# *IN-SITU* ENTRAPMENT OF LACCASE IN MESOPOROUS SILICA MICROPARTICLES FOR DEGRADATION OF OXYTETRACYCLINE

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*For a decade of my journey.*

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# ABSTRACT

A simple and reproducible method for *in-situ* entrapment of laccase in mesoporous silica microparticles (LSM) was studied. This involved the hydrolysis and condensation of tetraethyl orthosilicate (TEOS) via sol-gel route using one-step (base catalyst) and two-step (acid-base catalyst) methods followed by an ambient drying procedure. It was found that the one-step method was not suitable for *in-situ* entrapment as it left a significant amount of untrapped laccase in the reaction media which led to the inactivation of laccase due to its active site alteration by continuous contact with basic condition. Conversely, the laccase was entrapped entirely in the silica matrices which were synthesized using the two-step method with the highest specific catalytic activity of 434.71 U/g obtained from the 2-LSM15 sample. In addition, the LSM showed an improvement in stability towards pH and temperature compared to the free laccase and was able to retain more than 80% of its initial catalytic activity after one month of storage. The synthesis condition for laccase entrapment was then optimized using a 3-level-4-factor Box–Behnken experimental design to investigate the relationships of the starting material compositions towards the catalytic activity of the entrapped laccase. The optimal condition for laccase entrapment obtained from the response surface methodology (RSM) at $H_2O$/TEOS = 5.44 by molar, HCl = 2.52 mol $\times 10^{-6}$, TEA = 0.39 mol $\times 10^{-3}$ and Lac = 3.83 mg/ml. The predicted response of the maximum solution was 301.7 U/g and the experimental value was 298.36 U/g, respectively, under the optimal condition. Moreover, the sample was capable of retaining almost 90% of the original catalytic activity after 10 repeated recovery and uses. The application of the LSM was further investigated for the degradation of oxytetracycline (OTC). As the temperature increases, OTC component became unstable thus made the use of laccase for OTC degradation unnecessary. On the other hand, the OTC component turned out to be more stable as the pH increased. However, when LSM was applied, 68-88 % of OTC was degraded under previous circumstances. In the kinetic study, opposite pattern of the degradation kinetics rate constants was observed for free laccase and LSM as the amount of enzyme loading increases. The corresponding constant values for free laccase decreased, while the values for LSM experienced a decent escalation. The LSM with a dosage of 4:1 resulted in the highest turnover number ($K_{cat}$= 140136.99 $min^{-1}$) of OTC molecules converted to product per enzyme molecule per unit of time and with catalytic efficiency, $K_{cat}/K_m$= 814.75.

# ABSTRAK

Satu kaedah mudah dan boleh diulang untuk pemerangkapan *in-situ* lakase di liang meso pada silika berzarah mikro (LSM) telah dikaji. Ia melibatkan hidrolisis dan pemeluwapan tetraetil orthosilikat (TEOS) melalui kaedah sol-gel menggunakan satu langkah (pemangkin bes) dan dua langkah (pemangkin asid-bes) diikuti dengan pengeringan ambien. Kaedah satu langkah telah didapati tidak sesuai untuk tujuan pemerangkapan *in-situ* memandangkan ia telah meninggalkan sejumlah lakase yang ketara yang tidak terperangkap dalam media tindakbalas dan membawa kepada penyahaktifan lakase kerana pengubahan tapak aktif oleh pendedahan yang berterusan dengan keadaan bes. Sebaliknya, lakase terperangkap sepenuhnya dalam matriks silika yang disintesis menggunakan kaedah dua langkah dengan aktiviti spesifik setinggi 434.71 U/g diperolehi dari 2-LSM15. Di samping itu, LSM menunjukkan peningkatan terhadap kestabilan pH dan suhu berbanding lakase bebas dan dapat mengekalkan lebih 80% daripada aktiviti awal pemangkin selepas satu bulan tempoh penyimpanan. Keadaan sintesis untuk pemerangkapan lakase kemudian dioptimumkan menggunakan rekabentuk eksperimen Box-Behnken 3-peringkat-4-faktor untuk menyiasat hubungan antara komposisi bahan permulaan terhadap aktiviti pemangkin lakase yang terperangkap. Keadaan optimum untuk pemerangkapan lakase telah diperolehi melalui kaedah gerak balas permukaan (RSM) pada $H_2O$ / TEOS = 5.44 oleh molar, HCl = 2.52 mol × $10^{-6}$, TEA = 0.39 mol × $10^{-3}$ dan Lac = 3.83 mg/ml. Reaksi ramalan dari penyelesaian maksimum adalah 301.7 U/g dan nilai dari eksperimen adalah 298,36 U / g, masing-masing, di bawah keadaan yang optimum. Selain itu, sampel optimum mampu untuk mengekalkan hampir 90% daripada aktiviti pemangkin asal selepas 10 pemulihan berulang dan kegunaan. Aplikasi LSM untuk degradasi antibiotik kemudiannya dikaji menggunakan oksitetrasiklin (OTC) sebagai model antibiotik. Apabila suhu meningkat, komponen OTC menjadi tidak stabil seterusnya membuatkan penggunaan lakase untuk degradasi OTC tidak diperlukan. Sebaliknya, komponen OTC ternyata menjadi lebih stabil apabila pH meningkat. Walau bagaimanapun, dengan penggunaan LSM, OTC telah mendegradasi 68-88% di bawah keadaan sebelumnya. LSM juga menunjukkan keupayaan degradasi yang lebih tinggi untuk OTC berbanding lakase dalam bentuk bebas. Kadar tindak balas untuk degradasi OTC oleh LSM meningkat dengan dos yang semakin meningkat, sebaliknya nilai kadar tindak balas menurun dengan penggunaan lakase bebas. LSM dengan dos 4: 1 menghasilkan jumlah tertinggi perolehan ($K_{cat}$ = 140.136,99 $min^{-1}$) yang mana molekul OTC ditukar kepada produk per molekul enzim per unit masa dan dengan kecekapan pemangkin, $K_{cat}/K_m$ = 814,75.

# TABLE OF CONTENTS

| CHAPTER | TITLE | PAGE |
|---|---|---|
| | **DECLARATION** | ii |
| | **DEDICATION** | iii |
| | **ACKNOWLEDGEMENT** | iv |
| | **ABSTRACT** | v |
| | **ABSTRAK** | vi |
| | **TABLE OF CONTENTS** | vii |
| | **LIST OF TABLES** | xi |
| | **LIST OF FIGURES** | xii |
| | **LIST OF SYMBOLS** | xv |
| | **LIST OF ABBREVIATIONS** | xvi |
| | **LIST OF APPENDICES** | xviii |
| **1** | **INTRODUCTION** | **1** |
| | 1.1 Research Background | 1 |
| | 1.2 Problem Statement | 3 |
| | 1.3 Objectives of Research | 5 |
| | 1.4 Scopes of Research | 6 |
| | | 7 |

**2 LITERATURE REVIEW 7**

2.1 Laccases 7

2.1.1 Introduction to laccases 7

2.1.2 Chemistry of laccases 9

2.1.3 Laccase activity and stability 11

2.1.4 Laccase reaction pathways 12

2.1.5 Applications of laccase in biotechnology 15

2.1.6 Immobilization of laccases 17

2.2 Silica as Support Materials 23

2.2.1 Synthesis of mesoporous silica microparticles 25

2.2.1.1 Sol-gel technique 26

2.2.1.2 Sol preparation and gelation 29

2.2.1.3 Aging 33

2.2.1.4 Drying 35

2.2.2 Synthesis optimization 36

2.3 Removal of Antibiotics Residue 37

2.3.1 Sources and occurrence of antibiotics 38

2.3.2 Potential effects of antibiotics and bacterial resistance 41

2.3.3 Antibiotic removal systems 43

2.3.4 Removal processes 46

2.3.4.1 Nondestructive methods 46

2.3.4.2 Destructive methods 47

2.3.5 Oxytetracycline removal 48

**3 MATERIALS AND METHODS 51**

3.1 Materials 51

3.2 Synthesis of LSM 52

3.2.1 *In-situ* entrapment procedure 52

3.2.2 Catalytic activity assay 55

3.2.3 Stability assessment 56

3.3 Optimization of LSM Synthesis Condition using RSM 57
  3.3.1 Experimental design and statistical analysis 57
  3.3.2 Kinetic study and reusability 57
3.4 Oxytetracycline Degradation by using LSM 59
  3.4.1 Degradation procedure 59
  3.4.2 Oxytetracycline concentration assay 60
  3.4.3 Stability assessment 60
  3.4.4 Kinetic study and reusability 61
3.5 Sample Characterization 61

**4 RESULTS AND DISCUSSIONS 63**
4.1 Synthesis of LSM 63
  4.1.1 Effect of preparation methods 63
  4.1.2 Effect of starting material compositions 67
  4.1.3 Effect of enzyme loading 70
  4.1.4 Aging conditions 71
  4.1.5 Stability assessment 73
  4.1.6 Structural and spectroscopic analysis of LSM 76
4.2 Optimization of LSM Synthesis Conditions using RSM 82
  4.2.1 Model fitting 82
  4.2.2 Mutual effects of variables 85
  4.2.3 Kinetic study and reusability 93
  4.2.4 LSM characterization 95
4.3 Oxytetracycline Degradation by using LSM 98
  4.3.1 Effect of reaction time 98
  4.3.2 Effect of pH and temperature 99
  4.3.3 Kinetic study 102
  4.3.4 Reusability of LSM 105

