PRODUCTION OF MESOXALIC ACID FROM GLYCEROL OXIDATION BY LACCASE/2,2,6,6-TETRAMETHYLPIPERIDINE-1-OXYL (LACASSE/TEMPO) SYSTEM: EFFECT OF PROCESS PARAMETERS AND KINETIC STUDY

HONG CHI SHEIN

Thesis submitted in fulfilment of the requirements for the award of the degree of
Master of Engineering (Chemical)

Faculty of Chemical and Natural Resources Engineering
UNIVERSITI MALAYSIA PAHANG

SEPTEMBER 2016

TABLE OF CONTENTS

DECLARATION

LIST OF TABLES

LIST OF SCHEMES

LIST OF FIGURES

LIST OF SYMBOLS

ε	extinction coefficient
l	light path
A	absorbance
min	minute
hrs	hours
k	rate constant
°C	degree celcius
K	Kelvin
Cx	Concentration of compound x
cP	centipoise
ppm	part per million
M	mol/L
Pa	pascal
r	reaction rate
E_0	redox potential

LIST OF ABBREVIATIONS

ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
APHA	American Public Health Association
$BaCl_2$	Barium chloride
CI	Chilling injury
CPHM	4-chlorophenylhydrazone of mesoxalic acid
CV	Cyclic voltammetry
DAD	Diode Array detector
DHA	Dihydroxyacetone
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
FA	Formic acid
GA	Glyceric acid
GCE	Glassy carbon electrode
Gled	Glyceraldehyde
Gly	Glycerol
H_5IO_6	Periodic acid
HA	Hydroxylapatite
HIV	Human immunodeficiency virus
HPLC	High-performance liquid chromatography
ILA	Insulin-like activity
L-DOPA	L-3,4-dihydroxyphenylalanine
LMS	Laccase mediated system

MA	Mesoxalic acid
$Na_2C_3O_5$	Sodium mesoxalate
NHE	Normal hydrogen electrode
ODE	Ordinary differential equation
OECD-FAO	Organisation for Economic Co-operation and Development-Food and Agriculture Organization
pI	Isoelectric point
PLGA	Poly(lactic-co-glycolic acid)
RID	Refractive index detector
RNA	Ribonucleic acid
RT	Reverse transcriptase
SCE	Saturated calomel electrode
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
TA	Tartronic acid
TEMPO	2,2,6,6-Tetramethylpiperidine-1-oxyl
USDA	United States Department of Agriculture

PRODUCTION OF MESOXALIC ACID FROM GLYCEROL OXIDATION BY LACCASE/2,2,6,6-TETRAMETHYLPIPERIDINE-1-OXYL (LACASSE/TEMPO) SYSTEM: EFFECT OF PROCESS PARAMETERS AND KINETIC STUDY

HONG CHI SHEIN

Thesis submitted in fulfilment of the requirements for the award of the degree of
Master of Engineering (Chemical)

Faculty of Chemical and Natural Resources Engineering
UNIVERSITI MALAYSIA PAHANG

SEPTEMBER 2016

ABSTRAK

Kenaikan harga bahan api fosil telah mendorong industri minyak untuk mencari sumber tenaga boleh diperbaharui, biodiesel. Gliserol, bahan sampingan utama bagi pengeluaran biodiesel wujub sebagai bahan yang berkos rendah dan blok binaan yang sangat serba guna. Nyata sekali, gliserol telah dikanali sebagai sebatian yang menggalakkan dalam mendapatkan bahan kimia berharga melalui pengoksidaan. Dalam kajian ini, pengoksidaan gliserol dengan menggunakan 2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPO) dengan kehadiran laccase untuk menghasilkan satu bahan ubat anti HIV yang berpotensi iaitu asid mesoxalic telah dikaji. Kajian ini dijalankan dengan memberi tindak balas gliserol dengan TEMPO dan laccase dari *Trametes versicolor* dan menukarkan keadaan tindak balas seperti suhu (5 °C – 61 °C), pH (3.5 – 6.15), nisbah molar gliserol kepada TEMPO (1:0 - 100:3) dan nisbah TEMPO kepada laccase (mM: U/ml) (9:0 - 9:4).. Satu eksperimen awal telah dijalankan sebagai pemeriksaan sebelum penyiasatan keadaan tindak balas pada produk pengoksidaan di mana keadaan tindak balas telah ditetapkan pada 25 °C , pH 4.5, nisbah gliserol/TEMPO 10:3 dan nisbah TEMPO/laccase 9: 3. Kajian kinetik telah dilakukan untuk menyiasat kadar tindak balas. Bahan tindak balas pengoksidaan dan produk telah dinilai dengan menggunakan HPLC manakala aktiviti laccase telah ditentukan dengan menggunakan ujian ABTS. Keadaan tindak balas terbaik didapati ialah 19 °C, pH 5.5, nisbah 1:3 bagi gliserol kepada TEMPO dan nisbah 9:3 bagi TEMPO kepada laccase selepas menjalankan eksperimen. Model homogen telah digunakan untuk memadankan data kinetik melalui MATLAB. TEMPO dimangkinkan oleh kuprum-bergantung oxidase, laccase, kepada oxoammonium kation yang terlibat dalam pengoksidaan aerobik. Kehadiran laccases membenarkan pertumbuhan semula kation oxoammonium, dengan itu, proses pengoksidaan dapat diteruskan selagi laccases aktif. Laluan mekanistik yang berbeza ini disebabkan oleh perbezaan dalam potensi redoks antara TEMPO dan laccase. Tiada dihydroxyacetone dikesan dalam pengoksidaan terpilih gliserol dengan laccase/TEMPO. Ia menunjukkan bahawa kumpulan hidroksil primer telah terpilih untuk dioksidakan kepada gliseraldehid. Asid mesoxalic diperoleh adalah 0.0712 M pada keadaan tindak balas suboptimal. Ia adalah kenaikan dua-lipatan daripada keputusan awal. Ini jelas menunjukkan betapa pentingnya keadaan tindak balas ke arah pembentukan produk. Selain itu, kajian ini juga menunjukkan bahawa pembentukan asid mesoxalic mengguanakan tenaga yang kurang dalam keadaan yang terbaik berbanding dengan kaedah konvensional yang memerlukan suhu yang tinggi. Tenaga pengaktifan bagi pembentukan asid mesoxalic daripada asid tartronik adalah 107.17 kJ/mol yang menunjukkan tenaga minimum yang diperlukan untuk ia berlaku adalah yang paling tinggi berbanding dengan langkah-langkah lain. Nilai bagi faktor pra-eksponen dan pemalar kadar tindak balas yang tertinggi untuk langkah tindak balas ini mencadangkan bahawa perlanggaran molekul adalah yang tertinggi. Oleh yang demikian, suhu amat terjejas pada pembentukan asid mesoxalic.

