PRODUCTION OF BIO-DEGRADABLE POLYURETHANE FOAM FROM SUGARCANE BAGASSE AND BIODIESEL-DERIVED CRUDE GLYCERINE

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ABSTRACT: As the threat of the depletion of fossil based fuels becomes more of a reality, there is a need to find alternative carbon sources for the production of a wide range of chemicals, polymers and plastics. One plastic that is used in almost every aspect of everyday life is polyurethane foam. Currently it is produced through polyols and diisocyanates that are all obtained from fossil based resources such as crude oil and coal. Lignin is a by-product from the second generation bioethanol process and crude glycerol is a by-product of biodiesel production through transesterification. These two by-products can be combined to produce polyurethane foam of high quality. In this study, the influence of reaction parameters such as temperature, pH of the crude glycerol, and reaction time on the hydroxyl numbers and the properties of the produced foam was investigated. Foam properties such as density, cell size and compression strength was studied. Results showed that the hydroxyl numbers and thus the properties of the lignin and crude glycerin used as reagents have a significant influence on the foam density and rigidity. The foams were tested for biodegradability using a standard ASTM method and it was shown that the produced foams degraded faster than commercial rigid polyurethane foam. Polyurethane foams were also applied as ssDNA immobilization scaffolds by the DST/NWU Preclinical Drug Development Platform, on campus, to determine the potential to be used as part of a diagnostic system. Results showed that polyurethane foam is a viable starting material for immobilization scaffolds in the development of an affordable and practical point-of-care tuberculosis diagnostic system.

Keywords: biodegradable, polyurethane, glycerin, bagasse, lignin.

1 INTRODUCTION

Fossil fuels are used not only for the production of electricity or as sources of fuel, but also for the production of a large variety of products, especially plastic products. Unfortunately, fossil fuels are not renewable and because of this, a point will eventually be reached where it is no longer economically viable to extract fossil resources. Even at this point in time, as a result of the ever increasing demand for energy globally, the costs of fossil fuels, and by extension products produced from them, are increasing [1]. There is also an ever increasing level of concern about the impact that fossil fuel products have on the environment [1]. New carbon sources are thus necessary to produce plastics for everyday use and as alternative sources of energy. Biomass is known to be renewable and can be used as an alternative carbon source.

One biomass based product that has been receiving increasing attention as an alternative fuel to fossil based fuels, is biodiesel. However, the growth of the biodiesel industry has been hampered due to the relatively high cost of its production [2]. The most preferred industrial method for biodiesel production is transesterification [3] which produces large amounts of crude glycerin [4] that has little commercial value and is currently a liability to biodiesel producers [1].

Another biomass waste material that has been assessed for its potential as a raw material for production of commonly fossil based products, such as plastics, is lignin [5]. Lignin is most commonly produced by the paper and pulp industry through the use of the Kraft pulping process [6]. However, the current market for lignin is limited and the vast majority of the produced lignin gets burned to recover energy and pulping chemicals [7].

These sources of waste biomass have been used in the production of polyurethane in the past. In general, glycerin is used as a solvent [8] while lignin, preferably used after chemical modification is used to produce polyols for polyurethane production [6].

In some studies where lignin was chemically modified for the production of polyurethane, such as that of Borges da Silva *et al.* [6], polyurethanes with similar characteristics to those produced with petroleum based components were obtained.

If polyurethanes can be produced with the use of waste biomass materials such as lignin and crude glycerin, this will provide alternative options for the industries from whence these waste materials are obtained. Biodiesel producers will therefore have an additional source of income, which will make the production of biodiesel more economically viable and which will accelerate the growth of that industry. It would also reduce the dependence of the polyurethane industry on fossil based raw materials, which would reduce their carbon footprint and potentially result in more biodegradable polymers.

