# *IN SILICO* ANALYSIS ON DEGRADATION ORDER OF CELLULOSIC COMPONENTS IN OIL PALM LEAVES BY *Trichoderma asperellum* UC1 ENZYMES

AINA HAZIMAH BINTI BAHAMAN

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## DEDICATION

This thesis is dedicated to my parents and family, who taught me that the best kind of knowledge to have is that which is learned for its own sake. It is also dedicated to my lectures, whose taught me that even the largest task can be accomplished if it is done one step at a time and also my friends for their endless support and motivation.

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## ABSTRACT

Increasing interest towards the enzyme industry has led to studies exploring possible applications of new enzymes for improving different manufacturing processes. This study focuses on capitalizing the oil palm biomass rich in lignocellulosic residues such as lignin, cellulose, and hemicellulose, which have an array of biotechnological applications. Literature has shown that oil palm frond leaves (OPFL) can be transformed into nanocellulose (NC) by fungal lignocellulosic enzymes, particularly those produced by *Trichoderma* species. The study aimed to comprehend this aspect by *in silico* approach of molecular docking, molecular dynamics (MD) simulation and molecular mechanics Poisson-Boltzmann surface area (MM- PBSA) analysis to identify the catalytic mechanism and selectivity of fungal enzymes endocellulase, exocellulase, β-glucosidase, and xylanase degrading the polymeric structures of OPFL. The study also seeks to identify the most stable enzymes to catalyse the optimal degradation of OPFL to yield maximal production of NC. It is an alternative greener avenue by a biotechnological approach to enzymatically extract NC from OPFL in order to circumvent the environmentally unfriendly use of corrosive acids and bases to extract NC. Energy minimized fungal enzyme models revealed satisfactory scores of PROCHECK, Verify3D and ERRAT according to the requirement of the validation which are >90%, >80% and >50%, respectively. Catalytic residue prediction by blind docking, COACH meta-server and multiple sequence alignment indicated the catalytic triads for endocellulase, exocellulase, β-glucosidase and xylanase were Ser116-His205-Glu249, Ser382-Arg124-Asp385, Glu165-Asp226-Glu423 and Arg155-Glu210-Ser160, respectively. The binding affinity of endocellulase for the substrates are as follows: hemicellulose ($-6.0$ kcal $mol^{-1}$) > lignin ($-5.6$ kcal $mol^{-1}$) > cellulose ($-4.4$ kcal $mol^{-1}$), while exocellulase showed its preference on lignin ($-8.7$ kcal $mol^{-1}$) > cellulose ($-8.5$ kcal $mol^{-1}$) > hemicellulose ($-8.4$ kcal $mol^{-1}$). The binding affinity of β- glucosidase for the substrates are as follows: cellulose ($-8.1$ kcal $mol^{-1}$) > lignin ($-7.9$ kcal $mol^{-1}$) > hemicellulose ($-7.8$ kcal $mol^{-1}$), whereas xylanase showed a corresponding preference for hemicellulose ($-6.7$ kcal $mol^{-1}$) > cellulose ($-5.8$ kcal $mol^{-1}$) > lignin ($-5.7$ kcal $mol^{-1}$). Selectivity of the enzymes was reiterated by MD simulations where interactions between endocellulase-hemicellulose, exocellulase-lignin, β-glucosidase-cellulose and xylanase- hemicellulose were the strongest. Notably low free-binding energy (ΔGbind) of endocellulase- hemicellulose ($-141.50$ +/− 74.59 kJ/mol), exocellulase-lignin ($-149.73$ +/− 39.00 kJ/mol), β-glucosidase-cellulose ($-207.23$ +/− 47.13 kJ/mol) and xylanase-hemicellulose ($-131.48$ +/− 24.57 kJ/mol) were observed. The findings thus successfully identified the specific actions of sugar-acting enzymes for NC production and cellulose component selectivity of the polymer- acting endocellulase, exocellulase, β-glucosidase and xylanase of *T. asperellum* UC1.

