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Sequential low and medium frequency ultrasound assists biodegradation of wheat chaff by white rot fungal enzymes

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ABSTRACT

The consequences of ultrasonic pre-treatment using low (40 kHz) and medium (270 kHz) frequency (40 kHz followed by 270 kHz) on the degradation of wheat chaff (8 g 100 ml^{-1} acetate buffer, pH 5) were evaluated. In addition, the effects of the ultrasonic pre-treatment on the degradation of the wheat chaff when subsequently exposed to enzyme extracts from two white rot fungi (*Phanerochaete chrysosporium* and *Trametes* sp.) were investigated. Pre-treatment by sequential low and medium frequency ultrasound had a disruptive effect on the lignocellulosic matrix. Analysis of the phenolic-derived volatiles after enzymatic hydrolysis showed that biodegradation with the enzyme extract obtained from *P. chrysosporium* was more pronounced compared to that of the *Trametes* sp. The efficacy of the ultrasonic pre-treatment was attributed to increased enzyme accessibility of the cellulose fibrils due to sonication-induced disruption of the plant surface structure, as shown by changes in the microstructure.

1. Introduction

Lignocellulosic biomass is an abundant and renewable source of fuel, energy and organic chemicals and has potential application as an animal feed (Sun, 2010). Pre-treatment of lignocellulosic biomass is necessary to alter its physical and chemical structure for improved cellulose/hemicellulose accessibility (Volynets & Dahman, 2011; Weng, Li, Bonawitz, & Chapple, 2008; Zakzeski, Bruijnincx, Jongerius, & Weckhuysen, 2010). Current pre-treatment methods used to degrade lignocellulosic biomass involve the utilization of harsh chemicals (e.g. strong bases, concentrated sulphuric acid, oxidizing agents) (da Costa Sousa, Chundawat, Balan, & Dale, 2009) or energy-intensive technologies such as steam explosion (Josefsson, Lennholm, & Gellerstedt, 2001; Hana, Deng, Zhang, Bichod, & Wue, 2010). Biological pre-treatment of lignocellulosic biomass is an alternative more environmentally friendly approach (Leonowicz et al., 1999; Sánchez, 2009). The most promising microorganisms for biological pre-treatment are basidiomycetes, which degrade lignin to varying extents depending on the type of fungi and fermentation conditions used (Dinis et al., 2009). These fungi secrete one or more of three extracellular enzyme complexes that are fundamentally important for lignin degradation, predominantly lignin peroxidase, manganese peroxidase, and laccase (Hatakka, 1994; Jönsson & Nyman, 1992). Even so, biodegradation of lignin by white rot fungi alone is itself an impractically slow process. Alternative pre-treatment approaches, which not only reduce the energy input and the use of harsh chemicals but increase the total process efficiency of biomass transformation, are required.

Microwave irradiation has been investigated as an alternative means of pre-treating lignocellulosic material. For example, Binod et al. (2012) showed that microwave-based alkali pre-treatment of sugarcane baggase removed external fibres, increasing the accessibility of the cellulose to enzymes. Chen, Tu, and Sheen (2011) found that dilute acid pre-treatment of ground sugarcane bagasse using microwave heating markedly destroyed the lignocellulose structure, causing swelling and fragmentation of the bagasse particles. The ability of microwave to facilitate the extraction of soluble phenolic compounds from plant material has also been reviewed (Routray & Orsat, 2012).

Ultrasound has been used successfully in conventional processes of desizing, extraction and wool wax scouring (Hurren, Zhang, Liu, & Wang, 2006; Yachmenev, Bertoniere, & Blanchard, 2001; Yachmenev, Blanchard, & Lambert, 2004). Typically, in such

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applications, mechanically pre-comminuted material is placed in a medium containing either strong acid or alkali and subjected to a single low frequency over the range 20–40 kHz. Sun and Tomkinson (2002) demonstrated ultrasound (20 kHz) assisted alkali-dissolution of ground wheat straw. Similarly Velmurugan and Muthukumar (2001) applied ultrasonication (24 kHz) to assist alkali delignification of powdered sugarcane bagasse by increasing surface oxidation of lignin and hemicellulose. Li, Sun, & Sun (2010) showed that ultrasound treatment (35 kHz) of ball-milled, dewaxed bamboo culms increased the partitioning of lignin into ethanol-soluble extracts. Pre-treatment of sawdust with ultrasound (22 kHz) enhanced lignin degradation during subsequent solid-phase cultivation of *Panus (Lenitnus) tigrinus* (Kadimaliev, Revin, Atykyan, & Samuilov, 2003). It has also been established that ultrasound in combination with appropriate solvents can be used to recover lignin from hemicelluloses (García, Alriols, Llano-Ponte, & Labidi, 2011).

Ultrasound also has the potential to increase the activities of enzymatic processes. For example, ultrasound (16 and 20 kHz, up to 3 W cm^{-2}) has been shown to enhance the enzymatic bio-scouring of cotton (Yachmenev et al., 2004). While the disruptive effects of cavitation from low frequency ultrasound (20–100 kHz) physically tease the plant material apart and increase accessibility to the underlying cellulose, higher frequencies (>100 kHz) increase free radical generation and therefore may be expected to sonochemically oxidize components (Ashokkumar et al., 2008).