It is, however, important to note that varying production conditions for the chemical modification of lignin, would result is varying polyol, and by extension polyurethane, characteristics. As such it is important to determine how the production conditions influence the polyol and polyurethane characteristics. One possible application of biodegradable bio-based polyurethane is the medical industry. According to the World Health Organization [9], tuberculosis (TB) is a serious health problem with nearly 9 million new infections each year, and 1.5 million reported deaths in 2013. The diagnosis of TB is thus crucial in reducing the number of deaths due to TB infection [10]. Current testing methods are expensive and the commonly used tests are not time efficient [11]. Problems commonly experienced with the testing of TB are the state of the sample to be analyzed [12] and inhibitors present in the sample [13]. Low numbers of bacteria could also lead to false-negative results. The time taken to analyze samples influences the effectiveness of a method. Many methods cannot meet the requirements for clinical diagnosis [12].

The DST/NWU Preclinical Drug Development Platform (PCDDP) has developed a new testing method by means of rapid cell lysis in a lysis micro reactor (LMR) whereby clinical samples can be processed quickly and effectively [13].

Cell walls are weakened during cell lysis causing the cell to rupture, releasing the genetic material [14]. High temperatures, rapid mixing and the buffer solution aid in the cell lysis [13]. The scaffold may be used to concentrate the ssDNA that is present in the biological sample by means of interaction of the hydrophobic bases of ssDNA with the hydrophobic surface of the material[13]. The scaffold also acts as the transport mechanism between the different testing stages.

In this study the effectiveness of polyurethane synthesized from lignin and crude glycerin as scaffolds for TB DNA analysis is compared to that of scaffold prepared from poly-ethylene glycol (PEG) with a molecular weight of 200 g.mol^{-1} (hydroxyl number of 563). The influence of different synthesis parameters on the hydroxyl number and some properties of the foam were investigated as well as the biodegradability of the lignin-based foams compared to commercial polyurethane foam and starch.

2 MATERIALS AND METHODS

2.1 Materials

PEG200 (Average molecular weight of 190 – 210 g.mol^{-1}), purchased from Sigma Aldrich was used as synthetic polyol in this study. Dabco DC5357 (Air Products & Chemicals) was used as a silicone surfactant and N,N-dimethylcyclohexylamine was used as gelling catalyst. Methylene diphenyl diisocyanate (MDI, Bayer) with a NCO group content of 30% was used for all foam syntheses. All materials were used as received from the supplier without further purification.

2.2 Preparation of poly-ethylene glycerol scaffolds

Polyurethane foams were prepared according to the methods described by Hu *et al.* [15]. Polyol, surfactant and catalyst were added to a mixing cup and vigorously mixed using an electric stirrer for 10-15 s. Diisocyanate was then added to the polyol mixture, and vigorously mixed for a further 10-15 s. After the foaming process was complete, the foam was allowed to cure for 24 hours at room temperature. A band saw was used to cut the foam into disks with a width of approximately 1 cm. The scaffolds were cut from the disk using a laser cutter (Speedy 300, Trotec) and a scalpel. The dimensions of the scaffolds were 60 mm x 2 mm x 0.7 mm. The influence of isocyanate index (100 and 200) on the suitability of the polyurethane foam to be used as scaffolds were investigated using a catalyst loading of 2.5 and 5 g with a fixed gelling agent amount of 0.84 g.

2.3 Preparation of lignin-based scaffolds

2.3.1 Liquefaction of lignin in crude glycerin

Crude glycerin was obtained as by-product from the transesterification of virgin sunflower oil. A methanol to oil ratio of 6:1 was used with a reaction temperature and time of 60°C and 60 minutes respectively. The glycerin was allowed to settle from the reaction mixture by gravity and collected by decantation. The glycerin thus obtained contained 49.3% glycerol with a pH of 10.8 and was used without any further purification.

Commercial Kraft lignin was obtained from Sigma Aldrich chemical company and used as received from the supplier. A crude glycerin to lignin weight ratio of 15:1 was used for all experiments. Liquefaction was done in a closed glass flask using a hot oil bath to heat the mixture to the desired temperature and a magnetic stirrer (250 rpm) was used to ensure uniform mixing of the reaction mixture. The influence of reaction temperature (120-180°C), pH of the crude glycerin (7.5-8.5) and reaction time (1 to 2 hours) were investigated using a traditional linear experimental design. The pH of the crude glycerin was adjusted prior to liquefaction using a standard 0.1 M sulfuric acid solution.