ABSTRACT

The increase of fossil fuel price has prompted the oil industry to look for renewable energy sources, biodiesel. Glycerol, the main by-product of biodiesel production has the potential of being a low-cost and extremely versatile building block. Significantly, glycerol has been touted as a promising compound in obtaining valuable chemicals via oxidation route. In this study, oxidation of glycerol by using 2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPO) in the presence of laccase to produce a potential anti HIV drug ingredient which is mesoxalic acid was investigated. The study was conducted by reacting glycerol with TEMPO and laccase from *Trametes versicolor* and varying the reaction conditions such as temperature (5 °C – 61 °C), pH (3.50 - 6.15), molar ratio of glycerol to TEMPO (1:0 - 100:3) and ratio TEMPO to laccase (mM: U/ml) (9:0 - 9:4). A preliminary experiment in which the reaction conditions was fixed at 25 °C, pH 4.5, 10:3 of glycerol/TEMPO ratio and TEMPO/laccase ratio of 9:3 was conducted as screening before the investigation of reaction conditions on the oxidation products. Kinetic study was performed to investigate the reaction rate. Oxidation reactants and products were quantified by using HPLC whilst laccase activity was determined by using ABTS assay. The best reaction conditions after conducting experiments were found to be 19 °C, pH 5.5, ratio 1:3 of glycerol to TEMPO and ratio 9:3 of TEMPO to laccase. A homogeneous model was used to fit the kinetic data via MATLAB. TEMPO was catalysed by the copper-dependent oxidase, laccase to oxoammonium cations which involved in the aerobic oxidation. The presence of laccases allowed the regeneration of oxoammonium cations, thus, the oxidation process could proceed as long as laccase were active. This different mechanistic pathway was attributed to the difference in redox potential between TEMPO and laccase. The selective oxidation of glycerol by laccase/TEMPO results in no dihydroxyacetone detected. It demonstrated that the primary hydroxyl group had been selectively oxidised to glyceraldehyde. Mesoxalic acid attained was 0.0712 M at the suboptimal reaction conditions obtained. It was two-fold increment from that in the preliminary results. This clearly showes the importance of reaction conditions towards the products formation. Moreover, this study also shows that mesoxalic acid formation consumed less energy in the best condition compared to the conventional method which required high temperature. The activation energy for the formation of mesoxalic acid from tartronic acid was 107.17 kJ/mol which indicated the minimum energy required for it to occur was the highest compared to other steps. The highest in pre-exponential factor and rate constant for this reaction step suggested that the collision of molecules was the greatest. Hence, the temperature affected greatly on the formation of mesoxalic acid.

REFERENCES

Abadulla, E., Tzanov, T., Costa, S., Robra, K.H., Cavaco-Paulo, A. and Gubitz, G. M. (2000). Decolorization and detoxification of textile dyes with a laccase from *Trametes hirsuta*. *Applied and Environmental Microbiology, 66*, 3357-3362.

Abbadi, A. and Van Bekkum, H. (1996). Selective chemo-catalytic routes for the preparation of β-hydroxypyruvic acid. *Applied Catalysis, A, 148,* 113 - 122.

Ahmad, A. A., Othman, R., Yusof, F. and Wahab, M. F. A. (2011). Zinc laccase biofuel cell. *IIUM Engineering Journal, 12*, 153-160.

Alhanash, A., Kozhevnikova, E.F. and Kozhenikov, I.V. (2010). Gas phase dehydration of glycerol to acrolein catalysed by caesium heteropoly salt. *Applied Catalysis, 378*,11-18.

Anelli, P. L., Biffi, C., Montanari, F., and Quici, S. (1987). Fast and selective oxidation of primary alcohols to aldehydes or to carboxylic acids and of secondary alcohols to ketones mediated by oxoammonium salts under two-phase conditions. *Journal of Organic Chemistry, 52*, 2559-2562.

Anelli, P. L., Banfi, S., Montanari, F. and Quici, S. (1989). Oxidation of Diols with Alkali Hypochlorites Catalyzed by Oxammonium Salts under Two-Phase Conditions. *Journal of Organic Chemistry, 54*, 2970-2972.

Arends, I.W.C.E., Li, Y.X. and Sheldon, R.A. (2006). Stabilities and rates in the laccase/TEMPO-catalyzed oxidation of alcohols. *Biocatalysis and Biotransformation, 24*, 443-448.

Assavanig, A., Amornkitticharoen, B., Ekpaisal, N., Meevootisom, V. and Flegel, T.W. (1992). Isolation, characterization and function of laccase from Trichoderma. *Applied Microbiology and Biotechnology, 38*, 198-202.

Astuti, E., Supranto., R. and Prasetya, A. (2014). Kinetic Modelling of Nitration of Glycerol: Three Controlling Reactions Model. *Engineering Journal, 18,* 73-82.

Authayanun, S., Arpornwichanop, A., Paengjuntuek, W. and Assabumrungrat, S. (2010). Thermodynamic study of hydrogen production from crude glycerol autothermal reforming for fuel cell applications. *International journal of hydrogen energy, 35*, 6617-6623.

Ayoub, M. and Abdullah, A. Z. (2012). Critical review on the current scenario and significance of crude glycerol resulting from biodiesel industry towards more sustainable renewable energy industry. *Renewable and Sustainable Energy Reviews, 16*, 2671-2686.

Banat, I. M., Nigam, P., Singh, D. and Marchant, R. (1996). Microbial decolorization of textile-dye-containing effluents: a review. *Bioresource Technology, 58,* 217-227.

