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SCALING UP THE AQUEOUS EXTRACTION OF DEGRADATION PRODUCTS FROM
WHEAT STRAW AFTER *Pleurotus ostreatus* HARVESTING

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ABSTRACT

The cultivation of mushrooms on wheat straw can be considered a solid state fermentation, yet following harvest the residual, partially degraded straw is discarded. During cultivation, the degradation of lignocellulose in the straw takes place by the fungus under the action of enzymes releasing degradation products with small molecular weight, some of which are potentially valuable. These compounds may be extracted from straw after mushroom cultivation in two stages: an aqueous extraction followed by a solvent extraction. The present work is focused on the first stage of the process. The aqueous extraction releases water soluble compounds, such as sugars and phenolics with lower molecular weight, which are easily obtained. The partially degraded straw may then be treated with organic solvents to release water insoluble lignin breakdown products, such as fatty acids, phenolics and other aromatics.

It is important to conduct scale-up experiments at a scale that would reflect the amount of waste straw generated by a mushroom farm. A study was performed using a vessel of 300 L capacity with mixing impeller, by observing the influence of the temperature (20°C, 25°C, 40°C, 60°C and 80°C) and water-to-dry straw ratio (from 40:1 to 90:1) on the total extracted matter and especially on sugar and phenolic compounds yields. A microbial study of the aqueous extract was also performed at 20°C and 25°C to explain the high concentration of organic carbon in the extract under certain circumstances. The optimum extraction conditions were determined by taking into account the yield and the energy consumption of the process. The conclusion was that the extraction temperature can be conducted between 20°C and 25°C with good results for obtaining liquor which can be used in a biogas installation. The extraction should be conducted at 80°C to obtain greater yields of sugars and phenolics.

Keywords: *Pleurotus ostreatus*; Lignocellulose; Extraction; Scaling up.

1. Introduction

Increasingly, biomass and agricultural wastes are becoming targets for biorefinery operations to circumvent the negative implications of using the edible parts of plants for biofuel production (Kaparaju *et al*, 2009; Cheroubini and Ulgiati, 2010; Pilar Dorado *et al*, 2009; Naik *et*

al., 2010). Plant wastes consist predominantly of lignocellulose in the cell walls, they are recalcitrant and require high energy inputs to separate the cellulose, hemicellulose and lignin fractions.

The mushroom cultivation industry employs naturally occurring lignocellulose-degrading fungi in a solid state fermentation at ambient temperatures (Crawford and Crawford, 1980; Valmaseda *et al.*, 1991; Hammel, 1997; Pandey, 2003) to convert straw into a high value product, e.g. the oyster mushroom *Pleurotus ostreatus* (Bisaria *et al.*, 1987; Kerem *et al.*, 1992) or the button mushroom *Agaricus bisporus* (Durrant *et al.*, 1991). White rot fungi such as *P. ostreatus* efficiently degrade all the components of the lignocellulose composite, including lignin. Many thousands of tonnes of wheat and rice straw are used each year and, following cultivation, the partially degraded straw is generally discarded. An integrated biorefinery model may be applied not only to produce mushrooms, but also to extract useful compounds from the residual degraded straw, e.g. C₅ and C₆ sugars and phenolics for biofuel production, pharmaceutical use, polymers synthesis or as lubricants. We aim to develop a two stage extraction process to separate these compounds: an aqueous extraction followed by a solvent extraction, under mild conditions. This process can potentially be performed locally, at farm scale. In a previous work (Koncsag *et al.*, 2010), the optimum conditions of the extraction process were determined at the laboratory scale. However, scaling up the extraction process is essential to develop the optimum process at real scale, i.e. a mushroom farm dealing with the disposal of tonnes of straw.

The present work is focused on the first stage of the process, the aqueous extraction, the role of which is to recover organic compounds (e.g. sugars) from degradation of straw and to enhance the subsequent solvent extraction by removing the material which might prevent the solvent penetrating the substrate and releasing the high value lignin breakdown products. A study was performed using a vessel of 300 L capacity with mixing impeller, where the influence of the temperature (20°C, 25°C, 40°C, 60°C and 80°C), processing time and water-to-dry straw ratio (from 40:1 to 90:1) were assessed for the extraction of total extracted matter and especially sugar and phenolic compound yields. Optimum extraction conditions in terms of product release were identified and related to the amount of energy required to achieve these results, producing the basis of future scale up experiments.

