

MISKOLCI EGYETEM

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**MIKROORGANIZMUSOK ALKALMAZÁSA POLIURETÁN
BIOLÓGIAI LEBONTÁSÁBAN**

**APPLICATION OF MICROORGANISMS IN THE
BIODEGRADATION OF POLYURETHANE**

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1. Introduction

Polyurethane (PU) and other plastics play an important role in our daily lives. PU foam is used in a wide variety of products, which leads to accumulation of PU as waste in our environment (e.g., water, soil) [1]. There are many strategies for waste management, one of this is biodegradation, however, is relatively seldom considered. The advantage of biodegradation is that it does not require large energy investment and the plastic could be fully mineralized in the process. The disadvantages are toxic compounds produced potentially during degradation and the polymeric product could degrade earlier than it is supposed to [2]. Several studies have investigated the degradability of polyurethanes using microorganisms. In general, microorganisms are involved in the biodegradation of different polymers, including polyurethane. The most studied bacteria in the biodegradation of polyurethane belong to the families of *Pseudomonadaceae*, *Enterococcaceae*, *Corynebacteriaceae*, and *Comamonadaceae* [3]. The degradation capabilities of microorganisms are result of a prolonged period of adaptation to the polymers as energy source. To use polyurethane as an energy source, microorganisms need special enzymes. Three main types of enzymes are mentioned in the literature: lipase, urease, and esterase, of which the latter is considered to play a major role. In our research all the available bacterial esterase sequences will be collected and the homology between them will be determined using the BLASTp and MSA (Multi Sequence Alignment) programs. Then, those which has a 3D structure will be selected and used as host molecules for molecular docking. The enzyme structures will be prepared using standard protocols (e.g., adding hydrogens). At the same time, 3D structures of model urethanes will be prepared using the GaussView 6.0 software and optimized by the Gaussian 09 software package at the B3LYP/6-31G(d,p) level of theory and these will be used as ligands during molecular docking calculations. After the optimization of the ligand structures, they will be ready to be docked to the enzymes. The interactions between the enzymes and model urethanes will be determined and compared by using the AutoDock Vina software. The different ligand-esterase interactions will be assessed and quantified.

1.1 Objectives

The aim of the research was to investigate the theoretical degradation of polyurethane foam as a waste, for which the following subtasks were planned.

1. Identifying possible degradative enzymes based on literature.
2. Sequence homology search using BLASTp and MSA programs.
3. Identifying possible docking sites.
4. Construction of model urethane molecules using GaussView 6.0 software and docking of selected enzymes to possible docking sites using AutoDock Vina program.

2. Literature

2.1 Production of polyurethane foams

Polymers that contain several urethane units (**Figure 1**) independently of the rest of the molecule are called polyurethanes [4], [5]. Urethane groups were first synthesized by Otto Bayer in 1936.

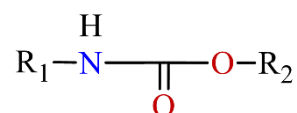


Figure 1 Urethane unit

Polyurethanes are macromolecular substances formed by the polyaddition reaction of di- or polyisocyanates with diols or polyols (**Figure 2**). The foam structure is formed by the carbon dioxide produced during the reaction when water or carboxylic acid is present in the mixture [5].

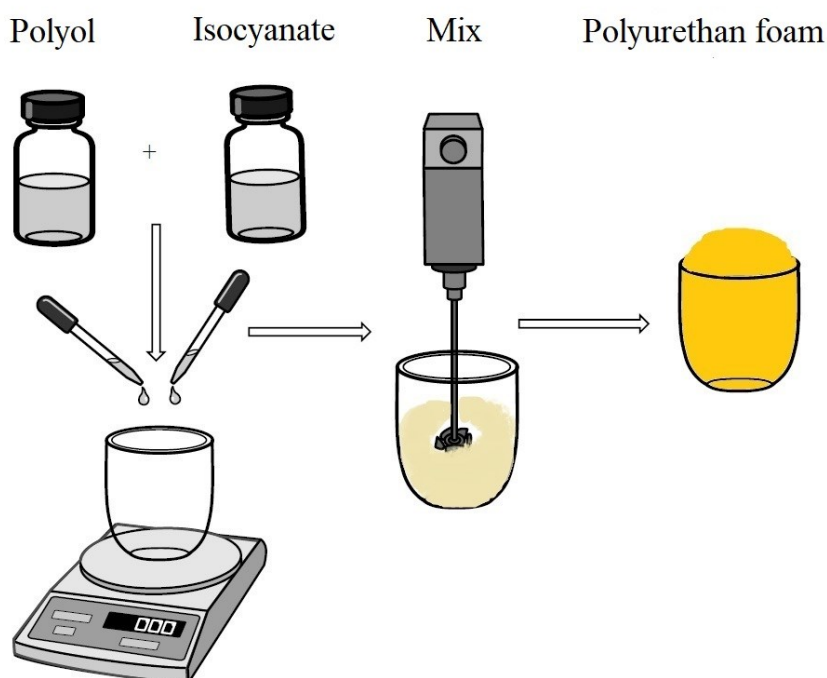


Figure 2 Polyurethane synthesis in laboratory.

PU foams can be divided into several groups according to their mechanical properties. The first group are the flexible foams, which are characterised by a sparse cross-linked structure, reversible deformability and low resistance to compressive stress. The next group is the integral foams, which have the special feature that no special protective

layer is required, since the protective layer is formed during production. They are characterised by low thermal conductivity, good mechanical and chemical properties, and wear resistance. Rigid foams have a closed cell structure, good adhesion, good mechanical properties and good thermal conductivity. Other polyurethanes used in industry include elastomers, fiber materials, film formers and adhesives [6].

2.2 Polyurethane degradation

Biological breakdown is influenced by two main factors, abiotic and biotic factors. Abiotic factors are sunlight, temperature, and air. In abiotic media, sunlight can induce decomposition through photolysis and oxidation, while water and chemical contaminants can trigger hydrolysis [7].

Biotic degradation can be divided into three parts, such as biological attack, fragmentation and finally mineralization. Biological attack represents the beginning of material decomposition where the structure of the material does not deteriorate, but different chemical reactions start [8]. In biofragmentation, microorganisms release larger amounts of enzymes that catalyse the degradation process. Fission of higher molecular weight units starts. Mineralisation involves the production of biomass, carbon dioxide and water, but mineral components can also include various inorganic gases and ammonium salts [9].

2.2.1 Polyurethane degradation with fungi

It was found that different properties, such as crystallinity, molecular orientation or the side chains attached to the molecule, have a strong influence on the enzymatic degradability of synthetic polymers. [10] Furthermore, if the chains of synthetic polymers show more repeats, the polymer chains could be clustered which results the formation of regular crystalline regions. These regular repeats limit the accessibility of the polymer chains to microbial enzymes. It was revealed that amorphous regions of polyurethane are more easily degraded by fungi, and that the bonds of amorphous regions are attacked first, followed by those of regular regions [11]. In addition, polyester PU is more susceptible to degradation by fungi [5]. Studies have identified several possible fungal species that exhibited polyurethane degradation properties - *Curvularia senegalensis*, *Fusarium solani*, *Aureobasidium pullulans* and *Cladosporium* sp. Within these fungi, *Curvularia senegalensis* showed the highest degradation activity. In the case of fungi, *endo* enzymes

were isolated, which randomly hydrolyze the polyurethane, so that the degradation results in a reduction in tensile strength and a minimal loss of mass. Besides *exo* enzymes have been isolated as well, which remove monomeric units from the end of the chain, but do not cause a large difference in tensile strength [12].

2.2.2 Polyurethane degradation with bacteria

By adding autoclaved 2x2 cm pieces of 250 mg PU film as sole carbon source to a minimal salt medium and inoculating it with soil samples, researchers were able to isolate and identify *Pseudomonas aeruginosa* MZA-85 strain as an organism capable to degrade PU. The cell number of the PU film containing media was compared with the cell number of the control sample using photometry at 600 nm. PU degradation enzyme activity was measured by addition of *p*-nitriphenol to the supernatants. Compounds resulting from degradation process were analyzed by GC-MS, FT-IR, and Strum test methods [13]. It was revealed that the isolated *Pseudomonas aeruginosa* MZA-85 strain could not only utilize polyurethane as a carbon source by breaking ester bonds, but also utilizes the intermediate products as a carbon source. Carbon utilization was verified by the CO₂ Strum test. The bacterial strain degraded the aliphatic part of polyurethane more efficiently than the aromatic part, which resulted the formation of adipic acid and 1,4-butanediol [14]. It was found that esterases interact with the polymer nearby the aliphatic ester bonds, and consequently it is assumed that this is the starting point for polyurethane chain degradation. In the supernatant of the reaction media, there were no compounds that indicated the degradation of polyisocyanate [13].

