**Mild Hydrogenation of Urban Biowaste Hydrolysates to Biopolymers with Improved Properties.**

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This work reports the hydrogenation of soluble biopolymers (SBP) obtained by hydrolysis of municipal biowaste anaerobic digestate (AD) and compost (CP). The SBP are mix of heterogenous molecules with 5-750 kDa molecular weight constitued by aliphatic and aromatic C moieties substitued by acid and basic functional groups. They are mutipurpose products for use in chemical industry as biosurfactants and plastics’ additives, in agriculture and in animal husbandry. Purpose of hydrogenation was to improve SBP properties, while keeping macromolecularity. Hydrogenation was carried out in 5 % water solution at pH 10 at 100 °C and 35 bar H2 pressure for 2-30 min. Results show hydrogenation of SPB aromatic moieties to products with more aliphatic C and different molecular weight distribution. Relative to the pristine SBP, the molecular weight of hydrogenated CP SBP and of AD SBP are, respectively, higher and lower. Surfactants properties improve upon hydrogenation. Hydrogenated AD SBP is better surfactants than hydrogenated CP SBP. Further improvement and uses of hydrogenated SBP are discussed.

**Introduction**

There are two major societal concerns nowdays. One is the depletion of fossil source as feedstock to produce fuels and chemicals. The other is the increasing production of wastes. Both have negative environmental impacts. One approach to alleviate both problems is using biowastes as renewable feedstock alternative to fossil sources. In this context, since 2004, the authors of the present work have carried R&D work focused on municipal biowastes (MBW). The reason for choosing MBW, and not biowaste from other sources, is that MBW is the only negative [1] cost feedstock, worldwide easibly available in every urban settlement.[2] Over this time, the authors have developed a chemical hydrolysis process converting MBW to soluble biopolymers (SBP). They have demonstrated the performance of SPB as mutipurpose products for use in the chemical industry as biosurfactants and for the manufacture of composite plastic materials, in agriculture as soil fertilizers and plant biostimulants, and in animal husbandry as diet supplement.[3]

Currently, biowastes are processed by fermentation, incineration or pyrolysis.[4, 5] These disrupt the polymeric structures of the native organic matter (NOM) and produce simple molecules to use as biofuel or as building blocks for the manufacture of chemical commodities and specialities. On the contrary, the above MBW hydrolysis process yields high molecular weight fragments in the range of 5 kDa to over 750 kDa. These save the memory of the polymeric nature and functionalities of NOM, which allow the multipurpose properties of SBP. In the authors concept, the SBP represent also a potential intermediate feedstock to produce advanced biobased chemical specialities and materials by applying organic chemistry reactions.

Organic chemistry is the base technology of oil refineries. These produce unsaturated hydrocarbons and aromatic molecules from which most commercial commodities and specialities are obtained by chemical reactions such as oxidation, hydrogenation, alkylation, halogenation, and polymerization. In principle, this approach represents a model strategy to follow also for the valorization of MBW as feedstock. However, MBW, as well as SBP, presents a major challenging criticality. Contrary to simple organic molecules having a definite well known chemical structure, MBW or SBP are complex mixes of molecules differing for chemical composition and molecular weight. Thus, both the reaction conditions and the characterization of the products require much research work before understanding whether they can compete for cost and performance with fossils’ processes and products.

The authors have already faced the challenges connected with the complexity of MBW and SBP chemical composition in previous work on the chemical hydrolysis[6] of MBW and chemical oxidation of SBP.[7] They have reported the following results. The hydrolysis of MBW yields a range of molecules with 5-750 kDa constitued by aliphatic and aromatic carbon C types, and various acid and basic functional groups. The oxidation of SPB alters significantly the molecular weight distribution, chemical composition and properties of SBP. It yields mostly products with molecular weight below 5 KDa, such as dicarboxylic acids, di- and tri-alcohols, and hydroxy acids as monomers and oligomers. Fractions with 200–500 kDa molecular weights fractions account for 12–29% of the total pristine SBP organic matter. The lower molecular weight fractions are interesting as potential biobased platform molecules or building blocks to produce fine, commodity and speciality chemicals. The products with 200-500 kDa molecular weight exhibit good surfactants properties. They latter ones are interesting as ready-for-use active principles in the formulation of many consumers’ finished products.

The above findings prospect that, by integrating biochemical and chemical reactions, a wide variety of potentially value-added products could be obtained from biowastes. The present work on the hydrogenation of SBP was undertaken to continue research in this direction, and find out which differences in the products nature and distribution the hydrogenation of MBW gave compared to oxidation. The authors are not aware of previous published work reporting a similar approach for the valorization of MBW as feedstock to produce biobased chemical specialities and materials.

**Results and Discussion**

**Sourcing materials**

The SBP for the hydrogenation reaction were available from previous work.[3,7] They were obtained by hydrolysis at pH 13 and 60 ° of two different samples taken from the process streams of Acea Pinerolese Spa MBW treatment plant located in Pinerolo (TO), Italy. The plant processes the biowaste through two integrated steps comprising anaerobic and aerobic fermentation.[3] Hereinafter, the SBP investigated in this work are named with the AD and CP abbreviations. The AD SBP were obtained from the anaerobic digestate of municipal kitchen wastes. The CP SBP were obtained from urban gardening vegetable residues and food wastes digestate, which were mixed, respectively, in 2/1 w/w ratio and composted for 110 days. In the present work, both SBP were hydrogenation at 100 °C and 35 bar H2 pressure for 2, 15 and 30 minutes, using a solution containing 5 w/w % SBP in water at pH 10.