After completion of the reaction, the reaction was quenched by placing the reaction flask in cold water. The reaction product was removed from the flask and weighed to determine the polyol yield. The polyol was used as recovered from the reaction flasks without further washing or purification.

2.3.2 Determination of hydroxyl number

The experimental procedure followed for determination of the hydroxyl numbers of the polyols used is presented in Figure 1. All reactions were done in 250 ml GL-45 laboratory glass bottles with blue pp screw caps and pouring rings.

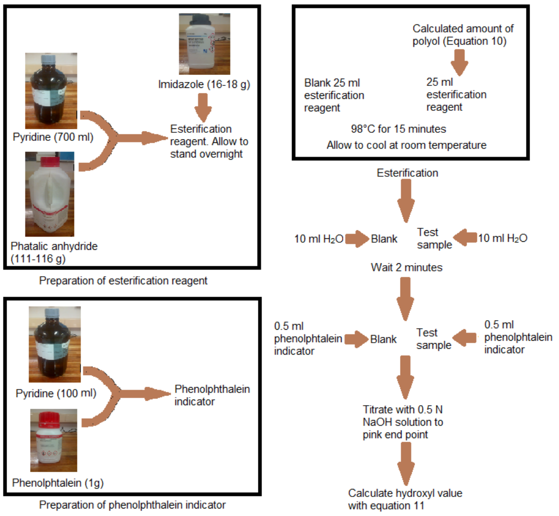

Figure 1: Experimental procedure for determination of hydroxyl number

The hydroxyl numbers of the polyols were determined with the use of method D (Imidazole-catalyzed phthalic anhydride pressure bottle), as described in the ASTM standard [16] for hydroxyl number determination of polyurethane raw materials. Foams were prepared as described for poly-ethylene glycol-based foams.

2.4 Characterization of foams

2.4.1 Microscopy analysis

Sample blocks, with a width and length of as close as possible to 1 cm and a thickness of approximately 0.3 cm, were cut from the produced polyurethane foams with the greater surface area being a side view of the foam.

The blocks were placed under a stereoscopic light microscope set to 10 times magnification and the focus of the microscope as well as the amount of light shone on the sample was set to obtain the optimal picture. A Nikon camera attachment along with a computer program was used to capture pictures of the samples.

These samples were used to determine the cell sizes of the produced foams by simply measuring the dimensions of numerous cells and determining its average dimension.

2.4.2 Density analysis

The produced foam was cut into blocks with dimensions as close to 4cm by 4 cm by 2.5 cm as possible. The final dimensions of the blocks were carefully measured in order to determine the volume of the blocks, after which the blocks were weighed with the use of a mass balance. The weight of the blocks was then divided by the volume to calculate the density of the foam in $kg \cdot m^{-3}$.

2.4.3 Compressibility analysis

A 100kN MTS Landmark servo hydraulic test system was used to run compression tests on the same foam sample blocks previously cut for density determination. The data obtained from these tests were subsequently used to determine both the compression strength and elasticity modulus of compression for the foams.

2.4.4 Biodegradability analysis

A soil mixture containing one third Culterra potting soil, one third a reddish sandy soil obtained approximately 15 meters from a stream (26°41’00.1”S 27°05’54.5”E) and one third soil obtained from a residential garden (26°42’00.8”S 27°04’54.4”E) was used for testing the biodegradability of the produced polyurethane foams.

The moisture holding capacity of the soil was determined by saturating a portion of the dry soil mixture with water and allowing it to drain over a period of 48 hours, after which the amount of water that was retained within the soil was determined.