The degradation of polyester polyurethane foam waste with *Pseudomonas chlororaphis* bacterial strain ATCC55729 was also investigated. The foam was cut into 0.5 x 0.5 x 0.5 cm³ pieces, washed, then dried at 90°C to constant weight. A portion of the sample (1 g) was autoclaved and added to 200 ml of culture medium than inoculated with 0.1 ml of bacterial suspension [15]. Flasks were placed in a shaking incubator and the digestion experiment was carried out with constant shaking at 150 rpm at 30 °C. Enzyme activity was detected from supernatants by *p*-nitriphenol spectrophotometrically at 405 nm. Colony count at the start of the experiment was determined at 2×10^9 cfu ml⁻¹ and then stagnation was observed, since the cell number of 5×10^7 cfu ml⁻¹ remained until the end of the test [15]. This indicates that *Pseudomonas chlororaphis* ATCC55729 might use the tested foam as energy source, but probably this substrate is not adequate for cell

proliferation. The other explanation could be, that substances from the degradation may have toxic effect on *Pseudomonas chlororaphis* ATCC55729 strain, and therefore no increase in colony number was observed during degradation experiment.

Some polyester polyurethane products tend to degrade in the presence of yeast extract in the incubation medium. Two microorganisms, *Corynebacterium sp.* and *Pseudomonas aeruginosa* were isolated as responsible species for this kind of polymer degradation. However, in experiments, without yeast extract, the used microorganisms did not grow, in physical tests, the decrease in tensile strength of the PU substrate was observed. ATR-FTIR (Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy) was used to investigate the degradation products, and it was found that the ester segments of the carbonyl group were hydrolysed [16]. Esterase activity was measured from the supernatant of the experimental medium, where the release of *p*-nitrophenol was monitored spectrophotometrically at 405 nm. This study also indicates that the enzyme produced by the microbes are extracellular proteins [17].

Comamonas acidovorans TB-35 strain, isolated from a solid polyurethane (PU) product was also investigated in PU degrading experiments. PU cube (2 x 2 x 1 mm³) containing reaction mixture was incubated for 7 days together with the isolated bacteria, and it was found that the isolated strain degraded 48% of the PU cube samples. It was also determined that *Comamonas acidovorans* TB-35 strain has two extracellular esterase enzymes. One of the enzymes is secreted out of the cell (into the reaction medium), while the other enzyme is bound to the cell membrane. The latter enzyme was shown to degrade the polyurethane. The amount of free diethylene glycol, as product of degradation was measured, and it was shown that while amount of diethylene glycol increased (0.5 mg), the mass of PU decreased (1.2 mg) [18].

The possible way of polyurethane biodegradation by fungi and bacteria is intensively studied and until now three different polyurethanases were described. One of these enzymes is PudA (polyurethanase-depolymerase). The PudA enzyme acts in two steps, first, it adheres to the surface of the polymer and after that it hydrolyses the ester bond [19]. In case of *Pseudomonas chlororaphis*, two other enzymes were identified which are possibly involved in the degradation of PU. One of these is encoded by the *pueA* (polyurethane-esterase A) gene and the other by the *pueB* (polyurethane-esterase B) gene. The PueA and PueB proteins have similarities with the extracellular lipases of *Pseudomonas fluorescens* and *Serratia marcescens* [20].

According to another study, PU-esterases have been distinguished according to their appearance in the cell. One of the PU-esterases is the membrane-bound enzyme, while the other is considered to be extracellular protein. These two types of enzymes have different roles in degradation. The membrane-bound enzyme makes the PU substrate accessible for degradation, while the extracellular one ensures its binding to the polymer surface and then hydrolyses the ester bond [5].

As we can see from the presented literatures, polyurethane degradation capacity of bacteria were demonstrated for several different strain including *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Corynebacterium sp.*, *Comamonas acidovorans*, and *Serratia marcescens* [17], [20]–[23] (**Table 1**).

Table 1 Microbial degradation of polyurethane.

Microorganism	Enzyme	Polymer type
<i>Pseudomonas aeruginosa</i>	esterase	polyester- PU
<i>Pseudomonas fluorescens</i>	esterase	PU
<i>Corynebacterium sp.</i>	esterase	PU
<i>Comamonas acidovorans</i>	esterase	polyester- PU
<i>Serratia marcescens</i>	esterase	PU

2.4 Enzymatic degradation of polyurethane

The enzymatic degradation of a polymers consists of three main steps:

1. Enzyme release from the cell.
2. Enzyme adherence to the polymer surface.
3. Diffusion of degradation products into the solution [24].

Enzymatic degradation is strongly influenced by the macromolecular composition of the polymer, and by abiotic and biotic factors. Together, these effects factors contribute to the fragmentation of polymer. The shredded plastic can be utilized by microorganisms thanks to their enzymes [25]. There is a wide literature on microbial enzymatic degradation of polymer molecules, and in case of PU degradation it is considered that hydrolases, namely esterases, ureases and proteases play the major role.

2.4.1 Biodegradation with esterases

The role of esterases in the degradation of polyurethane is mentioned in several literatures [20], [26]–[28], [29]. The esterases degrade polyester polyurethanes by hydrolysis, and in these reactions carboxylic acid or alcohol is produced, which can be easily detected by HPLC (**Figure 3**). Similarly, the degradation of the urethane bond produces carbamic acid and alcohol. Cregut and co-workers described a putative mechanism of bacterial polyurethane degradation, where first an urethanase type enzyme is activated, followed by an esterase-type enzyme [30]. The urethanase enzyme cleaves monomers from the surface of the polymer, while the esterase cleaves the polymer molecule along the ester bonds [30] (**Figure 3 (A)**).

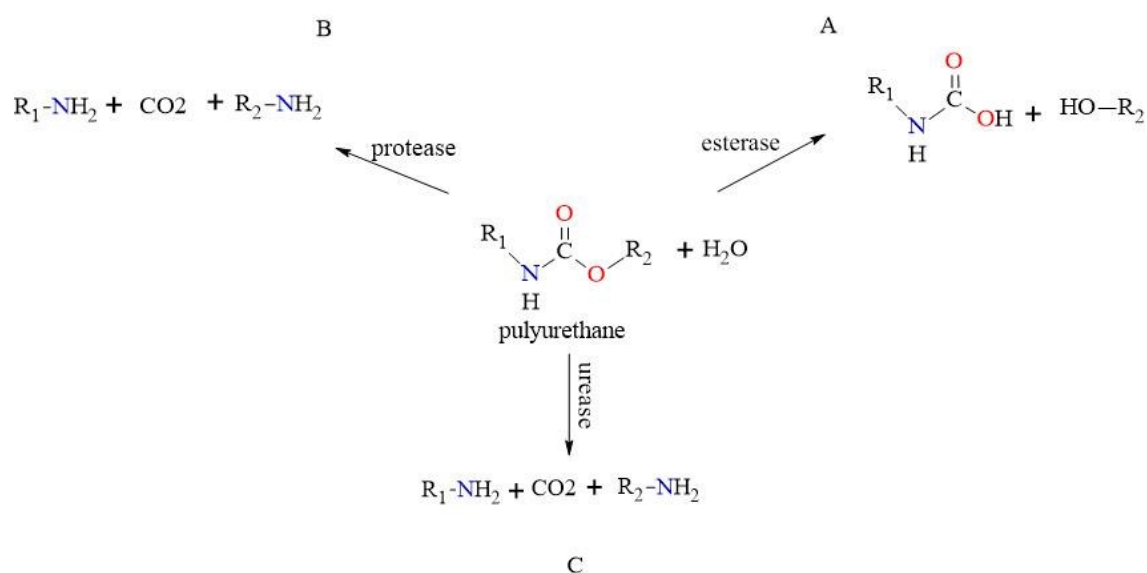


Figure 3 (A) Urethane degradation by an esterase **(B)** Urethane degradation by a protease
(C) Degradation by urease

As we can see on **Figure 3**, not only esterases, but also proteases play important role in the degradation of polyurethanes (**Figure 3 (B)**). During these reactions, amines, alcohols, and carbon dioxide are produced by hydrolysis of urethane bonds [2]. Ureases are also important in urethane degradation processes (**Figure 3 (C)**) since urease enzymes break down the aliphatic parts of PU more easily than the crystalline regions of the molecule [2].