To understand the choice of the hydrogenation experimental conditions, it must be considered first that the SBP, as materials of biological origin, are complex mixtures of molecules, differing for molecular weight and chemical nature.[3] The sourcing food and gardening residues composing the pristine MBW are constituted by the main proximates of vegetable and animal food species, i.e. cellulose, hemicelluloses, lignin, proteins, fats. The anaerobic digestate and compost of these bio-wastes contain residual organic moieties of the pristine bio-waste proximates, which have survived the anaerobic and/or aerobic fermentation process. The hydrolysis of the digestate and compost yields the SBP. These products are constitued by aliphatic organic and inorganic matter containing several mineral elements, likely bonded to the organic matter. Table 1 reports the chemical composition of the AD and CP SBP and of the sourcing anerobic digestate (AD MBW) and (CP MBW) compost. The AD MBW and CP MBW are much different one from the other. The SBP keep the chemical memory of the organic C types, functional groups and mineral elements present in the sourcing AD MBW and CP MBW. However, they have a different chemical composition. For example, the SBP have lower ash, higher C and N content and lower C/N ratio than the pristine MBW. Also, contrary to the pristine AD MBW and CP MBW, the SBP are well soluble at alkaline pH. The compositional differences between AD MBW and CP MBW are enhanced in the derived AD and CP SBP. This is particularly true for the organic matter. The higher Af/Ar ratio for D SBP, compared to CP SBP, evidences that the former has relatively more aromatic C. As shown hereinafter, this feature has resulted important to determine the different course of the hydrogenation reaction and nature of the products obtained.

**Table 1**. Chemical composition[a] of AD and CP SBP and sourcing D and CP MBW.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | pH | | | Ash, w/w % | | | | | | C, w/w % | | | | N, w/w % | | | C/N | |
| D MBW | 8.2 | | | 34.5 | | | | | | 29.99 | | | | 3.81 | | | 7.87 | |
| D SBP | 6.4 | | | 15.4 | | | | | | 45.07 | | | | 7.87 | | | 5.73 | |
| CPMBW | 8.2 | | | 56.1 | | | | | | 27.07 | | | | 2.45 | | | 11.05 | |
| CP SBP | 8.2 | | | 27.9 | | | | | | 38.25 | | | | 4.01 | | | 9.54 | |
|  | Mineral elements: Si, Fe, Al, Mg, Ca, K, Na as % w/w; Cu, Ni, Zn, Cr, Pb, Hg as ppm | | | | | | | | | | | | | | | | | |
|  | Si | Fe | Al | | Mg | Ca | | | K | Na | | Cu | | Ni | Zn | Cr | Pb | Hg |
| D MBW | 3.46 | 0.77 | 0.40 | | 0.88 | 7.16 | | | 0.53 | 0.22 | | 73 | | 33 | 263 | 20 | 46 | 0.40 |
| D SBP | 0.36 | 0.16 | 0.78 | | 0.18 | 1.32 | | | 9.15 | 0.39 | | 100 | | 27 | 185 | 11 | 44 | 0.23 |
| CPMBW | 10.7 | 1.07 | 0.71 | | 1.12 | 4.27 | | | 1.09 | 0.08 | | 249 | | 97 | 427 | 27 | 54 | 0.33 |
| CP | 2.55 | 0.77 | 0.49 | | 1.13 | 6.07 | | | 3.59 | 0.16 | | 202 | | 92 | 256 | 19 | 85 | 0.15 |
|  | C types and functional groups[b] as C % referred to total C | | | | | | | | | | | | | | | | () | (/) |
|  | Af | NR+OMe | | | OR | | OCO | Ph | | PhOY | COX | | C=O | | Af/Ar | |
| D MBW | 33.6 | 9.1 | | | 26.6 | | 6.0 | 8.9 | | 4.3 | 10.5 | | 1.0 | | 2.54 | |  |  |
| D SBP | 43.4 | 9.9 | | | 14.0 | | 3.4 | 9.6 | | 3.2 | 15.9 | | 0.6 | | 3.39 | | 188 | 1.4 |
| CPMBW | 37.3 | 9.7 | | | 28.1 | | 4.4 | 8.0 | | 5.2 | 6.7 | | 0.6 | | 2.83 | |  |  |
| CP SBP | 40.9 | 7.3 | | | 14.2 | | 3.8 | 12.3 | | 6.0 | 12.9 | | 2.6 | | 2.23 | | 77.5 | 3.5 |

[a]Data from previous work[3] obtained according to therein reported analytical methods: ash as residue after heating he sample to 105 and 650 °C; organic C and N by microanalysis; mineral elements by atomic absorption spectroscopy; concentration values referred to dry matter; weight () and number () average molecular weights (kDa) by multi-angle light scattering detector online to size exclusion chromatography system; C types and functional groups by solid-state 13C NMR spectroscopy. [b]Assignments to C types and functional groups, and percent values of each band area, relative to the total band areas obtained by integrating each band area in the reported chemical shift (, ppm) ranges:  0-53 for aliphatic and/or cycloaliphatic (Af), 53-63 for amine (NR) and methoxy (OMe), 63-95 for alkoxy (OR), 95-110 for anomeric (OCO), 110-140 for aromatic (Ph), 140-160 for phenol and phenoxy (PhOY, Y = H and/or R), 160-185 for carboxyl (COX, X = OH and/or NR), 185-215 for carbonly (C=O) C atoms; R = H, alkyl and/or aryl C, Ar = sum of Ph and PhOY C.

