
Theoretical and experimental investigation of the recyclability of TDI-based urethanes



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1. Introduction

1.1. Abstract

Polyurethane (PU) has one of the largest market shares in the plastics industry. Urethane bonds hold the polyurethane building blocks together. PU is synthesized via the addition of various polyisocyanate and polyol species. The use of polyurethanes depends on the variety of their mechanical and chemical properties. In terms of fields of use, polyurethane appears in construction, transportation furniture packaging, textiles, and many other products. It is obvious that because of the huge amount of polyurethane, which is getting produced from year to year, a large volume of waste is also generated which must be managed somehow. There are plenty of different polyurethane waste management methods. The most well-known types are landfilling and incineration of the material. Another less widespread is the degradation of polyurethane wastes. It can be stated that degradation is a general waste management method which includes several solutions to degrade polyurethane wastes, such as mechanical, thermal, biological, photo, and chemical degradation processes. In this work degradation was investigated as a combined chemical-biological system for the neutralization of polyurethane waste. To study this, the first step was the synthesis of a model diurethane because it made the glycolysis reaction mechanism easier to understand. For this model diurethane synthesis, 2,4-TDI and *n*-butanol (BuOH) as reactants were used. The most preferable reaction conditions were determined for the model diurethane synthesis. Moreover, by using computational chemistry methods, the thermodynamic properties of the catalyst-free diurethane synthesis were also investigated. After the diurethane synthesis, several glycolysis reactions were carried out, and based on the results the reaction conditions and equipment were optimized. Furthermore, the glycolysis product was used as a model substrate for enzyme detection via agar assay.

1.2. Objectives

The aim of the research was to investigate the theoretical and experimental degradation and recyclability of TDI-based urethane compounds, for which the following processes have taken place:

- Selection of the possible degradation methods which can be employed.
- Theoretical determination of the reaction mechanism for TDI-based urethane synthesis.
- Experimental feasibility of the TDI-based urethane formation and both degradation methods.

- Evaluation of the experiments and biodegradation of chemically obtained glycolysis products, detection of the enzyme involved in degradation.

2. Literature

2.1. Importance of polyurethanes

Polyurethanes (PU) are a class of polymers that have urethanes as monomers in their chemical structures. Polyurethanes can be categorized as carbamates since urethane connections are also known as carbamate linkages in organic chemistry, according to a number of studies. Due to the work of Otto Bayer, a German industrial chemist, polyurethanes have been known for more than a century. He proposed preparing these macromolecules using diisocyanates and diols. The first commercially accepted polyurethane is based on hexamethylene diisocyanate (HDI) and butanediol [1]. There are plenty of diisocyanate compounds that can be used as an initial polyisocyanate in the synthesis of polyurethanes such as methylene diphenyl diisocyanate (MDI), and toluene diisocyanate (TDI). Urethane linkages are formed when an isocyanate reacts with a hydroxyl group. Different types of polyols ensure the hydroxyl group, whereas the previously described diisocyanates (or polyisocyanates) can supply the isocyanate groups [2].

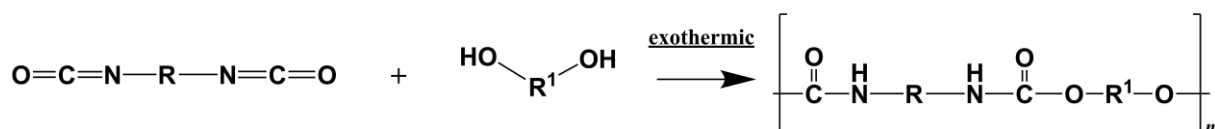


Figure 1 Schematic representation of polyurethane formation.

This particular addition reaction is extremely strongly exothermic (**Figure 1**). Polyurethane polymers with linear and flexible properties can be fabricated through the use of diisocyanates and diols, whereas polyisocyanates and polyols containing three or more cyanate or hydroxyl groups, yield polyurethanes with superior mechanical properties, such as increased rigidity and thermal stability [3]. It is evident that the choice of polyols and isocyanates utilized in the formation process has the greatest influence on the properties of polyurethanes. Different types of polyether polyols, aliphatic polyester polyols, aromatic polyester polyols, polycarbonate polyols, and polybutadiene polyols are the most commonly used polyols in the production of polyurethanes [4]. The aliphatic isocyanates such as hexamethylene diisocyanate are used in a few applications, but most often the aromatic type isocyanates are used for the formation of polyurethanes, because the aromatic types are more reactive than their aliphatic counterparts, and that is why the aromatic ones like MDI, and TDI are being used in most common

applications [4]. Furthermore, it is important to note that the properties of the resulting polyurethanes can be tailored to our preferences through the use of various additives. These include blowing agents, both physical and chemical, which affect the structure of the foam cell; dyes, which control the color of the polyurethane polymer product; fire/flame retardant additives, which lessen the flammability of the material; plasticizers, which lessen hardness; crosslinkers/chain extenders, which provide mechanical support; fillers, which increase stiffness and tensile strength, and so forth [5]. Based on how they behave under various temperature conditions, polyurethanes can be divided into two main groups: thermoset and thermoplastic polyurethanes. There are various types of polyurethanes, including elastomers, adhesives, rigid, flexible, and molded foams, according to the structure of the formed polyurethanes. However, the list is not exhaustive [6]. Based on the utilization of plastic and an examination of the plastics industry, it can be concluded that polyurethane currently holds one of the biggest market shares in the industry. Because a change in one of their mechanical or chemical properties can lead to the creation of new applications, the diversity of polyurethanes' properties is essential to their widespread use. As for the fields of use polyurethanes appears in building and construction, transport and marine, furniture and bedding, machinery and foundry, appliance, packaging, textiles, fiber and apparel, electronics, footwear, and many others according to the United States polyurethane production by end-use market [7].

2.2. Degradation of polyurethane

As previously mentioned, due to its extensive range of mechanical and chemical properties, polyurethane can be considered one of the most widely used type of polymer in the plastics industry. The demand for polyurethane-based products is increasing daily, as evidenced by the compound's 4 million metric tons produced in Europe and an astounding 25 million tons produced globally per year [2]. Even though polyurethane products greatly simplify daily living, the rising demand is also a major deterrent. Because of the high sustainability of these products, it is difficult to get rid of polyurethane products when they are no longer needed [7]. Additionally, due to their high demand, the production of polyurethane generates a significant amount of industrial and post-consumer waste that needs to be treated in order to preserve environmental protection and increase the recyclability of polyurethane production [4]. The production of polyurethane through recycling doesn't produce as much waste as it could if end-of-life polyurethane waste recycling wasn't taken into account [8]. Therefore, the primary objective is to recycle polyurethane wastes by employing various degradation mechanisms and thus maintaining control over the waste management of these polymers. With an appropriate

degradation method, even new raw materials, such as recycled isocyanates or recycled polyols, can be used in the production of new polyurethane products. This means that the degradation products of the polyurethane wastes present an opportunity to create new polyurethane products. [9] The degradation of polyurethane wastes can be achieved in various ways. Each degradation category has its advantages and disadvantages, so there is still no clear consensus on how to effectively apply different degradation options in waste management related to recycling [9]. The main possibilities for polyurethane degradation include mechanical, thermal, photo-, biological, and chemical degradation

2.2.1. Chemical degradation

Chemical degradation processes offer the most efficient methods of breaking down polyurethanes [6], [8]. Due to their extensive history aligning with the development of polymers, chemical degradation not only enables the elimination of polyurethane wastes but also facilitates recycling[4][6]. [6], [8][6] Recycling occurs in all chemical degradation processes because, following degradation, monomers or building blocks, polyols, isocyanates, and amines can be recovered in the case of polyurethanes[4], [6][6]. Recoverable polyols, isocyanates, or amines can be utilized to create newly formatted polyurethanes, minimizing waste, and providing an excellent environmental model[4][6]. Chemical degradation techniques can be categorized based on the kind of reaction that takes place during the process. In addition to the four frequently employed reaction techniques hydrolysis, hydrogenation, glycolysis, and aminolysis, acidolysis and alcoholysis should also be considered[9][6]. Due to the slow reactions and the need of specific conditions for the degradation reaction to occur, it was concluded that catalyst is always necessary. An overall representation of the different chemical degradation methods can be seen inFigure 2[6]:

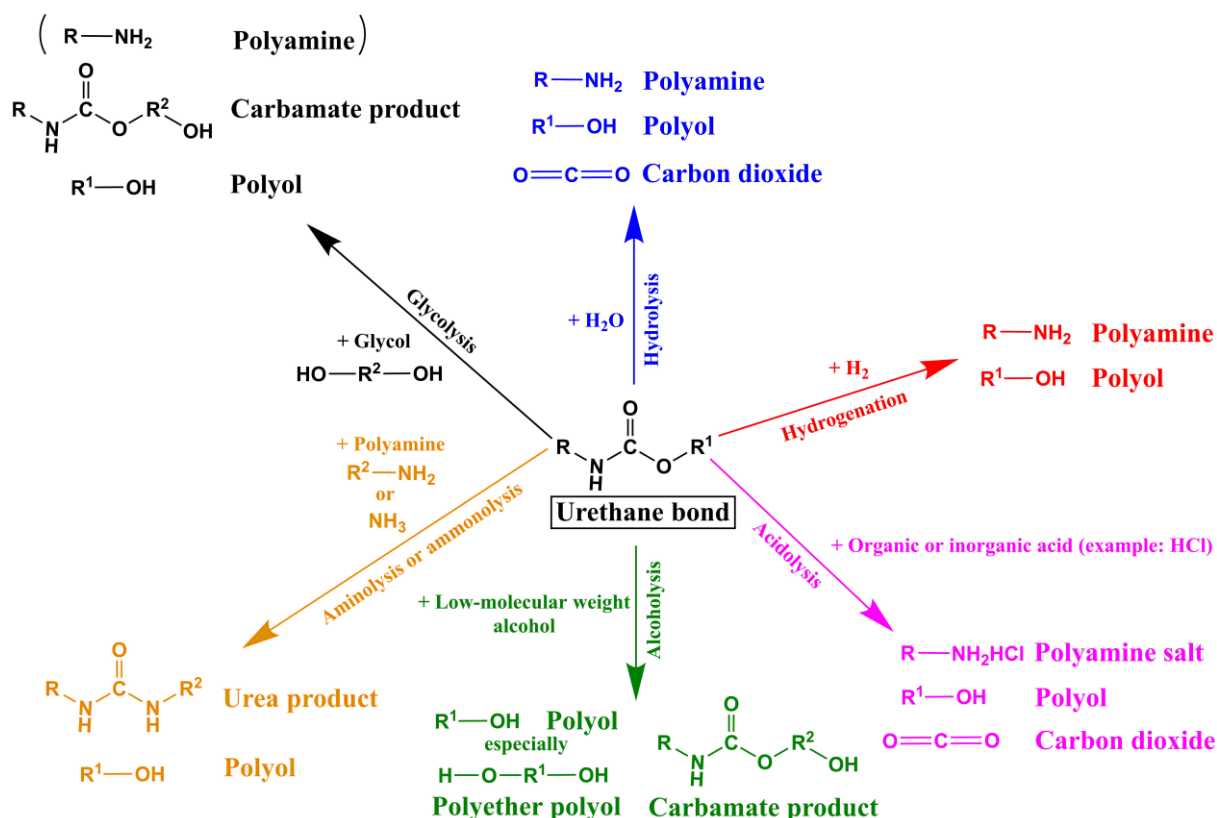


Figure 2 Chemical degradation pathways of polyurethane and the corresponding products.