The biodegradability test was set up in accordance with ASTM standard (D5988-12) [17]. 125 g of soil was added to 250 mL desiccators along with sufficient samples of the materials to be tested in powdered form or crushed to less than 1 mm in diameter, to ensure that 250 mg of carbon was added to all but the blank and technical control tests. Elemental analysis of the foams were used to determine the carbon content. Starch was used as the positive reference material and a commercial polyurethane foam (Rigifoam) was used for comparison.

A 4.72 $g.L^{-1}$ solution of ammonium phosphate was added to the soil for each test specimen to ensure a C:N ratio of 12.5:1. The same volume? of ammonium phosphate solution was also added to the blank tests.

Distilled water was added to the soil of all the test samples and blank tests to the extent of the soil reaching 80% of its water holding capacity.

A 150 mL beaker containing 100 mL of a 0.025 N barium hydroxide solution was placed within each of the desiccators in order to capture the CO_2 generated in each along with a 100ml beaker containing 50 mL of distilled water to ensure minimal moisture loss from the soil during the tests.

The desiccators were sealed using high vacuum grease and stored in a cool, dry and dark place.

The desiccators were carefully shaken periodically to break up the barium carbonate layer that forms on the barium hydroxide solution as it absorbs CO_2 to ensure effecting CO_2 absorption at all times.

The desiccators were removed and opened periodically for a period of approximately 20 minutes to refresh the air within the desiccators and during this time phenolphthalein was added to the barium hydroxide solution and the barium hydroxide solution was titrated with a 0.05 N hydrochloric acid solution to a clear end point. The amount of hydrochloric acid needed for the titration of each sample was used to calculate the amount of CO_2 generated by each sample and, by extension, the percentage of carbon lost by each of the test materials.

A fresh batch of barium hydroxide solution was added to each desiccator before it was resealed again.

2.5 DNA capturing

The suitability of polyurethane as ssDNA immobilization scaffold was tested through the novel TB diagnosis method set out by the DST/NWU Preclinical Drug Development Platform [13]. Scaffolds were placed in the lysis microreactor, with the reference H37 Rv mycobacterium cells and lysis buffer solution. The lysis conditions include temperatures of 95°C and mechanical stirring at 3600 rpm for 7 minutes. The scaffold was removed from the lysis microreactor and transferred to a PCR (polymerase chain reaction) cuvette. PCR mastermix and DNA primers for selected gene sequences of mycobacteria were added to the cuvette, which was placed in a Philisa rapid thermocycler (Streck Inc., Omaha, NE). 4 µl of Novel Juice was added to the samples and loaded into the wells of a agarose gel consisting of 2% agarose, 33 ml of 3x Gel-red dye, 10 ml of 10x Bionic Buffer and 47 ml ddH_2O. Gel electrophoresis on an agarose gel at 70V for 90 minutes was used to visualize the PCR products. A UV transilluminator was used to obtain digital images of the 2%, 33 ml Gel red, agarose gel.

3 RESULTS AND DISCUSSION

3.1 Effect of liquefaction variables on hydroxyl numbers

3.1.1 Effect of reaction time

The influence of reaction time on the hydroxyl values of polyols prepared from lignin at a constant reaction temperature of 150°C, a feed glycerin pH of 8 and a solvent to lignin ratio of 15:1 is presented in Figure 2.

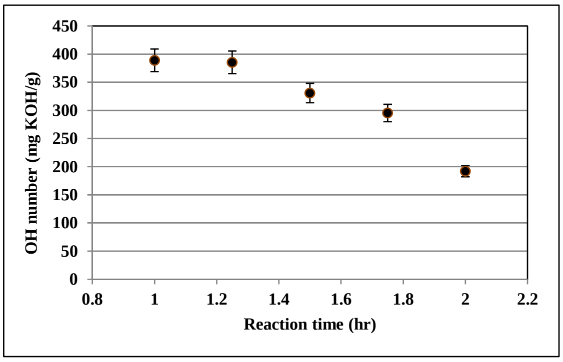

Figure 2: Effect of reaction time on hydroxyl numbers of Kraft-lignin based polyols

From Figure 2 it can be seen that the hydroxyl value decreases as reaction time increases. During liquefaction of lignin, ether and ester bonds between lignin units are cleaved [18] resulting in relatively high hydroxyl values at short reaction times. As liquefaction reaction time is increased, more of the liquefied hydrocarbons and glycerol within the solvent is thermally degraded or oxidized reducing the hydroxyl value [19]. Lim *et al.* [20] also determined that polyols with the greatest hydroxyl values had the lowest molecular weights.