**Choice of hydrogenation conditions of SBP.**

There are no previous examples in literature for the hydrogenation of materials similar to SBP. Much work has been published on the hydrogenation of lignin. As this is one of the major proximates contained in the SBP sourcing MBW, the work on lignin hydrogenation was considered to device the experimental conditions for the hydrogenation of SBP. Two recent papers report the hydrogenation of lignin, [8,9], or of lignin model compounds, at 130-280 °C and 20-35 atm in the presence of PD, Ni, Mo, Cu, Zn, Al, Si catalysts. Up to 50 % lignin to products conversions are reportedeven in the absence of catalyst.[9] Most lignin is converted to liquid phenol monomers within 2 hours reaction time. For SBP hydrogenation, the reaction experimental conditions were devised to find out wether a relatively low energy consumption with no added expensive catalysts could be carried out efficiently. To this end, hydrogenation was carried out at pH 10 to keep in solution the SBP organic matter substrate and the bonded mineral elements (Table 1) to act as catalysts. The mild 100 °C hydrogen temperature and relatively short 2-30 minutes reaction time were chosen to limit energy consumption. As SBP multipurpose performance[3] arises from its polymeric structure and multiple functional groups (Table 1), using mild reaction conditions was also a precaution for avoiding its depolimerization to the constituting monomers, while attempting to improve its properties and widen its potential applications.

**Hydrogenation material balance and products’ molecular weight distribution**

The hydrogenation of SBP and the products’ separation were carried out according to scheme 1

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150 °C, 35 bars, pH 10

SBP 5 % solution + H2  I + II (1)

II  Ri (i = 750, 150, 100, 50, 30, 5, 0.2) + P0.2 (2)

**Scheme 1**. SBP hydrogenation and products’ separation

Step 1 is the reaction yielding the solid product (I) and the liquid phase (II). These were separated by centrifugation. In step 2, the liquid phase II was filtered sequentially through polysulphone membrane with different molecular cut off. For example, by feeding II to the membrane with 750 kDa cut off, the retentate (Ri, i = 750) was separated from the corresponding permeate (P750). Repeating this operation over the other membranes with decreasing molecular cut off yielded the retentates R150, R100, R50, R30, R20, R5, R0.2 and the final permeate P0.2 through the membrane with 0.2 kDa cut off. All products were dried at 60 °C. The same fractionation step 2 was carried out for the pristine SBP. All materials were characterized by C and N microanalysis and 13 C solid state NMR spectroscopy.

Table 2 reports the total mass, C and N balance of the hydrogenation reaction carried out on the CP and AD SBP 5 % solution at pH 10, 150 °C and 35 bars for 2, 15 and 30 minutes. The data account for the total mass, C and N recovered with the insoluble product (I) and the Ri and P0.2 fractions of the soluble product (II). The insoluble product (I) accounted for 3.6-5.6 % and 20-24 % of the total mass and C and N recovered from the hydrogenation of AD and CP, respectively. Table 2 reports also the total mass, C and N data for the pristine CP and AD SBP processed according to the fractionation step 2. The data for the pristine CP and AD SBP are calculated based on the sum of the weights and on the C content measured for each fractions (R750 through P0.2) obtained in step 2 only. Experimental recoveries range from 96 to 110 %. The average value calculated over all mass, C and N % values for pristine CP and AD SBP is 103 ± 5 %. The variability of the experimental data for step 2 recoveries is reasonable, upon considering the number of filtrations, drying operations and analyses that were carried out to obtain the data reported in Table 2. It may therefore be concluded that the global mass, C and N recoveries for the pristine CP and AD SBP are well quantitative. This bodes well for the reliability of step 2 products’ recovery procedure and for the analytical methods applied to these complex substances. The average recovery for the hydrogenated SBP, calculated over all mass, C and N % values for hydrogenated CP and AD SBP in Table 2, is 73.8 ± 11.5. This is significantly lower than the value obtained for the pristine CP and AD SBP. A possible reason for the lower recovery values of the hydrogenated SBP is the formation gaseous products, which are formed during hydrogenation and lost in the gas phase upon opening the reaction flask.

**Table 2.** Total mass, C and N recovered (w/w %) with Ri, P0.2 and/or insoluble fraction I from pristine and hydrogenated SBP, relative to starting SBP mass, C and N.

|  |  |  |  |
| --- | --- | --- | --- |
| SBP[a] | Mass % | C % |  |
| Pristine CP | 97.1 | 110 | 104 |
| CP H2 2’ | 100 | 78.2 | 71.1 |
| CP H2 15’ | 87.1 | 72.4 | 68.1 |
| CP H2 30’ | 83.8 | 59.9 | 55.6 |
| Pristine AD | 96.4 | 106 | 103 |
| AD H2 2’ | 69.4 | 63.4 | 56.2 |
| AD H2 15’ | 81.3 | 78.8 | 70.6 |
| AD H2 30’ | 84.5 | 68.5 | 79.3 |

[a] Pristine non hydrogenated CP, hydrogenated CP for 2 (CP H2 2’), 15 (CP H2 15’) and 30 (CP H2 30’) minutes, and hydrogenated AD for 2 (AD H2 2’), 15 (AD H2 15’) and 30 (AD H2 30’) minutes

Figure 1a reports the total C relative distribution over the soluble pristine and hydrogenated CP fractions. It shows that pristine CP contains two main fractions, i.e. the R750 and R150, accounting for 41 and 43 % of the total C content, respectively. The CP hydrogenation changes significantly the pristine CP fractions’ distribution. The R750 fraction increases upon increasing the hydrogenation time, accounting for 80 % of the total C content at 30 minutes reaction time. All other fractions, accounting for the remaining total C and N content, decrease. The only exception are the P0.2 fractions of the hydrogenated products resulting higher than in pristine CP; i.e. 12-16 C % for P0.2 of 30 minutes hydrogenated CP vs 8 C % for P0.2 of pristine CP. The data indicate that, under the adopted mild reaction conditions, CP hydrogenation induces mainly polymerization of the organic matter in pristine CP, yielding the soluble R750 as main product and the insoluble (I) fraction. Some depolimerization also occurs, yielding a minor amount of the low molecular weight P0.2 fraction.