[10][4] In this work, the chemical degradation of TDI-based urethanes was carried out by catalysed glycolysis. Glycols belonging to the alcohol family, such as ethylene glycol, are the most widely used type of glycol for degradation of polyurethanes [10]. Ethylene glycol has a high boiling point, above 190 °C. In addition to simple glycols, other glycols, like polyethylene glycol can also be used to perform the degradation process, as mentioned in several studies [4]. A transcarbamation reaction between the polyol of the urethane / carbamate group and the glycol's hydroxyl group, which serves as the decomposing agent, can be seen during glycolysis. There are many different possible glycolysis setups because of the numerous studies that have been conducted in the past to determine the ideal polyurethane glycolysis conditions that yield the highest efficiency [9], [10]. One may consider two primary mechanisms of glycolysis, the single-phase and the split-phase glycolysis. Compared to the other mechanism, which is single-phase glycolysis, split-phase glycolysis has superior properties and can produce more pure products. As the name suggests, two phases can be obtained using split-phase glycolysis [10], [11]. While the bottom phase contains excess glycol that can be extracted and used in another split-phase glycolysis process, the upper layer contains recovered polyol that can be used in new polyurethane formation processes, reducing polyurethane waste, and improving the environmental friendliness of the degradation process. Other less interesting products found in

the bottom layer include various carbamate, urea, and even polyamine products [12], [13]. All four of the pillars (flexible solid, flexible foamed, rigid solid, and rigid foamed polyurethane) can be dismantled with the aid of glycolysis. Catalysts are required in the glycolysis processes because, even with their assistance, the reaction can take days. The most common catalysts for the glycolysis of polyurethane are various kinds of amines, sodium hydroxide (NaOH), potassium hydroxide (KOH), potassium acetate ($\text{CH}_3\text{CO}_2\text{K}$), and sodium acetate ($\text{C}_2\text{H}_3\text{NaO}_2$) [4], [10], [11]. Furthermore, it can be claimed that a number of reaction parameters affect the reaction temperature, such as the catalyst or even the glycolysis agent used.

2.2.2. Biological degradation

The growing demand of PU requires the formation of biodegradable polyurethanes, because the decomposition of these most commonly used polymers with conventional methods can take up years, resulting in a huge amount of wastes, which are most frequently landfilled. Biodegradable products are known for more than 50 years and carries a lot of advantages. The main aspect of the biodegradable polymers is that they decompose into carbon dioxide, methane, water, inorganic compounds, and biomass with the help of some microorganisms, which can be fungi, bacteria, and algae as well [9], [14]. The biodegradation process of the polyurethane wastes includes a fragmentation step, and a mineralization step with the help of aerobic or anaerobic microorganisms, according to whether the mineralization process during the biodegradation requires oxygen or not [9]. As for the mineralization step, [9], [14] [15] it requires different microbial enzymes, as it is necessary to break down different types of bonds in the polyurethane molecule [9], [14]. If the biodegradation of polyurethanes takes place in a laboratory environment, before the process is applied to the degradation of waste, it is advisable to conduct solid or liquid-medium assays, a sole carbon source assay, a carbon dioxide production assay, Fourier transform infrared spectroscopy analysis, GC-MS analysis, or spectrophotometric enzyme activity analysis. These analyses are essential for obtaining a comprehensive understanding of the degradation process and its critical parameters [15]. The characterization of the degradation products is important because biodegradation can play a role in the recycling of end-of-life polyurethanes.

The products of polyurethane glycolysis are carbamates which are also active pesticide ingredients. G. Rasul Chaudhry investigated the applicability of *Pseudomonas* sp. for the degradation of carbamates using carbofuran, aldicarb and carbaryl substrates. Substrate concentrations was 1000 $\mu\text{g/ml}$ and carbamate degradation was detected after 40 h [16]. T.A. Naqui and co-workers tested the bacterial strain *Pseudomonas aureginosa* for carbaryl and

carbofuran degradation. 15-day tests at different temperatures and pH were used to optimize the degradation of substrates. The end product of the degradations was carbofuran-7-phenol. This product could not be further degraded by the bacteria [17]. Namadev K. Pujar and co-workers investigated the degradation of chlorpropham [isopropyl N-(3-chlorophenyl) carbamate] (CIPC) by *Bacillus licheniformis* NKC-1 strain and the corresponding product detected was 4-chlorocatechol. The experiment was performed for 120 h at 40°C and pH 8.3 and enzyme presence was detected. The carbamate ester bonds are cleaved by a CIPC hydrolase to give 3-chloroaniline, isopropyl alcohol, carbon dioxide and chloride ions. This enzyme neutralizes the herbicide activity and thereby detoxifies the component [18]. Enzymes such as P450 monooxygenases, glutathione S-transferases, carbamate hydrolases and carboxylesterases play a prominent role in the detoxification of carbamates [19], [20]. The carbamate resistance of the insect *Helicoverpa armigera*, it been found that this phenomenon is related to the carbamate hydrolase enzyme [21], [22]. Phenylcarbamate hydrolase was isolated and purified from an *Arthrobacter oxydans* P52 bacterium and it was found that this enzyme is a monomer with a molecular weight of 55 kDa. The gene responsible for the degradation of phenylcarbamate is located in the plasmid of *A. oxydans* P52 (pHP52). The partial amino acid sequence of the gene has been documented after cloning and expressing it in *E. coli* [23]. The enzyme *N*-methylcarbamate hydrolase from *Pseudomonas* strain CRL-OK, which catalyses the hydrolysis of carbaryl, α -naphthyl acetate and phosphotriester, was also identified and purified. It was found that this enzyme is resistant to solvents and thermal denaturation [24]

The carbaryl hydrolase *cehA* and *cahA* genes have been detected in *Rhizobium* and *Arthrobacter* strains [24]. The presence of these genes has been previously identified in *Pseudomonas* and *Novosphingobium* strains [25], [26]. The *cehA* and *mcd* genes mainly encode the enzyme carbamate hydrolase and have been previously shown to play a role in oxamyl degradation [25]. The carbofuran-degrading CehA hydrolase has also been reported to be isolated from *Sphingobium* sp. strain CDS-1. More recently, the carbamate C-N hydrolase enzyme AmeH, which is involved in the degradation of methomyl pesticides, was also identified in the *Aminobacter aminovorans* strain MDW-2. Based on these results, it was concluded that the degradation of carbamate groups in pesticides is mainly carried out by hydrolases, and this knowledge may be useful for the remediation of contaminated areas in the environment [27].

Due to the model diurethane structure applied in the current study, the second component formed during glycolysis is *n*-butanol. The bacteria in the current studies should not only have esterase activity, but also butanol stress tolerance. There are literature data that several gram positive strains are tolerant to butanol such as *Bacillus subtilis* 168 or *Bacillus subtilis* GRSW2-

B1 strain. This is due to the up-regulation of SigB-positive regulator (RsbRA) and six SigB-controlled gene products in response to butanol stress, including ribonuclease R (*rnr* gene product) and a general stress protein 20U (*dps* gene product), all of which have been previously reported to be directly involved in the response to ethanol stress in *B. subtilis* [28]–[30].

3. Methods

3.1. TDI-based diurethane formation

The breakdown of the drastically increasing polyurethane wastes was initially the primary idea and focus of this work, but it is clear that studying a compound like polyurethane, which is composed of many monomers, is not an easy task. Thus, a model system was employed for the study of polyurethane degradation. We have selected a compound with two urethane bonds in its chemical structure to serve as the model system because it can accurately represent polyurethane and facilitate a clearer understanding of the chemical degradation process. The investigation of the kinetics and mechanism of the diisocyanate reactions with alcohols using computational chemistry methods served as the inspiration for the choice of starting materials. For the formation of the diurethane compound the starting alcohol was *n*-butanol, which is the third smallest first-rate alcohol, and the initial diisocyanate is 2,4-toluene diisocyanate (**Figure 3**). 2,4-TDI was selected because it is a frequently used species in urethane synthesis.

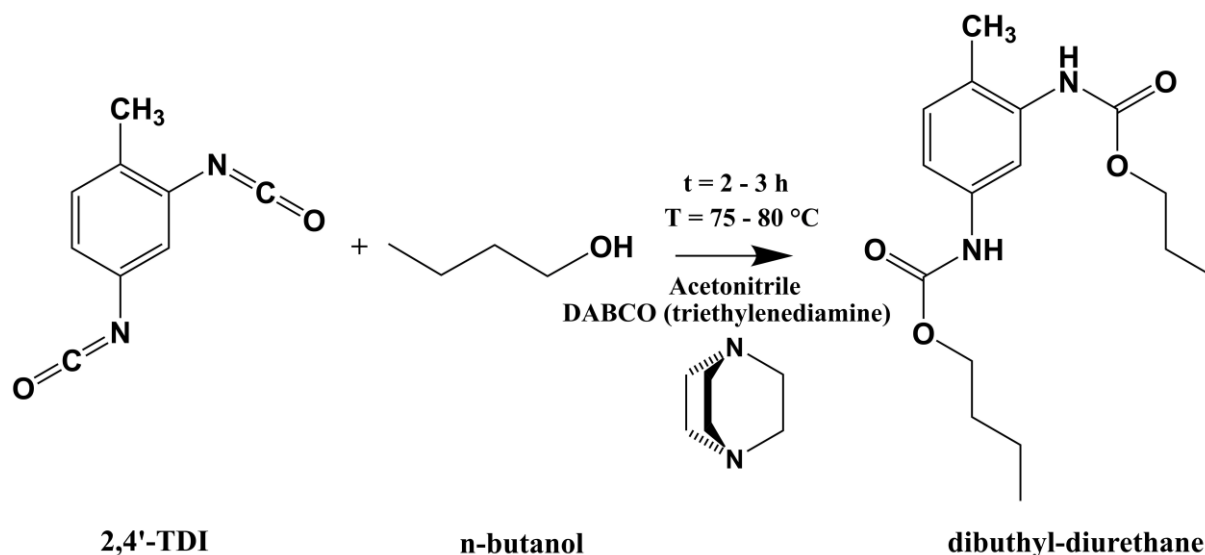


Figure 3 The synthesis of the model diurethane.