Behr, A., Jens, E., Ken, I., Julia, L. and Falk, L. (2008). Improved Utilisation of Renewable Resources: New Important Derivatives of Glycerol. *Green Chemistry, 10,* 13-30.

Beltrán-Prieto, J.C., Pecha, J., Kašpárková, V. and Kolomazník, K. (2013). Development of an HPLC method for the determination of glycerol oxidation products. *Journal of Liquid Chromatography & Related Technologies, 36,* 2758-2773.

Ben-Daniel, R., Paul Alsters, P. and Neumann, R. (2001). Selective Aerobic Oxidation of Alcohols with a Combination of a Polyoxometalate and Nitroxyl Radical as Catalysts. *The Journal of Organic Chemistry, 66,* 8650-8653.

Benfield, G., Bocks, S.M., Bromley, K. and Brown, B.R. (1964). Studies in fungal and plant laccases. *Phytochemistry, 3,* 79-88.

Bennett, G.N and San, K.Y. (2001). Microbial formation, biotechnological production and applications of 1,2-propanediol. *Applied Microbiology and Biotechnology, 55,* 1-9.

Bento, I., Martins, L.O., Lopes, G.G., Carrondo, M. A. and Lindley, P.F. (2005). Dioxygen reduction by multi-copper oxidases; a structural perspective. *Dalton Transactions.* 3507–3513.

Bernatchez, J.A., Paul, R. Tchesnokov, E.P., Ngure, M., Beilhartz, G.L., Berghuis, A.M.,Lavoie, R., Li, L.H., Auger, A., Melnyk, R.A., Grobler, J.A., Miller, M.D., Hazuda, D.J, Hecht, S.M. and Gotte, M. (2015). Derivatives of Mesoxalic Acid Block Translocation of HIV-1 Reverse Transcriptase. *The Journal of Biological Chemistry, 290,* 1474-1484.

Bewley, B.R., Berkaliev, A., Henriksen, H., Ball, D.B., & Ott, L.S. (2015). Waste glycerol from biodiesel synthesis as a component in deep eutectic solvents. *Fuel Processing Technology, 138,* 419-423.

Biofuel Digest. (2015). Brazilian biodiesel production up 85 % in 2015. Retrieved from http://www.biofuelsdigest.com/bdigest/2016/01/07/brazilian-biodiesel-production-up-85-in-2015 on 20 Jan 2016.

Bobbitt, J.M. and Flores, C.L. (1988). Organic nitrosonium salts as oxidants in organic chemistry. *Heterocycles, 27,* 509-533.

Bollag, J.M. and Leonowicz, A. (1984). Comparative studies of extracellular fungal laccases. *Applied and Environmental Microbiology, 48,* 849-854.

Bolm, C., Magnus, A. S. and Hildebrand, J.P. (2000). Catalytic Synthesis of Aldehydes and Ketones under Mild Conditions Using TEMPO/Oxone. *Organic Letters, 2,* 1173-1175.

Bourbonnais, R. and Paice, M.G. (1990). Oxidation of non-phenolic substrates. An expanded role for laccase in lignin biodegradation. *FEBS Letters, 267,* 99-102.

Bourbonnais, R. and Paice, M.G. (1992). Demethylation and delignification of kraft pulp by Trametes versicolor laccase in the presence of 2,2-azinobis(3-ethylbenzthiazoline-6-sulphonate). *Applied Microbiology and Biotechnology, 36,* 823-827.

Bourbonnais, R., Rochefort, D., Paice, M.G., Renaud, S. and Leech, D. (2000). Transition metal complexes: a new class of laccase mediators. *Tappi Journal, 83,* 68-78.

Brandner, A, Lehnert,K., Bienhonz, A., Lucas, M. and Claus, P. (2009). Production of biomass derived chemicals and energy: chemocatalytic conversion of glycerol. *Catalysis, 52,* 278-287.

Brown, D.A. (2001). Skin pigmentation enhancers. *Journal of Photochemistry and Photobiology B: Biology, 63,* 148-161.

Bub, G., Mosler, J., Sabbach, A., Kuppinger, F., Nordhoff, S. and Stochniol, G. (2006). International Patent WO092272A2.

Call, H.P. and Mucke, I. (1997). History, overview and applications of mediated lignolytic systems, especially laccase-mediator-systems. *Journal of Biotechnology, 53,* 163-202.

Calvo, A.M., Copa-Patiño, J.L., Alonso, O. and González, A.E. (1998). Studies of the production and characterization of laccase activity in the basidiomycete *Coriolopsis gallica*, an efficient decolorizer of alkaline effluents. *Archieve of Microbiology, 171,* 31–36.

Catalina, G(I). (2013). *Summary design of a new class of liposomes with potential osteotropic properties.* Phd Thesis. University of Medicine and Pharmacy of Craiova.

Cella, J.A., Kelley, J.A. and Kenehan, E.F. (1975). Nitroxide-catalysed oxidation of alcohols using m-chloroperbenzoic acid. A new method. *Journal of Organic Chemistry, 40,* 1860-1862.

Chavan, S. P. and Soni, P. (2004). A facile deprotection of oximes using glyoxylic acid in an aqueous medium. *Tetrahedron Letters,45*, 3161-3162.

Chellapandian, M. and Sastry, C.A. (1994). Immobilization of alkaline protease on nylon. *Bioprocess Engineering, 11,* 17-21.

Chetpattananondh, P. and Tongurai, C. (2008). Synthesis of high purity monoglycerides from crude glycerol and palm stearin. *Songklanakarin Journal of Science and Technology, 30,* 515-521.

Chi, Z., Pyle, D., Wen, Z., Frear, C. and Chen, S. (2007). A laboratory study of producing docosahexaenoic acid from biodiesel-waste glycerol by microalgal fermentation. *Process Biochemistry, 42*, 1537-1545.

Chiu, C., Dasari, M.A. and Suppes, G.J. (2006). Dehydration of Glycerol to Acetol via Catalytic Reactive Distillation, *AlChE Journal, 52,* 3543-3548.

