High Molecular Weight Biosurfactants from Mild Chemical Reactions of Fermented Municipal Biowastes

High Molecular Weight Biosurfactants Production via Mild chemical Reactions of Municipal Biowastes Digestate and Compost

Enzo Montoneri,*[a] Gloria Fabbri,[a] Pierluigi Quagliotto,[b] Andrea Baglieri,[c] Elio Padoan,[a] Valter Boero,[a] and Michèle Negre[a]

[a] *Prof. E. Montoneri, G. Fabbri, Dr. E. Padoan, Prof. V. Boero, Prof. M. Negre*
Università di Torino, Dipartimento di Scienze Agrarie, Forestali e Alimentari, Largo P. Braccini 2, 10095 Grugliasco (TO), Italy
E-mail: enzo.montoneri@gmail.com
[b] *Prof. P. Quagliotto*
Università di Torino, Dipartimento di Chimica, Via Giuria 7, 10125 Torino, Italy
[c] *Prof. A. Baglieri*
Università di Catania, Dipartimento di Scienze delle Produzioni Agrarie e Alimentari, Via S. Sofia 98, 95123 Catania, Italy
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Abstract

This work shows that mild chemical hydrolysis and oxidation of lignocellulosic matter may yield new high value added products.
Two hydrolysate obtained from anaerobic digestate and compost of a municipal bio-waste treatment plant hav been ozonizated at room temperature. This reaction yields two main products: biopolymers (30 % yield) whith molecular weights ranging from 100 to over 750 kDa, exhibiting remarkable surfactant properties, and small molecules with molecular weight ≤ 0.2 kDa. The ozonised biopolymers have significantly different molecular weight distribution and much better surfactant properties compared to the pristine biopolymers. Their potential market value is estimated from 1.5 to 150 € kg^{-1} by comparison with commercial products. The small molecules are the bio-based counterpart of commercial chemicals obtained from fossil sources. Their market value ranges from 0.6 to 3 € kg^{-1}. Perspectives are discussed for the development of a bio-based chemical industry built on the integration of biochemical and mild chemical technologies to convert biomass to value added products, and compared to the current model based on biochemical technology coupled to lignin incineration or pyrolysis.

Introduction

The valorisation of biomass as alternative feedstock to fossils for the production of fuel and chemicals is a currently a major scientific, technological and societal issue. It faces challenges connected to source and processes. So far, R&D has focused on plants from dedicated cultivations and on residues [1] from agriculture and food industry.[2] Exploitation of dedicated plants raises concern due to the land subtraction to food production.[3] Agriculture and food industry residues are not available everywhere. Collection and transportation costs are important criticalities for both dedicated plants and bio-wastes.[4] Process issues arise because, contrarily to fossils exploitation based on consolidated chemical technologies, biomass valorisation is mostly pursued by less mature fermentation and/or biotechnology-based processes. Figure 1 shows a synthetic scheme of the current technological paradigm.
Biomass is a complex mixture of polysaccharides (PS) and lignin (LG), as major components. The PS are easily biodegradable and can be fermented by the native microbial population or by selected and/or genetically modified microorganisms. Lignin is recalcitrant to chemical and biochemical attack and inhibits fermentation. Thus, biomass fermentation requires previous separation of

PS from LG (Step 1 in Figure 1). This is achievable by a number of physical, chemical and biological methods.[5, 6] Afterwards, the PS may be fermented to yield biogas, bioethanol or hydroxy acid monomers (Step 2 in Figure 1) to be polymerised in a second biochemical reaction step using selected or genetically modified microorganisms. Lignin could be burned to recover its heat value or pyrolysed (Step 5 in Figure 1) to small molecules that can be used as building blocks for the manufacturing of different products.

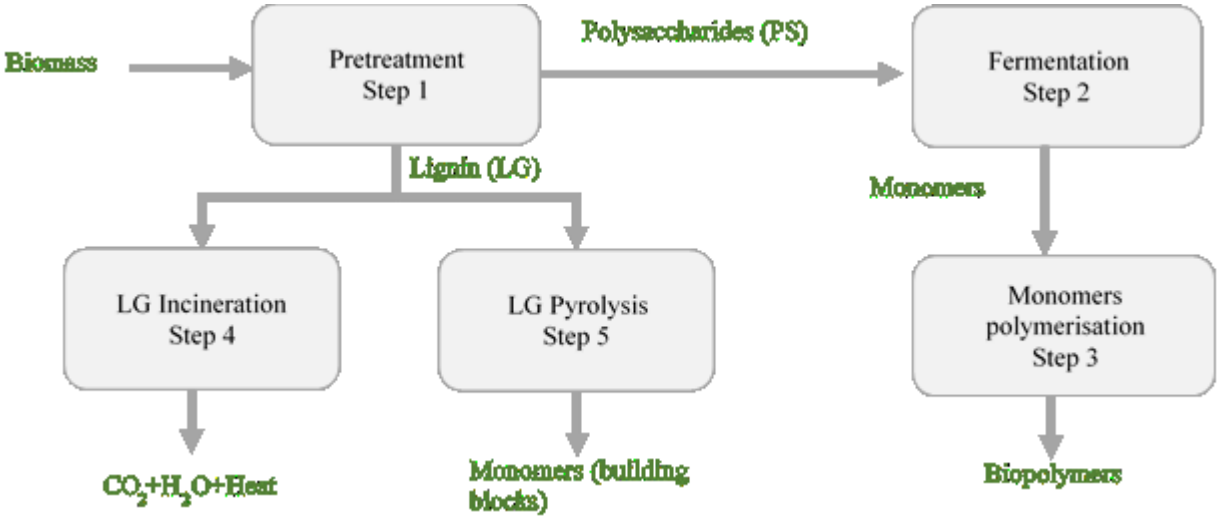

Figure 1. Multistep conventional scheme for biomass valorisation

The above biochemical-thermochemical paradigm has several criticalities. Firstly, fermentation requires the production and the support of the selected microorganisms. Then, LG incineration has much less value than the chemicals obtainable by pyrolysis while, on the other hand, pyrolysis requires energy consumption. Moreover, as drivers of bio-based chemicals development, major chemical companies are reluctant to undertake fermentation technology as core business.[2] Several major chemical companies are carrying on R&D work to improve the sustainability of the above technological paradigm. However, maintaining biological systems and optimizing biochemical processes require different procedures from those to optimise chemical reactions with which chemical companies are familiar.

In conclusion, technological factors, biomass collection costs, social concern on using crops for non-food production, and the attitude of major chemical companies hinder the implementation of bio-based chemicals economy to the desirable commercial level.

In the frame work of the Green Supply Chain Management (GSCM) and Sustainable Supply Chain Management (SSCM) concepts,[7] since 2004 the University of Torino has carried out research on bio-waste valorisation for the production of value added bio-based products[8]. The goal was to device a new approach based on a low energy consuming chemical technology, which could integrate the fermentation technological paradigm and attempt solving the feedstock and process criticalities. Mimicking the strategy of the petrochemical industry, the approach consists in choosing municipal bio-wastes (MBW) and processing it by hydrolysis and oxidation. A review[8] has recently been published containing references for previous papers describing specific processes for and products from MBW valorisation. In authors' vision, hydrolysis was the primary step to obtain soluble biopolymers (SBP) from the native lignocellulose matter in the bio-waste. The

oxidation purpose was the addition of functionalities to the SBP, obtaining oxidized biopolymers (SPBox) with improved properties tailored for specific applications.
The reason for choosing MBW was that it is a potentially negative cost[9] feedstock easily available worldwide, in every urban settlement.[10] Global MBW EU production is estimated at 90 Mt/yr.[11] Cities can provide MBW with reliable long-term sustainable availability, not likely to compromise food production as biomass from dedicated plants does. Collection and transportation costs are covered by citizens' taxes.
The reason for applying hydrolysis and oxidation under mild conditions is to obtain SBP and SBPox keeping the memory of the macro molecularity and functional groups in the pristine lignin matter in MBW, but becoming soluble and thus utilisable as such or further worked out to obtain chemical specialities for a wide variety of applications.
In the authors' research, the Acea Pinerolese MBW treatment plant has been taken as case study. The plant is located in Pinerolo, Italy. It collects 60,000 MBW t/yr from 150,000 inhabitants living in 47 municipalities. It represents a typical modern EU installation operating by two integrated anaerobic and aerobic fermentation stages yielding, respectively, anaerobic digestate (D) and compost (CP).