Acetonitrile was also present in the system as a solvent, because the reaction between the 2,4-TDI and *n*-butanol is highly exothermic, so the solvent made the diurethane compound production in a bigger scale safer than reacting the two materials without it. It can be concluded

that variations in the reaction's course were only evident in samples collected between one and two hours into the reaction; following that, the reaction showed no discernible differences. Because of this, using a catalyst could significantly accelerate the reaction. In the field of chemistry, a catalyst is a material that speeds up a chemical reaction without changing its chemical composition permanently. By lowering energy requirements like the activation energy, catalysts can speed up reactions. They can also improve selectivity and offer a variety of financial advantages. Since this was just the beginning of the degradation process in our case, as can be seen, a catalyst was needed to speed up the diurethane synthesis. If this had taken longer, there would have been less time for the degradation experiments, which are the primary focus of this study. Doing literature research we selected 1,4-diazabicyclo[2.2.2]octane, or in other names triethylenediamine or DABCO (in this work this catalyst will be referred to as DABCO). This compound has a three-dimensional, cage-like structure and is a bicyclic amine molecule. This chemical compound has a variety of applications, but the most advantageous one for us was that it is a widely recognized catalyst for a number of organic transformations. It is frequently used as a catalyst in the synthesis of polyurethanes because it helps the formation of urethane linkages from isocyanate and alcohol starting materials. Additionally, it has the ability to catalyze processes that results in N-protected amino acid synthesis, the Michael addition, or the Knoevenagel condensation as well. It could be said that using a temperature range of 75 to 80 °C and this catalyst, the reaction could end in about two or three hours. This was ideal and easily reproducible, and it helped the entire glycolysis processes by providing a quick way to produce glycolysis raw materials. As previously mentioned, the use of DABCO did not alter the theoretical reaction mechanism of the diurethane formation represented because catalysts do not undergo chemical changes during reactions.

Nevertheless, explaining the reaction conditions does not clarify how the formation of diurethane was tracked. Numerous analytical techniques, including spectrophotometry, titration, and chromatography, can be employed to monitor a chemical reaction. In our research to follow the diurethane formation FTIR was used. The primary objective of the reaction tracking was to ensure that the peak, which is associated with the isocyanate (-NCO) bond's resonance frequency, disappeared. This signifies that all of the 2,4-TDI had reacted, and that the reaction could be terminated since all of the initial isocyanates had been transformed into the dibutyl diurethane product. It occurred around two or three hours into the reaction, as was previously mentioned when employing DABCO as a catalyst and higher reaction temperatures. Even though the degradation of the generated diurethane model compound is the primary focus of this work, the process of producing this material was not sufficiently explained in earlier

studies, so it was necessary to investigate what instruments were required for the diurethane synthesis. The equipment for the diurethane synthesis contained a 500 cm³ reaction flask, a drip funnel, spiral cooler, a magnetic induction heater, and an electric thermometer. We started by dropping butanol into the reaction flask gradually after adding the butanol and DABCO mixture to the drip funnel. To prevent a sudden temperature rise, and achieve a safer synthesis reaction, we only added a small amount of butanol droplets to the reaction mixture as opposed to adding the entire amount to the reaction flask, which had the TDI solution, which had a mass percentage of 15% or 30%. After the production, diurethane has been cleaned using atmospheric distillation and rotary evaporation.

3.2. Glycolysis

There was sufficient model system for the chemical degradation process to proceed with glycolysis following the diurethane formation and cleaning process. Ethylene glycol is a widely used glycol that has been chosen for glycolysis. The primary rationale behind choosing this particular compound was to maintain the reaction's simplicity and avoid overcomplicating the entire idea. The chemical structure of the starting diurethane compound was already shown, and the molecular model of the ethylene glycol is also well-known so the reaction mechanism of the glycolysis can be represented (**Figure 4**).

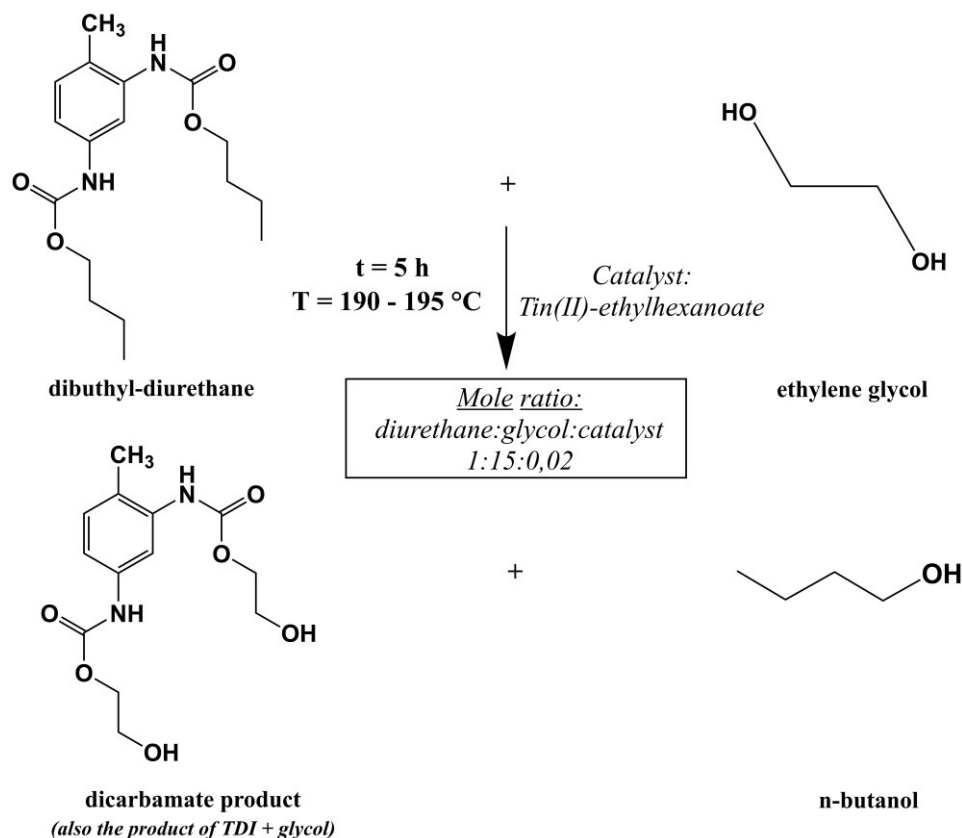


Figure 4 The studied glycolysis reaction mechanism.

Figure 4 illustrates why chemical degradation can be a viable solution for managing polyurethane waste. The glycolysis reaction can recover the raw material polyol fraction, which is used to form urethane, as a byproduct. The main goal of this study was to determine whether the glycolysis and biological degradations are feasible, to demonstrate that they can be applied to the recycling of urethane, and also to compare the two different degradation methods. According to the literature, the general idea was to perform glycolysis using the tin(II)-ethylhexanoate catalyst and the mole ratios mentioned above. Since the distillate formation, or the production of *n*-butanol, ceased after five hours in both the test and main experiments, all of the glycolysis reactions were examined for five hours. The temperature was chosen in order to distillate the excess butanol that was left on the diurethane product after the model compound was cleaned and the *n*-butanol that was being created during the glycolysis reaction. It was crucial to avoid reaching the ethylene glycol's boiling point of 197.3 °C, because doing so could have caused the reaction to shut down. Instead, the temperature was maintained between 190 and 195 °C. It's also important to note that during the test experiments, it was observed that glycolysis did not begin (no distillate formation occurred) until the temperature reached the chosen 190 °C, and earlier studies have also indicated that glycolysis requires this temperature. As was partially discussed in the previous chapter, we conducted some testing experiments to determine the ideal reaction conditions, including the ideal temperature and reaction time, as well as whether a catalyst is necessary for glycolysis. The choice of condenser was the first crucial step. There were two possibilities available to us. The Liebig condenser, which was also utilized for product cleaning, and the Marcusson condenser. To determine which condenser aids in glycolysis and which does not, we performed reactions with both. The amount of distillate formation was the primary factor because the glycolysis reactions could be monitored by tracking changes in the distillate volume over time. For this reason, we required glycolysis equipment that facilitates the easy condensation of boiling *n*-butanol, resulting in rapid distillate formation and, consequently, an appropriate tracking method for monitoring the glycolysis's progress. We used a glycolysis reaction test to evaluate the two condensers. The Liebig condenser aids in the tracking of glycolysis and the distillation of *n*-butanol, according to theoretical calculations, because the observed distillate volume was more in line with the theoretically predicted distillate volume in this particular case. The equipment for the glycolysis contained a magnetic induction heater, two thermometers, test tube with marks on each volume to track the distillate volume, a reaction flask, and the above-mentioned Liebig condenser.

3.3. Computational chemistry methods

However, laboratory experiments have been done in order to determine the optimal reaction circumstances such as temperature, time, appropriate equipment and also the right catalyst, and mole ratios during the glycolysis and the diurethane synthesis as well, we wanted to investigate the reaction mechanism, and different effects on the diurethane synthesis and glycolysis using computational chemistry methods with the help of the below mentioned softwares.

3.3.1. GaussView 6.0 [31]

The first step during the computational chemistry calculations was the creation of the reactants, corresponding complexes, intermediates, and products in the case of diurethane synthesis and glycolysis. The 3D models of these compounds were created by using the Gaussview 6.0. This software is a user-friendly program which gives the opportunity to create the 3D model of any molecules with a very clean and easy-to-use user interface. By starting the GaussView 6.0, a blank window, and the user interface can be seen (**Figure 5**). In the blank window, the molecule of interest can be built by using the user interface.

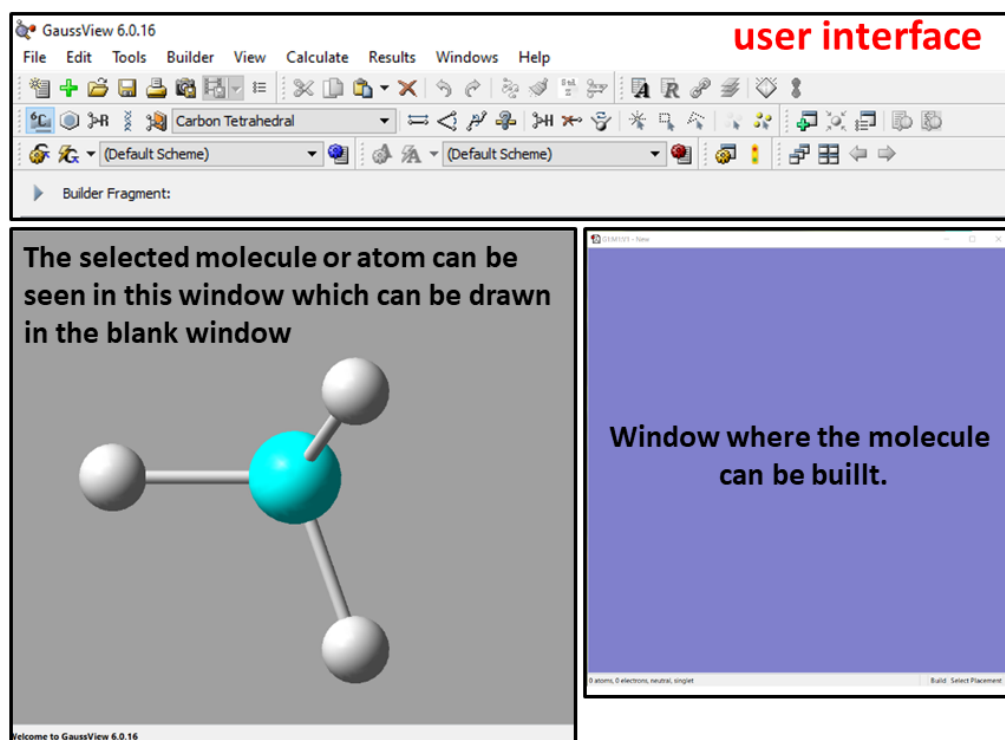


Figure 5 Opening windows of Gaussview 6.0

After the structure in question is created it can be saved as a Gaussian input file. This input file is very important because it contains information about what kind of calculation must be done, on a given molecule. The created input file can be opened in a text editor software such as TextPad or Notepad++. When opened in a text editor the following can be seen (**Figure 6**):