2. Material and methods

2.1. Raw material

The raw material for the study consisted of wheat straw degraded by fungus *Pleurotus ostreatus* grown in 20 kg batch in plastic bags under standard cultivation conditions at the WHRI, University of Warwick, Bioconversion Unit. Briefly, steam treated straw was inoculated with *P.ostreatus* (strain HK35) at a rate of 1.5 kg grain spawn to 100 kg straw, the fungus was incubated in the dark at 25 °C for 24 days before fruiting was induced by illumination, splitting the growth bags and temperature reduction (15 °C). Mushrooms were harvested continually over a 28 day period. The weight of bags decreased from 20-23 kg to 5-8 kg during incubation and mushroom harvesting. Periodically, the straw was watered with an unmeasured quantity of water to keep the mushroom crop moist, therefore, bag to bag moisture levels was variable at the end of harvesting.

2.2. Equipment

The equipment used in this experiment was a 300L biofermentor installation from Brunswick Co. with a cylindrical vessel (H:D= 3:1) and agitation system (Rushton turbine with three impellers and variable rotation speed 45-450 rpm). The vessel had an electronic weighing scale and an external jacket for warming/cooling. The main working parameters were controlled pneumatically. For this experiment, only the temperature in the vessel and the rotation speed were controlled. Water for extraction was tap water warmed-up to the desired temperature $\pm 1^{\circ}\text{C}$ with a mix of water and steam circulating in the jacket.

2.3. Work procedure

4-22 kg straw partially degraded by *P. ostreatus*, with moisture in range of 53.5-82.2%, was placed in the biofermentor vessel and 100-210 kg water was added. Different ratios of water: straw were analyzed in this study. The vessel was closed, agitation and warming processes started, and the desired temperature was reached in minutes (maximum 32 min, at 80°C).

Sampling from aqueous extract was carried out hourly up to 5 hours and a final sample was collected after 24 hours from the start. Samples were filtered through synthetic muslin and the Easy Oxidizable Organic Carbon (EOOC) was measured. Samples were freeze dried under vacuum to obtain the yield of total dry matter (kg DM/kg dry straw). This yield is an expression

of the degree of straw degradation combined with the efficiency of the extraction process. The total dry matter means all the inorganic and organic material extracted, including fibre. For sugars and phenolics determination, the samples were filtered supplementary through glass fiber cellulose-free filters pore size 7.0 μm (GFA 21cm discs, Whatman).

2.4. Analysis methods

2.4.1. Determination of Easy Oxidizable Organic Carbon in aqueous extract

The titrimetric Walkley-Black analysis (Chacon *et al.* 2002) for Easy Oxidizable Organic Carbon (EOOC) in soils was modified to obtain a qualitative indication of the total organic matter obtained in the aqueous extract. The method is based on the reaction between carbon in organic compounds (oxidation state zero) with dichromate ions in acid environment. The reaction of carbon in the organic compound is not complete because not all compounds undergo facile oxidation. Therefore this method is not quantitative but has proved to be sensitive enough to indicate extraction efficiency.

A sample of aqueous extract (10mL) was poured in a 500 mL Erlenmeyer flask. 10 mL volume of $\text{K}_2\text{Cr}_2\text{O}_7$ 1N was added; 20 mL H_2SO_4 94-98% was then added, mixed for 1 minute and allowed to stand for 30 minutes. After this time, the mixture was diluted with water (200 mL), and 10 mL of concentrated H_3PO_4 was added, with NH_4F (cca.0.2 g) and Ph_2NH indicator (10 drops). The excess of $\text{K}_2\text{Cr}_2\text{O}_7$ was titrated with 0.5 N $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2$ solution prepared with H_2SO_4 (20 mL to 1 litre $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2$ solution). The colour of the mixture changes from dull green to a dark blue in the proximity of the end point of the titration and shifts to brilliant green at the end point. A water blank was prepared and titrated in parallel.

