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The impact of conditioning of barley straw on the inhibition of *Scenedesmus* using chemostats

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16 **Abstract:** The current paper investigates the role of barley straw conditioning on
17 inhibiting the alga *Scenedesmus*. Fresh, pre-rotted and white rot fungi (WRF) augmented
18 straw was tested in a series of chemostat experiments over 15 weeks. All three systems
19 were effective at inhibiting the alga with differences observed in the lag time before
20 inhibition occurred and the rate of alga decline. Lag times of 8, 4 and 1 week(s) were
21 recorded for the fresh, rotted and fungi-treated straws respectively, with a maximum
22 inhibition rate of >7 x10^4 cells.week^{-1} observed for the fungi pre-treated system. Overall,
23 the results indicate that pre-treatment is a viable method to enable barley straw to be used
24 in a more reactive manner. Explanation is postulated that during pre-treatment no
25 alternative sources of nitrogen are available thereby leading to greater bacterial
26 decomposition of straw lignin to release inhibitory substances. The principle of utilising
27 an engineered pre-treatment by inoculating barley straw with WRF to enhance the impact
28 of the straw on algal inhibition has been clearly demonstrated. Further work is required to
29 understand how the straw pre-treatment stage can be reduced to minimise its duration
30 whilst maximising the inhibitory effect of adding barley straw.
31 **Key Words**: Algal control, Barely straw, Chemostat, *Scenedesmus*
32

33 **INTRODUCTION**

34 Algae are photosynthetic, aquatic plants that are ubiquitous in surface waters.
35 While at low concentrations algae do not pose a significant problem, during periods of
36 seasonal algal blooms surface waters can become very polluted disrupting recreational
37 use and significantly impairing drinking water production. Reported bloom
38 concentrations in the UK can be as high as $2x10^6$ cells.ml^{-1}. Usually these blooms occur
39 between February and November and typically may last for a few weeks up to several
40 months for taxa including: *Microcystis, Aphanizomenon, Asterionella, Melosira,*
41 *Anabaena, Cyclotella, Pediastrum, Coelastrum* and *Scenedesmus* (Henderson *et al.*,
42 2008a). The impact of such blooms regarding water production is seen both in terms of
43 increased operating costs through reduced filter run times or increased coagulant demand
44 as well as reduction in product water quality. For example, alga can lead to deterioration
45 of water colour, taste and odour as well as an increase in the formation of disinfection by-
46 products (Henderson *et al.*, 2008a). While surface water treatment plants can largely cope
47 with blooms through appropriate control of the coagulation process (Henderson *et al.*,
48 2008b) source control remains a key strategy to preserve costs and resources. Included in
49 such source control techniques are bubble curtains, chemical dosing and exposure to
50 ultrasound or barley straw (Purcell *et al.*, 2008). Barley straw has been used in the UK
51 since the 1970s at a range of scales ranging from application in household ponds to larger
52 systems such as canals, streams and potable water reservoirs. Reports on the use of barley
53 straw indicate it is highly effective, consistent and produces no associated damage to
54 desired species such as invertebrates and fish (Everall and Lees, 1997; Barrett *et al.*,
55 1999). Barley straw is normally applied as loosely bundled bails at key locations in the

56 reservoir at dose rates from 5 g.m^{-3} (Barrett, 1994) up to 40 kg.m^{-3} (Gibson, 1990)
57 depending on whether localised or overall average concentrations are reported. In
58 laboratory trials where extract from rotted barley straw was applied, effective inhibition
59 occurred at equivalent straw concentrations approximating 1-2 kg.m^{-3} (Waybright *et al.*,
60 2008). Fresh straw has been shown to be ineffective so addition of the barley straw bails
61 is recommended several months before the blooms are expected so that the barley straw
62 has degraded and can be effective before or during exponential growth phases of the
63 algae.

64 To date most work carried out to understand the application of barely straw in
65 inhibiting algal growth has focussed on demonstrating the efficacy of the technology.
66 This has led to the hypothesis that chemicals produced during decay of the straw are
67 responsible for inhibition (Everall and Lees, 1996). Further work has identified these
68 chemicals to be phenolic in nature (Everall and Lees, 1996 & 1997; Waybright *et al.,*
69 2008). These phenols are derived from lignin in the barley straw that are produced as the
70 plant material decays rather than directly from the microbial species responsible for the
71 decay (Pillinger *et al.,*1993 & 1994; Barrett, 1994). Lignin has a rigid insoluble
72 polymeric molecular structure that is difficult to degrade under most natural conditions.
73 However microorganisms such as white rot fungi (WRF) communities are able to
74 colonise barley straw and degrade lignin. In doing so phenolic compounds may be
75 liberated (Garrotel *et al.,* 1999; Demirbas, 2005; Rogalinski *et al.,* 2008).