1	#BHandHLYP/6-31G(d) opt Freq SCRF(SMD,Solvent=Acetonitrile)	<u>Method and basis set</u>	
2			
3	n_butanol	<u>Title card</u>	
4			
5	0 1	<u>Charge and multiplicity</u>	
6	C	-6.03123422	1.35987874 6.28702332
7	H	-6.38769467	2.36875729 6.28694077
8	H	-4.96123425	1.35966007 6.28710541
9	C	-6.54481229	0.63408875 7.54442797
10	H	-6.18835041	-0.37478930 7.54451036
11	H	-7.61481227	0.63430589 7.54434529
12	C	-6.03142885	1.36001664 8.80183250
13	H	-6.38789218	2.36889418 8.80174995
14	H	-4.96142888	1.35980102 8.80191459
15	O	-6.50832086	0.68606743 9.96942253
16	H	-6.18851741	1.13827239 10.75353760
17	C	-6.54461973	0.63395231 5.02961880
18	H	-6.18957532	1.13950047 4.15596868
19	H	-6.18636876	-0.37429132 5.02860374
20	H	-7.61461793	0.63227054 5.03063315
21			
22			

Coordinate of atoms which
build up the molecule

Figure 6 The structure of a Gaussian input file.

The input can be divided into three sections: route section, title section, and molecule specification (charge, multiplicity, atom types, and the corresponding coordinates). The route section contains the level of theory, the basis set and the commands (e.g., Opt or Optimization). In this case the level of theory was BHandHLYP/6-31G(d). In the title section, a description or title can be added to the input file in question. The molecule specification includes the charge and multiplicity of the molecule, and the corresponding atoms with their respective coordinates.

3.3.2. Gaussian 09 [32]

After preparing the 3D models of the molecules, optimization and frequency calculations have been carried out with the help of the Gaussian 09 program package. The corresponding zero-point energy (E_0), enthalpy (H), and Gibbs free energy (G) of the structures were collected, and the thermodynamic quantities which describe the reactions were computed. As soon as the input files were ready, the above-mentioned calculations could be carried out by using the Gaussian software. The calculations could take as long as a few seconds or several hours or even more. During the calculations, the software generate output files which could be opened by using a text editor or GaussView. If the calculations went well, the output files contained a final structure. Furthermore, the output file revealed whether the calculation was done, because if it could not run properly then an error message showed up at the end of the output file. However, as it was mentioned before, even if the calculation seemingly terminated normally, to avoid false results, visual inspection of the structure in GaussView was necessary.

```

HE WHO LAUGHS LAST PROBABLY DIDN'T GET THE JOKE.
Job cpu time:      0 days  0 hours  5 minutes  1.1 seconds.
Elapsed time:      0 days  0 hours  0 minutes 25.8 seconds.
File lengths (MBytes): RWF=      42 Int=      0 D2E=      0 Chk=      6 Scr=      1
Normal termination of Gaussian 16 at Fri Oct  6 17:16:15 2023.

```

Figure 7 Example of a normal termination message in the output file.

If the structure was correct, the thermodynamic parameters (e.g. zero-point energy) could be collected from the output file (Figure 7, Figure 8):

```

Zero-point correction=          0.142101 (Hartree/Particle)
Thermal correction to Energy=    0.148852
Thermal correction to Enthalpy=   0.149796
Thermal correction to Gibbs Free Energy= 0.112060
Sum of electronic and zero-point Energies= -233.379479 ← E0
Sum of electronic and thermal Energies= -233.372728 ← ET
Sum of electronic and thermal Enthalpies= -233.371783 ← H
Sum of electronic and thermal Free Energies= -233.409519 ← G

          E (Thermal)          CV          S
          KCal/Mol          Cal/Mol-Kelvin  Cal/Mol-Kelvin
Total          93.406          22.534  Entropy 79.422

```

Figure 8 Thermochemistry in a Gaussian output file.

These steps had been followed and thereafter, the collected thermodynamic properties were sorted in Excel and used in further evaluation.

3.4. Microbiological methods

Microorganisms with hydrolytic or esterase activity are often used to degrade urea products. Therefore, strains available in our laboratory were tested on agar plates containing Impranil DLN SD, where Impranil was the carbon source, according to the detection methods known in the literature for esterase enzyme activity [15], [33], [34]. Microbial strains having esterase activity will be useful for further analysis of urethane degradation. We also used glycolysis products as substrates to map the hydrolytic enzymes of our bacterial strains, where the clearing zones on agar plates indicated positive enzyme activity.

Table 1 Bacterial strains used to degrade glycolysis products.

Species	Strains
<i>Pseudomonas fluorescens</i>	ATCC13525
<i>B. subtilis</i>	N11-PM12
<i>B. velensis</i>	GZCB6
<i>B. subtilis</i>	MD28

Screening microbial ester hydrolysis by LB agar plate assays [35], [36]

Luria-Bertani (LB) medium was prepared by adding 5 g yeast extract, 10 g NaCl, 10 g tryptone, and 13 g agar to 1000 ml dH₂O, and finally this medium was complemented with 3-6 ml Impranil DLN SD. The cultured plates were incubated for 5-10 days at 30°C.

Screening microbial ester hydrolysis by MSM agar plate assays

0.9 g Na₂HPO₄, 0,7 g KH₂HPO₄, 2 g NaCl, 0,4 g MgSO₄, 0.1 CaCl₂, 2 g NH₄Cl, 2 ml Element solution (per 100 ml: 0.1 g Cu(NO₃), 0.02 FeSO₄, 0.3 g Mn(NO₃)₂ 0.4 g Zn(NO₃)₂), 13 g agar and 0.1 % ml Impranil DLN SD, 0.01% methyl red indicator was completed by 1000 ml dH₂O. The incubation of the cultured plates was 1 day at 30°C.

Screening microbial hydrolysis of glycolysis products using LB agar plates

1000 ml of the conventional Luria-Bertani (LB) medium was complemented with 0,5% of glycolysis product (30 m/m% 2,4 TDI diurethane glycolysis product) and the plates were incubated 30 days at 30°C.

Screening microbial hydrolysis of glycolysis product by MSM agar plate assays

0.9 g Na₂HPO₄, 0.7 g KH₂HPO₄, 2 g NaCl, 0,4 g MgSO₄, 0.1 CaCl₂, 2 g NH₄Cl, 2 ml Element solution (per 100 ml: 0.1 g Cu(NO₃), 0.02 FeSO₄, 0.3 g Mn(NO₃)₂ 0.4 g Zn(NO₃)₂), 13 g agar and 0.1 % glycolysis product (30 m/m% 2,4 TDI diurethane glycolysis product), 0.01% methyl red indicator was supplemented with 1000 ml dH₂O. The incubation period was 1-10 day at 30°C.

4. Results

4.1. Effects of the change of TDI solution m/m% on diurethane synthesis

The first thing which was interesting to investigate was connected to the first step of this research which was diurethane synthesis. As it was said, we had to make a solution from the 2,4-TDI to keep the diurethane synthesis safe in a bigger scale. However, we wanted to observe what happens if we change the starting TDI solution's m/m%, from 15 m/m%, which was recommended in several literatures, to 30 m/m%. Without DABCO catalyst addition the reaction took 1-2 days to fulfil, and the **TDI solution m/m% does not affect the reaction**

time without catalyst. With the use of catalyst, it could be discovered in both cases (15 m/m% and 30 m/m%) that after 2-3 hours there were no 2,4-TDI left in the reaction mixture according to IR analysis. Although the reaction time cannot be influenced with the change of the starting TDI solution concentration the other thing which had to be looked at was the change of temperature during the *n*-butanol addition, because after that the reaction mixtures were heated to reflux temperature (**Figure 9**):

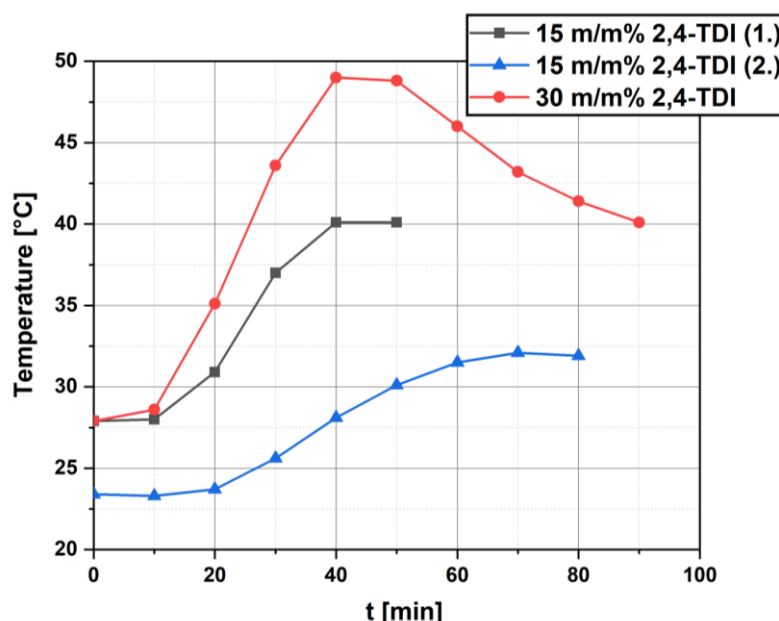


Figure 9 Temperature change during the *n*-butanol addition.

It can be seen in **Figure 9** that the highest temperature rise was occurred when the most concentrated (30 m/m%) 2,4-TDI solution was used as a starting diisocyanate solution. It was expected because the more concentrated a starting solution the more heat gets produced during an exothermic reaction. In conclusion a more concentrated 2,4-TDI solution than 15 m/m% was not necessary, because the same reaction time could be observed during the glycolysis, while the temperatures were much higher during the alcohol addition, which led to a longer *n*-butanol dosage process in the case of 30 m/m% 2,4-TDI solution. That is the reason why the diurethane from 15 m/m% 2,4-TDI was produced twice when we ran out of diurethane rather than using 30 m/m% TDI solution. The handling and measurement of the diurethane so the reproducibility of the reactions was the same because the diurethane products must have been cleared from the excess acetonitrile, and butanol compounds.

4.2. “Test tube” experiments (diurethane synthesis)

We made experiments to demonstrate what happens during the diurethane synthesis reaction when the 2,4-TDI and *n*-butanol were reacted in different ratios, without using any acetonitrile

solvent. **Table 2** sums up the moles, and masses of the starting materials in the three different solvent free experiments:

Table 2 Amount of starting compounds.