Calculation:

$$EOOC, \% = (B - S) \cdot 0.5 \cdot \frac{12}{4000} \cdot \frac{1}{g} \cdot 100$$

where:

B- volume of ferrous solution for the titration of the blank, mL

S- volume of ferrous solution for the titration of the sample, mL

g – grams of sample

12/4000- equivalent weight of carbon, g

0.5N- molarity of the ferrous solution

2.4.2. Determination of sugars and phenols from aqueous extract

The sugars were determined as glucose, with dinitrosalicylic acid (DNS) (Miller, 1959; Bailey, 1988) by UV-VIS absorption spectrometry, measuring the levels of reducing sugar (absorbance at $\lambda=540$ nm). The phenols were determined as gallic acid with Folin Ciocalteu reagent (Singleton *et al*, 1999), through a colorimetric method, reading absorbance at $\lambda=765$ nm.

2.5. Microbiological study

P. ostreatus is not grown under sterile conditions, therefore, it was assumed that the straw cultures would contain a background microflora and that this microflora might grow in the extraction medium at ambient temperatures (20 °C and 25 °C). Experiments were conducted to determine the effect of extraction temperature and incubation time on the background microflora. Samples were taken from the 20 °C and 25 °C extraction experiments at 1, 2, 3, 4, 5 and 24 hours incubation and serially diluted in 0.01M phosphate buffered saline (PBS, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4, Sigma Chemical Co.). Diluted samples were spread plated onto nutrient agar medium (Difco) and direct colony counts were conducted following 72 hours incubation at 25 °C.

2.6. Energy consumption determination

The optimum extraction conditions were determined by taking into account the yields and the energy consumption in the process. Thermal energy consumption was calculated from steam parameters (temperature, pressure, flow). Electric power was consumed for water pump and impeller motor. A detailed calculation of energy requirement was made on the basis of mixing equations and diagrams (Rushton *et al*, 1950). Then, a comparison of energy consumption for running at different temperatures was allowed.

3. Results and discussion

The total matter extracted, the organic carbon and the concentration of sugars and phenols were measured and compared in different extraction conditions. Also, the energy consumption was calculated in every case.

3.1. The influence of temperature

In order to observe the effect of temperature on the release of sugars and water soluble phenolics, extractions were performed at 20°C, 25°C, 40°C, 60°C and 80°C. The mixing speed was kept low, at 45-90 rotations/minute, because of the high volume fraction of straw in suspension. This speed was enough to create a turbulent regime thus ensuring good mixing of the straw and water during the test.

The effect of the temperature on sugar and phenol yield was determined when comparing results for the same water-to-straw ratio: 60:1 wt/wt. Samples were compared at the end of the extraction process (after 24 h running). The continuous increase in concentration of Easy Oxidizable Organic Carbon (EOOC) in the extracts with higher temperatures (Figure 1) indicates that the Total Organic Carbon (TOC) would have the same tendency. This can be explained by the increase in solubility of organic compounds in water at higher temperature. The phenols concentration in the extract also increased with temperature, as seen in Figure 2, and reached the solubility limit for each temperature assessed.

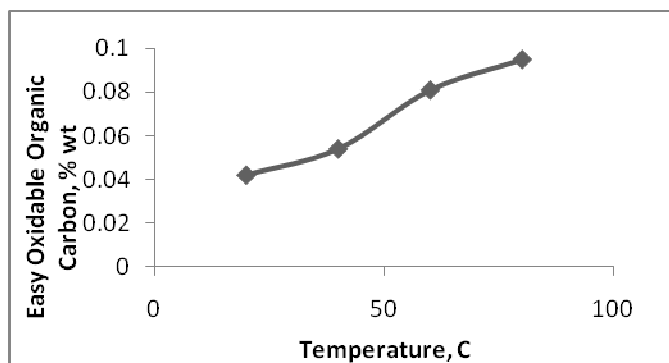


Figure 1. The influence of the extraction temperature on the Easy Oxidizable Organic Carbon (EOOC) content (% wt) in the aqueous extract of *P. ostreatus*-colonised straw (water-to-straw ratio =60:1 kg/ kg dry straw).