D'Amico, S., Gerda, C. and Feller, G. (2003). Temperature Adaptation of Proteins: Engineering Mesophilic-like Activity and Stability in a Cold-adapted a-Amylase. *Journal of Molecular Biology, 332,* 981-988.

D'Annibale, A., Stazi, S.R., Vinciguerra, V. and Sermanni, G.G. (2000). Oxirane-immobilized *Lentinula edodes* laccase: stability and phenolics removal efficiency in olive mill wastewater. *Journal of Biotechnology, 77*, 265-273.

De Nooy, A.E.J., Besemer, A.C. and van Bekkum, H. (1995). Selective oxidation of primary alcohols mediated by nitroxyl radical in aqueous solution. Kinetics and mechanism. *Tetrahedron, 51,* 8023-8032.

De Nooy, A.E.J., Besemer, A.C. and van Bekkum, H. (1996). On the use of stable organic nitroxyl radicals for the oxidation of primary and secondary alcohols. *Synthesis*, 1153-1174.

Demirel, S., Lehnert, K., Lucas, M. and Claus, P. (2007). Use of renewables for the production of chemicals: Glycerol oxidation over carbon supported gold catalysts. *Applied Catalysis B: Environmental, 70*, 637-643.

Díaz-âlvarez, A.E., and Cadierno, V. (2013). Glycerol: A promising Green Solvent and Reducing Agent for Metal-Catalyzed Transfer Hydrogenation Reactions and Nanoparticles Formation. *Applied Science, 3*, 55-69.

Diaz, J.A., Skrzynska, E., Girardon, J-S, Ftouni, J., Capron, M., Dumeignil, F. and Fongarland, P. (2016). Kinetic modelling of the quasi-homogeneous oxidation of glycerol over unsupported gold particles in the liquid phase. *European Journal of Lipid Science and Technology, 118*, 72-79.

Dijksman, A., Arends, I.W.C.E. and Sheldon, R.A. (2001). A comparison of the activity of polymer immobilised TEMPO (PIPO) with MCM-41 and silica supported TEMPO as heterogeneous catalysts for the oxidation of alcohols. *Synlett, 1*, 102-104.

Dijksman, A., Arends, I.W.C.E. and Sheldon, R.A. (2003). Cu(II)-nitroxyl radicals as catalytic galactose oxidase mimics. *Organic & Biomolecular Chemistry, 1,* 3232–3237.

Donkin, S.S. (2008). Glycerol from Biodiesel Production: The New Corn for Dairy Cattle. *Revista Brasileira de Zootecnia, 37*, 280-286.

Dubois, J. L., Duquenne, C. and Holderich, W. (2006). Patent WO2006087083.

Edens, W. A., Goins, T. Q., Dooley, D. and Henson, J. M. (1999). Purification and characterization of a secreted laccase of *Gaeumannomyces graminis* var. *tritici. Applied and Environmental Microbiology, 65*, 3071–3074.

Eriksson, C.J.P., Saarenmaa, T.P.S., Bykov, I. L. and Heino, P.U. (2007). Acceleration of ethanol and acetaldehyde oxidation by d-glycerate in rats. *Metabolism: Clinical and Experimental, 56*, 895–98.

Fabbrini, M., Galli, C., Gentilli, P. and Macchitella, D. (2001). An oxidation of alcohols by oxygen with the enzyme laccase and mediation by TEMPO. *Tetrahedron Letter, 42,* 7551-7553.

Fabbrini M, Galli, C. and Gentili, P. (2002). Comparing the catalytic efficiency of some mediators of laccase. *Journal of Molecular Catalysis B: Enzymatic, 16,* 231–240.

Fan, X., Burton, R. and Zhou, Y. (2010). Glycerol (byproduct of biodiesel production) as a source for fuels and chemicals. Mini review. *The Open Fuels & Energy Science Journal, 3,* 17-22.

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. *Canadian Journal of Microbiology, 46*, 189–194.

Faure, D., Bouillant, M.L. and Bally, R. (1994). Isolation of Azospirillum lipoferum 4T Tn5 Mutants Affected in Melanization and Laccase Activity. *Applied and Environmental Microbiology, 60*, 3413–3415.

Fogel, R. and Limson, J.L. (2013). Electrochemically predicting phenolic substrates suitability for detection by amperometric laccase biosensors. *Electroanalysis, 25*, 1237–1246.

Fordham, P., Besson, M. and Gallezot, P., (1995). Selective catalytic oxidation of glyceric acid to tartronic and hydroxypyruvic acids. *Applied Catalysis A, General, 133*, 179-184.

Franceschi, V. R. and Nakata, P. A. (2005). Calcium oxalate in plants: Formation and function. *Annual Review of Plant Biology, 56,* 41–71.

Fukuoka, T., Habe, H., Kitamoto, D. and Sakaki, K. (2011). Bioprocessing of glycerol into glyceric Acid for use in bioplastic monomer. *Journal of Oleo Science, 60,* 69–73.

Gallezot, P. (1997). Selective oxidation with air on metal catalysts. *Catalysis Today, 37*, 405–418.

Galli, C. and Gentili, P. (2004). Chemical messengers: mediated oxidations with the enzyme laccase. *Journal of Physical Organic Chemistry, 17*, 973-977.

Ghindilis, A. L., Gavrilova, V. P. and Yaropolov, A. I. (1992). Laccase based biosensor for determination of polyphenols: determination of catechols in tea. *Biosensors and Bioelectronics, 7*(2), 127–131.

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

Giovanelli, G. and Ravasini, G. (1993). Apple juice stabilization by combined enzyme-membrane filtration process. *LWT-Food Science and Technology, 26*(1), 1–7.

Givaudan, A., E€osse, A., Faure, D., Potier, P., Bouillant, M.-L. and Bally, R. (1993). Polyphenol oxidase in Azospirillum lipoferum isolated from rice rhizosphere: Evidence for laccase activity in non-motile strains of *Azospirillum lipoferum. FEMS Microbiology Letters, 108*, 205-210.

Grasemann, M.and Laurenczy, G. (2012). Formic acid as a hydrogen source – recent developments and future trends. *Energy & Environmental Science, 5,* 8171-8181.