Results and Discussion

Structure of the results presentation, discussion, and rationale

The SBP and their oxidation products (SBPox) are complex mixtures of molecules with molecular weights distributed over a very wide range from over 750 kDa to less than 0.2 kDa. In each SBP, the molecular composition differ one another regarding both the molecular weight and the chemical composition. Thus, the biopolymers described in this work cannot be characterized as synthetic polymers made by repeating units of well-known chemical composition. Under these circumstances, a great amount of work was carried out to fractionate the SBP and SBPox through ultrafiltration membranes with different molecular cut off (see Experimental Section in Supporting Information, SI). Each obtained retentate (R_i) and permeate (P0.2) fraction contain groups of molecules with different ranges of molecular weights: above 750 kDa in R750, between 750 and 150 kDa in R150, 150-100 kDa in R100, 100-50 kDa in R50, 50-30 kDa in R30, 30-20 kDa in R20, 20-5 kDa in R5, 5-0.2 kDa in R0.2, and below 0.2 kDa in P0.2.
Each fraction was analysed for carbon types content and functional groups determined by ^{13}C solid-state NMR spectroscopy. This analytical technique allows identifying aliphatic and aromatic carbon types and several functional groups. However, given the organo-inorganic complex nature of SBP, NMR signals were quite broad. They allowed identifying carbon moieties present in the SBP sample, but not the assessment of their distribution over the product molecular pool and/or selective precise structural features. As essential part of the SPB and SBPox characterisation, measurements of surface tension in water solution were performed. These measures allowed the assessment of the solution behaviour of the different R_i fractions, as well as predicting their potential uses. For the implementation of SBP and SBPox production at industrial and commercial level, chemical composition and surface tension data may be used as control tool for the release of products with constant specifications.
Under the above circumstances, the present Results and Discussion section is organized to treat the following relevant aspects:

- the state of art of lignin chemical processes;
- the course and the material balance of SBP oxidation;
- the SBP and SBPox chemical characterisation and surfactant properties comparison;
- the SBPox comparison with commercial surfactants.

For clarity, the results are presented in subsections, consistently with the authors' strategy to carry on a step-by step experimental plan. Each step allows selecting the products

and analyses to carry on in the next step. The last subsection discusses the relevance of the experimental SBPox reaction yield, chemical nature and properties in comparison with data for commercial surfactants.

Lignin Chemical Processes

Hydrolysis

After cellulose, lignin is the most abundant proximate in vegetables. The exploitation of renewable feedstock by the new bio-based chemical industry as alternative to the petrochemical industry, will generate huge amount of lignin.[12] Several works have applied chemical hydrolysis by hydrothermal treatment at 200-400 °C to solubilise lignin contained in wheat,[13 triticale straw,[14] walnut shell,[15] sawdust and rice husk,[16] rice bran,[17] and other biomass residues.[18] Under these conditions, extensive depolymerisation of lignin occurred, with formation of small phenolic molecules. These are the building blocks obtained also through lignin pyrolysis in the biochemical-thermochemical paradigm (Figure 1) for the valorisation of biomass.

The production of water soluble lignin-like bio macromolecules was reported for the first time using low temperature hydrolysis.[8] The soluble biopolymers (SBP) were obtained by alkaline hydrolysis of the D and CP fermented MBW at 60 °C. The SBP are a mix of molecules with molecular weights ranging from 5 to over 750 kDa. They contain aliphatic and aromatic C, substituted by variety of acid and basic functional groups bonded to a variety of mineral elements (see Table S1 in the SI file). These C moieties and mineral elements are memories of the polysaccharide, protein, fat and lignin proximates of the sourcing digestate and compost. The SBP are proven multipurpose products for use in the chemical industry as bio surfactants and for the manufacture of composite plastic materials, in agriculture as soil fertilizers and plant biostimulants, and in animal husbandry as dietary supplement. The D and CP SBP have very different chemical composition (Table S1). The D SBP has a higher aliphatic /aromatic C ratio, relatively more N (lower C/N ratio) and less mineral elements than CP SBP. Performance-wise, D and CP SBP rank differently depending on the intended application. For example, D SBP is a better surfactant than CP SBP, while CP SBP performs better as fertilizer and biostimulant on the tested plants.[8]

Oxidation

One of the main reasons to carry out the oxidation in this work is to try to improve the surfactant properties of SBP but both D and CP SBP are very dark coloured. The color limits the range of applications of these products as SBP have been used as auxiliaries for textile washing or dyeing.[8] Thanks to their property of sequestering oily material or dyes, they have been proven capable to enhance the transfer of dirt from the fabric to the aqueous washing medium. In the case of textile dyeing, they help the controlled release of the dye from the dyeing bath to the fabric, to achieve colour uniformity. However, the colour memory of the SBP remains in the treated fabric in form of a yellow dark light nuance. This weakens the competitiveness of the SBP with commercial surfactants.

The black colour of SBP is due to the chromophores of the aromatic conjugated rings present in the product as memories of the parent native lignin matter. The same colour problem is well known in the paper and pulp industry. In this case, oxidation by ozone is carried out to remove residual lignin from the cellulose pulp in order to bleach it.[19]

Recently, Montoneri et al. [20] have reported a preliminary study on the ozonisation of CP SBP. The reaction was carried out by flowing ozone in the aqueous SBP solution at pH 9.7. During the reaction, the pH was kept constant through alkali addition. The reaction was stopped after 48h, when further ozone flowing did not cause any more pH decrease. The products were oxidized biopolymers (SBPox) with molecular weight above 35 kDa obtained with a 30 % yield. The other products were smaller molecules with molecular weight below 5 kDa, such as dicarboxylic acids, di- and tri-alcohols, and hydroxy acids monomers and oligomers. The SBPox showed lighter

colour and enhanced capacity to lower the water surface tension, compared to the pristine SBP. The products with molecular weight below 5 kDa had no surfactant properties. Compared to the oxidized products, the pristine SBP contained 80 % molecules with molecular weight above 35 kDa. The results indicated that significant depolymerisation of the pristine SBP occurred by ozonisation under the above conditions.

For the authors' purpose, the depolymerisation reaction is undesirable. On the other hand, this is a problem inherent to the oxidation of organic molecules. CO_2 and small molecules are thermodynamically more stable than the SBP biopolymers. Numerous cases of biomass oxidation are reported in literature, which can help devising oxidation experimental conditions for oxidation according to the desired products.

Studies on chemical oxidation of biomass with classic metal catalysts used in the petrochemical industry have been focused so far on the production of sugars from cellulose and hemicellulose and their oxidate derivatives, and of aromatic compounds from lignin. These methods follow the same criteria of the fermentation paradigm in Figure 1, modifying the native structure of the biomass polymeric proximates to obtain small molecules[21] to use in place of petrochemical counterparts. Biomass oxidation is carried out preferably in water, as most eco-friendly solvent. Mixed methanol-water media are used in some studies.[22] Reaction temperature is well above 90 °C, up to 180°C. Most metal catalysts, except transition metal catalysts, are unstable in water. Biomass oxidation requires high energy consumption, and also recovery and handling of metal catalysts, both for economic and environmental reasons.[22, 23] Studies on the oxidation of biomass proximates, such as commercial cellulose[23] and starch, lignin from the waste liquor of the paper and pulp industry, and related model compounds have been published. Different products are obtained depending on the starting substrate, organo-metal catalyst, pH, oxidizing reagent, temperature and solvents. A comprehensive review[24] on the oxidation of lignin employed in the paper making industry reports the formation of phenolic compounds, dicarboxylic acids and quinones. However, it does not mention formation of any soluble biopolymers as the SBP and SBPox obtained from the low temperature hydrolysis[8] of CP and ozonisation[20] of its hydrolysate.

It stems from the above state-of-art that the authors' approach to valorise biomass by chemical reactions under mild conditions, which did not destroy the macro molecularity of the lignocellulosic matter in the pristine bio-waste, is rather unique. The chemical properties and/or performance of the SBP and SBPox products encouraged to continue further R&D work along this route.

The present paper reports further work addressing the following aspects, which were not covered before. In the previous work,[20] ozonisation of CP SBP was carried in alkaline water solution only at 48 h gas-liquid contact time while ozonisation of D SBP was not investigated. The comparison of the ozonisation substrates was particularly important as D SBP was expected to yield better surfactants. For the ozonisation of both D and CP SBP, the authors report now the effect of the gas-liquid contact time on the products' nature and distribution. Studying the effects of the contact time aimed to find the optimum experimental conditions to increase the yield of the SBPox, relatively to that of the small molecules.

The course and material balance of the SBP oxidation reaction

Ozonisation of SBP gave a water-soluble and a water insoluble product. The formation of the insoluble product was observed also in the preliminary ozonation work[20] and from authors[25] studying the ozonisation of pine kraft lignin in alkaline solution. This fact was attributed to the dehydrogenative coupling of phenolic degraded fragments by active oxygen radicals and to the extensive cross-linking between ozonised molecules. This mechanism could also explain the formation of the insoluble product in our preliminary work.[20]

Table S2 in SI file, Figure 2 and Figure 3 report, respectively, the data on the mass balance, the alkali consumption and the crude

products distribution as a function of the reaction time. Table S2 report the total soluble and insoluble products mass recovery relatively to the starting mass, averaging 90.7±6.3 %. The starting mass is nearly quantitatively recovered with the reaction products and there is no definite trend with the reaction time. This fact validates the reliability of the sampling and of the analytical procedure (in SI file) used to calculate the reaction mass balance.

Figure 2 shows that, for both D and CP SBP, the reaction proceeds with increasing alkali consumption due to the formation of acid functional groups; i.e. up to 11.2 and 20 acid meq per g of pristine D or CP, respectively. Upon increasing the reaction time over the experimental range, the alkali consumption increases linearly and tends to taper off to a plateau at 60 h for D and 48 h for CP, indicating the end of the reaction. These data offered scope to assess whether and how the quality of the products changed upon increasing the ozone-SPB contact time.