3.1.2 Effect of reaction temperature

The influence of reaction temperature on the hydroxyl values of polyols prepared from lignin at a constant reaction time of 1.5 hours, a feed glycerin pH of 8 and a solvent to lignin ratio of 15:1 is given in Figure 3.

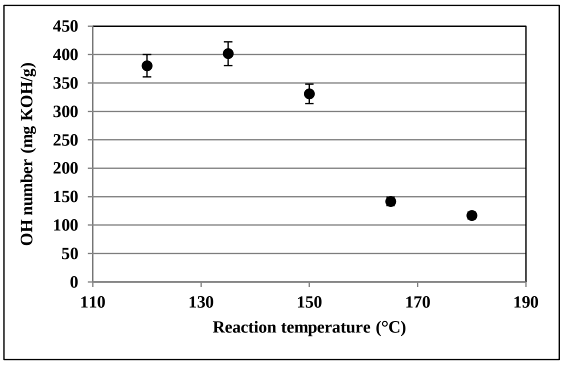

Figure 3: Effect of reaction temperature on hydroxyl numbers of Kraft-lignin based polyols

According to Hu *et al.* [8] the decrease in hydroxyl value as observed during liquefaction is a result of oxidation, degradation and condensation reactions taking place during liquefaction, and that reduces the number of hydroxyl groups within the reaction mixture. These reactions occur preferentially at low temperatures, which explains why polyols prepared at lower temperatures have higher hydroxyl values. As the reaction temperature increases however, the hydroxyl values of the produced polyols decreases, flattening out at approximately 165°C. The latter may be due to the fact that as the oxidation, degradation and condensation reactions continue, there are less material readily capable of undergoing these reactions, resulting in the observed decrease in hydroxyl value with an increase in reaction temperature becoming less significant.

3.1.3 Effect of crude glycerin pH

The influence of crude glycerin pH on the hydroxyl values of polyols prepared from lignin at a constant reaction time of 1.5 hours, a reaction temperature of 150°C and a solvent to lignin ratio of 15:1 is given in Figure 4.

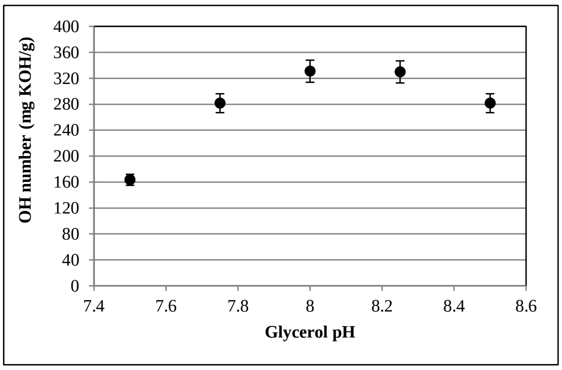

Figure 4: Effect of crude glycerol pH on hydroxyl numbers of Kraft-lignin based polyols

Mahmood *et al.* [21] determined that, at a relatively low pH, liquefaction intermediates will repolymerize, resulting in polyols with higher molecular weights. This implies that an increase in pH would result in more alkaline polyols that have lower molecular weights. Mahmood *et al.* [21] however determined that these changes would have no impact on the overall conversion. The observed decrease in molecular weight with an increase in pH was deemed to be as a result of ether linkages being attacked and broken by water or hydroxyl ions. This means that a point would be reached where there would be no more linkages to break, resulting therein that a further increase in pH would have no further impact on the polyol molecular weights. The low hydroxyl numbers of polyols produced with Kraft lignin at low pH values is likely due to the repolymerization of liquefaction intermediates [21]. As the pH increases the hydroxyl value of the produced polyols also increases due to lower degrees of repolymerization.