4.3.5 OTC proposed degradation pathway and toxicity study 106

**5 CONCLUSIONS AND RECOMMENDATIONS 109**

**REFERENCES 112**

Appendices A – G 134-158

# LIST OF TABLES

| TABLE NO. | TITLE | PAGE |
|---|---|---|
| 2.1 | Classes of enzymes. | 8 |
| 2.2 | Enzymes immobilization techniques. | 18 |
| 2.3 | Various sol-gel preparations. | 30 |
| 3.1 | Detail composition of the starting material for the one-step method. | 53 |
| 3.2 | Detailed composition of the starting material for the two-step method. | 54 |
| 3.3 | A 3-level-4-factor Box-Behnken experimental design. | 58 |
| 3.4 | Detailed composition of substrate concentration for OTC degradation by free laccase and LSM at dosage of 0.5:1, 2:1, and 4:1. | 61 |
| 4.1 | Effect of storage duration on the specific catalytic activity of entrapped laccase. | 76 |
| 4.2 | Peak summary for FTIR spectra (Appendix A.3). | 77 |
| 4.3 | Textural properties of 2-LSM3, 2-LSM8, 2-LSM 11, and 2-LSM15. | 80 |
| 4.4 | Analysis for joint test of all independent variables. | 84 |
| 4.5 | Kinetic constants for OTC degradation by free laccase and LSM at dosage of 0.5:1, 2:1, and 4:1. | 103 |

# LIST OF FIGURES

| FIGURE NO. | TITLE | PAGE |
|---|---|---|
| 2.1 | Schematic representation of copper coordination centers, including interatomic distances among all relevant ligands. | 10 |
| 2.2 | Catalytic cycle of laccase. | 13 |
| 2.3 | Catalytic cycle of a laccase oxidation system and with presence of mediator. | 15 |
| 2.4 | Various industrial and biotechnological applications of laccases. | 16 |
| 2.5 | The reactions of silica gels synthesis. | 27 |
| 2.6 | Hydrolysis by acid and base catalyzed conditions. | 28 |
| 2.7 | Ternary phase diagram of the system TEOS-ethanol-water at 25 °C . | 32 |
| 2.8 | The sol-gel process and its various products. | 35 |
| 2.9 | Origin and principal contamination routes of human and veterinary antibiotics. | 39 |
| 2.10 | Process flow diagram of advanced wastewater treatment plant using microfiltration/reverse osmosis for antibiotics removal assessment. | 45 |
| 2.11 | The structure and characteristics of oxytetracycline. | 49 |
| 3.1 | Schematic representation for synthesis of LSM. | 52 |

4.1 SEM images of LSM synthesized via one-step method. (a) $18x10^{-3}$ mol $NH_4OH$, (b) $25x10^{-3}$ mol $NH_4OH$, (c) $18x10^{-3}$ mol TEA, and (d) $25x10^{-3}$ mol TEA. 65

4.2 SEM images of starting material composition synthesized by two-step method. (a) TEOS/$H_2O$ = 0.5/0.6; HCl = $5x10^{-6}$ mol; TEA = $0.72x10^{-3}$ mol, (b) TEOS/$H_2O$ = 1.5/0.6; HCl = $5x10^{-6}$ mol; TEA = $0.72x10^{-3}$ mol, (c) TEOS/$H_2O$ = 1.5/0.6; HCl = $1.5x10^{-6}$ mol; TEA = $0.72x10^{-3}$ mol, and (d) TEOS/$H_2O$ = 1.5/0.6; HCl = $5x10^{-6}$ mol; TEA = $1.8x10^{-3}$ mol. 68

4.3 Effect of starting material compositions: (a) TEOS; (b) HCl; (c) TEA; and (d) laccase loading on the specific activity of the LSM. 70

4.4 Effect of pH on the specific catalytic activity of free laccase and LSM. 74

4.5 Effect of temperature on the specific catalytic activity of free laccase and LSM. 75

4.6 Nitrogen adsorption/desorption isotherm and pore size distribution of (a) 2-LSM3, (b) 2-LSM8, (c) 2-LSM11, and (d) 2-LSM15. 79

4.7 (a) SEM and (b) TEM images of 2-LSM15. 81

4.8 Optimization plot of the predicted versus observed values. 83

4.9 Pareto chart of the ISV optimization. 88

4.10 Laccase specific catalytic activity ($A_s$) response surface contour plot of the $X_2$ and $X_1$ at constant (TEA) = $1.5x10^{-3}$ mol and (Lac) = 3 mg/g. 87

4.11 Laccase specific catalytic activity ($A_s$) response surface contour plot of the $X_3$ and $X_1$ at constant (HCl) = $2x10^{-6}$ mol and (Lac) = 3 mg/g. 88

4.12 Laccase specific catalytic activity ($A_s$) response surface contour plot of the $X_4$ and $X_1$ at constant (HCl) = $2x10^{-6}$ mol and (TEA) = $1.5x10^{-3}$ mol. 88
4.13 Laccase specific catalytic activity ($A_s$) response surface contour plot of the $X_3$ and $X_2$ at constant ($H_2O$/TEOS) = 5 and (Lac) = 3 mg/g. 90
4.14 Laccase specific catalytic activity ($A_s$) response surface contour plot of the $X_4$ and $X_2$ at constant ($H_2O$/TEOS) = 5 and (TEA) = $1.5x10^{-3}$ mol. 91
4.15 Laccase specific catalytic activity ($A_s$) response surface contour plot of the $X_4$ and $X_3$ at constant ($H_2O$/TEOS) = 5 and (HCl) = $2x10^{-6}$ mol. 92
4.16 Reusability of LSM for 10 cycles in the presence of 1 mM ABTS in 100 mM sodium acetate buffer (pH 5) for 1 h at room temperature (30 °C). 94
4.17 (a) SEM and (b) TEM images of LSM. 96
4.18 Nitrogen adsorption/desorption isotherms and pore size distribution of LSM. 97
4.19 Effect of reaction time on degradation of OTC. 99
4.20 Effect of pH on degradation of OTC. 100
4.21 Effect of temperature on degradation of OTC. 101
4.22 Dependence of the initial reaction rate of free laccase and LSM on the initial substrate concentration. 103
4.23 Relative degradation of OTC by LSM in subsequent processes. 106
4.24 Proposed reaction pathway for OTC degradation by laccase. 107

# LIST OF SYMBOLS

$A$ - Catalytic activity (U)

$A_s$ - Specific catalytic activity (U/g)

$C_o$ - Initial concentrations

$C_t$ - Residual concentrations of after t minutes

$K_m$ - Kinetic activator constant (mM)

$K_{cat}$ - Catalytic constant ($min^{-1}$)

$K_{cat}/K_m$ - Catalytic efficiency

$V$ - Reaction volume (L)

$V_{max}$ - Theoretical maximum velocity (µM/min)

$\varepsilon$ - Molar absorption coefficient ($M^{-1}$ $cm^{-1}$)

$l$ - Thickness of the sample (cm)

$\Delta t$ - Reaction time (min)

$\Delta A$ - Increase in absorbance at 436nm

$\Delta A/\Delta t$ - Reaction rate

# LIST OF ABBREVATIONS

| | | |
|---|---|---|
| 2,6-DMP | - | 2,6-dimethoxyphenol |
| ABTS | - | 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonate) |
| ANOVA | - | Analysis of variance |
| AOP | - | Advanced oxidation processes |
| APD | - | Ambient pressure drying |
| ATR | - | Attenuated Total Reflectance |
| BBD | - | Box-Behnken design |
| BET | - | Brunauer-Emmet-Teller |
| BJH | - | Barret–Joyner–Halenda |
| CCD | - | Central composite designs |
| DM | - | Doehlert matrix |
| FTIR | - | Fourier transform infrared |
| HBT | - | Triazole 1-hydroxybenzotriazole |
| IPA | - | Isopropanol |
| ISV | - | Independent synthesis variables |
| LSM | - | Laccase entrapped in mesoporous silica microparticle |
| $NH_4OH$ | - | Ammonia solution |
| OFAT | - | One-factor-at-a-time |
| OTC | - | Oxytetracycline |
| PSD | - | Pore size distribution |
| RSM | - | Response surface methodology |
| SEM | - | Scanning electron microscope |
| SMZ | - | Sulfamethoxazole |
| STZ | - | Sulfathiazole |
| TEA | - | Triethylamine |
| TEM | - | Transmission electron microscopy |
| TEOS | - | Tetraethyl orthosilicate |