The hydrogenation of AD exhibits opposite trend. Figure 1b reports the total C relative distribution over the soluble pristine and hydrogenated AD fractions. It shows that pristine AD contains two main fractions, i.e. the R750 and R150, accounting for 65 and 21 % of the total C, respectively. The AD hydrogenation decreases the relative content of the pristine R750 and R150 fractions to 53 and 16 C %, respectively, already at 2 minutes reaction time, and to even lower amounts upon increasing the reaction time to 30 minutes. This results in the increase of the lower molecular weight fractions from R100 to P0.2. Nonetheless, complete depolimarization to the P0.2 fraction is still limited. In essence, the P0.2 fractions in 2-30 minutes hydrogenated AD accounts for 16-24 % of the total C content, compared to 7 C % in pristine AD SBP. The depolimerization of the pristine R750 and R150 fractions upon AD hydrogenation is consistent also with the lower yield of the insoluble product I (3.6-5.6 %), compared to the yield of I (20-24 %) obtained in the hydrogenation of CP.

**Figure 1**. Distribution of C among fractions of pristine and hydrogenated SBP for 2, 15 and 30 min, obtained by filtration with membranes of different molecular cut off. Figure 1a: data for pristine and hydrogenated CP SBP. Figure 1b: data for pristine and hydrogenated AD SBP.

The presence of higher molecular weight fractions in hydrogenated CP SBP, compared to pristine CP SBP, suggests occurrence of condensation reactions during hydrogenation. In the hydrocracking of coal and petroleum, these reactions are known as Scholl condensation reactions involving aromatic hydrocarbons. Salim and Bell [10] have published data on the hydrogenation of three-ring aromatic structures related to coal carried out under mild conditions. They have demonstrated that the relative yields of hydrogenated products and condensed higher molecular weight products depend much on the type of catalyst and the hydrocarbon structure. The pristine SBP are likely to contain similar hydrocarbon moieties (Table 1). However, pristine CP and AD are much different one from the other for organic and mineral composition. Thus, the difference between the effects of hydrogenation on the molecular weight distribution of the two SBP is not surprising.

**Chemical composition and surface activity property of SBP hydrogenated products**

***C, N and surface activity data***

For the objective of the present work to limit SBP depolimerization upon hydrogenation, it was also important to characterize, in additon to the molecular weight distribution, the chemical composition and properties of the recovered fractions and properties. Due the large number of samples and the complexity of the chemical composition of pristine and hydrogenated SBP, only the main Ri fractions were investigated. Pristine and hudrogenated SBP were first compared to assess differences based on C and N content, and on surface tension properties. For pristine SBP, the C/N ratio, more than the C and N contents, has been found a useful parameter indicating chemical composition differences between products, [11] and thus to select samples to be investigated further by more complex, time consuming and expensive analytical methods and techniques. Also, for these complex substances of biological origin, the capacity to lower the surface tension of water has been found a basilar property to measure. It allows prospecting a wide number of potential applications in many sectors of industrial chemistry.[12]

Surface tension measurements are performed over a range of added product concentration in water to obtain surface tension versus product concentration plots, and determine the product critical micellar concentration and the corresponding surface tension. In the case of SBP, the critical micellar concentration has been found around 2 g L-1. Thus, in the present work, the products showing significant differences in the C/N parameter were also investigated by surface tensione measurements at 2 g L-1 concentration.

**Table 3**. C, N, and surface tension data[a] for pristine SBP and hydrogenated main fractions.

|  |  |  |  |
| --- | --- | --- | --- |
| SBP[b] | C (w/w %) | C/N w/w | mN/m) at 2 g L-1 |
| CP R750 | 43.9 ±0.42 ac | 8.57±0.045 a | 67.7±2.53 abc |
| CP R750 H2 2’ | 39.3 ±1.18 bc | 8.32±0.134 a | 71.00.07 b |
| CP R750 H2 15’ | 40.2±0.56 c | 8.53±0.059 a | 63.40.22 c |
| CP R750 H2 30’ | 35.1±0.74 d | 8.53±0.135 a | 70.80.07 b |
|  |  |  |  |
| CP R150 | 38.6±0.68 abc | 8.72±0.05 a | 66.1±0.36 a |
| CP R150 H2 2’ | 40.1±0.29 b | 8.19±0.15 b | 63.6±0.55 b |
| CP R150 H2 30’ | 38.4±0.11 c | 8.13±0.06 b |  |
|  |  |  |  |
| AD R750 | 51.31±0.16 a | 6.58±0.054 a | 56.8±0.81 a |
| AD R750 H2 2’ | 39.9±0.36 b | 6.86±0.090 a | 50.50.46 bcd |
| AD R750 H2 15’ | 44.1±0.48 c | 7.06±0.111 a | 49.40.30 c |
| AD R750 H2 30’ | 44.5±0.25 c | 7.54±0.155 a | 52.20.17 d |
|  |  |  |  |
| AD R150 | 48.5±0.64 a | 5.31±0.05 a | 57.3±0.03 a |
| AD R150 H2 2’ | 43.1±0.16 b | 6.22±0.06 a | 55.0±0.95 b |
| AD R150 H2 30’ | 42.8±0.18 b | 5.72±0.06 a |  |
|  |  |  |  |
| AD R100 | 47.1±0.76 a | 4.67±0.13 a | 54.2±0.66 a |
| AD R100 H2 2’ | 41.5±0.50 bcd | 6.60±0.27 bc | 55.6±0.52 a |
| AD R100 H2 15’ | 40.5±0.63 c | 6.12±0.104 cd |  |
| AD R100 H2 30’ | 43.2±0.055 d | 5.66±0.034 d |  |