The primary objective of this work was to evaluate the impact of low and medium frequency ultrasound on wheat chaff and its subsequent biodegradation by enzyme extracts obtained from cultivation of two white rot fungi, *Trametes* sp. and *Phanerochaete chrysosporium*. It was hypothesized that the use of a combination of frequencies would be more effective than a single frequency for disrupting plant biomass, thereby improving enzyme access to the underlying cellulose. The justification for using 40 kHz as the initial pre-treatment frequency in the sequential process presented here was based on the scouring of wool wax, where 35–45 kHz was found to be optimal for cracking the waxy layer and stripping away the scaly surface (Hurren et al., 2006). Additionally targeting of the cracks in the waxy cuticle and potential lifting of the waxy surface by micro-streaming could be expected. The second frequency of 270 kHz was selected to provide enhanced oxidizing potential (Ashokkumar et al., 2008) and specifically erode the closed lip crevice of the microscopic respiratory stomata, and thereby enhance accessibility of enzymes to the internal tissue structure. Based on previous literature findings using microwave (Binod et al., 2012; Chen et al., 2011), a brief high temperature heat treatment of the wheat chaff prior to ultrasonic pre-treatment was also evaluated for its ability to enhance sonication-induced disruption of the lignocellulosic material and subsequent enzymatic hydrolysis.

2. Materials and methods

2.1. Growth of the fungal isolates and preparation of the enzyme extracts

The *Trametes sp.* strain 2403 and *P. chrysosporium* strain 16543 were obtained from the CSIRO Collection of Wood Decaying Fungi. The medium used to grow the fungal isolates contained (per litre): 3 g $NaNO_3$; 1 g K_2HPO_4; 0.5 g KCl; 0.5 g yeast extract (BD Bacto) and 0.01 g $FeSO_4 \cdot 7H_2O$. After formulation the medium was adjusted to pH 5 using 5 M HCl and sterilized by autoclaving at 121 °C. After cooling, 10 ml of 50 g L^{-1} $MgSO_4 \cdot 7H_2O$ solution was added to the medium.

2.1.1. Growth conditions

Wheat chaff was obtained from a local supplier (Country Link Rural Supplies, Lara, Victoria 3212, Australia). The fungal strains were subcultured on malt extract agar (Oxoid) plates incubated at 25 °C. Hyphal mat plugs from the malt extract plates were used to inoculate 50 ml minimal media containing 4% (w/v) wheat chaff in 250 ml Erlenmeyer flasks. The wheat chaff was not treated prior to inoculation. Flasks were incubated at 25 °C on an orbital shaker set at 180 rpm. After 11 days there was visible hyphal mass in the flasks and fungal growth appeared to have softened and partially digested but not completely solubilized the wheat chaff.

2.1.2. Crude enzyme preparation

A portion (20 ml) of the supernatant (included hyphal fragments) from the 250 ml flasks was used to inoculate 750 ml of minimal media containing 4% (w/v) wheat chaff in 2 l Erlenmeyer flasks. The flasks were incubated at 25 °C on an orbital shaker set at 120 rpm for 16 days. Cultures were sieved through 2 mm mesh to remove larger solids, and then centrifuged at 4 °C for 15 min at 12,227 × *g* to pellet smaller solids and fungal hyphae. The supernatant, containing the extracellular fungal enzymes, was decanted and stored at 4 °C.

2.2. Constituent analysis

Acid detergent lignin (ADL), acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined on triplicate samples by Agrifood Technology Pty Ltd, Werribee, VIC 3000, Australia, using the methods supplied (ANKOM Technology). From the NDF (composed primarily of cellulose, hemicellulose and lignin) and ADF (composed primarily of cellulose and lignin), hemicellulose was obtained, whereas cellulose was obtained from the ADF and ADL. The raw wheat chaff contained hemicelluloses (27.8 ± 0.3%), cellulose (21.4 ± 0.5%) and lignin (1.7 ± 0.3%) on a dry matter basis.