3.2 Foam characterization

3.2.1 Microscopy analysis

The of liquefaction conditions on the cell size of PUF foams are shown in Figure 5.

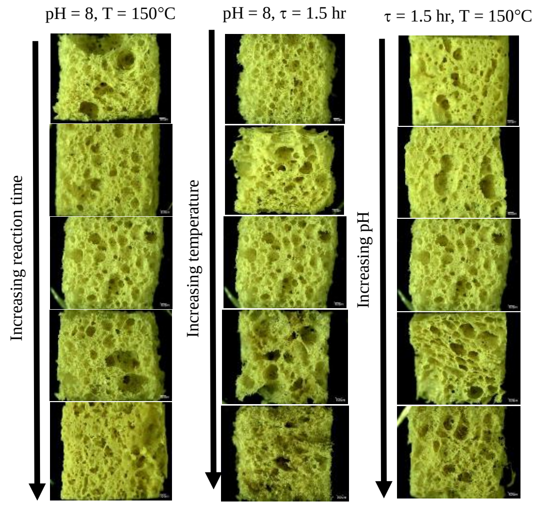

Figure 5: Microscope images at 10 times magnification of different Kraft-lignin based PUF prepared from polyols that were prepared at different conditions

Thirumal *et al.* [22] found that lower density foams were generally found to have greater cell sizes. Hakim *et al.* [23] suggested that polyols with higher hydroxyl values would produce foams with smaller cells sizes.

The average cell size of foams produced from Kraft lignin polyols was found to be comparatively small. This is likely due to the higher hydroxyl values of the Kraft lignin polyols, which would lead to a higher crosslink density within the foam, making cell growth more difficult and thereby reducing the average cell size of these foams.

It was also observed that, contrary to results from literature [24], no correlation between the average cell sizes of the produced foams or their cell size distributions and the hydroxyl values of the polyols used to produce them could be drawn. The results obtained in relation to the comparison between average cell size and hydroxyl value can be seen in Figure 6.

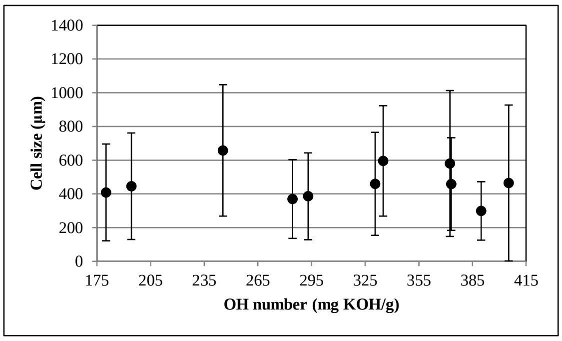

Figure 6: Effect of hydroxyl number of average cell size of foams prepared from Kraft-lignin based polyols

3.2.2 Density analysis

The effect of hydroxyl number on the density of foams prepared from Kraft-based lignin is presented in Figure 7.

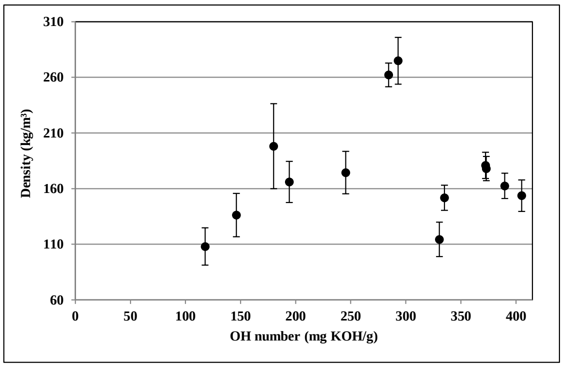

Figure 7: Effect of hydroxyl number on density of foams prepared from Kraft-lignin based polyols

Although there were some outliers, the general trend is an initial increase in density up to a hydroxyl number of approximately 300, after which the density decreases. According to Aroúja *et al.* [25] the aromaticity of the polyols play an important role in the density of the produced foams. Initially, the increase in hydroxyl groups with an increase in hydroxyl number leads to greater crosslinking of the polyols, resulting in an increase in the density of the produced foams, as was also observed by Hakim *et al.* [23]. As crosslinking is increased further, the aromaticity of the produced foam is also increased, leading to low foam mobility and larger expansion, resulting in foams with reduced density.

