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Polyurethanes preparation using proteins obtained from microalgae

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Abstract It is widely believed that the biofuels can be sustainably produced using microalgae that are known to convert CO_2 from the atmosphere to lipids, in the presence of nutrient and accumulate them as their body mass. However, when algal biofuels are produced using thermochemical route, ~30–65 % of proteins present in algae are lost due to decomposition and some of the nitrogen from amino acids is incorporated into the biofuels. The algal protein is a valuable resource that can bring additional revenue to the biorefinery by converting this co-product to high-value polyurethanes. In this work, we have demonstrated a one-step removal of proteins from algae through hydrolysis of the proteins to smaller peptides and amino acids using environment friendly flash hydrolysis (FH) process. Subcritical water was used as a reactant and as a reaction media for hydrolyzing the algae proteins via FH. *Scenedesmus* spp., slurry in water (3.8 %), was used as the algal feed stock during the FH process which was run at 280 °C for a residence time of 10 s. The soluble amino acids and peptides were separated from the other insoluble algal biomass components (cell wall and lipids) by filtration followed by freeze-drying. The product was then characterized by ion chromatography and Fourier transform ion cyclotron resonance mass spectrometry to determine its composition. The freeze-dried peptide and amino acids were then reacted with diamine and ethylene carbonate to produce polyols that were further processed to produce polyurethane. The relatively high hydroxyl value of these amino acid-based polyols and their compatibility with other commercially available polyols made them particularly suitable for producing rigid polyurethane foams. Due to the presence of amines and secondary amines in these polyols, the polymerization process was self-catalytic and the resulting foams are less flammable than conventional rigid polyurethane foams. The conversion of algal proteins to high-value industrial products by a relatively simple process greatly improves the value of proteins extracted from algae.

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Introduction

Microalgae can be used as sustainable feedstock to produce a wide range of next generation biofuels and bioactive compounds [1, 2]. Several novel approaches have been undertaken to economically produce the microalgae in large scale [3, 4]. Microalgae are primarily comprised of three major constituents, namely proteins, carbohydrates and lipids [5, 6]. The protein fraction of microalgae has been reported to be in the range from 30 to 65 wt%, depending on the strain used and the environment conditions under which they were grown [7]. When microalgae are subjected to thermochemical conversions (pyrolysis or hydrothermal liquefaction) to produce biocrude, most of the proteins are decomposed and some of these nitrogen atoms from protein are incorporated into biofuels [8]. Since protein is one of the major constituents of microalgae, it makes more sense to extract them and transform them to

high-value biobased plastics or biochemicals. In order to make algal biorefineries cost-competitive, one has to consider fractionating microalgae into proteins, lipids and cell wall that could be used as feedstock for different applications instead of using conventional biofuels processing steps [9].

In recent years, there has been a growing interest to manufacture biobased plastics, fuels and chemicals from renewable resources [10]. Switching from petroleum feedstock to biorenewable carbon-based feedstock offers an inherent "value proposition" that reduces carbon emissions which has a positive impact on the environment [11, 12]. There is a growing public awareness to use environmentally friendly materials that are produced from renewable resources, and this has led to intensified research aimed at developing plastic materials using biobased feedstocks. One notable example is the production of polyurethanes from renewable resources.

Polyurethanes are prepared by polycondensation of polyols and isocyanates to yield products such as flexible foams that are used in upholstered furniture, rigid insulation foams used in walls and roofs, thermoplastic materials for medical devices as well as coatings, adhesives, sealants and elastomers that are used in various applications [13]. Several new biobased polyols have been recently introduced into the polyurethane market. Essentially, all these new biopolyols are derived from lipids extracted from seeds (primarily soybean triglycerides, but some from non-edible triglycerides) [14, 15].

The first-generation biorefinery using sugarcane juice and corn starch to produce bioethanol; rapeseed and soy oil to biodiesel has been well established and has reached saturation point. Several efforts are underway to establish the advanced biorefinery that will produce biofuels using lignocellulosic biomass and algal biomass as feedstock [16]. As the second-generation industry gets established, proteins will be produced as co-product that is derived from microbes (yeast and bacteria). It will be advantageous to use these "byproduct" proteins for high-value polyols that can be used for various industrial applications. Very few reports are available in the literature where proteins are used to produce biobased products. Soy protein isolate was incorporated as filler into flexible polyurethane foam formulations to increase the biomass content up to 40 % [17, 18]. However, these polyurethane foams were inferior to conventional polyurethane foams derived from petrochemicals. The amines in the algae proteins can be converted to urethanes which can be utilized in the production of polyols for poly(amide-urethane) foams [19]. These amino acid-based polymers are expected to be non-toxic, biocompatible and biodegradable [20].

