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Study of Xylose as Product Inhibitor in Xylanase from *Aspergillus niger*, *Bacillus subtilis*, and *Trichoderma reesei*: Insilico Approach

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ABSTRACT

Bioinformatics is helpful for solving molecular biology problems computationally with the advantage of being safe, free from chemical waste, secure, cost-effective, and can shorten research time. The issues that arise can be in the form of degradation of the xylanase enzyme using some microorganisms like *Aspergillus niger*, *Bacillus subtilis*, and *Trichoderma reesei* on lignocellulose bonds. To predict the optimum conditions for this enzymatic reaction has used bioinformatics applications through substrate enzymes obtained from protein data banks. The purpose of this study was to determine the optimum conditions for obtaining xylanase enzymes from the microorganisms *Aspergillus niger*, *Bacillus subtilis*, and *Trichoderma reesei* by bioinformatics (in silico). This research was conducted in bioinformatics using a RCSB Protein Data Bank database and PubChem. The programs used to see the interaction between substrate enzymes are PyMol, PyRx, and LigPlot. The best conditions based on the results of bioinformatics simulations will form the basis for producing xylanases on a laboratory scale. In this study, the results of interaction data between *Bacillus subtilis* and D-xylose, *Aspergillus niger* with D-xylose, and *Trichoderma reesei* with D-xylose have a binding affinity value of -5.2 kcal/mol; -5.1 kcal/mol; and -4.3 kcal/mol respectively.

Keywords: *Aspergillus niger*, *Bacillus subtilis*, in Silico, *Thricodherma reesei*, xylanase.

INTRODUCTION

In most areas of Indonesia, lignocellulosic waste is still considered as waste. This lignocellulosic waste is produced from agriculture such as rice straw, corncob, sawdust and sugarcane bagasse. So far, the use of lignocellulosic waste is limited to fodder and household fuel for cooking; besides that, there is no other utilization that can be optimally utilized for its lignocellulose content. Lignocellulose as old plant waste, its cell walls have undergone further lignification to form complex bonds, including cellulose and hemicellulose. Lignocellulose still has added value if further processing is carried out. The development of biotechnology using microorganisms can be used for processing this waste. The waste is processed to produce useful products [1].

Utilization of lignocellulosic waste in the waste bioconversion process can be carried out to obtain added value from the wreckage into other products such as fertilizers, bioethanol, biogas and so on. The use of lignocellulosic materials is more attractive than starchy materials because it does not compete in service for food purposes [2].

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Utilization of lignocellulosic waste by using microorganisms can produce extracellular enzymes that can degrade lignocellulosic materials into their constituent fractions [3]. The substrate used in the fermentation process affects the activity and productivity of the enzyme. The presence of specific substrates in the production medium can stimulate microorganisms to secrete their cellular metabolites. To open the hemicellulose structure to make it easier for enzymes to break down polysaccharides into monosaccharides [4].

Xylanase enzymes can be produced by various types of bacteria, yeast, moulds, protozoa, insects and snails [5]. Xylanase made from microorganisms such as *Aspergillus niger* is stable at temperatures less than 45^{o}C, has an optimum temperature of 45-60^{o}C and is stable at a pH of 4-8. *Trichoderma reesei* is stable at temperatures less than 50^{o}C, has an optimum temperature of 50-60^{o}C, is stable at pH 3.5-6.5. *Bacillus subtilis* is stable at temperatures less than 50 ^{o}C, has an optimum temperature of 30-55^{o}C, is stable at pH 5-7 [6] xylanase has an essential role in the industrial world, such as the paper, pharmaceutical, feed and food industries [7]. The use of enzymes in the free form is expensive because enzymes are easily damaged and difficult to separate from the reaction mixture so that they cannot be used repeatedly [8]. The work of the enzyme is influenced by several factors, namely the formulation of the media for growth and the production of fermentation, which is an essential step in designing experiments on a laboratory scale. Efforts to find substrates for enzyme production continue, in addition to the use of hemicellulose waste [9]. Substrate temperature, pH, cofactors and inhibitors [5]. One way to predict optimal enzyme working factors requires technology in the form of bioinformatics which reduces the cost of experiments on a laboratory scale.

Bioinformatics is a combination of biology and informatics engineering or computer science [10]. Along with the rapid development of molecular biology, data on the results of wet-lab experiments have become increasingly abundant. So, we need a piece of new knowledge to process this data into useful information [11].