3. Methods

3.1 BLAST- Basic Local Alignment Search Tool

The Basic Local Alignment Search Tool (BLAST) was developed in 1990 by Stephen Altschul. This program uses a heuristic algorithm to find the most similar protein sequence for a sequence retrieved from a database. The algorithm searches for segments with the lowest possible gap between related sequences, using pairwise comparisons in each block. This pairwise comparison helps to filter out related and unrelated sequences from the database [31], [32].

3.1.1 E score

The fitting process of the protein sequences is carried out as follows. First, it creates a list of words from the template sequence. Each word consists of triplets. The list contains all possible words from the template. Next, the program searches the database for occurrences of these words. Here the algorithm matches the matching words with the words in the database. The matches are scored according to a substitution matrix. A match is defined as a score above a certain threshold. This is followed by pairwise matching, where words are matched in 2 directions according to the same substitution matrix. If the score decreases, then the matching ends. This results in a GAP-free sequence with the highest score according to our scoring matrix. Since protein databases are growing and pairwise comparisons using significance scores help to distinguish between related and unrelated sequences, but this leads to errors, there may be many random alignments of unrelated sequences. Therefore, a new statistical measure of expectation (E) has been introduced. This number shows whether the alignments from the search are random. The smaller the E value, the better the fit. It is determined by the following formula:

$$E = m * n * P$$

Where m is the total number of residuals in the database, n is the number of residuals in the template sequence, and P is the probability that the result of a high scoring pairs (HSP) match which is the result of a randomness [33] [34].

If $E < 1e^{-50}$ (1×10^{-50}), then the probability that the database match is homologous is extremely high. If E is between 0.01 and $1e^{-50}$ then the match is considered homologous. If

E is between 0.01 and 10, the match is not considered significant, but may indicate a presumed distant homology relationship [31], [34], [35].

3.1.2 Bit score

The bit score is another statistical indicator used alongside the E value in BLAST. The bit score characterizes sequence similarity independent of template sequence length and database size and is calculated by taking the raw pairwise alignment score into account. The bit score (S') is given by the following scheme:

$$S' = \lambda * S - \frac{\ln K}{\ln 2}$$

λ is the Gumble distribution constant, S is the raw matching score, and K is the constant associated with the scoring matrix used. Obviously, the bit score (S') is linearly related to the raw alignment score (S). Thus, the higher the bit score, the greater the significance of the match. The bit score provides a constant statistical indicator for searching databases of different sizes, or for searching the same database at different times as the database grows [31].

3.1.3 P score

The number of random HSPs with score $\geq S$ is described by a Poisson distribution. This means that the probability of finding exactly one HSP with score $\geq S$ is given by

$$e^{-E} * \frac{E^a}{a!}$$

where E is the value of S given by E in the above equation. Specifically, the probability of finding a zero HSP with score $\geq S$ is e^{-E} , *i.e.* the probability of finding at least one such HSP.

$$P = 1 - e^{-E}$$

This is the P -value associated with the score S . For example, if we expect to find three HSPs with a score $\geq S$, the probability of finding at least one is 0.95. BLAST programs give the E -value instead of the P -value because it is easier to understand the difference between, for example, 5 and 10 E -values than between 0.993 and 0.99995 P -values. However, if $E < 0.01$, then the P and the E are almost the same [36].

3.2 MSA - Multi sequence alignment

Multi Sequence Alignment (MSA) refers to the alignment of multiple sequences, which indicates evolutionary relationships between genes. Its task is to search for patterns in sequences and find conserved units. Sequence alignment is done by algorithms, with the possibility of progressive approaches and dynamic and heuristic approximations. In this research, the aim was to explore structural and functional similarities between proteins. Enzymes are nothing more than proteins with a function, built up by amino acids. These amino acids are coded by nucleobases such as adenine, guanine, thymine, cytosine. The structure of these units and amino acids influences the process they catalyze and the function they perform. Their spatial structure also determines where the binding site can be located, where the active site or active center is found. This is where the substrates, in our case the polyurethane bind. The reason for looking for similarity is that structurally related molecules often bind to the same binding site, *i.e.* similar molecules may have similar binding sites which is called molecular mimicry [37].

3.3 Accepted Point Mutation (PAM 250)

PAM, an acronym derived from Accepted Point Mutation. It is a probabilistic model for amino acid substitution, derived from a comparison of the frequency of substitution in closely related sequences and the frequency expected for completely random substitution of amino acids. The basis of this scoring system is that the evolution of protein sequences is not a random process - *i.e.* the substitution of some amino acids is not random. They occur frequently, especially in related sequences. Amino acid substitutions also tend to retain charge, size and hydrophobicity, among other characteristics [38].

The PAM 250 scoring matrix means that there could be about 250 mutations per 100 amino acids, so only very similar sequences will achieve higher alignment scores. The PAM matrices contain positive and negative values depending on whether the alignment score is greater than zero, the sequences are considered related (similar in terms of the alignment matrix used), if the score is negative, they are assumed to be unrelated. Relatedness can also refer to the evolution and functionality of proteins. The choice of the matrix also affects the result, so we need to make an assumption about the similarity of the sequences to get a useful result: sequences that are quite distant will not produce a good match [39].

3.4 Preparation of model urethanes

There were two main parts of this research within which computational chemistry tools were used to describe the interactions between the model urethanes to the two different polyurethane esterase molecules (**Figure 4**).

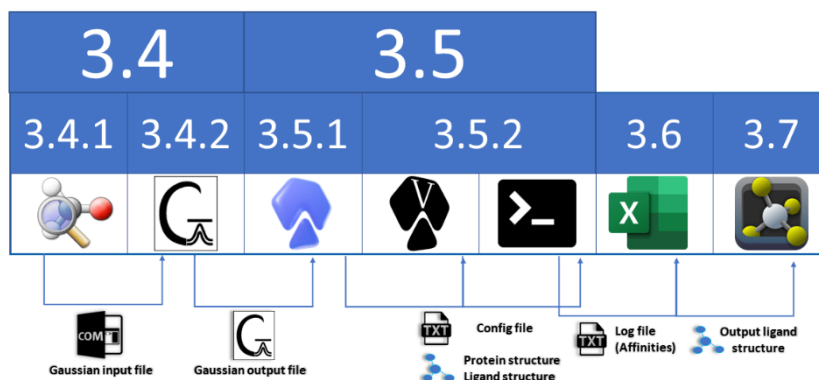


Figure 4 Schematic representation of the applied workflow.

3.4.1 GaussView 6.0

3D structures of five model urethanes were created. These are mono-urethane models, PU-HDI, PU-HMDI, PU-MDI, PU-TDI24, and PU-TDI26 and represents various industrially important polyurethane types [40]. The 3D model of the structures in question were created by using the GaussView 6.0 software [41]. In this software any molecular structure can be created in 3D. By starting the GaussView 6.0, a blank window, and the user interface can be seen (**Figure 5**).

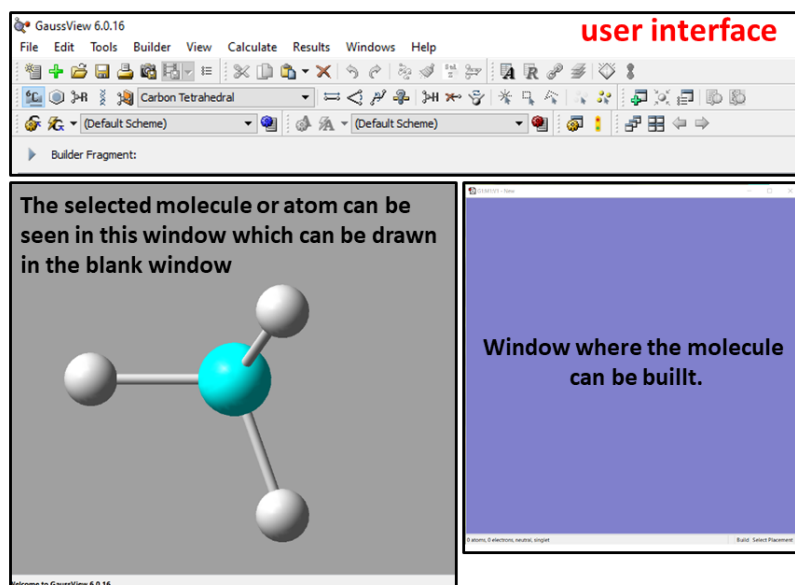


Figure 5 Opening windows of GaussView 6.0.

In the blank window the user has the opportunity to place different types of atoms, or pre-built structures like methane, water, or cyclic compounds as well such as cycloalkanes, or benzene. In this window the structures can also be arranged locally, or it is possible to rotate the atoms, and molecules around different axes. The window next to this blank window is the user interface, where the molecules can be seen which will be placed in the blank window after the selection of the right atom or molecule. Also, by using the user interface, and selecting the right button, there are options to modify such things like bond distances, angles, dihedrals and it is possible to delete or select a single atom, or more atoms by using the selection tool in different shapes.