2.3. Treatment of wheat chaff

The experimental design is outlined in Fig. 1. The wheat chaff consisted of roughly chopped pieces (~1 cm). Briefly, the wheat chaff (8%, w/v) was immersed in an aqueous solution of sodium acetate buffer (2%, w/v, pH 5) in a 250 ml volume conical flask. The samples were then microwave treated on high power (650 W for 1 min) and immediately cooled in iced-water. Ultrasound pre-treatment was performed using a Blackstone-Ney Ultrasonics, MultiSONIK-2™ 7 frequency, Ultrasonic Generator (Blackstone-Ney Ultrasonics Inc, Jamestown, New York, USA, 900 cm^2 flat transducer plate, programmable duty cycle, output power and frequency) at 40 kHz, 0.5 W cm^{-2}, 35 °C, for 10 min followed immediately by 270 kHz, 0.5 W cm^{-2}, 35 °C for a further 10 min. To obtain a balance between possible loss of enzyme activity and improved mass transfer at high temperature, a temperature of 35 °C was used throughout pre-treatment and enzymatic hydrolysis. A short ultrasonic pre-treatment (5–10 min) has been shown to be sufficiently long to modify lignocellulosic substrates (Chen et al., 2011; Kadimaliev et al., 2003; Li et al., 2010) and increase the biomass biodegradation (Kadimaliev et al., 2003). Hence, a 10 min treatment time was selected for both the initial and second steps of the sequential ultrasonic treatment. Such a brief treatment time is also considered a practical time limit for a batch process that is intended to be ultimately converted into a continuous process (Ashokkumar et al., 2010; Lee et al., 2011).

To assess the efficacy of pre-treatment on biodegradation, all pre-treated samples were subsequently inoculated independently with the crude enzyme extract (100 ml) obtained from either *Trametes* sp. or *P. chrysosporium*. An aliquot (~1 ml) of the aqueous phase was immediately removed (0 h), and then kept at 4 °C, prior to

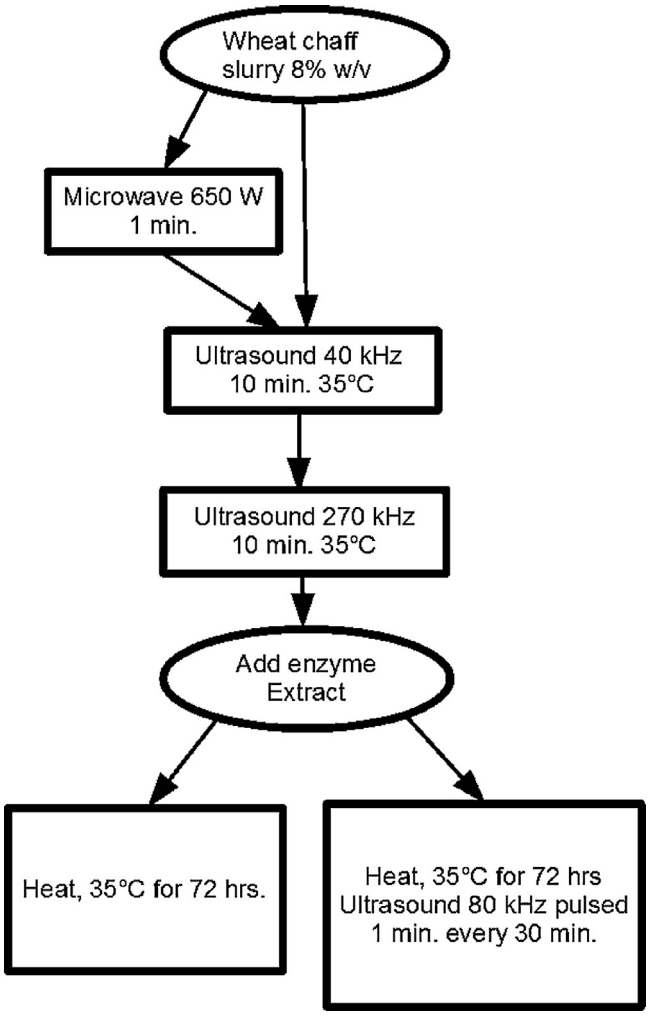

Fig. 1. Outline of experimental design. P = *P. chrysosporium*. T = *Trametes* sp. US = ultrasound.

further analysis. The remaining wheat chaff suspension was further incubated at 35 °C for 72 h. During this time they were subjected to acoustic pulses of 80 kHz (50% power, 0.25 W cm^{-2}) for 1 min every 30 min. The medium frequency pulsed ultrasound (80 kHz) was applied to enhance the activity of the enzymes, by facilitating mass transfer. An aliquot (~1 ml) of the aqueous phase was removed at 24, 72 h, and treated as per the 0 h aliquot. After 72 h incubation, the remaining solids were filtered (metal sieve), rinsed well with deionized water, frozen and freeze-dried. Control samples (i.e. no pre-treatment) were incubated simultaneously under otherwise equivalent conditions. All experiments were performed in duplicate.