Bioinformatics is useful for solving molecular biology problems computationally. The issues that arise can be in the form of basic things such as solving enzyme mechanisms, protein metabolism or identifying microbes. In this problem, the data obtained needs to be processed with a unique approach to becoming useful information [10]. This information can generally be stored in a database, which can be accessed using a computer and an internet network. So that research on microorganisms such as *Aspergillus niger*, *Bacillus subtilis*, and *Trichoderma reesei* in the field of bioinformatics can be carried out only by using standard computers, internet networks and sufficient knowledge of bioinformatics. This process reduces the unnecessary steps of the wet experiment, which helps to save costs and uses technology that makes it easier to carry out results with higher accuracy.

This research will characterize the work factors of the xylanase enzyme, which is optimum for hemicellulose. Furthermore, after obtaining the optimum data, the data will be used for wet-lab experiments. Molecular docking is part of computational chemistry. This computational chemistry has the advantage of being safe, free from chemical waste, easy, cost-effective and can shorten research time [12]. Molecular docking is a method used to find the exact orientation of one or more molecules at the active site of a protein. In other words, molecular docking is the study of how two or more molecules, such as ligands and proteins whose interactions match [13].

Xylanase enzyme is a biocatalyst for the hydrolysis of xylan (hemicellulose) into reducing sugars. Xylan is a xylose polymer with -1,4 bonds with 30-100 monomer units [14]. The use of xylanase enzymes in the industrial world has a very important role, for example in

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paper making, xylose sugar production, food and beverage production, animal feed production, bread quality improvement, water absorption and biofuel production [15].

Xylanase enzymes can be produced by several microorganisms such as: *Aspergillus niger, Bacillus subtilis, Trichoderma reesei, Cryptococcus, Penicillium, Aureo-basidium, Fusarium, Rhizomucor, Humicola* [16]. All microorganisms require a medium containing a carbon source for their growth. Xylanase-producing microorganisms require xylan as a carbon source. Because xylan has an expensive price, the use of pure compounds directly in the production media requires quite a high cost.

EXPERIMENT

Chemicals and instrumentation

Aspergillus niger; Bacillus subtilis and *Trichoderma reesei* database and D-xylose database. The variables used in this study are Ligands and microorganisms.

Bioinformatics Stage

The database of enzymes and substrates have been downloaded. The enzyme database was obtained from the RCSB Protein Data Bank (https://www.rcsb.org/). The substrate or lignin database was obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/). And then modify proteins or enzymes with the PyMol program. At this stage, the cleaning of the enzyme sequence was carried out to produce an enzyme with a pure sequence.

then modelled using a homology modelling approach with the SWISS-MODEL webservice. 3D models of glucose substrate were obtained from the PubChem database. Structures in the SDF format were saved and used for further analysis. To find out the binding position of the enzyme and substrate so that the mechanism of action is known, a molecular docking study is performed. The interaction simulation between enzymes and substrate was studied using the Molecular Docking PyRx 0.8 program. The docking process uses autodock vina and is carried out specifically on the active site of the target protein. Molecular docking can predict the binding affinity of a compound to a specific target protein [17]. The higher the binding affinity of the substrate, the more accurate the interaction prediction will be. The molecular docking process is also based on control of the ligands that have previously interacted. Interactions between substrates and enzymes were analyzed with the Discovery Studio V.4.0 program. The purpose of this analysis is to understand the types of amino acids involved in interactions, so it can be concluded the active side and mechanism of action of the enzyme [17-18].

RESULT AND DISCUSSION

Xylanase preparation

Lignocellulose is biomass consisting of cellulose (40–60%), hemicellulose (20–40%), and lignin (10–30%). Endo-B-1,4-xylanase is the primary carbohydrate polymer in lignocellulosic biomass, which is converted into fermentable sugars for energy production. Xylan is the second most abundant component of hemicellulose in nature. Endoxylanase (EXN) hydrolyzes xylan to a xylooligosaccharide mixture. One of the stages of the enzyme production process is pretreatment; the pretreatment process is to modify the structure of lignocellulose by removing lignin and changing the structure of cellulose and hemicellulose [19].