76 To date, little work has focussed on the role of lignin decay and whether it can be
77 enhanced to provide a more controlled response within water treatment applications. The
78 current study complements the previous work and attempts to understand the role of pre-

79 treatment with WRF by conducting trials in continuously operated chemostats over
80 timescales appropriate to the applications in question. As part of the trial, barley straw
81 pre-treatment with three common WRF species (*Phanerochaete chrysosporium,*
82 *Pleurotus ostreatus* and *Trametes versicolor)* has been conducted to establish the
83 potential for engineered enhancement when using barley straw.
84

85 **MATERIALS AND METHODS**

86 ***Algae preparation***

87 Cultures of unialgal *Scenedesmus* were chosen due to their robustness and ease of
88 growth in laboratory conditions. Pure batch cultures of *Scenedesmus* were produced by
89 adding 5 ml of unialaga S*cenedesmus* culture to 100 ml Jaworski's media (J. media) in a
90 sterile 250 ml Erlenmeyer flask under aseptic conditions inside a laminar flow cabinet.
91 The flask was then sealed with a cotton wool bung. Cultures were grown on an orbital
92 shaker (Barloworld Scientific, Staffordshire, UK) at 110 rpm at room temperature (18-
93 20°C) under the constant light of two 15W tube lights (Hagen Sun-Glo, Maidenhaed
94 Aquatics, Woburn Sands, UK). Cultures were maintained by weekly sub-culturing by
95 transferring 5 ml culture to 100 ml fresh J. media following the same method as above.
96 Cultures were inspected microscopically every 2 weeks to ensure purity and used as
97 required.
98

99 ***Fungi preparation***

100 The three species of WRF; *P. chrysosporium DSMZ 1556*, *P. ostreatus DSMZ*
101 *1833* and *T. versicolor DSMZ 11269* were obtained from Deutsche Sammlung von

102 Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany). These species
103 were selected as they have been shown to be particularly good producers of the lignin and
104 complex phenol degrading laccase enzyme (Hou *et al.,* 2003). All three species were
105 maintained in pure cultures on malt extract peptone agar.

106 Fungi were grown by transferring a 1 cm^2 section of the culture onto the agar with
107 an ethanol washed flame sterilised scalpel under aseptic conditions in a laminar flow
108 cabinet. The plate was then sealed with parafilm and kept in a sealed environment
109 chamber (Sanyo MLR-450H, Sanyo, Japan) at 20°C, 100% humidity and in the dark.
110 Fungi were re-cultured until required, which was typically every month or when the plate
111 was completely covered with growth.

112

113 ***Chemostat preparation***

114 Prior to use, the 100 l chemostats were thoroughly cleaned and then filled with
115 dechlorinated tap water and sealed with cling film to prevent invasion by external
116 organisms and chemostats were allowed to stand for 2 days before use. The *Scenedesmus*
117 cultures were used to seed the chemostats The chemostats were kept in a temperature
118 control room at 20 ± 2°C. Light was controlled by using two 20W aquarium lights
119 equipped with a light timer set to a 16h light:8h dark cycle. These conditions were chosen
120 to coincide with typical bloom conditions seen in the field: long summer time daylight
121 hours and warm temperatures (Everall and Lees, 1996). Maintenance of a stable
122 population of the cultures was carried out by diluting the cultures in the chemostat by
123 10% d^{-1} with dechlorinated tap water using a peristaltic pump (Watson-Marlow Bredel
124 Pumps, UK). No media addition was used as preliminary work had shown that the algae

125 could grow consistently in just tap water. The tanks were kept mixed with aerating
126 aquarium pumps (Maidenhead Aquatics, Woburn Sands, UK). The algae cultures were
127 allowed one month to acclimatise and then blank data was collected for three weeks
128 before straw was added. In total, two tanks were prepared for each of the straw conditions
129 tested, which were: i) fresh barley straw; ii) pre-rotted rotted barley straw and iii) fungal
130 pre-treated barley straw. Four further tanks were kept as controls. A chemostat was also
131 prepared to analyse fungal metabolites (acid digestible fibre (ADF) and ergosterol) and
132 their impact on algae growth. This was prepared and maintained identically to the fresh
133 straw chemostats.
134
135 ***Straw preparation***
136 Three sources of barley straw were used:
137 (i) Fresh straw (obtained from a local pet shop).
138 (ii) Four month old rotted straw (obtained from a surface water reservoir in
139 the Yorkshire region).
140 (iii) Fungal-treated straw.
141 Fungal-treated straw was prepared by placing 25 g of fresh straw into a 1.5 l acid washed
142 beaker sealed with tin foil. The straw was then autoclaved at 121 °C and 50 atm for 15
143 mins (PriorClave, London, UK). Once autoclaved the straw was stored in darkness
144 overnight in an ethanol-cleaned sealed environment chamber (Sanyo MLR-450H, Sanyo,
145 Japan) at 100% humidity. The cooled straw was opened under aseptic conditions inside
146 the laminar flow cabinet. Three 1 cm^2 squares of *P. chrysosporium, P. ostreatus* or *T.*
147 *versicolor* were placed at the bottom, middle and top of the straw. Once the fungi were
148 added the straw was resealed and placed in the environment chamber where it was kept in