Figure 3 shows that, upon increasing the reaction time, the D SBP soluble product (D1) increases, while the insoluble fraction (D2) decreases. By comparison, in CP ozonisation, the changes in products yield are less evident. In the 32-88 h reaction time range, the relative yield of D2 is well lower than that of CP2.

These data are relevant as they point out a marked difference in the behaviour of the pristine D and CP SBP versus the ozone-SBP contact time. The difference in D2 and CP2 yields is likely related to the difference in composition between the materials. As shown in Table S1, the CP SBP has 43 % more phenyl and phenoxy C, compared to D SBP. This fact seems consistent with the dehydrogenative coupling mechanism reported in the ozonisation of Kraft lignin.[25] This mechanism is likely to operate more efficiently in the more aromatic CP SBP causing cross linking of phenyl moieties up to the formation of the insoluble D2 product.

Due to the water solubility properties and to their higher reaction yield, the D1 and CP1 soluble ozonised products were further investigated for their chemical nature and for the surfactant activity of their fractions separated using membrane ultrafiltration (UF). The insoluble D2 and CP2 ozonised products were not investigated further. They were considered secondary products, offering less interesting perspectives of potential uses, compared to the soluble ozonised products.

The data in this subsection allowed a first selection of products to proceed to the next step of the experimental plan.

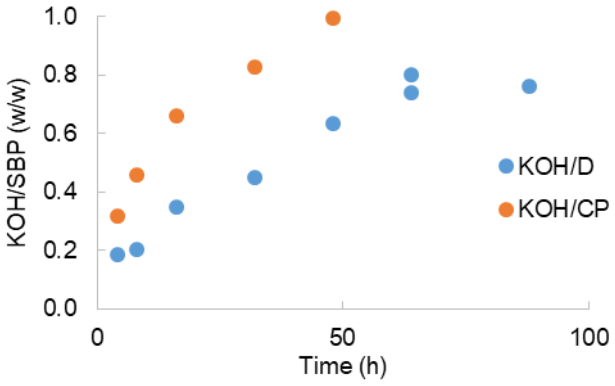

Figure 2. Alkali consumption vs. reaction time in the ozonisation of D and CP SBP.

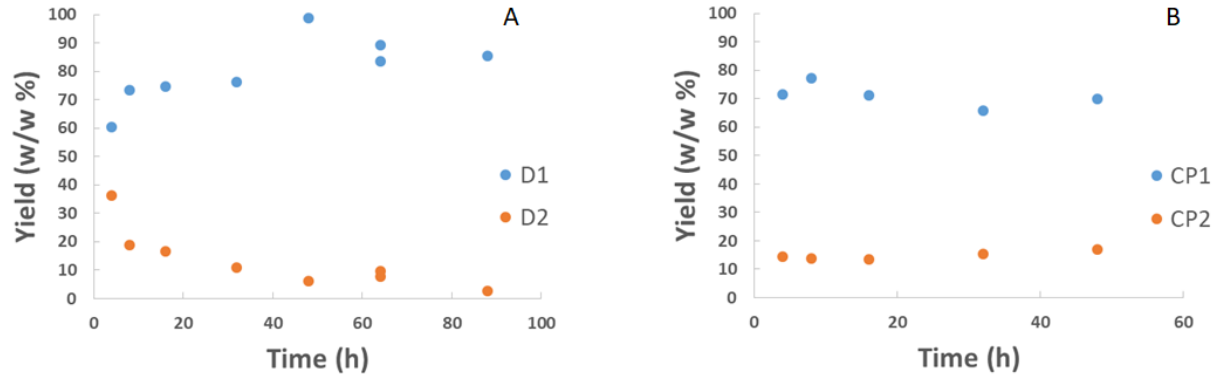

Figure 3. Distribution vs. ozonisation reaction time for soluble D1 and insoluble D2 (Figure 3A) and for soluble CP1 and insoluble CP2 products (Figure 3B).

Characterisation of soluble ozonised products

On one hand, the complex molecular pool constituting the SBP is a positive feature. It is the reason of their multiple properties. It allows using them in diversified applications.[8] On the other hand, it poses the problem of composition reproducibility and performance replicability. The SBP originate from biological waste streams, which are generally non-homogeneous and site-specific. This fact may affect product quality. It might compromise his industrial scale up and commercialisation. Identifying and isolating, from the SBP molecular pool, components performing as active principles in the intended application can help to enhance their performance, guaranteeing their production with reproducible specifications and increasing their value. It is a worthwhile rather challenging task, also necessary to meet the objectives of the EU products standardisation/regulation/certification policy. Separation of pristine and ozonised SBP based on molecular weight has been attempted in the present work. UF through membranes with different molecular weight (see SI file) allowed obtaining nine fractions from above 750 kDa (i.e. R750) to less than 0.2 kDa (P0.2) from each sample. Over the investigate reaction time range eight D1 and five CP1 samples were produced. Each sample could in principle produce eight R_i (with i=750-0.2) and one P0.2 fraction. These bring up to one hundred seventeen the number of samples to analyse for chemical composition and properties. The characterisation implied obtaining data for the thirty-two analytes listed in Table S1. In addition, the surfactants properties of the products had been measured. The capacity to lower the surface tension of water was assessed as in a previous work.[8] It depends from the chemical composition and its solution behaviour. It allows envisaging the product potential performance in a wide range of applications where commercial surfactants are employed.

A strategy to limit the number of samples without missing relevant information was adopted. In the second step of the experimental plan, the crude D1 and CP1 obtained after 48 h, when the progress of the reaction was near to its end, were filtered starting with the membrane with 30 kDa cut off. This allowed separating the R30 retentate, containing molecules with molecular weight above 30 kDa, and the P30 permeate. The P30 was then filtered sequentially through the membranes with lower molecular weight cut off to yield the R20, R5, R0.2 and P0.2 kDa. These products were characterized for chemical composition and surface tension in water solution. They were compared one to the other in order to select the products to investigate further in the third step of the experimental plan.

Comparison of D1 and CP1 SBP

Molecular weight distribution

Figure 4 gives the relative distribution of organic C among the UF fractions of the crude soluble ozonised CP1 and D1 products. Values were calculated using the weight of each fraction and their relative C content. The data at 0 h reaction time show that pristine CP and D SBP contain mainly molecules with molecular weight above 30 kDa. These account for over 80 % of the total C recovered in the pristine SBP fractions. Ozonisation drastically changes the molecular weight distribution. Generally, upon increasing the ozonisation time, R30 fraction decrease while P0.2 fraction increase. The intermediate molecular weight fractions account for only few percent of the total recovered C. The decrease of the R30 fraction is more marked for the ozonised CP (Fig.4B). For ozonised D, the R30 fraction decreases to 30 % after 8 h of reaction, and remains constant at longer reaction times. The differences in the relative yields for D and CP is likely due to the relatively higher aromatic C content of the latter (see Af/Ar in Table S1), consistently with the idea of ozonisation occurring mainly at aromatic C moieties (see below Chemical composition of pristine and 4-64 h ozonised D SBP by ^{13}C NMR solid state spectroscopy).

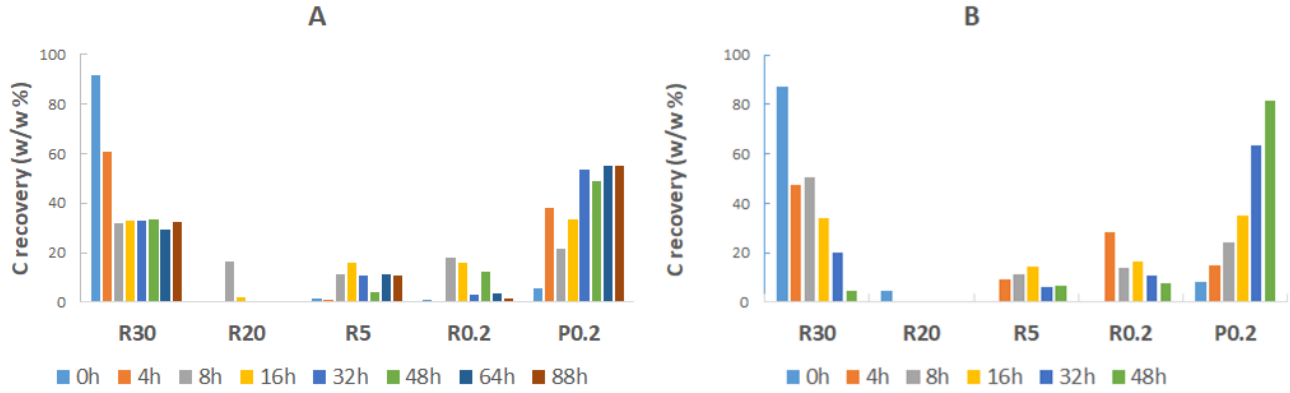

Figure 4. Relative organic C w/w % recovery with fractions isolated by ultrafiltration of ozonised D1 SBP (Figure 4A) and ozonised CP1 SBP (Figure 4B) at different ozonisation time. Ri (i = 30-0.2) and P0.2 are the retentates and permeate fractions obtained by ultrafiltration on different cut-off membranes. R30 contains molecules with molecular weight above 30 kDa. Ri (i = 20-0.2) and P0.2 contain fractions with molecular weight from 30 to 20 kDa for R20, from 20 to 5 kDa for R5, from 5 to 0.2 kDa for R0.2, below 0.2 kDa for P0.2.