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Following the development of bioinformatics technology, this software can predict the interaction of ligands with amino acid proteins. Bioinformatics can also be used to study hemicellulose monomers (D-xylose) with microorganisms (*A. niger*, *B. subtilis*, and *T. reesei.*). This program is to reduce time and costs in wet lab research; this study uses a simulation of the interaction between macromolecules (ligands) and macromolecule (protein) targets called molecular docking.

According to [20] the database of the xylanase enzyme was obtained from the RCSB PDB (Research Collaboratory for Structural Bioinformatics Protein Data Bank). Xylanase structure from *Aspergillus niger*, xylanase from *Bacillus subtilis*, and xylanase from *Trichoderma reesei* with PDB ID of (1UKR), (1IGO) and (1QOZ) can be seen ini Figure 1.

Figure 1. Xylanase from *Aspergillus niger*, *Bacillus subtilis*, and *Tricodherma reesei* (Source : Data Bank RCSB PDB)

The database of the substrate or xylan was obtained from different data banks [20] obtained the xylan database from PubChem with ID 125409, the NMR database used is one of the constituent structures of D-xylose (Figure 2.).

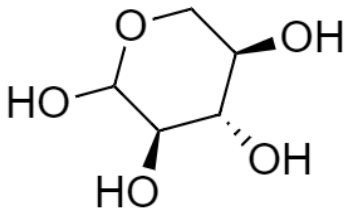

Figure 2. Structure of D-xylose

The docking process involves a large molecule (protein) with a small molecule (ligand). In general, this method is called a lock and key, which means that during the docking process, the ligand is positioned in a cavity on the surface of the protein [21]. Research on thermostable enzymes or proteins can provide new knowledge about the factors that determine the thermal stability of enzymes. An understanding of the determinants of the thermal stability of a protein or enzyme can be used as a reference for engineering proteins and avoiding the mistargeting of mutations that can have fatal consequences for enzyme stability and activity.

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Amino Acid Interaction in the PyMol Program

The first step in the docking stage uses PyMOL software, which is to modify the protein to get a pure sequence and remove other compounds such as water and other metals. This modification is to alleviate the ligand-protein interactions. PyMOL software can also visualize chemical compounds in 3D from a micromolecule or macromolecule, while for protein-ligand docking interactions, Autodock Vina is integrated into PyRx. In the simulation carried out between D-xylose and *Aspergillus niger* can be seen in Figure 3.

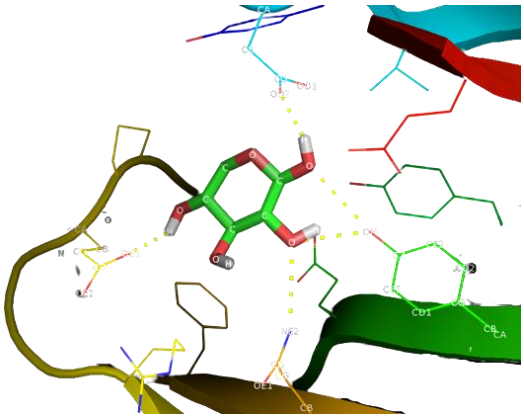

Figure 3. 3D Visualization of D-xylose with Amino Acid Residues of xylanase from *Aspergillus Niger*

Binding Affinity Value and RMSD Value

The best docking simulation results are based on the lowest binding affinity of -5.1 kcal/mol and supported by the lowest RMSD upper bound and lower-bound values, namely the RMSD value of 0. This binding affinity value shows that the more negative the binding affinity value is, the more binding interaction between proteins ligands getting bigger. In the relationship between binding affinity and RMSD, the RMSD value depends on the interaction of molecular bonds and the bond energy between proteins and ligands, so RMSD is used to measure the quality of the conformational interactions of the molecules. However, the RMSD value cannot be used as the best evaluation of the molecular conformation model but only reflects the similarity of the sequence [22]. RMSD upper bound matches each atom in one conformation with itself in another conformation, while for RMSD, the lower bound is matching each atom in one conformation with the nearest atom of the same type of element in another conformation.

In the docking simulation, the Ligplot program is used to visualize the active side interactions of the interactions between ligands and proteins that occur. Some symbols on the Ligplot to facilitate analysis of the interactions that occur between D-xylose and *Aspergillus niger* can be seen in Figure 4.