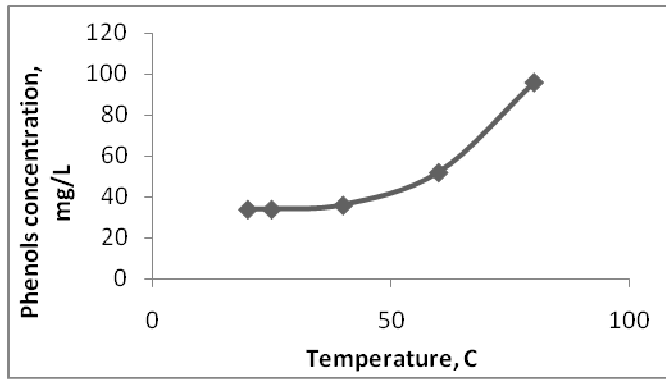


Figure 2. The influence of the temperature extraction on the phenols concentration (mg/L) in the aqueous extract of *P. ostreatus*-colonised straw (water-to-straw ratio =60:1 kg/ kg dry straw).

The sugar level in the extracts could not be correlated with temperature (Figure 3), but when extracted sugar was expressed as mg/kg dry straw, a clear correlation may be seen (Figure 4). Sugars extracted at 60-80 °C as seen in Figure 4 are presumed to be the whole sugars formed from the degradation of cellulose and hemicellulose which remained in the substrate after mushroom cultivation, expressed here as *sugars recovered*.

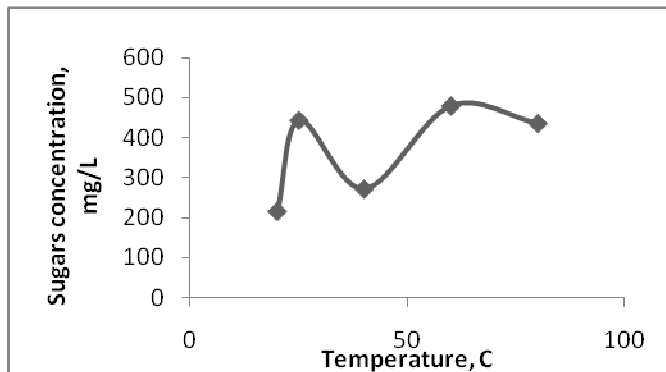


Figure 3. The influence of the extraction temperature on the sugars concentration in the extract (water-to-straw ratio =60:1 kg/ kg dry straw).

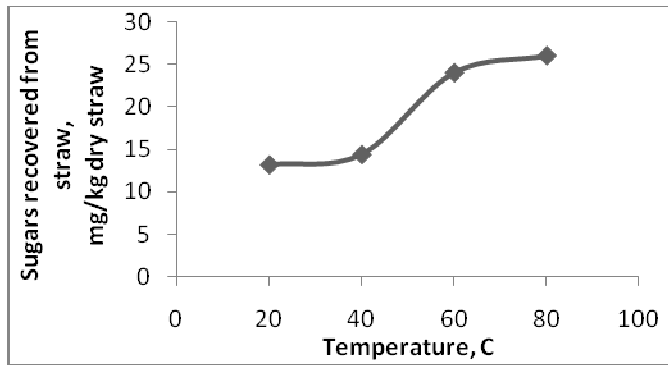


Figure 4. The influence of the extraction temperature on the sugars recovered from straw (water-to-straw ratio =60:1 kg/ kg dry straw). Sugars are calculated as mg recovered per kg straw on dry basis.

The weight of total extracted dry matter (DM) (see section 2.3) demonstrated an unexpected decreasing trend with increasing temperature, (Figure 5).