TMCS - Trimethylchlorosilane
WWTP - Wastewater treatment plant

# LIST OF APPENDICES

| APPENDIX | TITLE | PAGE |
|---|---|---|
| A | Data Collection for Synthesis of LSM. | 134 |
| A.1 | Data effect of pH on the specific activity of free laccase and LSM. | 134 |
| A.2 | Data effect of temperature on the specific activity of free laccase and LSM. | 134 |
| A.3 | FTIR spectra of (a) free laccase, (b) denatured free laccase, (c) hydrophilic LSM, (d) hydrophobic LSM and (e) denatured LSM samples. | 135 |
| B | Data Collection for Optimization of LSM Synthesis Condition using RSM. | 136 |
| B.1 | Data of 3-level-4-factor Box-Behnken experimental results. | 136 |
| B.2 | Data reusability of LSM for 10 cycles. | 137 |
| C | Data Collection for Degradation of OTC. | 138 |
| C.1 | Standard calibration curve for oxytetracycline. | 138 |
| C.2 | Data effect of reaction time on degradation of OTC. | 139 |
| C.3 | Data Effect of pH on degradation of OTC. | 139 |
| C.4 | Data effect of temperature on degradation of OTC. | 139 |
| C.5 | Data dependence of the initial reaction rate on the initial substrate concentration for kinetic study. | 140 |
| C.6 | Data relative degradation of OTC by LSM in subsequent processes. | 140 |
| D | Nitrogen Adsorption-desorption (NAD) Isotherm. | 141 |
| D.1 | NAD for 2-LSM3. | 141 |
| D.2 | NAD for 2-LSM8. | 142 |
| D.3 | NAD for 2-LSM11. | 143 |

| | | |
|---|---|---|
| D.4 | NAD for 2-LSM15. | 144 |
| D.5 | NAD for LSM (optimum). | 145 |
| E | Pore Size Distribution. | 146 |
| E.1 | Pore size distribution for 2-LSM3. | 146 |
| E.2 | Pore size distribution for 2-LSM8. | 147 |
| E.3 | Pore size distribution for 2-LSM11. | 148 |
| E.4 | Pore size distribution for 2-LSM15. | 149 |
| E.5 | Pore size distribution for LSM (optimum). | 150 |
| F | Recent Developments in Laccase Immobilization. | 151 |
| G | List of Publications. | 158 |

# CHAPTER 1

# INTRODUCTION

## 1.1 Research Background

Laccases (benzenediol: oxygen oxidoreductase; EC 1.10.3.2) belongs to the superfamily of multicopper oxidases. Among the oxidative enzymes, laccases have received a lot of attention from researchers due to their peculiar catalytic properties, offering great potential for biotechnological and environmental applications (Bollag, 1992). This oxidoreductase enzyme is classified based on its oxidation-reduction reaction. Laccases are the oldest and most studied enzymatic systems which are widely present in nature. Yoshida first described laccase in 1883 when he extracted it from the exudates of the Japanese lacquer tree, *Rhus vernicifera.* The biological roles of laccase are diverse in nature (Mayer and Staples, 2002). In fungi, laccases carry out a variety of physiological roles including morphogenesis, fungal plant pathogen/ host interaction, stress defense, and lignin degradation (Thurston, 1994; Gianfreda *et al.*, 1999). In plants, laccases have been found in the wood and cellular walls of herbaceous species, where they participate in lignin biosynthesis (Sato *et al.*, 2001). Bacterial laccases appear to have a role in morphogenesis (Sharma *et al.*, 2007), in the biosynthesis of the brown spore pigment and protection afforded by the spore coat against UV light and hydrogen peroxide, and also in copper homeostasis. While the main function of the laccase-type proteins in insects is believed to be sclerotization of the cuticle in the epidermis (Dittmer *et al.*, 2004).

In the function of substrate specificity, laccases are remarkably non-specific as to their reducing substrates, but the range of substrates oxidized varies from one laccase to another. Laccases has broad substrates specificity including organic pollutants such as chlorinated phenol and polycyclic aromatic hydrocarbons (PAHs) (Forootanfar *et al.*, 2012; Dehghanifard *et al.*, 2013) and synthetic dyes (Gholami-Borujeni *et al.*, 2011; Ashrafi *et al.*, 2013; Mirzadeh *et al.*, 2014). The ability of laccases to oxidize some pharmaceutical agents such as diclofenac, naproxen, ketoprofen, oseltamivir, tetracyclines, sulfonamides, erythromycin, and estrogenic hormones has been reported as well (Lloret *et al.*, 2010, 2013; Rodríguez-Rodríguez *et al.*, 2012; Sathishkumar *et al.*, 2012; Suda *et al.*, 2012). The use of laccase has been explored for wide applications including the detoxification of industrial effluents, mostly from paper and pulp (Crestini and Argyropoulos, 1998; Wesenberg *et al.*, 2003), textile and petrochemical industries, medical diagnostics and as a bioremediation agent to clean up herbicides, pesticides, and certain explosives in soil. Laccase was also used as cleaning agents for certain water purification systems and waste water treatment, as catalysts for the manufacture of anti-cancer drugs and even as ingredients in cosmetics. Besides that, laccase also has the capacity to remove xenobiotic substances (Dur′an and Esposito, 2000; Torres *et al.*, 2003), to transform antibiotics and steroids, as well as produce polymeric products which makes them a useful tool for bioremediation purposes (Rodriguez Couto and Herrera, 2006).

Even though free laccases are effective in various industrial and biotechnological applications, there are still many constraints on their application in real effluents. The non-reusability of free laccase and its deactivation by temperature, pH, and inhibitors are the setback which consequently will reduce their activity and limit their usefulness. These limitations however can be overcome by the immobilization of enzyme and it is the most straightforward way to implement enzyme-based processes (Lloret *et al.*, 2011). Immobilization is achieved by fixing enzymes to or within solid supports, as a result of which heterogeneous immobilized enzyme systems are obtained. The major advantages of laccase immobilization are the increase in the thermostability of the enzyme and its resistance to extreme conditions and chemical reagents (Fernández-Fernández *et al.* 2012). In addition, immobilized laccases may be easily separated from the reaction products, allowing the enzymes to be employed in continuous bioreactor operations (Arica *et al.*, 2009; Georgieva *et al.*, 2008).

## 1.2 Problem Statement

Development of a simple and reliable procedure for enzyme immobilization is always an important aspect of biotechnology. Formulation is a key step because it determines to a large extent the biocatalyst performance, the immobilization yield and the contribution of the biocatalyst to the total cost of a bioprocess (Tufvesson *et al.*, 2010). In addition to this, the enzyme demands mild experimental conditions (pressure, temperature, pH etc.) must be considered in the design as well. In some cases, although laccase has been successfully immobilized, the immobilization yield was less than 50% of the initial laccase concentration (Annibale *et al.*, 1999). Even though there is stability enhancement of the immobilized laccase, the catalytic activity appeared to be lower than laccase in the free form (Brandi *et al.*, 2006). Some studies have also reported a complex and multistep procedure which takes a few days to complete (Qiu and Huang, 2010; Machado *et al.*, 2012), this will be a waste of time and may affect the total production cost subsequently.

Several techniques may be applied to immobilize laccases. They are mainly based on *ex-situ and in-situ* immobilization technique. The *ex-situ* immobilization involves preparation of the support material followed by either adsorption or covalent binding between enzyme and silica support surfaces. The adsorption of laccase onto a support is based on ionic and/or other weak forces of attraction, whereas covalent binding utilizes activation of chemical groups on the support surface with nucleophilic groups on the laccase. Laccases have been reported with stability improvement by *ex-situ* immobilization on numerous supports, such as porous and non-porous glass, agarose, amorphous silica, organic gels or kaolinite, graphite, and chitosan (see review by Durán *et al.*, 2002; Fernández-Fernández *et al.* 2012). However, apart from the stability improvement, *ex-situ* immobilization often resulted in lower immobilization yield and may be attributed to leaching due to the weakening of binding strength between the matrix and the immobilized enzyme from repeated use (Singth *et al.*, 2014). The covalent binding may perturb the enzyme native structure and lead to reduction of enzyme activity (Duran *et al.*, 2002). Besides, the ex-situ procedure becomes disadvantageous since the process is somehow time consuming with separate preparation of support matrix and the immobilization procedure which could lead to an upsurge cost (Huang *et al.*, 2006; Huang *et al.*, 2007).

On the other hand, *in-situ* immobilization technique involves entrapment of the enzyme within a polymer lattice or its encapsulation in an organic or inorganic polymer (membranes). In this technique, the preparation time could be lessens since the support material and enzyme immobilization are prepared simultaneously. It is basically a controlled of enzyme loading and may provide relatively small perturbation of the enzyme native structure and function (Durán *et al.*, 2002). However, the main drawback of these immobilization methods is mass transfer limitation (Brady and Jordaan, 2009). Another method considered as *in-situ* technique is self-immobilization, it is a carrier-free immobilization which did not depends on any support material. It utilizes bifunctional cross-linkers to form enzyme aggregates, but their major drawback is the high purity required for the crystallization of the enzyme (Fernández-Fernández *et al.* 2012).

Therefore, in approaching this issue, the present study was conducted to develop a simple and reproducible method for laccase immobilization. The *in-situ* immobilization technique using entrapment method has been chosen in order to simplify the procedure and to reduce the processing time. The usage of harsh chemical and harsh condition as well as fancy equipment (such as sonicator, autoclave, or freeze dryer) is not implemented in developing this procedure. Laccase was immobilized in mesoporous silica microparticles to encounter the mass transfer limitations (Carlsson *et al.*, 2014) and air dried under ambient condition to preserve the immobilized laccase. The developed immobilization procedure was further optimized to find the best condition for laccase entrapment, followed by degradation of oxytetracyline (an antibiotic) to demonstrate the applicability of the immobilized laccase. From previous studies, removal of OTC using photo-irradiation (Shaojun *et al.*, 2008) and ozonation (Li *et al.*, 2008) results in higher toxic level in the after treatment solution. Thus, utilization of environmental friendly process using laccase through enzymatic treatment for removal of OTC is introduced in this study.

## 1.3 Objectives of the Research

The objectives of the research are:

a) To synthesis and characterize laccase entrapped in mesoporous silica microparticle (LSM).

b) To optimize the synthesis condition for LSM using response surface methodology (RSM).

c) To investigate LSM biodegradation performance using oxytetracyline.