3.2.3 Compressibility analysis

The influence of hydroxyl number on the compressibility of the prepared foams per unit of density is given in Figure 8.

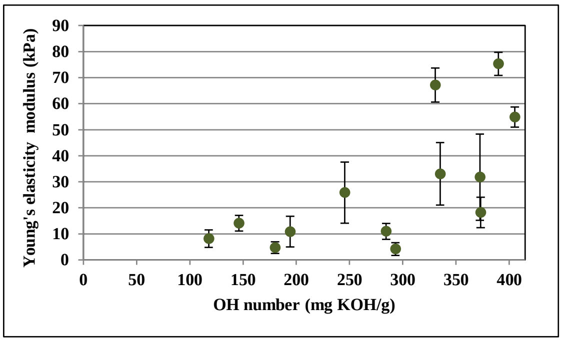

Figure 8: Effect of hydroxyl number on Young's elasticity modulus for the foams prepared from Kraft-lignin based polyols

Young's elasticity modulus is dependent on the density of the foam as well as the thickness of the cells' walls. An increase in density with an increase in hydroxyl number does not influence the elasticity of the foam, because the cell walls of the foam is still relatively thin, which results in a low elasticity. As the hydroxyl number increases further, the density of the foam decreases as a result of larger air bubbles in the foam. As can be seen from the microscopy analysis, the foams with a low density has thick cell walls, which results in higher elasticity moduli. This result correlated with the density and microscopy analysis.

3.2.4 Biodegradability analysis

The carbon loss over time of the foams prepared from Kraft-lignin based polyols is compared to the carbon loss of starch as reference materials and commercial polyurethane foam in Figure 9.

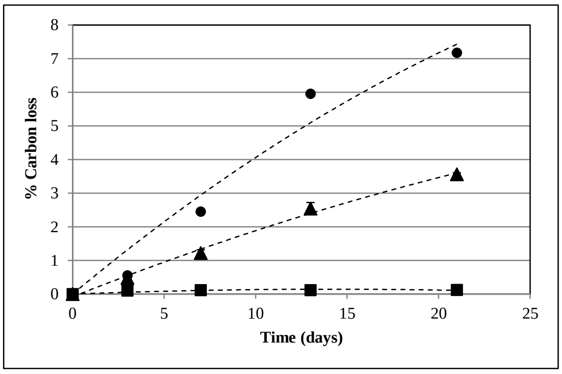

Figure 9: Carbon loss (wt %) of prepared foams (▲), commercial foam (■) and starch (●)

After a 121 day soil incubation of the samples, the final carbon loss % for Kraft lignin based foams was 5.77±0.4%. The final carbon loss of the commercially obtained Rigifoam and the starch were 1.48±0.13% and 6.64±0.92% respectively.

From these results it can clearly be seen that the foams produced from the polyols produced during this study are indeed more degradable than their commercial counterpart, but not as much as the starch reference material. The greater level of biodegradability for foams produced during this study is likely a result of the glycerol and lignin containing polyols produced foams with structures more similar to the natural food of the microbes within the soil, enabling them to degrade the foam more easily.

3.2.5 ssDNA capturing

The properties of the prepared PEG200 foams used as reference material for ssDNA capturing showed that the foams were similar in structure to rigid foams and had a density of 210 kg.m^{-3}.