Golubev, V. A., Rozantsev, E. G. and Neiman, M. B. (1965). Izv. Akad. Nauk SSSR. Ser. Khim., 1927 [*Bulletin of the Academy of Sciences of the USSR. Division of chemical sciences.* (Engl. Transl.), 1898].

Gonzalez-Pajuelo, M., Meynial-Salles, I., Mendes, F., Andrade, J.C., Vasconcelos, I. and Soucaille, P. (2005). Metabolic engineering of Clostridium acetobutylicum for the industrial production of 1,3-propanediol from glycerol. *Metabolic Engineering,7*, 329-336.

Haag, R., Sunder, A. and Stumbe, J.(2000). An approach to glycerol dendrimers and pseudo-dendritic polyglycerols. *Journal of the American Chemical Society, 122,* 2954–2955.

Habe, H., Fukuoka, T., Sato, Shun., Kitamoto, D. and Sakaki, K. (2011). Synthesis and evaluation of dioleoyl glyceric acids showing antitrypsin activity. *Journal of Oleo Science, 60,* 327–331.

Handa, S., Handa, A.K., Hasegawa, P.M. and Bressan, R.A. (1986). Proline accumulation and the adaptation of cultured plant cells to water stress. *Plant Physiology, 80,* 938-945.

Hansen, C. F. A., Hernandez, A. A., Mullan, B. P. B., Moore, K. B. and King, R. H. C. (2009). A chemical analysis of samples of crude glycerol from the production of biodiesel in Australia and the effects of feeding crude glycerol to growing- finishing pigs on performance, plasma metabolites and meat quality at slaughter. *Animal Production Science, 49*, 154–161.

Hazimah, A.H., Ooi, T.L. and Salmiah, A. (2003). Recovery of glycerol and diglycerol from glycerol pitch. *Journal of Oil Palm Research, 15*, 1–5.

Heinzkill, M., Bech, L., Halkier, T., Schneider, P. and Anke, T. (1998). Characterization of laccases and peroxidases from wood-rotting fungi (family Coprinaceae). *Applied and Environmental Microbiology, 64*, 1601–1606.

Hou, H., Zhou, J., Wang, J., Du, C. and Yan, B. (2004). Enhancement of laccase production by Pleurotus ostreatus and its use for the decolorization of anthraquinone dye. *Process Biochemistry, 39,*1415–1419.

Hoyt, H. E. and Manninen T. H. (1951). U. S. Patent: 2558520.

Hu, W.B., Lowry, B. and Varma, A. (2011). Kinetic study of glycerol network over Pt-Bi/C catalyst. *Applied Catalysis B: Environmental, 106*, 123-132.

Jacques, A. and Nadege, L-G. (1998). Hetero Diels-Alder Reaction with Aqueous Glyoxylic Acid: An Experiment in Organic Synthesis and 2-D NMR Analysis for Advanced Undergraduate Students. *Journal of Chemical Education, 75*, 1285.

Jin, P., Zhu, H., Wang, L., Shan, T. and Zheng, Y. H. (2014). Oxalic acid alleviates chilling injury in peach fruit by regulating energy metabolism and fatty acid contents. *Food Chemistry, 161*, 87–93.

Johannes, C. and Majcherczyk, A. (2000). Natural mediators in the oxidation of polycyclic aromatic hydrocarbons by laccase mediator systems. *Applied and Environmental Microbiology, 66*, 524-528.

Johnson, D.T. and Taconi, K.A. (2007). The glycerine glut: options for the value-added conversion of crude glycerol resulting from biodiesel production. *Environmental Progress and Sustainable Energy, 26*, 338–48.

Kataoka, M., Sasaki, M., Hidalgo, A.R., Nakano, M. and Shimizu, S. (2001). Glycolic acid production using ethylene glycol-oxidizing microorganisms. *Bioscience, Biotechnology, and Biochemistry, 65,* 2265–2270.

Katryniok, B., Kimura, H., Skrzynska, E., Girardon, J-S., Fongarland, P., Capron, M., Ducoulombier, R., Mimura, N., Paul, S. and Dumeignil, F. (2011). Selective catalytic oxidation of glycerol: perspectives for high value chemicals. *Green chemistry, 13,* 1960-1979.

Kim B.H. and Gadd GM. (2008). *Bacterial Physiology and Metabolism.* Cambridge University Press.

Kim, S.J., Park, J.G., Kim, J.H., Heo, J.S., Choi, J.W., Jang, Y.S. , Yoon, J., Lee, S.J., Kwon, I.K. (2011). Development of a biodegradable sirolimus-eluting stent coated by ultrasonic atomizing spray. *Journal of Nanoscience and Nanotechnology, 11*, 5689–5697.

Kim, S. S., Nehru, K., Kim, S. S., Kim, D. W. and Jung, H. C. (2002). A mild and highly efficient oxidation of sulfides to sulfoxides with periodic acid catalyzed by $FeCI_3$. *Organic Synthesis, 17*, 2484-2486.

Ko, E. M., Leem, Y. E., and Choi, H. T. (2001). Purification and characterisation of laccase isozymes from the white rot basidiomycete *Ganoderma lucidum. Applied Microbiology and Biotechnology, 57*, 98-102.

Koivistoinen, O.M., Kuivanen, J., Barth, D, Turkia, H., Pitkanen, J.P., Penttila, M. and Richard, P. (2013). Glycolic acid production in the engineered yeasts Saccharomyces cerevisiae and Kluyveromyces lactis. *Microbial Cell Factories, 12*, 82–97.

Koiwa, I., Okabe, Y., Matsubara, H., Osaka, T. and Goto, F. (1985). Effect of Tartronic Acid on Magnetic Properties of Electroless Plated Co-Ni-Re-P Alloy Films for a Perpendicular Recording Medium. *IEEE Translation Journal on Magnetics in Japan, 1,* 443-449.

Korolev,Y.A., Greish, A.A., Kozlova, L.M., Kopyshev, M.V., Litvin, E.F. and Kustov, L.M.(2010). Glycerol dehydroxylation in hydrogen on a Raney cobalt catalyst. *Catalysis in Industry, 2*, 287–289.