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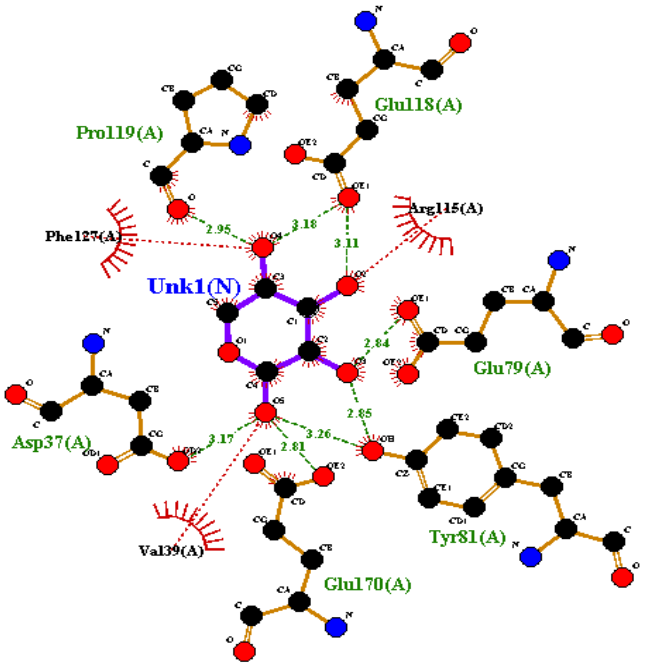

Figure 4. Visualization of interaction of D-xylose with Amino Acid Residues of xylanase from *Aspergillus Niger*

Interaction of D-xylose with xylanase

The green dotted line indicates the hydrogen bond, the green dotted number indicates the bond strength, the red half-sun curve indicates the brown bond line indicates the hydrophobic bond, the amino acid residue that binds to the hydrogen atom, the bond line blue indicates ligands, blue circle symbol indicates nitrogen atom, red circle symbol indicates oxygen atom, black circle symbol indicates carbon atom.

Hydrogen bonding is an intermolecular attraction that occurs between the interactions of hydrogen atoms (covalent) that are bonded to highly electronegative atoms (N, O, or F) and the lone pairs of other highly electronegative atoms. This bond appears as the N-H, O-H, and F-H bonds are very polar, where the positive partial charge on H and the negative partial charge on the electronegative atom (N, O, or F) [23]. The ligand-receptor interaction has a maximum distance of 5 Å if more than this maximum distance is not analyzed because it indicates that it does not affect the ligand-receptor interaction. The closer the hydrogen bond is, the stronger the bond energy is formed; this distance is divided into three types, namely strong at 2.2 - 2.5 Å, medium for 2.5 - 3.2 Å, weak at 3.2 - 4 Å [24].

In the interaction of *Aspergillus niger* with D-xylose, there are ligands and amino acid residues; the ligand atoms are marked with a blue bond while the amino acid atom is marked with a brown bond. The LigPlot analysis provides data on the interaction of hydrogen bond groups with green dotted lines that occur in D-xylose, which corresponds to the amino acid residue, namely glutamate (Glu118), the O_2 group interacts with D-xylose on the OH group on the 4 side of medium strength with an interaction length of 3.11 Å and in glutamate (Glu118) the O_2 group interacts with D-xylose on the OH group 2 side medium with an interaction length of 3.18 Å, glutamate (Glu79) the O_2 group interacts with D-xylose on the OH group 3 side medium with an interaction length of 2.84 Å, tyrosine (Tyr81) OH group interacts with D-xylose on the OH group on side 3 medium strength with an interaction length of 2.85 Å on and

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tyrosine (Tyr81) OH group interacts with D-xylose on the OH group on the weak side 5 with an interaction length of 3.26 Å, at glutamate (Glu170) OH group interacts with D-xylose on the OH group 5 side medium strength with an interaction length of 2.81 Å, aspartate (Asp37) group us OH interacts with D-xylose on the OH group of side 5 medium strength with an interaction length of 3.17 Å, of all these amino acids are polar so that it is easy to interact with these ligands. In contrast to the amino acid proline (Pro119), the O_2 group interacts with D-xylose on the 4-side OH group, which is non-polar with medium strength with an interaction length of 2.95 Å. These amino acid residues are very helpful for determining the catalytic activity of enzymes (Nauli, 2014). The hydrophobic bond is represented by the red half of the sun, namely Arginine (Arg115) interacts with the 2-side OH group, Phenylalanine (Phe127) interacts with the 4-side OH group, and Valine (Val39) interacts with the 5-side OH group, of which the three amino acids are. Non-polar so that it is not easy to interact with other groups [25]. From Figure 4, it can be seen in Table 1.