[a]Values are given as average of triplicate measurements ± standard deviation. Values for hydrogenated products have been compared to assess significant differences relative to the pristine SBP. Within each group of pristine SBP and its hydrogenated products, values followed by different letters indicate significant differences at p ≤ 0.05. [b]Pristine CP R750, CP R150, AD R750, AD R150 and Ad R100 fractions, and corresponding fractions hydrogenated for 2 (H2 2’), 15 (H2 15’) and 30 (H2 30’) min.

Table 3 reports the results of the C, N and surface tension measurements for the main fractions of the pristine and hydrogenated SBP. It shows that the C/N value of the pristine CP R750 and R150 fractions (including the R100 fraction non reported in Table 3) is 8.6-8.7 against 4.7-6.6 for the pristine AD R750, R150 and R100 fractions. This is consistent with the relatively higher N content in raw unfractionated AD compared to raw unfractionated CP (Table 1). The wider range of C/N values for pristine AD R750, R150 and R100 compared to the range of C/N values for pristine CP R750 and R150 fractions indicates higher compositional variability among AD fractions than among CP fractions. Also, the wider range of C/N values for the AD hydrogenated products (C/N 5.7-7.5), compared to the range of C/N values for the CP hydrogenated products (C/N 8.2-8.5) indicates that hydrogenation affects the composition of the pristine AD fractions more than that of CP fractions. Significant differences between pristine and hydrogenated SBP are shown for C/N values of hydrogenated CP R150 fractions (C/N 8.1-8.2) compared to the pristine CP R150 fraction (C/N 8.7), and for the hydrogenated AD R100 fractions (C/N 5.7-6.6) compared to the pristine AD R100 fraction (C/N 4.7). The C/N differences are not always associated to significant differences in surface tension value. Table 3 shows that the hydrogenated CP R150, AD R750 and AD R100 yield signficantly lower surface tension calues that corresponding pristine CP and AD fractions with the same molecular weight. This is a significant property improvement achieved by hydrogenation under mild conditions without destroying the macromolecularity of the pristine SBP.

The P0.2 low molecular weight fractions are not included in Table 3. The C/N ratio of the pristine and hydrogenated P0.2 fractions ranged from 11 to 19 in the case of CP SBP and from 8 to 11 in the case of AD SBP hydrogenation. These fractions did not exhibit any surfactant property, as they had no effect on the surface tension of plain water. Within the scope of the present work to improve the SBP properties without destroying the pristine macromolecularity, the P0.2 fractions were considered undesirable products and were not investigated further.

***13 C NMR spectroscopic data***

**Table 4**. Data from 13C NMR spectroscopy: signal chemical shift (ppm), assignment to C type[a] and integrated relative band area % for pristine and hydrogenated SBP.[b]

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| ppm | 0-53 | 53-63 | 63-95 | 95-110 | 110-140 | 140-160 | 160-185 | 185-215 |  |
| Product/C type | Af | NR+OME | OR | OCO | Ph | PhOY | COX | C=O | Af/Ar |
| CP R750 | 45.9 | 6.8 | 8.5 | 2.6 | 12.1 | 5.2 | 17.5 | 1.4 | 2.65 |
| CP R150 | 33.2 | 7.5 | 10.8 | 4.2 | 17.1 | 8.3 | 17.7 | 1.2 | 1.31 |
| CP R150 H2 2’ | 33.9 | 7.3 | 12.7 | 4.5 | 13.8 | 7.7 | 17.5 | 2.6 | 1.58 |
| AD R750 | 49.4 | 7.9 | 13.2 | 4.2 | 6.5 | 2.0 | 14.5 | 2.1 | 5.77 |
| AD R150 | 42.0 | 9.8 | 14.6 | 3.9 | 8.2 | 2.9 | 17.9 | 0.7 | 3.80 |
| AD R100 | 39.3 | 10.2 | 10.8 | 2.9 | 9.4 | 3.5 | 21.2 | 2.6 | 3.03 |
| AD R750  H2 30’ | 53.5 | 7.9 | 14.6 | 3.7 | 5.6 | 1.3 | 12.8 | 0.6 | 7.79 |
| AD R100  H2 30’ | 39.5 | 9.8 | 11.4 | 3.5 | 9.1 | 3.4 | 20.6 | 2.7 | 3.15 |

[a]Data for aliphatic and/or cycloaliphatic (Af), amine (NR), methoxy (OMe), alkoxy (OR), anomeric (OCO), aromatic (Ph), phenol and phenoxy (PhOY, Y = H and/or R), carboxyl (COX, X = OH and/or NR) C atoms, R = H, alkyl and/or aryl C, Ar = sum of Ph and PhOY C . [b] Legends as in Table 2 and 3.