After the structures were created, by arranging the atoms, and molecules in the right order and fine tuning the bond angles, distances, and types (single, double, or triple binding), it can be saved as a Gaussian input file. The received input file can be opened in a text editor (*e.g.*, TextPad) (**Figure 6**).

# B3LYP/6-31G(d,p) Opt Freq SCRF(SMD,Solvent=Water)				Method and basis set
PU	HDI	Title		
0	1	Charge and multiplicity		
O		-6.20867693	0.32987877	-0.23536304
H		-6.52913152	1.23481290	-0.23360831
C		-4.77867693	0.32987877	-0.23536304
H		-4.42201058	0.83428177	-1.10901441
H		-4.42201008	0.83428157	0.63828825
C		-4.26534406	-1.12204732	-0.23536285
H		-4.62203146	-1.62647209	-1.10899306
H		-4.62196899	-1.62644312	0.63830959
C		-2.72534406	-1.12201451	-0.23541740
H		-2.36871941	-0.61934273	-1.11008302
H		-2.36865707	-0.61586728	0.63721618
C		-2.21198015	-2.57392679	-0.23254729
H		-2.56645194	-3.07583386	0.64343161
H		-2.57081728	-3.08083189	-1.10385845
O		-0.78198461	-2.57390486	-0.23612045
C		-0.30460955	-3.92186621	-0.23247911
O		0.94538607	-3.92121922	-0.23572546
C		-0.82082247	-4.65264188	-1.48591085
H		-1.81835190	-4.99833522	-1.31175243
H		-0.81663205	-3.97998008	-2.31802175
C		0.09263035	-5.85400740	-1.79238243
H		-0.41067437	-6.52421685	-2.45751674
H		0.32776611	-6.36467142	-0.88197954
C		1.39204558	-5.35579753	-2.45185721
H		2.18955444	-5.39018956	-1.73932875
H		1.25783672	-4.34909235	-2.78865484
C		1.73680417	-6.25699181	-3.65210318

**Coordinates of the
molecule's atoms**

Figure 6 Structure of a Gaussian input file.

The input file contains three main parts: the route section, the title section, and the section which includes molecule specification details, such as charge, multiplicity, atom types, and the corresponding coordinates. The route section contains the level of theory, which is used, and the commands which must be performed during the calculation (*e.g.* Opt - Optimization) process. In this work B3LYP/6-31G(d,p) level of theory was used to optimize the structure. In the title section, a short description or title is optional to be associated with the input file for easier recognition. The molecule specification includes the charge and multiplicity of the molecule, the atoms which make up the whole structure, and the coordinates according to the atoms in question.

3.4.2 Gaussian 09

The calculation process can be carried out by using the Gaussian 09 [42] program package. It is the step in which the drawn molecules are getting optimized, and different thermodynamic properties are also calculated. If the calculation is done the software generates an output file (**Figure 7**) which can be opened using a text editor, or by using GaussView.

```
ABSOLUTISM, OBSOLETISM -- IF IT WORKS, IT'S OUT OF DATE
-- STAFFORD BEER
Job cpu time:      0 days  4 hours  7 minutes 53.8 seconds.
File lengths (MBytes):  RWF=   4250 Int=      0 D2E=      0 Chk=    63 Scr=     1
Normal termination of Gaussian 09 at Fri Oct 14 21:34:10 2022.
```

Figure 7 A screenshot of the last section of a normal terminated Gaussian output file.

By using the text editor, different values according to the optimized molecules can be extracted, however by using GaussView the optimized structure can be seen in 3D. If the calculation normal terminated the output file contains the final structure. The optimized model compounds were used in the docking process in which the binding affinity of these species to two different esterases were determined.

3.5 Docking ligands to enzymes

3.5.1 AutoDock Tools (preparing the structures for docking)

After the monomer structures were well prepared, and properly optimized they could be used in the second step, which is the docking process. The main aim of this step to determine the binding affinities of different urethane models towards polyurethane esterases [43]. The structures were prepared by using AutoDock Tools. If the AutoDock Tools is opened a window welcomes the user (**Figure 8**).

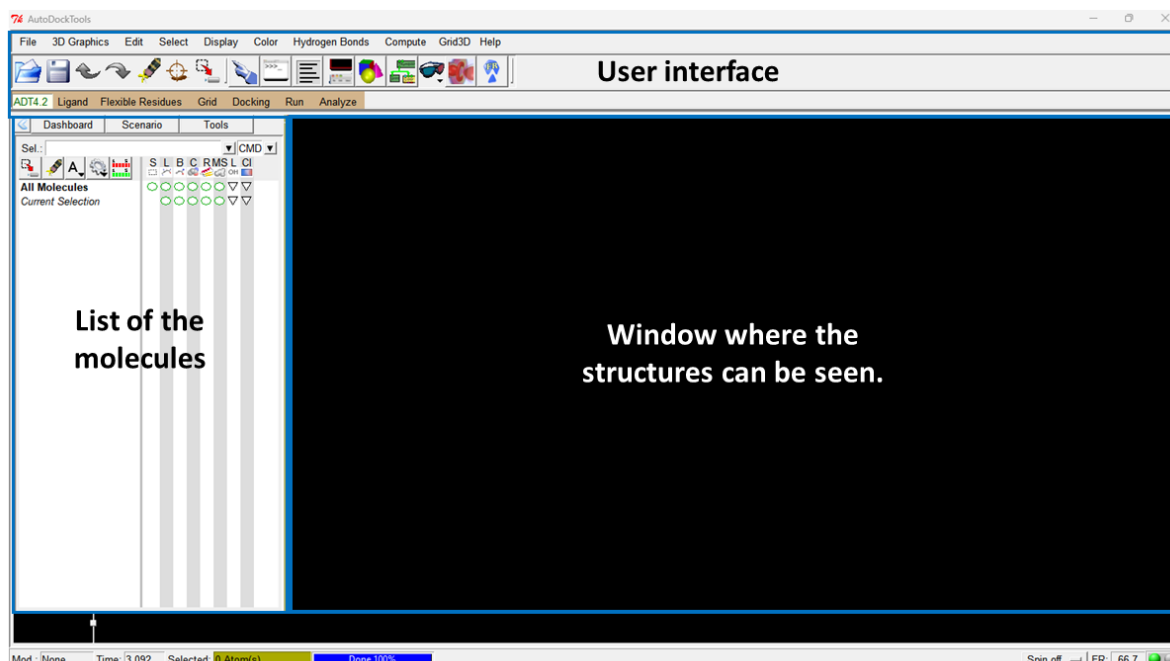


Figure 8 Opening window of AutoDock Tools.

This window includes three parts. The first part is a black section of the opening window, here the molecules can be dragged in which takes part in the docking process, and also it is possible to see the 3D structures of the molecules that have been placed in this part of the window. At the left of this, the list of the molecules can be seen which appear in the black screen. In this part of the window the appearance of the molecules can be set by using different buttons. Also, the most important part of the opening window are the options in the user interface that can help the user to perform changes in the above-mentioned molecules.

First, the protein esterase molecules were added. After placing the esterase molecules in the black part of the opening window the preparation of the protein structures could be done by using different options. Deleting waters was the first step of the protein preparation process which could be performed by using the Edit – Delete Water (**Figure 9**) option [44].

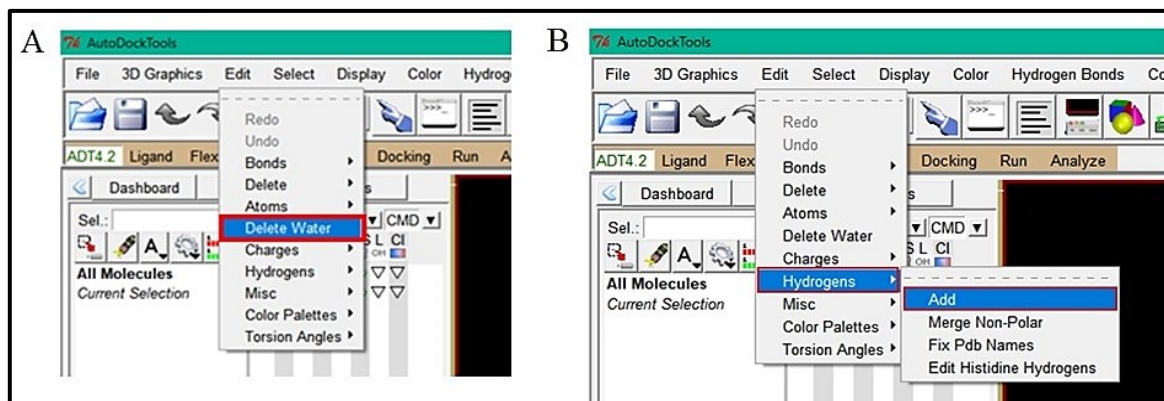


Figure 9 (A) Delete water option in AutoDock Tools. **(B):** Add hydrogens option in AutoDock Tools.