The main issue inhibiting progress in producing biobased products using proteins is related to the difficulties associated with processing the proteins and the high sensitivity to moisture [21]. The strategy we used to overcome these problems was to hydrolyze the proteins to a mixture of amino acids and low molecular weight peptides which are much easier to handle. Furthermore, we then protected the carboxylic acids and converted the amines to carbamates (urethanes) by reacting them with ethylene carbonate. The products thus obtained were low molecular weight hydroxyl-terminated (e.g., polyols) pre-polyurethanes. These polyols were then characterized and used to prepare rigid polyurethane foams.

Microbial growth and lipid production profile has shown that microalgae *Scenedesmus* sp. is one of the ideal candidates that could be used as feedstock for biofuel production [22]. We have already reported that flash hydrolysis (FH) of microalgae in subcritical water will solubilize proteins as water-soluble peptides and amino acids and the remaining insoluble material which comprises lipids and algal cell can be filtered and used for producing biofuels [23]. FH is carried out using wet algae slurry in a continuous flow reactor with a short residence time (few seconds). Our previous study has shown that 30–66 % of nitrogen could be extracted (dry weight basis) depending on the temperature used in the FH process [16]. Previously, it has been reported that poly(hydroxy-urethane)s were prepared by the reaction of a-ω alkylamines and ethylene carbonate. This approach eliminates the need for toxic isocyanates and corrosive phosgene that is required to produce isocyanates [19]. Algae proteins are of great interest here since they do not compete with vegetable proteins that are used as food and feed, thus, avoiding social issues related to diverting food to industrial raw materials.

In this study, FH was used to extract proteins (as peptides and amino acids) from algae biomass in an aqueous phase, separated from insoluble materials (rich in lipids and algal cell wall) and freeze-dried to fine powders. The freeze-dried solid samples, composition and properties were evaluated using elemental analysis (EA), ion chromatography (IC) for amino acid profiles, and molecular (formula) composition by electrospray ionization (ESI) coupled to Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). Subsequently, the polyurethane foams were prepared by hydrolyzing the algae hydrolyzate (freeze-dried solids) to amino acids, followed by amidation with ethylene diamine. The amine-terminated intermediates were then reacted with cyclic ethylene carbonate to yield hydroxyl-terminated pre-polyurethane which was eventually used to prepare open cell structure foams. This highly porous structure is important for many potential applications and so understanding and controlling the structure of the polyol and the mechanism of cell formation is of considerable interest. In order to gain an insight into the chemistry of the polyol preparation process

and better understand the reaction mechanism governing its formation, a common amino acid found in algae proteins (e.g., glycine) was also used as a model compound.

Materials and methods

Microalgae

Monocultures of *Scenedesmus* spp. cultivated at Old Dominion University using photobioreactors maintained at pH between 9 and 10, and a modified BG-11 growing media are used to maintain optimal growing conditions [24, 25]. The elemental compositions (dry basis) such as carbon, nitrogen, hydrogen and ash contents of the harvested algae used in this study were 51.95, 9.65, 6.2 and 4.23 wt%, respectively.

FH of microalgae for protein extraction

All the experiments conducted for FH were performed using the same algae species. The experimental setup and process of FH was the same as described in the previous paper and patent application [23, 26]. A set of eleven experiments were conducted for the protein extraction. The optimum FH conditions that solubilized maximum proteins were found to be subcritical water, 280 °C, and 10 s residence time. For this FH hydrolysis experiment, 80 mL of algae slurry with 3.8 wt% solids content was used for each experiment. The product after FH was collected and the total volume was measured using a graduated cylinder. Solid and liquid phases were separated by centrifugation, followed by filtration. The hydrolyzate (liquid) was freeze-dried using a Labconco Benchtop FreeZone® 1L until fully dried. The composition of the homogenized product was determined by elemental analysis. It was then stored in a desiccator at room temperature until further use.

Chemicals

Glycine (Gly), 1,2-diaminoethane (ED), ethylene carbonate (EC) and sodium hydroxide (NaOH) as well as all the reagents required for the titrations (e.g., butanol, pyridine, acetic anhydride, hydrochloric acid, hydroxide potassium, phenolphthalein and bromophenol blue) were purchased from Sigma-Aldrich and used as received unless otherwise noted.

Elemental analysis

The elemental composition of the dry algae biomass and freeze-dried algae hydrolyzate used in the study was determined by a Thermo Finnigan Flash 1112 Elemental Analyzer using a nicotinamide standard for calibration. Approximately 1–2 mg of each solid sample was placed in a 3.3 × 5 mm tin capsule for combustion. Typical analysis consisted of placing the sample in a furnace at 900 °C oven at 75 °C using helium as the carrier gas at 91 mL/min. The moisture content and elemental analysis were performed in triplicate, and the reported values are the average of these three values with a standard deviation (SD) less than 5 % (moisture content SD = 3.8 %; elemental analysis SD = 1.9 %).

Ion chromatography

To identify individual amino acids in the algal hydrolyzate, liquid samples were hydrolyzed with 6 M HCl for 17 h in a heating block set at 110 °C [27]. The samples and blanks were cooled to ambient temperature and transferred to 0.5-mL vials where the HCl was evaporated to dryness. The samples and the blanks were reconstituted in 300 µL purified deionized water and analyzed using a Dionex ICS-5000 AAA-Direct™ system equipped with an AminoPac PA10 column and column guard. Standard 18 amino acids from Sigma-Aldrich were used for this analysis.

