**Analysis and optimisation of a novel “bio-brewery” approach: Production of bio-fuels and bio-chemicals by microwave-assisted, hydrothermal liquefaction of Brewers’ Spent Grains**

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**Abstract**

This work firstly explores the feasibility of using a novel microwave-assisted, catalysed, hydrothermal process for the valorisation of brewers’ spent grains (BSGs), examining the effects of the temperature (180-250 ºC), pressure (50-130 bar), reaction time (0-2 h) and catalyst amount (Ni-Co/Al-Mg, 0-0.25 g cat/g biomass). This route turned out to be a very promising approach for the production of bio-fuels (bio-oil and bio-char) and platform chemicals (sugar rich aqueous solutions) in a single unit, helping the development of an innovative bio-refinery around BSGs. The overall conversion and the yields to gas, aqueous fraction and bio-oil varied by 31-68%, 10-33%, 9-48% and 4-14%, respectively. The bio-oil was made up a complex mixture of phenols (0-26%), ketones (0-80%), aldehydes (0-57%), carboxylic acids (0-18%) and nitrogen compounds (0-76%). The proportions of C, H, N and O in the bio-oil varied as follow: 15-61 wt.%, 5-10 wt.%, 1-6 wt.% and 26-77 wt.%, respectively, which shifted its higher heating value (HHV) between 9 and 27 MJ/kg. The liquid fraction comprised a mixture of DP>6 oligosaccharides (67-98 C-wt.%), DP2-6 oligosaccharides (0-10 C-wt.%), saccharides (0.2-7 C-wt.%), carboxylic acids (0-7 C-wt.%) and furans (0-27 C-wt.%). The spent solid after the experiments resembled an energetic like bio-char product, whose proportions of C, H, O and N varied by 35-72 wt.%, 4-8 wt.%, 1-4 wt.% and 18-57 wt.% and its HHV shifted between 9 and 32 MJ/kg. The optimisation of the process revealed that using a temperature of 250 ºC and a pressure of 125 bar for 2 h, it is possible to convert the original material into high-energy biofuels: (8%) bio-oil (26 MJ/kg) and (35%) bio-char (32 MJ/kg); together with a (31%) saccharide-rich (>99 C-wt.%) aqueous solution, thus converting this process into a very promising approach to achieve an environmentally-friendly and integral valorisation of brewers’ spent grains.

**Keywords:** brewers’ spent grains, hydrothermal processes, microwave heating, bio-fuels, value-added chemicals.

**Highlights (85 characters including spaces)**

- Environmentally friendly bio-refinery concept developed around brewers’ spent grains

- Production of high-energy biofuels (bio-oil & bio-char) and sugar rich solutions

- Detailed effect of process conditions: temperature, pressure, time & catalyst loading

- Optimum conditions for a complete BSGs valorisation: 250 ºC, 125 bar, 2 h

**1. Introduction**

Brewers’ Spent Grain (BSG) is a lignocellulosic solid material obtained during the production of beer [1]. It is a by-product from the mashing and filtration of malting grains during maltose production and largely consists of barley malt grain husk as well as part of the pericarp and seed coat layers. On average, BSGs contain around 70 wt.% of moisture (largely water) and 30 wt.% of solid matter. This high proportion of water hinders their transportation, storage and disposal due to possible microbial growth [2]. The composition of brewers’ spent grains varies with barley variety, time of harvest, characteristics of hops and other adjuncts added, and brewery technology [3]. On average, the dried solid fraction contains 12-25 wt.% cellulose, 22-40 wt.% hemicellulose, 4-28 wt.% lignin, 14-27 wt.% proteins and 4-13 wt.% lipids [4]. Roughly, during the production of 100 L of beer, around 45 kg of BSGs is generated, which is equivalent to 87 million tonnes a year of BSGs produced worldwide [5, 6]. Therefore, as a result of environmental concerns and the increasing beer production worldwide, BSGs management has become an important issue and new methodologies, strategies and management processes have to be developed for the valorisation of this feedstock.

Along this line, three alternative methods have been addressed over the past few years for the valorisation of BSGs. The first relies on the use of this solid material as a promotor or co-feeding material in several applications, such as, biomedical [7], brick component [8], paper manufacturing [9], and adsorbents for cadmium [10] and chromium [11]. The second includes its direct application in the food and chemical industries without valorisation; while the third consists on the production of energy and chemicals using several thermochemical and biological processes. With respect to the use of BSGs in the food industry, this material has been primarily used as a cattle feed for a wide range of animals [12]. In this regard, Tsirgogianni et al. [13] reported that milk production increased without significantly affecting the health or fertility of the animals, Kaur et al. [14] observed that the addition of 30 wt.% BSG on carps daily diet led to an increase in their body weight without significant health repercussions. Besides, BSGs have also been used as a flour substitute during the manufacturing of bread, biscuits and snacks [15]. For example, Steinmacher et al. [16] addressed the use of BSGs for the production of bread with better physical properties by means of an innovative extrusion-bioconversion process. Nascimento et al. [17] studied the effect of the addition of BSGs into rice to produce snacks. Nevertheless, BSGs can only be used for the production of off-white products due to their flavour and dark colour. Besides, BSGs should be always added in small quantities to prevent the alteration of some of the physical properties of the final product.

As regards the production of chemicals and energy, several biological and thermochemical processes have been addressed to recover valuable components from this residue as well as for the production of value-added chemicals and energy. Biological routes include the production of ethanol, butanol, hydrogen, xylitol, lactic acid and biodiesel via fermentation of the carbohydrate content of BSGs, however, the valorisation of the lignin content was not addressed. White et al. [18] produced ethanol from BSGs by means of an acid hydrolysis (0.16N HNO3) followed by fermentation using *Pichia stipitis* and *Kluyveromyces marxianus*. Xiros et al. [19] reported the production of ethanol using an alkaline pre-treatment under microaerobic conditions followed by a fermentation step with *Fusarium oxysporum*. Plaza et al. [20] obtained 75 g butanol/kg BSG during the ABE (acetone, methanol, ethanol) fermentation with *Clostridium beijerinckii* of BSGs previously treated with sulfuric acid. Though, these works used mineral acids such as nitric and/or acetic acid and, therefore, a post treatment step was needed to treat the liquid effluent produced. Zhang et al. [21] used a calcined red mud (CRM) pre-treatment to produce hydrogen. A yield of 199 mL of hydrogen/g volatile solid was reported when BSG was treated with 10 g/L of CRM. Mussatto et al. [22] produced xylitol from BSGs by means of an acid hydrolysis followed by fermentation with *Candida guilliermondii*. Hofvendahl et al. [23] used the glucose produced from BSG as a substrate to product lactic acid using *Lactobacillus delbrueckii.* Mallen et al. [6] developed an acid catalysis decomposition coupled with an in situ transesterification method for biodiesel production. With this process, the triglycerides present in BSGs were converted to biodiesel in a yield of 83 % under optimum conditions (60 °C, 7.2 mL/g methanol/BSG and 0.95 M H2SO4 for 300 min). Despite these good results, these works only considered the valorisation of the carbohydrate or lipid contents of the BSG without reporting possible uses for the other structural components of the material. Therefore, more investigations are still needed towards an integral valorisation of this by-product.

Thermochemical processes have been used for the production of energy and value-added chemicals from BSG. Okamoto et al. [24] and Sato et al. [25] examined the production of high energy dense charcoal (27 MJ/kg) from BSG. However, the major problem was the high levels of NOx and dust particle emissions released during the combustion of the charcoal produced [26]. As regards value added products, the use of thermochemical processes for BSGs valorisation has been focused on the conversion of the lignin fraction of the solid for the production of phenolic compounds. In particular, Mussatto et al. [27] extracted ferulic and p-coumaric acids by alkaline hydrolysis. Ferulic and p-coumaric acids yields of 9.65 mg/g lignin and 9.22 mg/g lignin were respectively obtained using a reaction temperature of 120 ºC and 2 wt.% NaOH for 90 minutes. Meneses et al. [28] addressed the production of antioxidant phenolic compounds from BSGs by solvent extraction using acetone/water mixtures. They found that the antioxidant capacity of the extracts was strongly correlated to their flavonoids content and that optimum conditions took place with 60 vol.% acetone in the solvent. Socaci et al. [29] used acetone-ethanol/water solvent systems for the production of antioxidants from BSGs, evaluating the antimicrobial and antimutagenic activities of the extracts. Optimum conditions were 60/40 vol.% and 40/60 vol.% for the ethanol/water and acetone/water system, respectively.

Another interesting option for the management and valorisation of BSGs that has not been addressed before is the catalytic hydrothermal liquefaction (HTL) of this residue for the simultaneous production of value-added chemicals and biofuels in a single process aiming to build up a sustainable and environmentally friendly bio-refinery concept around this residue: a novel “bio-brewery” approach. In addition, to the best of the authors’ knowledge, thermochemical processes have never been used for the complete valorisation of this material; i.e. a one-pot efficient method to completely valorise the cellulose, hemicellulose, lignin and protein contents of the solid in a single step. In this regard, HTL is a thermochemical process allowing the conversion of biomass using liquid hot compressed water at subcritical conditions: moderate temperatures (150-400 ºC) and relatively high pressures (5-25 MPa) [30-35]. These conditions dispense with the need to vaporise the water and/or dry the raw material, thus helping to improve the economic aspects and energetic profitability of the process. This is of paramount interest for upgrading watery feedstocks, such as BSG. A considerable amount of literature is available addressing the use of this technology for the valorisation of wet biomass; however, this technology has never been used for the valorisation of BSGs. Besides, the fact that microwave heating represents a potentially faster, more efficient and selective process for the thermal treatment of biomass [36-39]. The use of microwave reactors to conduct pressurised hydrothermal reactions at moderate temperature and elevated pressure is an emerging technology and the number of publications using microwave, pressurised reactors is very scarce. Bearing in mind that water is very effective in microwave energy absorption; the combination of hydrothermal liquefaction along with microwave assisted heating offers a very promising new technology for the valorisation and management not only of BSGs but also of many other types of biomass by-products [40-42]. In particular, microwave heating, i.e. converting electromagnetic radiation into heat energy within the target material is considered one of the most up-and-coming methodology to replace conventional heating in biomass valorisation [37, 40, 43-48] as microwave heating allows using much lower temperatures and helps improve the controllability of the process. Hydrothermal liquefaction of biomass provides a viable route to liquid biofuels. However, a subsequent upgrading of the bio-oil might be required to come up with a bio-oil with suitable fuel properties. A possible solution is the use of a catalyst during the hydrothermal process. This is intended to improve the process efficiency aiming to improve the yield and the physicochemical properties of the bio-oil [34]. In this work, a heterogeneous Ni-Co/Al-Mg catalyst has been used to improve the yield and physicochemical properties of the reaction products.

Herein, this work firstly addresses the valorisation of BSGs by Microwave-Assisted Hydrothermal Liquefaction (MA-HTL) for the simultaneous production of value-added chemicals and liquid and solid biofuels, examining the effects of the temperature (180-250 ºC), pressure (50-130 bar), reaction time (0-2 h) and catalyst amount (Ni-Co/Al-Mg; 0-0.25 g catalyst/g dry BSG) on the process. This includes the overall conversion, the yields to value-added products (gas, liquid and bio-oil) as well as some of their most important physicochemical properties, such as their chemical and elemental compositions and calorific values. The fact that Microwave-Assisted Hydrothermal Liquefaction has never been reported for the management and upgrading of BSGs before, together with the results provided by the in-depth parametric analysis and the optimisation conducted, demonstrates that this work represents a novel investigation in this field, which can help to develop novel, environmentally-friendly and energy efficient routes for the valorisation not only of BSGs but also for other types of biomass.

**2. Experimental**

**2.1 Feedstock characterisation**

Brewers’ spent grains (BSGs) were supplied by MAHOU San Miguel (Spain). The characterisation of the original material revealed that it contained 70-80 wt.% moisture (largely water) and a HHV of 7.30 MJ/kg. Therefore, to avoid the degradation and decomposition of the feedstock during the experimentation, water was removed using a freeze dryer for 24 h. Afterwards, the dried BSG was characterised by means of proximate, ultimate and fibre (cellulose, hemicellulose and lignin) analyses, calorific value and ash content. Proximate and ultimate analyses were performed according to standard methods (ISO-589-1981 for moisture, ISO-1171-1976 for ash and ISO-5623-1974 for volatiles). Fibre characterisation (cellulose, hemicellulose and lignin) was performed by thermogravimetric (TG) analysis considering the decomposition temperature of each structural component [49]. The amount of lipids has been calculated by means of a liquid-solid extraction with cyclohexane using a solid/liquid ratio of 1/10. Thermograms were recorded on a thermogravimetric analyzer Q50 (TA Instruments). The analyses were done under a N2 atmosphere increasing the temperature from 100 ºC to 900 ºC at a temperature rate of 10 ºC/min. The protein content was calculated following the experimental procedure developed by Meneses et al. [28] using the amount of N calculated by elemental analysis. Mass balances close to 100 wt.% were obtained in the experimental fibre analysis determination and these data have been standardised to sum up to 100 wt.%. Elemental analysis was carried out using an Exeter Analytical (Warwick, UK) CE440 Elemental Analyser. Table 1 lists the characterisation results of the dried BSGs used in this work. The characterisation of the lipid fraction by 1H NMR, 13C NMR and 31 P NMR revealed that this fraction contained oleic, linoleic and palmitic acids, sterols (largely cholesterol) and mono and di-glycerol. Fairly similar values to those reported by Mussatto et al. [12] were obtained.