# ABSTRAK

Minat yang meningkat terhadap industri enzim telah membawa kepada kajian penerokaan aplikasi enzim baharu untuk menambah baik pelbagai proses pembuatan. Kajian ini tertumpu kepada memanfaatkan biomas kelapa sawit yang kaya dengan sisa lignoselulosa misalnya lignin, selulosa, dan hemiselulosa, yang mempunyai pelbagai aplikasi bioteknologi. Literatur telah menunjukkan bahawa daun pelepah kelapa sawit (OPFL) boleh ditukar menjadi nanoselulosa (NC) oleh enzim kulat lignoselulosa, terutamanya yang dihasilkan oleh spesies *Trichoderma*. Kajian ini menggunakan pendekatan *in silico* kemasukan molekul, simulasi dinamik molekul (MD) dan analisis mekanik molekul keluasan permukaan Poisson-Boltzmann (MM-PBSA) untuk mengenal pasti mekanisma pemangkin dan kepilihan bagi enzim kulat endoselulase, eksoselulase, β-glukosidase, dan xilanase menguraikan struktur polimer OPFL. Kajian ini juga bertujuan untuk mengenal pasti enzim yang paling stabil untuk memangkinkan penguraian optimum OPFL untuk pengeluaran hasil NC yang maksimum. Ia merupakan jalan alternatif yang lebih hijau dengan pendekatan bioteknologi untuk mengekstrak NC daripada OPFL untuk mengelak penggunaan asid dan bes yang tidak mesra alam sekitar dan bersifat menghakis. Model enzim kulat tenaga minimum menunjukkan skor yang memuaskan bagi PROCHECK, Verify3D dan ERRAT mengikut keperluan pengesahan iaitu masing-masing >90%, >80% dan >50%. Ramalan residu mangkin oleh pemasukan molekul, COACH meta-server dan pelbagai penjajaran urutan menunjukkan triad mangkin bagi endoselulase, eksoselulase, β-glukosidase dan xilanase adalah Ser116-His205-Glu249, Ser382-Arg124- Asp385, Glu165-Asp226-Glu423 dan Arg155-Glu210-Ser160, masing-masing. Tenaga pengikatan endoselulase dengan substrat adalah seperti berikut: hemiselulosa (−6.0 kcal $mol^{-1}$) > lignin (−5.6 kcal $mol^{-1}$) > selulosa (−4.4 kcal $mol^{-1}$), manakala eksoselulase menunjukkan keutamaan pada lignin (−8.7 kcal $mol^{-1}$) > selulosa (−8.5 kcal $mol^{-1}$) > hemiselulosa (−8.4 kcal $mol^{-1}$). Tenaga pengikatan β-glukosidase dengan substrat adalah seperti berikut: selulosa (−8.1 kcal $mol^{-1}$) > lignin (−7.9 kcal $mol^{-1}$) > hemiselulosa (−7.8 kcal $mol^{-1}$), sedangkan xilanase menunjukkan keutamaan yang sepadan untuk; hemiselulosa (−6.7 kcal $mol^{-1}$) > selulosa (−5.8 kcal $mol^{-1}$) > lignin (−5.7 kcal $mol^{-1}$). Kepilihan enzim telah diulang menggunakan simulasi MD di mana enteraksi antara endoselulase-hemiselulosa, eksoselulase-lignin, β-glukosidase-selulosa dan xilanase-hemiselulosa adalah terkuat. Tenaga pegikatan bebas (ΔGbind) yang paling rendah bagi endoselulase-hemiselulosa (−141.50 +/− 74.59 kJ/mol), eksoselulase-lignin (−149.73 +/− 39.00 kJ/mol), β-glukosidase-selulosa (−207.23 +/− 47.13 kJ/mol) dan xilanase-hemiselulosa (−131.48 +/− 24.57 kJ/mol) telah diperhatikan. Dengan demikian, penemuan ini berjaya mengenal pasti tindakan spesifik enzim yang bertindak terhadap gula untuk pengeluaran NC dan kepilihan komponen selulosa bagi endoselulase, eksoselulase, β-glukosidase dan xilanase daripada *T. asperellum* UC1 yang bertindak terhadap polimer.