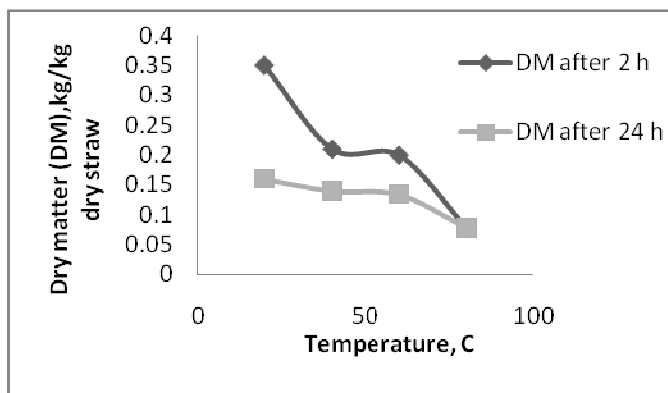


Figure 5. Dry matter extract from straw degraded by *P.ostreatus* and treated with water at different temperatures. Measurements were recorded after 2 and 24 hours extraction

The dry matter extracted was expected to increase with increasing water temperature , as observed with the organic matter extracted (Figure 1), but experimental data ran counter this hypothesis. The reasons for this observation are not clear and further experimentation should be conducted to determine the cause, particular focus on solubility, microbial activity and deposition of salt at higher temperatures should be considered.

3.2.The influence of the water-to-straw ratio on the release of soluble products

The water-to-straw ratio was altered from 40:1 to 60:1 kg/kg dry straw at 20°C and 25°C and the concentration of EEOC, sugar, phenols and DM were measure over a 24 hour period (Figure 6).

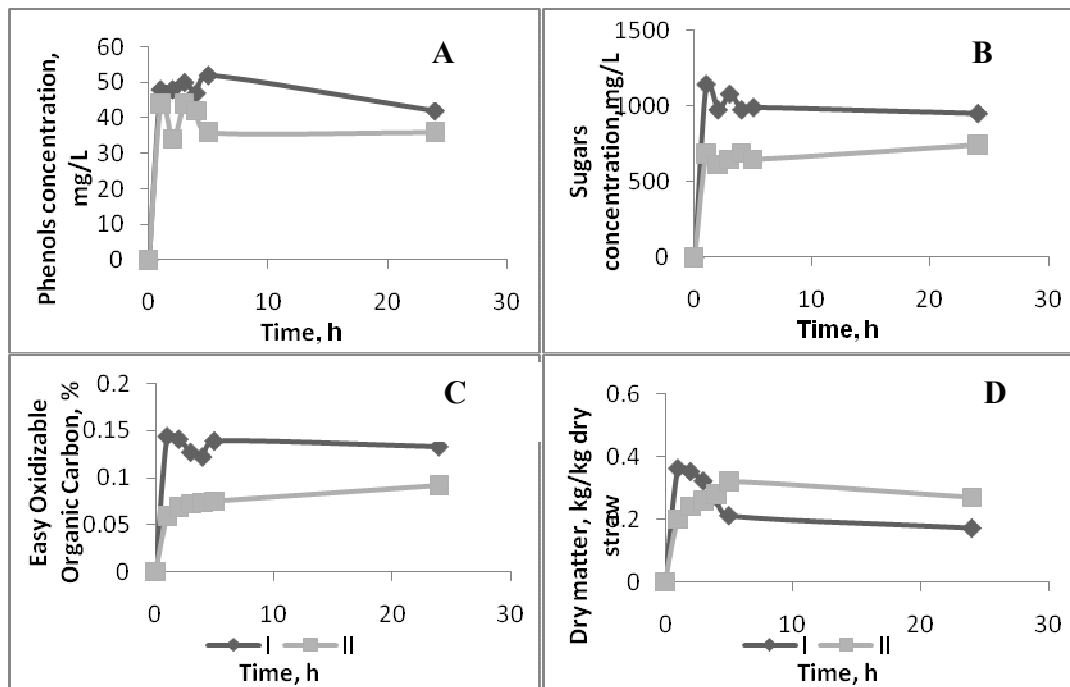


Figure 6. The influence of the water-to-straw ratio on the extraction efficiency at 20°C. Legend: I-ratio 40:1 kg water/kg dry straw, II-ratio 60:1 kg water/kg dry straw; A- the influence on the Easy Oxidizable Organic Carbon content (%wt) in the extract ; B- the influence on the total extracted matter (kg dry matter/kg dry straw); C- the influence on the sugars concentration in the extract (mg/L); D- the influence on the phenols concentration in the extract (mg/L)