Table 1: Dried Brewers’ spent grains characterisation

|  |  |  |  |
| --- | --- | --- | --- |
| ***Proximate analysis (wt.%)*** |  | ***Elemental analysis (wt.%)*** |  |
| Moisture | 1.38 ±0.31 | C | 50.28±0.33 |
| Ash | 7.60±0.29 | H | 6.37±0.27 |
| Volatiles | 62.95±0.17 | N | 3.93±0.04 |
| Fixed carbon | 24.41±0.47 | Oa | 39.17±0.56 |
| ***Fibre analysis (wt.%)*** |  | **Ash composition (wt. %)** |  |
| Cellulose | 23.55±0.22 | Ca | 33.46 ± 1.11 |
| Hemicellulose | 15.52±0.15 | Fe | 2.47 ± 0.67 |
| Lignin | 25.20±0.20 | K | 2.13 ± 0.23 |
| Proteins | 23.37±0.22 | Mg | 7.82 ± 0.21 |
| Lipids | 4.76±0.31 | Na | 1.13 ± 0.20 |
| Ash | 7.60±0.29 | P | 28.18 ± 1.39 |
| **HHV (MJ/kg)** | 20.95±0.49 | S | 20.73 ± 0.88 |

a Oxygen was calculated by difference.

**2.2 Experimental system**

The experiments were carried out in a small bench scale, batch, microwave-assisted hydrothermal reactor using a co-precipitated Ni-Co/Al-Mg catalyst. This catalyst was selected having regard to its good performance and stability during lignocellulosic bio-oil upgrading in sub and supercritical water [50] and its good preparation reproducibility. It was prepared by adding a solution of NH4OH to a solution containing Ni(NO3)2·6H2O, Al(NO3)3·9H2O, Mg(NO3)2·6H2O and Co(NO3)2·6H2O dissolved in milli-Q water, at 40 ºC and moderately stirred, until a pH of 8.2 was reached. The hydrated precursor produced in the coprecipitation step was filtered, washed at 40 ºC and dried overnight at 105 ºC. Afterwards, it was ground and sieved to a particle size ranging from 100 to 160 µm, calcined in an air atmosphere up to a temperature of 750 ºC for 3h and reduced with H2 at 650 ºC during 2 h. It has 28% Ni expressed as Ni/(Ni+Co+Al+Mg) an atomic Mg/Al ratio of 0.26 and an atomic Co/Ni ratio of 0.10, with a BET surface area of about 132 m2/g [51]. Further information about the preparation and characterisation of the catalyst can be found elsewhere [51].

The reactor (Figure 1) is a Milestone Synth-Wave microwave-assisted, high pressure, batch reactor. It consists of a 1L water-cooled high-grade stainless steel reactor that can be operated at high temperature and pressure (up to 300 ºC and 199 bar respectively). Microwave radiation is generated by a magnetron, which is connected to the bottom of the pressure vessel by a waveguide. The pressure vessel is made of special stainless steel and is closed with a clamping device. A thermowell and a pressure sensor are located inside the reactor. The former is used for the automatic display and control the internal temperature of the pressure vessel and the latter monitors the total vessel internal pressure. The microwave power is controlled with the aid of a PID control, while a burst disc protects the system in case of any possible and unexpected pressure increase. For the experiments, firstly, 20 g of biomass, 400 mL of deionized water and different amounts of catalyst were placed into the reactor. Then, the reactor was purged with N2 to create an inert atmosphere and finally an external and known amount of N2 (based on a home-made calibration) was added to the reactor at room temperature in order to achieve the experimental pressure at reaction conditions. A ramp time (time to reach the temperature of the experiment from room temperature, 25 ºC) of 15 minutes was used for all the experiments. This varied the heating rate between 11 and 15 ºC/min depending on the temperature of the experiment. After reaction, the reactor was cooled down to 60 ºC at a rate of around 12 ºC/min. The reaction time shifted between 0 and 2 h according to the experimental design. A 0 h reaction only includes the initial heating time and the cooling time, which is the same for all the experiments. Therefore, the experiments conducted using a 0 h reaction time are fairly comparable and the reaction time can be adequately analysed, as a wide interval (0-2 h) was selected to analyse the effect of the reaction time.

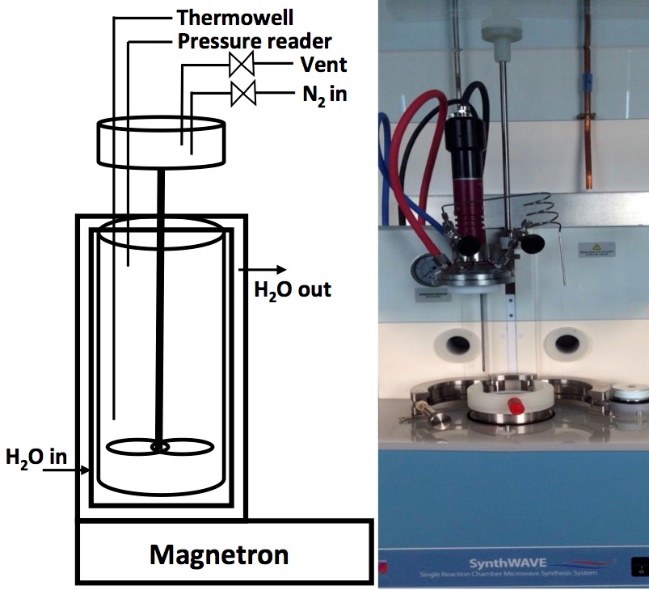


Figure 1: Microwave-assisted hydrothermal reactor

After the reaction, once the reactor was cool and the pressure had been released, the cover was opened. Then, the reaction products (the bio-oil and water fractions and the spent biomass and catalyst) were centrifuged and strained with a Buchner. The solid fraction (spent catalyst and biomass) was dried overnight in an oven at 105°C and the bio-oil was extracted from the water by means of a two-step liquid-liquid extraction, following the method recently developed and optimised by Ren et al. [52], firstly with chloroform to extract phenols, alcohols, furans and ketones and secondly with ethyl acetate to extract carboxylic acids. Then, the solvents were removed using a rotary evaporator and the bio-oil was dried under vacuum and weighted to calculate the bio-oil yield. The catalyst was recovered from the dried spent solid with the aid of a magnetic stirrer. Finally, the reaction products, including the bio-oil, water liquid fraction, as well as the solid fraction (spent biomass) were stored for offline analyses. The amount of gas produced was calculated gravimetrically by weight difference. The whole reactor vessel was weighted before (containing the biomass, water and catalyst) and after (after releasing the gas used to pressurise the reactor and the gaseous products produced in the reaction, and therefore containing the spent catalyst, biomass, bio-oil and water) the experiment. At the operating temperature and pressure at which HTL is conducted, the formation of H2 and CH4 is not thermodynamically favoured and the gas phase largely comprises CO2 [35, 53].

**2.3 Experimental design and data analysis**

The experiments were planned with a 2 level 4 factor Box-Wilson Central Composite Face Centred (CCF, α: ±1) design to analyse the influence of temperature (180-250 °C), pressure (50-130 bar), time (0-2 h) and catalyst loading (0-0.25 g catalyst/g biomass). The intervals of variation for the temperature and pressure were chosen having regard to the operating conditions commonly used in the literature [30-35], taking into account the limitations of the microwave reactor used in the experimentation (maximum operating temperature and pressure: 280 ºC and 200 bar, respectively), ensuring a wide interval of variations for these variables to properly study the effects of the operating conditions. The reaction time was varied between 0 and 2 h in order to ensure both a wide interval of variation, and a much longer reaction time in comparison to the ramp time. The amount of catalyst was selected to analyse non-catalytic and catalytic processes. This design corresponds to a full factorial design, where k indicates the number of factors studied (in this case 4 variables) and represents the numbers of runs. Moreover, 8 axial experiments were carried out to study non-linear effects and interactions and 4 replicates at the centre point (centre of the variation interval of each factor) were also conducted to evaluate the experimental error. These 28 experiments allow understanding the influence of all the operating variables and the possible interactions between them. The experimental results were analysed with an analysis of variance (ANOVA) with 95% confidence and the cause-effect Pareto principle. The ANOVA analysis helped for the selection of the operating variables and interactions that significantly influence the response variables under consideration, while the cause-effect Pareto principle was used to calculate the relative importance of the operating variables in the response variables. The higher the Pareto percentage, the greater is the influence of the operating variable on the response variable. The ANOVA analysis of the results was used to develop the interaction figures to analyse the effects of the operating conditions on the experimental results. In these figures, the evolution of the variables obtained from the ANOVA analysis of all the experiments was represented. In addition, some experimental points were added to the figures in some cases.

**2.4 Response variable and analytical methods**

The response variables used to analyse the effect of the operating conditions on the process include the overall conversion and the yields to gas, liquid (aqueous) fraction and bio-oil and the most important physicochemical properties of each product. Table 2 summarises these response variables and the analytical methods used for their determination. The bio-oil was analysed by elemental analysis and gas chromatography, the water liquid fraction was analysed by means of High Performance Liquid Chromatography (HPLC), while the spent solid was characterised by elemental analysis and calorific value. The bio-oil composition was analysed by Gas chromatography-mass spectroscopy (GC-MS). The bio-oils were solubilised in a chloroform/methanol (2:1) solution, and the 1.5µl of this solution was injected in a Perkin Elmer Claus 500 gas chromatograph coupled to a Perkin Elmer Claus 560s mass spectrometer. A non-polar ZB-5HT (30m×0.25mm id × 0.25µm film thickness) column from Phenomenex (UK) was used. The total amount of compounds present in the bio-oil that can be identified by GC-MS usually accounts for the 30 wt.% of the crude bio-oils [54], as many lignin-derived compounds cannot be analysed due to their high molecular masses. However, useful trends can be retrieved from this analysis, and a comparison can be established to analyse the effects of the conditions on the bio-oil chemical composition. The liquid fraction was analysed by High Performance Liquid Chromatography (HPLC). These analyses were conducted with an Agilent 1260 Infinity HPLC equipped with Agilent Hi-Plex H (300 x 7.7mm, 8um particle size) and ACE C18 (250 x 4.6mm, 5um particle size) columns and 1660 DAD WR UV/UV-VIS and 1660 Infinity Refractive Index (RI) detectors was used for the HPLC analyses. The elemental analyses were conducted with Exeter Analytical (Warwick, UK) CE440 Elemental Analyser. The calorific value of the spent solid was measured by means of an automatic calorimeter (6400 Parr instrument company).

Table 2: Response variables and analytical methods

|  |  |  |
| --- | --- | --- |
| **Product** | **Response variable** | **Analytical method** |
| **Bio-oil** |  | Gravimetric |
|  | Elemental Analysis |
| HHV (MJ/kg) = 0.3491 C (wt.%) + 1.1783 H (wt.%) – 0.1034 O (wt.%) – 0.015 N (wt.%) + 0.1005 S (wt.%) | Estimated |
|  | GC/MS |
| **Liquid (aqueous)** |  | Balance |
|  | HPLC |
|  | Elemental Analysis |
| **Solid** |  | Gravimetric |
| HHV (MJ/kg) | Calorimetric |
|  | Elemental Analysis |
| **Gas** |  | Gravimetric |

**3. Results and discussion**

Table 3 lists the operating conditions used in the experiments and the experimental results. These include the global results (overall solid conversion and the yields to gas, liquid aqueous fraction and bio-oil), together with the bio-oil chemical and elemental compositions and HHV, the chemical composition of liquid (aqueous) fraction and the elemental and HHV of the spent solid.