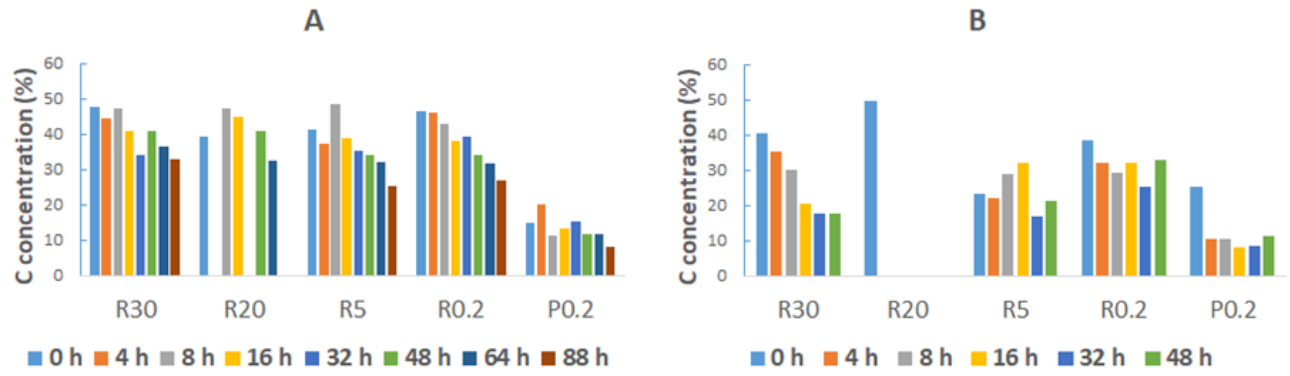

Figure 5. Organic C w/w % concentration in Figure 5A and Figure 5 B, respectively, for Figure 4A D1 SBP and Figure 4B CP1 SBP products.

Chemical composition

For each product, Figures 5 and 6 give the organic C w/w % content and C/N ratio, respectively.
The C/N ratio is a useful parameter indicating possible differences in the chemical composition of the organic matter present in each fraction of SBP, [8] as C concentrations (Figure 5) are affected by the different ash content of the products. For each SBP, fractions obtained with membranes with the same cut off were compared to assess significant differences of C/N values.
Figure 6 shows that, for CP fractions, significant C/N differences are observed within the R0.2 and P0.2 groups. For D fractions, significant C/N differences are present within all groups except the R0.2. Particularly relevant are the high C/N values of the 64 h and 88 h ozonised R30 fractions. These values indicate that the chemical nature of 64 and 88 h ozonised D is significantly different from that of all other D fractions (see also below Characterisation of D1 obtained at 64-88 h reaction time).

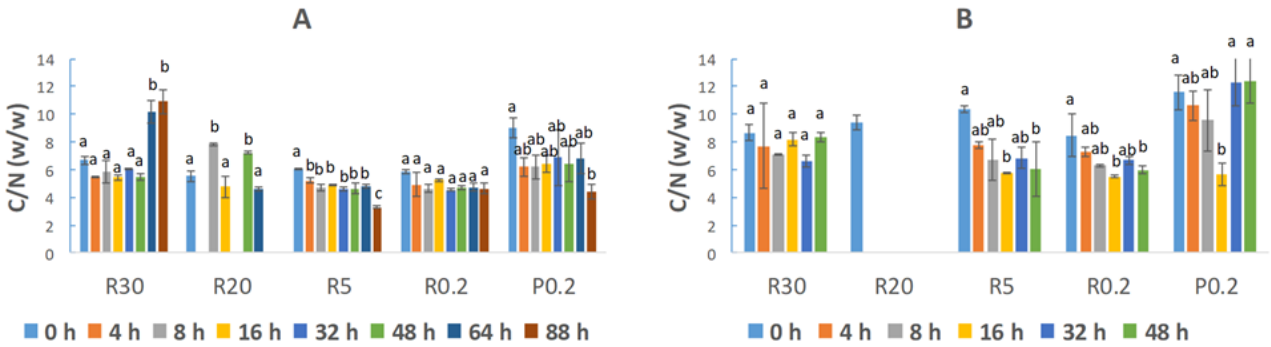

Figure 6. C/N ratio in Figure 6A and Figure 6B, respectively, for Figure 4A D1 SBP and Figure 4B CP1 SBP products. Within each group of fractions with the same Ri (i= 0.2-30) value or within the P0.2 group, column C/N values with different letters are significantly different at $P < 0.05$ level. Values followed by two letters are not significantly different from values having one of the two letters in common.

With the perspective to scale up the ozonisation to industrial production level, data in Figure 4-6 are highly relevant, allowing to assess readily the yield and chemical composition of the obtainable products as a function of the reaction time. Particularly, the C/N ratio appear as a sensitive and easily analysed parameter that may allow clearance of the product for its commercial release.

Surface tension in water solution a 2 g L^{-1} concentration

Previous works[8] on SBP indicated three features needing improvements, connected with the use of SBP as surfactants. These are the capacity to lower the surface tension of water, the critical micellar concentration and the colour of the solutions. The critical micellar concentration of pristine SBP has been reported about 2 g L^{-1}. SBP in the solid state are black and, unfortunately, 2 g L^{-1} solutions are still dark. Herein after, the soluble ozonised fractions are evaluated and compared based on water surface tension and colour at 2 g L^{-1} concentration.
Surface tension measurements showed clearly that only R30 fractions lower the water surface tension. Specifically, only 48 h ozonised CP R30 (Table 1) lower significantly water surface tension compared to starting material. Conversely, ozonised D SBP fractions show surface tensions significantly lower than pristine D SBP starting from 16 h reaction time, with the 48 h ozonised fraction reaching the lowest value.

Table 1. Surface tension (γ, $mN.m^{-1}$) for the 2 g L^{-1} solutions of the R30 fractions of ozonised CP SBP and D SBP versus reaction time (t, h).[a]

t, h	0	4 h	8 h	16 h	32 h	48 h
CP	67.7±0.43a	61.8±0.32ab	62±0.20ab	64.6±0.70ab	67.6±4.12a	59.2±0.40b
D	56.8±1.23ac	60.3±0.77b	55.8±0.56c	52.1±0.68d	45.4±0.09e	43.4±0.46e

[a]Within each row values with different letters are significantly different at $P < 0.05$ level. Values followed by two letters are not significantly different from values having one of the two letters in common.

Figure 7 shows the bleaching effect of ozonisation on the R30 fractions isolated from the reaction at 0, 4, 8, 16, 32, 48 h reaction time. For CP SBP, significant bleaching is observed at 16 h reaction time. For D SBP, significant bleaching is observed already at 4 h reaction time. The black colour of pristine CP and D SBP is likely due to the chromophore aromatic moieties, memory of the lignin-like proximates present in compost and digestate. Other authors[19] report that ozone reacts with lignin aromatic rings, destroying the chromophore structures to form aliphatic carboxylic moieties. As the darker pristine CP SBP contains 40 % more aromatic C than the lighter D SBP (Table S1), D SBP is bleached at shorter reaction times. In addition, the ozonised D SBP (D1) exhibits better surfactant properties than the oxidized CP SBP.

Based on these results, D1 obtained at 64-88 h reaction time was selected for further investigation in the third stage of the experimental plan.

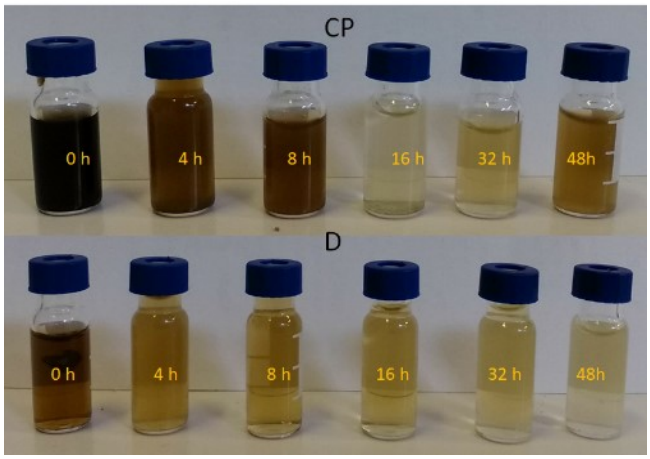

Figure 7. Colour of the 2 g L^{-1} solutions of the R30 fractions of ozonised CP and D SBP recovered from the reaction at 0, 4, 8, 16, 32, 48 h reaction time

Characterisation of D1 obtained at 64-88 h reaction time

Molecular weight distribution and C/N ratio

For pristine and for 64 and 88 h soluble ozonised D SBP, Figure 8 shows the organic C distribution over the fractions obtained through sequential UF using membranes with molecular cut-offs from 750 to 0.2 kDa. It is readily evident that ozonisation drastically changes the molecular weight distribution of pristine D SBP. No statistical differences appear between the ozonised D samples.