2.4. Analysis of headspace volatiles

The volatile compounds generated during enzymatic degradation of lignocellulose in wheat chaff were analyzed by Agilent GCMS (GCMS-6890N model GC and 5975B model MSD) system equipped with the combiPAL robotic autosampler (CTC Analytics AG, Switzerland), according to Ying et al. (2010). The aqueous sample (1.5–2.0 g), taken at pre-determined times, was mixed with an internal standard solution (containing 2-methyl-3-heptanone and 2-ethyl butyric acid in methanol) in a 22 ml screw cap headspace vial. Volatile metabolites in the sample vial were extracted/concentrated on the solid-phase micro-extraction (SPME) fibre (carboxen/polydimethylsiloxane, CAR/PDMS; 85 μm film thickness; 10 mm long; Supelco, Bellefonte PA 16823, USA) for 30 min at 50 °C. After 30 min of SPME sampling of volatiles, the SPME fibre was retracted back into the needle sleeve and removed from the vial. SPME sampled volatiles were thermally desorbed in the GC inlet (PTV inlet- 260 °C, set in splitless mode, splitless time 1 min) and chromatographed on a VF-WAXms column (30 m × 0.32 mm × 1.0 μm; Varian Inc, Mulgrave VIC 3170. Australia) using a temperature gradient of 35–225 °C at 10 °C min^{-1} with initial and final hold times of 3 and 10 min, respectively. Eluted compounds were analyzed by MS detector (MSD). MSD conditions were as follows: capillary direct interface temperature, 260 °C; ionization energy, 70 eV; mass range, 30–300 amu; scan rate, 5 scans s^{-1}. Duplicate analyses were performed on each sample (the variation in duplicate analysis was less than 5%).

Compounds were identified by comparison of mass spectra with the spectra in the NIST08 database (National Institute of Standards Technology mass spectral search programme; NIST, United States of America) and linear retention indices (determined using a set of saturated alkanes C_7 to C_{22}). Quantitative analysis was performed using the internal standard.

2.5. Microscopy

Samples were visualized using confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM).

2.5.1. Confocal laser scanning microscopy

Fluorescence of the freeze-dried wheat chaff solids was visualized using an Argon laser with excitation at 488 nm.

2.5.2. Scanning electron microscopy

Freeze-dried wheat chaff was mounted directly on double-sided, conductive carbon tape affixed to the aluminium, SEM sample stub. A small portion of silver paste was used to secure the sample and to aid conductivity during scanning. Prepared samples were provided with a thin, 1 nm platinum-palladium metal coating (in the Cressington 208HR sputter coater) to enhance image scanning. The samples were imaged in the Hitachi S-4300 SE/N Field Emission, Schottky Scanning Electron Microscope, operated at a 1.2 kV accelerating voltage, to provide surface characteristics.

3. Results and discussion

The physical effects of ultrasound were observed in the disruption of the treated wheat chaff (Figs. 2–4). GCMS of headspace volatiles confirmed that numerous compounds including phenolic compounds, alcohols, acids and esters were obtained due to the effects of the ultrasound pre-treatment followed by enzymatic treatment of the wheat chaff (Fig. 5).

3.1. Microscopy

Various components, such as wax, lignin, cellulose, phenolics, chlorophyll and amino acids in the wheat chaff display fluorescence. A decrease in fluorescence may therefore be linked to an increase in degradation of the wheat chaff. There was a relative decrease in fluorescence of the wheat chaff samples following pre-treatment by ultrasound, as visualized by CLSM (Fig. 2). There was a noticeable decrease in relative fluorescence in the ultrasonically pre-treated wheat chaff samples incubated without addition of the enzyme extract (Controls – Compare Fig. 2A with B). When wheat chaff pre-treated by ultrasound was inoculated with the enzyme extract obtained from *P. chrysosporium*, a further decrease in the relative fluorescence was observed (compare Fig. 2B with D). Similar results were found for the ultrasonically pre-treated wheat chaff inoculated with the enzyme extract obtained from *Trametes*