Table 3. Experimental conditions and results obtained in the experiments

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Run** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17-20** | **21** | **22** | **23** | **24** | **25** | **26** | **27** | **28** |
| **T (°C)** | 180 | 250 | 180 | 250 | 180 | 250 | 180 | 250 | 180 | 250 | 180 | 250 | 180 | 250 | 180 | 250 | 215 | 180 | 250 | 215 | 215 | 215 | 215 | 215 | 215 |
| **P (bar)** | 50 | 50 | 130 | 130 | 50 | 50 | 130 | 130 | 50 | 50 | 130 | 130 | 50 | 50 | 130 | 130 | 90 | 90 | 90 | 50 | 130 | 90 | 90 | 90 | 90 |
| **t (h)** | 0 | 0 | 0 | 0 | 2 | 2 | 2 | 2 | 0 | 0 | 0 | 0 | 2 | 2 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 |
| **Wcat/Wbio (g/g)** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.13 | 0.13 | 0.13 | 0.13 | 0.13 | 0.13 | 0.13 | 0.00 | 0.25 |
| ***Global results*** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Overall Conversion (%) | 32.13 | 56.31 | 37.70 | 56.38 | 53.08 | 64.54 | 55.32 | 63.47 | 34.84 | 59.20 | 35.21 | 59.97 | 56.47 | 67.27 | 66.34 | 67.53 | 57.67±0.56 | 56.01 | 68.36 | 56.51 | 56.96 | 63.58 | 57.07 | 55.70 | 56.66 |
| Gas yield (%) | 14.09 | 19.26 | 12.96 | 18.18 | 22.94 | 31.52 | 19.00 | 25.01 | 15.70 | 21.71 | 10.74 | 17.36 | 26.08 | 32.58 | 13.58 | 25.32 | 22.39±0.92 | 16.69 | 28.03 | 24.53 | 18.25 | 21.84 | 24.52 | 19.72 | 22.94 |
| Liquid yield (%) | 9.29 | 30.21 | 16.39 | 31.58 | 24.90 | 25.37 | 31.16 | 30.00 | 15.29 | 31.15 | 19.15 | 35.61 | 25.46 | 25.59 | 47.53 | 33.12 | 28.07±1.43 | 36.07 | 34.26 | 24.54 | 31.49 | 28.51 | 25.47 | 29.24 | 26.52 |
| Bio-oil yield (%) | 8.75 | 6.85 | 8.35 | 6.62 | 5.23 | 7.65 | 5.15 | 8.46 | 3.86 | 6.34 | 5.32 | 7.00 | 4.93 | 9.10 | 5.23 | 9.08 | 7.21±0.90 | 3.25 | 6.06 | 7.45 | 7.21 | 13.23 | 7.09 | 6.74 | 7.20 |
| ***Chemical composition of the bio-oil (% Area)*** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Phenols | 0.00 | 4.41 | 0.00 | 0.00 | 2.90 | 25.94 | 5.63 | 11.28 | 0.00 | 9.11 | 0.00 | 7.13 | 15.81 | 1.49 | 4.75 | 1.52 | 6.14±1.67 | 9.91 | 5.65 | 7.03 | 0.00 | 0.00 | 6.79 | 5.76 | 7.68 |
| Ketones | 0.00 | 20.49 | 0.00 | 12.38 | 3.91 | 29.72 | 6.83 | 49.60 | 0.00 | 17.58 | 0.00 | 16.07 | 29.45 | 66.13 | 27.69 | 44.30 | 65.29±2.41 | 26.23 | 40.84 | 67.59 | 80.14 | 0.00 | 43.32 | 49.32 | 54.84 |
| Aldehydes | 0.00 | 6.79 | 0.00 | 7.14 | 9.73 | 0.00 | 56.97 | 0.00 | 0.00 | 10.38 | 0.00 | 10.34 | 15.79 | 7.17 | 7.43 | 7.13 | 2.82±0.19 | 13.49 | 1.53 | 10.91 | 0.00 | 0.00 | 1.54 | 2.93 | 3.29 |
| Carboxylic Acids | 0.00 | 3.94 | 0.00 | 10.13 | 7.76 | 0.00 | 8.62 | 1.31 | 0.00 | 14.09 | 0.00 | 10.06 | 9.76 | 1.49 | 14.86 | 1.92 | 13.36±0.76 | 10.07 | 1.88 | 15.00 | 8.62 | 0.00 | 1.99 | 3.61 | 18.13 |
| Nitrogen compounds | 0.00 | 62.96 | 0.00 | 65.99 | 75.71 | 35.97 | 21.96 | 36.28 | 0 | 48.63 | 0.00 | 52.08 | 29.19 | 20.43 | 45.27 | 42.03 | 22.39±2.94 | 40.30 | 45.46 | 14.48 | 6.88 | 0 | 46.36 | 34.86 | 16.05 |
| **Elemental analysis and HHVof the bio-oil** | | | | | | | | | | | | | | | | | | | | | | | | | |
| C (wt.%) | 15.09 | 57.71 | 27.12 | 58.03 | 58.50 | 60.88 | 58.28 | 61.01 | 26.44 | 58.42 | 14.6 | 54.07 | 58.66 | 61.21 | 58.55 | 61.37 | 59,35±0.64 | 59.12 | 61.28 | 59.57 | 57.92 | 34.82 | 60.00 | 59.36 | 59.65 |
| H (wt.%) | 9.30 | 5.89 | 7.97 | 5.50 | 5.42 | 6.70 | 5.11 | 6.95 | 7.89 | 6.01 | 9.58 | 6.41 | 5.76 | 7.17 | 5.97 | 7.21 | 6,23±0.17 | 6.06 | 6.93 | 6.75 | 6.23 | 7.06 | 6.88 | 6.04 | 6.62 |
| N (wt.%) | 0.96 | 2.82 | 1.64 | 2.58 | 2.73 | 5.48 | 2.64 | 5.50 | 1.38 | 3.19 | 0.91 | 3.30 | 3.11 | 5.60 | 2.96 | 5.44 | 4,62±0.11 | 2.73 | 5.60 | 4.45 | 4.65 | 2.56 | 5.01 | 3.76 | 4.26 |
| O (wt.%) | 76.65 | 33.58 | 63.26 | 33.89 | 33.36 | 26.94 | 33.96 | 26.53 | 64.29 | 32.37 | 75.36 | 36.21 | 32.43 | 26.01 | 32.52 | 25.98 | 29,81±0.81 | 32.09 | 26.19 | 29.22 | 62.39 | 55.56 | 26.61 | 30.79 | 29.47 |
| HHV (MJ/Kg) | 8.49 | 23.57 | 12.29 | 23.20 | 23.31 | 26.28 | 22.82 | 26.66 | 11.85 | 24.08 | 8.58 | 22.64 | 23.87 | 27.04 | 24.06 | 27.15 | 24,90±0.48 | 24.42 | 26.77 | 26.66 | 21.04 | 14.69 | 26.23 | 24.60 | 25.51 |
| **Chemical composition of the liquid aqueous fraction (dry basis, C-wt.%)** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Oligosaccharides >DP6 | 98.23 | 57.90 | 93.02 | 66.63 | 89.69 | 93.08 | 97.65 | 98.14 | 99.35 | 80.06 | 99.49 | 97.85 | 95.93 | 97.25 | 95.45 | 97.34 | 98.39±0.20 | 90.79 | 97.62 | 98.47 | 98.47 | 87.40 | 98.43 | 96.06 | 98.73 |
| Oligosaccharides DP2-6 | 1.42 | 1.05 | 5.81 | 1.88 | 0.63 | 0.00 | 0.13 | 0.00 | 0.01 | 4.98 | 0.01 | 0.09 | 1.57 | 0.00 | 1.91 | 0.00 | 0.01±0.00 | 6.06 | 0.00 | 0.01 | 0.01 | 9.86 | 0.00 | 0.00 | 0.02 |
| Saccharides | 0.24 | 7.01 | 0.76 | 5.36 | 1.71 | 0.74 | 0.68 | 0.35 | 0.28 | 4.83 | 0.21 | 0.86 | 0.46 | 0.43 | 0.54 | 0.38 | 0.27±0.07 | 1.03 | 0.42 | 0.27 | 0.31 | 1.31 | 0.20 | 0.23 | 0.30 |
| Carboxylic Acids | 0.07 | 7.07 | 0.13 | 3.01 | 1.39 | 0.55 | 0.20 | 0.15 | 0.08 | 4.87 | 0.10 | 0.50 | 0.53 | 0.42 | 0.58 | 0.25 | 0.30±0.09 | 0.35 | 0.24 | 0.23 | 0.25 | 0.13 | 0.16 | 0.20 | 0.26 |
| Furans | 0.05 | 26.97 | 0.28 | 23.12 | 6.58 | 5.52 | 1.34 | 1.31 | 0.28 | 5.27 | 0.19 | 0.71 | 1.51 | 1.84 | 1.53 | 1.99 | 1.03±0.20 | 1.77 | 1.69 | 1.02 | 0.96 | 1.30 | 1.20 | 3.49 | 0.69 |
| **Elemental composition and HHV of the spent solid** | | | | | | | | | | | | | | | | | | | | | | | | | |
| C (wt.%) | 50.80 | 58.50 | 50.48 | 58.40 | 59.30 | 71.14 | 59.53 | 71.54 | 35.29 | 46.96 | 45.89 | 42.93 | 48.49 | 40.57 | 50.49 | 45.04 | 50.03±2.68 | 46.41 | 52.06 | 47.70 | 41.98 | 36.73 | 46.74 | 61.28 | 40.50 |
| H (wt.%) | 7.00 | 7.26 | 6.86 | 7.25 | 7.38 | 7.01 | 7.43 | 7.04 | 5.07 | 5.91 | 6.32 | 5.35 | 6.15 | 4.34 | 6.36 | 4.75 | 5.75±0.28 | 6.04 | 5.48 | 5.44 | 4.82 | 4.92 | 5.20 | 7.01 | 4.75 |
| N (wt.%) | 3.64 | 2.64 | 3.91 | 2.50 | 2.44 | 3.45 | 2.50 | 3.41 | 3.03 | 1.82 | 2.62 | 1.96 | 2.025 | 1.98 | 1.92 | 2.16 | 1.75±0.31 | 2.40 | 2.32 | 1.91 | 1.72 | 2.34 | 1.87 | 2.3 | 1.58 |
| O (wt.%) | 38.54 | 31.59 | 38.75 | 31.84 | 30.87 | 18.38 | 30.52 | 17.99 | 56.61 | 45.31 | 45.17 | 49.75 | 43.33 | 53.1 | 41.23 | 48.03 | 42.96±2.80 | 45.15 | 40.13 | 44.94 | 51.47 | 56.01 | 46.175 | 29.4 | 53.16 |
| HHV (MJ/Kg) | 22.59 | 26.82 | 21.76 | 26.60 | 26.50 | 31.75 | 26.61 | 31.89 | 17.89 | 17.47 | 18.50 | 20.45 | 20.08 | 17.74 | 20.61 | 18.34 | 22.11±0.45 | 20.45 | 23.38 | 20.60 | 19.95 | 19.23 | 21.31 | 27.19 | 19.96 |

**Table 4: Relative influence of the operating conditions on yields to gas, bio-oil, liquid yields and overall conversion according to ANOVA analysis and cause-effect Pareto Principle**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **R2** | **Indep** | **T** | **P** | **t** | **W** | **TP** | **Tt** | **TW** | **Pt** | **PW** | **tW** | **Wt T2** | **T2** | **P2** | **t2** | **W2** | **TPt** | **TPW** | **TtW** | **PtW** | **T2P** | **T2t** | **T2W** | **TP2** | **TPtW** | **T2P2** |
| **Gas yield (%)** | 0.99 | 22.56 | 5.67  (21) | -2.67  (15) | 1.34  (22) | 1.61  (1) | n.s | 0.61  (3) | n.s | -1.17  (6) | -1.03  (5) | n.s | n.s | n.s | -1.07  (6) | n.s | -1.13  (3) | n.s | 0.52  (3) | n.s | n.s | n.s | 2.79  (5) | -1.60  (3) | -2.18  (4) | 0.45  (2) | n.s |
| **Bio-oil yield (%)** | 0.94 | 7.27 | 0.95  (14) | n.s | -3.08  (4) | n.s | n.s | 0.83  (12) | 0.63  (9) | n.s | n.s | 0.62  (9) | n.s  (11) | -2.99 | n.s | 2.51  (16) | n.s | n.s | n.s | -0.34  (5) | n.s | n.s | 3.18  (15) | -0.39  (5) | n.s | n.s | n.s |
| **Liquid yield (%)** | 0.97 | 27.84 | n.s | 3.57  (13) | n.s | n.s | -1.33  (5) | -5.21  (19) | -1.09  (4) | 1.48  (5) | 1.16  (4) | n.s | n.s | 7.33  (0) | n.s | n.s | n.s | n.s | n.s | n.s | 1.18  (4) | n.s | 3.40  (12) | 2.13  (8) | 3.34  (12) | -1.20  (4) | -8.18  (10) |
| **Overall Conversion (%)** | 0.99 | 57.10 | 6.17  (22) | n.s | -3.25  (19) | n.s | -1.13  (3) | -3.78  (10) | n.s | n.s | n.s | 0.91  (3) | n.s | 5.08  (5) | n.s | 3.22  (3) | n.s | -0.49  (1) | n.s | -0.87  (2) | 0.84  (2) | 1.13  (3) | 10.89  (10) | 1.74  (5) | 1.55  (1) | -0.76  (2) | -11.30  (7) |

*T= temperature, P=pressure, t= time, W= catalyst/biomass ratio. n.s: Non significant with 95% confidence. Numbers in brackets indicate the percentage Pareto influence of each factor on the response variable. Response = Indep. + Coefficient T·T + Coefficient P·P + Coefficient C·C + Coefficient W·W + Coefficient TC·T·C + Coefficient TW·T·W+ Coefficient PC·P·C + Coefficient PW·P·W + Coefficient CW·C·W + Coefficient T2·T2 + Coefficient P2·P2 + Coefficient C2·C2 + Coefficient W2·W2 + Coefficient TPC·T·P·C + Coefficient TPW·T·P·W + Coefficient TCW·T·C·W + Coefficient PCW·P·C·W + Coefficient T2P·T2·P + Coefficient T2W·T2·W + Coefficient TP2·T·P2 + Coefficient TPtW·T·P·t·W*