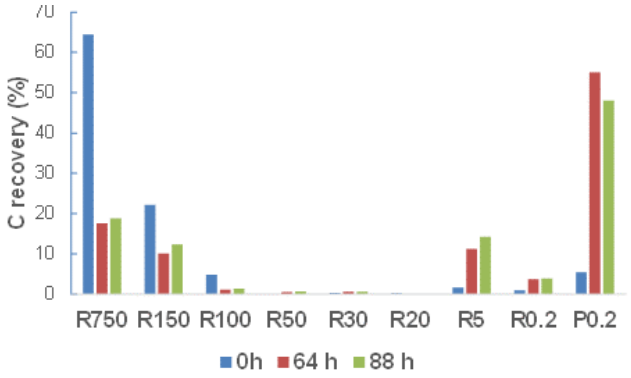

Figure 8. Relative organic C recovery (w/w %) with fractions isolated by ultrafiltration of pristine and 64 and 88 soluble ozonised D SBP. Ri (i = 750-0.2) and P0.2 are the retentates and permeate fractions containing molecules in the following molecular weight ranges: over 750 kDa for R750, from 750 to 150 kDa for R150, from 150 to 100 kDa for R100, from 100 to 50 kDa for R50, from 50 to 30 kDa for R30, from 30 to 20 kDa for R20, from 20 to 5 kDa for R5, from 5 to 0.2 kDa for R0.2, below 0.2 kDa for P0.2.

Figure 9 reports the organic C concentrations and C/N values of the fractions. The difference with the data in Figure 4-6 is that Figure 8 and 9 show the further breakdown of the pristine and 64 and 88 h ozonised R30 D fractions into the higher molecular weight fractions. This further breakdown is highly relevant, as it will be shown below (Table 2) that only the fractions with molecular weight above 100 kDa exhibit surfactant properties.

From Figure 9B, emerge that the higher C/N values of the 64 and 88 h ozonised R30 D in Figure 6 are due mainly to the contribution of the higher molecular weight R750, R150 and R100 fractions.

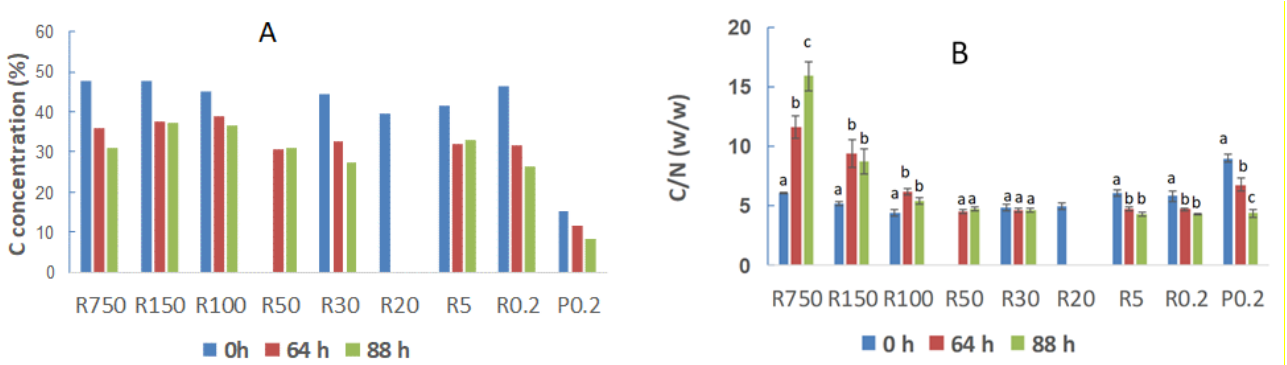

Figure 9. Organic C w/w % concentration (Figure 9A) and C/N ratio (Figure 9B) for Figure 8 products. Within each group of fractions with the same Ri (i= 0.2-30) value or within the P0.2 group, column C/N values with different letters are significantly different at $P < 0.05$ level. Values followed by two letters are not significantly different from values having one of the two letters in common.

Surface tension of R750, R150 and R100 fractions

Table 2 reports the surface tension (γ, mN m^{-1}) for the 2 g L^{-1} solutions of the R750, R150 and R100 fractions of pristine and ozonised D SBP at 64 and 88 h. The data confirm the superior performance of the 64 h ozonised fractions, compared to others. The 64 h ozonised R150 fraction yields the lowest surface tension value while a longer ozonisation time (88 h) seems to worsen the product performance.

Table 2. Surface tension (γ, mN m^{-1})[a] for the 2 g L^{-1} solutions of the R750, R150 and R100 fractions of pristine and ozonised D SBP at 64 and 88 h.

Reaction time, h	0	64	88
R750 γ	56.8±0.8a	39.5±1.0b	49.0±0.3c
R150 γ	57.3±0.0a	36.8±0.6b	58.6±0.2a
R100 γ	54.2±0.7a	43.6±2.3b	

[a]Within each row values with different letters are significantly different at $P < 0.05$ level.

Figure 10 shows the colour of Table 2 solutions. There seems to be an interesting correlation between colour and surface tension. The products with $\gamma > 49$ are darker. Based on the lowest surface tension at 2 g L^{-1} (Table 2) and colour whiteness (Figure 10), the 64 h ozonised R150 fraction was selected for further investigation on the behaviour of its macromolecules in solution. This required measurements of the surface tension as function of the product concentration in water. The results are shown in Figure 11.

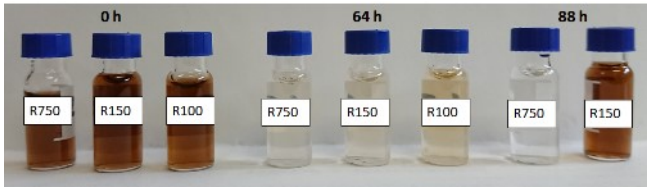

Figure 10. Color of the 2 g L^{-1} solutions listed in Table 2

The pattern of the plot of surface tension versus concentration in Figure 11 is typical of conventional small molecule surfactants.[26] At low concentrations, the surfactant molecules lie at the air-water interface to expose the lowest possible hydrophobic surface to water. At higher concentrations, when the air-water interface is saturated, the excess surfactant molecules aggregates, forming micelles in the bulk water phase. In this form, several molecules are held together by intermolecular forces to yield spherical or pseudo-spherical clusters where hydrophobic surfaces remain in the inner micellar core whereas polar heads are directed toward the water phase. In a typical case, the premicellar and the post micellar regimes are defined by two linear surface tension (γ)-added surfactant concentration (C) with different slopes. The intersection of the two lines gives the critical micellar concentration (cmc). The slope change is because, above the cmc, the effect of the added surfactant molecules on the air-water surface interfacial tension is much lower, since they mainly increase the bulk phase molecular aggregates.

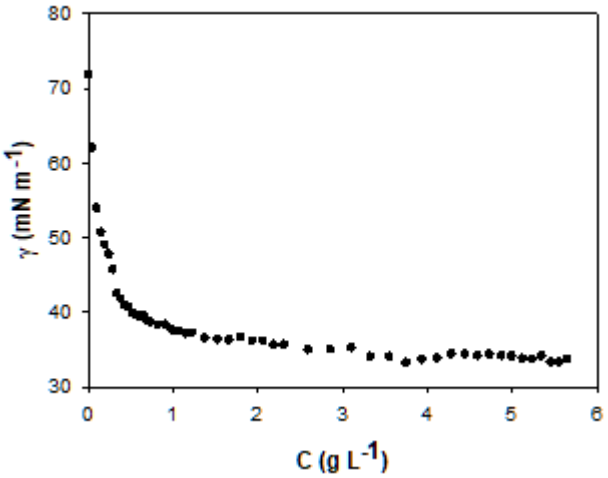

Figure 11. Surface tension (γ) of R150 fraction isolated from the 64 h ozonised D SBP vs. added product concentration.

Figure 11 shows that upon increasing the product concentration from 0.1 to 0.38 g L^{-1}, the surface tension decreases linearly with a steep slope. At higher concentrations, the decrease of the surface tension is significantly less steep and it tends to flatten out from 3.1 g L^{-1}. The experimental data in the 0.1 to 0.38 g L^{-1} and 1.1 to 3.1 g L^{-1} concentration ranges are well interpolated by the linear equation:

$\gamma = a - b\ C$ (1),

where γ is the surface tension, C is the product concentration.

The linear regressions yields a = 57.6 ± 0.6, b = 42.0 ± 2.5, r (correlation coefficient) = 0.99 calculated over the 0.1-0.38 g L^{-1} range, and a = 38.6 ± 0.3 , b = 1.3 ± 0.2, r = 0.93 calculated over the 1.1-3.1 g L^{-1} range.

The change of slope from 42 to 1.3 is attributed to the formation of micelle constituted by aggregates of the polymeric molecules. The calculated values for the critical micellar concentration (cmc) and for the surface tension at the cmc value (γ_{cmc}) are, respectively, 0.47 g L^{-1} and 38 mN m^{-1}. The cmc and γ_{cmc} values suggest the R150 fraction of the 64 h ozonised D SBP as a remarkable surfactant (see below R&D perspectives).