149 the dark at 20°C and 100% humidity for 2 months until the fungi had thoroughly spread
150 throughout the straw. For each of the three straw systems tested, two 25 g bundles of
151 straw were prepared inside the laminar flow cabinet. Each tank received one 25 g bundle
152 of straw suspended from the top of the tank by plastic string to keep the straw at the water
153 surface.
154
155 ***Algae sample analysis***
156 Water samples were collected weekly from the main body of each tank for the
157 duration of the test using a sterile 50 ml syringe. All water samples were analysed using
158 standard methods for dissolved oxygen, temperature and pH. Alkalinity was measured by
159 standard titration with 0.1 M H_2SO_4 using bromcresol green indicator. Dissolved organic
160 carbon (DOC) was measured using a Shimadzu TOC-5000A TOC Analyzer (Shimadzu,
161 Milton Keynes, UK). Cell counts were obtained using a Spectramax plus 384 96 plate
162 reader (Molecular Devices, Sunnyvale, USA), reading optical density at 687 nm (OD_{687}).
163 The OD_{687} was compared against a standard curve of cell number against OD_{687} to
164 determine the cell count. Cell count numbers were compared against haemocytometer
165 counts to maintain counts within 10% of one another.
166
167 ***Acid digestible fibre (ADF)***
168 Samples were collected for ADF analysis from straw after 0, 1, 3, 5 and 9 months.
169 The lignin, cellulose and fibre content of the straw samples were analysed according to
170 the acid–detergent method described by Rowland and Roberts (1994). Briefly, this
171 involved simmering 0.7 g (W1) of a straw sample in a 100 ml solution of certyltrimethyl

172 ammonium bromide (ACROS Chemicals, Geel, Belgium) in a 250 ml Erlenmeyer flask.
173 The top of the flask was plugged with an inverted 50 ml volumetric flask to prevent
174 evaporation. Samples were simmered for 1 hour, followed by immediate transfer of the
175 straw to a no. 2 porosity sinter (W2). The straw was then rinsed with 50 ml aliquots of
176 boiling water followed by acetone until no more colour was removed. The sample was
177 filtered through the sinter under vacuum. Once rinsed the samples were dried at 105°C
178 for 2 h, then weighed once cooled (W3). Once weighed the sinter was half-filled with
179 cooled (15°C) 72% H_2SO_4 and stirred with a glass rod. The acid was allowed to drain
180 away under gravity and continuously topped up with fresh acid for 3 h. After 3 h the acid
181 was filtered off under vacuum and the sample rinsed with boiling water followed by
182 acetone until colour was no longer removed. The sinter was then dried at 105°C for 2 h
183 and weighed (W4) followed by 2 h at 550°C and then re-weighed (W5).
184 The weights were then used to calculate the following:
185 % Acid digestible fibre = (W3 – W2) x 100 / W1
186 % Lignin = (W4 – W5) x 100 / W1
187 % Cellulose = (W3 – W4) x 100 / W1
188 ***Ergosterol***
189 The fungal population was quantified using ergosterol analysis according to the rapid
190 ultrasonication method described by Ruzicka *et al.* (1995). Samples were taken at 3, 6
191 and 9 months from both the bulk water in the chemostat and from water extracted from
192 within the straw bundle. Ergosterol was quantified by taking the filtrates from these
193 samples through a 0.7 μm filter paper and adding it to a 50 ml polyethylene tube. A
194 duplicate was used for each sample, pre-spiked using a variable volume pipette with 100