Kulys, J. and Vidziunaite, R. (2005). Kinetics of laccase-catalysed TEMPO oxidation. *Journal of Molecular Catalysis B: Enzymatic, 37*, 79–83.

Kurniawati, S. (2005*). Kinetics of the laccase-catalyzed oxidation of aqueous phenol.* PhD Thesis. McGill University, Canada.

Kurniawati, S. and Nicell, J. A. (2008). Characterization of *Trametes versicolor* laccase for the transformation of aqueous phenol. *Bioresource Technology, 99*, 7825–7834.

Kurosaka, T., Maruyama, H., Naribayashi, I. and Sasaki, Y. (2008). Production of 1,3-propanediol by hydrogenolysis of glycerol catalyzed by $Pt/WO_3/ZrO_2$. *Catalysis Communications, 9*, 1360.

Kuznetsov, B. A., Shumakovich, G. P., Koroleva, O. V. and Yaropolov, A. I. (2001). On applicability of laccase as label in the mediated and mediatorless electroimmunoassay: effect of distance on the direct electron transfer between laccase and electrode. *Biosensors and Bioelectronics, 16,* 73–84.

Landt, M. (2000). Glyceraldehyde Preserves Glucose Concentrations in Whole Blood Specimens. *Clinical Chemistry, 46,* 1144–1149.

Leontievsky, A., Myasoedova, N., Pozdnyakova, N. and Golovleva, L. (1997). "Yellow" laccase of *Panus tigrinus* oxidizes non-phenolic substrates without electron-transfer mediators. *FEBS Letter, 413,* 446–448.

Levy, S.B. (1992). Dihydroxyacetone-containing sunless or self-tanning lotions. *Journal of the American Academy of Dermatology. 27*, 989–993.

Li, P.Y., Yin, F., Song, L. J. and Zheng, X. L. (2016). Alleviation of chilling injury in tomato fruit by exogenous application of oxalic acid. *Food Chemistry, 202*, 125–132.

Liebminger, S., Siebenhofer, M. and Guebitz, G. (2009). Oxidation of glycerol by 2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPO) in the presence of laccase. *Bioresource Technology, 100*, 4541–4545.

Liu, X., Li, S. S., Liu, Y. M. and Cao, Y. (2015). Formic acid: A versatile renewable reagent for green and sustainable chemical synthesis. *Chinese Journal of Catalysis, 36*, 1461-1475.

Ma, Y., Loyns, C., Price, P. and Chechik, V. (2011). Thermal decay of TEMPO in acidic media via an N-oxoammonium salt intermediate. *Organic and Biomolecular Chemistry, 9,* 5573–5578.

Maier, G., Dietrich, H. and Wucherpfenning, K. (1990). Winemaking without SO2-with the aid of enzymes? *Weineirtschaft-Technik,126,* 18–22.

Mario, P and Michele, R. (2010). Future of glycerol. 2nd ed. Royal Society of Chemistry. London.

Marzorati, M., Danieli, B., Haltrich, D. and Riva, S. (2005). Selective laccase-mediated oxidation of sugar derivatives. *Green Chemistry, 7*, 310-315.

Mathiasen, T.E. (1995). Laccase and beer storage. PCT international application, WO 9521240 A2.

Matijosyte, I. (2008). *Mechanistic studies of Laccase/TEMPO catalyzed aerobic oxidation of alcohols*. Master Thesis. Vilnius University, Lithuania.

McKay, G. (1979). Waste color removal from textile effluents. *American Dyestuff Reporter, 68,* 29–34.

McMorn, P., Roberts, G. and Hutchings, G.J. (1999). Oxidation of glycerol with hydrogen peroxide using silicalite and alumina phosphate catalysts. *Catalysis Letters, 63*,193–197.

Minussi, R.C., Pastore, G.M. and Durán, N.(2002). Potential applications of laccase in the food industry. *Trends in Food Science and Technology, 13*, 205–216.

Miranda, R. C., Gomes, E. B., Pereira, N. J., Marin-Morales, M. A., Machado, K. M. and Gusmao, N. B. (2013). Biotreatment of textile effluent in static bioreactor by *Curvularia lunata* URM 6179 and *Phanerochaete chrysosporium* URM 6181. *Bioresource Technology, 142,* 361–367.

Miyazawa, T., Endo, T., Shiihashi, S. and Okawara, M. (1985). Selective Oxidation of Alcohols by Oxoaminium Salts ($R_2N=O^+X^-$). *The Journal of Organic Chemistry, 50,* 1332-1334.

Mohtar, Y., Tang, T.S. and Salmiah A. (2001). Quality of basic oleochemicals produced in Malaysia. *Informatics, 12,* 529-536.

Morozova, O.V., Shumakovich, G.P., Gorbacheva, M.A., Shleev, S.V. and Yaropolov, A.I. (2007). "Blue" Laccases. *Journal of Biochemistry, 72,* 1136-1150.

Mu, Y., Tend, H., Zhang, D., Wang, W. and Xiu, Z. (2006). Microbial production of 1,3-propanediol by *Klebsiella pneumoniae* using crude glycerol from biodiesel preparations. *Biotechnology Letters, 28,* 1755-1759.

Nakagawa, Y.,Shinmi, Y.,Shuichi, K.and Tomishige, K. (2010). Direct hydrogenolysis of glycerol into 1,3-propanediol over rhenium-modified iridium catayst. *Journal of Catalysis, 272,* 191–194.

Ngo, T.A., Nguyen, T.H. and Bui B.T.V. (2012). Thermophilic fermentative hydrogen production from xylose by *Thermotoga neapolitana* DSM 4359. *Renew Energy, 37,* 174–179.

Nguyen, B.C. and Kochevar, I.E. (2003). Factors influencing sunless tanning with dihydroxyacetone. *British Journal of Dermatology, 149,* 332–340.

Ninomiya, R., Miura, Y., Kosaka, K.,TSakao, K. and Okinak, S. (1966). Stimulatory Effect of Chelating Agents and Mesoxalate on the In Vivo Release of Insulin in the Pancreas of the Dog. *Diabetes, 15,* 1.