For the scope of the present work, these surface tension data (Table 1, 2 and Figure 11) were highly relevant. The availability of the different SBP and SBPox fractions gave the opportunity to study for these new biopolymers the relationship of the surface tension with their chemical nature. Assessing this relationship is essential for all practical purposes connected to the implementation of SBP and SBPox production at industrial and commercial level (see subsection "structure of the results' presentation and discussion"). It constitutes the fourth step of the experimental plan, which is described in the next subsection.

Chemical composition of pristine and 4-64 h ozonised D SBP using ^{13}C NMR solid state spectroscopy

Table 3 reports chemical shifts, assignments to C types and functional groups, and percent values of each band area of the ^{13}C NMR solid state spectra, obtained by integrating each band area in the reported chemical shift ranges. Resonance bands assignments are according to a previous works.[8] In addition to the SBPox data, pristine SBP have been analyzed to increase the data pool, allowing to have a statistically more reliable correlation of surface tension versus chemical composition (see Figure 12).

In the evaluation of Table 3 ^{13}C data, it must be considered that the band area % values are only estimates of the true C mol/mol % of the different C type giving rise to the resonance bands. In H poor moieties, such as polycyclic aromatic hydrocarbons, C is underestimated due to poor polarisation of ^{13}C far from ^{1}H nuclei. Vice versa, protonated C groups may be overestimated. For 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 (COOM, M = H or metal)), ester (COOR) and amide (CON) C bonded to O. For SBP, obtained under alkaline conditions, presence of COOR is excluded. The band at 140-160 ppm arises from phenol and phenoxy C bonded to O.

The data show that Table 3 products have a variety of lipophilic and hydrophilic C moieties, and that the relative ratios of these moieties vary considerably over the different products. As an example, the aliphatic C content of the 64 h ozonised R750, R150 and R100 fractions is much higher than that of all other fractions while they differ largely for carboxyl (COX) content. Generally, all the ozonised fractions have a higher aliphatic/aromatic (Af/Ar) C ratio. This was an expected result of the ozonisation proces. Cataldo[27] has studied the reactivity of different proteins with ozone, reporting that aromatic amino acids are the most sensitive to ozonisation. Oxidation occurs at various degrees including lysis of the aromatic rings. Vice versa, the polyamide bond of the protein main chain is not degraded. Other authors report that ozone reacts with lignin aromatic rings,[28] converting aromatic carbon to aliphatic carboxyl carbon.[29]

Prescinding from the reasons leading to the data in Table 3, from the statistical point of view the large compositional variability allows testing over a wide range of values the correlation shown in the next subsection (Figure 12), therefore guaranteeing for its reliability.

Table 3. ^{13}C NMR spectroscopy chemical shifts (δ, ppm) of pristine and 4-64 h ozonised D SBP fractions.Assignment to C types and functional groups[a] calculated as C % referred to total C.[b]

δ, ppm	**0-53**	**53-63**	**63-95**	**95-110**	**110-140**	**140-160**	**160-185**	**185-215**	
Product/C type	Af	NR	OR	OCO	Ph	PhOY	COX	C=O	Af/Ar
R750/0h	49.4	7.9	13.2	4.2	6.5	2.0	14.5	2.1	5.8
R150/0h	42.0	9.8	14.6	3.9	8.2	2.9	17.9	0.7	3.8
R100/0h	39.3	10.2	10.8	2.9	9.4	3.5	21.2	2.6	3.0
R30/4h	44.1	9.4	15.4	3.9	4.3	1.5	20.9	0.6	7.6
R30/8h	47.4	9.0	13.2	2.9	2.8	0.59	22.6	1.4	14
R30/16h	42.3	4.9	9.5	2.2	5.6	5.2	25.5	4.7	3.9
R30/32h	57.1	7.0	10.0	2.1	2.2	0.1	20.6	1.0	25
R30/48h	45.5	5.2	11.8	3.0	7.6	2.1	22.9	1.8	4.6
R750/64h	74.4	1.1	3.1	2.8	4.6	3.9	5.7	4.5	8.7
R150/64h	72.7	2.5	1.4	2.5	3.8	3.7	7.9	5.4	9.7
R100/64h	49.6	6.2	7.7	0.4	3.8	3.4	26.5	2.4	6.9

[a]Data for aliphatic and/or cycloaliphatic (Af), amine (NR), alkoxy (OR), anomeric (OCO), aromatic (Ph), phenol and phenoxy (PhOY, Y = H and/or R), amide and carboxylic (COX, X = NR, H, Metal) and cabonyl (C=O) C atoms. R = H, alkyl and/or aryl C. Ar = sum of Ph and PhOY C. [b]Products identified by the fraction number (Ri)/time (h) of the reaction from which they were isolated.

Surface activity properties versus chemical composition for pristine and 4-64 h ozonised D SBP

The capacity of surfactants to lower water surface tension depends on their hydrophilic lipophilic balance (HLB). This empirical parameter allows predicting the performance of a surfactant. It is calculated based on the proportion between the weight percentages of functional groups,[30] and it is related to the oil and water solubility of the compound under investigation. It depends also from the configuration of the molecular structure in solution. For synthetic small molecule and polymeric surfactants of well-known chemical composition and structure, calculation of HLB is feasible. In the case of SBP, establishing a relationship between products surface activity and chemical nature is hard. Yet, such relationship may help to identify a target SBP chemical composition and drive further research for optimizing its performance as surfactant.

Any attempt to establish a correlation between surface tension values in Table 1 and 2 and Table 3 raw % values fails. Better results (Figure 12) are obtained by calculating the HLB value for each product.

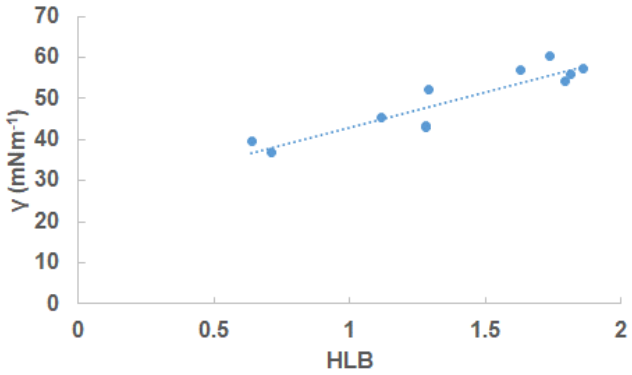

Figure 12. Plot of surface tension (γ) values (Table 3 and 4) vs. HLB interpolated by equation γ = a + b HLB (5), where a = 26.0 ± 3.5, b = 17.1 ± 2.4, r (correlation coefficient) = 0.91.

The HLB values in Figure 12 are calculated according to the following equations:

$Ci = C_{bai}\, C_{orgdm}\, C_{dm}/10^4$ (2)

$HLB_i = Ci\ gn_i$ (3)

$HLB = \Sigma\ HBL_i$ (4)

In Equations 2-4, Ci is the content of the individual C type (listed in Table 3) in the 2 g L^{-1} solution used for the surface tension measurements; C_{bai} is the individual C type % band area (reported in Table 5); C_{orgdm} is the product organic C concentration (reported in Fig 5 and 9); C_{dm} is the 2 g L^{-1} dry matter content of the product in the solution used to obtain the surface tension values in Table 1 and 2; gn_i are the individual group number values for each C type (Table 3S); HBL_i is the contribution of the individual C type to the total HBL value calculated for the product containing it.

The plot in Figure 12 is interpolated according to the equation:

$\gamma = a + b\ HLB$ (5),

where a = 26.0 ± 3.5, b = 17.1 ± 2.4, r = 0.91. The high correlation coefficient is quite remarkable, considering the complexity of pristine and ozonised SBP, and that the values reported in Figure 12 derive from different experimental measurements and the use of gni values calculated by different authors (see SI). The HLB values in Figure 12 account for the contribution of all the different C types and functional groups, which could be identified. Omitting one or more C types and functional groups from the calculation of the products HLB yields very poor surface tension-HLB correlations.

Characterisation of the P0.2 fraction of the 64h ozonised D SBP

Although the most valuable products are the SBPox (see Perspectives subsection below), the P0.2 fraction deserved to be analysed for a complete estimation of the potential value of the ozonisation process under investigation.
Based on a previous work on the ozonisation of CP SBP,[20] a large number of products was expected in the P0.2 fractions of each ozonisation run. Carrying on a qualitative and quantitative characterisation of the composition of each P0.2 fraction require a large amount of analytical work and, for the present work scope, it was unreasonable. On the other hand, the ozonisation of D SBP at 64 h reaction time gave very promising products for their values as surfactants (Table 2). Further investigation on the qualitative composition of the 64 h ozonised P0.2 fraction seemed therefore worthy to understand better the potential and criticalities of ozonisation to obtain sustainable valued added products from MBW.
Table 4 reports the list of the main compounds found in the P0.2 fraction of the 64h ozonised D SBP. These were identified (see Experimental in SI file) by LC-MS/MS in negative or positive ionisation mode. Some products analysed in positive ionisation mode were not fully identified. They have in common odd molecular weight value, likely due to the presence of an N atom, and a characteristic fragmentation pattern leading to loss of m/z 60 and m/z 46 fragments. They might contain alkyne and alkene chains substituted with one or two hydroxyl and oxime groups. Formulas given in Table 4 have C/N values ranging from 2 to 7. The C/N value for the 64 h ozonised P0.2 fraction (Figure 9) is about 7. Thus, the compounds listed in Table 4 with C/N 7 must be the main constituents of the P0.2 fraction. Similar compounds might be present also in pristine D SBP, having C/N = 8. However, the P0.2 fraction content in the ozonised product (Figure 8) is 10 times higher than in pristine D SBP. This suggests that the ozonisation of D SBP at 64 h reaction time converts a large part of the pristine biopolymers with molecular weight above 750 kDa into the small molecules listed in Table 4.

