Bioresour Technol., 101:89428947.

De Carvalho, C. C. C. R. (2011). Enzymatic and whole cell catalysis: Finding new strategies for old processes. Biotechnol. Adv., 29: 7583.

Dhingra, V., Gupta, M., Andacht, T. & Fu, Z. F. (2005). New frontiers in proteomics research: a perspective. Int. J. Pharm., 299: 1-18.

D'Souza, D. T., Tiwari, R., Sah, A. K. & Raghukumar, C. (2006). Enhanced production of laccase by a marine fungus during treatment of coloured effluents and synthetic dyes. Enzyme Microb. Technol., 38:50411.

Eberl, A., Heumann, S., Kotek, R., Kaufmann, F., Mitsche, S. & Cavaco-Paulo, A. (2008). Enzymatic hydrolysis of PIT polymers and oligomers. J, Biotechnol., 135: 4551.

Eijsink, V. G. H., Bjerk, A., Gaseidnes, S., Sirevag, R., Synstad, B. & Van Den Burg, B. (2004). Rational engineering of enzyme stability. J. Biotechnol., 113: 10520,

Enaud, E., Trovaslet, M,, Bruyneel, F,, Billottet, L., Karaaslan, R. & Sener, M. E. (2010). A novel azoanthraquinone dye made through innovative enzymatic process. Dyes and Pigments, 85: 99-108.

EnayatBamii[1], K., Tabandeh, F,, Yakhchali, B., AUkhanJL H. A, & RodriguezCouto, S. (2009). Assessment of the joint effect of laccase and cellobiose dehydrogenase on the decolouration of different synthetic dy*s, J, Hazard Mater., 169: 17681.

, P, (1995). Cytochrome P450 proteins and potential utilization in biodegwtdation. Environ Health Perspect.

KM, 0. *I,* Kim, H. fcChiang, P. C. (2000). npcolouritation of wastcwater. Crit. Rev. Environ Sci, Teehnol, 30; 449505.

v, C, F., Carmichael, A. B., Allan, F. K , England, 1[J]. A., Kouch, *D.* A. & Wong, L. L. (5000), Prutuiii yiiffiiwHriiij/ uf cyLachiome Pl&Ocam (CYP101) for the oxidation of pulyryrlir /iriimatlc hydrocarbons. Protein. BnR.Dw.S«l.[f] 13: 121128.

HufrHiti?r[r]'M.[r] Ullriclv R-, Pecyna, M., Liers, C. & Lundgll, T, (2010). Nuw and classic families of accreted fungal hemc peroxidases. Appl. , 87: 871897.

Kapoor, M., Beg, Q. K., Bhushan, B., Singh, K., Dadhich, K. S. & Hoondal, G. S. (2001). Application of an alkaline and thermostable polygalacturonase from Bacillus sp. MG-cp-2 in degumming of ramie (Boehmeria nivea) and sunn hemp (Crotalaria jvincea) bast ?bres. Process Biochem., 36:803807.

Karim, Z. & Husain, Q. (2009). Redox-mediated oxidation and removal of aromatic amines from polluted water by partially puri?ed bitter gourd (Momordica charantia) peroxidase. Int. Biodet. Biodeg., 63:587593.

Katuri, K. P., Venkata Mohan, S., Sridhar, S., Pati, B. R. & Sarma, P. N. (2009). Laccase-membrane reactors for decolourization of an acid azo dye in aqueous phase: process optimization. Water Res., 43:36473658.

Khouni, L, Marrot, B. & Ben Amar R. (2010). Decolourization of the reconstituted dye bath effluent by commercial laccase treatment: optimization through response surface methodology. Chem. Eng. J., 156: 12133.

Khouni, I., Marrot, B,, Moulin, P. & Ben Amar, R. (2011). Decolourization of the reconstituted textile ef?uent by different process treatments: Enzymatic catalysis, coagulation/?occulation and nano?ltration processes. Desalination, 268: 2737.

Kunamneni, A., Camarero, S., Garcia-Burgos, C, Plou, F., Ballesteros, A. & Alcalde. M. (2008). Engineering and applications of fungal laccases for organic synthesis. Microb. Cell Fact, 7:32.

Liu, J. Z., Wang, T. L. & Ji, L. N. (2006). Enhanced dye decolourization ef ?ciency by citraconic anhydride-modi?ed hors.eradish peroxidase. J. Mol. Catal. B: Enzym., 41: 8186.

Lutz, S. (2010). Beyond directed evolution semi-rational protein engineering and design. Curr. Opin. Biotechnol., 21:734743.

Maalej-Kammoun, M., Zouari-Mechichi, H, & Belbahri, L., (2009) . Woodward S, Mechichi T. Malachite green decolonization and detoxi?cation by the laccase from a newly isolated strain of Trametes sp. Int. Biodet. Biodeg., 63: 600606.