To delete waters was necessary because their appearance could lead to false results. Thereafter hydrogens atoms were added to the proteins [44] (**Figure 9**).

The addition of hydrogen atoms was an essential step because the 3D structures of the proteins do not include hydrogens. The structures were determined by X-ray crystallography, and as a result of the small size of the hydrogen atoms, their position was not determined and thus, they do not include in the 3D models. After adding the hydrogens with AutoDock Tools, the protonation state of the sidechains of the amino acids within the protein should be determined. After these steps the prepared structure has to be selected as a protein molecule using the Grid – Macromolecule – Choose Protein option and the structure has to be exported into a pdbtq file, which can be used in the final step of the docking process [44].

After all these changes in the protein molecule, there were some things to do with the urethane models as well before carrying out the docking. Using GaussView 6.0 software the models had to be exported into a pdb format. Next, these structures had to be imported into AutoDock Tools, next to the protein molecule and had to be selected as a ligand molecule by using the Ligand – Input – Choose – Ligand option path. As a result, a pdbtq file of the ligand molecule could be received, which could be used in the final step of the docking process [44]. If the pdbtq files of the ligand and protein molecules are ready the docking site had to be defined. Two types of docking were carried out, blind docking [45], and guided docking [46]. Using the blind docking method, no specific position on the protein was selected and the whole structure was targeted by the ligand molecule. However, as one grid box was not enough to cover the whole structure, thus, several grid boxes were used which overlap with each other [45]. In case of the guided docking, a specific location or locations could be determined where the ligand should be docked the

interactions can be determined. These locations were determined for the two esterases and the docking sites were narrowed down to these locations and smaller grid boxes were enough to be used. For this the corresponding active center should be appointed as the location of the docking itself [46]. The grid box (as the name reveals) has a shape of a box (**Figure 10**). Different parameters of the grid box could be changed, such as the number of points in x, y, or z-dimension (this changed the size of the grid box), the spacing in angstrom (this defined the volume of the box by changing the 3 sides at once), and the center of the grid box (by this, the box could be moved around in the x, y, and z axes). When the grid box that has the appropriate size, and location was being made for the docking process, the last step has been reached.

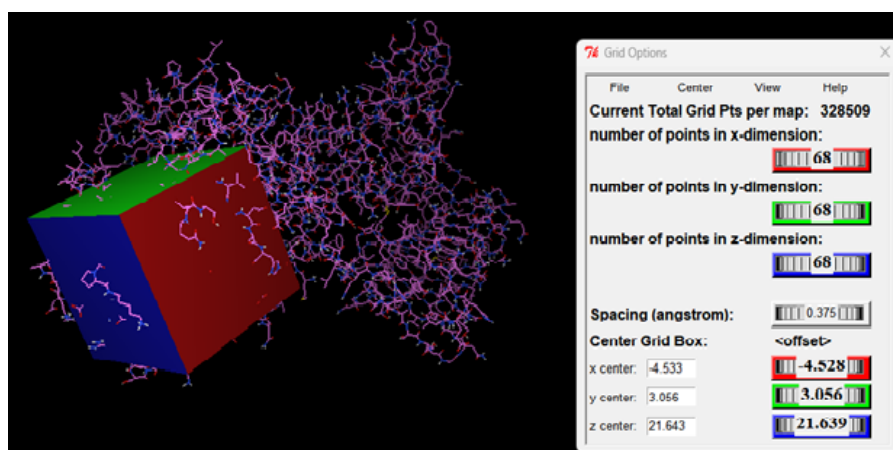


Figure 10 Grid box with PueA esterase

The final step of the docking process was to prepare the config file and start the docking calculation. The config file which contains the docking parameters has 4 different sections (**Figure 11**).

```
receptor = PueA_model1.pdbqt
ligand = PU_HDI.pdbqt

center_x = 5.551
center_y = 11.639
center_z = 22.588

size_x = 34
size_y = 34
size_z = 34

energy_range = 4
exhaustiveness = 8
```

Figure 11 Config file example for AutoDock Vina.

The first section is where the receptor (protein esterase) molecule and the ligand molecule are defined by giving their file names. The next part includes the parameters of the grid box, or as it can be said the location where the docking should take place. The last 2 parts provides information of the energy range and exhaustiveness (*i.e.* precision) of the docking process.

3.5.2 Autodock Vina

After the config file is ready, the docking process could be fulfilled using Autodock Vina through the command prompt. Thus, it is possible to predict how the molecules interact with each other in a bound state [47]. The command which had to be used can be seen in **Figure 12**.

```
cd C:\(The place where the config file is stored)

"C:\Program Files (x86)\The Scripps Research Institute\Vina\vina.exe" --receptor protein.pdbqt --ligand ligand.pdbqt --config config.txt --log log.txt --out output.pdbqt
```

Figure 12 Command to start the docking process with AutoDock Vina.

After the docking process correctly terminated an output file, and a log file were generated. The log file could be opened by using a text editor and it contain the different docking modes, the affinities and distances from the best mode for all cases respectively. The output file could be opened using PyMol and the structure how the molecules interact with each other in a bound state could be seen in a 3D model, including the names of the residues which interact with the ligand and also the type of interaction between the two molecules in each state (**Figure 13**).



Figure 13 Docked ligand at various sites of the protein in PyMol.

3.6 Analysis of the docking scores

After the output log files were generated, the affinities of each docking processes and each docking modes could be compared. The affinity values were collected, sorted and compared. Lower the value better the interaction between the protein and ligand.

3.7 PyMol

In each docking processes a representation was made about how the ligand molecules in their best docking modes are located compared to the protein, and the docking mode which had the best docking score (lowest affinity) have been selected from the others and it was shown in a zoomed representation how the ligand molecule is located punctually, and how does it interact with the protein. For the structural analysis PyMol was used in order to find the right parts, and interactions of the molecules.

4. Results and Discussion

4.1 BLAST results of PueA

In case of 3D modelling the first step is to select a template, which can be a protein of known 3D structure, or a protein with unknown structure. If latter is the case at least the amino acid sequence of the protein with polyurethane-degrading activity has to be known (described in the literature). The UniProt database was searched for the enzymes described. It is also known that the binding sites of polyurethane-degrading enzymes include serine (SER), histidine (HIS), and aspartic acid (ASP) residues, and it has been also reported that the most important part of the protein's active site is a SER residue [48]. Therefore, after comparing amino acid sequences and searching for conserved regions, in polyurethane-degrading enzymes SER with nearby of HIS and ASP residues has to be located. As is the case in the 3D structures of PueA and PueB (**Figure 14**), which have known structures and can be used for further protein search. BLAST software was used to search the UniProt database for enzymes with similar amino acid sequences to PueA or PueB. The resulting sequences were aligned and AliView was applied for this multi alignment process or the alignment function included in UniProt. The most similar enzymes were selected for further studies and the least similar enzymes were moved from the alignment.

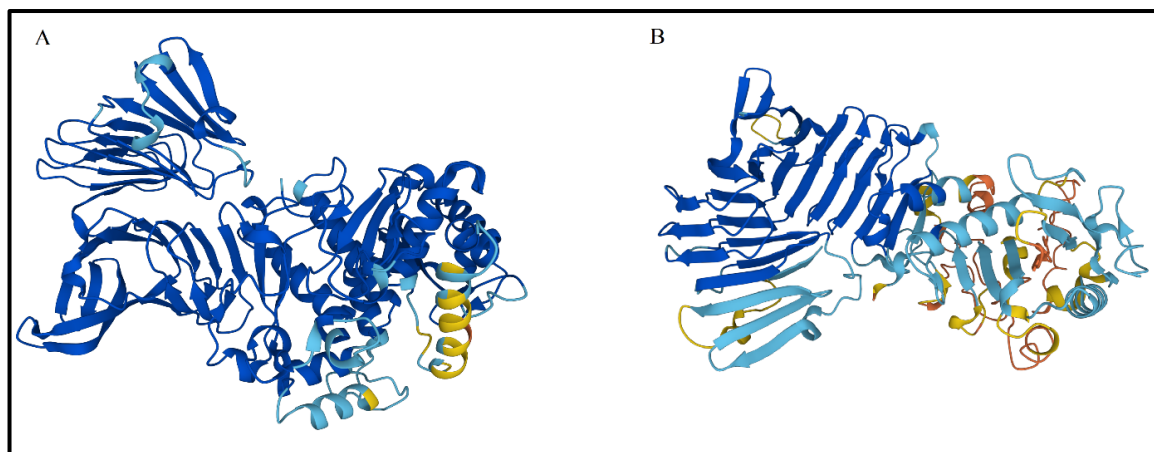


Figure 14 3D structures of polyurethanase esterase A (PueA) [49] – **A** and polyurethanase esterase B (PueB) [50]– **B**. Their entry numbers are Q9X3C0 and Q9R9H2, respectively. Both enzymes can be found in *Pseudomonas chlororaphis*.