FT-ICR-MS analysis

The hydrolyzate from the 280 °C treatment (FH) was diluted in methanol to produce a final solution with dissolved organic carbon (DOC) concentration of 30 ppm in 1:1 (v/v) methanol/water. No adjustment of pH was needed for the analysis of the samples. The analysis was performed on a Bruker Daltonics 12 Tesla Apex Qe FT-ICR-MS. The instrumental parameters for sample analysis were similar to those reported previously [28]. Elemental formulae were calculated using an in-house developed MATLAB program with an accuracy of less than 1.0 ppm, and final assignment choices were aided using a series of conservative rules inserted into a program developed using MATLAB (7.14.0.739 (R2012a), the Mathworks Inc., Natick, MA). For each peak, a single formula (atoms of CHON and S) is assigned and atomic ratios (H/C and O/C) are defined.

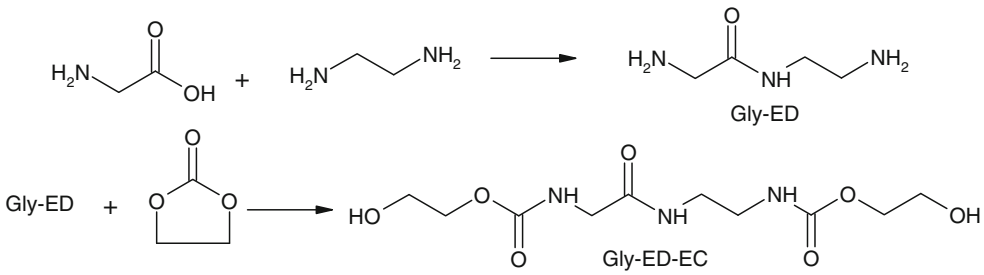
Nuclear magnetic resonance (^{1}H-NMR)

^{1}H-NMR spectra were recorded on a 500-MHz NMR spectrometer (Varian Inc., USA, Unity Plus 500 MHz) using the solvent peak as an internal standard.

Attenuated total reflection–Fourier transform infrared (ATR–FTIR)

ATR–FTIR spectra were acquired on a FT-IR (Shimadzu Co., Tokyo, Japan, IRAffinity-1) equipped with a single reflection ATR system (PIKE Technologies, Madison, USA, MIRacle ATR).

Scheme 1 Preparation of polyols model compound from glycine



End group analysis

Acid values, amine values and hydroxyl values were determined according ASTM D1980-87 [29], ASTM D2073-92 [30] and ASTM D1957-86 [31], respectively.

Glycine-based polyol synthesis

Glycine (Gly) was used as a model compound to better understand the reaction and optimize the process. A typical synthesis is comprised of two sequential steps carried out in the same flask (Scheme 1). In the first step, Gly was condensed with ethylene diamine (ED) to produce the amine-terminated intermediate (Gly-ED). This intermediate was then reacted with ethylene carbonate (EC) to yield the desired hydroxyl-terminated pre-polyurethanes (Gly-ED-EC). It is important to note that although the synthesis involved two distinct reaction steps, they were all done sequentially using a "single-pot" approach. A detailed description of each of these steps is given below.

Synthesis of Gly-ED intermediate

Glycine (272.9 g, 3.63 mol), excess ED (616.9 g, 10.26 mol) and NaOH (5.4 g, 2 wt%) were charged into a polycondensation reactor (PAAR, Series 4530, 2000 mL) equipped with a mechanical stirrer, a thermocouple, a nitrogen inlet and a Dean–Stark trap. The mixture was first flushed with nitrogen for 20 min. Then, the temperature was set at 110 °C and the pressure was increased at 10 bars. The pressure increased progressively to 13 bars and became constant after 2 h of reaction. The pressure was then released and the reaction was allowed to run for additional 17 h at 110 °C under nitrogen. At the end of the reaction, high vacuum (0.004 bars) was applied for 2 h to remove unreacted ED. Samples were taken periodically and were titrated to determine the acid and amines values. The structure of the product was further confirmed by ^{1}H-NMR and FTIR.

Gly-ED

^{1}H-NMR (500 Hz, C_2D_6OS, δ in ppm): 3.96 (s,–(O)$CHNCH_2C(O)OH$); 3.82 (s, –$C(O)NHCH_2C(O)NH$–); 3.42 and 3.39 (t, –$C(O)NHCH_2CH_2C(O)NH$–); 3.39 (s, –$NH_2CH_2NHC(O)$–); 3.35 (s, –$NHC(O)CH_2NH_2$–); 3.28 (t, $H_2NCH_2C(O)OH$); 2.78 (t, $NH_2CH_2CH_2NHC(O)$–).