Mat£, D., Garcla-Burgos, C., Garda-Ruiz, E., Ballesteros, A. O_v Camarero, 5. & Alcalde, M. (2010). Laboratory evolution of high-redox potential laccascs. Chem. Biol,, 17: 10301041.

Ma«o_f M. & Husain, Q. (2009). Decolourization of direct dyes by immobilized turnip peroxidase in batch and continuous processes. Ecotoxicol Environ Saf., 72: 965971.

Murmecke, D. M. (1976). Enzymatic hydrolysis of organophosphate insecticides, a possible pesticide disposal method. Appl. Environ Microbiol.,32:7-13.

Murugan, K. & Al-Sohaibani, S. A. (2010). i Biocompatible removal of tannin and associated colour from tannery ef?ucnt using the feloma^s and tannin acyl hydrolasw (B.CAl A.20) enzymes of mango industry solid watJW isolate Aspergillus candidus MTTC 9628. Res. J. Microbiol., 5: 262271.

, K., Kin\ Y. M., Jeon, J. R. & Chang, Y. S. (2009). Effect of metal ions on reactive dye decwlwm iiation by laccase from **(ianndcrma lUcidUm. J, Hazard Mater...** 168:523529,

Lbrnn, J. p.. TOCa -Hwrwa, J L- *• RodriguCB COUto, 5. (5010) RindcgradattOn Of a simulated textile CftueiW by immnhiliacd-COatCd laborniory-acale reactors. Appl.

Pavlova, M., Klvanai M., Prukup, *7,r* Chaloupkova/ R, H^nax P- & Otyepka, M. (5009). Redesigning dwlu*lngpnasc access tunnels na ft atrategy for Upgrading an iuuluupngrnic aubstate. Nat. Chcm "Rinl., 5:727733.

Pirillo, S., Einschlag, F. S. G., Ferreira, M. L. & Rueda, E. H. (2010). Eriochrome Blue Black R and Fluorescein degradation by hydrogen peroxide oxidation with horseradish peroxidase and hematin as biocatalysts. J. Mol. Catal. B: Enzym., 66: 6371.

Rodgers, C. J., Blanford, C. F., Giddens, S. R., Skamnioti, P., Armstrong, F. A. & Gurr, S. J. (2010). Designer laccases: A vogue for high-potential fungal enzymes. Trends Biotechnol.,28:6372.

Sanchez, S. & Demain, A. L. (2010). Enzymes and bioconversions of industrial, pharmaceutical, and biotechnological signi?cance. Org. Process Res. Dev., 15: 22430.

Singh, S., Kang, S. H., Mukhandani, A. & Chen, W. (2008). Bioremediation: environmental clean-up through pathway engineering. Curr. Opin. Biotechnol., 19:437444.

SOPHIED European FP6 project. Novel sustainable bioprocess for European colour industries. Http://www.sophied.net/ about-colour-industry/ dye-maker/2011. October 17.

Steele, H. L., Jaeger, K. E., Daniel, R. & Streit, W. R. (2009). Advances in recovery of novel biocatalysts from metagenomes. J. Mol Microbiol. Biotechnol., 16:2537.

Stolz A. (2001). Basic and applied aspects in the microbial degradation of azo dyes. Appl. Microbiol, Biotechnol., 56:6980.

Vanhulle, S., Enaud, E., Trovaslet, M., Nouaimeh, N., Bols, C. M. & Keshavarz, T. (2007). Overlap of laccases/cellobiose dehydrogenase activities during the decolourisation of anthraquinonic dyes with close chemical structures by Pycnoporus strains. Enzyme Microb. Technol., 40:17231731.

Vanhulle, S., Enaud, E., Trovaslet, M., Billottet, L., Kneipe, L. & Jiwan, J. L. H. (2008). Coupling occurs before breakdown during biotransformation of Acid Blue 62 by white rot fungi. Chemosphere, 70:10971107.

Wesenberg, D., Kyriakides, I. & Agathos, S. N. (2003). White-rot fungi and their enzymes for the treatment of industrial dye effluents. Biotechnol. Adv., 22:16187.

Wu, J. H., Wang, Z. &'Xu, *S*, Y. (2008). Enzymatic production of bioactive peptides from sericinrecovered from silk industry wastewater. Process Biochem., 3:4807.

Zhang, Y. H. P., Himmel, M. E. & Mielenz, J. R. (2006). G*utlook for cellulase improvement: screening and selection strategies. Biotechnol. Adv., 24:452481.

Zille, A., Munteanu, F. D., Gtibitz, G. M. & Cavaco-Paulo A. (2005). Laccase kinetics of degradation and coupling reactions. J.Mol. Catal. B: Enzym., 33:238.

Zumarraga, M., Bulter, T., Shleev, *S.*, Polaina, J., Martinez-Arias, A. & Plou, F.J. (2007). In vitro evolution of a fungal laccase in high concentrations of organic cosolvents. Chem.Biol., 14:105264.