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| | | |
|---|---|---|
| 3D | - | Three-dimensional |
| A or Ala | - | Alanine |
| AFM | - | Atomic Force Microscopy |
| BC | - | Bacterial cellulose |
| C | - | Carbon |
| C or Cys | - | Cysteine |
| CAZy | - | Carbohydrate-Active enZYmes |
| Cel5A | - | Endocelulase Family 5 |
| CNC | - | Cellulose nanocrystals |
| CNF | - | Cellulose nanofibrils |
| D or Asp | - | Aspartic acid |
| DNA | - | Deoxyribonucleic acid |
| Da | - | Daltons |
| E or Gly | - | Glutamic acid |
| EC | - | Enzyme commission number |
| EFB | - | Empty Fruit Bunch |
| F or Phe | - | Phenylalanine |
| FESEM | - | Field Emission Scanning Electron Microscopy |
| FTIR | - | Fourier-transform Infrared Spectroscapy |
| G or Gly | - | Glycine |
| GH | - | Glycosidase Hydrolases |
| GH-5 | - | Glycosidase Hydrolases Family 5 |
| GH-A | - | Glycosidase Hydrolases Clan A |
| GRAVY | - | Grand average of hydropathy |
| GROMACS | - | GROningen Machine for Chemical Simulations |
| GT | - | Glucosyl-transferases |
| H | - | Hydrogen |
| H or His | - | Histidine |
| I or Ile | - | Isoleucine |
| ID | - | Identification or identity |

| | | |
|---|---|---|
| K or Lys | - | Lysine |
| L or Leu | - | Leucine |
| M | - | Amino; represented by either A or C |
| MD | - | Molecular Dynamic |
| MM-PBSA | - | Molecular mechanics Poisson-Boltzmann surface area |
| MPOC | - | Malaysian Palm Oil Council |
| N | - | Any base; A or C or G or T |
| N | - | Nitrogen |
| N or Asn | - | Asparagine |
| NS | - | Nanosilica |
| NC | - | Nanocellulose |
| NCBI | - | National Centre for Biotechnology Information |
| NMR | - | Nuclear Magnetic Resonance |
| O | - | Oxygen |
| OPFL | - | Oil Palm Frond Leaves |
| P or Pro | - | Proline |
| PDB | - | Protein Data Bank |
| PS | - | Polysulphur |
| Q or Glu | - | Glutamine |
| R or Arg | - | Arginine |
| RCSB | - | Research Collaboratory for Structural Bioinformatics |
| RMSD | - | Root-mean Square Deviation |
| RMSF | - | Root-mean Square Fluctuation |
| RSM | - | Response Surface Methodology |
| S | - | Sulphur |
| S or Ser | - | Serine |
| SSF | - | Solid-state fermentation |
| T or Thr | - | Threonine |
| TGA | - | Thermogravimetric analysis |
| V or Val | - | Valine |
| W or Trp | - | Tryptophan |
| XRD | - | X-ray Diffraction |
| Y or Tyr | - | Tyrosine |

## LIST OF SYMBOLS

| | | |
|---|---|---|
| ΔG | - | Gibbs energy |
| °C | - | Degree celsius |
| Å | - | Armstrong |
| Φ | - | Phi |
| Ψ | - | Psi |
| % | - | Percent |
| cal | - | Calorie |
| K | - | Kelvin |
| kcal | - | Kilocalorie |
| kDa | - | Kilodaltons |
| kg | - | Kilogram |
| kJ | - | Kilojoule |
| mM | - | Milimolar |
| mol | - | Molar |
| nm | - | Nanometre |
| ns | - | Nanosecond |
| pH | - | Potential of hydrogen |
| pI | - | Isoelectric point |
| pKa | - | Association constant |
| ps | - | picosecond |

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# CHAPTER 1

# INTRODUCTION

## 1.1 Background of Study

Fungi of the genus *Trichoderma* are a very large group of microorganisms which role has been investigated in different field of areas, for instance, as biological control agents as well as producers of commercially relevant lignocellulosic degrading enzymes (Błaszczyk *et al.*, 2014). Silva *et al.* (2011) claimed that some species of the genus *Trichoderma* are cost-effectively significant due to their versatility in the production of antibiotics and industrial enzymes (e.g., cellulases, hemicellulases and xylanases). Similarly, lignocellulosic enzymes of *Trichoderma* species are popular bioremediation agents for the biodegradation and biotransformation of biomass into commercially important platform chemicals (Hoseinzadeh *et al.*, 2017). In recent study, Ezeilo *et al.* (2019b) has reasserted that *Trichoderma asperellum* is an excellent producer of cellulase and xylanase. Unlike most other enzymes, cellulase is a complex of three enzymes which are endo-β-1,4-glucanases (EC 3.2.1.4), exo-β-1,4-glucanases (EC 3.2.1.91) and β-glucosidases (EC 3.2.1.21) that work synergistically to attack native cellulose (Raghuwanshi *et al.*, 2014). On the contrary, xylanase (EC 3.2.1.8) has a cellulose-binding domain which preferentially degrades the linear polysaccharide β-1,4-xylan into xylose. This action, thus breaks down the hemicellulose, which forms the major component of plant cell walls (Chen & Wang, 2017; Mello *et al.*, 2017). In actual fact, the task to rid-off enormous quantities of post-harvest agricultural lignocellulosic biomass presents an uphill battle, of which is faced by many nations worldwide (Thushari *et al.*, 2019).