Synthesis of Gly-ED-EC polyol

The Gly-ED monomer thus prepared (240 g, amine value equals to 771.7 mg KOH/g) was heated to 70 °C and EC (320 g, 3.63 mol) that was previously melted in an oven at 60 °C was charged into the reaction vessel. The ratio of the total amine equivalents to the total carbonate equivalents was kept close to 1. The reaction was carried out in the bulk at 70 °C for 2 h. The final product Gly-ED-EC was analyzed by titration (amine, acid and hydroxyl values), ^{1}H-NMR and FTIR.

Gly-ED-EC

^{1}H-NMR (500 Hz, C_2D_6OS, δ in ppm): 7.88 (s, –$C(O)NHCH_2CH_2NHC(O)$–); 7.26 (s, –$NHC(O)CH_2NHC(O)O$–), 7.12 (s, –$C(O)NHCH_2CH_2NHC(O)$–); 4.47 (s, –$(O)COCH_2CH_2OCO(O)$– in ethylene carbonate); 3.95 (t; –$NHC(O)OCH_2CH_2OH$); 3.53 (–$NHC(O)OCH_2CH_2OH$) and (–$NHC(O)CH_2NHC(O)O$–); 3.35 ($HOCH_2CH_2OH$); 3.10 (–$OC(O)NHCH_2CH_2NHC(O)$–); 3.01 (–$OC(O)NHCH_2CH_2NHC(O)$–).

Algae protein-based polyol synthesis

Algae hydrolyzates (25 g) were added to a solution of 100 mL water and 3 N HCl in a 250-mL 3-necked round-bottom flask fitted with a mechanical stirrer, condenser and a nitrogen inlet. The reaction mixture was heated under reflux at 100 °C for 36 h while stirring to effectively hydrolyze the proteins to amino acids. The acidic hydrolyzate was neutralized using NaOH to a pH 6 and then vacuum distilled to remove the water. The hydrolyzate mixture thus obtained was reacted with a large excess of ethylenediamine (0.55 mol, 33 g) in the same setup used for the Gly model compound at 110 °C for 6 h. Unreacted ethylene diamine was removed by distillation to yield the amido-amine-terminated intermediate. This intermediate was then reacted with ethylene carbonate (0.48 mol, 38 g)

Table 1 Typical foaming reaction profiles

	Reference foam	Algae-based polyol foam
Mix time (s)	10	8
Cream time (s)	11	9
Gel time (s)	12	10
Rise time (s)	37	30
Tack-free time (s)	49	46
End of rise (s)	84	70

at 80 °C for 2 h to yield the hydroxyl-terminated urethane pre-polymer.

Hard foam preparation

Water-blown pour-in-place foams were prepared from the algae polyols. The properties of these foams were compared to foams prepared with a commercial polyols having a hydroxyl value in the range of 60–360 mg KOH/g and used as controls.

In a typical process, the polyol component of the polyurethane system was prepared by blending a predetermined amount of the algae protein-based polyol (up to 5 %) with the other formulation components (plant oil-based polyols, surfactants, water) using a high-torque mixer. The polyol side was then mixed with an isocyanate and poured into a paper box, and the foaming reaction profile was recorded (Table 1). Additionally, foams were prepared in a mold to determine the core density, resiliency, tensile strength, elongation at break and dry compression according to ASTM D 3574-11.

Scanning electron microscopy (SEM) images

The morphology and internal structure of the produced macroporous polymers were investigated using variable pressure scanning electron microscopy (SEM) (Zeiss, EVO LS25, Oberkochen, Germany). The accelerating voltage used was 15 kV and the beam current was 290 pA. The samples were cut with a foam knife into approximately 1-cm cubes. The SEM was operated in variable pressure mode, and the chamber pressure was set to 30 Pa with nitrogen gas. Because the images were taken in variable pressure mode, no sample pretreatments (metal coating, etc.) were necessary.

Results and discussion

Algae hydrolyzate characterization

The elemental carbon, hydrogen and nitrogen contents (dry basis) of algae hydrolyzate (freeze-dried powder) recovered after FH of *Scenedesmus* sp. at 280 °C was 48.1, 7.1 and 11.4 wt%, respectively. The high nitrogen content in the freeze-dried powder indicated the presence of a majority of nitrogen-derived compounds, e.g., peptides and amino acids. Figure 1 shows the product images obtained after FH and the overall material balance. On average (with SD <5 %), 3.05 g of algae solids (dry basis) were loaded into the continuous flow reactor and 0.87 g were recovered as insoluble (lipid-rich) solids and 1.75 g recovered as soluble solids after freeze-drying the hydrolyzate. In previous studies using FH under similar experimental conditions, it was demonstrated that up to 66 % of the nitrogen content in *Scenedesmus* sp. could be extracted as soluble peptides and amino acids [23]. During FH, algae proteins are hydrolyzed to peptides and amino acids in subcritical water. If the reaction is not stopped quickly, these water-soluble compounds may degrade under the reaction conditions and can form free stable radical anions via the Maillard reaction where they could combine with carbohydrates [32].