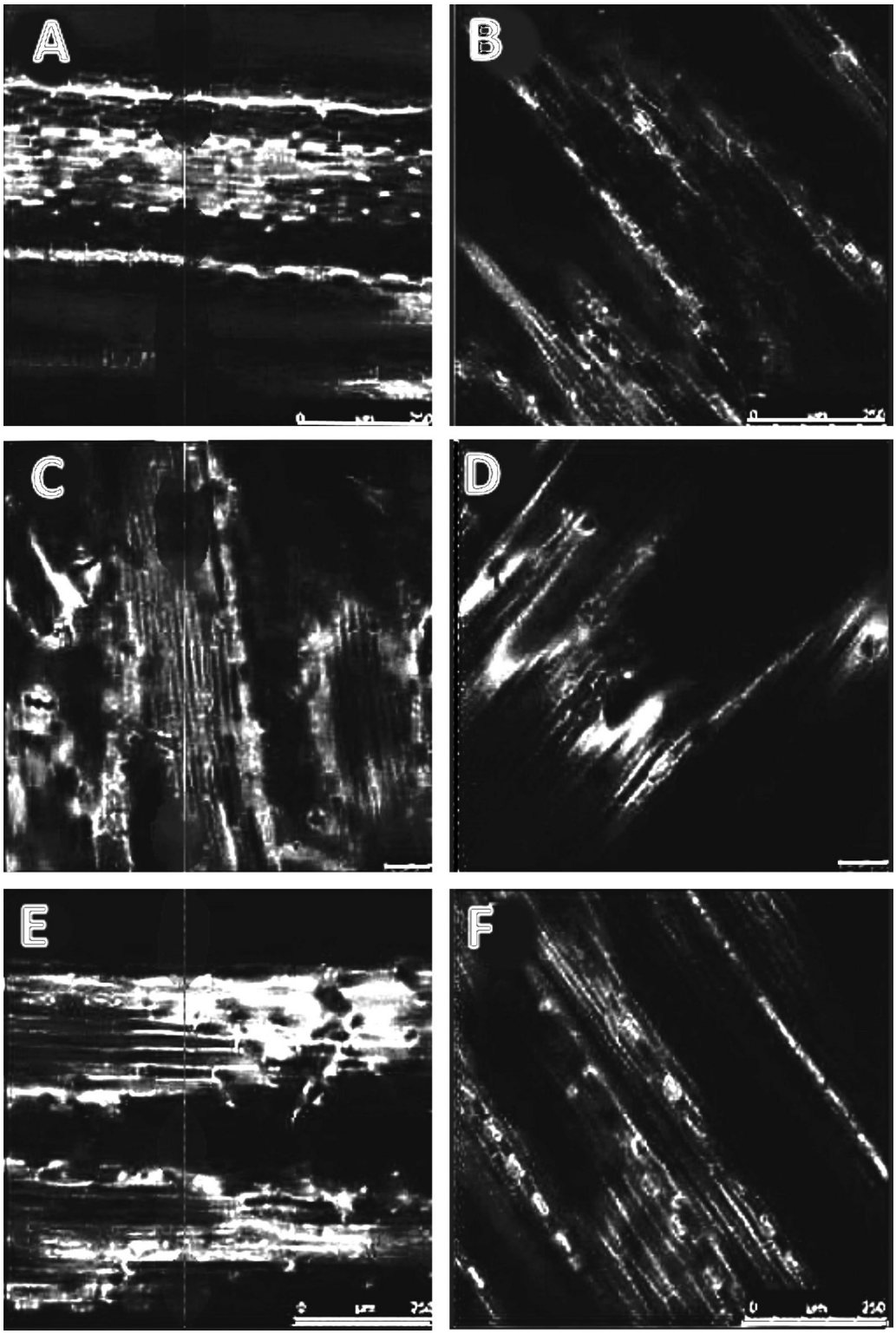

Fig. 2. Fluorescence at 488 nm of freeze-dried wheat chaff solids following pre-treatment and incubation for 72 h at 35 °C. The fungal extract was obtained from *P. chrysosporium*. All Images 817 μm × 817 μm. Control A and B, enzyme treated C and D and E and F with microwave and enzyme). A, C and E no ultrasonic treatment and B, D and F with ultrasonic treatment. Magnification bar 250 μm.

sp. A comparison between pre-treatment with ultrasound alone (Fig. 2B) and a sequence of microwave then ultrasound (Fig. 2F) indicated that addition of the microwave treatment did not assist in the biodegradation of wheat chaff. This perhaps suggests that irradiation may have partially destroyed the natural microflora inherently associated with the wheat chaff, which had contributed to its degradation. This could explain why sequential microwave and ultrasonic pre-treatment (Fig. 2E) was not as effective as ultrasonic pre-treatment alone (Fig. 2A). In addition, microwave heating may have caused condensation reactions to produce structures that resist enzymatic degradation. Other workers (Binod et al., 2012; Chen et al., 2011) found using acid or alkali in combination with