**Table 5: Relative influence of the operating conditions on the bio-oil properties according to the ANOVA analysis and cause-effect Pareto Principle**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **R2** | | **Indep** | **T** | **P** | **t** | **W** | **TP** | **Tt** | **TW** | **Pt** | **PW** | **tW** | **WtT2** | **T2** | **P2** | **t2** | **W2** | **TPt** | **TPW** | **TtW** | **PtW** | **T2P** | **T2t** | **T2W** | **TP2** | **TPtW** | **T2P2** |
| **Chemical composition (C-wt.%)** | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Nitrogen Compounds** | 0.98 | 23.00 | n.s | n.s | 23.18  (8) | -4.44  (5) | 4.12  (6) | -16.70  (15) | n.s | n.s | 5.71  (7) | n.s | n.s | 21.28  (8) | -10.92  (7) | n.s | n.s | 3.33  (9) | -3.02  (3) | 2.59  (3) | 5.68  (4) | n.s | -18.37  (7) | n.s | 12.03  (12) | -3.05  (6) | n.s |
| **Phenols** | 0.94 | 6.69 | n.s | -2.02  (10) | 3.08  (15) | n.s | n.s | n.s | -2.15  (10) | -1.04  (5) | n.s | -2.13  (10) | n.s | n.s | -3.18  (2) | -3.30  (1) | n.s | n.s | 1.93  (9) | -3.63  (17) | n.s | n.s | n.s | n.s | 1.99  (9) | 1.63  (7) | 5.41  (6) |
| **Ketones** | 0.99 | 65.29 | 11.28  (12) | 6.28  (0) | 21.66  (14) | 4.66  (5) | n.s | 3.46  (4) | n.s | n.s | -2.49  (3) | 4.80  (5) | n.s | -31.76  (22) | 8.57  (2) | -43.63  (15) | -13.21  (2) | n.s | -1.90  (2) | n.s | -3.31  (3) | -6.93  (2) | -9.71  (3) | n.s | n.s | -2.73  (3) | 35  (4) |
| **Aldehydes** | 0.99 | 2.80 | -5.98  (5) | -5.45  (3) | 0.77  (7) | n.s | -2.60  (4) | -7.08  (12) | 3.85  (7) | 2.22  (4) | -3.69  (6) | -2.44  (4) | n.s | 4.72  (6) | 2.66  (2) | -2.02  (1) | 0.32  (0) | -2.64  (4) | 3.26  (6) | 3.00  (5) | -3.64  (6) | 7.71  (5) | 3.39  (2) | -1.59  (2) | 3.23  (2) | 3.31  (6) | n.s |
| **Carboxylic acids** | 0.97 | 12.35 | -4.10  (2) | -3.19  (1) | n.s | 7.26  (9) | n.s | -4.66  (20) | n.s | n.s | n.s | n.s | n.s | -6.37  (13) | n.s | -11.35  (12) | n.s | n.s | -0.96  (4) | -1.01  (4) | 0.85  (4) | 3.81  (6) | n.s | -5.99  (9) | 4.22  (6) | n.s | 10.62  (11) |
| **Elemental Analysis and HHV** | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **C (wt.%)** | 0.99 | 59.13 | n.s | n.s | 12.59  (25) | n.s | n.s | -8.41  (19) | n.s | n.s | -1.78  (4) | n.s | n.s | 1.49  (9) | n.s | -11.30  (11) | n.s | n.s | 1.19  (3) | n.s | 1.79  (4) | n.s | -2.15  (2) | n.s | 9.72  (21) | -1.21  (3) | n.s |
| **H (wt.%)** | 0.97 | 6.35 | 0.44  (8) | n.s | n.s | 0.21  (7) | n.s | 1.04  (32) | n.s | n.s | 0.26  (8) | n.s | n.s | n.s | n.s | 0.47  (9) | n.s | n.s | -0.18  (6) | n.s | -0.22  (7) | n.s | -0.52  (16) | n.s | -0.76  (8) | n.s | n.s |
| **N (wt.%)** | 0.99 | 4.54 | 1.43  (28) | n.s | 1.06  (26) | 0.11  (3) | n.s | 0.22  (5) | n.s | n.s | n.s | n.s | n.s | -0.29  (15) | n.s | -0.67  (9) | -0.45  (4) | n.s | 0.088  (2) | -0.13  (3) | n.s | n.s | n.s | n.s | -0.34  (3) | -0.10  (2) | n.s |
| **O (wt.%)** | 0.99 | 29.72 | -2.95  (18) | 16.59  (4) | -14.48  (21) | n.s | n.s | 7.17  (13) | n.s | n.s | 1.62  (3) | n.s | n.s | n.s | 16.08  (10) | 11.37  (2) | n.s | n.s | -1.13  (2) | n.s | -1.63  (3) | -16.33  (9) | 3.49  (2) | n.s | -7.57  (4) | 1.24  (2) | -16.46  (7) |
| **HHV (MJ/Kg)** | 0.99 | 25.11 | 1.17  (21) | -2.31  (2) | 5.77  (24) | n.s | n.s | -2.45  (13) | n.s | n.s | -0.48  (2) | n.s | n.s | n.s | -1.76  (10) | -4.65  (8) | n.s | n.s | 0.31  (2) | n.s | 0.53  (3) | 2.24  (4) | -1.61  (3) | n.s | 2.91  (5) | -0.44  (2) | 2.29  (3) |

*T= temperature, P=pressure, t= time, W= catalyst/biomass ratio. n.s: Non significant with 95% confidence. Numbers in brackets indicate the percentage Pareto influence of each factor on the response variable. Response = Indep. + Coefficient T·T + Coefficient P·P + Coefficient C·C + Coefficient W·W + Coefficient TC·T·C + Coefficient TW·T·W+ Coefficient PC·P·C + Coefficient PW·P·W + Coefficient CW·C·W + Coefficient T2·T2 + Coefficient P2·P2 + Coefficient C2·C2 + Coefficient W2·W2 + Coefficient TPC·T·P·C + Coefficient TPW·T·P·W + Coefficient TCW·T·C·W + Coefficient PCW·P·C·W + Coefficient T2P·T2·P + Coefficient T2W·T2·W + Coefficient TP2·T·P2 + Coefficient TPtW·T·P·t·W*

**3.1 Effect of the operating conditions on the overall conversion and products distribution**

The overall BSG (solid) conversion and the yields to gas, liquid aqueous fraction and bio-oil vary as follow: 31-68%, 10-33%, 9-48%, 4-14%, respectively. The relative influence of the operating variables on these responses according to the ANOVA analysis and the cause-effect Pareto principle is listed in Table 4. This analysis shows that the temperature and reaction time are the operating variables with the highest influence on the overall conversion and gas and bio-oil yields, while the liquid yield is highly influenced by the pressure; this latter variable also exerts a high influence on the liquid and gas yields. The coefficients of the codec models developed for these variables (positive or negative) indicate that an increase in the temperature not only does increase (positive term) the overall biomass conversion, but also leads to an increase in the yields to products (gas, liquid and bio-oil). Conversely, increasing the reaction time diminishes the bio-oil yield and overall conversion (negative terms) and upsurges the gas yield (positive term), while augmenting the pressure leads to an increase in the aqueous fraction yield and a decrease in the gas yield. In addition, the overall conversion and the bio-oil and aqueous fraction yields are strongly influenced by the interaction between the temperature and reaction time. These variations are in good agreement with the literature addressing the hydrothermal liquefaction of biomass using conventional heating [31, 55-57].

The effects of the operating variables and the most important interactions detected with the statistical analysis of the results (ANOVA analysis) are plotted in Figure 2. In particular, Figures 2 a and b show the effects of the temperature on the overall conversion at low and high pressure (50 and 130 bar) in the absence of the catalyst, using a reaction time of 0 and 2 h, respectively. Figures 2 c and d show these effects for the highest catalyst/biomass ratio used in this work (0.25 g cat/g biomass). Figures 2 e-h and Figures 2 i-l plot these effects for the gas and liquid yields, respectively. Due to the negligible influence of the pressure (between 50 and 130 bar) on the bio-oil yield, the effect of the temperature on this product for short and long (0 and 2 h) reaction times using 0 and 0.25 g cat/g biomass is shown in Figures 2 m and n.



Figure 2. Interaction plots showing the effects of the operating conditions on the overall conversion (a-d), gas yield (e-h), liquid yield (i-l) and bio-oil yield (m-n), obtained from the ANOVA analyses. Bars are LSD intervals with 95% confidence.

**3.1.1 Overall biomass conversion**

The effects of the temperature on the overall biomass conversion significantly depend on the reaction time and catalyst amount. Regardless of the amount of catalyst, when a short reaction time (0 h) is used, an initial increase in the temperature between 180 and 220 ºC leads to an increase in the biomass conversion. This is followed by a subsequent decrease with further increasing the temperature up to 250 ºC. Biomass hydrothermal liquefaction largely comprises three steps. Firstly, an initial biomass depolymerisation, secondly, the formation of reactive fragment by the subsequent decomposition of the biomass monomers by cleavage, dehydration, decarboxylation and deamination leading to the formation of reactive fragments that can evolve towards bio-oil and/or gas formation and, thirdly, the recombination and/or repolymerisation of these reactive fragments, which produces more bio-oil together with complex solid species such as char and coke [33]. Thus, the initial increase observed in the overall biomass conversion is accounted for by the positive kinetic effect of the temperature on the process, which increases the reaction rates of all reactions involved (depolymerisation, deamination, decomposition and re-polymerisation) [31, 33, 34, 53]. However, high temperatures also favour the formation of solid species via recombination and depolymerisation [33]; thus increasing solid formation. Hence, as the overall conversion was gravimetrically calculated considering the spent solid recovered at the end of the experiment, an artificial decrease in the overall biomass conversion (to gas, liquid and bio-oil) is observed; i.e. an increase in the solid yield. Other authors addressing biomass hydrothermal valorisation using conventional heating also observed this decrease in the conversion (or an increase in the solid yield) at elevated temperature due to solid formation (largely char and coke) [31, 33, 53].

Increasing the reaction time (Figures 2 a/c vs. 2 b/d) has different consequences for the process depending on the temperature. While, at low (180 and 200 ºC) and high (230-250 ºC) temperature an increase in the reaction time from 0 to 2 h leads to a substantial increase in the overall biomass conversion, a small decrease is observed with increasing the reaction time at medium (200-230 ºC) temperatures. These developments are the consequence of the prevalence of some reactions over others. At low temperature the increase in the biomass conversion is the result of the transformation of biomass into water-soluble liquids, bio-oil and gaseous products due to the positive kinetic effect of the reaction time on the process [31, 33, 34, 53]. Increasing the reaction time at medium-high temperature favours the formation of solid species such as coke, char and humins via recombination and repolymerisation of the reactive fragments produced from the initial depolymerisation of biomass [31, 33, 53]. This leads to a decrease in the overall biomass conversion at medium temperature due to the increase in solid production as described earlier. The small increase in conversion observed at high temperature and long reaction times is the consequence of two counteracting effects: solid production via recombination and repolymerisation and solid removal due to the greater range of decarboxylation and thermal decomposition reactions taking place when high temperatures are used for long reaction times [31, 33, 34, 53]. This allows the transformation into gases of the biomass reactive fragments as well as part of the bio-oil and solid products, such as char and coke [31, 58].

The effects of the catalyst amount and pressure on the overall biomass conversion are much weaker. In particular, the catalyst exerts a negligible effect on the overall conversion when a short (0 h) reaction time is used. For a long reaction time (2 h) an increase in the catalyst amount from 0 to 0.25 g cat/g biomass leads to a small increase in the overall conversion at low temperature and high pressure (180-200 ºC and 130 bar), however, these variations are not very important from a practical point of view. This is accounted for by the heterogeneous nature of the catalyst, which hampers the intimate contact between the biomass and the catalyst, resulting in mass transfer limitations [31, 34, 53]. In addition, other authors have also reported the negligible effect of the pressure on the overall biomass conversion when conventional heating was used in hydrothermal liquefaction [31, 33, 58]. However, the catalyst and the pressure have an important influence on the gas, aqueous phase and bio-oil yields as well as on the properties of these fractions, as will be explained next.