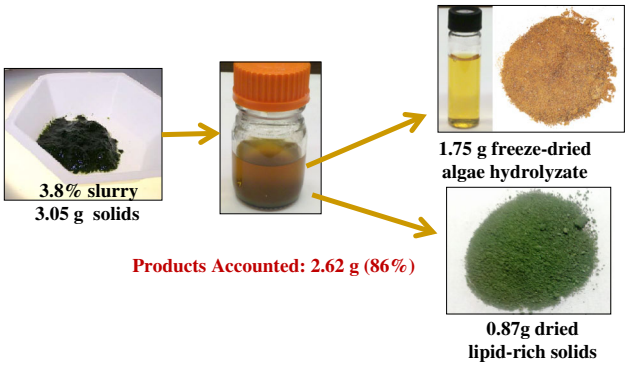


Fig. 1 Material balance of fast hydrolysis of *Scenedesmus* spp. at 280 °C and 10 s residence time. At the end of the process, the hydrolyzed peptides are soluble in water and the cell membrane and lipids forms insoluble precipitate that are filtered out. The soluble peptides were then freeze-dried to get algal peptide powder. The hydrolyzed peptides are further process to produce polyols to produce polyurethane foam

Algae hydrolyzates recovered after FH were analyzed directly for free amino acid content by IC. Only arginine was identified as free amino acid in the hydrolyzate. However, after the HCl hydrolysis of the hydrolyzates, again an amino acid profile was obtained and it showed a mixture of different amino acids. The data clearly indicated that the FH released water-soluble proteins and peptides; and with further HCl digestion, individual amino acids could be produced. A total of fifteen amino acids were identified using IC methods, using eighteen amino acid standards. These include arginine (Arg), lysine (Lys), alanine (Ala), threonine (Thr), glycine (Gly), valine (Val), serine (Ser), proline (Pro), isoleucine (Ile), leucine (Leu), histidine (His), phenylalanine (Phe), glutamate (Gle), aspartate (Asx) and tyrosine (Tyr) as shown in Fig. 2.

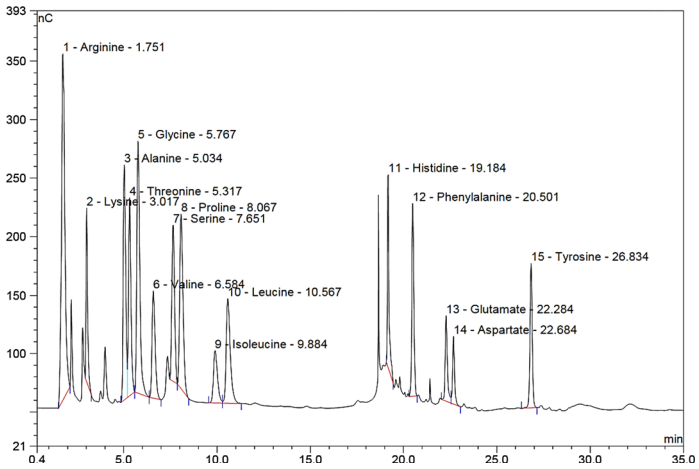


Fig. 2 Amino acids profile and their % composition in algae hydrolyzate recovered after FH at 280 °C and 10 s residence time [23]

Amino Acids	Ala	Arg	Asx	Glu	Gly	His	Ile	Leu	Lys	Phe	Pro	Ser	Thr	Tyr	Val
Composition (%)	8.1	20.0	4.1	9.0	8.3	3.5	4.8	9.8	4.2	4.3	5.8	3.4	4.0	4.1	6.8

The FT-ICR-MS analysis was used to get the insight into the molecular formulas, e.g., carbon (C), hydrogen (H), nitrogen (N), oxygen (O), phosphorous (P) and sulfur (S) of algae hydrolyzate constituents. The results confirmed that the algae hydrolyzate recovered after FH was rich in amino acids and peptides with molecular weight distribution ranging between 200 and 800 mass units (*m*/*z*). We were able to assign molecular formulas to 98 % of the total peaks in the FT-ICR-MS spectrum with the majority of the formulas having CHON, CHO, CHONS, CHONP, CHOS atoms. In addition, 94 % of all the identified molecular formulas had only CHON atoms. The van Krevelen plot (Fig. 3) shows that most of the data plot within the O/C range of 0.2–0.5 and H/C of 1–2. CHON composition peaks were predominantly plotted in that area which is mostly associated with protein like molecular formulas. About 97 % of all the identified CHON formulas have masses that are similar to those of peptides with amino acid chain lengths ranging between 2 and 5 amino acids. This data show that the majority of the original proteins in the algae that are not decomposed are broken down into smaller peptide chains after the FH treatment. The IC results where 15 amino acids are identified (Fig. 2) further supports this conclusion.