In this study, the hydrolytic enzymes (endocellulase, exocellulase, β-glucosidase, and xylanase) produced by *T. asperellum* UC1 were expected to cleave the lignocellulosic component of oil palm frond leaves (OPFL) that consist cellulose, hemicellulose, and lignin, into shorter precursor biopolymer to subsequently obtain

nanocellulose (NC). Like any other lignocellulosic wastes from agro-industrial biomass, the OPFL is made up of three different components, with cellulose being the major constituent (35-50%), ensued by hemicellulose (20-35 %) and lignin (10-25 %) (Ezeilo *et al.*, 2019c; Limkar *et al.*, 2019). The inherently high cellulose content in OPFL is a reservoir for the fabrication of different technologically important NC products (Elias *et al.*, 2017; Ariffin *et al.*, 2018). The current popular route to extract NC from biomass, are far from green, due to a heavy dependence on corrosive acids and bases to breakdown the cell wall of the plant material to eliminate the interfering hemicellulose and lignin components. This has created serious long-term environmental concerns, especially when the processes are scaled up for mass treatment of the agricultural cellulose feedstock.

In light of the aforementioned matter, the enzymatic route to harvest the cellulosic materials from OPFL, the largest contributing biomass in Malaysia (Ezeilo *et al.*, 2019c; Limkar *et al.*, 2019), into biopolymer precursors to produce NC, may prove useful in alleviating environmental problems as a consequence of surplus OPFL in the environment. Moreover, scientifically effective enzyme-assisted extraction and purification methods to obtain the cellulosic component from OPFL is a technological avenue that is yet to be fully explored. Thus, the feasibility of this approach using cellulases from *T. asperellum* UC1 as the bioremediation agent, remains unknown. Since the topic of NC is gaining popularity and discussed in many research fields (Elias *et al.*, 2017; Elias *et al.*, 2018b; Elias *et al.*, 2019b), economical and sustainable protocols using greener processing pathways for its extraction, should be developed.

Although literature on empirical enzymatic hydrolysis of cellulases from different microbial sources is abundantly available, it is important to note that selectivity of a particular source of cellulase to degrade or hydrolyze the different lignocellulosic components may differ from microorganism to another (Luterbacher *et al.*, 2013).The same can be assumed for the preferential action of the *T. asperellum* UC1 cellulases and xylanase on OPFL. Thus, to better understand the structure-to-function of *T. asperellum* fungal cellulases and xylanase to digest OPFL, this study focused on the *in silico* or computational investigation using molecular docking and molecular dynamics (MD) simulations, to uncover the preferential action of the fungal

cellulases and xylanase on the different lignocellulosic components. Firstly, the initial *in silico* investigation would begin with the docking of target substrates (lignocellulosic components) into the active sites of the cellulases; endocellulase, exocellulase, β-glucosidase, and xylanase. This step provides preliminary data to this study to identify the selectivity of each enzyme to cleave the three lignocellulosic components in OPFL, founded on the estimated binding energies of the amino acid residues docked with the substrates. In principle, substrate docking enables the prediction of the preferred orientation of one molecule to a second when bound to each other to form a stable complex, alongside predicting the binding conformation of small molecule ligands to the appropriate target binding site (Farzaneh *et al.*, 2016; Ciemny *et al.*, 2018; Jin *et al.*, 2018). By docking cellulose, hemicellulose and lignin into the active site of the fungal cellulases, the strength and type of signals from the generated conformation of the catalytic triad that interacted with the enzymes, can be estimated. The next step in this *in silico* investigation involves molecular dynamic (MD) simulation to elucidate the flexibility and to computationally investigate the structural stability of the target protein-ligand (enzyme-substrate) complexes (Saadhali *et al.*, 2016; Kumar *et al.*, 2019a).