Ratio	2,4-TDI		n-butanol	
	<u>n [mole]</u>	<u>m [g]</u>	<u>n [mole]</u>	<u>m [g]</u>
1 : 2	0.0201	3.5072	0.0395	2.9292
1 : 5	0.0081	1.4039	0.0396	2.9350
1 : 18,6	0.0045	0.7865	0.0837	6.2067

After reacting the two starting compound three different diurethane products were created with different textures:

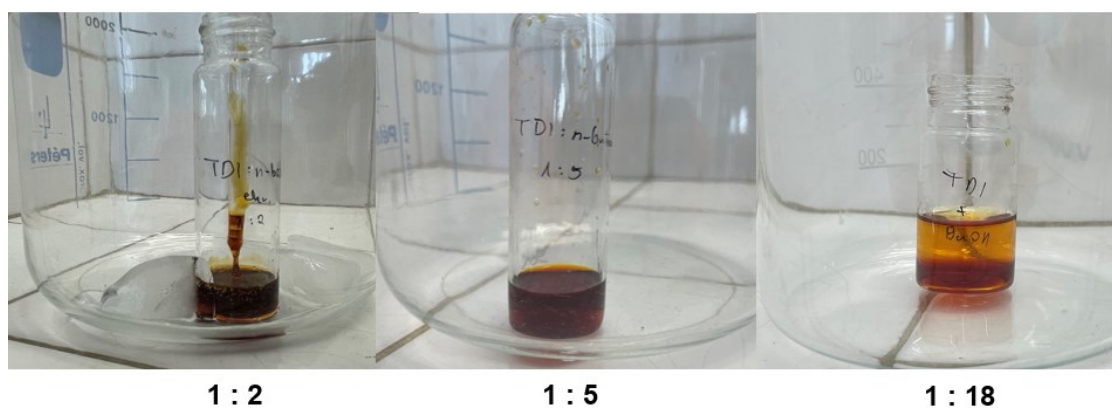


Figure 10 Diurethane products of the test tube experiments.

As it can be seen in **Figure 10** the more butanol was used the less viscose the material was, which was expected. Looking at the IR spectra of these compounds it could be said that the more butanol was used the less 2,4-TDI did the reaction mixture contain, so that is the reason why the diurethane synthesis needs excess butanol during the process. The color changing was the same as in the case of diurethane synthesis with solvent. For homogeneity all the three reaction mixtures must have been stirred gently, because the reaction is very exothermic without acetonitrile. Also, it could be observed that during the reaction of the two starting compound CO_2 gas was formed. The formation of the CO_2 gas could be associated with the reaction between the 2,4-TDI with the small amount of water which the starting *n*-butanol had in it. After a couple days the diurethane formed via the 1 : 2 ratio became drier and had a sticky consistency like a “bubble gum after chewing”. For the validation of the glycolysis reaction this 1 : 2 diurethane product was used in a glycolysis reaction because there were no excess *n*-butanol or acetonitrile solvent in the diurethane product so if *n*-butanol was formed during the process it could be only associated with the progress of the glycolysis.

4.3. Determining the needed diurethane, and the theoretical distillate volume

After discussing the results connected to the diurethane synthesis the observations taken from the glycolysis step need to be described. The glycolysis setup was tested and finetuned and the appropriate reaction conditions and the amount of diurethane required for the glycolysis were also determined. The tracking of the glycolysis reaction was carried out by paying attention to the distillate volume change in the progress of time. Therefore, we had to think of a distillate amount which we wanted to get after the end of the glycolysis and by that volume we could calculate the required diurethane to get the distillate volume desired. We thought that 5 cm³ distillate volume was enough for the investigation of the glycolysis reaction. However we expected 5 cm³ and calculated the required diurethane amount for that distillate volume, with these preliminary calculations we only took into account the volume that is created during the glycolysis rather than calculating with both the volume formed during the glycolysis and the volume that was distilled from the diurethane product as well, but after calculating the needed starting diurethane, the expected butanol volume after the end of the glycolysis was supplemented with the excess butanol volume distilled from the measured diurethane.

With the help of preliminary calculations, we could calculate the required diurethane mass for every glycolysis carried out with the diurethane. When the 15 m/m% 2,4-TDI solution was used to synthesis the diurethane, for 5 cm³ of butanol distillate formation during the glycolysis 20.8139 grams of diurethane required, while in case of the other system prepared from 30 m/m% 2,4-TDI solution, 24.005 grams were needed. These calculations were carried out by Excel and Excel's add-on called Solver to shorten and simplify the calculation process of the required diurethane mass and the theoretical butanol volume gotten after the glycolysis.

4.4. Glycolysis reactions

We made two test experiments and nine glycolysis experiments (Table 3, Table 4). All of the glycolysis reactions were studied for 5 hours, because in the test experiments, and the main experiments as well, it could be seen that after 5 hours the distillate formation (formation of the *n*-butanol) stopped.

Table 3 Details of the reactions (pt. 1).

Reactions	G1 (test)	G2 (test)	G3	G4	G5
TDI : <i>n</i> -BuOH	1 : 5	1 : 5	1 : 5	1 : 2	1 : 5
<i>m</i> _{diurethane} [g]	18.2	16.8851	20.6559	3	21.0553

m_{glykol} [g]	29.9464	31.7514	64.6410	21.6982	60.1460
diurethane : glycol	~ 1 : 15	~ 1 : 15	~ 1 : 15	~ 1 : 15	~ 1 : 15
catalyst	Tin(II)-ethylhexanoate	Tin(II)-ethylhexanoate	Tin(II)-ethylhexanoate	Tin(II)-ethylhexanoate	Tin(II)-ethylhexanoate
V_{theoretical, BuOH} [cm³]	17.3	16.1	20.4	1.7	20.1
V_{distillate} [cm³]	10.5	11.2	13.6	1.6	13.8
Distillate/theoretical [%]	60.6	69.7	66.5	94.0	72.8

Table 4 Details of the reactions (pt. 2).

G6	G7	G8	G9	G10	G11
1 : 5	1 : 5	1 : 5	1 : 5	1 : 2	1 : 5
22.5545	24.97	21.1373	12.8227	12.47	12.47
69.7239	66.53	20.2118	72.8166	36.36	36.01
~ 1 : 15	~ 1 : 15	~ 1 : 5	~ 1 : 30	~ 1 : 15	~ 1 : 15
DABCO	-	Tin(II)-ethylhexanoate	Tin(II)-ethylhexanoate	DABCO	-
22.33	24.72	16.14	9.79	9.52	9.52
13.8	15.6	12.0	7.4	6.2	5.2
61.8	63.1	74.4	75.6	65.1	54.6

In these reactions we investigated the effect of the catalyst (G3, G6, G7 – G5, G10, G11), the diurethane – glycol ratio (G5, G8, G9), the glycolysis temperature and time of the glycolysis, and, we had the opportunity in the case of G4 reaction to validate the feasibility of the glycolysis reaction with these materials, and circumstances. After the correction of the expected and distillate volumes, the effects, and another validation method will be discussed.

4.5. Correction of distillate volumes using gas chromatography

The calculations which were described above were appropriate for defining the required diurethane as well as the theoretical distillate volume, but those calculations had to be corrected with the help of gas chromatography to calculate the real expected distillate volume by calculating the analytically corrected excess butanol volume in the diurethane. It can be said that the glycolysis distillate did not only contain butanol, so the volume of the measured

distillate had to be corrected as well only for determining the butanol volume in the distillate. With the help of the correction, we could correct the theoretical (expected) volumes by calculating the real butanol amount produced during glycolysis, and the real excess butanol amount in the diurethane. Also, we could calculate how much of the distillate was actually butanol. The corrected values can be seen in **Table 5** and **Table 6**:

Table 5 Corrected volumes associated with the glycolysis reactions (pt. 1).

Number	G1 (test)	G2 (test)	G3	G4	G5
TDI : n-BuOH	<i>1 : 5</i>	<i>1 : 5</i>	<i>1 : 5</i>	<i>1 : 2</i>	<i>1 : 5</i>
diurethane : glycol	<i>~ 1 : 15</i>	<i>~ 1 : 15</i>	<i>~ 1 : 15</i>	<i>~ 1 : 15</i>	<i>~ 1 : 15</i>
catalyst	Tin(II)-ethylhexanoate	Tin(II)-ethylhexanoate	Tin(II)-ethylhexanoate	Tin(II)-ethylhexanoate	Tin(II)-ethylhexanoate
V_{expected} [cm³] (corrected)	<i>10.67</i>	<i>9.90</i>	<i>11.94</i>	<i>1.70</i>	<i>12.35</i>
V_{distillate} [cm³] (corrected)	9.05	9.65	11.12	1.07	12.27
Distillied /expected butanol [%]	84.82	97.47	93.13	63.9	99.35

Table 6 Corrected volumes associated with the glycolysis reactions (pt. 2).

G6	G7	G8	G9	G10	G11
<i>1 : 5</i>	<i>1 : 5</i>	<i>1 : 5</i>	<i>1 : 5</i>	<i>1 : 2</i>	<i>1 : 5</i>
<i>~ 1 : 15</i>	<i>~ 1 : 15</i>	<i>~ 1 : 5</i>	<i>~ 1 : 30</i>	<i>~ 1 : 15</i>	<i>~ 1 : 15</i>
DABCO	-	Tin(II)-ethylhexanoate	Tin(II)-ethylhexanoate	DABCO	-
<i>13.04</i>	<i>14.43</i>	<i>12.39</i>	<i>7.52</i>	<i>7.31</i>	<i>7.31</i>
11.84	13.34	10.21	5.63	5.34	4.34
90.80	92.43	82.41	74.87	73.51	59.37

It can be said based on these values that during the glycolysis in most cases more than 90 % of the expected butanol volume were formed, which means (after calculating what percentage of the expected volumes were excess butanol distilled from the diurethane) that glycolysis had taken place in those reactions. However, there are some reactions where the ratio was less than 90 %, it is obvious from the analytical results that glycolysis also appeared to be during those reactions as well, because the measured distillate volumes are far more than the excess butanol

which could have been distilled from the diurethane product. In the next chapters the effects of different circumstances will be discussed by looking at the change of the ratio between the distillate and expected butanol versus the change of time.