The concentration of sugars and organic carbon in the extract was higher at the ratio 40:1 (1.114 – 0.952 g/L sugar and 0.133 % EEOC.), (where the quantity of straw processed was greater) compared with the 60:1 ratio (0.612 – 0.744 g/L sugar, and EEOC 0.092%). After 24 h, the phenol concentration for both ratios of straw had a value around 40 mg/L, which corresponds to their solubility limit at this temperature. The total dry matter extracted per kg straw showed a slight decreasing trend over time.

A similar trend was observed at 25°C (data not shown in figures), with sugars increasing from 0.476 g/L to 0.804 g/L when water: straw ratio was altered from 60:1 to 40:1 wt/wt respectively. EEOC values increased from 0.09% to 0.14% when altering water-to-straw ratio

from 60:1 to 40:1 but phenols remained constant at 36 mg/L in every case, this corresponding approximately to their solubility limit at 25°C . The data also suggest that the optimal extraction values (equilibrium) was achieved between 4 to 5 hours extraction (Figure 6), therefore, longer extraction times are unnecessary. A similar result was observed at each extraction temperature examined (data not presented).

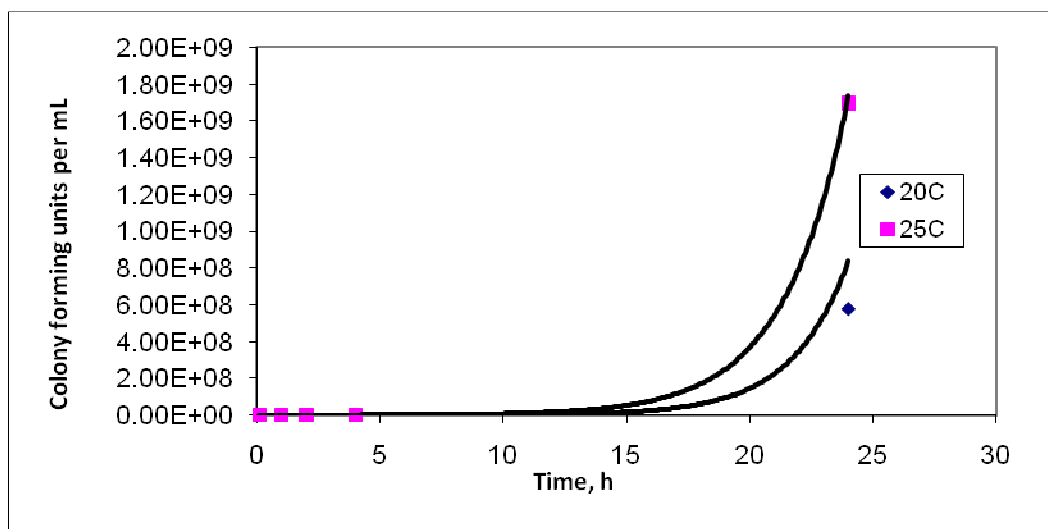


Figure 7. Microbial population density (colony forming units) in the extraction solution from *P. ostreatus*-colonised straw substrate over 24 hours at 20°C and 25°C. The density increases exponentially in time (correlation factor $r^2=0.882$ at 20°C and $r^2=0.994$ at 25°C respectively, for the exponential equation describing these curves).

The effect of the extraction process at ambient temperatures (20 °C and 25 °C) on the microflora (bacterial and yeast) in each experiment was monitored by serial dilution and direct plating over a 24 hour period (Figure 7). Low levels and a limited increase in colony forming units (cfu’s) were detected in the first 5 hours of sampling. After 24 hours, the number of cfu’s had increased 518 fold and 2317 fold in the 20 °C and 25 °C extractions respectively. It is possible that the increased microbial activity could increase the breakdown of the straw substrate, but it is more likely that the bacteria and yeast would utilise the soluble sugars in the extraction solution. This supports the previous conclusion that optimal extraction values were obtained after 4 to 5 hours incubation. In this time the natural microflora from the substrate would have a low population density and would be unlikely to affect the products obtained from the extraction.