Novita, F. J., Lee, H.Y and Lee, M.Y. (2015). Self-heat recuperative dividing wall colun for enhancing the energy efficiency of the reactive distillation process in the formic acid production process. *Chemical Engineering and Processing: Process Intensifiaction, 97,* 144-152.

OECD/FAO. (2015). *OECD-FAO Agricultural Outlook.* OECD Agriculture statistics (database).

Osma, J.F. (2009). *Production of laccase by the white rot fungus Trametes pubescence for their potential application to synthetic dye treatment.* Phd Thesis, Universitat Rovira Virgili.

Pachauri, N. and He, B. (2006).Value-added utilization of crude glycerol from biodiesel production: a survey of current research activities. *American Society of Agricultural and Biological Engineers*. Paper number:066223.

Palmer, T. (1995). Understanding Enzymes. Prentice Hall.

Palmieri, G., Giardina, P., Bianco, C., Scaloni, A., Capasso, A. and Sannia, G. (1997). A novel white laccase from *Pleurotus ostreatus*. *The Journal of Biological Chemistry, 272*, 31301-31307.

Palmore, G. T. R. and Kim, H.H. (1999). Electro-enzymatic reduction of dioxygen to water in the cathode compartment of a biofuel cell. *Journal of Electroanalytical Chemistry, 464*(1), 110–117.

Papanikolaou, S.,Ruiz-Sanchez, P.,Pariset, B.,Blanchard, F. and Michel, F. (2000). High production of 1,3-propanediol from industrial glycerol by a newly isolated *Clostridium butyrium* strain. *Journal of Biotechnology, 77*, 191–208.

Pawar, S.S, Dekhane, D. V., Shingare, M.S. and Thore, S. N. (2008). Glyoxylic acid as catalyst: A simple selective synthesis of 1,2-disubstituted benzimidazoles in aqueous media. *Chinese Chemical Letters. 19*, 1055–1058.

Porta, F. and Prati, L. (2004). Selective oxidation of glycerol to sodium glycerate with gold-on-carbon catalyst: An insight into reaction selectivity. *Journal of Catalysis, 224*, 397–403.

Raghukumar, C. (2000). Fungi from marine habitats: an application in bioremediation. *Mycological Research, 104,* 1222–1226.

Ritter, G., Maier, G., Schoepplein, E. and Dietrich, H. (1992). The application of polyphenoloxidase in the processing of apple juice. *Bulletin de Liaison-Groupe Polyphenols, 16*, 209–212.

Rodr´ıguez Couto, S., Sanrom´an, M. and G¨ubitz, G.M. (2005). Influence of redox mediators and metal ions on synthetic acid dye decolourization by crude laccase from *Trametes hirsuta. Chemosphere, 58*, 417–422.

Rogalski, J. and Leonowicz. Laccase. In Pandey, A. (ed), *Concise Encyclopedia of Bioresource Technology,* Food Products Press, Haworth Reference Press, New York, 533-542.

Roscoe, H. E. (1888). A Treatise on Chemistry, Volume 3, Organic Chemistry, 161. D. Appleton and Co., New York.

Rubtsov, Y.L and Kazakov, A. I. (1997). Equilibrium constants of nitration of alcohols and thermal stability of their nitrates. *Russian Chemical Bulletin, 46*, 1707-1709.

Sayyari, M., Valero, D., Babalar, M., Kalantari, S., Zapata, P. J. and Serrano, M. (2010). Prestorage oxalic acid treatment maintained visual quality, bioactive compounds, and antioxidant potential of pomegranate after long-term storage at 2 °C. *Journal of Agricultural and Food Chemistry, 58,* 6804–6808.

Schmid, Rolf D. and Urlacher, Vlada. (2007). *Modern Biooxidation: Enzymes, Reactions and Applications.* Germany: Wiley-VCH Verlag GmbH Weinheim.

Segura, Enrique T. (1958): Effects of calcium mesoxalate on metalloxan diabetes in the rabbit. *Revista de la Sociedad Argentina de Biología (Rev Soc Argentina Biol), 8,* 276-282.

Sharma, K.K., Shrivastava, B., Sastry, V. R. B. , Sehgal, N. and Kuhad, R.C. (2013). Middle-redox potential laccase from *Ganoderma sp.*: its application in improvement of feed for monogastric animals. *Scientific Reports 3,* Article number: 1299.

Sheldon, R.A. and Arends, I.W.C.E. (2006). Catalytic oxidations mediated by metal ions and nitroxyl radicals. *Journal of Molecular Catalysis A: Chemical, 251,* 200-214.

Singhabhandhu, A. and Tezuka, T. (2010). A perspective on incorporation of glycerin purification process in biodiesel plants using waste cooking oil as feedstock. *Energy, 35,* 2493-2504.

Skorobogat'ko, O.V., Gindilis, A.L., Troitskaya, E. N., Shuster, A. M., and Yaropolov, A.I. (1994). Laccase as new enzymatic label for enzyme immunoassay. *Analytical Letters, 27,* 2997-3012.

Slinn M, Kendall K, Mallon C, Andrews J.(2008). Steam reforming of biodiesel by-product to make renewable hydrogen. *Bioresource Technology, 99,* 5851- 5858.

Slomczynski, D., Nakas, J. P. and Tanenbaum, S.W. (1995). Production and characterization of laccase from *Botrytis cinerea* 61–34. *Applied and Environmental Microbiology, 61,* 907–912.

Soares, G. M. B., Costa-Ferreira, M. and Pessoa de Amorim, M. T. (2001). Decolorization of an anthraquinone-type dye using a laccase formulation. *Bioresource Technology, 79,* 171–177.

Solomon, E.I., Chen, P., Metz, M., Lee, S.K. and Palmer, A.E. (2001). Oxygen binding, activation, and reduction to water by copper proteins. *Angewandte Chemie International Edition, 40,* 4570–4590.

Speight, J.G. (2002). *Chemical process and design handbook.* McGraw-Hill Professional. United States.

Stanko, R.T., Robertson, R.J., Spina, R.J., Reilly Jr., J.J., Greenawalt, K.D. and Goss, F.L. (1990). Enhancement of arm exercise endurance capacity with dihydroxyacetone and pyruvate. *Journal of Applied Physiology, 68*, 119–124.