## 1.4 Scopes of Research

The scopes of research are presented to specify in details the objectives of research that stated above:

a) In-situ entrapment of laccase in mesoporous silica microparticles which involved hydrolysis and condensation of tetraethyl orthosilicate (TEOS) was studied via sol-gel route using one-step (base catalyst) and two-step (acid-base catalyst) methods followed by an ambient drying procedure. The influence of the methods used, the compositions of the starting material and the aging conditions towards polymeric structure and catalytic activity of the laccase entrapped in mesoporous silica microparticles (LSM) were investigated. . In order to characterize the LSM, their catalytic activity and stability will be observed as well as their physical properties such as particle morphology, specific surface area, average pore volume, size, and determination of the functional group.

b) The synthesis condition for LSM was further optimized in this study to obtain the optimal condition for laccase immobilization. The response surface methodology (RSM) based on a 3-level-4-factor Box–Behnken experimental design was employed to establish the relationships among the independent synthesis variables (ISV) as well as to search for an optimal synthesis condition for laccase entrapped in mesoporous silica microparticles (LSM). The ISV comprise of $H_2O$/TEOS molar ratio ($H_2O$/TEOS), hydrochloric acid loading (HCl), triethylamine loading (TEA), and laccase loading (Lac) were evaluated towards the laccase specific catalytic activity ($A_s$) response as the dependent variable.

c) Several parameters which are reaction temperature, reaction pH and reaction time were varied in order to investigate the biodegradation performance of free laccase and LSM using oxytetracyline as substrate. The degradation kinetic study and reusability were carried out afterward.

lessens. In some cases, the removal of the parent compound was successful. However, the process yielded toxic intermediates with harmful effects on the organisms. Growth inhibition of standard microbial strains (for example, *Bacillus megaterium, E. coli*, and *Saccharomyces cerevisiae*) is one of the most commonly applied methods for such evaluation. The measurement of $BOD_5$ and COD were also significant for the evaluation of the biodegradability. Hopefully these findings will contribute to the body of knowledge on subject concerning laccase immobilization as well as their potential applications for future research.

# REFERENCES

Abellán, M.N., Bayarri, B., Giménez, J. and Costa, J. (2007). Photocatalytic Degradation of Sulfamethoxazole in Aqueous Suspension of $TiO_2$. *Appl. Catal. B*, 74, 233-241.

Adams, C., Asce, M., Wang, Y., Loftin, K. and Meyer, M. (2002). Removal of Antibiotics from Surface and Distilled Water in Conventional Water Treatment Processes. *J. Environ. Eng.,* 128, 253-260.

Alexy, R., Sommer, A., Lange, F.T. and Kümmerer, K. (2006). Local Use of Antibiotics and Their Input and Fate in a Small Sewage Treatment Plant – Significance of Balancing and Analysis on a Local Scale vs. Nationwide Scale. *Acta Hydroch. Hydrob*., 34, 587-592.

Aminov, R.I., Chee-Sanford, J.C., Krapac, I.J., Garrigues-Jeanjean, N. and Mackie, R.I. (2001). Occurrence and Diversity of Tetracycline Resistance Genes in Lagoons and Groundwater Underlying Two Swine Production Facilities. *Appl. Environ. Microbiol*., 67(4), 1494-1502.

Anbia, M. and Lashgari, M. (2009). Synthesis of Amino-Modified Ordered Mesoporous Silica As A New Nano Sorbent For The Removal of Chlorophenols From Aqueous Media. *Chem. Eng. J.*,150, 555–560.

Arica, M.Y., Altintas, B. and Bayramoglu, G. (2009). Immobilization of Laccase onto Spacer-Arm Attached Non-Porous Poly(GMA/EGDMA) Beads: Application For Textile Dye Degradation. *Bioresour. Technol*., 100, 665–9.

Ashrafi, S.D., Rezaei, S., Forootanfar, H., Mahvi, A.H. and Faramarzi, M.A. (2013). The Enzymatic Decolorization And Detoxification of Synthetic Dyes By The Laccase From A Soil-Isolated Ascomycete, *Paraconiothyrium variabile. Int. Biodeterior. Biodegrad.*, 85, 173-181.

Attrassi, B., Saghi, M. and Flatau, G. (1993). Multiple Antibiotic-Resistance of Bacteria in Atlantic Coast (Marocco). *Environ. Technol.*, 14, 1179-1186.

Bailón-Pérez, M.I., García-Campaña, A.M., Cruces-Blanco, C. and del Olmo Iruela, M. (2008). Trace Determination of Β-Lactam Antibiotics in Environmental Aqueous Samples Using Off-Line and On-Line Preconcentration in Capillary Electrophoresis. *J. Chrom. A,* 1185(2), 273-280.

Balcióglu, I.A. and Ötker, M. (2003). Treatment of Pharmaceutical Wastewater Containing Antibiotics by $O_3$ and $O_3/H_2O_2$ Processes. *Chemosphere*, 50, 85-95.

Baldrian, P. (2006). Fungal Laccases Occurrence and Properties. *FEMS Microbiol. Rev.*, 30 (2), 215–242.

Bautitz, I.R. and Nogueira, R.F.P. (2007). Degradation of Tetracycline by Photo-Fenton Process-Solar Irradiation and Matrix Effect. *J. Photochem. Photobiol. A,* 187, 33-39.

Bayramoglu, G., Yilmaz, M. and Arica, M.Y. (2010). Reversible Immobilization of Laccase to Poly(4-vinylpyridine) Grafted and Cu(II) Chelated Magnetic Beads: Biodegradation of Reactive Dyes. *Biores. Technol.*, 101, 6615–6621.

Bogush, G.H. and Zukoski Iv, C.F. (1991). Uniform Silica Particle Precipitation: An Aggregative Growth Model. *J. Colloid Interf. Sci.*, 142, 19-34.

Bollag, J.M. (1992). Decontamination Soil with Enzymes. *Environ. Sci. Technol.* 26, 1876–1881.

Brady, D. and Jordaan, J. (2009). Advances in Enzyme Immobilisation. *Biotechnol. Lett.*, 31, 1639–50.

Brandi, P., Annibale, A.D., Galli, C., Gentili, P., Sofia, A., and Pontes, N. (2006). In Search for Practical Advantages from The Immobilisation of an Enzyme : The Case of Laccase. *J. Mol. Cat. B: Enzym.*, 41, 61–69.

Call, H.P. and Mücke, I. (1997). History, Overview and Applications of Mediated Lignolytic Systems, Especially Laccase-Mediator-Systems (Lignozym®process). *J. Biotechnol.*, 53, 163–202.

Carlsson, N., Gustafsson, H., Thörn, C., Olsson, L., Holmberg, K., and Åkerman, B. (2014). Enzymes Immobilized in Mesoporous Silica : A Physical – Chemical Perspective. *Adv. in Colloid and Interface Sci.*, 205, 339–360.

Christian, T., Schneider, R.J., Färber, H.A., Skutlarek, D., Meyer, M.T. and Goldbach, H.E. (2003). Determination of Antibiotic Residues in Manure, Soil, and Surface Waters. *Acta Hydroch. Hydrob.*, 31, 36-44.

Chung, T. -W., Yeh, T. -S. and Yang, T. C. K. (2001). Influence of Manufacturing Variables on Surface Properties and Dynamic Adsorption Properties of Silica Gels. *J. Non-Cryst. Solids,* 279(2–3), 145-153.

Claus, H. (2004). Laccases: Structure, Reactions, Distribution. *Micron*, 35, 93–96.

Crestini, C. and Argyropoulos, D.S. (1998). The Early Oxidative Biodegradation Steps of Residual Kraft Lignin Models with Laccase. *Bioorg. Med. Chem.*, 6, 2161–9.

D'Annibale, A., Stazi, S.R., Vinciguerra, V., Di Mattia, E. and Sermanni, G.G. (1999). Characterization of Immobilized Laccase From *Lentinula edodes* And Its Use in Olive Mill Wastewater Treatment. *Process. Biochem.*, 34, 697–706.

Dai, Y., Niu, J., Liu, J., Yin, L. and Xu, J. (2010). In Situ Encapsulation of Laccase in Microfibers by Emulsion Electrospinning: Preparation, Characterization, and Application. *Biores. Technol.,* 101, 8942-8947.

De Stefano, L., Rea, I., De Tommasi, E., Rendina, I., Rotiroti, L. and Giocondo, M. (2009) Bioactive Modification of Silicon Surface Using Self-Assembled Hydrophobins from *Pleurotus ostreatus*. *Eur. Phys. J. E.*, 30, 181–5.

Dehghanifard, E., Jonidi, A.J., Rezaei, R.K., Mahvi, A.H., Faramarzi, M.A. and Esrafili, A. (2013). Biodegradation of 2,4-dinitrophenol With Laccase Immobilized on Nano-porous Silica Beads. *J. Environ. Health Sci. Eng.*, 10, 25.

Deng, M., Zhao, H., Zhang, S., Tian, C., Zhang, D., Du, P. and Li, H. (2015). High Catalytic Activity of Immobilized Laccase on Core–Shell Magnetic Nanoparticles By Dopamine Self-Polymerization. *J. Mol. Cat. B: Enzym.*, 112, 15–24.

Díaz-Cruz, M.S., López de Alda, M.a.J. and Barceló, D. (2003). Environmental Behavior and Analysis of Veterinary and Human Drugs in Soils, Sediments and Sludge. *Trac-Trend. Anal. Chem.,* 22(6), 340-351.