Further details on the products’ chemical composition, as related to the C/N and surface tension data, were sought by C13 NMR spectroscopy. Table 4 reports chemical shifts (ppm), assignments to C types and functional groups, and percent values of each band area, relative to the total band areas obtained by integrating each band area in the reported chemical shift ranges. Resonance bands’ assignments are according to previous work carried out on pristine AD and CP SBP.[11]

The band area % values in Table 4 are estimates of the true C mole % of the different C type giving rise to the resonance bands. For example, C in H poor moieties, such as polycyclic aromatic hydrocarbons, is underestimated due to poor polarization of 13C far from 1H nuclei. Vice versa, protonated C groups may be overestimated. In the case of the pristine and hydrogenated SBP, bands are very broad and, in most cases, do not allow a selective assignment. For instance, the band at 160-185 ppm may arise from free carboxylic acid (COOH), carboxylate (COOM, M = metal), ester (COOR) and amide (CON) C bonded to O. In the case of SBP, obtained by alkaline hydrolysis of MBW, the presence of ester groups and free COOH is excluded. The band at 140-160 ppm arises from phenol and phenoxy C bonded to O.

A qualitative estimate of the reliability of the 13C data arises from the consistency with C/N data obtained by elemental microanalysis. The 13C band possibly arising from C bonded to N are those recorded at 53-63 ppm assignable to C bonded to amine or methoxyl groups and at 160-185 ppm assignable to CON and COOM. Table 5 shows that the sum of these band areas accounts for C well in excess of the C bonded to N as determined by elemental microanalysis. The opposite would be unequivocal proof of inconsitency of spectroscopic data with elemental microanalytical data. It should therefore be concluded that, for each product listed in Table 5, the difference (reported in Table 5 column 4) between the sum of band areas % at 53-63 ppm and 160-185 ppm (reported in Table 5 column 3) and the N/C mol/mol % (reported in Table 5 column 2) represents the total OMe and COOM C mol/mol %.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Product | N/C mol/mol % from elemental microanalysis | Sum of band areas % at 53-63 ppm and 160-185 ppm assigned to C bonded to N and to O | Sum of band areas % at 53-63 ppm and 160-185 ppm assignable to C bonded to O | OMe and COOM C mol/mol % |
| CP R750 | 10.0 | 24.3 | 14.3 | 7.5 |
| CP R150 | 9.8 | 25.2 | 15.4 | 7.9 |
| CP R150 H2 2’ | 10.5 | 24.8 | 14.3 | 7.0 |
| AD R750 | 13.0 | 22.4 | 9.4 | 1.5 |
| AD R150 | 11.4 | 27.7 | 16.3 | 6.5 |
| AD R100 | 16.1 | 31.4 | 15.3 | 5.1 |
| AD R750 H2 30’ | 18.4 | 20.7 | 2.3 | - 4.6 |
| AD R100 H2 30’ | 15.1 | 30.4 | 15.3 | 5.5 |

**Table 5.** Consistency ofelemental microanalytic dataand 13C NMR spectroscopy bands comprising resonance assignable to C bonded to N.

The data in Table 4 show also that the hydrogenate fractions CP R150 H2 2’, AD R750 H2 30’ and AD R100 H2 30’ contain relative less aromatic C and more aliphatic and/or cycloaliphatic C than the pristine SBP fraction (CP R150, AD R750 and AD R100) with the same molecular weight. This is reflected in the Af/Ar ratio values calculated for each hydrogenated fraction, compared to the pristine SBP fraction with the same molecular weight. Consistently with published data on the hydrogenation of three-ring aromatic structures related to coal, [10] the resultssupport the belief that hydrogenation under the conditions of the present work involves primarily the SBP aromatic moieties as reactive sites.

***Surface activity property versus chemical composition***

The capacity of surfactants to lower water surface tension depends on the hydrophilic lipohilic balance (HLB). This is an empirical parameter, which allows predicting the performance of a surfactant. The HLB is calculated based on the proportion between the weight percentages of functional groups, for which group numbers are available.[13] It is related to the oil and water solubility of the compound under investigation. It depends also on the configuration of the molecular structure in solution. Provare correlazione con equazione HLB = Sum of hydrophilic groups – Sum of liphophilic groups, oppurre HLB = Sum of liphophilic groups/Sum of hydrophilic groups

For synthetic small molecule[13] and polymeric surfactants[14] of well known chemical composition and structure, calculation of HLB is feasible. In the case of SBP, establishing a relationship between the products’ surface activity properties and chemical nature is very hard. Surface tension data for pristine unfractionated SBP with molecular weight in the 5-750 kDa range have been published.[12] Attempts to establish surface activity versus chemical composition properties have not been successful, due to their complex chemical nature. The fractionation procedure adopted in this work by filtration with membrane with molecular cut off at 0.5, 5, 20, 30, 50, 100, 150, 750 kDa cut off and the availability of the corresponding retentate and permeate fractions for pristine and hydrogenated SBP offers now the opportunity to attempt studying again products’ chemical composition-property relationship. The data in Table 4 and 5 comprise C types and functional groups that, in principle, have very different hydrophilic/lipophilic property. Based on their relative contents, the authors have calculated a range of empirical parameters, potentially capable to represent the change of the hydrophilic/lipophilic balance value across the listed products. Each parameter gave a range of values across the listed products. These were analyzed for correlation with the surface activity of each product as indicated by the  values in Table 3. The results showed that the surface tension of the product’s 2 g L-1 solutions did not correlate with any of the individual C types and functional groups listed in Table 4. The best correlation coefficient (R = 0.89) was obtained by fitting the  vs. Af/Ar ratio (Table 3) values’ plot (Figure 2) with the Boltzman equation. A slightly better correlation coefficient (R = 0.90) was obtained for the plot (Figure 2) of  values vs. the parameter LH2 = (100 - COOH - PhOY)/(COOH + PhOY).