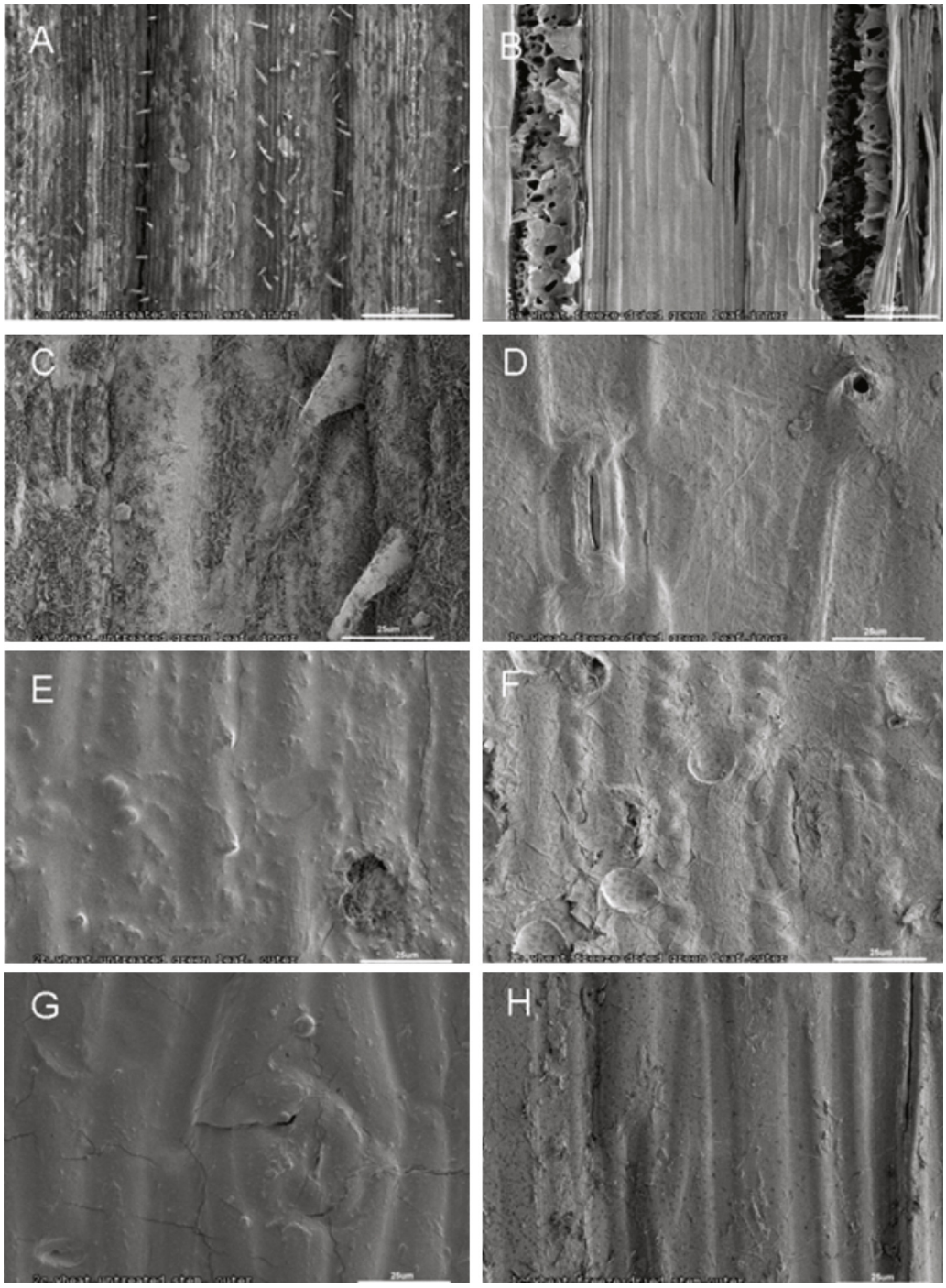

Fig. 3. SEM micrographs of the lower surface (A–D), and upper surface (E and F), of wheat chaff leaves and surface of stem (G and H). Wheat chaff (8% (w/v) solids in 2% sodium acetate buffer, pH 5) was incubated at 35 °C, in the absence (A, C, E, G) and presence (B, D, F, H) of ultrasound (40 kHz, 10 min). Magnification bar A and B 250 μm and C–G 25 μm.

microwaves, was more effective for delignification than microwaving alone. The apparent reason for the difference between our work and that of others (Binod et al., 2012; Chen et al., 2011) is because in our work the indigenous microflora presumably contributed to the enzyme degradation process whereas when an acid or alkali pre-treatment was used, the indigenous microflora were inactivated.

The lack of visible structural detail of the wheat chaff pretreated by ultrasound alone, as obtained by confocal imaging using fluorescence (Fig. 2), and the relatively greater increase in visual browning of the aqueous phase of these samples following subsequent enzymatic hydrolysis suggest that the sequential ultrasonic pre-treatment enhanced the enzyme accessibility to the lignocellulose matrix.

Removal/redistribution of the wax by the initial ultrasound step was evident in the SEM images (Compare Fig. 3A with B, and C with D). Lipophilic materials and surface irregularities attract the attachment of cavitation bubbles, thus, maximizing the effect of bubble-induced micro-jetting towards these points and maximizing removal of the lipophilic material and damage to the surface substructure (Gale & Busnaina, 1995; Leighton, 1994). The dense network of platelet structure of epicuticular wax on the wheat leaf