For such an investigation, the three-dimensional (3D) structures of the four fungal enzymes must be solved, in order to obtain the molecular organization and function of conserved enzymes' residues that will interact with the different OPFL components (Bienert *et al.*, 2017). The use of bioinformatics has gradually taken centre stage to aid for *in silico* studies of proteins to modulate their properties. However, the use of molecular docking and MD simulation to predict the selectivity of the enzyme-substrate in this study, may not be adequate. Literature have should that an additional calculation to validate data from earlier assessments is needed. Many studies have employed molecular mechanics Poisson-Boltzman Surface Area (MM-PBSA) analysis to estimate the binding affinities of the complexes. The MM-PBSA data have been proven useful for reproducing and rationalizing experimental findings, alongside improving the results of virtual screening and docking (Karami *et al.*, 2017; Wang *et al.*, 2017; Kumar *et al.*, 2019a).

## 1.2 Problem Statement

In view of the greener biotechnological approach of using cellulases and xylanase from *T. asperellum* UC1 extract and purify cellulose from OPFL, the feasibility of this approach should therefore be assessed. This is because selectivity of a particular source of microbial cellulases and xylanase to degrade or hydrolyze the different lignocellulosic components may differ from microorganism to another. To expedite understanding on the feasibility of the approach and improve comprehension on the enzymatic action that occur on the lignocellulosic components in the OPFL, this study proposed the use of a computational approach.

The work was aimed to carry out *in silico* assessment of endocellulase, exocellulase, β-glucosidase, and xylanase of *Trichoderma asperellum* UC1, to hydrolyze the lignocellulosic components in the OPFL. This approach may be a useful preliminary study to understand the selectivity of the fungal enzymes, before embarking on laborious and time-consuming empirical studies. To the best of our knowledge, an *in silico* study to identify the order of selectivity of the hydrolysis of the lignocellulosic components of OPFL by endocellulase, exocellulase, β-glucosidase, and xylanase of *Trichoderma asperellum* UC1, remains unreported.

It is hypothesized that production of NC can be expedited by identifying the preference of each enzyme for the cellulose, hemicellulose or lignin components. So much so, the solid-state fermentation (SSF) conditions of the fungus can be modulated to enable the production of a large quantity of a particular enzyme into the growth supernatant. Only then, the right order of cellulase-containing supernatant can be added at intervals to the SSF system, to enzymatically remove the interfering lignin and hemicellulose components from OPFL. Higher yields of NC is expected if the dominant acting enzymes first cleaves the interchain β-glycosidic bonds, as α-glycosidic acting enzymes will mostly yield monomers of sugar subunits, and not the nanosized cellulose (Saai Anugraha *et al.*, 2016).

## 1.3 Objectives of the Study

As the study aimed to predict the specific order of action for the fungal enzymes to cleave the multi cellulosic polymer layer in OPFL, the specific objectives of this study are therefore, as follows:

1. To evaluate the interaction of xylanase, endocellulase, exocellulase and β-glucosidase with lignin, cellulose and hemicellulose by substrate docking and MD simulation.
2. To estimate free binding energies ($\Delta G_{binding}$) of lignin, hemicellulose and cellulose with xylanase, endocellulase, exocellulase and β-glucosidase using MM/PBSA method.

## 1.4 Scopes of Study

This study begins with the use of AutoDock version 4.2.6 and AutoTools 1.5.6 software for both blind docking and binding site-based docking which involves the substrate (cellulose, hemicellulose, lignin) with the fungal enzymes in order to resolve the interaction energy of protein-ligand complexes. This technique is used to determine the active site residues of the tested substrates (cellulose, hemicellulose, lignin) that act upon during the programs i.e. molecular docking that can predict how a ligand interacts with the amino acid residues binding site of a receptor in order to enable catalysis. Next, AutoDock Vina was used as a successor docking analysis of AutoDock as it significantly improves the accuracy and performance compared to Lamarckian Genetic Algorithm. For the MD simulation, protonation of enzymes model surface residues (endocellulase, exocellulase, β-glucosidase, and xylanase from *T. asperellum* UC1) to a molecular dynamic simulation by GROningen MAchine for Chemical Simulations (GROMACS) version 2018.6 were used to identify the reaction of enzyme-substrate complex in order to understand macromolecular structure-to-function relationships, MD simulations allows the motion of the enzymes to be simulated in defined conditions on the basis of classical

molecular dynamics. Moreover, protein modelling might be the only way to obtain structural information where the experimental techniques are inapplicable.