3.3. Energy consumption

The energy consumption with mixing and heating was calculated for running at different temperatures and duration of the operation for a batch consisting of 22 kg straw (approx. 4 kg dry straw) and 210 L water (Table 1).

Table 1. Energy consumption with mixing and heating during the aqueous extraction at different temperatures of 22 kg straw (4.04 kg dry) with 210 L water

Power and energy consumption	Temperature			
	20°C	40°C	60°C	80°C
Power consumption for mixing, W	116	115	114	113
Power consumption for heating (average), W	0	158	562	1283
Total energy used up in 1 h, kWh	0.1	3.7	13.3	30.5
Total energy used up in 2 h, kWh	0.2	3.9	13.4	30.6
Total energy used up in 3 h, kWh	0.3	4.1	13.6	30.8
Total energy used up in 4 h, kWh	0.5	4.2	13.8	30.9
Total energy used up in 5 h, kWh	0.6	4.4	13.9	31.1
Total energy used up in 24 h, kWh	2.8	6.6	16.2	33.5

As expected, by operating at higher temperature, the energy consumption increased significantly. The energy consumption was limited by operating at environmental temperature (around 20°C) and by shortening the mixing time as much as possible. Operating at 20 °C for 5 hours uses 7.33 and 51.8 times less energy than operating for the same time at 40 °C or 80 °C respectively. This is in contrast with an approximate two fold increase in sugar release between 20 °C and 80 °C (Figure 4). Careful consideration must be applied to ensure that the benefits of increased in yield by applying greater extraction temperature is not lost by the energy required to obtain such temperatures.

The energy consumption varies slightly with the quantity of straw (data not shown), therefore, from an energetic point of view it is wise to process as much as possible in a batch. The limit is given by the quantity held in suspension; if too much straw is loaded, some of it floats during the mixing, so the efficiency of the extraction might be negatively affected; it was observed that this limit was around approximately 4 kg dry straw per batch.

4. Conclusions

This study aimed to consider whether the partially degraded straw remaining following the cultivation of the oyster mushroom, *Pleurotus ostreatus*, could be exploited to release

lignocellulose breakdown products on a scale relevant to the mushroom cultivation industry. The three main polymers constituting the lignocellulose composite of straw, cellulose, hemicelluloses and lignin are degraded by *P. ostreatus* releasing sugars and aromatic compounds which may have potential application in the production of biofuels or provision of platform chemicals (Karunanandaa *et al* , 1992, Hadar *et al*, 1993). In this study a vessel of 300 L capacity was used, reflecting the scale of the process when dealing with *P. ostreatus* cultivation. While focusing on water extractable compounds, the authors recognize the need for further study to target the non-water soluble lignin breakdown products which is likely to contain valuable aromatic platform chemicals.

The present study investigated varying parameters (temperature, extraction time and water-to-straw ratio) to optimize the extraction of useful products from the partially degraded substrate. The influence of the temperature and water-to-dry straw ratio was assessed by measuring the total extracted matter and especially the yield of sugars and phenolic compounds. The energy consumption was also estimated.

The conclusions of the study are the following:

- The aqueous extraction can be performed at the farm at a reasonable scale (a 300L capacity vessel) for the processing of around 22 kg (4 kg dry) straw degraded remaining following the cultivation of the oyster mushroom;
- Higher water-to-straw ratios are not recommended because the concentration of extractable in the water decreases when increasing this ratio;
- The aqueous extraction time should be limited to 4 hours because no important supplementary extractable is obtained after this and background microflora activity is low;
- The optimum temperature for the extraction is the room temperature (around 20°C) from the energetic point of view, but also when targeting the total organic carbon extracted; Higher temperature extraction provide greater product release, but this advantage may be negated by the energy required to generate the higher temperatures

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