4.6. Validation of glycolysis

4.6.1. Validation with G4 reaction

There were a lot of preliminary calculations which was later supported by analytic measurements, but it was important to identify that the distillate which was formed during the glycolysis could be originated from the glycolysis and do not contain any other compound in a big amount or excess butanol which could have been distilled from the starting diurethane. For this purpose, to validate that the distillate contained (almost) only butanol and which could only form during the glycolysis we used a special diurethane system. The making of this special diurethane compound was described earlier in **Chapter 4.2** (*“Test tube” experiments (diurethane synthesis)*) but for revision we describe the most important properties. It was mentioned before in general for the diurethane synthesis, we used butanol excess to provide enough butanol compound for the 2,4-TDI to react with, but this caused that additional butanol remained in the system containing diurethane after all of the isocyanate groups have reacted. Also, for safety reasons we made a solution for the 2,4-TDI because the reaction between the diisocyanate and the alcohol is highly exothermic. In the formation of the starting diurethane compound which we used in this G4 glycolysis we did not make a solution out of the starting 2,4-TDI (that is why we made it in a test tube only for representative reasons). This eliminated the possibility that during the glycolysis of this diurethane acetonitril could get into the distillate, because it was not present in the system. But what if during the glycolysis reactions only the excess butanol from the not totally clean diurethane product caused distillate formation? For answering this question and to validate the glycolysis apart from the solvent free method, we used equimolar ratios at the diurethane formation so for 1 mole of 2,4-TDI 2 moles of n-butanol was measured into, because the TDI has two isocyanate functional groups which can react with the butanol. That eliminated the possibility that during the G4 reaction, the distillate would contain only butanol from the distillation of the diurethane starting compound because there was not any excess butanol on the starting diurethane. By the G4 experiment it can be said that distillate formation took place in the glycolysis reaction, which could only occur due to the glycolysis because the starting diurethane did not have excess butanol or acetonitril, so the butanol could only arise from the reaction which can be seen in **Figure 4**. As a consequence it can be stated that the G4 reaction showed that glycolysis can be

carried out using 190 – 195 °C temperature, 1 : 15 diurethane – glycol ration, and tin(II)-ethylhexanoate catalyst, and the reaction progresses as **Figure 4** shows.

4.6.2. Validation with standard dicarbamate + HPLC

The glycolysis was appropriately validated with the G4 reaction, but in that case only the distillate so the butanol formation was considered. It can be also interesting to investigate whether the remaining glycolysis products contained the dicarbamate compound which can be seen among the products of the glycolysis reaction represented in **Figure 4**. As for the investigation we made a standard dicarbamate compound by reacting the 2,4-TDI with ethylene glycol, in the same reaction circumstances which was used in the diurethane synthesis. The reaction from which the dicarbamate compound formed can be seen in **Figure 11**:

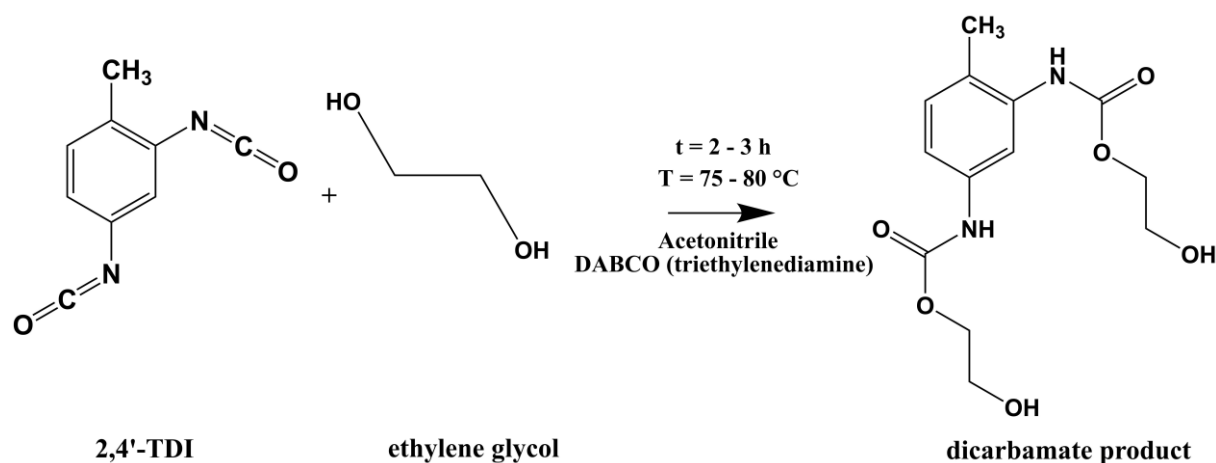


Figure 11 Reaction to produce the dicarbamate product.

After producing enough dicarbamate compound we selected the G3, G4, G8, and G9 glycolysis products to subject them to analytical measurements with the help of HPLC (High Performance Liquid Chromatography) qualitative determination. First, we diluted the standard and the selected products to 2000x, because if the substance is too concentrated than the chromatogram is useless because the peaks cannot be separated from each other and cannot form a gaussian curve at different retention times, which can be associated with the present of different compounds, that can be found in the substance. As for the HPLC method the moving phase (eluent) was the mixture of H₂O and acetonitrile in a ratio of 1 : 1. The reason why the ratio was 1 : 1 is because if the ratio is different, then the polarity of the eluent can change causing the different compounds to be detected in the very early stage of the chromatography process and that can cause also a useless chromatogram because the peaks can be too close to each other. The right polarity was a key because the separation was based on polarity differences, and that's why different compounds was present at different retention times, because they could

react with the stationary phase differently until they hit the detector at different times (retention times). After the HPLC examination it could be said that in every investigated glycolysis product the carbamate product was present, but the interesting fact was that it was present in different forms, because during the glycolysis **oligomerization** took place, which was evidenced by the peaks appearing at different retention times. The more time it took for the oligomer to be detected the more ring number did it contain:

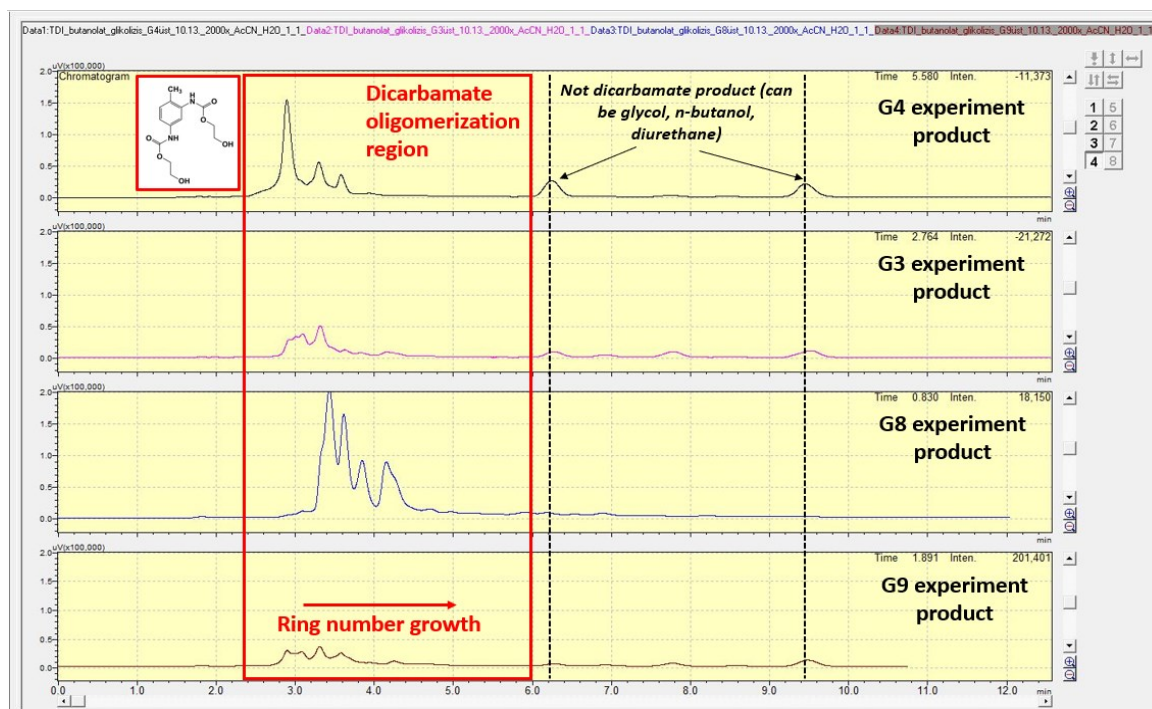


Figure 12 Chromatograms of different glycolysis products.

Looking at the chromatograms it can be said that in most cases the original single ring dicarbamate was present in the smallest extent, while there were two and more ring numbered dicarbamate oligomers which were present to a considerable extent. Also it can be seen that changing the diurethane : glycol ratio had an effect on the oligomerization because in the case where the ratio was 1 : 5 there was a notable amount of single ring dicarbamate, but in the case of the 1 : 30 ratio glycolysis the peak which could be associated with that the compound was almost absent, and the multiple ring numbered oligomers' amount started to rise so the least glycol excess was within which during the glycolysis the least oligomerization took place. Also, it could not be missed to investigate whether the peaks on the chromatograms could be associated with the different oligomers. This investigation was carried out with the “spiking” technique. During this technique we selected the G4 dicarbamate product diluted to 2000x with acetonitrile and we added 10 µl, 20 µl, and 40 µl standard dicarbamate to this mixture. To our greatest delight it could be stated that the above-mentioned determination method was

appropriate, and the examined peaks could have been linked with the different oligomers of the dicarbamate product, because the peaks have risen in the effect of the growing amount of standard compound.

4.7. Computational chemistry results

4.7.1. Thermodynamic properties of diurethane formation (steric hindrance)

It was interesting that in the case of catalyst-free diurethane synthesis after the first hour a mentionable reduction in the isocyanate groups presence was observed using the IR spectrum, but after the first hour the isocyanate peak on the spectrum did not really decrease much. Also, it could be said that without a catalyst several days were needed for the reaction to take place. This got our interest considering the mechanism and energetic conditions of diurethane synthesis without the use of catalyst. The energetical investigation was carried out using computational chemistry mentioned before in the **Methods** section. There were two different possible reaction pathways according to the catalyst-free diurethane synthesis, which can be seen in **Figure 13**:

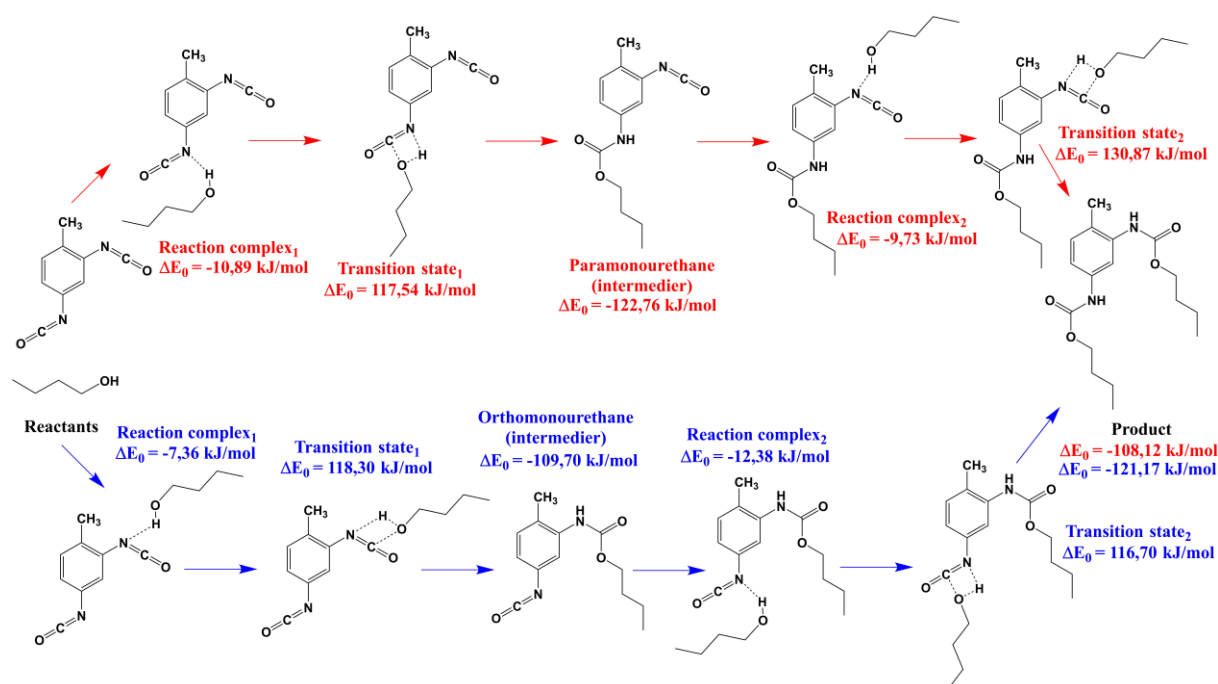


Figure 13 Two possible reaction mechanisms of catalyst-free diurethane formation.