In the final objective of the study, the estimation of ligand-binding affinities by molecular mechanics Poisson-Boltzmann surface area (MM-PBSA) was used to reproduce and rationalize experimental findings and to improve the results of virtual screening and docking. Gibbs free energy was calculated using GROMACS and the method adapted from an open-source software MM-PBSA. In this study, MM-PBSA method was utilized to compute of the average free energies of solvation ($\Delta G_{binding}$) between the fungal enzymes (endocellulase, exocellulase, β-glucosidase and xylanase) and set of designed ligands i.e. cellulose, hemicellulose, lignin. Thus, it shows the final values of free binding energies of enzyme-substrate complex, and will validate the data seen in MD simulation studies protein complexes with cellulosic materials *viz.* cellulose, hemicelluloses and lignin.

## 1.5 Significance of Study

The information derived by this study is valuable for comparing the tendencies and efficacies of each cellulase and xylanase in *T. asperellum* UC1 in removing the interfering lignin and hemicellulose, before pure cellulose can be obtained from OPFL. The findings of this study may contribute to the body of knowledge, in terms of identifying the correct order to add different fungal cellulases and xylanase obtained from SSF fermentation, to produce NC using OPFL as the starting biomass.

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1. Aina Hazimah Bahaman, Roswanira Abdul Wahab. (2019). Molecular Dynamic Simulation and Substrate Docking to Predict Degradation Order of Cellulosic in Oil Palm Leaves by Fungal Cellulases. 3rd Asia International Multidisciplinary Conference (AIMC) 2019. ISBN: 978-93-88786-10-2

### Journal with Impact Factor

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2. Bahaman, A. H., Wahab, R., Abdul Hamid, A. A., Abd Halim, & K. B., Kaya, Y. (2020). Molecular docking and molecular dynamics simulations studies on β-glucosidase and xylanase *Trichoderma asperellum* to predict degradation order of cellulosic components in oil palm leaves for nanocellulose preparation. Journal of Biomolecular Structure and Dynamics, 1-17. doi: 10.1080/073911 02.2020.1751713. (Accepted at Journal of Biomolecular Structure and Dynamics, Q2, IF: 3.31).

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# Substrate docking and molecular dynamic simulation for prediction of fungal enzymes from *Trichoderma* species-assisted extraction of nanocellulose from oil palm leaves

Aina Hazimah Bahaman[a,b], Roswanira Abdul Wahab[a,b], Azzmer Azzar Abdul Hamid[c], Khairul Bariyyah Abd Halim[c], Yilmaz Kaya[d,e] and Mohamed Faraj Edbeib[f]

[a]Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, Johor, Malaysia; [b]Enzyme Technology and Green Synthesis Group, Universiti Teknologi Malaysia, Johor, Malaysia; [c]Research Unit for Bioinformatics and Computational Biology (RUBIC), Kulliyyah of Science, International Islamic University Malaysia (IIUM), Kuantan, Pahang, Malaysia; [d]Department of Agricultural Biotechnology, Faculty of Agriculture, Ondokuz Mayis University, Samsun, Turkey; [e]Department of Biology, Faculty of Science, Kyrgyz-Turkish Manas University, Kyrgyzstan; [f]Department of Animal Production, Faculty of Agriculture, Baniwalid University, Baniwalid, Libya