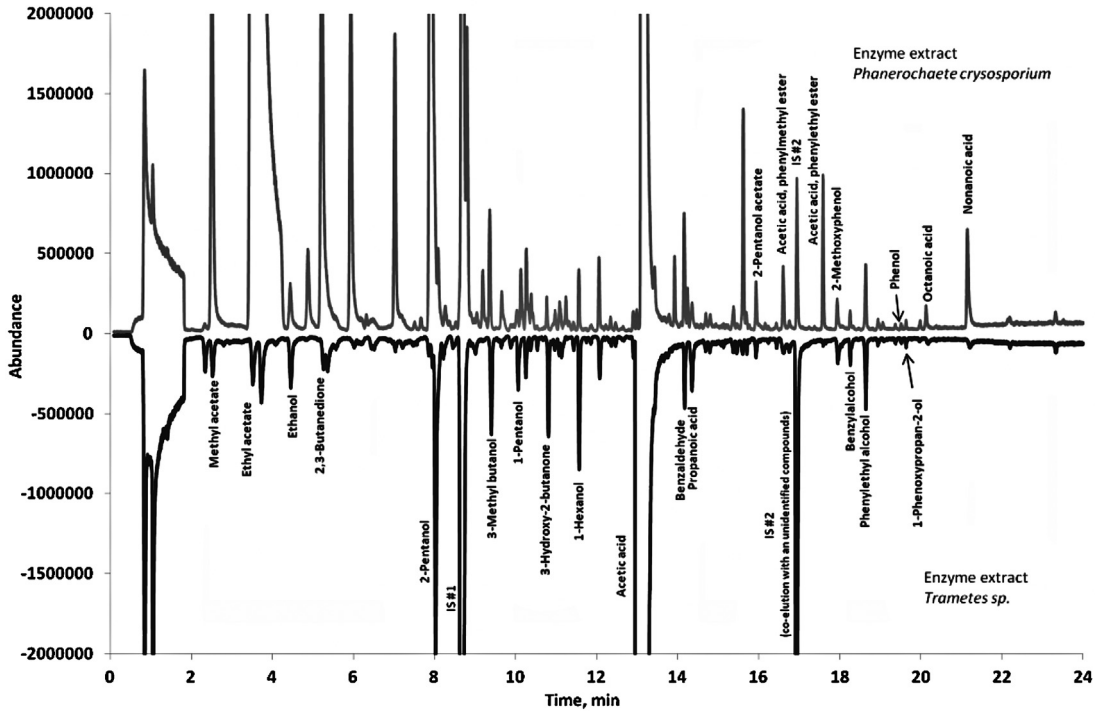

Fig. 4. Typical GC profile of volatiles produced from wheat chaff after sequential ultrasonication (40 kHz, 10 min/270 kHz, 10 min) and incubation (72 h at 35 °C) with enzyme extracts obtained from *P. chrysosporium* and *Trametes* sp. Internal standards IS#1 2-methyl-3-heptanone, IS#2 2-ethyl butyric acid.

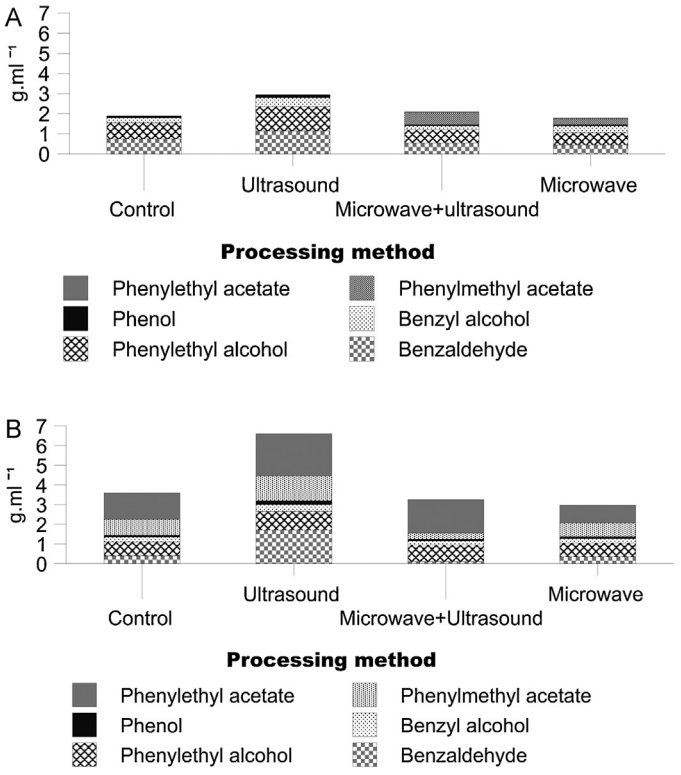

Fig. 5. Quantitative data on dominant headspace phenolic-derived compounds above treatment liquors (benzaldehyde, benzyl alcohol, phenol, phenylethyl alcohol, phenylmethyl acetate, phenylethyl acetate) produced from wheat chaff after sequential ultrasonication (40 kHz, 10 min/270 kHz, 10 min, microwave at 650 W and sequential ultrasonication, microwave alone) and incubation (72 h at 35 °C) with enzyme extracts obtained from *Trametes* sp. (A) and *P. chrysosporium* (B). Values represent concentrations in the treated liquor derived from GCMS headspace gas spectra using equipment software internal reference standards. Internal standards IS#1 2-methyl-3-heptanone, IS#2 2-ethyl butyric acid.

blade (Koch et al., 2006) could potentially have attracted cavitation bubbles in a similar way.

In addition to removal of the wax, ultrasonication caused small implosions (Compare Fig. 3C with D), and pitting (Compare Fig. 3E with F) on the surfaces of the plant material, due to degradation of the cell wall components. Enhanced visualization of the underlying cellulose fibrils after ultrasound by SEM (Compare Fig. 3G with H) provided further evidence that ultrasound had caused erosion of the wheat chaff.