**Figure 2.** Plot of  values (Table 2) vs. Af/Ar values (Table 3) and vs LH2 (see text).



**Figure 2.1** Plot of  values (Table 2) vs. HLBT2cor; y = a + bx, a = 112.5 ± 4.5, b = - 36.7 ± 3.2,

R = 0.975; Nel file SI inserire i dettagli dei calcoli

The LH2 parameter was calculated from Table 4 data under the following assumptions: the 53-63 ppm band area is contributed mainly by amine bonded C and MeO C is relatively negligible; thus, subtracting the 53-63 ppm band area % from N/C mol/mol % value obtained by elemental microanalysis (Table 5) yields amide carboxyl C (CON) moles; subtracting CON moles from total carboxyl (COX) C moles yields COOM mol/mol %. The  values did not correlate at all with the HLB parameter calculated without the breakdown of the COX % band area into CON and COOM groups, i.e LH3 = (100 - COX - PhOY)/(COX + PhOY).

The resultindicate that, for the pristine and hydrogenated SBP, the aliphatic C contribute lipophilicity, while the aromatic C moieties are more polar and contribute hydrophilicity. Hydrophilicity of aromatic C moieties is very likely contributed by phenyl carboxylic acid and phenol groups. It is likely that further breakdown of PhOY into phenol and phenoxy C yielded beter correlation. However, no experimental data are available in this work on the direct measurement of carboxylic acid and phenol groups.

**Perspectives for improvement and uses of hydrogenated SBP.**

As anticipated in the Introduction section, there are basically two approaches for processing biomass and/or biowaste materials, i.e. by destructing or by maintaining the macromolecularity of the native poysaccharide and lignin main proximates. The destructive approach relies on fermentation, incineration, pyrolysis and, in general, on chemical reactions under high temperature conditions. These techniques have been applied so far to biomass from cultivation for non-food purpose and agriculture or agroindustrial residues. Municipal biowastes (MBW) are currently processed only by anaerobic and aerobic fermentation. Since 2004, the authors have concentrated their attention on chemical processes to convert MBW to value added chemical products. Contrary to current technology applied to biomass and agriculture residues, the authors have always followed the macromolecularity non destructive approach. This is the case of the hydrolysis of MBW under mild conditions yielding the water soluble SBP. The hydrogenation now reported has been carried out following the same non-destructive principle. The results demonstrate that it is possible to carry on the hydrogenation of SBP under mild conditions, maintain the macromolecularity of the pristine SPB, still affect its chemical composition and obtain products with improved properties compared to the pristine SBP. In the present work, the capacity of the reaction products to lower water surface tension has been chosen as probe to assess property improvement. Yet, the increased aliphatic content of the hydrogenated product offers perspectives also for the development of improved biopolymers for the manufacture of eco-compatible or biodegradable plastics.

Aromaticity in SBP is the chemical memory of the native lignin proximate in MBW. The authors have already reported that destruction of SBP aromatic moieties by ozonization yields products with more aliphatic and/or cycloaliphatic C moieties and improved surfactant properties.[7] The conversion of aromatic to aliphatic and cycloaliphatic moieties, as obtained in the present work by hydrogenation, seems also a way to go for improving the surfactants properties of SBP. Glycolipids are bacterial surfactants with remarkable properties and high commercial value (30-150 € kg-1).[7] The four most important rhamnolipids are constitued by one or two rhamnose molecules attached to one or two β-hydroxydecanoic acids. They are capable to lower the surface tension of water from 70 down to 28 mN m-1 at the critical micellar concentration of 0.8-2 g L-1. Similar C moieties are present in SBP. Table 4 shows that either pristine and hydrogenated SBP contain alihatic (Af) C together with alkoxy (OR) and anomeric (OCO) C in approximately 3-4 OR/OCO ratio. Also, the sum of Af, OR and OCO C in the hydrogenated SBP is higher by 3-9 % relatively to that in the pristine SBP fractions with the same molecular weight.

Aromaticity in SBP is also responsible of poor processability for the manufacture of plastics.

The authors have demonstrated that composites made from synthetic polyethylenes and AD SBP are better processable to yield plastics with better properties than the composites made with CP SBP. [15]

The present work prospects that hydrogenation may yield SBP improvements by converting aromatic to aliphatic and cycloaliphatic moieties. The results offer highly worthwhile scope for further hydrogenation trials at higher temperature and/or for longer reaction time to assess whether more improvements of SBP potential may be obtained, while saving the macromolecularity of the pristine material.

**Conclusions**

The chemical composition of SBP depends much on the sourcing MBW material. The SBP obtained from MBW anaerobic digestate (AD) contains more aliphatic C than that obtained from composted MBW (CP). The AD SBP is a better surfactant than CP SBP. Hydrogenation of aromatic C moieties in SBP yields the more liphophilic aliphatic C moieities and improves the surfactant property of the pristine biopolymer. The results encourage further reseach to process MBW sourced SBP and, generaly biowastes from other sources, by chemical organic reactions under mild conditions to produce commodity and speciality chemicals and materials that keep the macromolecularity and functional groups’ memory of the pristine native biopolymers.