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**ABSTRACT**

Fungi of the *Trichoderma* species are valued industrial enzymes in support of the 'zero-waste' technology to convert agro-industrial biomass into valuable products, i.e. nanocellulose (NC). In this study, an *in silico* approach using substrate docking and molecular dynamic (MD) simulation was used to predict the order of which the multilayers of cellulosic polymers, i.e. lignin, hemicellulose and cellulose in oil palm leaves (OPL) are degraded by fungal enzymes, endocellulase and exocellulase. The study aimed to establish the catalytic tendencies of the enzymes to optimally degrade the cellulosic components of OPL for high yield production of NC. Energy minimized endocellulase and exocellulase models revealed satisfactory scores of PROCHECK (90.0% and 91.2%), Verify3D (97.23% and 98.85%) and ERRAT (95.24% and 91.00%) assessments. Active site prediction by blind docking, COACH meta-server and multiple sequence alignment indicated the catalytic triads for endocellulase and exocellulase were Ser116–His205–Glu249 and Ser382–Arg124–Asp385, respectively. Binding energy of endocellulase docked with hemicellulose (−6.0 kcal $mol^{-1}$) was the most favourable followed by lignin (−5.6 kcal $mol^{-1}$) and cellulose (−4.4 kcal $mol^{-1}$). Exocellulase, contrarily, bonded favorably with lignin (−8.7 kcal $mol^{-1}$), closely followed by cellulose (−8.5 kcal $mol^{-1}$) and hemicellulose (−8.4 kcal $mol^{-1}$). MDs simulations showed that interactions of complexes, endocellulase–hemicellulose and the exocellulase–cellulose being the most stable. Thus, the findings of the study successfully identified the specific actions of sugar-acting enzymes for NC production.

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**KEYWORDS**
*Trichoderma*; docking; cellulase; nanocellulose; molecular dynamics

## 1. Introduction

Lignocellulosic wastes from agro-industrial biomass are made up of three different components, with cellulose being the major constituent (35–50%), ensued by hemicellulose (20–35%) and lignin (10–25%) (Ezeilo, Lee, Huyop, Zakaria, & Wahab, 2019; Limkar, Pawar, & Rathod, 2019). While the microbial-assisted transformation of complex lignocellulolytic carbohydrates has gained considerable scientific attention (Ezeilo, Wahab et al., 2019), the processing output is far from satisfactory. This is because lignocellulosic sources and structure, composition, method of pre-treatment and reactor design are the key determining factors (Ezeilo, Lee et al., 2019; Ezeilo, Wahab et al., 2019). Consistently, the enormous quantity of unwanted lignocellulosic source from oil palm biomass such as oil palm leaves (OPL) in Malaysia, remains uncharted for biotechnological applications. In fact, OPL constitute a good renewable starting material to produce value-added products, for instance, the nano-sized cellulose, i.e. nanocellulose (NC) (Ariffin et al., 2018; Elias et al., 2018). Moreover, current chemical route to extract NC from biomass are far from sustainable due to the heavy dependence on corrosive acids and bases that corrode reactors, and require tedious downstream treatments (Elias et al., 2018; Elias, Wahab, Chandren, Abdul Razak, & Jamalis, 2019). Since the topic of NC is gaining popularity and discussed in many research fields (Elias et al., 2017, 2018, 2019), economical and sustainable protocols using greener processing pathways for its extraction, should be considered.

Having said that, an alternative biotechnological approach must be developed so as NC can be effectively extracted from OPL. One way is to extract NC using a bio-enzymatic approach whereby fungal cellulases from the glycoside hydrolase group are used for degrading the cellulosic material in OPL. Glycoside hydrolase is a large group of enzymes that hydrolyzes glycosidic bonds between two or more carbohydrates, or between carbohydrates and a non-carbohydrate subdivision (Falck, Linares-Pasten, Karlsson, & Adlercreutz, 2018). In general,

CONTACT Roswanira Abdul Wahab roswanira@kimia.fs.utm.my Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Malaysia

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# Molecular docking and molecular dynamics simulations studies on β-glucosidase and xylanase *Trichoderma asperellum* to predict degradation order of cellulosic components in oil palm leaves for nanocellulose preparation

Aina Hazimah Bahaman[a,b], Roswanira Abdul Wahab[a,b], Azzmer Azzar Abdul Hamid[c,d], Khairul Bariyyah Abd Halim[c,d] and Yilmaz Kaya[e,f]

[a]Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, UTM Johor Bahru, Johor, Malaysia; [b]Enzyme Technology and Green Synthesis Group, Universiti Teknologi Malaysia, UTM Johor Bahru, Johor, Malaysia; [c]Department of Biotechnology, Kuliyyah of Science, International Islamic University Malaysia, Kuantan, Malaysia; [d]Research Unit for Bioinformatics and Computational Biology (RUBIC), Kulliyyah of Science, International Islamic University Malaysia, Pahang, Malaysia; [e]Department of Agricultural Biotechnology, Faculty of Agriculture, Ondokuz Mayis University, Samsun, Turkey; [f]Department of Biology, Faculty of Science, Kyrgyz-Turkish Manas University, Bishkek, Kyrgyzstan