In one case the *n*-butanol could react with the isocyanate group of the 2,4-TDI in the ortho position, creating an ortho-monourethane, and after that this intermediate could react with another *n*-butanol forming the dibutyl diurethane model system, while in the other case the *n*-butanol could react with the isocyanate group of the 2,4-TDI in the para position, creating a para monourethane, and after that this intermediate could react with *n*-butanol forming the

dibutyl diurethane as well. The two reactions have different energetical properties, so different activation energy values. The activation energy is the energy value which the reactants must reach to be transformed into a product. From this it can be said that the lower the activation energy the more reactant molecules can be transformed into product molecules so the reaction can be carried out faster. The change of the zero-point corrected energy as a function of the reaction coordinate (the reaction coordinate means the progress of the reaction) can be seen in **Figure 14** representing the different energy values:

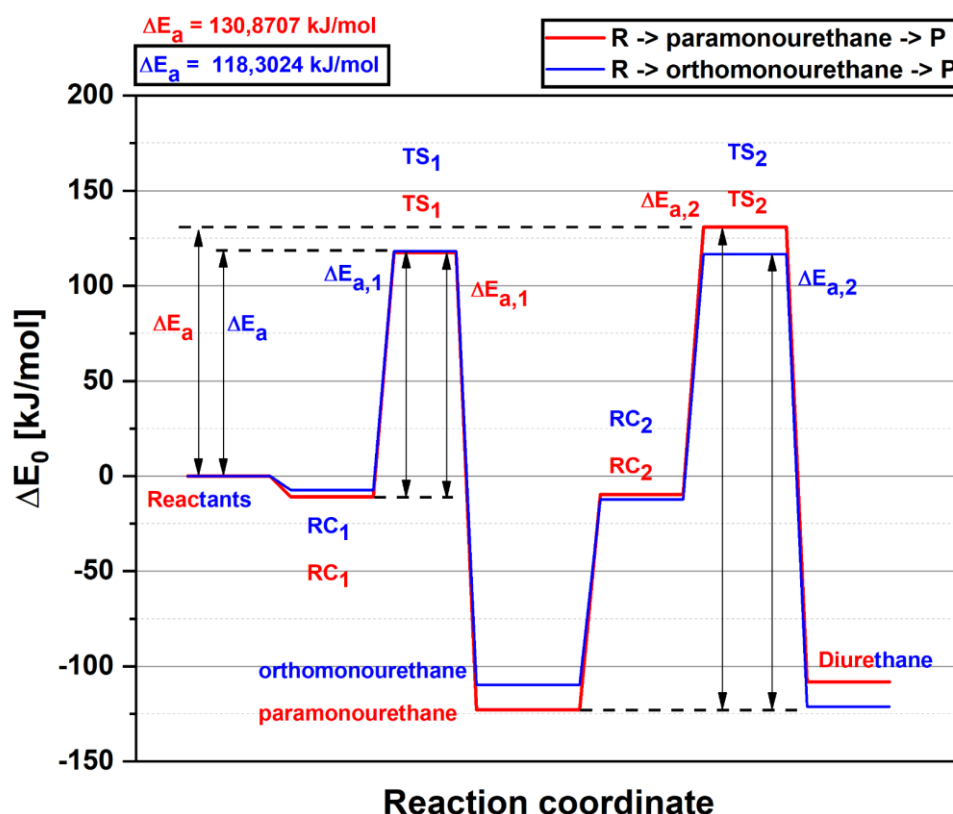


Figure 14 Energy change during the two different pathways leading to diurethane formation.

Figure 14 shows that the activation energy leading to the formation of ortho-monourethane intermedier occurred in the catalyst-free diurethane synthesis is lower. But para-monourethane is more stable compared to the ortho form. Further studies are needed and the catalytic diurethane formation, and glycolysis will be investigated.

4.8. Biological degradation results

The microbial degradation of polymers typically involves the use of multiple enzymes, with esterases playing a crucial role. Therefore, testing our bacterial strains for the presence of esterase enzymes is essential. Several esterase screening methods were employed, but the most successful outcomes were obtained using 0.1% Impranil DLN SD supplemented LB agar plates

(Figure 15, Figure 16). As we can see from the plate images, the size of the clearing zone around the bacterial colony indicates the presence of enzyme activity. This implies that if the studied bacterium has the enzyme needed to break down the substrate, it can utilize the substrate in the culture medium as an energy source for division. As a result, the substrate around the developing colonies is consumed, as indicated by the appearance of a clearing zone. The larger the clearing zone, the more efficient was the degradation of the applied substrate.

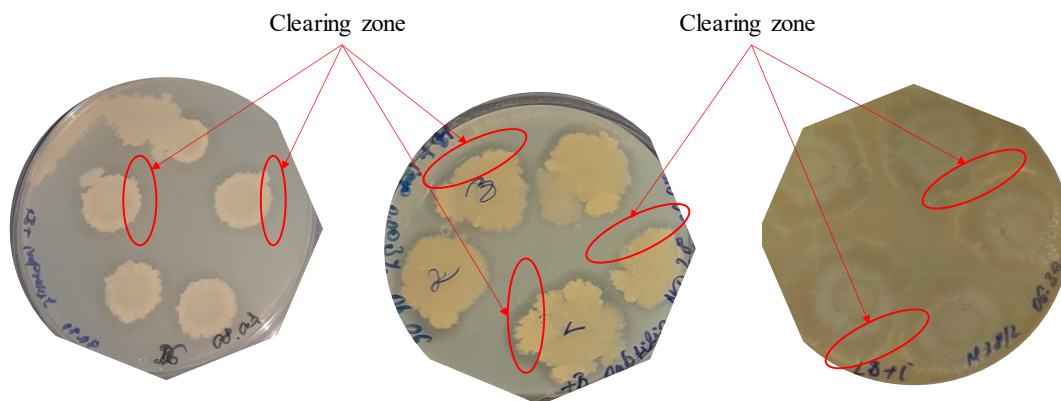


Figure 15 Esterase enzyme detection for Impranil DLN SD substrate on LB agar.

Figure 16 shows that the *B. velensis* GZCB6 strain showed the highest enzyme activity even after 5 days on LB agar plates supplemented with 0.1% Impranil.

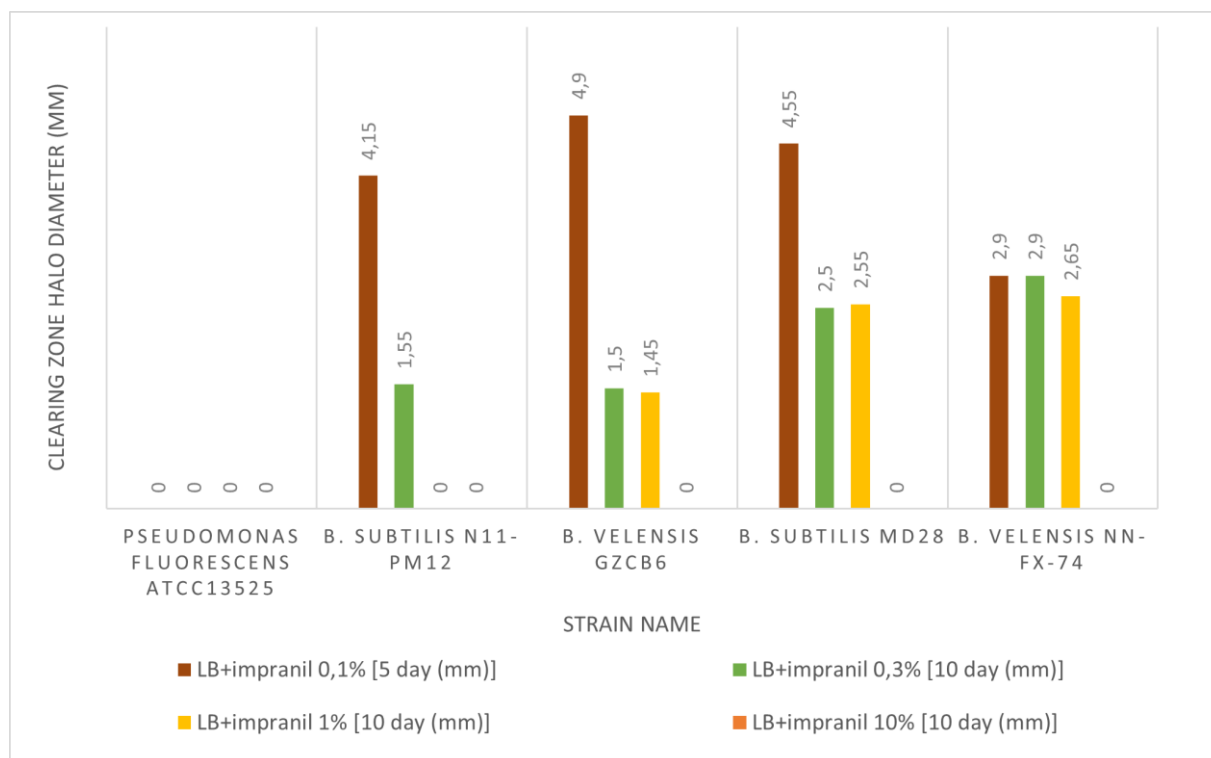


Figure 16 Esterase enzyme detection for Impranil DLN SD substrate on LB agar.

In order to confirm the observed esterase activity, Impranil DLN SD substrate was added to MSM nutrient solution. MSM culture medium is a carbon-free mineral salt medium, which is frequently used for the enrichment or isolation of microbes with specific enzymes, as well as for the detection of particular enzyme activities. This assay yielded results after 2 days, with faintly visible clearing zones (**Annex 2**). In this assay (**Figure 16**), we used methyl red as an indicator, which is a pH indicator; it is red below pH 4.4, yellow above pH 6.2 and orange in between. As you can see, the colour of the plates changed around bacterial colonies that had esterase enzymes. The larger this discolored ring, the more intense the enzyme activity.