**Supporting Information summary**

The Supporting Information file contains experimental details.

**Abbreviations**

AN SBP Soluble biopolymer obtained from MBW digestate

Af Aliphatic carbon

Ar Aromatic carbon

COY carboxyl carbon

CON amide carbon

C=O Soluble biobased substances isolated from the alkaline hydrolysate of composted home gardening and park trimming residues

CP Soluble biopolymer obtained from composted mix of home gardening and park trimming residues and digestate of the anaerobic fermentation of the MBW digestate

 Chemical shift of nuclear magnetic resonance signal from TMS

 Water surface tension

MBW Municipal biowastes

MN and MW Number and weight average molecular weights, respectively

NMR Nuclear magnetic resonance

NR Amine carbon

OCO Anomeric carbon

OMe Methoxy carbon

P0.2 fraction isolated from the permeate obtained by filtration through 0.2 kDa cut off

Ri, i = 5-750 fractions isolated from the retentates obtained by filtration through membranes with 5-750 kDa cut off.

RO Alkoxy carbon

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**Keywords:** Biomass, Biowastes, Green chemistry, Hydrolysis, Oxidation, Hydrogenation, Surfactants

[1] G. A. Sheldon-Coulson*, Production of Levulinic Acid in Urban Biorefineries, Master of Science Thesis in Technology and Policy Institute of Technology* **2011**, to be found under <https://dspace.mit.edu/bitstream/handle/1721.1/68450/769021899-MIT.pdf?sequence=2>.

[2] Ellen MacArthur Foundation, *Report “Cities in the circular economy: an initial exploration”* **2017,** to be found under <https://www.ellenmacarthurfoundation.org/assets/downloads/publications/Cities-in-the-CE_An-Initial-Exploration.pdf>.

[3] E. Montoneri, [Municipal Waste Treatment, Technological Scale up and Commercial Exploitation: The Case of Bio-waste Lignin to Soluble Lignin-like Polymers](http://eproofing.springer.com/books/mainpage.php?token=4jgijSi4t8-PhnRUamKB3h5SakpdjkKEl6BYXgaC1V8), *In: Food Waste Reduction and Valorisation,* Chapter 6, **2017**, Morone, P., Papendiek, F., Tartiu, V. E. (Eds.) Springer, doi:10.1007/978-3-319-50088-1.

[4] G. Q. Chen, Microbial Cell Factories 2012, 11:111, 1-3, to be found under <http://www.microbialcellfactories.com/content/11/1/111>. 2.

[5] E. Tsagaraki, E. Karachaliou, I. Delioglanis, E. Kouzi, Document D2.1 **2017,** to be found under <http://www.bioways.eu/download.php?f=150&l=en&key=441a4e6a27f83a8e828b802c37adc6e1>

[6] M. Negre, E. Montoneri, M. Antonini, G. Grillo, S. Tabasso, P. Quagliotto, S. Berto, R. Mendichi, G. Cravotto, A. Baglieri. *J. Cleaner Production* **2018**, 170, 1484-1492, to be found under <https://doi.org/10.1016/j.jclepro.2017.09.196>.

[7] E. Montoneri, D. Rosso, G. Bucci, S. Berto, A. Baglieri, R. Mendichi, P. Quagliotto, M. Francavilla, D. Mainero, M. Negre, *Chemistry Select* **2016**, 1, 1613-1629, doi: 10.1002/slct.201600339

[8] R. Shu, Y. Xu, P. Chen, L. Ma, Q. Zhang, L. Zhou, C. Wang, *Energy&Fuels* **2017**, 31 (7), 7208–7213, doi: 10.1021/acs.energyfuels.7b00934.

[9] Y. Y. Wang, L. L. Ling, H. Jiang, *Green Chem*. **2016**,18, 4032-4041, doi:10.1039/C6GC00247A.

[10] S. S. Salim, A. T. Bell, Fuel 1984, 63, 469-476

[11] D. Rosso, J. Fan, E. Montoneri, M. Negre, J. Clark, D. Mainero, Green Chem. 2015, 17, 3424–

3435.

[12] E. Montoneri, V. Boffa, P. Savarino, D. G. Perrone, C. Montoneri,R. Mendichi,E. J. Acosta, S.

Kiran, *Biomacromolecules* **2010**, 11, 3036-3042.

[13] R. Sowada, J. C. McGowan, *Tenside Surf. Det*. **1992,** 29 (2),109-113.

[14] [X](https://www.ncbi.nlm.nih.gov/pubmed/?term=Guo%20X%5BAuthor%5D&cauthor=true&cauthor_uid=16414065). Guo, Z. Rong, X. [Ying,](https://www.ncbi.nlm.nih.gov/pubmed/?term=Ying%20X%5BAuthor%5D&cauthor=true&cauthor_uid=16414065) **[*J Colloid Interface Sci.*](https://www.ncbi.nlm.nih.gov/pubmed/16414065) **2006,** 298(1), 441-450, to be found under

<https://doi.org/10.1016/j.jcis.2005.12.009>.

[15] F. Franzoso, C. Vaca-Garcia, A. Rouilly, P. Evon, E. Montoneri, P. Persico, R. Mendichi, R.

Nisticò, M. Francavilla, *J. Appl. Polym. Sci*. **2016**, 133, issue 9, start page 2110, doi:

10.1002/APP.43009.



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