Taken together the CLSM and SEM data suggested that ultrasonic pre-treatment disrupted the lignocellulose matrix and stripped away/degraded the waxy surface components. Ultrasound-induced disruptive effects to plant material have been previously observed (Sun & Tomkinson, 2002; Yachmenev, Condon, Klasson, & Lambert, 2009). These structural alterations are expected to facilitate the rate of enzymatic hydrolysis of plant tissue through increasing substrate accessibility. The observation that wax associated with the wheat chaff were removed by ultrasonic pre-treatment is in line with the observations of others who showed that the removal of hydrophobic non-cellulosics (i.e. waxes, pectins, proteins and fats) could be facilitated with use of ultrasound in combination with enzyme processing (Yachmenev et al., 2004). Alteration of the epicuticular wax ultrastructure of grape berry fruits was recently observed by Fava et al. (2011).

3.2. Headspace volatiles

To ascertain the effect of pre-treatment on biodegradation of wheat chaff by white rot enzyme extracts GC profiles of the headspace volatiles from the hydrolyzed pre-treated samples was obtained. Typical GC profiles of the headspace volatiles produced after ultrasonic pre-treatment of wheat chaff and its subsequent incubation for 72 h, with crude white rot fungal enzyme extracts, are shown in Fig. 4. Numerous compounds including phenolic compounds, alcohols, acids and esters, associated with degradation of the wheat chaff were identified after enzyme

hydrolysis. The phenolic-derived products are likely to arise from both degradation of lignin and also the enzymatic hydrolysis of lignocellulose-derived components during fermentation. The major lignin degradation products, such as variously substituted guaiacols and syringols (Martínez et al., 2005), have relatively high boiling points and are more polar, and for these reasons were not detected in the headspace profile using the GC conditions applied. Moreover, liginases produced by white rot fungi such as *P. chrysosporium* can cause demethylation of guaiacyl and syringyl derivatives (Huynh & Crawford, 1985; Karstens, Tien, Kalyanararnan, & Kirk, 1985) and additionally the higher frequency of 270 kHz we used may have generated free radicals that acted preferentially on the guaiacyl and syringyl compounds. The alcohols (ethanol, 2-pentanol, 3-methyl butanol, 1-pentanol, 1-hexanol), acids (acetic, propanoic, hexanoic, octanoic and nonanoic acids) and ester compounds (methyl acetate, ethyl acetate) identified in the headspace analysis are typical products derived from microbial fermentation of cellulose and hemicelluloses (Ashraf, Linforth, Bealin-Kelly, Smart, & Taylor, 2010). The most likely reason that ethanol and other products typical of a fermentation process were found is that the microflora associated with the wheat chaff (and possibly also from the environment) grew on the more readily biodegradable components that had been released from the wheat chaff (e.g. soluble carbohydrates) and either solubilized in the presence of water or released by the ultrasonic pre-treatment. Some fatty acids (e.g. hexanoic acid, octanoic acid, nonanoic acid) were also present in the headspace. These compounds could arise from degradation of the long chain fatty acids present in the plant cuticle and/or cell wall.

3.2.1. Quantification of volatile phenolic-derived compounds

A range of phenolic-derived compounds, including benzaldehyde, phenyl methyl acetate, phenyl ethyl acetate, benzyl alcohol, phenylmethyl alcohol and phenol, were quantified (Fig. 5). The type and concentration of the volatiles produced depended on the type of pre-treatment that had been applied to the wheat chaff.

Generally, greater levels of phenolic-derived volatiles were detected for wheat chaff pre-treated by ultrasound, compared to pre-treatment by microwave, combined microwave and ultrasound, or control samples (i.e. incubation without any physical treatment, under otherwise under comparable conditions). The inclusion of a microwave pre-treatment, with or without ultrasound, generally resulted in lower levels of phenolic-derived products. The detrimental effect of a microwave treatment may be related to the destructive effect of microwaves on the natural microflora inherently associated with the wheat chaff, which assisted in its biodegradation. The effect of delignification was less pronounced when the ultrasonic pre-treatment was followed by hydrolysis with the enzyme extract obtained from *Trametes* sp. (Fig. 5A) compared to *P. chrysosporium* (Fig. 5B). These differences may be related to the different types and activities of the enzymes produced by the white rot fungi. Multiple laccase genes are recognized in the lignin-degrading basidiomycetes belonging to the genera *Trametes*, and several genes proposed to encode lignin peroxidase have been reported in *T. versicolor* (Jönsson & Nyman, 1992). In contrast, *P. chrysosporium* is widely cited as a white rot fungus that does not produce laccase, but its production of lignin peroxidase and manganese peroxidase, is well established (Hatakka, 1994).

4. Conclusion

Modification of wheat chaff by low intensity sequential low and medium frequency ultrasound enhanced disruption of the lignocellulose matrix and may be recommended for accelerating subsequent enzymatic degradation of lignocellulosic substrates. The fungus from which the hydrolytic enzymes were derived influenced the type and quantity of phenolic-derived volatiles generated. Microwave pre-treatment led to less phenolic-derived volatiles being produced, suggesting that the microbial consortia inherently associated with the wheat chaff also contributed to its biodegradation. Further study is needed to optimize conditions with respect to treatment time and temperature, ultrasonic frequencies and power intensities for degrading the wheat chaff and recovering the myriad of potentially useful products that can be isolated from lignocellulose products.

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