The BLAST of PueA protein search resulted 250 sequences with some degree of similarity, which can be seen in **Annex 1**. The lysate was filtered by using the *E* value, which meant that proteins with an *E* value higher than 1×10^{-50} were filtered out, because *E* above this value does not indicate presumptive homology. Then, as a second filtering step, the bit score was considered, which meant filtering up to 200 points. Third, the *P* value was applied to find the best possible homologies [51]. Similarity search resulted one protein showing 99.4% similarity to the template polyurethane esterase A, while 4 proteins showed 70-90%, 6 proteins showed 60-70% similarity, 5 were 50-60% similar, and 3 proteins ended up in the 40-50% similarity. 231 proteins showed similarity below 40% based on the results of the UniProt database (**Annex 1**). The proteins homologue to PueA are also collected (**Annex 4**). Protein: protein alignments with *E* values < 0.001 can reliably be used to infer homology.

4.2 MSA results of PueA

Based on the *E* and *P* values, and bit number, several proteins were selected (**Annex 2**) for further analysis. The selected sequences were aligned by using UniProt MSA (**Annex 5**). This analysis clearly showed which are the conserved sequences in the proteins. During evolution certain amino acids became conserved and determined certain properties of the protein. The longest conserved amino acid regions found in the aligned sequences were, NIGYENDPVFRAL, SATNNIVNFNDHYAS, SKDSTIVVAN, and TFIIGSD. But the

most important was to find the GXSD and GGXGDXXX conserved regions, as these motifs are present in serine hydrolases. If a sequence analysis of a protein shows these motifs one can assume polyurethane degradation activity of the given protein. In the case of PueA and the other aligned proteins this conserved region is present (**Annex 5**). Thus, it can be concluded that the selected proteins belong to the same protein family. The similarities between the proteins (usually expressed in percentage similarity) were also calculated by the system based on the *E* and *P*-values and the sequence length (**Figure 15**).



Figure 15 Similarity between aligned sequences.

4.4 BLAST results of PueB

After screening the database for proteins that are similar to PueB, 250 proteins were found with similar sequences to the template PueB. The best similarity was 79%. One other protein was found with more than 50% similarity to the template. For seven proteins, the similarities were between 40% and 50%. The remaining matches all had similarities below

40%. From 250 protein, 22 proteins show homology. The BLAST result is presented in **Annex 3** with the E values. For the proteins that are still defined as homologous $E < 1e^{-50}$ (1×10^{-50}) was true for E . It is also observed that fewer identity matches were found between PueB and other proteins than for PueA.

4.5 MSA results of PueB

For the MSA assay, the most similar protein sequences were used. In this case conserved region assigning a serine hydrolase activity was also found during sequence analysis. Permutation of Ser-Asp-His linkage was not observed. Due to the low hit rate, only the proteins with 0 E value were rearranged (**Annex 6**).

Better homology matching can be detected using MSA. However, it is important to note that in this case the conserved clusters responsible for the degradation of polyurethane are found as well. The similarities between the proteins in % were also calculated by the system based on the E and P -values and the length of the sequence (**Figure 16**).

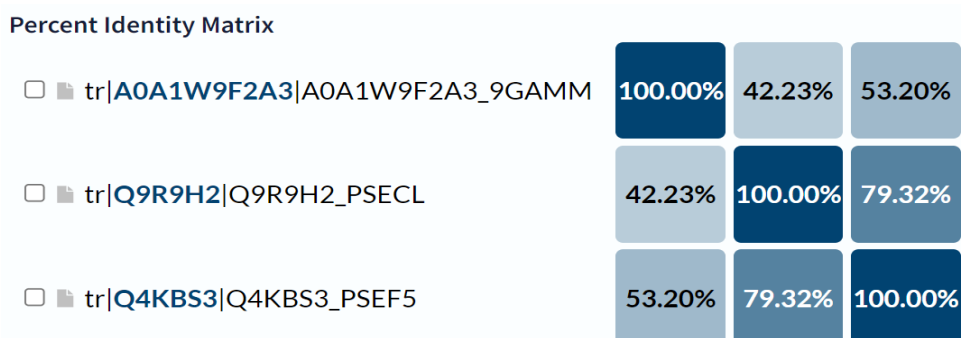


Figure 16 Similarity between aligned sequences with PueB (A0A1W9F2A3 polyesterase B, Q9R9H2 polyurethanase B, Q4KB53 polyurethanase B).

4.6 Binding affinity results

Taking into account the results obtained from the sequence alignment, docking was performed on the template proteins using urethane models representing different industrial polyurethanes. The relationship between the conserved sequence and the serine aspartame was considered.

For polyurethane esterase A, the studied binding sites were SER93, SER242, SER294, SER301, SER582, SER207, and SER270, while for polyurethane esterase B, the binding sites were SER83, SER58, SER212, SER253, SER401, SER416, and SER554.

In the case of PueA, the best binding affinity for the HDI model molecule belonged to the SER207 binding site with the best affinity result of -5.2 kcal/mol while the best blind docking result was only -4.6 kcal/mol. This shows that the blind docking did not give as good result as the directed one. The best binding affinity for HMDI was also for SER207. Here the affinity value was -7.1 kcal/mol compared to -4.1 kcal/mol for the blind docking, but the worst directional docking was also -4.6 kcal/mol. For MDI, the best result was obtained for SER207, which was -7.6 kcal/mol. For TDI24, the best affinity value was -6.7 kcal/mol for SER207, but the best value for TDI26 was obtained at the same location, which was also -6.7 kcal/mol (**Figure 17 A; Appendix 7**).

For PueB, the best affinity result for HDI were -4.4 kcal/mol for sites SER212, SER253, and SER83. While the blind docking value was -4.0 kcal/mol. The best binding affinity for HMDI was experienced at the SER401 site. Here the affinity value is -5.5 kcal/mol while for the corresponding blind docking site -4.6 kcal/mol. For MDI the best affinity result was found at SER416, -5.0 kcal/mol where the blind docking value was -5.1 kcal/mol. For TDI24, the best affinity value was -4.8 kcal/mol for SER212 and SER401 respectively, but also for TDI26, the best value was obtained for SER401, which was -5.6 kcal/mol (**Figure 17 B; Appendix 8**).

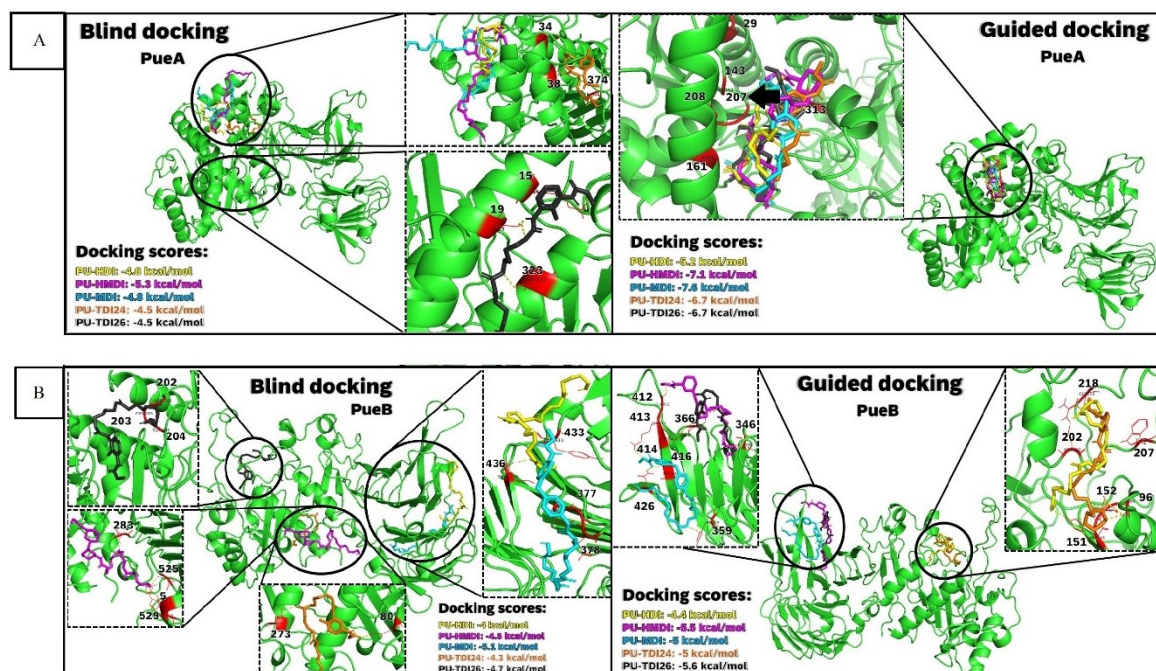


Figure 17 A: Results of blind and guided docking of PueA; **B:** Results of blind and guided docking of PueB.