Dittmer, N.T., Suderman, R.J., Jiang, H., Zhu, Y.-C., Gorman, M J. and Kramer, K.J. (2004). Characterization of cDNAs Encoding Putative Laccase-Like Multicopper Oxidases and Developmental Expression in The Tobacco

Dodor, D.E., Hwang, H. and Ekunwe, S.I.N. (2004). Oxidation of Anthracene and Benzo[α]pyrene by Immobilized Laccase from *Trametes versicolor*. *Enzyme Microb. Tech.,* 35, 210–7.

Doi, A.M and Stoskopf, M.K. (2000). The Kinetics of Oxytetracycline Degradation in Deionized Water under Varying Temperature , pH , Light , Substrate , and Organic Matter. *J. Aquat. Anim. Health*, 246–253.

Durán, N. and Esposito, E. (2000). Potential Applications of Oxidative Enzymes and Phenoloxidase-Like Compounds in Wastewater and Soil Treatment: A Review. *Appl. Catal. B: Environ.,* 28, 83–99.

Durán, N., Rosa, M. A., Annibale, A. D. and Gianfreda, L. (2002). Applications of Laccases And Tyrosinases ( Phenoloxidases ) Immobilized on Different Supports : A Review. *Enzym. and Microb. Technol*., 31, 907–931.

Dwivedi, U.N., Singh, P., Pandey, V.P., and Kumar, A. (2011). Structure–function Relationship among Bacterial, Fungal and Plant Laccases. *J. Mol. Catal. B: Enzym.,* 68(2), 117-128.

Farnet, A.M., Criquet, S., Tagger, S., Gil, G. and Le Petit, J. (2000). Purification, Partial Characterization, and Reactivity with Aromatic Compounds of Two Laccases from *Marasmius quercophilus* strain 17. *Can. J. Microbiol.*, 46(3), 189–194.

Farré, M.l., Pérez, S., Kantiani, L. and Barceló, D. (2008). Fate and Toxicity of Emerging Pollutants, Their Metabolites and Transformation Products in The Aquatic Environment. *Trac-Trends Anal. Chem.,* 27(11), 991-1007.

Fernández-Fernández, M., Sanromán, M.Á. and Moldes, D. (2012). Recent Developments and Applications of Immobilized Laccase. *Biotechnol. Adv., 31, 1808-1825.*

Fernando Bautista, L., Morales, G. and Sanz, R. (2010). Immobilization Strategies for Laccase from *Trametes versicolor* on Mesostructured Silica Materials and The Application to The Degradation of Naphthalene. *Biores. Technol.,* 101, 8541–8.

Ferreira, S.L.C., Bruns, R.E., Ferreira, H.S., Matos, G.D., David, J.M., Brandão, G.C. and dos Santos, W.N L. (2007). Box-Behnken Design: An Alternative for The Optimization of Analytical Methods. *Anal. Chim. Acta*, 597(2), 179–86.

Fey, P.D., Safranek, T.J, Rupp, M.E, Dunne, E.F, Ribot, E, Iwen, P.C, Bradford, P.A, Angulo, F.J and Hinrichs, S.H. (2000). Ceftriaxone-resistant Salmonella Infection Acquired By A Child From Cattle. *N. Engl. J. Med.*, 342, 1242–1249.

Forootanfar, H., Movahednia, M.M., Yaghmaei, S., Tabatabaei-Sameni, M., Rastegar, H., Sadighi, A. and Faramarzi, M.A. (2012). Removal of Chlorophenolic Derivatives by Soil Isolated Ascomycete of Paraconiothyrium variabile And Studying The Role of Its Extracellular Laccase. *J. Hazard. Mater*. 209-210, 199-203.

García-Galán, M.J., Rodríguez-Rodríguez, C.E., Vicent, T., Caminal, G., Díaz-Cruz, M. S. and Barceló, D. (2011). Biodegradation of Sulfamethazine By *Trametes versicolor*: Removal from Sewage Sludge and Identification of Intermediate Products by UPLC–QqTOF-MS. *Sci. Total Environ.,* 409(24), 5505-5512.

Georgieva, S., Godjevargova, T., Portaccio, M., Lepore, M. and Mita, D.G. (2008). Advantages in Using Non-Isothermal Bioreactors in Bioremediation of Water Polluted By Phenol By Means of Immobilized Laccase From Rhus vernicifera. J. Molec. Catal. B, 55, 177–84.

Gholami-Borujeni, F., Mahvi, A.H., Naseri, S., Faramarzi, M.A., Nabizadeh, R. and Alimohammadi, M. (2011). Application of Immobilized Horseradish Peroxidase For Removal And Detoxification of Azo Dye From Aqueous Solution. *Res. J. Chem. Environ.*, 15, 217-222.

Gianfreda, L., Xu, F. and Bollag, J.-M. (1999). Laccases: A Useful Group of Oxidoreductive Enzymes. *Bioremediat. J., 3*(1), 1-26.

Giardina, P., Faraco, V., Pezzella, C., Piscitelli, A., Vanhulle, S., and Sannia, G. (2010). Laccases: A Never-Ending Story. *Cell. Mol. Life Sci.,* 67, 369–385.

Göbel, A., McArdell, C.S., Joss, A., Siegrist, H. and Giger, W. (2007). Fate of Sulfonamides, Macrolides, and Trimethoprim in Different Wastewater Treatment Technologies. *Sci. Total Environ.*, 372(2–3), 361-371.

Gregg, S.J. and Sing, K.S.W. (1982). Adsorption, Surface Area and Porosity. London: Academic Press.

Gurav, J.L., Nadargi, D.Y., and Rao, A.V. (2008). Effect of Mixed Catalysts System on TEOS-Based Silica Aerogels Dried at Ambient Pressure. *Appl. Surf. Sci.,* 255, 3019–3027.

Hæreid, S., Anderson, J.M., Einarsrud, M.-A., Hua, D.W. and Smith, D.M.J. (1995). Preparation and Properties of Monolithic Silica Xerogels from TEOS-Based Alcogels Aged in Silane Solutions. *J. Non-Cryst. Solid*, 185, 221.

Hamscher, G., Pawelzick, H.T., Höper, H. and Nau, H. (2005). Different Behavior of Tetracyclines and Sulfonamides in Sandy Soils After Repeated Fertilization With Liquid Manure. *Environ. Toxicol. Chem.,* 24(4), 861-868.

Halling-Sørensen, B., Lykkeberg, A., Ingerslev, F., Blackwell, P. and Tjørnelund. J. (2003). Characterization of The Abiotic Degradation Pathways of Oxytetracyclines In Soil Interstitial Water Using LC– MS–MS. *Chemosphere,* 50, 1331–1342.

Hamscher, G., Sczesny, S., Höper, H. and Nau, H. (2002). Determination of Persistent Tetracycline Residues in Soil Fertilized with Liquid Manure by High-Performance Liquid Chromatography with Electrospray Ionization Tandem Mass Spectrometry. *Anal. Chem.,* 74(7), 1509-1518.

Heinzkill, M., Bech, L., Halkier, T., Schneider, P. and Anke, T. (1998). Characterization of Laccases And Peroxidases From Wood Rotting Fungi (Family *Coprinaceae*). *Appl. and Environ. Microb.*, 64(5), 1601-1606.

Hilonga, A., Kim, J., Sarawade, P.B. and Taik, H. (2009). Low-Density TEOS-Based Silica Aerogels Prepared at Ambient Pressure Using Isopropanol as The Preparative Solvent. *J. Alloys Compd*., 487, 744–750.

Hirsch, R., Ternes, T., Haberer, K. and Kratz, K.L. (1999). Occurrence of Antibiotics in the Aquatic Environment. *Sci. Total Environ*. 225, 109-118.

Hoegger, P.J., Kilaru, S., James, T.Y., Thacker, J. R. and Kües, U. (2006). Phylogenetic Comparison and Classification of Laccase and Related Multicopper Oxidase Protein Sequences. *FEBS J.,* 273(10), 2308-2326.

Homem, V. and Santos, L. (2011). Degradation and Removal Methods of Antibiotics from Aqueous Matrices – A Review. *J. Environ. Management,* 92(10), 2304-2347.

Hornworm, Manduca sexta, and The Malaria Mosquito, Anopheles gambiae. Insect Biochem. Molec., 34(1), 29-41.

Honn, K.V. and Chavin, W. (1975). An Improved Automated Biuret Method for The Determination of Microgram Protein Concentrations. *Anal. Biochem*., 68(1), 230–235.

Huang, J., Liu, C., Xiao, H., Wang, J., Jiang, D. and Gu, E. (2007). Zinc Tetraaminophthalocyanine-$Fe_3O_4$ Nanoparticle Composite for Laccase Immobilization. *Int. J. Nanomedicine*, 2, 775-784.

Huang, J., Xiao, H., Li, B., Wang, J. and Jiang, D. (2006). Immobilization of *Pycnoporus Sanguineus* Laccase on Coppert-Aminophthalocyanine-$Fe_3O_4$ Nanoparticle Composite. *Biotechnol. Appl. Biochem*., 44, 93-100.

Husevhg, B., Lunestad, B.T., Johannessen, P.J., Enger, Ø. and Samuelsen, O.B. (1991). Simultaneous Occurrence of *Vibrio salmonicida* and Antibiotic-Resistant Bacteria in Sediments at Abandoned Aquaculture Sites. *J. Fish Dis.,* 14(6), 631-640.