Sun, B., Gu, C.J., Ma, J. H. and Liang, B.R. (2005). Kinetic study on TEMPO-mediated selective oxidation of regenerated cellulose. *Cellulose, 12,* 59-66.

Tan, H. W., Abdul Aziz, A. R. and Aroua, M. K. (2013). Glycerol production and its applications as a raw material: A review. *Renewable and Sustainable Energy Reviews, 27,* 118–127.

Taspinar, A. and Kolankaya, N. (1998). Optimization of enzymatic chlorine removal from Kraft pulp. *Bulletin of Environmental Contamination and Toxicology, 61*, 15–21.

Thakker, G.D., Evans, C.S. and Rao, K.K. (1992). Purification and characterization of laccase from *Monocillium indicum* Saxena. *Applied Microbiology and Biotechnology, 37*, 321–323.

The Soup and Detergent Association. (1990). *Glycerine: an overview.*

Thompson, J.C. and He, B.B. (2006). Characterization of crude glycerol from biodiesel production from multiple feedstocks. *Applied Engineering in Agriculture, 22*, 261–265.

Tišma, M., nidaršiè-Plazl, P., Plazl, I., Zelic, B. and Vasic-Racki, D. (2008). Modelling of L-DOPA oxidation catalyzed by laccase. *Chemical & Biochemical Engineering Quarterly, 22*, 307–313.

Tortolini, C., Rea, S., Carota, E., Cannistraro, S. and Mazzei, F. (2012). Influence of the immobilization procedures on the electroanalytical performances of *Trametes versicolor* laccase based bioelectrode. *Microchemical Journal, 100*, 8–13.

USDA. (2014). *Malaysia Biofuel Annual Report.* Gain Report No. MY4011.

Viikari, L., Niku-Paavola, M.L., Buchert, J., Firssell, P., Teleman, A. and Kruus, K. (1999). Method of producing oxidized starch. Patent No: WO1999023240 A1.

Villa, A., Campione, C. and Prati, L. (2007). Bimetallic gold/palladium catalysts for the selective liquid phase oxidation of glycerol. *Catalysis Letters, 115*, 133–136.

Villa, A., Campisi, S., Mohammed, K.M., Dimitratos, N., Vindigni, F., Manzoli, M., Jones, W., Bowker, M., Hutchingse, G.J. and Prati, L. (2015). Tailoring the selectivity of glycerol oxidation by tuning the acid–base properties of Au catalysts. Catalysis Science & Technology, 5, 1126-1132.

Wang, K., Hawley, M.C. and Deathos, S.J. (2003). Conversion of glycerol to 1,3-propanediol via selective dehydroxylation. *Industrial and Engineering Chemistry Research, 42*, 2913–2923.

Wang, F.F., Shao., S., Liu, C.L., Xu, C.L., Yang, R.Z. and Dong, W.S., 2015. Selective oxidation of glycerol over Pt supported on mesoporous carbon nitride in base-free aqueous solution. *Chemical Engineering Journal, 264*, 336–343.

Watanabe, M., Iida, T., Aizawa, Y., Aida, T.M. and Inomata, H. (2007). Acrolein síntesis from glycerol in hot-compressed water. *Bioresource Technology, 98*, 1285–1290.

Werpy, T., Frye, J., Zacher, A. and Miller, D. (2002) Hydrogenolysis of 6-carbon sugars and other organic compounds. US Patent : 6841085B2.

Wolfson,A., Litvak, G., Dlugy, C., Shotland, Y. and Tavor, D. (2009). Employing crude glycerol from biodiesel production as an alternative green reaction medium. *Industrial Crops and Products, 30*, 78–81.

Worz, N., Brandner, A. and Claus, P. (2010). Platinum-Bismuth-Catalyzed Oxidation of Glycerol: Kinetics and the Origin of Selective Deactivation. *The Journal of Physical Chemistry C, 114*, 1164–1172.

Xu, F. (1996). Oxidation of Phenols, Anilines, and Benzenethiols by Fungal Laccases: Correlation between Activity and Redox Potentials as Well as Halide Inhibition. *Biochemistry, 35*, 7608–7614.

Xu, F. (1997). Effects of redox potential and hydroxide inhibition on the pH activity profile of fungal laccases. *The Journal of Biological Chemistry, 272*, 924–928.

Xu, F. (1999). Laccase. In Flickinger, M.C. and Drew, S. W. (eds.), *Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis, Bioseparation*. John Wiley & Sons Inc., New York, 1545-1554.

Yaropolov, A.I., Skorobogatko, O.V., Vartanov, S.S. and Varfolomeyev, S.D. (1994). Laccase-properties, catalytic mechanism and applicability. *Applied Biochemistry and Biotechnology, 49*, 257-280.

Yaver, D.S., Overjero, M.D.C., Xu, F., Nelson, B.A., Brown, K.M., Halkier, T., Bernauer, S., Brown, S.H. and Kauppinen, S.K. (1999). Molecular characterization of laccase genes from the basidiomycete *Coprinus cinereus* and heterologous expression of the laccase lcc1. *Applied and Environmental Microbiology, 65*, 4943-4948.

Ying, G.G., Williams, B. and Kookana, R. (2002). Environmental fate of alkylphenols and alkylphenol ethoxylates- a review. *Environment International, 28*, 215–226.

Yoon, S.J., Choi, Y-C., Son, Y-Il., Lee, S-H. and Lee, J-G. (2010). Gasification of biodiesel by-product with air or oxygen to make syngas. *Bioresource Technology, 10*, 1227–1232.

Zander, Z., Yagloski, R., DeCoste, J., Zhang, D. and DeLacy, B. G. (2016). One-pot synthesis of high aspect ratio titanium dioxide nanorods using oxalic acid as a complexing agent. *MaterialsLetters, 163*, 39–42.

Zhang, Z.Y., Xin, L. and Li, W.Z. (2012). Electrocatalytic oxidation of glycerol on Pt/C in anion-exchange membrane fuel cell: Cogeneration of electricity and valuable chemicals. *Applied Catalysis B: Environmental, 119-120*,40-48.