**3.1.2 Yields to gas, liquid aqueous fraction and bio-oil**

With respect to the yields of gas and liquid (aqueous phase), temperature effects depend on the catalyst, reaction time and pressure, while for the bio-oil yield such effects are only dependent on the reaction time. For a short reaction time (0 h), the temperature has a similar influence on the gas, liquid and bio-oil yields regardless of the amount of catalyst. Particularly, in the absence of the catalyst (Figures 2 e, i and m), an initial increase in the temperature between 180 and 220 ºC leads to increases in the gas, liquid and bio-oil yields. Conversely, a subsequent increase in the temperature up to 250 ºC has different consequences for these variables. While the gas and liquid yields are not significantly modified and steady values for these variables are observed, the bio-oil yield substantially decreases. This decrease takes place along with the depletion observed for the overall biomass conversion due to solid formation, thus suggesting the transformation of part of the bio-oil into solid species. In addition, gas formation could also be produced from the thermal decomposition of the proteins present in the solid via deamination [59]. The hydrothermal liquefaction of biomass is usually endothermic at low temperatures and exothermic at high temperatures [34, 60]. Consequently, the gas, liquid and bio-oil yields increase with temperature and reach a point after which an increase in temperature prevents liquefaction. In addition, the decrease observed in the bio-oil yield might be accounted for by the transformation of this liquid into other products. This includes gas formation (by cracking and/or pyrolysis), and/or char production (by condensation, crystallisation and/or repolymerisation) [31, 33, 58].

Increasing the amount of catalyst does not substantially modify the evolution of these variables with the temperature and similar profiles (Figures 2 e/i/m vs. g/k/n) are observed; i.e. the initial increase between 180 to 220 ºC as well as the subsequent steady state or decay observed between 220 and 250 ºC. However, several variations are observed for the gas, liquid and bio-oil yields (these variations depending on the temperature and pressure) when the catalyst/biomass ratio is increased from 0 to 0.25 g cat/g biomass. This is believed to be a consequence of the positive effect of the catalyst on the product distribution. At low temperature (180-215 ºC), an increase in the catalyst amount decreases the bio-oil yield regardless of the pressure. At low pressure (50 bar), such variation is accompanied with increases in the gas and liquid yields, while at high pressure (130 bar), the liquid yield increases and the gas yield is influenced less (Figures 2 e/i/m vs. g/k/n). An increase in the catalyst amount produces a greater spread of pyrolysis, decarboxylation and deamination reactions [31, 34, 58], thus increasing gas production. In addition, part of the bio-oil can be transformed into gas via pyrolysis, thermal decomposition and cracking reactions [31, 33, 58]. Furthermore, increasing the amount of catalyst increases the overall biomass conversion, resulting in a moderate increase in the liquid yield. At high temperature (215-250 ºC), augmenting the amount of catalyst in the reactor does not substantially modify the yields to these products, as the positive kinetic effect of the temperature may mask the effect of the catalyst when very short reaction times (0 h) are used. Only a small increase in the gas yield along with a moderate decrease bio-oil yield, are observed at low pressure (50 bar).

The effects of changing the reaction time depend on the temperature and catalyst amount. For non-catalytic experiments (0 g cat/g biomass) increasing the reaction time from 0 to 2 h significantly decreases the bio-oil yield for the whole temperature period considered in this work. However, the reaction time has different consequences for the gas and liquid yield depending on the temperature. At low temperature, this increase in time leads to a small increase in the gas yield together with a substantial increase in the liquid yield. At low temperature, biomass hydrolysis and depolymerisation, leading to the formation of water-soluble species, is favoured over gas formation via pyrolysis and/or decarboxylation [31, 33, 58]. Therefore, increasing the reaction time helps promote the formation of water-soluble species favoured at early reaction stages, thus increasing the liquid (aqueous fraction) yield. Conversely, at high temperature, a substantial increase in the gas yield is observed, while the liquid aqueous fraction yield is unaffected due to the prevalence of pyrolysis, decarboxylation and deamination reactions, favoured at high temperature [31, 33, 58]. As a result, for a 2 h reaction time and 0 g cat/g biomass (Figures 2 g, k and m) regardless of the pressure, an initial increase in the temperature between 180 and 220 ºC does not substantially modify the gas, liquid or bio-oil yield. Conversely, a further increase in the temperature up to 250 ºC results in a substantial increase in the gas yield together with a small increase in the bio-oil yield, the liquid yield being unaffected. These variations thus suggest a progressive transformation of the bio-oil into gases at high temperature.

When the greatest amount of catalyst (0.25 g cat/g biomass) is used, an increase in the reaction time decreases the bio-oil yield regardless of the pressure, while different outcomes depending on the pressure are observed for the gas and aqueous fraction (liquid) yields. At low pressure (50 bar), an increase in the reaction time increases the liquid and gas yields, the variations observed for this latter variable being particularly marked between 200 and 250 ºC. At high pressure (130 bar), the liquid yield and the gas yield also show increases with increasing the reaction time, although the variations are less pronounced. However, a substantial increase is observed for the liquid yield at high pressure and low temperature (180-220 ºC). Increasing the pressure decreases the density of water which also diminishes its dielectric loss factor [61], leading to a decrease in the effectiveness of microwave heating [42]. This lower efficiency increases the proportion of species produced at early reaction stages, such as water-soluble compounds derived from the hydrolysis and depolymerisation of biomass and, therefore, hinders gas formation. This is in good agreement with the increases and decreases observed for the liquid and gas yield, respectively, when the pressure increases from 50 to 130 bar. These variations also modify the effect of the reaction time on the yield to products when a 2 h reaction time is used. Particularly, under these conditions (0.25 g cat/g biomass and 2 h), regardless of the pressure, an increase in the temperature substantially increases the gas and bio-oil yields and decreases the liquid yield due to the progressive transformation of the water-soluble species into bio-oil and gases.

**3.2 Effect of the operating conditions on bio-oil properties**

The effects of the operating conditions on the bio-oil chemical and elemental compositions and Higher Heating value (HHV) according to the ANOVA and cause-effect Pareto analysis are listed in Table 5.

**3.2.1 Bio-oil elemental composition and Higher Heating Value (HHV)**

The relative amounts of C, H, N and O in the bio-oil vary as follow: 15-61 wt.%, 5-10 wt.%, 1-6 wt.% and 26-77 wt.%, respectively. These variations shift the bio-oil Higher Heating Value (HHV) between 9 and 27 MJ/kg. The elevated nitrogen concentration in the bio-oil in some cases is the consequence of the high protein content of the original solid, which is in good agreement with the work of Mussatto et al. [1]. According to the cause-effect Pareto analysis, the temperature and the reaction time are the operating variables with highest influence on the bio-oil HHV and the proportions of N and O, while the relative amount of C is highly influenced by the reaction time. The interaction between temperature and reaction time also has a substantial influence on these variables. The proportion of H is substantially affected by the interaction between the temperature and reaction time. Figure 3 shows the effects of the operating conditions and interactions detected with the ANOVA analysis. Particularly, Figures 3 a and b show the effects of the temperature on the relative amount of C at low and high pressure (50 and 130 bar) in the absence of the catalyst, using a reaction time of 0 and 2 h, respectively. Figures 3 c and d plot these effects for the highest catalyst/biomass ratio used in this work (0.25 g cat/g biomass). These effects for the relative amounts of H, O, N and HHV of the bio-oil are shown in Figures 3 e-h, i-l, m-p and q-t, respectively.

The effect of the temperature on the bio-oil elemental composition and HHV significantly depends on the reaction time and pressure. For a short reaction time (0 h), increasing the temperature leads to different consequences for the bio-oil elemental composition and HHV depending on the pressure. Regardless of the amount of catalyst, at low pressure (50 bar), an increase in the temperature between 180 and 250 ºC increases the relative amounts of C and N in the bio-oil and decreases the proportion of O; thus, leading to an increase in the bio-oil HHV (Figures 3 a/c, e/g, i/k, m/o and q/s). Increasing the pressure from 50 to 130 bar does not significantly modify the evolution with the temperature of the relative amounts of C, H and N, and the same variations are observed at 130 bar; i.e. increases in the relative amounts of C and N together with a decrease in the proportion H. These developments are accounted for by a greater spread of deoxygenation, deamination and thermal cracking reactions [62-65], which are enhanced at high temperature. Conversely, at 130 bar the relative amount of O increases between 180 and 215 ºC and decreases with a further increase in the temperature up to 250 ºC. As a result, the HHV display a steady evolution between 180 and 215 ºC, followed by a sharply upsurge when the temperature increases up to 250 ºC.

In addition, (for a short reaction time 0 h), the effect of the pressure depends on the amount of catalyst and vice versa. For non-catalytic experiments, an increase in the pressure from 50 to 130 bar leads to an increase in the relative amounts of C and O together with a decrease in the proportion of H; the relative amount of N and HHV being not substantially affected. An increase in the pressure decreases the density of water which also diminishes its dielectric loss factor [61], leading to a decrease in the effectiveness of microwave heating [42] as described earlier. This thermodynamic inhibitory effect increases the proportions of C and O of the bio-oil as the possible deoxygenation (decarboxylation and decarbonylation) and dehydrogenation reactions might occur to a lesser extent. In addition, an increase in the relative amount of catalyst from 0 to 0.25 g cat/g biomass has different consequences for the bio-oil elemental composition and HHV depending on the pressure. On the one hand, at low pressure (50 bar), this increase in the amount of catalyst leads to an increase in the proportion of C together with reductions in the concentrations of H and O, without significantly modifying the bio-oil N content or HHV. These variations are accounted for by the positive effect of the catalyst promoting the deoxygenation (decarboxylation and decarbonylation) and hydrodeoxygenation reactions occurring under hydrothermal conditions. These effects have been reported by other authors during bio-oil upgrading in sub-critical water with Ni [66] or metals based [62, 63, 65, 67] catalysts. On the other hand, at 130 bar, increasing the amount of catalyst produces the opposite effect; i.e. a decrease in the proportion of C as well as increases in the relative amounts of H and O. As a consequence of these variations, when the highest amount of catalyst (0.25 g cat/g bio-oil) is used, increasing the pressure from 50 to 130 bar decreases the proportion of C and HHV and increases the relative amounts of H and O in the bio-oil. This is believed to be the consequence of the thermodynamic inhibitory effect of the pressure, thus hindering deoxygenation and hydrodeoxygenation reactions.



Figure 3. Interaction plotws showing the effects of the operating conditions on the bio-oil elemental composition: C (a-d), H (e-h), O (i-j) and N (m-p) and HHV (q-t) obtained from the ANOVA analyses. Bars are LSD intervals with 95% confidence.

The effect of the reaction time on the bio-oil elemental composition and HHV depends on the temperature. Regardless of the pressure or catalyst amount, an increase in the reaction time from 0 to 2 h results in a substantial increase in the proportions of C and N together with a decrease in the relative amounts of H and O in the bio-oil, which leads to a substantial increase in the HHV. These variations might be the consequence of the longer exposure of the material to microwave heating; thus, promoting the development of decarboxylation and deoxygenation reactions. In addition, this also increases the amount of proteins removed from the original biomass via deamination and therefore increases the proportion of N in the bio-oil. These variations are notably marked at low temperature and allow us to obtain the same bio-oil composition and HHV at low temperature using the longest (2 h) reaction as those produced at high temperature using a very short reaction time (0 h). As a consequence, for a 2 h reaction the effect of the temperature on the bio-oil elemental composition and HHV is much weaker and the effect of the catalyst is not significant, as the use of long reaction times might mask the effect of the temperature. Specifically, the temperature does not affect the proportion of C in the bio-oil, while small variations are observed for the relative amounts of H, O and N and HHV.

Regardless of the pressure, an increase in the temperature increases the proportions of H (especially between 180 and 220 ºC) and N in the bio-oil. Conversely, two different outcomes are observed depending on the pressure for the concentration of O. At low pressure (50 bar) the relative amount of O decreases slightly, while at high pressure (130 bar), a pronounced increase is observed between 180 and 215 ºC, followed by a decrease on further the temperature increases up to 250 ºC. The inhibitory thermodynamic effect of the pressure might hinder bio-oil deoxygenation at low temperature, leading to an increase in the amount of O in the bio-oil. A subsequent increase in the temperature promotes deoxygenation reactions, which leads to a decrease in the amount of O. This is in good agreement with the lower quantity of gas formation at low pressure compared to higher pressures, as described earlier. These variations have a significant influence on the HHV of the bio-oil, however the changes observed are not very important from a practical point of view. The bio-oil produced using a 2 h reaction time has a HHV between 24 and 27 MJ/kg regardless of the other conditions.

**3.2.2 Bio-oil chemical composition**

The bio-oil is made up a mixture of phenols (0-26%), ketones (0-80%), aldehydes (0-57%), carboxylic acids (0-18%) and nitrogen compounds (0-76%). Phenols include phenol and 3,5-dimethoxy-phenol. Ketones comprise 2,4(1H,3H)-pyrimidinedione, 1,3,5-trimethyl, 4-hydroxy-6-methyl-2(1H)-pyridinone and dicyclopropyl-methanone, while aldehydes include vanillin. Carboxylic acids are made up of 3,5-dihydroxy benzoic acid, 2,2-dimethyl,3-butenoic acid, propionic acid and gluconic acid. Nitrogen compounds include 3-dimethylaminoacrylonitrile, 3-(1,3,6-trimethyl-4-oxo-3-piperidinyl)propanenitrile and 1H-1,2,3-triazol-1-amine, 4-(4-phenyl)-N-(phenylmethylene). The detailed chemical composition calculated by GC-MS is shown in Table S1. The presence of these compounds is in good agreement with previous pathways addressing biomass decomposition under hydrothermal conditions. The process mechanism involves the hydrolysis of biomass bio-polymers into water soluble oligomers followed by their breakup into reactive fragments comprising a mixture of sugars, aldehydes and phenols as well as the decomposition of the biomass protein content into N-derived species [34, 68].