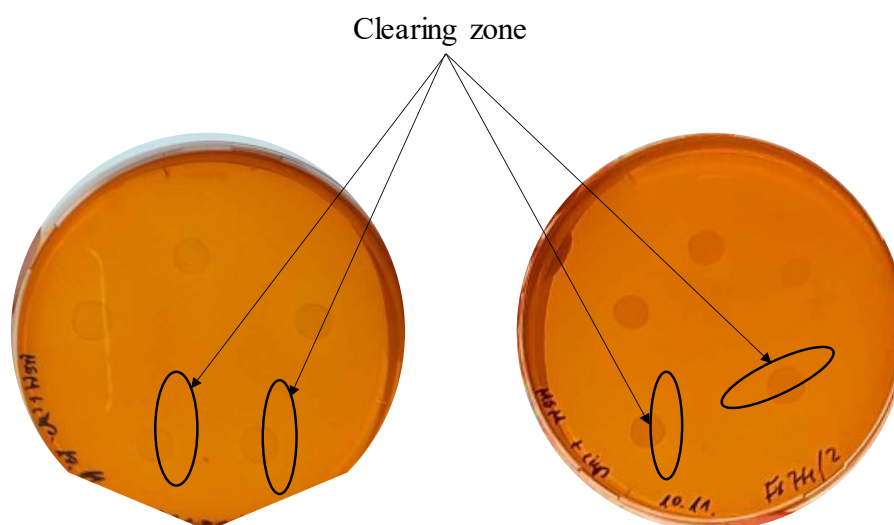


Figure 17 Enzyme activity amplification on MSM + methyl red agar plates with 0,1% Impranil DLN SD substrate supplementation.

From previous experiments, it has been confirmed that the tested bacteria possess esterase activity. As the next step, we further investigated them using glycolysis products as substrates to identify which strains might be the most effective in polymer (ultimately the PU) degradation. Initially, we added 0.1% glycolysis product to LB agar because this substrate quantity proved to be the most favorable in esterase activity tests with Impranil. However, in this case, we could not detect a clearing zone on the plates, instead, we observed intensive growth of the bacteria. This was likely due to the fact, that application of 0.1% glycolysis product not adequately stain the agar (see **Annex 3**), and thus preventing the successful detection of enzyme activity associated with a change in the color of the plates. To overcome this, we added 0.5% glycolysis product to the LB agar.

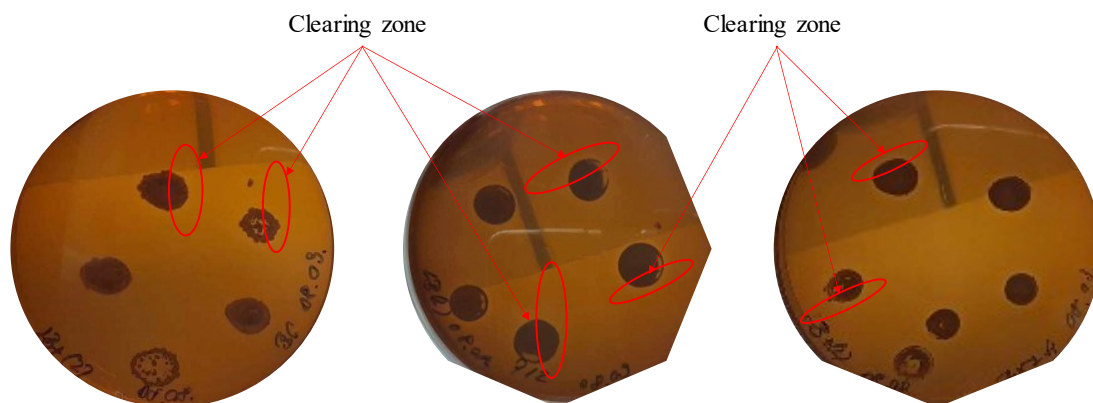


Figure 18 LB agar supplemented with a glycolysis product.

As we can see in **Figure 18**, clearing zone was observed on the LB+0.5% glycolysis product plates, and the diameters of these zones was shown in the subsequent diagram (**Figure 19**). The best results were achieved with the *Bacillus velensis* GZCB6, as it showed the largest diameter of the clearing zone.

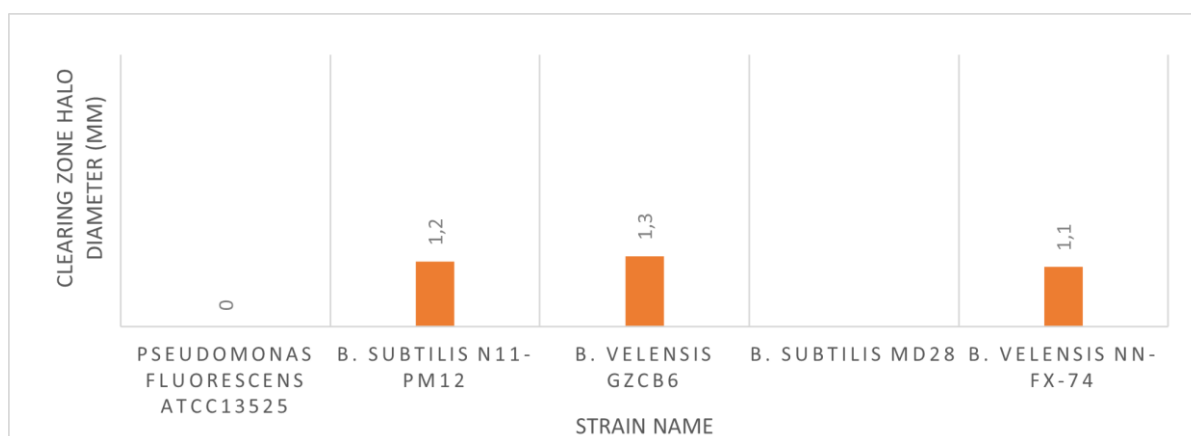


Figure 19 Enzyme activity amplification with glycolysis product agar.

To determine whether the enzyme is activated even when the glycolysis product is the sole carbon source, we performed the agar test by supplementing the MSM minimal salt medium with glycolysis products (**Figure 20**) (as LB itself is a complete medium containing other carbon sources).

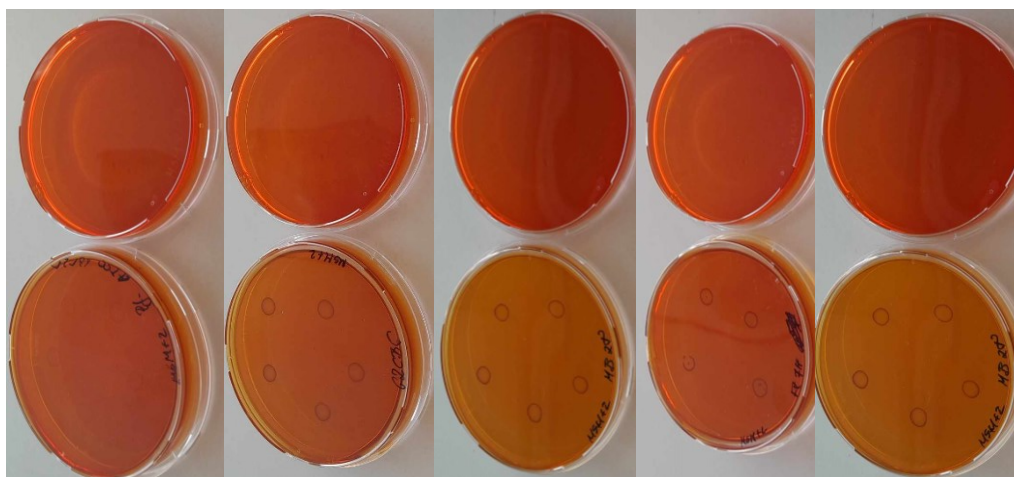


Figure 20 MSM agar supplemented with a glycolysis product and methyl red (1 day starter).

The results show that the bacteria (listed in **Table 1**) have an enzyme, different from *Pseudomonas fluorescens* ATCC 13525 strain, does not have an isomerase activity, because in none of the cases did it degrade Impranil used as a substrate. In other words, it was not able to degrade the glycolysis product of 2,4-TDI diurethane. The study was repeated with a 2-day starter with an OD600 value of approximately 0.6 (Appendix 4). In general, the 2-day starter gave visual results for both the Impranil tests and the glycolysis supplement at higher concentrations (**Annex 4**).

5. Conclusion

Polyurethane (PU) holds one of the largest market shares in the plastics industry. The use of polyurethanes depends on the variety of their mechanical and chemical properties. In terms of fields of use, polyurethane appears in construction, transportation, furniture, packaging, textiles, and many other products. Due to the large quantities of polyurethane produced every year, a large amount of waste is also generated and needs to be managed in one way or another. There are different methods for managing polyurethane waste. It can be said that decomposition is a general waste management method that includes several solutions for decomposing polyurethane waste, such as mechanical, thermal, biological, photo and chemical degradation processes. In this work, polyurethane degradation was investigated by using a combined chemical-biological system for the neutralization of PU waste. To study this, the first step was to synthesize a diurethane model compound based on 2,4-TDI and *n*-butanol (BuOH) as reactants. The most suitable reaction conditions were determined for the diurethane model synthesis. Moreover, by using computational chemistry tools, the thermodynamic properties of the catalyst-free diurethane synthesis were also investigated. After diurethane synthesis, several glycolysis reactions were performed and based on the results, the reaction conditions and

equipment were optimized. During our work, we searched for microorganisms that possess hydrolyzing enzymes, primarily esterases, capable of breaking down glycolysis products (oligomer carbamates). This is essential because microorganisms capable of utilizing glycolysis products as the sole carbon source may prove useful in the degradation processes of more complex polymers (e.g., PU) in the future. To test enzyme activities, we used the enzyme activity testing methods developed by Molitor *et al.* for *Pseudomonas* species, examining the appearance of clearing zones on LB agar supplemented with various esterase substrates. However, in the case of *Pseudomonas fluorescens* ATCC 13525 available in our laboratory, we were unable to detect enzyme activity [37]. In the M9 medium used by Biffinger *et al.*, no degradation was observed in any of the bacteria, but esterase activity was detected in a medium containing salt and trace elements (MSM minimal medium) with indicator supplementation.. Overall, it can be concluded that some of the bacteria we examined (especially those belonging to *Bacillus* genus) have esterase activity, and their esterase enzyme activity was observed on carbon-rich media (LB) supplemented with Impranil DLN SD. Our results were corroborated with minimal medium experiments, where Impranil DLN SD was the sole carbon source [38]. In terms of objectives, a selection of applicable degradation methods has been made. Theoretical determination of the reaction mechanism of TDI-based urethane synthesis. Experimental feasibility of TDI-based urethane formation and both degradation methods. Evaluation of the experiments and detection of the enzyme involved in the degradation of the chemically obtained glycolysis product. Future goals include studying the effect of different factors on glycolysis. In particular, the theoretical study of glycolysis. Furthermore, the biodegradation of polyurethane glycolysis products and the mapping of degradation products.

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