Table 1. Results of Hydrogen and Hydrophobic Interactions from *Aspergillus niger* Docking

Microorganisms	Enzyme	Hydrogen Interaction			Hydrophobic interaction	
		Quantity	length (Å)	Amino Acid Residue	Quantity	Amino Acid Residue
Hemiselulosa-*Aspergillus niger*	Xilanase	2	3.18	Glu118	1	Arg115
			3.11		1	Phe127
		1	2.95	Pro119	1	Val39
		1	2.84	Glu79		
		2	3.26 2.85	Tyr81		
		1	2.81	Glu170		
		1	3.17	Asp37		

Based on table 1, the hydrogen interactions that occur in the *Aspergillus niger* microorganism with D-xylose, there are six amino acid residues (Aspartate37, Glutamate118, Glutamate170, Glutamate79, Prolin119, Tyrosin81) with different interaction distances, the average interaction strength of amino acid residues moderate strength. The closest hydrogen interaction distance is found in the amino acid Tyrosin81 residue with a distance of 2.81 Å. While the hydrophobic interactions that occur in the *Aspergillus niger* microorganism with D-xylose, there are three interactions of amino acid residues (Arginin 115, Phenilalanin127, Valin39). In the simulation carried out between D-xylose and *Bacillus subtilis* can be seen in Figure 5.

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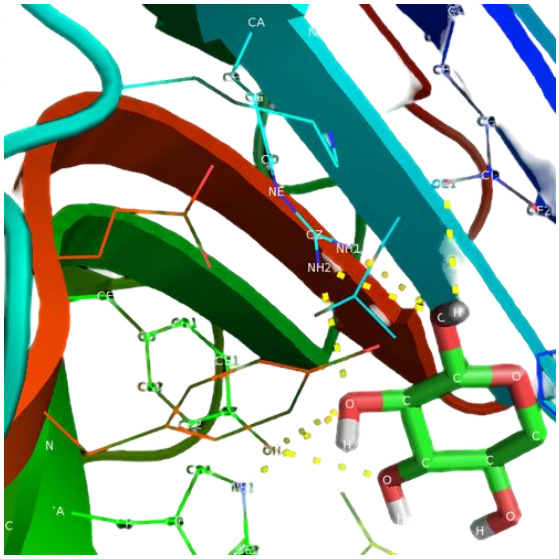

Figure 5. 3D Visualization of D-xylose with Amino Acid Residues of xylanase from *Bacillus subtilis*

In the docking results, data binding affinity is obtained and the RMSD value can be seen in table 2.

Table 2. Results of *Bacillus subtilis* Docking

Ligan	Binding Affinity(kcal/mol)	Rmsd upper Bound	Rmsd lower Bound
D-xylose - Bacillus Subtilis	-5.2	0	0
	-5	2.053	1.073
	-5	2.975	1.417
	-4.9	7.63	6.634
	-4.9	6.704	5.093
	-4.9	5.348	4.324
	-4.9	11.109	9.491
	-4.7	3.28	1.438

Based on Table 2, the best docking simulation results are shown based on the lowest binding affinity of -5.2 kcal/mol and supported by the lowest RMSD upper bound and lower-bound values, namely the RMSD value of 0. This binding affinity value shows that the more negative the binding affinity is, the more binding interaction between proteins ligands getting bigger. In the Ligplot + simulation to visualize the active side interactions of the ligand-protein interactions that occur. The following is the interaction that occurs between D-xylose and *Bacillus subtilis* can be seen in Figure 6.