Table 4. Compounds, corresponding precursors and main product ions identified in negative or positive ionisation mode in the analysis of the P0.2 fraction of the 64 h ozonised D SBP.

Negative ionisation mode				**Positive ionisation mode**			
compound	formula	m/z precursor	m/z product	compound	formula	m/z precursor	m/z product
Oxalic acid	$C_2H_2O_4$	89.0	60.8	Acetylenediol	$C_2H_2O_2$	59.0	-
Malonic acid	$C_3H_4O_4$	102.8	58.9	Butadiynediol	$C_4H_2O_2$	83.0	42.2
Succinic acid	$C_4H_6O_4$	116.9	72.8	Glycerol	$C_3H_8O_3$	93.0	61.7
Malic acid	$C_4H_6O_5$	133	70.8	ni[b]	$C_2H_3NO_3$	89.9	44.2
Glutaric acid	$C_5H_8O_4$	130.7	86.8	ni[b]	$C_3H_3NO_3$	102.0	60.0
Hydroxyglutaric acid	$C_5H_8O_5$	147	128.7	ni[b]	$C_3H_5NO_3$	103.8	44.2
Carboxysuccinic acid	$C_5H_6O_6$	162.8	102.7	ni[b]	$C_4H_5NO_3$	115.8	56.0
Propantricarboxylic acid	$C_6H_8O_6$	174.7	130.8	ni[b]	$C_4H_7NO_3$	117.8	59.2
Adipic acid	$C_6H_{10}O_4$	145.0	100.7	ni[b]	$C_4H_9NO_3$	119.8	60.0
Propylene glycol	$C_3H_8O_2$	74.8	31.3	ni[b]	$C_5H_7NO_3$	129.8	70.0
Acetaldehyde[a]	C_2H_4O	222.9	186.7	ni[b]	$C_5H_9NO_3$	131.8	72.2

Propynal[a]	C_3H_2O	232.8	196.7	ni[b]	$C_6H_{13}NO_3$	147.8	87.9
Ketomalonic acid[a]	C_3H2O_5	297	181.6	ni[b]	$C_7H_{11}NO_3$	158.0	98.0
Acroleine[a]	C_3H_4O	234.8	196.6	ni[b]	$C_7H_{14}NO_3$	161.2	101.9
Oxaloacetic acid[a]	C_4H4O_5	311	181.6				
Methylbuthenyloxy[a]	C_5H_5O	260	196.8				
Hydroxybutanone[a]	C_4H_8O	267	181.8				
Hydroxyhexanone[a]	$C6H_{12}O_2$	295	181.6				

[a]Identified after dinitrophenylhydrazine derivatisation. [b]Compounds not fully identified.

Perspectives

The major products obtained in the present work are the ozonised R750, R150 and R100 fractions containing new biopolymers with molecular weight above 100 kDa and the P0.2 fractions containing a mix of small molecules. These biopolymers do not have known commercial counterparts. The P0.2 fractions contain a mix of commercial chemicals, falling under the category of platform molecules[31] and/or building blocks.[11]

Under the adopted ozonisation experimental conditions, the R750, R150 and R100 polymeric fractions are obtained with a lower yield than the P0.2 fractions (Figure 8). Yet, the former ones have a high potential as new value added chemical specialities, arising from the macromolecular structure and surfactants properties. The two following subsection are dedicated to the discussion of the potential value and of the R&D perspectives for the biopolymers and small molecules.

Biopolymers potential value

The surface tension and the cmc value of the biopolymers are two important parameters prospecting commercial applications as they have important effects on the surfactant value. Rates and pathways of many kinds of chemical reactions can be altered by performing the reaction in micellar media instead of aqueous solution.[32] The formation of micelles at low cmc means that a surfactant can perform its action at low concentrations. Thus, the surfactant consumption and the process costs decrease. Low surface tension is particularly important in commercial applications where the surfactant is used as wetting agent.[33] These are, for example, flotation, detergency, enhanced oil recovery, surface paint, emulsion. A major result of the present work is the bleaching (Figure 10) and the improvement of the surfactants properties of pristine SBP through room temperature oxidation. Current bio surfactants[11] are produced using plants or animal fats, or microorganisms. The global bio surfactants demand is 400,000 t/yr. Raw materials costs accounts for 10-30% of the total production costs. Rhamnolipids and sophorolipids are key products in the microbial bio surfactants market. They are glycolipids produced by *Pseudomonas aeruginosa*, amongst other organisms, frequently cited as the best-characterized bacterial surfactants. The four most important rhamnolipids comprise either one or two rhamnose molecules attached to one or two b-hydroxydecanoic acids. These wander molecules 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}. Due to the exceptional performance [34] their price range is 30-150 €/kg[35], much higher than the 1.5-5.5 €/kg price[36] of fossil sourced synthetic or oleochemical natural surfactants.

In comparison with the bacterial surfactants, the R150 D SBP exhibits colour, cmc, and γ_{cmc} values, competitive with glycolipids for performance. However, the D SBP production cost has been estimated 0.1-0.5 € kg^{-1}[20], much below the bacterial surfactant cost. With this in mind, even if the ozonised SBP was produced in low yields, its high potential value could

make the ozonisation process worthy of further development.

A second relevant result of the present work is the production of a range of pristine and ozonised products (Figure 4, 5, 7-10) with different chemical composition and surface tension values. This has allowed achieving the remarkable correlation in Figure 12 between product chemical composition and capacity to lower the water surface tension. The correlation shows that the product surface tension property depends on the balance between the different C-types and functional groups. Particularly, it suggests that the surfactant properties of the complex SBP molecular pool may be improved by oxidising the aromatic C moieties to achieve lysis of the aromatic ring and yield lipophilic C moieties.

For SBP, Rhamnolipids bacterial surfactants represent a model target structure to be achieved by further chemical reactions on SBP. The SBP contain aliphatic and anomeric C (Table 3), which suggest the presence of analogous moieties as in rhamnolipids. The SBP and SBPox contain also aromatic C. Total aromatic C runs 8.5-12.7 C in pristine SBP and 7.2-8.5 in 64 h SPBox (Table 2), because of SBP ozonisation conversion of aromatic rings to aliphatic carboxylic acids. This change in the distribution of total C in the pristine and ozonised SBP is associated with the better surface tension properties of ozonised SBP (Table 3). Ozonisation has been carried out also on sophorolipids with similar structural properties to rhamonolipids to increase their hydrophilic character and make them more efficient in detergency applications.[37] The comparison between data obtained in this work and in previous works on rhamnolipids and sophorolipids encourages further R&D on the oxidation under mild conditions to increase the yield and quality of the SBPox to obtain bio surfactants matching the above glycolipids properties.

Small molecules value

The potential value of the P0.2 fractions is both environmental and economical. They are obtained from renewable sustainable sources and contain a mix of compounds. The products listed in Table 4, could replace commercial counterpart products from fossil sources. They belong to a list of over 200 value-added bio-based compounds,[31] which can be derived from lignocellulose biomass (dedicated plants or agriculture residues) using fermentation or pyrolysis. They are referred to as building blocks or platform molecules to be used for the fabrication of materials and finished consumers' products. Most bio-based chemicals have higher production costs than their counterpart from fossil source. Market prices for bio based chemicals range from 0.62 €/kg for acetic acid to 2.94 €/kg for succinic acid and over 3 €/kg for 1,4 butanediol.[11] By comparison, prices for fossil sourced chemicals range from 0.62 €/kg for acetic acid to 2.50 €/kg for succinic acid and 1.8 €/kg for 1,4 butanediol. Cost competition between bio based and fossil derived chemicals depends largely on the price of the sourcing feedstock. To this respect, MBW, as negative cost feedstock,[9] is certainly favoured over agriculture biomass and crude oil.

The value of the P0.2 fractions arise mostly from the presence of the N containing products listed in Table 4 under the positive ionisation mode subheading. They represent the major constituents of the P0.2 fractions and they likely belong to the class of organic oximes. These compounds and their derivatives are important intermediates in organic synthesis and[38] they are also of interest as biologically active molecules. They are known as fungicides, products sold up to hundreds $/kg[39] in a market with 15 billion $/yr turnover.[40] The fungicide properties of the pristine D and CP SBP have been reported,[41] but not assigned to any component of these products. The finding that the ozonised SBP contain the potential fungicide oximes may prospect enhanced fungicide power by ozonised P0.2 fractions, compared to the pristine SBP containing 10 times lower amount of P0.2 compounds (Figure 8).