Model compound glycine-based polyol

The reaction of glycine with 1,2-diaminoethane was followed by ^{1}H-NMR, and the spectrum of the product Gly-ED is shown in Fig. 4. It is apparent that the amidation reaction was successful (Structure A in Scheme 1). The ED protons appear as triplets at $\delta_{H3} = 3.35$ and 2.78 ppm for $–CH_2–NHC(O)–$ and $NH_2–CH_2–CH_2–$, respectively. It is apparent from these data that the amidation reaction led to some oligomerization as indicated by the structures listed in the Fig. 4. The relative concentrations of these oligomers were determined by normalizing the areas corresponding to protons $–NH–C(O)$ $–CH_2–NH_2$. These data indicate that the overall conversion of glycine was 91 % and the product distribution was 86, 7, 1 and 6 % for structures A, B, C and D, respectively.

The structure of the major product was further confirmed by ATR–FTIR (Fig. 5). The main changes that were observed between the glycine and the Gly-ED spectra were the appearance of two new peaks at 1651 and 1271 cm^{-1}, corresponding to C=O deformation and C–O deformation of the amide groups, respectively. The peak corresponding to N–H of the primary amine at 1500 cm^{-1} was shifted to 1527 cm^{-1} and is attributed to the transformation of amine into amide. Finally, the intensity of the peak corresponding at COO^- at 1404 cm^{-1} in the glycine spectrum significantly decreased and shifted to 1436 cm^{-1}, indicating that the environment of the salt was changed.

The amine and acid values (Table 2) clearly indicate that essentially, all the carboxylic groups were protected and converted to amides. The amine value was found to be close to the expected value, indicating the reaction between glycine and ethylene diamine was completed successfully.

Gly-ED was then reacted with ethylene carbonate to give Gly-ED-EC (Fig. 5). The formation of urethane in this reaction is confirmed by the appearance of a peak corresponding to carbonyl stretching of the urethane groups at 1687 cm^{-1}. Moreover, no peak of ethylene carbonate is detectable in the final FTIR spectrum indicating that all the EC was reacted. ^{1}H-NMR spectrum of Gly-ED-EC (Fig. 6) confirms these results; new peaks appeared at $\delta_{H9} = 3.95$ ppm and $\delta_{H10} = 3.53$ ppm, corresponding of protons