4.7 Conclusion

The research was aimed to identify potential new polyurethane degrading enzymes using the sequences of PueA and PueB proteins as template. These known polyurethane degrading enzymes have special conserved regions and conserved amino acids which are the key structural elements for substrate binding at the active site of the enzyme. BLAST and MSA methods were used for sequence and homology searches. Recognizing the conserved clusters of PueA or PueB in other proteins give us the first step in the way of understanding the properties and the mechanism of these polyurethane degrading enzymes.

In case of homology search results, for polyurethane esterase A, the program found statistically 250 similar proteins, of which 15 had an *E* value of exactly 0.0, as shown in **Appendix 1**. These 15 proteins and the template protein were aligned using MSA and some sequence similarities of several bits were detected. The found conserved regions corresponds to the conserved motif of serine hydrolases (GXSD; GGXGDXXX), but it was also found that these regions are not highly conserved. In case of PueA homologues SER93, SER242, SER294, SER301, SER582, SER207, and SER270 were used to identify possible connection points for molecular docking.

The results of the homology search for polyurethane esterase B indicated also 250 statistically similar proteins, but only 2 of them have an *E* value of exactly 0.0 as it is shown in **Appendix 3**. These 2 proteins and the template protein were matched by MSA. In this case also several sequence repeats, among others, motifs for serine hydrolases were found and in this case these regions were highly conserved. Possible active sites were identified as well, here SER83, SER58, SER212, SER253, SER401, SER416, and SER554 were considered.

As a result of docking, polyurethane esterase A generally gave better results for guided docking than for blind docking. For the PueA enzyme, we obtained the most favorable affinity results for the SER207 site. This is located in the middle of a highly localized region, and the sequence of the region (GHSLGG) is similar to the region of serine hydrolases. The underlined amino acid indicates the difference from the expectation. The best results for polyurethane esterase B were obtained for the **SER401** site. This amino acid is also located in a conserved region (SHET).

Considering our future plans these results are very important as they can help us understanding the degradation processes of polyurethanes, establishing new reaction

mechanism for degradation process or improve the known ones, and serve as a basis for planning and realizing laboratory experiments.

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5. References

- [1] W. Yang, Q. Dong, S. Liu, H. Xie, L. Liu, and J. Li, "Recycling and Disposal Methods for Polyurethane Foam Wastes," *Procedia Environ Sci*, vol. 16, pp. 167–175, 2012, doi: 10.1016/j.proenv.2012.10.023.
- [2] A. Kemona and M. Piotrowska, "Polyurethane Recycling and Disposal: Methods and Prospects," *Polymers 2020, Vol. 12, Page 1752*, vol. 12, no. 8, p. 1752, Aug. 2020, doi: 10.3390/POLYM12081752.
- [3] B. Y. Peng *et al.*, "Biodegradation of Polystyrene by Dark (*Tenebrio obscurus*) and Yellow (*Tenebrio molitor*) Mealworms (Coleoptera: Tenebrionidae)," *Environ Sci Technol*, vol. 53, no. 9, pp. 5256–5265, May 2019, doi: 10.1021/ACS.EST.8B06963/SUPPL_FILE/ES8B06963_SI_001.PDF.
- [4] K. M. Zia, H. N. Bhatti, and I. Ahmad Bhatti, "Methods for polyurethane and polyurethane composites, recycling and recovery: A review," *Reactive and Functional Polymers*, vol. 67, no. 8, pp. 675–692, Aug. 2007. doi: 10.1016/j.reactfunctpolym.2007.05.004.
- [5] G. T. Howard, "Polyurethane Biodegradation," 2012, pp. 371–394. doi: 10.1007/978-3-642-23789-8_14.
- [6] Péter Gröb, "A Gyártástechnológiai Paraméterek Hatása Poliuretán Integrálhab Termékek Tulajdonságaira", Accessed: Sep. 02, 2022. [Online]. Available: <chrome-extension://efaidnbmnnnibpcajpcgclefindmkaj/https://repozitorium.omikk.bme.hu/bitstream/handle/10890/743/ertekezes.pdf?sequence=1>
- [7] F. Xie, T. Zhang, P. Bryant, V. Kurusingal, J. M. Colwell, and B. Laycock, "Degradation and stabilization of polyurethane elastomers," *Prog Polym Sci*, vol. 90, pp. 211–268, Mar. 2019, doi: 10.1016/J.PROGPOLYMSCI.2018.12.003.
- [8] K. Skleničková, S. Abbrent, M. Halecký, V. Kočí, and H. Beneš, "Biodegradability and ecotoxicity of polyurethane foams: A review," <https://doi.org/10.1080/10643389.2020.1818496>, vol. 52, no. 2, pp. 157–202, 2020, doi: 10.1080/10643389.2020.1818496.
- [9] Y. Tokiwa, B. P. Calabria, C. U. Ugwu, and S. Aiba, "Biodegradability of Plastics," *International Journal of Molecular Sciences 2009, Vol. 10, Pages 3722-3742*, vol. 10, no. 9, pp. 3722–3742, Aug. 2009, doi: 10.3390/IJMS10093722.
- [10] A. Banerjee, K. Chatterjee, and G. Madras, "Enzymatic degradation of polymers: a brief review," <https://doi.org/10.1179/1743284713Y.0000000503>, vol. 30, no. 5, pp. 567–573, 2014, doi: 10.1179/1743284713Y.0000000503.
- [11] M. Mochizuki, M. Hirano, Y. Kanmuri, K. Kudo, and Y. Tokiwa, "Hydrolysis of polycaprolactone fibers by lipase: Effects of draw ratio on enzymatic degradation," *J Appl Polym Sci*, vol. 55, no. 2, pp. 289–296, Jan. 1995, doi: 10.1002/APP.1995.070550212.
- [12] D.S. Wales and B.F. Sagar, "Mechanistic Aspects of Polyurethane Biodeterioration," vol. 7, 1987, pp. 351–358.
- [13] Z. Shah, F. Hasan, L. Krumholz, D. Atkas, and A. A. Shah, "Degradation of polyester polyurethane by newly isolated *Pseudomonas aeruginosa* strain MZA-85 and analysis of degradation products by GC-MS," *Int Biodeterior Biodegradation*, vol. 77, pp. 114–122, 2013, doi: 10.1016/J.IBIOD.2012.11.009.
- [14] R. Wilkes, L. Aristilde, C. Ludmilla Aristilde, and R.-R. Hall, "Degradation and metabolism of synthetic plastics and associated products by *Pseudomonas* sp.:

- capabilities and challenges,” *J Appl Microbiol*, vol. 123, no. 3, pp. 582–593, Sep. 2017, doi: 10.1111/JAM.13472.
- [15] R. Gautam, A. S. Bassi, E. K. Yanful, and E. Cullen, “Biodegradation of automotive waste polyester polyurethane foam using *Pseudomonas chlororaphis* ATCC55729,” *Int Biodeterior Biodegradation*, vol. 60, no. 4, pp. 245–249, Jan. 2007, doi: 10.1016/J.IBIOD.2007.03.009.
- [16] A. E. Stepien *et al.*, “Assessment of the impact of bacteria *Pseudomonas denitrificans*, *Pseudomonas fluorescens*, *Bacillus subtilis* and yeast *Yarrowia lipolytica* on commercial poly(ether urethanes),” 2017, doi: 10.1016/j.polymertesting.2017.08.038.
- [17] M. J. Kay, R. W. McCabe, and L. H. G. Morton, “Chemical and Physical Changes Occurring in Polyester Polyurethane during Biodegradation,” 1993.
- [18] Y. Akutsu, T. Nakajima-Kambe, N. Nomura, and T. Nakahara, “Purification and properties of a polyester polyurethane-degrading enzyme from *Comamonas acidovorans* TB-35,” *Appl Environ Microbiol*, vol. 64, no. 1, pp. 62–67, 1998, doi: 10.1128/AEM.64.1.62-67.1998/FORMAT/EPUB.
- [19] G. T. Howard, “Biodegradation of polyurethane: a review,” 2002. [Online]. Available: www.elsevier.com/locate/ibiod
- [20] G. T. Howard, C. Ruiz, and N. P. Hilliard, “Growth of *Pseudomonas chlororaphis* on a polyester polyurethane and the purification and characterization of a polyurethanase-esterase enzyme,” pp. 7–12, 1999.
- [21] J. C. Biffinger *et al.*, “The applicability of Impranil® DLN for gauging the biodegradation of polyurethanes,” *Polym Degrad Stab*, vol. 120, pp. 178–185, Jul. 2015, doi: 10.1016/j.polymdegradstab.2015.06.020.
- [22] A. K. Urbanek, J. Rybak, M. Wróbel, K. Leluk, and A. M. Mironczuk, “A comprehensive assessment of microbiome diversity in *Tenebrio molitor* fed with polystyrene waste,” *Environmental Pollution*, vol. 262, Jul. 2020, doi: 10.1016/j.envpol.2020.114281.
- [23] S. S. Yang *et al.*, “Biodegradation of polystyrene wastes in yellow mealworms (larvae of *Tenebrio molitor* Linnaeus): Factors affecting biodegradation rates and the ability of polystyrene-fed larvae to complete their life cycle,” *Chemosphere*, vol. 191, pp. 979–989, Jan. 2018, doi: 10.1016/j.chemosphere.2017.10.117.
- [24] H. S. Azevedo and R. L. Reis, “2 Understanding the Enzymatic Degradation of Biodegradable Polymers and Strategies to Control Their Degradation Rate,” 2005.
- [25] A. Magnin, E. Pollet, and L. Av, “Characterization of the enzymatic degradation of polyurethanes,” doi: 10.1016/bs.mie.2020.12.011.
- [26] N. Mahajan and P. Gupta, “New insights into the microbial degradation of polyurethanes,” *RSC Advances*, vol. 5, no. 52. Royal Society of Chemistry, pp. 41839–41854, 2015. doi: 10.1039/c5ra04589d.
- [27] E. M. Christenson, S. Patel, J. M. Anderson, and A. Hiltner, “Enzymatic degradation of poly(ether urethane) and poly(carbonate urethane) by cholesterol esterase,” *Biomaterials*, vol. 27, no. 21, pp. 3920–3926, Jul. 2006, doi: 10.1016/J.BIOMATERIALS.2006.03.012.
- [28] Z. Shah, F. Hasan, L. Krumholz, D. Atkas, and A. A. Shah, “Degradation of polyester polyurethane by newly isolated *Pseudomonas aeruginosa* strain MZA-85 and analysis of degradation products by GC–MS,” *Int Biodeterior Biodegradation*, vol. 77, pp. 114–122, Feb. 2013, doi: 10.1016/J.IBIOD.2012.11.009.
- [29] T. Nakajima-Kambe, Y. Shigeno-Akutsu, N. Nomura, F. Onuma, and T. Nakahara, “Microbial degradation of polyurethane, polyester polyurethanes and polyether

- polyurethanes,” *Applied Microbiology and Biotechnology* 1999 51:2, vol. 51, no. 2, pp. 134–140, 1999, doi: 10.1007/S002530051373.
- [30] M. Cregut, M. Bedas, M. J. Durand, and G. Thouand, “New insights into polyurethane biodegradation and realistic prospects for the development of a sustainable waste recycling process,” *Biotechnol Adv*, vol. 31, no. 8, pp. 1634–1647, 2013, doi: 10.1016/J.BIOTECHADV.2013.08.011.
- [31] Jin. Xiong, *Essential bioinformatics*. Cambridge University Press, 2006.
- [32] “Historical Introduction and Overview.” [Online]. Available: <http://www-nbrf.georgetown.edu/pir>
- [33] A. Morgulis, G. Coulouris, Y. Raytselis, T. L. Madden, R. Agarwala, and A. A. Schäffer, “BLAST+ features,” *Bioinformatics*, vol. 24, no. 16, pp. 1757–1764, Mar. 2009, doi: 10.1093/bioinformatics/btn322.
- [34] “E-values and Bit-scores in BLAST - Ricardo Avila.” <https://ravilabio.info/notes/bioinformatics/e-value-bitscore.html> (accessed Oct. 27, 2022).
- [35] “Metagenomics - Multi-FASTA format.” <https://www.metagenomics.wiki/tools/fastq/multi-fasta-format> (accessed Oct. 27, 2022).
- [36] “The Statistics of Sequence Similarity Scores.” <https://www.ncbi.nlm.nih.gov/BLAST/tutorial/Altschul-1.html> (accessed Oct. 27, 2022).
- [37] “Sci-Hub | | 10.1007/3-540-29623-9_5460.” https://sci-hub.se/10.1007/3-540-29623-9_5460 (accessed Oct. 26, 2022).
- [38] D. Wheeler, “Selecting the Right Protein-Scoring Matrix,” *Curr Protoc Bioinformatics*, vol. 00, no. 1, pp. 3.5.1–3.5.6, Jan. 2003, doi: 10.1002/0471250953.BI0305S00.
- [39] “Scoring matrix - Bioinformatics.Org Wiki.” https://www.bioinformatics.org/wiki/Scoring_matrix (accessed Oct. 28, 2022).
- [40] V. P. do Canto, C. E. Thompson, and P. A. Netz, “Polyurethanases: Three-dimensional structures and molecular dynamics simulations of enzymes that degrade polyurethane,” *J Mol Graph Model*, vol. 89, pp. 82–95, Jun. 2019, doi: 10.1016/J.JMGM.2019.03.001.
- [41] R. Dennington, T. Keith, and J. Millam, “GaussView, Version 5.,” *Semichem Inc., Shawnee Mission, KS*, 2009.
- [42] M. J. Frisch *et al.*, “Gaussian 09 Revision A.1.,” *Gaussian Inc.*, 2009.
- [43] P. L. Kastiris and A. M. J. J. Bonvin, “On the binding affinity of macromolecular interactions: Daring to ask why proteins interact,” *J R Soc Interface*, vol. 10, no. 79, Feb. 2013, doi: 10.1098/RSIF.2012.0835.
- [44] R. Huey, G. M. Morris, and S. Forli, “Using AutoDock 4 and AutoDock Vina with AutoDockTools: A Tutorial,” 2012, Accessed: Oct. 29, 2022. [Online]. Available: <http://autodock.scripps.edu>.
- [45] N. M. Hassan, A. A. Alhossary, Y. Mu, and C. K. Kwok, “Protein-Ligand Blind Docking Using QuickVina-W with Inter-Process Spatio-Temporal Integration,” *Sci Rep*, vol. 7, no. 1, Dec. 2017, doi: 10.1038/S41598-017-15571-7.
- [46] X. Fradera and J. Mestres, “Guided docking approaches to structure-based design and screening,” *Curr Top Med Chem*, vol. 4, no. 7, pp. 687–700, Mar. 2004, doi: 10.2174/1568026043451104.

- [47] O. Trott and A. J. Olson, “AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading,” *J Comput Chem*, vol. 31, no. 2, p. NA-NA, 2010, doi: 10.1002/JCC.21334.
- [48] V. P. do Canto, C. E. Thompson, and P. A. Netz, “Polyurethanases: Three-dimensional structures and molecular dynamics simulations of enzymes that degrade polyurethane,” *J Mol Graph Model*, vol. 89, pp. 82–95, Jun. 2019, doi: 10.1016/J.JMGM.2019.03.001.
- [49] “pueA - Polyurethanase esterase A - *Pseudomonas chlororaphis* (*Pseudomonas aureofaciens*) | UniProtKB | UniProt.”
<https://www.uniprot.org/uniprotkb/Q9X3C0/entry> (accessed Nov. 09, 2022).
- [50] “pueB - Polyurethanase B - *Pseudomonas chlororaphis* (*Pseudomonas aureofaciens*) | UniProtKB | UniProt.”
<https://www.uniprot.org/uniprotkb/Q9R9H2/entry> (accessed Nov. 09, 2022).
- [51] “Sci-Hub | An Introduction to Sequence Similarity (‘Homology’) Searching. Current Protocols in Bioinformatics, 42(1), 3.1.1–3.1.8 | 10.1002/0471250953.bi0301s42.”
<https://sci-hub.se/10.1002/0471250953.bi0301s42> (accessed Nov. 15, 2022).