Jara, C.C., Fino, D., Specchia, V., Saracco, G. and Spinelli, P. (2007). Electrochemical Removal of Antibiotics from Wastewater. *Appl. Catal. B*, 70, 479-487.

Jiao, S., Zheng, S., Yin, D., Wang, L. and Chen, L. (2008). Aqueous Photolysis of Tetracycline and Toxicity of Photocatalytic Products to Luminescent Bacteria. *Chemosphere,* 73*,* 377-382.

Jones, O.A., Lester, J.N. and Voulvoulis, N. (2005). Pharmaceuticals: A Threat to Drinking Water?. *Trends Biotechnol.,* 23(4), 163-167.

Ju, H.-Y., Kuo, C.-H., Too, J.-R., Huang, H.-Y., Twu, Y.-K., Chang, C.-M. J. and Shieh, C.-J. (2012). Optimal Covalent Immobilization of A-Chymotrypsin on $Fe_3O_4$-Chitosan Nanoparticles. *J. Mol. Catal. B: Enzym.*, 78, 9–15.

Judenstein, P., Titman, J., Stamm, M. and Schmidt, H. (1994). Investigation of Ion-Conducting Ormolytes: Structure-Property Relationships. *Chem. Mater*. 6, 127-134.

Jung, S.H.H. (2007). Effective Preparation of Crack-Free Silica Aerogels via Ambient Drying, *J. Sol-Gel Sci Technol.*, 139–146.

Karthikeyan, K.G. and Meyer, M.T. (2006). Occurrence of antibiotics in wastewater treatment facilities in Wisconsin, USA. *Sci. Total Environ*., 361(1-3), 196–207.

Kawachi, Y., Kugimiya, S., Nakamura, H. and Kato, K. (2014). Enzyme Encapsulation in Silica Gel Prepared By Polylysine And Its Catalytic Activity. *Appl. Surf. Sci.*, 314, 64–70.

Kay, Blackwell, P.A. and Boxall, B.A. (2004). Fate of Veterinary Antibiotics in a Macroporous Tile Drained Clay Soil. *Environ. Toxicol. Chem*., 23, 1136-1144.

Kemper, N. (2008). Veterinary Antibiotics in The Aquatic and Terrestrial Environment. *Ecol. Indic.,* 8(1), 1-13.

Kim, S.H., Shon, H.K. and Ngo, H.H. (2010). Adsorption Characteristics of Antibiotics Trimethoprim on Powered and Granular Activated Carbon. *J. Ind. Eng. Chem,.* 16, 344-349.

Kiraz, N., Burunkaya, E. and Asiltu, M. (2010). Effect of Amine Catalysts on Preparation of Nanometric $SiO_2$ Particles and Antireflective Films via Sol – Gel Method, *J. Sol-Gel Sci.,* 56, 167–176.

Kirkbir, F., Murata, H., Meyers, D., Ray Chaudhuri, S. and Sarkkar, A. (1996). Drying and Sintering of Sol-Gel Derived Large $Sio_2$ Monoliths. *J. Sol-Gel Sci. Technol.* 6, 203-217.

Klauson, D., Babkina, J., Stepanova, K., Krichevskaya, M. and Preis, S. (2010). Aqueous Photocatalytic Oxidation of Amoxicillin. *Catal. Today*, 151, 39-45.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B. and Buxton, H.T. (2002). Pharmaceuticals, Hormones, And Other Organic Wastewater Contaminants In US Streams, 1999–2000: A National Reconnaissance. *Environ. Sci. Technol.*, 36, 1202-1211.

Kunamneni, A., Ballesteros, A., Plou, F. J. and Alcalde, M. (2007). Fungal Laccase – A Versatile Enzyme For Biotechnological Applications. *Appl. Microb.*, 233–245.

Laguna, M. and Estella, J. (2007). Effects of Aging and Drying Conditions on The Structural and Textural Properties of Silica Gels, *Micropor. Mesopor. Mat.*, 102, 274-282.

Lee, H.S. and Hong, J. (2000). Kinetics of Glucose Isomerization To Fructose By Immobilized Glucose Isomerase: Anomeric Reactivity of D-glucose in Kinetic Model. *J. Biotechnol.*, 84, 145–153.

Leff, L.G., Dana, J.R., McArthur, J.V. and Shimkets, L.J. (1993). Detection of Tn5-like Sequences in *Kanamycin*-Resistant Stream Bacteria and Environmental DNA. *Appl. Environ. Microbiol.*, 59(2), 417–421.

Lei, C.H., Shin, Y., Magnuson, J.K., Fryxell, G., Lasure, L.L. and Elliott, D.C. (2006). Characterization of Functionalized Nanoporous Supports for Protein Confinement. *Nanotechnology*, 17, 5531-5538.

Lei, Z. and Jiang, Q. (2011). Synthesis and Properties of Immobilized Pectinase onto The Macroporous Polyacrylamide Microspheres. *J. Agric. Food Chem.*, 59, 2592-2599.

Li, D., Yang, M., Hu, J., Ren, L., Zhang, Y. and Li, K. (2008). Determination and Fate of Oxytetracycline and Related Compounds in Oxytetracycline Production Wastewater and The Receiving River. *Environ. Toxicol. Chem.*, 27(1), 80–86.

Li, K., Yediler, A., Yang, M., Schulte-hostede, S. and Hung, M. (2008). Ozonation of Oxytetracycline and Toxicological Assessment of Its Oxidation By-Products, *Chemosphere,* 72, 473–478.

Liu, Y., Guo, C., Wang, F., Liu, C. and Liu, H. (2008). Preparation of Magnetic Silica Nanoparticles and Their Application in Laccase Immobilization. *Chin. J. Process Eng.*, 8, 583–8.

Lloret, L., Eibes, G., Lu-Chau, T.A., Moreira, M.T., Feijoo, G. and Lema, J.M. (2010). Laccase- Catalyzed Degradation Of Anti-Inflammatories And Estrogens. *Biochem. Eng. J.*, 51, 124-131.

Lloret, L., Eibes, G., Feijoo, G., Moreira, M. T. and Lema, J. M. (2011). Immobilization of Laccase by Encapsulation in A Sol – Gel Matrix and Its Characterization and Use for the Removal of Estrogens. *Biotechnol. Prog.*, *27*, 1570–1579.

Lloret, L., Eibes, G., Moreira, M.T., Feijoo, G. and Lema, J.M. (2013). On The Use of A High- Redox Potential Laccase As An Alternative For The Transformation of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs*). J. Mol. Catal. B: Enzym.*, 97, 233-242.

Loke, M.L., Jespersen, S., Vreeken, R., Halling-Sørensen, B. and Tjørnelund, J. (2003). Determination of Oxytetracycline And Its Degradation Products By High-Performance Liquid Chromatography–Tandem Mass Spectroscopy In Manure-Containing Anaerobic Test Systems. *J. Chromatogr. B*, 783, 11–23.

Machado, A., Tavares, A. P. M., Rocha, C. M. R., Cristóvão, R. O., Teixeira, J. A. and Macedo, E. A. (2012). Immobilization of Commercial Laccase on Spent Grain. *Process Biochemistry*, 47(7), 1095–1101.

Madhavi, V. and Lele, S.S. (2009). Laccase: Properties and Applications. *Biores. Technol.,* 4(4), 1694–1717.

Malintan, N.T. and Mohd, M.A. (2006). Determination of Sulfonamides in Selected Malaysian Swine Wastewater by High-Performance Liquid Chromatography. *J. Chrom. A,* 1127(1–2), 154-160.

Malkin, R., Malmström, B. G. and Vänngård, T. (1969). The Reversible Removal of one Specific Copper(II) from Fungal Laccase. *Eur. J. Biochem.,* 7(2), 253-259.

Mayer, A.M. and Staples, R.C. (2002). Laccase: New Functions for an Old Enzyme. *Phytochemistry,* 60: 551–565.

Méndez-Díaz, J.D., Prados-Joya, G., Rivera-Utrilla, J., Leyva-Ramos, R., Sánchez-Polo, M., Ferro-García, M.A. and Medellín-Castillo, N.A. (2010). Kinetic Study of The Adsorption of Nitroimidazole Antibiotics on Activated Carbons in Aqueous Phase. *J. Colloid Interf. Sci.*, 345, 481-490.

Messerschmidt, A. and Huber, R. (1990). The Blue Oxidases, Ascorbate Oxidase, Laccase and Ceruloplasmin Modelling and Structural Relationships. *Eur. J. Biochem.,* 187(2), 341-352.

Mezza, P., Phalippou, J. and Sempere, R. (1999). Sol–gel Derived Porous Silica Films. *J. Non-Cryst. Solids,* 243, 75–79.

Mirzadeh, S.-S., Khezri, S.-M., Rezaei, S., Forootanfar, H., Mahvi, A.H. and Faramarzi, M.A. (2014). Decolorization of Two Synthetic Dyes Using The Purified Laccase of Paraconiothyrium Variabile Immobilized on Porous Silica Beads. *J. Environ. Health Sci. Eng.,* 12, 6.

Mohidem, N.A. and Mat, H.B. (2009). The Catalytic Activity of Laccase Immobilized in Sol-Gel Silica. *J. Appl. Sci.*, 9, 3141-3145.

Mohidem, N.A. and Mat, H.B. (2012). Catalytic Activity and Stability of Laccase Entrapped in Sol–Gel Silica with Additives. *J. Sol-Gel Sci. Technol.,* 61(1), 96-103.