Table 5 shows that the proportions of ketones, aldehydes and nitrogen compounds in the bio-oil are strongly influenced by the temperature, the reaction time and the interaction between both variables, while the concentration of phenols is mostly influenced by the pressure and the catalyst-temperature and catalyst-time interactions. Figure 4 shows the effect of the operating variables and interactions on the bio-oil chemical composition. More specifically, Figures 4 a and 4b show the effects of the temperature on the relative amount of phenols at low and high pressure (50 and 130 bar) in the absence of the catalyst, using a reaction time of 0 and 2 h, respectively. Figures. 4 c and 4 d show these effects for the highest catalyst/biomass ratio used in this work (0.25 g cat/g biomass). Figures 4 e-h, i-l m-p and q-t show these effects for the relative amounts of ketones, aldehydes, carboxylic acids and nitrogen compounds respectively in the bio-oil.



Figure 4. Interaction plots showing the effects of the operating conditions and interactions on the bio-oil chemical composition obtained with the ANOVA analyses. Bars are LSD intervals with 95% confidence.

The effect of the temperature on the bio-oil chemical composition depends on the reaction time. For a short reaction time (0 h) similar evolutions with the temperature are observed regardless of the pressure and catalyst amount; these two latter variables exerting a very weak influence on the bio-oil chemical composition. In particular, within the whole temperature interval considered (180-250 ºC) and regardless of the catalyst amount (from 0 to 0.25 g cat/ g biomass), the bio-oil has a negligible amount of phenols and aldehydes. In addition, at low temperature (180-215 ºC), the proportions of ketones, carboxylic acids and nitrogen compounds are also very low; however, increasing the temperature from 215 to 250 ºC increases the relative amount of ketones and very sharply increases the proportions of both carboxylic acids and nitrogen compounds in the bio-oil. These results suggest that the bio-oil produced using a short reaction time is largely made up of high molecular weight compounds that are not detectable by gas chromatography. An increase in the temperature promotes hydrolysis, depolymerisation and deamination reactions, which accounts for the increase observed in the proportions of ketones, carboxylic acids and nitrogen compounds. In addition, the decomposition of the proteins of the solid into liquid and gaseous products via decarboxylation and deamination reactions is favoured at high temperature, thus increasing the relative amount of N compounds in the bio-oil [59, 69].

Increasing the reaction time results in different consequences for the bio-oil chemical composition depending on the pressure and catalyst amount. For non-catalytic experiments (0 g cat/g biomass), the effect of the reaction time depends on the pressure with different outcomes taking place (Figures 4 a, e, i, m and q vs. b, f, j, n and r). At low pressure (50 bar), an increase from 0 to 2 h substantially increases the proportion of phenols and ketones, while aldehydes, carboxylic acids and nitrogen compounds increase between 180 and 220 ºC and decrease between 220 and 250 ºC. Under hydrothermal conditions, the polysaccharide content (cellulose and hemicellulose) of BSG decomposes towards the formation of phenols via isomerisation, cyclisation and dehydration reactions, the lignin content depolymerises to give alkyl-substituted phenols, while the protein content decomposes leading to the formation of N substituted ketones and N derived compounds [34, 70]. An increase in the reaction time kinetically promotes these reactions, which explains the increases observed in the proportions of these species. The decrease occurring for the relative amounts of carboxylic acids and N compounds might be accounted for by their transformation into gases via decarboxylation and deamination reactions [59, 69], when long reaction times are applied at high temperature. This also explains the decreases observed in these fractions with further increases in temperature.

The effect of the pressure is not significant when a short reaction time (0 h) is used. Conversely, for a 2 h reaction the effect of the pressure is significant. In general, regardless of the pressure when a reaction time of 2 h is used in the absence of the catalyst, an increase in the temperature between 180 and 250 ºC leads to an increase in the proportions of phenols and ketones together with a decrease in the relative amounts of aldehydes and carboxylic acids. These variations are the result of the positive effect of the temperature on via isomerisation, cyclisation and dehydration reactions (increasing the proportions of phenols and ketones) and decarboxylation, deamination and thermal decomposition (decreasing the relative amount of carboxylic acids, aldehydes and in some cases, N compounds). At low temperature, the bio-oil has a high proportion of low molecular weight aromatic aldehydes, carboxylic acids and N compounds. An increase in the temperature kinetically promotes biomass depolymerisation, thus increasing the relative amounts of phenols and aromatic ketones in the bio-oil and decreasing the proportion of aldehydes, carboxylic acids and N compounds (at low pressure).

Increasing the pressure from 50 to 130 bar has two different outcomes depending on the temperature. While at low temperature (180-215 ºC) this increase leads to a substantial increase in the proportion of aldehydes together with a decrease in the relative amount of nitrogen compounds, at high temperature (215-250 ºC) a decrease and an increase in the proportions of ketones and phenols take place, respectively. This is believed to the result of the microwave thermodynamic inhibitory effect of the pressure on the efficiency of the microwave heating, as described earlier, which hampers biomass depolymerisation and deamination together with the extraction of proteins at low temperature. As a result, at high pressure and using a long reaction time (130 bar and 2 h), augmenting the temperature leads to a greater increase in the proportion ketones and a more marked decrease in the relative amount of aldehydes, especially between 180 and 220 ºC, while the concentration of N compounds increases slightly.

When the highest amount of catalyst (0.25 g cat/g bio-oil) is used, the effect of the reaction time depends on the temperature and pressure. For a short reaction time (0 h), the bio-oil produced at low temperature might contain a great amount of high-molecular weight species undetectable using gas chromatography, especially at low temperature. An increase in the temperature leads to an increase in the concentrations of carboxylic acids and N compounds as described earlier. An increase in the reaction time has a significant influence on the bio-oil composition, with different developments observed depending on the temperature and pressure. On the one hand, at low pressure (50 bar), increasing the reaction time from 0 to 2 h leads to a substantial increase in the relative amount of ketones in the bio-oil, while different outcomes are observed for the proportions of phenols, aldehydes, carboxylic acids and nitrogen compound depending on the temperature. Between 180 and 230 ºC the relative amounts of phenols, aldehydes, carboxylic acids and N compounds increase due to the positive effect of the reaction time on the process, leading to a greater spread of isomerisation, cyclisation and dehydration reactions; thus producing a bio-oil with compounds having a lower molecular weight and, therefore, detectable with gas chromatography. Between 230 and 250 ºC decreases are observed for the proportions of phenols, aldehydes, carboxylic acids and nitrogen compounds due to the substantial increase occurring in the concentration of nitrogen derived ketones produced from the decomposition of the protein content of the original biomass as well as the transformation into gases of some species in the bio-oil, such as carboxylic acids [62-65]. On the other hand, when a high pressure (130 bar) is used, the effect of the reaction time on the proportions of phenols and aldehydes is less important and substantial variations are not observed. Conversely, the proportions of ketones and nitrogen compounds increase markedly, especially between 180 and 220 ºC, while the relative amount of carboxylic acids increases between 180 and 215 ºC and decreases between 215 and 250 ºC.

These variations modify the effect of the temperature on the bio-oil chemical composition when a long reaction time and a high catalyst amount are used. In particular, for 2 h and using 0.25 g cat/g bio-oil, the effect of the temperature depends on the pressure. At 50 bar, increasing the temperature from 180 to 250 ºC decreases the proportions of phenols (especially between 180 and 220 ºC), aldehydes and carboxylic acids and increases the relative amount of ketones. When the highest amount of catalyst is used, increasing the temperature promotes the decomposition of carboxylic acids and the extraction of nitrogen-derived ketones from the bio-oil. An increase in the pressure from 50 to 130 bar leads to a significant depletion in the proportions of phenols (between 180 and 215ºC) and aldehydes as well as to an increase in the relative amount of nitrogen compounds and ketones (between 200 and 250 ºC). As a result, when a high pressure (130 bar) is used, the temperature does not substantially modify the proportions of phenols and nitrogen compounds in the bio-oil. The proportions of ketones and aldehydes increases and decreases, respectively, between 180 and 215 ºC, while a significant decrease in the relative amount of carboxylic acids is observed due to the positive effect of the temperature in the deoxygenation and decarboxylation reactions [62-65].

**3.3 Effect of the operating conditions on the chemical composition of the liquid phase**

The liquid phase consists of the aqueous fraction produced after bio-oil extraction and comprises a mixture of DP>6 oligosaccharides (98-67 C-wt.%), DP2-6 oligosaccharides (0-10 C-wt.%), saccharides (0.2-7 C-wt.%), carboxylic acids (0-7 C-wt.%) and furans (0-27 C-wt.%). Saccharides include glucose, xylose, fructose, rhamnose, mannose, arabinose and levoglucosan. Carboxylic acids comprise lactic, formic, acetic, levulinic, glucuronic and galacturonic acids, while furans are made up of 5-hydroxymethylfurfural (HMF) and furfural. The detailed chemical composition calculated by HPLC is shown in Table S2. The ANOVA analysis and the cause-effect Pareto principle (Table 6) reveal that the temperature, reaction time and the interaction between these two variables strongly influence the relative amounts of DP>6 oligosaccharides and saccharides in the liquid phase. The concentrations of DP2-6 oligosaccharides and carboxylic acids are highly influenced by the reaction time and the interaction between the reaction time and temperature. The proportion of furans is highly influenced by the catalyst/biomass ratio and the interaction between the reaction time and temperature. Figure 5 illustrates the effect of these variables and the most important interactions on the chemical composition of the liquid phase detected with the ANOVA analysis. Figures 5 a and b show the effects of the temperature on the relative amount of DP>6 oligosaccharides at low and high pressure (50 and 130 bar) in the absence of the catalyst, using a reaction time of 0 and 2 h, respectively. Figures. 5 c and d show these effects for the highest catalyst/biomass ratio used in this work (0.25 g cat/g biomass). Figures 5 e-h, i-l, m-p and q-t respectively show these effects for the relative amounts of DP2-6 oligosaccharides, saccharides, carboxylic acids and furans in the liquid phase.

The effect of the temperature on the chemical composition of the liquid phase depends on the reaction time and catalyst amount. For a short reaction time (0 h) and in the absence of a catalyst, the liquid phase is largely made up of DP>6 oligosaccharides produced at early reaction stages. Regardless of the pressure, an increase in the temperature leads to a substantial decrease in the proportions of DP>6 oligosaccharides due to the positive effect of the temperature on the process. This promotes the depolymerisation and hydrolysis of DP>6 oligosaccharides and leads to a substantial increase in the proportion of DP2-6 oligosaccharides between 180 and 215 ºC. A further increase in the temperature from 215 to 250 ºC decreases the concentration of DP2-6 oligosaccharides and increases the relative amount of saccharides. This is accounted for by the progressive transformation of the former into the latter due to the positive influence of the temperature on hydrolysis and depolymerisation reactions [71]. Additionally, between the 215 and 250 ºC increasing the temperature also increases the proportion of carboxylic acids and furans due to the positive effect of the temperature on secondary reaction leading to sugar decomposition via secondary reactions [72-74]. Under these conditions, the pressure exerts a statistically significant influence on the liquid chemical composition. Though, the variations observed when the pressure increases from 50 to 130 bar are not very important from a practical point of view. Particularly, at low temperature (180-215 ºC), the pressure influences the proportions of DP>6 oligosaccharides and DP2-6 oligosaccharides; an increase from 50 to 130 bar substantially increases and decreases the concentration of former and the latter. At higher temperature (215-250 ºC) this increase in pressure slightly decreases the proportions of saccharides, carboxylic acids and furans and increases the proportion of DP>6 oligosaccharides. These variations are believed to be the consequence of the thermodynamic inhibitory effect of the pressure [61], decreasing the efficiency of the microwave process [42]. This hinders the development of depolymerisation and hydrolysis reactions, thus augmenting the amount in the liquid phase of species produced at early reaction stages.

Increasing the catalyst/biomass ratio significantly modifies the composition of the liquid phase as well as the effects that the temperature and pressure have on this fraction. At low temperature (180-200 ºC), the liquid phase is also largely made up of DP>6 oligosaccharides regardless of the catalyst/biomass ratio. This suggests that hydrolysis and depolymerisation reactions do not take place to a substantial extent at low temperature and/or the catalytic activity for these reactions at low temperature is low. Conversely, at high temperatures (215-250 ºC), increasing the catalyst from 0 to 0.25 g cat/g biomass increases the proportion of DP>6 oligosaccharides and decreases the concentration of furans in the liquid regardless of the pressure. These variations might be accounted for by the positive effect of the catalyst on biomass depolymerisation (increasing the amount of oligomers) and deoxygenation (decreasing the proportions of furans).