FTIR spectra for the PEF200 foams showed urethane linkages at approximately 3300 cm^{-1} (NH bonds) [25], 2870 cm^{-1} (CH_2 and CH_3 bonds) [26] and 1720 cm^{-1} (CO bonds) [27]. The presence of a peak at 1600 cm^{-1} indicated the presence of aromatic ring structures, which contributed to the aromaticity of the foams [28]. The presence of isocyanurate rings were observed at approximately 1415 cm^{-1} [29]. The presence of an NCO group at 2270 cm^{-1} [25] indicated that the isocyanate was not completely consumed in the reaction [28].

The results of the ssDNA immobilization tests for the PEG200 foams and the Kraft-lignin based foams are shown in Figures 10 and 11 respectively. Three scaffolds were tested for each foam. A positive and negative control were used to ensure that the process progressed correctly. A DNA ladder was used to size the amplicons.

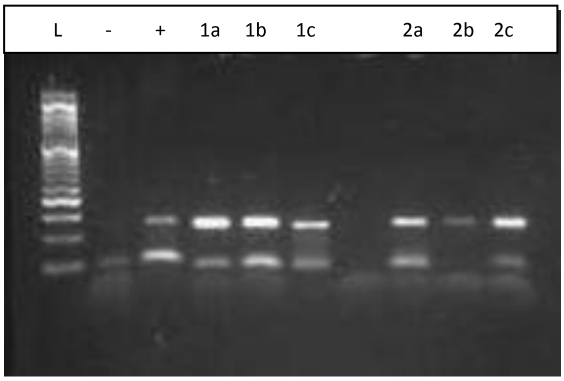

Figure 10: Amplification of PEG200 foams for capturing of TB ssDNA, 1 is PEG 200+ excess MDI, 2 is PEG 200.

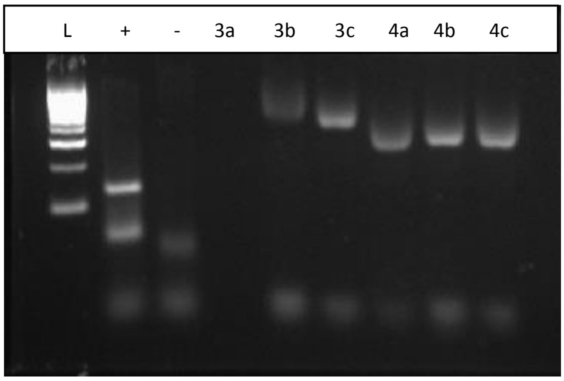

Figure 11: Amplification of Kraft-lignin based foams for capture of TB ssDNA. 3 cut by laser, 4 cut by scalpel.

The ssDNA fragments immobilized by the scaffolds were 123 base pairs in size. Effective ssDNA immobilization was characterized qualitatively with a bright band at the desired DNA amplicon value. PEG200 with excess MDI followed by PEG 200 showed potential in immobilizing ssDNA although repeatability needs to be improved. No bands were observed in the amplification of the Kraft-lignin based foams. The potential of PEG 200 with excess MDI could be explained possibly by the unreacted diisocyante present that supplied excess NCO groups. The presence of NCO groups were indicated by FTIR. NCO is an electrophilic group that can interact with the nucleophilic sites of ssDNA[30]. The negative result obtained for the Kraft-based lignin is mostly due to the high porosity and hydrophilicity of the scaffolds that resulted in the scaffolds absorbing all the liquid in the PCR vial.

4 CONCLUSIONS

The results from this study showed that polyurethane scaffolds can be used to capture TB ssDNA by means of a combination of hydrophobic-hydrophobic and electrophilic-nucleophilic interactions. Although Kraft-lignin based scaffolds were not successful in capturing ssDNA, it was proven that the direct relationship between the foams characteristics and the hydroxyl number of the Kraft-lignin based polyols makes it possible to tailor make the foams for ssDNA capture. The hydroxyl numbers are directly related to the polyol preparation conditions and thus it is possible to predict the properties of the foam from the reaction conditions.

Biodegradability tests showed that the lignin based foams were more degradable than commercial rigid polyurethane over a period of 121 days. This is a significant result as it shows the viability of improving the environmental impact that plastics have by replacing fossil based plastics with biomass based counterparts.

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