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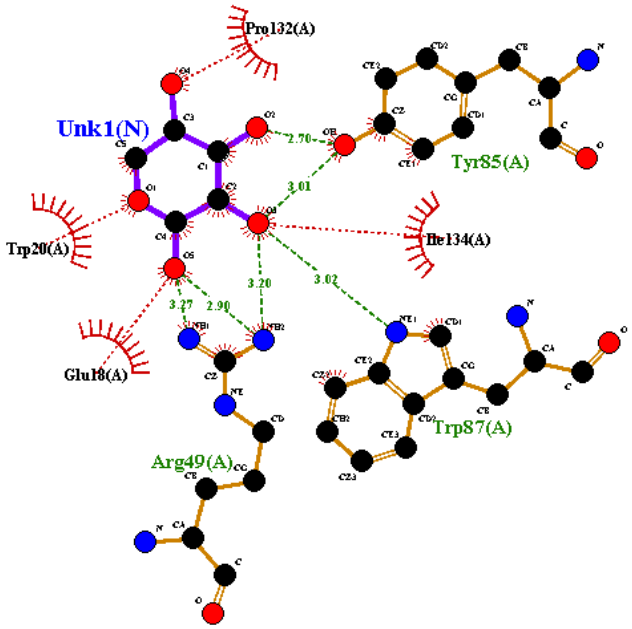

Figure 6. Visualization of interaction of LigPlot with Amino Acid Residues from *Bacillus subtilis*

In the interaction between *Bacillus subtilis* and D-xylose, there are ligands and amino acid residues, and the ligand atoms are marked with blue bonds. In contrast, the amino acid atoms are marked with brown bonds. The LigPlot analysis provides data on the interaction of hydrogen bond groups with green dotted lines that occur in D-xylose, which corresponds to the amino acid residue, namely tyrosine (Tyr85), the OH group interacts with D-xylose on the OH group 2 side, which is polar, so it is easy to interact with medium strength and 2.70 Å interaction length and tyrosine (Tyr85) OH group interacts with D-xylose on OH group 3 side medium with 3.01 Å interaction length, while tryptophan (Trp87) NH group interacts with D-xylose on OH group 3 side is wrong one non-polar amino acid with medium strength with a long interaction length of 3.02 Å, Arginine (Arg49) NH group interacts with D-xylose on the OH group of medium strength with an interaction length of 2.90 Å, while Arginine (Arg49) NH2 group interacts with D -xylose on the OH group on the 5-side weak strength with an interaction length of 3.27 Å and Arginine (Arg49) the NH group interacting with D-xylose on the OH group on side 3 is weak with an interaction length of 3.20 Å. These amino acid residues are very helpful for determining the catalytic activity of enzymes [26]. For hydrophobic bonds with the red half-sun symbol, Proline (Pro132) interacts with the 4-side OH group, Isoleucine (Ile134) interacts with the 3-side OH group, Tryptophan (Trp20) binds to the 1-side OH group, and Glutamate (Glu18) interacts with the OH group OH side 5 of the four acids is non-polar, so it is not easy to interact with other groups [25]. From Figure 5, it can be seen in Table 3.

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Table 3. Hydrogen and Hydrophobic interaction between D-xylose and xylanase from *Bacillus subtilis*

Microorganism	Enzyme	Hydrogen Bonding			Hydrophobic Interaction	
		Quantity	Length (Å)	Amino acid Residue	Quantity	Amino acid Residue
Hemiselulosa-*Bacillus subtilis*	Xilanase	2	2.70	Tyr85	1	Pro132
			3.01		1	Ile134
		1	3.02	Trp87	1	Trp20
			3.27	Arg49	1	Glu18
		3	3.20			
			2.90			

Based on table 3, the hydrogen interactions that occur in the microorganism *Bacillus subtilis* with D-xylose, there are three amino acid residues (Tyrosin85, Tryptophan87, Arginin49) with different interaction distances, the average interaction strength of the amino acid residues is of moderate strength. The closest hydrogen interaction distance is found in the amino acid Tyrosin85 residue with 2.70 Å. While the hydrophobic interactions that occur in the microorganisms *Bacillus subtilis* with D-xylose, there are four interactions of amino acid residues (Glutamate18, Isoleucine134, Prolin132, Tryptophan20, Phenilalanin127,).

In the simulation conducted between D-xylose and Tricodherma, reesei can be seen in Figure 7.

