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

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

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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
E₀  redox potential
LIST OF ABBREVIATIONS

ABTS 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
APHA American Public Health Association
BaCl₂ 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
H₅IO₆ 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
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<tr>
<td>MA</td>
<td>Mesoxalic acid</td>
</tr>
<tr>
<td>Na$_2$C$_3$O$_5$</td>
<td>Sodium mesoxalate</td>
</tr>
<tr>
<td>NHE</td>
<td>Normal hydrogen electrode</td>
</tr>
<tr>
<td>ODE</td>
<td>Ordinary differential equation</td>
</tr>
<tr>
<td>OECD-FAO</td>
<td>Organisation for Economic Co-operation and Development-Food and Agriculture Organization</td>
</tr>
<tr>
<td>pI</td>
<td>Isoelectric point</td>
</tr>
<tr>
<td>PLGA</td>
<td>Poly(lactic-co-glycolic acid)</td>
</tr>
<tr>
<td>RID</td>
<td>Refractive index detector</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>RT</td>
<td>Reverse transcriptase</td>
</tr>
<tr>
<td>SCE</td>
<td>Saturated calomel electrode</td>
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<tr>
<td>SDS-PAGE</td>
<td>Sodium dodecyl sulfate polyacrylamide gel electrophoresis</td>
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<tr>
<td>TA</td>
<td>Tartronic acid</td>
</tr>
<tr>
<td>TEMPO</td>
<td>2,2,6,6-Tetramethylpiperidine-1-oxyl</td>
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<tr>
<td>USDA</td>
<td>United States Department of Agriculture</td>
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ABSTRAK

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