Theoretically , market allocation of the compounds in the P0.2 ozonised fractions would add value to the MBW ozonisation process. However, the commercial production of the P0.2 fraction poses the problem of the separation of the many compounds obtained in

the ozonisation reaction. Separation of complex mixtures of products is a commont problem most oxidation processes of fossil hydrocarbons. Mature separation technology is available from the conventional chemical industry.[42]

Conclusions

The results obtained in the authors' previous[8, 20] and present work show that mild chemical hydrolysis and oxidation at room or 60 °C temperature may be well integrated with biochemical processes to valorise biomass as renewable alternative to fossil feedstock more than incineration or high temperature hydrothermal hydrolysis, oxidation and pyrolysis. In the present work, focusing on fermented MBW, it is shown that room temperature oxidation allows improving the performance and value of biopolymers obtained by 60 °C hydrolysis.
Oxidation plays a fundamental role in the petrochemical industry[43]. Generally, oxidation is known to be a non-selective reaction. Selective oxidation processes have been developed starting from simple hydrocarbon molecules and using different oxidising reagents and catalysts. In the case of the complex macromolecular pool constituting SBP, selective oxidation is much more complicated. Yet, the potential value of the products obtained by ozonisation encourages further process and product development. This should be carried out along parallel directions. It should include the use of different oxidizing agents and catalysts and the quantitative determination of the P0.2 fraction composition, in order to proceed toward optimisation of the separation process. This work prospects a long time research but the potential value of the obtained polymers and monomers makes worthwhile to proceed in this direction.

Supporting Information Summary
The experimental section include the description and chemical composition of the pristine wastes, the characterization methods, apparatus and details of all the procedures described in the manuscript.

Conflict of Interest
The authors declare no conflict of interest.

Keywords: Bio-waste · Fermentation · Hydrolysis · Ozonolysis · Bio-surfactants · Bio-based chemicals

References

[1] A. C. Neto, M. J. O. C. Guimaraes, E. Freire, *J. Clean. Prod.* **2018,** *184*, 168-178.
[2] A. Iles, A. N. Martin, *J. Clean. Prod.* **2013,** *45*, 38-49
[3] Food and Agriculture Organization of the United Nations (FAO), *The State of Food and Agriculture*, FAO, Rome, **2008**, p. 138.
[4] G. Q. Chen, *Microb. Cell Fact.* **2012,** *11*, 1-3.
[5] F. R. Amin, H. Khalid, H. Zhang, S. Rahman, R. Zhang, G. Liu, C. Chen, *AMB Express* **2017**, 7, Article number 72.
[6] R. C. Sun, *BioResources* **2008,** *4*, 452-458.
[7] U. R. de Oliveira, L. S. Espindola, I. R. da Silva, I. N. da Silva, H. M. Rocha, *J. Clean. Prod.* **2018**, *187*, 537-561.
[8] E. Montoneri in *Food Waste Reduction and Valorisation, Chapter 6* (Eds.: P. Morone, F. Papendiek, V. E. Tartiu) Springer, Cham, **2017**, 79–120.
[9] G. A. Sheldon-Coulson, Production of Levulinic Acid in Urban Biorefineries Master of Science Thesis, Technology and Policy Institute (USA), **2011.**
[10] Ellen MacArthur Foundation, Report "Cities in the circular economy: an initial exploration" to be found under https://www.ellenmacarthurfoundation.org/assets/downloads/publications/Cities-in-the-CE_An-Initial-Exploration.pdf (accessed October 5, 2019), **2017**.
[11] E. Tsagaraki, E. Karachaliou, I. Delioglanis, E. Kouzi, "Document D2.1" to be found under http://www.bioways.eu/download.php?f=150&l=en&key=441a4e6a27f83a8e828b802c37adc6e1 (accessed October 26, 2019), **2017.**
[12] J. Irmer, *Bioeconomy BW* to be found under https://www.biooekonomie-

bw.de/en/articles/dossiers/lignin-a-natural-resource-with-huge-potential (accessed November 6, 2019), **2017**.

[13] I. Janker-Obermeier, V. Sieber, M. Faulstich, D. Schieder, *Ind. Crops Prod.* **2012**, *39*, 198– 203.

[14] F. Monteil-Rivera, G. Hai Huang, L. Paquet, S. Deschamps, C. Beaulieu J. Hawari, *Bioresour. Technol.* **2012**, *104*, 775–782.

[15] A. Liu, Y. Park, Z. L. Huang, B. W. Wang, R. O. Ankumah, P. K. Biswas, *Energy Fuels* **2006**, *20*, 446-454

[16] S. Karagoz, T. Bhaskar, A. Muto, Y. Sakata, *Fuel* **2005**, *84*, 875-884.

[17] O. Pourali, F. S. Asghari, H. Yoshida, *Chem. Eng. J.* **2010**, *160*, 259-266.

[18] B. Zhang, H. J. Huang, S. Ramaswamy, *Appl. Biochem. Biotechnol.* **2008**, *147*, 119-131.

[19] S. P. Mishra, D. Lachenal, C. Chirat, *Pulp & Paper International* to be found under https://technology.risiinfo.com/chemicals/west-europe/benefits-ozone-bleaching (accessed November 8, 2019), **2011.**

[20] E. Montoneri, D. Rosso, G. Bucci, S. Berto, A. Baglieri, R. Mendichi, P. Quagliotto, M. Francavilla, D. Mainero, M. Negre, *ChemistrySelect* **2016**, *1*, 1613-1629.

[21] S. R.Collinson, W. Thielemans, *Coord. Chem. Rev.* **2010**, *254*, 1854-1870.

[22] T. Lou, Y. C. Hou, W. Z. Wu, M. G. Niu, S. H. Ren, Z. Q. Lin , V. K. Ramani. *Fuel* **2018**, *216*, 572-578.

[23] A. E. Modvig, Selective Oxidation of Biomass-Derived Chemicals PhD thesis, Technical University of Denmark (DK), **2017**.

[24] R. Ma, Y. Xu, X. Zhang, *ChemSusChem* **2015**, *8*, 24-51.

[25] R. Wang, C. L. Chen, J. S. Gratz, *Holzforschung* **2004**, *58*, 622–630.

[26] M. J. Rosen, *Surfactants and Interfacial Phenomena* **1989**, 2nd ed., Wiley, New York.

[27] F. Cataldo, *Polym. Degrad. Stab.* **2003**, *82*, 105–114.

[28] K. Niemel, R. Alen, E. Sjostrom, *Holzforschung* **1985**, *39*, 167 –172.

[29] G. Brunow, K. Lundquist, G. Gellerstedt in *Analytical Methods in Wood Chemistry, Pulping and Papermaking* (Eds: E. Sjostro, R. Alen), Springer Verlag, Germany, **1998.**

[30] R. Sowada, J. C. McGowan, *Tenside Surf. Det.* **1992**, *29*, 109–113.

[31] F. H. Isikgor, C. R. Becer, *Polym. Chem.* **2015**, *6*, 4497-4559.

[32] J. Du, B. Jiang, J. Xie, X. Zeng, *J. Disper. Sci. Technol.* **2001**, *22*, 529–533.

[33] S. K. Shah, A. Bhattarai, S. K. Chatterjee in *Modern Trends. in Science and Technology* (Eds.: D. Adhikari, S. K. Rai, K. P. Limbu, Nepal Biological Society, Nepal Physical Society (Eastern Chapter) and Research Council of Science and Technology), **2013**, 147-158.

[34] K. K. S. Randhawa, P. K. S. M. Rahman, *Front. Microbiol.* **2014**, *5*, 454.

[35] H. E. Connolly, P. K. S. M. Rahman, I. M. Banat, R. A. Lord in *Trends in Bioremediation and Phytoremediation* (Ed.: G. Plaza), Research Signpost, India, **2010**, 157-172.

[36] D. Rust, S. Wildes, *Surfactants: a market opportunity study update,* to be found under https://soynewuses.org/wp-content/uploads/Surfactants-MOS-Jan-2009.pdf (accessed December 4, 2019), **2008.**

[37] I. A. Van Bogaert, W. Soetaert, Sophorolipids in *Biosurfactants, Vol. 20* (Ed.: G. Soberón-Chávez), Springer, Germany, **2011**, 179–210.

[38] E. Abele, E. Lukevics, *Org. Prep. Proced. Int.* **2000,** *32*, 235-264.

[39] Ebiochem, *Wholesale 2,1,3-Benzothiadiazole* to be found under http://www.ebiochem.com/product/2-1-3-benzothiadiazole-71535 (accessed October 15, 2019), **2016**.

[40] B. C. Gerwick, T. C. Sparks, *Pest Manage. Sci.* **2014**, *70*, 1169-1185.

[41] B. Jindrichova, L. Burketova, E. Montoneri, M. Francavilla, *J. Clean. Prod.* **2018,** *183*, 335-342

[42] National Research Council, *Separation technologies for the industries of the future*, The National Academies Press, Washington, DC, **1998**.

[43] M. G. Clerici, M. Ricci, F. Rivetti, *Enciclopedia degli idrocarburi,* Istituto della Enciclopedia Italiana Fondata da

Giovanni Treccani S.p.A, Rome, **2006**, pp. 615-686.

Entry for the Table of Contents

Different urban biowastes were fermented to yield anaerobic digestate and compost. These were hydrolyzed to yield soluble biopolymers (SBP) and oxidated through ozonisation to produce small molecules to be used as building blocks and biopolymers with lower molecular weight, improved surfactants properties and a lighter colour, compared to pristine SBP.