**Table 6: Relative influence of the operating conditions on the liquid phase properties according to the ANOVA analysis and cause-effect Pareto Principle**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **R2** | **Indep** | **T** | **P** | **t** | **W** | **TP** | **Tt** | **TW** | **Pt** | **PW** | **tW** | **Wt T2** | **T2** | **P2** | **t2** | **W2** | **TPt** | **TPW** | **TtW** | **PtW** | **T2P** | **T2t** | **T2W** | **TP2** | **TPtW** | **T2P2** |
| **Oligosaccharides DP>6 (C-wt.%)** | 0.99 | 98.42 | 3.41  (9) | n.s | 5.52  (10) | 1.34  (9) | 1.83  (4) | 5.92  (12) | 2.82  (6) | -0.55  (1) | n.s | -3.35  (7) | n.s | -4.21  (8) | n.s | -5.50  (4) | -1.02  (0) | -2.12  (4) | 0.45  (1) | -2.90  (6) | -1.74  (4) | 2.13  (4) | -1.02  (1) | 2.94  (2) | -8.45  (6) | n.s | 3.39  (1) |
| **Oligosaccharides DP 2-6 (C-wt.%)** | 1 | 0.011 | -3.03  (5) | n.s | -4.93  (11) | 0.009  (1) | -0.52  (5) | -0.31  (3) | 0.41  (4) | -0.031  (0) | -0.58  (5) | 0.49  (4) | n.s | 3.02  (2) | n.s | 4.92  (6) | n.s | 0.54  (5) | -0.14  (1) | -0.75  (7) | 0.68  (6) | 0.011  (0) | 4.24  (13) | -0.16  (0) | 2.81  (8) | 0.030  (0) | -6.73  (14) |
| **Saccharides (C-wt.%)** | 0.99 | 0.28 | -0.31  (10) | n.s | -0.55  (11) | n.s | -0.35  (4) | -1.13  (14) | -0.32  (4) | 0.24  (3) | -0.092  (1) | 0.34  (4) | n.s | 0.45  (8) | n.s | 0.48  (4) | n.s | 0.41  (5) | -0.16  (2) | 0.45  (6) | 0.27  (3) | -0.41  (5) | -0.34  (1) | -0.55  (7) | 1.25  (5) | 0.06  (1) | 0.35  (1) |
| **Carboxylic Acids (C-wt.%)** | 0.99 | 0.27 | n.s | n.s | n.s | n.s | -0.50  (7) | -1.02  (14) | -0.27  (4) | 0.41  (6) | 0.07  (1) | 0.26  (4) | n.s |  | n.s | -0.13  (8) | n.s | 0.57  (8) | -0.08  (1) | 0.32  (5) | 0.11  (2) | -0.63  (9) | -0.73  (10) | -0.33  (5) | 0.86  (12) | -0.045  (1) | 1.10  (5) |
| **Furans (C-wt.%)** | 0.99 | 1.07 | n.s | n.s | n.s | -1.40  (12) | -0.46  (2) | -3.47  (13) | -2.65  (10) |  | 0.54  (2) | 2.25  (8) | n.s | 0.66  (8) | n.s |  | 1.02  (4) | 0.61  (2) | n.s | 2.88  (10) | 0.67  (2) | -1.10  (4) | -2.20  (8) | -1.84  (2) | 3.43  (12) | n.s | 2.16  (2) |

*T= temperature, P=pressure, t= time, W= catalyst/biomass ratio. n.s: Non significant with 95% confidence. Numbers in brackets indicate the percentage Pareto influence of each factor on the response variable. Response = Indep. + Coefficient T·T + Coefficient P·P + Coefficient C·C + Coefficient W·W + Coefficient TC·T·C + Coefficient TW·T·W+ Coefficient PC·P·C + Coefficient PW·P·W + Coefficient CW·C·W + Coefficient T2·T2 + Coefficient P2·P2 + Coefficient C2·C2 + Coefficient W2·W2 + Coefficient TPC·T·P·C + Coefficient TPW·T·P·W + Coefficient TCW·T·C·W + Coefficient PCW·P·C·W + Coefficient T2P·T2·P + Coefficient T2W·T2·W + Coefficient TP2·T·P2 + Coefficient TPtW·T·P·t·W*

**Table 7: Relative influence of the operating conditions on the elemental composition and HHV of the spent solid according to the ANOVA analysis and cause-effect Pareto Principle**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **R2** | **Indep** | **T** | **P** | **t** | **W** | **TP** | **Tt** | **TW** | **Pt** | **PW** | **tW** | **Wt T2** | **T2** | **P2** | **t2** | **W2** | **TPt** | **TPW** | **TtW** | **PtW** | **T2P** | **T2t** | **T2W** | **TP2** | **TPtW** | **T2P2** |
| **C (wt.%)** | 0.93 | 50.05 | 2.25  (9) | n.s | 3.75  (15) | -8.05  (32) | n.s | n.s | -2.76  (10) | n.s | n.s | -1.86  (7) | n.s | n.s | -5.20  (7) | -8.31  (0) | n.s | n.s | n.s | -1.89  (7) | n.s | n.s | n.s | n.s | n.s | n.s | 15.68  (13) |
| **H (wt.%)** | 0.91 | 5.79 | -0.23  (10) | n.s | n.s | -0.85  (35) | n.s | -0.29  (11) | -0.22  (8) | n.s | n.s | n.s | n.s | n.s | -0.65  (14) | -0.73  (5) | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | 1.94  (17) |
| **N (wt.%)** | 0.94 | 1.82 | -0.12  (7) | n.s | -0.15  (8) | -0.43  (24) | n.s | 0.40  (21) | n.s | n.s | n.s | n.s | n.s | 0.53  (25) | n.s | 0.28  (7) | n.s | n.s | n.s | -0.14  (8) | n.s | n.s | n.s | n.s | n.s | n.s | n.s |
| **O (wt.%)** | 0.94 | 42.46 | -1.89  (7) | n.s | -3.55  (13) | 9.32  (33) | n.s | n.s | 3.05  (10) | n.s | n.s | 1.99  (7) | n.s | n.s | 5.74  (9) | 8.63  (1) | n.s | n.s | n.s | 2.15  (7) | n.s | n.s | n.s | n.s | n.s | n.s | -18.02  (14) |
| **HHV(MJ/Kg)** | 0.98 | 22.05 | 1.08  (10) | n.s | 1.31  (12) | -3.93  (35) | n.s | n.s | -1.42  (12) | n.s | 0.35  (3) | -1.03  (9) | n.s | n.s | -1.77  (3) | -1.78  (1) | 1.53  (9) | n.s | n.s | -0.47  (4) | n.s | n.s | n.s | n.s | n.s | n.s | 2.82  (6) |

*T= temperature, P=pressure, t= time, W= catalyst/biomass ratio. n.s: Non significant with 95% confidence. Numbers in brackets indicate the percentage Pareto influence of each factor on the response variable. Response = Indep. + Coefficient T·T + Coefficient P·P + Coefficient C·C + Coefficient W·W + Coefficient TC·T·C + Coefficient TW·T·W+ Coefficient PC·P·C + Coefficient PW·P·W + Coefficient CW·C·W + Coefficient T2·T2 + Coefficient P2·P2 + Coefficient C2·C2 + Coefficient W2·W2 + Coefficient TPC·T·P·C + Coefficient TPW·T·P·W + Coefficient TCW·T·C·W + Coefficient PCW·P·C·W + Coefficient T2P·T2·P + Coefficient T2W·T2·W + Coefficient TP2·T·P2 + Coefficient TPtW·T·P·t·W*

The microwave radiation not only does break the compact fibre structure but also might promote the penetration of the catalyst into the core solid structure [75]. However, different trends are observed for the proportions of DP2-6 oligosaccharides, saccharides and carboxylic acids depending on the pressure. At low pressure (50 bar), the proportions of DP 2-6 oligosaccharides and saccharides increases and decreases respectively, while non-significant variations are observed for the relative amount of carboxylic acids due to the positive effect on the hydrolysis and depolymerisation of DP>6 oligosaccharides. An increase in the pressure from 50 to 130 bar has a significant influence on the chemical composition of the aqueous phase when a temperature between 215 and 250 ºC is used. Within this temperature interval, this increase in pressure increases the proportions of DP>6 oligosaccharides, and diminishes the relative amounts of DP 2-6 oligosaccharides, saccharides, carboxylic acids and furans due to the inhibitory thermodynamic effect of the pressure. As a result, at 130 bar the effect of the temperature does not greatly modify the proportions carboxylic acids and furans while small variations occur for the proportions of oligomers (DP>6 and DP 2-6). Particularly, between 180 and 215 ºC the proportions of DP>6 oligosaccharides and DP 2-6 oligosaccharides decrease and increase, respectively. A further increase in the temperature up to 250 ºC has the opposite influence on these families; i.e. an increase in the proportion of DP>6 oligosaccharides along with a decrease in the relative amount of DP 2-6 oligosaccharides.

Increasing the reaction time from 0 to 2 h leads to a substantial increase in the proportion of DP>6 oligosaccharides regardless of the catalyst amount or pressure; this increase being marked at high temperature (215-250 ºC), thus augmenting the formation of high molecular weigh oligosaccharides. In addition, the proportions of DP2-6 oligosaccharides, saccharides, carboxylic acids and furans decrease due to the longer exposure of the material to microwaves, leading to the decomposition of these species to gaseous products. As a result, when a reaction time of 2 h is used, the liquid phase is largely made up of DP>6 oligosaccharides for the whole temperature interval consider in this work, regardless of the pressure or catalyst amount. Increasing the reaction time promotes gas production through the thermal cracking and deoxygenation of small oxygenates, thus increasing the relative amount of DP>6 oligosaccharides in the liquid phase as long reaction times favours CO2 production by the decarboxylation of low molecular weight oxygenates produced through from cellulose and hemicellulose [76, 77].



Figure 5. Interaction plots showing the effects of the operating conditions and interactions on the chemical composition of the aqueous liquid phase obtained with the ANOVA analysis of the results. Bars are LSD intervals with 95% confidence.

**3.4 Effect of the operating conditions on the spent solid elemental composition and HHV**

The relative amount of C, H, N and O in the spent solid produced after the experiments vary by 35-72 wt.%, 4-8 wt.%, 1-4 wt.% and 18-57 wt.%, respectively; the HHV of this product shifting between 9 and 32 MJ/kg. The statistical analysis of the results (Table 7) revealed that the relative amounts of C and O and HHV of the spent solid are strongly influenced by the catalyst amount and the reaction time. In addition, the temperature also has an important influence. The amount of catalyst also has an important influence on relative amounts of H and N in the spent solid. The pressure and the temperature play an important role in the relative proportion of H and N, respectively. In addition, the elemental composition and HHV of this solid is significantly influenced by several interactions between the operating variables. Figure 6 shows the effects on the operating variables and the most important interactions detected with the ANOVA analysis. Precisely, Figures 6 a/e/i/m show the effect of the reaction time on the proportions of C/H/O/ and HHV at medium temperature and pressure (215 ºC and 90 bar) with the lowest and highest catalyst/biomass ratio used in this work (0 and 0.25 g cat/g biomass). Figures 6 b, c and d show the effect of the temperature in the absence using the lowest and highest amount of catalyst (0 and 0.25 g cat/g biomass) for a reaction time of 1 h using a system pressure of 50, 90 and 130 bar, respectively. Figures 6 f-h, j-l and n-p plot these effects for the proportions of H, O and HHV of the spent solid, respectively. Figures 6 q-r show the effect of the temperature on the relative amount of N for the highest and lowest amount of catalyst (0 and 0.25 g cat/g biomass) at 90 bar using a reaction time of 0, 1 and 2 h, respectively.

The reaction time has a similar influence on the proportions of C, H and O and HHV of the spent solid regardless of the temperature or the pressure. Figures 6 a/e/i/m show that increasing the reaction time from 0 to 1 h leads to a significant increase in the proportions of C and H and solid HHV along with a decrease in the relative amount of O in the spent solid regardless of the amount of catalyst. A further increase in the reaction time up to 2 h does not greatly modify the elemental composition or HHV of the spent solid. These variations might be the consequence of the longer exposure of the material to microwave heating, which promotes the development of decarboxylation, deoxygenation reactions, increasing the proportions of C and H and decreasing the relative amount of O in the spent solid. In the absence of the catalyst, the variations observed for the proportions of C, H, O and HHV are not statistically significant. When the highest amount of catalyst (0.25 g cat/g biomass) is used, increasing the reaction time from 1 to 2 h slightly decreases the proportions of C and H and increases the relative amount of O; these variations leading to a small reduction in the HHV of the solid. However, these developments are not very important for a practical point of view. The levelling off observed between 1 and 2 h might be accounted for by the competition between cracking and pyrolysis reactions and condensation, crystallisation and re-polymerisation reactions increasing char formation [31, 58].