Mompelat, S., Le Bot, B. and Thomas, O. (2009). Occurrence and Fate of Pharmaceutical Products and By-Products, from Resource to Drinking Water. *Environ. Int.,* 35(5), 803-814.

Moner-Girona, M., Roig, A. and Molins, E. (2003). Sol-Gel Route to Direct Formation of Silica Aerogel Microparticles Using Supercritical Solvents. *J. Sol-Gel Sci. Technol*., 26:645–649.

Moore, D.E. and Zhou, W. (1994). Photodegradation of Sulfamethox- azole: A Chemical System Capable of Monitoring Seasonal Changes in UVB Intensity. *Photochem. Photobiol*, 59: 497– 502.

Morozova, V., Shumakovich, G.P., Gorbacheva, M.A., Shleev, S.V. and Yaropolov, A. I. (2007). “Blue” Laccases. *Biochem-Moscow*, 72, 1136–50.

Nicolaon, G.A. and Teichner, S.J. (1968). Preparation of Silica Aerogels From Methyl Orthosilicate in Alcoholic Medium, and Their Properties. *Bull. Soc. Chim. Fr.*, 1906-1911.

Nyanhongo, G.S., Gomes, J., Gübitz, G., Zvauya, R., Read, J.S. and Steiner, W. (2002). Production of Laccase by a Newly Isolated Strain of Trametes modesta. *Biores. Technol.,* 84(3), 259-263.

Nygaard, K., Lunestad, B.T., Hektoen, H., Berge, J.A. and Hormazabal, V. (1992). Resistance to Oxytetracycline, Oxolinic Acid and Furazolidone in Bacteria from Marine Sediments. *Aquaculture,* 104(1–2), 31-36.

Ong, D.L I., Ang, M.I.N.Y., Ianying, J.H.U., En, L.I.R. and Hang, Y.U Z. (2008). Determination And Fate of Oxytetracycline And Related Compounds In Oxytetracycline Production Wastewater And The Receiving River. *Environ. Toxicol. Chem.*, 27(1), 80–86.

Park, H. and Choung, Y.-K. (2007). Degradation of Antibiotics (Tetracycline, Sulfathiazole, Ampicillin) Using Enzymes of Glutathion S-Transferase. *H. Ecol. Risk Assess.,* 13(5), 1147-1155.

Pierre, A C and Rigacci, A. (2011). *Aerogels Handbook* (1st ed.), Springer.

Piontek, K., Antorini, M. and Choinowski, T. (2002). Crystal Structure of a Laccase from The Fungus *Trametes versicolor* at 1.90-Å Resolution Containing a Full Complement of Coppers. *J. Biol. Chem.*, 277(40), 37663-37669.

Qiting, J. and Xiheng, Z. (1988). Combination Process of Anaerobic Digestion And Ozonization Technology For Treating Wastewater From Antibiotics Production. *Water Treat.*, 3, 285–291.

Qiu, L. and Huang, Z. (2010). The Treatment of Chlorophenols With Laccase Immobilized on Sol-Gel Derived Silica. *World J. Microbiol. Biotechnol.*, 775–781.

Rahman, I.A., Jafarzadeh, M. and Sipaut, C.S. (2009). Synthesis of Organofunctionalized Nanosilica via A Co-Condensation Modification Using c-aminopropyltriethoxysilane (APTES). *Ceram. Int.*, 35, 1883–1888.

Rahmani, K., Faramarzi, M.A., Mahvi, A.H., Gholami, M., Esrafili, A., Forootanfar, H., and Farzadkia, M. (2015). Elimination and Detoxification of Sulfathiazole and Sulfamethoxazole Assisted By Laccase Immobilized on Porous Silica Beads. *Int. Biodeter. Biodegr.*, 97, 107–114.

Rao, A.V. and Bhagat, S.D., (2004). Synthesis and Physical Properties of TEOS-based Silica Aerogels Prepared by Two Step (Acid–Base) Sol–Gel Process. *Solid State Sci.*, 6, 945.

Rao, A.V. and Haranath, D. (1999). Effect of Methyltrimethoxysilane As A Synthesis Component on The Hydrophobicity And Some Physical Properties of Silica Aerogels. *Micropor. Mesopor. Mater.*, 30, 267–273.

Rao, A.P., Pajonk, G.M. and Rao, A.V. (2005). Effect of Preparation Conditions on The Physical and Hydrophobic Properties of Two Step Processed Ambient Pressure Dried Silica Aerogels. *J. Mater. Sci.,* 40, 3481–3489.

Rao, A.V., Bhagat, S.D., Hirashima, H. and Pajonk, G.M. (2006). Synthesis of Flexible Silica Aerogels Using Methyltrimethoxysilane (MTMS) Precursor. *J. Colloid Interface Sci.*, 300, 279–285.

Rao, A.V., Nilsen, E. and Einarsrud, M. A. (2001). Effect of Precursors, Methylation Agents and Solvents on The Physicochemical Properties of Silica Aerogels Prepared by Atmospheric Pressure Drying Method. *J. Non-Cryst. Solids,* 296(3), 165-171.

Rao, A.V., Rao, A.P. and Kulkarni, M.M. (2004). Influence of Gel Aging and $Na_2SiO_3/H_2O$ Molar Ratio on Monolithicity and Physical Properties of Water-Glass-Based Aerogels Dried at Atmospheric Pressure. *J. Non-Cryst. Solids.*, 350, 224-229.

Rao, A.V.,Wagh, P.B., Haranath, D., Risbud, P.P. and Kumbhare, S.D. (1999). Influence of Temperature on The Physical Properties of TEOS Silica Xerogels. *Ceram. Int.*, 25, 505.

Rhodes, G., Huys, G., Swings, J., McGann, P., Hiney, M., Smith, P. and Pickup, R.W. (2000). Distribution of Oxytetracycline Resistance Plasmids between Aeromonads in Hospital and Aquaculture Environments: Implications of Tn1721 in Dissemination of The Tetracycline Resistance Determinant. *Tet A. Appl. Environ. Microbiol.*, 66(9), 3883-3890.

Rodriguez Couto, S. and Herrera, J. L. T. (2006). Industrial and Biotechnological Applications of Laccases: A Review. *Biotechnol. Adv,.* 24, 500–513.

Rodríguez-Rodríguez, C.E., Jesús García-Galán, M., Blánquez, P., Díaz-Cruz, M.S., Barceló, D. and Caminal, G. (2012). Continuous Degradation of a Mixture of Sulfonamides by *Trametes versicolor* and Identification of Metabolites from Sulfapyridine and Sulfathiazole. *J. Hazard. Mater.,* 213–214(0), 347-354.

Rubert, K. and Pedersen, J.A. (2006). Kinetics of Oxytetracycline Reaction With a Hydrous Manganese Oxide. *Environ. Sci. Technol*., 40, 7216–7221.

Sadighi, A. and Faramarzi, M.A. (2013). Congo Red Decolorization by Immobilized Laccase Through Chitosan Nanoparticles on The Glass Beads. *J. Taiwan Inst. Chem. Eng*., 44, 156-162.

Samuelsen, O.B., Torsvik, V. and Ervik, A. (1992). Long-range Changes in Oxytetracycline Concentration And Bacterial Resistance Towards Oxytetracycline in A Fish Farm Sediment After Medication. *Sci. Total Environ.*, 114(0), 25-36.

Sandaa, R.-A., Torsvik, V.L. and Goksøyr, J. (1992). Transferable Drug Resistance in Bacteria from Fish-Farm Sediments. *Can. J. Microbiol.,* 38(10), 1061-1065.

Santalla, E., Serra, E., Mayoral, A., Losada, J., M Blanco, R. and Diaz, I. (2003). In-situ Immobilization of Enzymes in Mesoporous Silicas. *Solid State Sci.*, 13, 691-697.

Sarawade, P.B., Kim, J., Hilonga, A. and Taik, H. (2010). Production of Low-Density Sodium Silicate-Based Hydrophobic Silica Aerogel Beads by A Novel Fast Gelation Process and Ambient Pressure Drying Process. *Solid State Sci.*, *12*(5), 911–918.

Sarmah, A.K., Meyer, M.T. and Boxall, A.B.A. (2006). A Global Perspective on The Use, Sales, Exposure Pathways, Occurrence, Fate and Effects Of Veterinary Antibiotics (Vas) in The Environment. *Chemosphere*, 65(5), 725-759.

Sathishkumar, P., Chae, J.-C., Unnithan, A.R., Palvannan, T., Kim, H.Y., Lee, K.-J., Cho, M., Kamala-Kannana, S. and Oh, B.-T. (2012). Laccase-polylactic-co-glycolic Acid (PLGA) Nanofiber: Highly Stable, Reusable, and Efficacious for The Transformation of Diclofenac. *Enzyme Microb. Technol.*, 51, 113-118.

Sato, Y., Bao, W.L., Sederoff, R. and Whetten, R. (2001). Molecular Cloning and Expression of Eight Laccase cDNAs in Loblolly Pine (*Pinus taeda*). *J. Plant Res.,* 114, 147–155.

Schwartz, T., Kohnen, W., Jansen, B. and Obst, U. (2003). Detection of Antibiotic-Resistant 493 Bacteria and Their Resistance Genes in Wastewater, Surface Water, and Drinking Water 494 Biofilms. *FEMS Microbiol. Ecol.*, 43, 325-335.

Sergio, R. (2006). Laccases: Blue Enzymes for Green Chemistry. *Trends Biotechnol.,* 24(5), 219-226.