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**ABSTRACT**

Literature has shown that oil palm leaves (OPL) can be transformed into nanocellulose (NC) by fungal lignocellulosic enzymes, particularly those produced by the *Trichoderma* species. However, mechanism of β-glucosidase and xylanase selectivity to degrade lignin, hemicellulose and cellulose in OPL for NC production remains relatively vague. The study aimed to comprehend this aspect by an *in silico* approach of molecular docking, molecular dynamics (MD) simulation and Molecular-mechanics Poisson-Boltzmann surface area (MM-PBSA) analysis, to compare interactions between the β-glucosidase- and xylanase from *Trichoderma asperellum* UC1 in complex with each substrate. Molecular docking of the enzyme-substrate complex showed residues Glu165-Asp226-Glu423 and Arg155-Glu210-Ser160 being the likely catalytic residues of β-glucosidase and xylanase, respectively. The binding affinity of β-glucosidase for the substrates are as follows: cellulose (−8.1 kcal $mol^{-1}$) > lignin (−7.9 kcal $mol^{-1}$) > hemicellulose (−7.8 kcal $mol^{-1}$), whereas, xylanase showed a corresponding preference for; hemicellulose (−6.7 kcal $mol^{-1}$) > cellulose (−5.8 kcal $mol^{-1}$) > lignin (−5.7 kcal $mol^{-1}$). Selectivity of both enzymes was reiterated by MD simulations where interactions between β-glucosidase-cellulose and xylanase-hemicellulose were the strongest. Notably low free-binding energy ($\Delta G_{bind}$) of β-glucosidase and xylanase in complex with cellulose (−207.23 +/− 47.13 kJ/mol) and hemicellulose (−131.48 +/− 24.57 kJ/mol) were observed, respectively. The findings thus successfully identified the cellulose component selectivity of the polymer-acting β-glucosidase and xylanase of *T. asperellum* UC1.

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Trichoderma; molecular docking; MM-PBSA; nanocellulose; molecular dynamics simulation

## 1. Introduction

Lignocellulosic materials from agro-industrial activities remains the most abundant natural polymer on Earth while the chemically/physically augmented cellulose derivative, the nanocellulose (NC) is prized for its intrinsic functionality, as well as reliability and sustainability of production (Chieng et al., 2017; Chen et al., 2018). It is worth mentioning here that NC may be prepared from oil palm leaves (OPL) as the plant cell wall has a lignocellulosic content that is 50% cellulose, 25% hemicellulose and 25% lignin (Financie et al., 2016; Tan et al., 2018). Nonetheless, the current approach to produce cellulosic nanomaterials from biomass are non-eco-friendly, predominantly relying on corrosive acids and bases for extraction and purification of the biomaterial. Other processing complications also include corrosion of reactors, and tedious downstream treatments. Since the subject of NC is gaining acceptance in many research fields (Elias et al., 2017; Elias et al., 2018; Elias et al., 2019), a more economical and greener alternative protocol to prepare this nanomaterial should therefore be developed. In this milieu, an enzyme-assisted preparation of NC from OPL using fungal glycoside hydrolase enzymes, may prospectively be a greener option. Not only that, it can alleviate environmental due to the surplus of OPL by converting the biomass into technologically functional materials.

We previously cultivated the fungus *Trichoderma asperellum* UC1 under solid-state fermentation with success, using OPL as its sole carbon source to produce appreciable amounts of cellulase and xylanase (Ezeilo et al., 2019a; Ezeilo et al., 2019b). The *Trichoderma* fungal genus is a well-documented prolific producer of lignocellulolytic enzyme cocktails (Druzhinina & Kubicek, 2017), and the *T. asperellum* UC1 is reportedly a hyper cellulase and xylanase producer when cultivated in raw OPL (Ezeilo et al., 2019c). The fungus secretes β-glucosidase (EC 3.2.1.21) and xylanase (EC 3.2.1.8) that

CONTACT Roswanira Abdul Wahab roswanira@kimia.fs.utm.my Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, UTM Johor Bahru, 81310 Malaysia