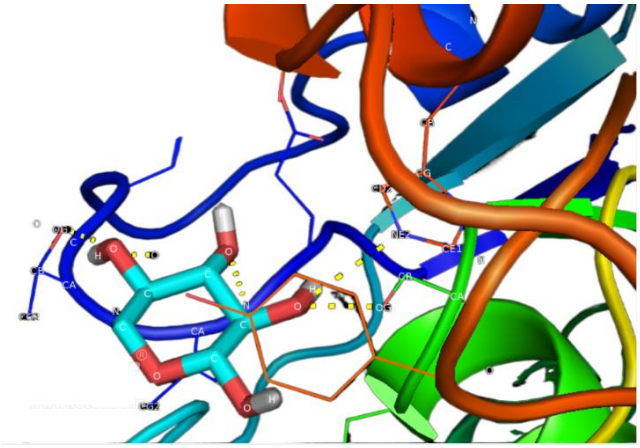

Figure 7. 3D Visualization of D-xylose with Amino Acid Residues of xylanase from *Tricodherma Reseei*

Where from the docking results, data binding affinity is obtained and the RMSD value. the same docking simulation data is obtained, which is shown at the binding affinity value of -4.3 kcal/mol, but the RMSD value is different. However, the lowest RMSD data is the RMSD value of 0, which occurs in the first data with a binding affinity value of -4.3 kcal/mol with a super bond and lower bond RMSD of 0. This binding affinity value shows that the more negative the value of the binding interaction between protein-ligands. The greater it is in the Ligplot + simulation to visualize the active side interactions of the ligand-protein interactions

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that occur. The following is the interaction that occurs between D-xylose, and Tricodhema reesei can be seen in Figure 8.

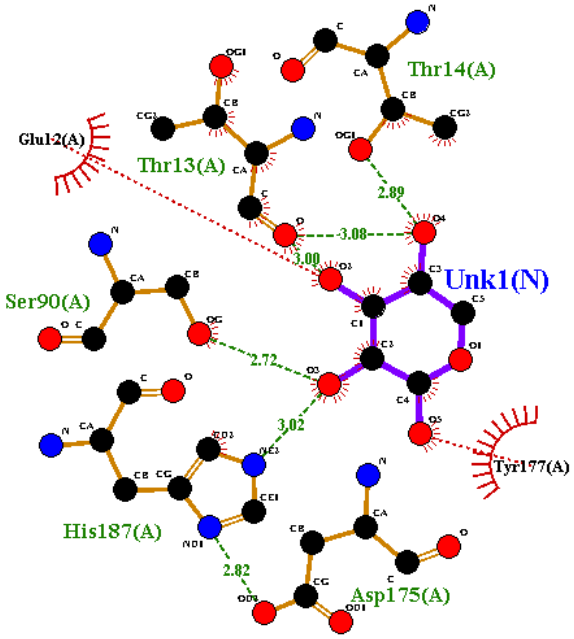

Figure 8. Visualization of LigPlot with Amino Acid Residues from *Tricodherma reseei*

In the Tricodherma Reseei interaction with D-xylose, there are ligands and amino acid residues, and the ligand atoms are marked with blue bonds. In contrast, the amino acid atoms are marked with brown bonds. The LigPlot analysis provides data on the interaction of hydrogen bond groups with green dotted lines that occur in D-xylose, which corresponds to the amino acid residue, namely threonine (Thr13), the O2 group interacts with D-xylose on the OH group on the two sides of medium strength with an interaction length of 3 Å and (Thr13) the O2 group interacts with D-xylose on the 4-side OH group medium strength with an interaction length of 3.08 Å, threonine (Thr14) OH group interacts with D-xylose on the 4-side OH group medium strength with an interaction length of 2.89 Å, serine (Ser90) OH interacts with D-xylose on the 3-side OH group of medium strength with an interaction length of 2.72 Å, the histidine (His187) NH group interacts with D-xylose on the medium 3-side OH group with an interaction length of 3.02 Å and the histidine (His187) group N interacts with OH groups with medium strength aspartic acid (Asp175) residues with an interaction length of 2.82 Å. Of all the amino acids are polar, so it is easy to interact with these ligands. These amino acid residues are very helpful for determining the catalytic activity of enzymes [26]. For hydrophobic bonds with a red half-sun symbol, Glutamate (Glu12) binds to the OH group 3 sides, and Tyrosine (Tyr177) binds to the five side OH groups. From Figure 5, it can be seen in Table 4.