Shaojun, J., Shourong, Z., Daqiang, Y.I.N., Lianhong, W. and Liangyan, C. (2008). Aqueous Oxytetracycline Degradation and The Toxicity Change of Degradation Compounds in Photoirradiation Process. *J. Environ. Sci.,* 20, 806–813.

Sharma, P., Goel, R. and Capalash, N. (2007). Bacterial Laccases. *World J. Microbiol. Biotechnol*., 23, 823-832.

Shen, J., Zhang, Z., Wu, G., Zhou, B., Ni, X. and Wang, J. (2006). Preparation and Characterization of Silica Aerogels Derived from Ambient Pressure. *J. Mater. Sci. Technol.*, 22, 798-802.

Shewale, P.M., Rao, A.V. and Rao, A.P. (2008). Effect of Different Trimethyl Silylating Agents on The Hydrophobic And Physical Properties of Silica Aerogels. *Appl. Surf. Sci*., 254, 6902–6907.

Shuler, M.L. and F. Kargi, (2005). Bioprocess Engineering. 2nd Edn., Prentice Hall, New York, ISBN: 100130819085.

Silva, C., Silva, C. J., Zille, A., Guebitz, G. M. and Cavaco-Paulo, A. (2007). Laccase Immobilization on Enzymatically Functionalized Polyamide 6,6-fibres. *Enzyme Microb. Technol.,* 41, 867–75.

Singh, N., Srivastava, G., Talat, M., Raghubanshi, H., Srivastava, O. N. and Kayastha, A. M. (2014). Cicer α-galactosidase Immobilization onto Functionalized Graphene Nanosheets Using Response Surface Method and Its Applications. *Food Chem.*, 142, 430–8.

Smitha, S., Shajesh, P., Aravind, P.R., Rajesh Kumar, S., Krishna Pillai, P. and Warrier, K.G.K. (2006). Effect of Aging Time and Concentration of Aging Solution on The Porosity Characteristics of Subcritically Dried Silica Aerogels. *Micropor. Mesopor. Mater*., 91, 286–292.

Soleimani Dorcheh, A. and Abbasi, M. H. (2008). Silica Aerogel; Synthesis, Properties and Characterization. *J. Mater. Process. Tech.,* 199(1–3), 10-26.

Solomon, E.I., Baldwin, M.J. and Lowery, M.D. (1992). Electronic Structures of Active Sites in Copper Proteins: Contributions to Reactivity. *Chem. Rev.*, 9, 521-542.

Stackelberg, P.E., Gibs, J., Furlong, E.T., Meyer, M.T., Zaugg, S. D. and Lippincott, R.L. (2007). Efficiency of Conventional Drinking-Water-Treatment Processes in Removal of Pharmaceuticals and Other Organic Compounds. *Sci. Total Environ.,* 377(2–3), 255-272.

Stöber, W., Fink, A. and Bohn, E. (1968). Controlled Growth of Monodisperse Silica Spheres in The Micron Size Range. *J. Colloid Interf. Sci.*, 26, 62-69.

Strøm, R.A., Masmoudi, Y., Rigacci, A., Petermann, G., Gullberg, L., Chevalier, B. and Einarsrud, M.-A. (2007). Strengthening and Aging of Wet Silica Gels for Up-Scaling of Aerogel Preparation. *J. Sol-Gel Sci. Technol.*, 41, 291–298.

Suda, T., Hata, T., Kawai, S., Okamura, H. and Nishida, T. (2012). Treatment of Tetracycline Antibiotics by Laccase In The Presence of 1-hydroxybenzotriazole. *Biores. Technol.*, 103(1), 498–501.

Thurston, C. F. (1994). The Structure and Function of Fungal Laccases. *Microbiology,* 140, 19-26.

Torres, E., Bustos-Jaimes, I. and Le Borgne, S. (2003). Potential Use of Oxidative Enzymes for The Detoxification of Organic Pollutants. *Appl. Catal. B: Environ.*, 46, 1-15.

Tufvesson, P., Lima-Ramos, J., Nordblad, M. and Woodley, J.M. (2010). Guidelines And Cost Analysis For Catalyst Production in Biocatalytic Processes. *Org. Process Res. Dev.*, 15, 266–274.

Vega, A. J. and Scherer, G.W. (1989). Study of Structural Evolution of Silica Gel using 1H and 29Si NMR. *J. Non-Cryst. Solid*, 111, 153–166.

Vieno, N.M., Härkki, H., Tuhkanen, T. and Kronberg, L. (2007). Occurrence of Pharmaceuticals in River Water and Their Elimination in a Pilot-Scale Drinking Water Treatment Plant. *Environ. Sci. Technol.*, 41(14), 5077-5084.

Wang, Y., Zheng, X. and Zhao, M. (2008). Study of Immobilization of Laccase on Mesoporous Molecular Sieve MCM-41. *J. Chem. Eng. Chin. Univ.*, 22, 83-7.

Watkinson, A.J., Murby, E.J. and Costanzo, S.D. (2007). Removal of Antibiotics in Conventional and Advanced Wastewater Treatment: Implications for Environmental Discharge and Wastewater Recycling. *Water Res.,* 41(18), 4164-4176.

Weng, S.-S., Ku, K.-L. and Lai, H.-T. (2012). The Implication of Mediators for Enhancement of Laccase Oxidation of Sulfonamide Antibiotics. *Biores. Technol.*, 113(0), 259-264.

Wesenberg, D., Kyriakides, I. and Agathos, S. N. (2003). White-Rot Fungi and Their Enzymes for The Treatment of Industrial Dye Effluents. *Biotechnol. Adv.,* 22(1–2), 161-187.

Westerhoff, P., Yoon, Y., Snyder, S. and Wert, E. (2005). Fate of Endocrine-Disruptor, Pharmaceutical, And Personal Care Product Chemicals During Simulated Drinking Water Treatment Processes. *Environ. Sci. Technol.*, 39 (17), 6649–6663.

Williams, S. (2002). Antibiotic Resistance: Not Just for Human Anymore. *J. Young Invest.,* 6(3).

Wilson, B.A., Smith, V.H., deNoyelles, F. and Larive, C.K. (2003). Effects of Three Pharmaceutical and Personal Care Products on Natural Freshwater Algal Assemblages. *Environ. Sci. Technol.*, 37(9), 1713-1719.

Wollenberger, L., Halling-Sørensen, B. and Kusk, K. O. (2000). Acute and Chronic Toxicity of Veterinary Antibiotics to Daphnia Magna. *Chemosphere*, 40(7), 723-730.

Wu, G.,Wang, J., Shen, J., Yang, T., Zhang, Q., Zhou, B., Deng, Z., Bin, F., Zhou, D. and Zhang, F. (2000). Properties of Sol-Gel Derived Scratch-Resistant Nano-Porous Silica Films by A Mixed Atmosphere Treatment. *J. Non-Cryst. Solids*, 275, 169-174.

Wu, J., Zhang, H., Oturan, N., Wang, Y., Chen, L. and Oturan, M.A. (2012). Application of Response Surface Methodology to The Removal of The Antibiotic Tetracycline by Electrochemical Process Using Carbon-Felt Cathode and DSA (Ti/$RuO_2$-$IrO_2$) Anode. *Chemosphere*, 87, 614–620.

Xu, F. (1997). Effects of Redox Potential and Hydroxide Inhibition on The pH Activity Profile of Fungal Laccases. *J. Biol. Chem.*, 272, 924–928.

Xu, W.-h., Zhang, G., Zou, S.-c., Li, X.-d. and Liu, Y.-c. (2007). Determination of Selected Antibiotics in The Victoria Harbour and The Pearl River, South China Using High-Performance Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry. *Environ. Pollut.*, 145(3), 672-679.

Ye, Z., Weinberg, H. S. and Meyer, M. T. (2007). Occurrence of Antibiotics in Drinking Water, *Anal. Bioanal. Chem.*, 387, 1365–1377.

Yildirim, D., Tükel, S. S., Alptekin, Ö. and Alagöz, D. (2014). Optimization of Immobilization Conditions of Mucor Miehei Lipase onto Florisil via Polysuccinimide Spacer Arm Using Response Surface Methodology and Applicationo Immobilized Lipase in Asymmetric Acylation of 2-Amino-1-Phenylethanols. *J. Mol. Cat. B: Enzym.*, 100, 91–103.

Youn, H.-D., Kim, K.-J., Maeng, J.-S., Han, Y.-H., Jeong, I.-B. and Jeong, G. (1995). Single Electron Transfer by an Extracellular Laccase from The White-Rot Fungus *Pleurotus ostreatus*. *Microbiology,* 141(2), 393-398.

Zhang, D., Yuwen, L. and Peng, L. (2013). Parameters Affecting the Performance of Immobilized Enzyme, *J. Chem*., 2013, 1-7.

Zhang, X. and Huang, S. (2001). Single Step On-Column Frit Making for Capillary High-Performane Liquid Chromatography Using Sol-Gel Technology. *J. Chromatogr. A*, 910, 13-18.

Zhou, B., Shen, J., Yuehua, W., Wu, G. and Ni, X. (2007). Hydrophobic Silica Aerogels Derived From Polyethoxydisi- Loxane And Perfluoroalkylsilane. *Mater. Sci. Eng*., 27, 1291–1294.

Zhou, Z., Hartmann, M. (2012). Recent Progress in Biocatalysis with Enzymes Immobilized on Mesoporous Hosts. *Top Catal*., 55, 1081–100.