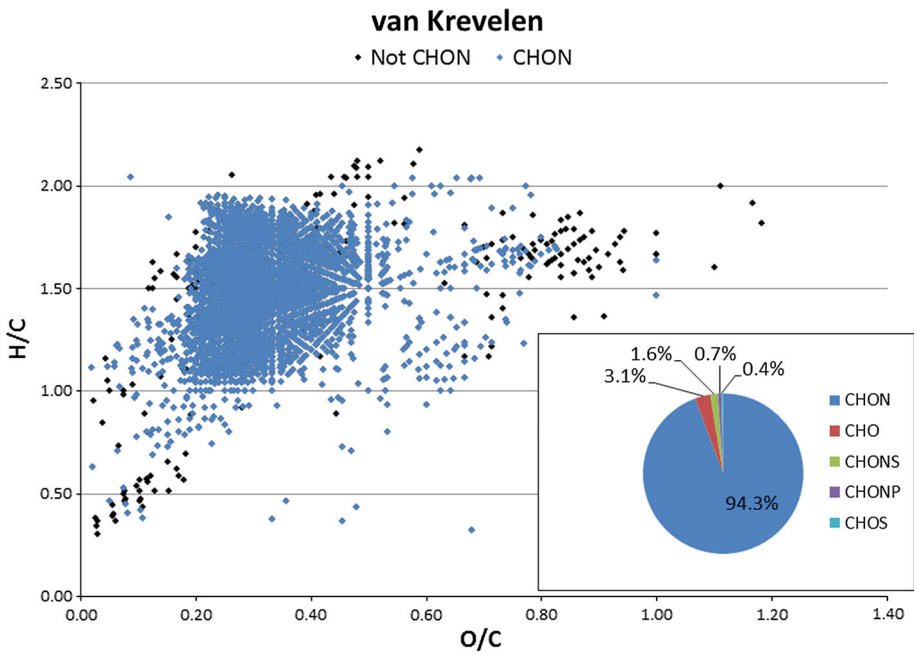


Fig. 3 Van Krevelen plot derived from FT-ICR-MS analysis of algae hydrolyzate

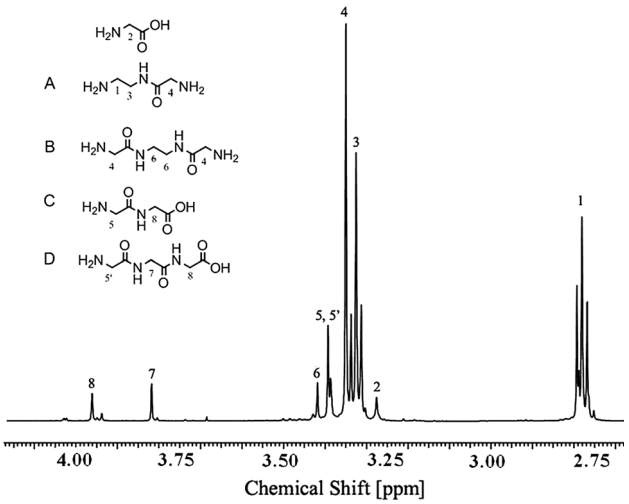


Fig. 4 ^{1}H-NMR of Gly-ED

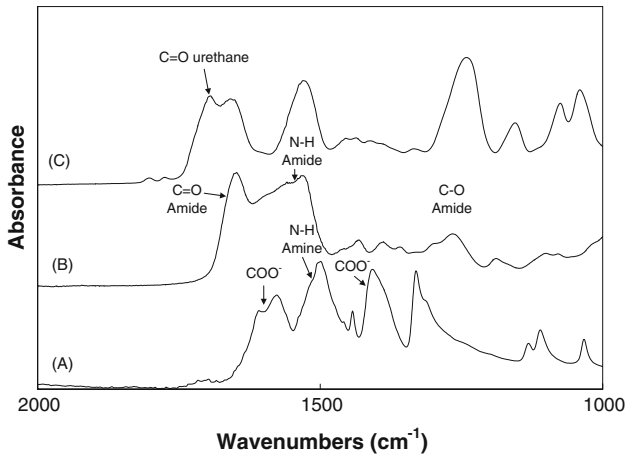


Fig. 5 ATR–FTIR of **a** glycine, **b** Gly-ED, **c** Gly-ED-EC

between urethanes and the hydroxyl groups that were obtained from the ring opening of ethylene carbonate. Furthermore, this reaction proceeded to completion as indicated by the disappearance of the resonance peaks corresponding to protons next to the amines.

The amine, acid and hydroxyl values of the polyol Gly-ED-EC are listed in Table 1. The change in the amine values from Gly-ED to Gly-ED-EC indicates that the yield of conversion of the amines to the hydroxyl-terminated urethane was 93 %. This high reaction yield was further confirmed by the hydroxyl value that is very close to the expected value calculated for complete conversion.

Algae protein-based polyol

The process that was developed for the reaction of Gly as a model compound to the hydroxyl-terminated urethane prepolymer was then used to prepare the urethane-polyol from the algae proteins. Amino acids from the algae proteins were first obtained by acidic hydrolysis following the well-known procedure reported in the literature for hydrolysis of proteins [33]. The hydrolyzate intermediate was neutralized with NaOH, and excess water was removed by vacuum distillation. The amino acid mixture was reacted with ethylene diamine to produce the amido-amine intermediates and then reacted with ethylene carbonate as described previously. The amine, acid, hydroxyl values of all intermediates and the

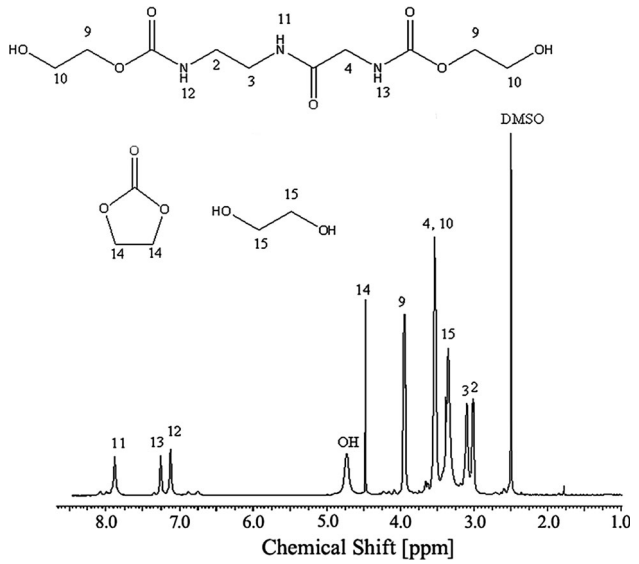


Fig. 6 ^{1}H-NMR of Gly-ED-EC

final polyol are summarized and compared to the results obtained with glycine in Table 2.

This algae polyol was used in combination with other polyols, surfactant, water and isocyanate to prepare polyurethane foams, and typical foam is shown in Fig. 7a. These foams are characterized by an open cell porous structure consisting of highly irregular, spherical pores. Although the overall structure consists of irregular pores, their size distribution is fairly narrow with an average pore size of 0.6 ± 0.23 mm (Fig. 7b). The presence of large numbers of pore throats in each cell suggests that the overall morphology of these foams is highly porous with a large number of interconnected pores. The cellular structure was highly dependent on the type and concentration of the surfactant that was used. It is generally agreed that above a critical concentration, the surfactant film becomes so thin that it will eventually rupture. Pore throats are formed at the interconnecting points separating two adjacent bubbles as the foam expands. Foams prepared with several concentrations of algae-based polyols were compared to commercial foam using polyether polyol in a