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Table 4. Results of Hydrogen and Hydrophobic Interactions on *Tricodherma reesei*

Microorganism	Enzyme	Hydrogen interaction			Hydrophobic interaction	
		Amount	Length (Å)	Amino Acid Residue	Amount	Amino Acid Residue
Hemiselulosa-*Tricodherma reesei*	Xilanase	2	3	Thr13	1	Glu12
			3.08		1	Tyr177
		1	2.89	Thr14		
		1	2.72	Ser90		
		1	3.26	His187		

Based on table 4, the hydrogen interactions that occur in the microorganism Tricodherma reesei with D-xylose, there are three amino acid residues (Histidine187, Serin90, Tryptophan87) with different interaction distances, the average interaction strength of the amino acid residues is of medium strength. The hydrogen interaction distance is closest to the amino acid residue Serin90 with a distance of 2.72 Å. While the hydrophobic interactions that occur in the microorganism Tricodherma reesei with D-xylose, there are two interactions of amino acid residues (Glutamate12 and Tyrosin177).

Based on the results of the docking data from the three microorganisms, the interaction between *Tricodherma Reesei* and D-xylose has a Gibbs free energy of -4.3 kcal/mol. *Aspergillus niger* with D-xylose has a Gibbs free energy value of -5.1 kcal/mol. Meanwhile, *Bacillus subtilis* docking data with D-xylose has Gibbs free energy of -5.2 kcal/mol. Based on the results of the docking between the ligand and enzyme's obtained from the conformation of the ligand with the smallest energy. Binding affinity is a measure of the enzyme's ability to bind to the substrate. The smaller the binding affinity value, the higher the affinity between the receptor and the ligand and vice versa if the greater the binding affinity value, the lower the affinity between the receptors [27]. Based on these data, the best interaction was shown by *Bacillus subtilis*. The molecular conformation of *Bacillus subtilis* can show the active side position of the enzyme because of the broad bond energy of the ligands to the protein and the closest hydrogen interaction distance to the amino acid residue occurs at Tyrosin81 with a distance of 2.70 Å.

Docking Validation

Redocking is a docking validation method to test the validity of the value generated from the initial docking. This validation uses the initial protein-ligand docking results with the D-xylose ligand; the results from the initial docking with the redocking results will be compared. Green ligands are the initial docking ligands, while magenta ligands are the result of redocking. Redocking is carried out using the comprehensive docking method because this method is used when the position of the active site of the enzyme is not known [28]. The parameters used in redocking use the RMSD (Root Mean Square Deviation) value, which can measure the magnitude of the deviation value in protein-ligand interactions with an average value of 1.5 to 2 Å. In the table, the initial interaction distance between docking and redocking has a change value but not too significant; the redocking ligand has a good position because the initial

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docking and redocking RMSD values are 0, so the ligand position is not too far from the initial docking ligand [24].

Table 5. *Bacillus* subtilis Docking Results

Ligan	Binding Affinity(kcal/mol)	RMSD upper Bound	RMSD lower Bound
D-xylose -Bacillus Subtilis	-5.2	0	0
	-5	3.197	0.664
	-5	1.948	1.24
	-4.9	3.611	1.304
	-4.9	2.797	1.164
	-4.5	9.81	8.197
	-4.5	10.986	9.402
	-4.5	3.605	2.582
	-4.4	4.302	2.515

Based on the results of research on microorganisms that have a strong interaction with D-xylose. Besides, to ensure that the bioinformatics results are proven by the results of a wet lab experiment, it can be seen in the table below.

Table 6. Value of Enzyme Activity

Xylanase source	Media	Enzyme activity (U/ml)	Source
Aspergillus niger	Rice husks	3.541	(Soliman, 2012)
	Rice straw	0.055	(Pangesti, 2012)
Bacillus subtilis	Rice husks	6.088	(Ardiansya,dkk 2014)
	Corn husks	0.2	(Erika, 2016)
Tricodherma reesei	Corn husks	1.210	(Mardiana H., dkk., 2014)
	Corn husks	2.973	(Sukmana, 2014)

In the table above, the most extensive enzyme activity data occurs in *Bacillus subtilis* compared to *Aspergillus niger* and *Trichoderma reesei*.

CONCLUSION

The effect of the interactions that occur is the effect of hydrophobic bonds that plays a role in stabilizing the interaction between ligands and proteins where the most hydrophobic bonds are found in *Bacillus subtilis* with D-xylose has a binding affinity value of -5.2 kcal/mol. This research can be developed further by comparing the results of insilico predictions with the results of research on a laboratory scale.

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