The effect of the temperature depends on the reaction pressure and the catalyst amount. At low pressure (50 bar), an initial increase in the reaction temperature between 180 and 215 ºC leads to a decrease in the proportions of C and H and HHV together with an increase the proportion of O of the spent solid. A further increase in the temperature up to 250 upsurges the proportions of C and H and HHV and decreases the relative amount of O. An increase in the pressure from 50 to 90 bar decreases the proportions of C and H and HHV and increases the relative amount of O in the solid residue at low (180-200 ºC) and high (230-250 ºC) temperature. These variations modify the effect of the temperature on the proportions of C, H and O and HHV of the spent solid. As a result, in the absence of the catalyst, the proportion of C and O increases and decreases very slightly, respectively, while the relative amount of H is not significantly affected. These variations are weaker when a catalyst/biomass ratio of 0.25 g cat/g biomass is used and a steady evolution with the reaction temperature is observed for the proportions of C and O in the spent solid.

**

Figure 6. Interaction plots showing the effects of the operating conditions and interactions on the elemental composition and HHV of the spent solid obtained with the ANOVA analyses of the results. Bars are LSD intervals with 95% confidence.

In addition, the effect of the temperature on the HHV also depends on the amount of catalyst. On the one hand, in the absence of a catalyst, increasing the temperature between 180 and 250 ºC increases the HHV. On the other, a steady evolution for the HHV takes place between 180 and 250 ºC when the highest amount of catalyst (0.25 g cat/g biomass) is used. A further increase in the pressure up to 130 increases the proportions of C and H and decreases the relative amount of O. As a result, the same evolutions with the temperature take place at 50 than at 130 bar. The effect of the temperature on the proportion of N in the spent solid depends on the reaction time regardless of the pressure or catalyst amount. Between 0 and 1 h, an initial increase in the temperature between 180 and 215 ºC decreases the relative amount of N in the solid, while a steady evolution takes place with a further increase up to 250 ºC. Conversely, the opposite trend is observed for a long reaction time (2 h); i.e. a steady evolution between 180 and 215 ºC followed by an increase between 215 and 250 ºC. Increasing the temperature and/or the reaction time might produce the degradation of the proteins present in the original solid by deamination (resulting in the formation of ammonia) and/or decarboxylation (which produces carboxylic acids and amines) [59, 78-80]. In addition, regardless of the operating conditions, an increase in the catalyst amount from 0 to 0.25 g cat/g biomass lead to increases in the relative amounts of C and H and the HHV of the spent solid and decreases the proportions of O and N. This is believed to be a consequence of the positive effect of the catalyst on decarboxylation and deamination reactions [31, 58].

**3.5 Theoretical optimisation**

Optimum operating conditions were sought for the selective conversion of BSGs into liquid and solid value-added products using the empirical models developed with the statistical analysis of the experimental results. Four different scenarios were considered. The first optimisation is directed towards the production of a bio-oil with adequate fuel properties and therefore, it comprises the maximisation of the biomass conversion, bio-oil yield and HHV as well as the minimisation of the N content in the bio-oil. The second aims at obtaining a fermentable oligo/saccharide-rich liquid a broth via the maximisation of the biomass conversion and liquid yield together with the minimisation of the possible fermentation inhibitors; i.e. carboxylic acids and furans. The third includes bio-char production by minimising the overall conversion (maximising the spent solid yield) and maximising the HHV of the spent solid. The fourth is directed towards a one-step selective BSG valorisation into high-energy bio-oil and bio-char together with a fermentable saccharide-rich aqueous solution. It considers the maximisation of the biomass conversion, bio-oil and liquid yields as well as the HHVs of the bio-oil and the spent solid, along with the minimisation of the proportions of carboxylic acids and furans in the liquid product. In these optimisations, a relative importance (1-5) has been given to each restriction to come up with a solution that satisfies all the criteria. Table 8 lists the optimisation conducted as well as the optimum values obtained in the optimisations.

**Table 8. Theoretical optimisation: objectives, relative importance and optimum values.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Optimisation 1** | | **Optimisation 2** | | **Optimisation 3** | | **Optimisation 4** | |
|  | Objective (importance) | Value | Objective (importance) | Value | Objective (importance) | Value | Objective (importance) | Value |
| **T (°C)** |  | 250 |  | 180 |  | 180 |  | 250 |
| **P (bar)** |  | 130 |  | 107 |  | 50 |  | 125 |
| **t (h)** |  | 2 |  | 2 |  | 0.7 |  | 2 |
| **Wcat/Wbio (g/g)** |  | 0.25 |  | 0.25 |  | 0 |  | 0 |
| **Global results** | | | | | | | | |
| Overall Conversion (%) | Maximise (3) | 67.1 | Maximise (3) | 73.7 | Minimise (5) | 36.2 |  | 65.3 |
| Gas yield (%) | Minimise (3) | 24.7 |  | 17.0 | Minimise (3) | 16.7 | Minimise (3) | 25.8 |
| Liquid yield (%) |  | 34.4 | Maximise (5) | 49.1 |  | 13.8 | Maximise (3) | 30.7 |
| Bio-oil yield (%) | Maximise (5) | 9.2 |  | 5.1 |  | 5.1 | Maximise (3) | 8.2 |
| **Bio-oil chemical composition (% Area)** | | | | | | | | |
| Phenols |  | 3.3 |  | 7.4 |  | 4.3 |  | 11.8 |
| Ketones |  | 45.3 |  | 9.5 |  | 43.1 |  | 37.6 |
| Aldehydes |  | 4.1 |  | 10.3 |  | 5.2 |  | 0 |
| Carboxylic Acids |  | 1.5 |  | 7.9 |  | 13.1 |  | 0 |
| Nitrogen compounds |  | 44.4 |  | 58.9 |  | 27.7 |  | 37.0 |
| **Bio-oil elemental analysis and HHV** | | | | | | | | |
| C (wt.%) |  | 61.1 |  | 66.4 |  | 40.3 |  | 59.1 |
| H (wt.%) |  | 7.1 |  | 5.6 |  | 7.3 |  | 7.2 |
| N (wt.%) | Minimise (3) | 5.5 |  | 2.8 |  | 2.2 |  | 5.6 |
| O (wt.%) |  | 26.7 |  | 27.2 |  | 49.7 |  | 28.1 |
| HHV (MJ/Kg) | Maximise (5) | 26.6 |  | 25.5 |  | 18.1 | Maximise (5) | 26.1 |
| **Chemical composition of the liquid aqueous fraction (dry basis, C-wt.%)** | | | | | | | | |
| Oligosaccharides DP>6 |  | 97.3 |  | 85.9 |  | 100 |  | 98.9 |
| Oligosaccharides DP2-6 |  | 0.0 |  | 9.6 |  | 0 |  | 0.8 |
| Saccharides |  | 0.4 |  | 1.3 |  | 0 |  | 0.1 |
| Carboxylic Acids |  | 0.3 | Minimise (5) | 0.4 |  | 0 | Minimise (5) | 0 |
| Furans |  | 2.2 | Minimise (5) | 2.5 |  | 0 | Minimise (5) | 0.4 |
| **Elemental composition and HHV of the spent solid** | | | | | | | | |
| C (wt.%) |  | 43.6 |  | 39.8 |  | 61.7 |  | 70.7 |
| H (wt.%) |  | 4.8 |  | 5.2 |  | 7.8 |  | 6.6 |
| N (wt.%) |  | 2.2 |  | 1.9 |  | 3.1 |  | 3.3 |
| O (wt.%) |  | 49.9 |  | 53.3 |  | 27.6 |  | 19.3 |
| HHV (MJ/Kg) |  | 18.7 |  | 19.3 | Maximise (5) | 25.7 | Maximise (5) | 31.6 |

Taking these restrictions into consideration, a good compromise between bio-oil yield and HHV (9.1 % and 27 MJ/kg) (Opt.1) takes place at high temperature (250 ºC), elevated pressure (130 bar) and using 0.25 g cat/g biomass for a long reaction time (2 h). The production of either a fermentable liquid broth or a high-energy bio-char are maximised at low temperature (180 ºC). In particular, Opt. 2 reveals that 49% of the original solid can be transformed into a high pure saccharide liquid (95 C-wt.%) solution at high pressure, using a long reaction time (2 h) and a high catalyst loading (0.25 g cat/g biomass). Opt. 3 shows that low pressures (50 bar) combined with short (0.7 h) reaction times in the absence of a catalyst allows converting the original solid into a high energy (26 MJ/kg) bio-char. A good compromise between bio-oil, bio-char and fermentable liquid production with suitable chemical properties to be used as energy and fermentable carriers, respectively (Opt. 4), is achieved at high temperature (250 ºC), elevated pressure (125 bar) using a long reaction time (2 h). Under such conditions, the original BSG is transformed into high-energy (8% yield, 26 MJ/kg) bio-oil and bio-char (36 % yield, 32 MJ/kg) and a high pure oligo-/saccharide aqueous liquid fraction (31% yield, 99 C-wt.%). These calorific values are greater than that of the original material (7.30 MJ/kg as received and 21 MJ/kg dried). These promising results represents a major contribution to rendering the entire bio-refinery for BSGs more economically and environmentally viable. Future work could include the investigation on the effect of the brewers’ spent grain composition on this process at optimum conditions.

**4. Conclusions**

This work addresses a novel bio-refinery concept for the valorisation of brewer’s spent grains via microwave-assisted, catalysed, hydrothermal liquefaction, analysing the effects of the operating conditions on the process. The most important conclusions are summarised as follows.

1. The operating conditions exerted a significant influence on the process, allowing the selective production of bio-oil, bio-char and sugar rich solutions from brewers’ spent grains. The overall solid conversion and the yields to gas, liquid aqueous fraction and bio-oil varied by 31-68%, 10-33%, 9-48% and 4-14%, respectively. The temperature and reaction time are the operating variables with the highest influence on the overall conversion and gas and bio-oil yields, while the liquid yield is highly influenced by the pressure. Increasing the temperature increased the overall biomass conversion and the yields to gas, bio-oil and liquid, while an increase in the reaction time promoted gas and solid formation.

2. The bio-oil was made up a complex mixture of phenols (0-26%), ketones (0-80%), aldehydes (0-57%), carboxylic acids (0-18%) and nitrogen compounds (0-76%). The proportions of C, H, N and O in this liquid varied as follow: 15-61 wt.%, 5-10 wt.%, 1-6 wt.% and 26-77 wt.%, respectively, these variations shifting the bio-oil HHV between 9 and 27 MJ/kg. In general, increasing the temperature, reaction time and catalyst amount increased the proportions of C, N and H of the bio-oil, decreasing its relative amount of O due to the development of deoxygenation, deamination and cracking reactions. These reactions also modifed the chemical composition of the bio-oil. At low temperatures and/or short reaction times, the bio-oil contained high proportions of high molecular weight compounds. Increasing the temperature, reaction time and catalysts amount decreased the proportion of these high molecular weight species, resulting in increases in the proportions of phenols, ketones, aldehydes, carboxylic acids and furan compounds; their relative proportions in the bio-oil depending on the operating conditions.

3. The liquid fraction consisted of a mixture of DP>6 oligosaccharides (98-67 C-wt.%), DP2-6 oligosaccharides (0-10 C-wt.%), saccharides (0.2-7 C-wt.%), carboxylic acids (0-7 C-wt.%) and furans (0-27 C-wt.%). Decreases in the proportions of DP>6 oligosaccharides occurs together with increases in the relative amounts of DP2-6 oligosaccharides and saccharides due to the transformation of the former into the latter via hydrolysis. These reactions are favoured increasing the reaction time, temperature and catalysts amount. Oligosaccharides were produced at early reaction stages and can be subsequently transformed into more depolymerised oligo- and saccharides as well as secondary products, such as carboxylic acids and furans via hydrolysis and dehydration reactions. The extension of these reactions depended on the operating conditions, which significantly influenced the composition of the aqueous fraction.

4. The spent solid produced after the experiments consisted of a bio-char like material. The proportions of C, H, O and N in this solid fraction varied by 35-72 wt.%, 4-8 wt.%, 1-4 wt.% and 18-57 wt.%, respectively, while its HHV shifted between 9 and 32 MJ/kg. Increasing the reaction time led to an initial increase in the proportions of C, H and HHV together with a decrease in the relative amount of O. A levelling off was observed using long reaction times due to the competition between several reactions (cracking, pyrolysis, condensation, crystallisation and re-polymerisation reactions). These reactions were also influenced by the temperature, pressure, reaction time and catalyst amount, which resulted in several variations in the composition of the bio-char and its HHV depending on the precise operating conditions used in the process.

5. Depending on the operating conditions, the process can be directed towards the production of bio-oil, bio-char or sugar-rich solutions. The production of a bio-oil with a high calorific value is favoured at high temperature, elevated pressures and using a high catalyst loading for a long reaction time. The productions of bio-char and sugar rich solutions are favoured at low temperature. On the one hand, elevated pressures and long reaction times favoured the production of sugar-rich solutions; on the other, short reaction times favoured the production of a high-energy bio-char. A good compromise between all these products can be achieved at a temperature of 250 ºC, and a pressure of 125 bar for 2 h. These conditions allow transforming the original material into a (8%) high-energy bio-oil (26 MJ/kg) and (35%) bio-char (32 MJ/kg) together with a (31%) saccharide-rich (>99 C-wt.%) aqueous solution. These values are higher than that of the dried BSG and much higher than the HHV of the raw BSG.

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