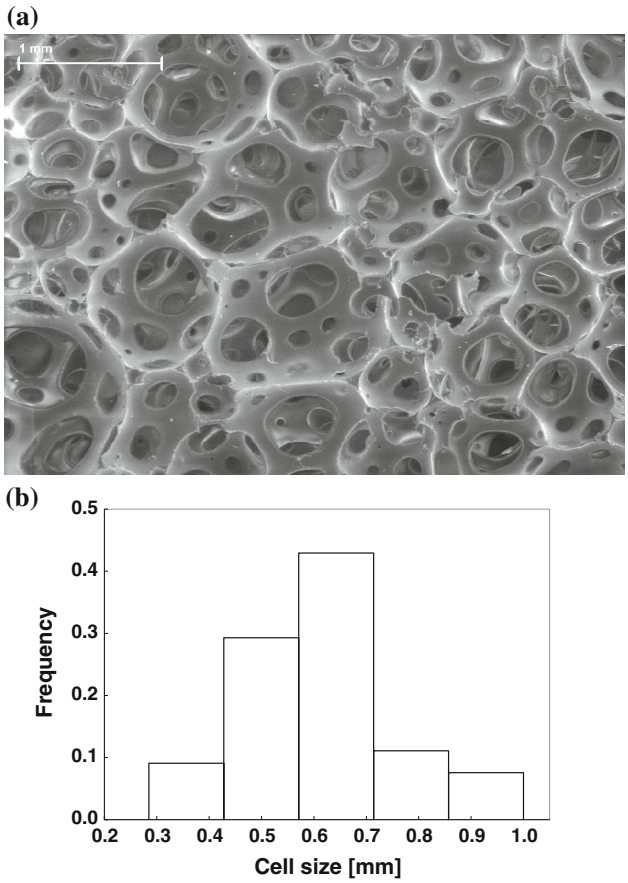


Fig. 7 Properties of polyurethane foam containing 5 wt% algae protein-based polyol. Here, **a** scanning electron micrograph picture of the foam; and **b** pore size distribution of foams

conventional formulation. The reaction profiles of these foams (cream time, gel time, rise time and tack-free time) were monitored (Table 1), and key physical properties of the resulting foams were recorded and compared (Table 3).

During the foam formulations, one of the most noticeable properties in the reaction profile of foams formulated with the algae-based polyol was a self-catalytic property. This property was further confirmed by recording parameters related to the reaction profile (Table 1). The results indicate that all were significantly shorter for foams

Table 2 Amine, acid and hydroxyl values of glycine, Gly-ED, Gly-ED-EC and the final PU

		Amine value (mg KOH/g)		Acid value (mg KOH/g)		Hydroxyl value (mg KOH/g)	
		Calc.	Exp.	Calc.	Exp.	Calc.	Exp.
Glycine	Gly-ED	957	771 ± 3	0	12 ± 3	–	–
	Gly-ED-EC	0	52 ± 1	0	10 ± 2	383	373 ± 2
Algae proteins	Hydrolyzate	–	78 ± 6	–	63 ± 3	–	–
	Al-ED	–	524 ± 5	–	6 ± 1	–	–
	Al-ED-EC	–	26 ± 2	–	3 ± 1	–	422 ± 7

Table 3 Physical properties of foams prepared

	Reference foam	Algae-based polyol foam
Core density, (kg/m^3)	53	53
Resiliency (%)	59	53
Tensile strength (kPa)	187	130
Elongation (%)	116	45
Dry compression set (%)	8.7	8.01

prepared with the algae-based polyol compared to the reference foam. Foams prepared with the algae-based polyol exhibited similar properties compared with the reference foam. Only the elongation at break was found to be somewhat lower than the reference foam. This property could easily be improved by optimizing the cell structure through some adjustment in the formulation. The open cell structure of these foams makes them suitable candidates as cushion protection foams, sound damping and scaffolding materials, as well as foams used in filtration, separation and extraction applications.

Conclusions

The high protein contents of microalgae make it a potential candidate for protein extraction and use for amino acid based biopolymers. FH, a green process, was used to hydrolyze proteins in aqueous phase while preserving the lipids in solid phase. The algae hydrolyzate obtained via FH was mostly comprised of water-soluble peptides. The FT-ICR-MS analysis confirmed the molecular weight distribution of these peptides in the range of 200–800 mass units and chain lengths ranging between 2 and 5 amino acids. This algae hydrolyzate was reacted with ethylene diamine and then ethylene carbonate to produce low molecular weight urethane polyols. These polyols were supplemented (up to 5 %) with other polyols and further polymerized to produce polyurethane foams. Using bio-based raw materials for producing hard forms is highly sustainable and is known to reduce greenhouse gas emissions. The conversion of algal proteins to industrial products like polyurethane can potentially add value to upcoming algae biorefineries. Polyurethane forms with increasing concentration of algal protein-based polyols are being pursued with improved form properties and reduced production cost.

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