195 µg of ergosterol (Sigma-Aldrich Co, Dorset, UK) in a 1 ml n-hexene-propan-2-ol (98:2)
196 solution (Acros Chemicals, Geel, Belgium). After 15 min, a 10 ml mixture of methanol
197 and ethanol at a ratio of 4:1 was added to all samples; samples were then cooled to 4°C
198 for two hours. After 2 h, 20 ml n-hexane-propan-2-ol was added to the samples, or 19 ml
199 in the case of the spiked samples. Samples were ultrasonicated at 150W for 200s using an
200 ultrasound probe (Virsonic 600, VirTis, NY, USA), while kept on ice. The samples were
201 then allowed to settle for 30s and the top 2 ml was transferred to a microfuge tube and
202 centrifuged for 10 min at 10,000 rpm. The top 1.5 ml was then used to calculate the
203 ergosterol concentration using high performance liquid chromatography (HPLC).
204 Ergosterol quantification was determined using a Shimadzu SCL-10A HPLC
205 (Shimadzu, Milton Keynes, UK) using a Licrosorb Si 60 (10 µm) column (Phenomex,
206 Macclesfield, UK) following a 4 x 3.0 mm guard cartridge (Phenomex, Macclesfield,
207 UK). The columns were flushed with n-hexane-propan-2-ol at 1.5 ml min^{-1}. For sample
208 analysis 100 µl was injected at 1.5 ml min^{-1} and absorbance measured at 282 nm.
209 Unless stated otherwise, all chemicals and glassware were from Fisher Scientific
210 (Loughborough, UK).
211
212 **RESULTS**
213 Reduction in the concentrations of *Scenedesmus* due to the application of barley
214 straw was observed to follow a three stage profile characterised by: (1) a lag phase where
215 no algal inhibition took place, (2) a declining phase where the concentration of algae
216 decreased over time and (3) a steady state phase where a new stable concentration of
217 algae existed (Fig. 1). The initial lag phase lasted for 4 and 8 weeks for the rotted and

218 fresh straw, respectively, indicating the importance of pre-treatment. The control
219 concentration increased during the equivalent time period showing that the inhibitory
220 effect was not due to the environmental conditions over the three week period.

221 In the case of fresh straw, *Scenedesmus* decreased from an average of 1.3-1.5 $x10^5$
222 $cells.ml^{-1}$ during the lag phase to 8.58 (±3.43) $x10^4$ $cells.ml^{-1}$ in week 9 and further to
223 4.89 (±23.67) $x10^4$ $cells.ml^{-1}$ by week 10 (Fig. 1). In comparison, in the case of rotted
224 straw, the algal population decreased from a lag phase concentration of 1.4 $x10^5$ $cells.ml^{-1}$
225 to 8.58 (±1.94) $x10^4$ $cells.ml^{-1}$ in week 6 and 2.98 (±0.01) $x10^4$ $cells.ml^{-1}$ by week 7.
226 Consequently, the new steady state population sizes were established 3 and 4 weeks after
227 the lag phase, indicating that pre-treatment influenced the rate of cell decline. The decline
228 rates of *Scenedesmus* during this phase were approximately 3 $x10^4$ and 4 $x10^4$ $cells.week^{-}$
229 ^{1} for the fresh and rotted straw, respectively (Fig. 1). First order kinetics yields a rate
230 constant of 0.45 $week^{-1}$ for the fresh straw and 0.52 $week^{-1}$ for the rotted straw. Reported
231 growth rates of *Scenedesmus* in controlled conditions for maximum growth are around 1
232 $x10^6$ $cells.week^{-1}$ with a rate constant approximating 0.5-1.5 d^{-1} indicating that
233 suppression of new growth was the controlling factor in the successful use of barley straw
234 (Mohammed and Markert, 2006).

235 The average alga concentration during the inhibited steady state phase were
236 similar for these two treatments, varying between 3.2-4.5 $x10^4$ $cells.ml^{-1}$ for the rotted
237 straw and 3.3-4.7 $x10^4$ $cells.ml^{-1}$ for fresh straw. Overall this demonstrates that the
238 application of barley straw at a dose of 0.25 $kg.m^{-3}$ produced significant but not complete
239 inhibition of the alga independent of pre-treatment.

240 Further investigation into the role of straw pre-treatment confirmed its importance
241 during the trial with WRF pre-treated straw. The lag phase was observed to last only one
242 week, which was three weeks shorter than that observed with pre-rotted straw. The
243 impact of the straw was very rapid with the population decreasing from 1.24 (±0.16) $x10^4$
244 $cells.ml^{-1}$ before the addition of straw to 6.32 (±0.53) $x10^4$ $cells.ml^{-1}$ within the first
245 week, representing a decline rate of 7.0 $x10^4$ $cells.week^{-1}$ and a first order rate constant of
246 0.67 $week^{-1}$ (Fig. 2). A second reduction to 2.69 (±0.20) $x10^4$ $cells.ml^{-1}$ was observed
247 after 11 weeks which then returned to the previous steady state value. The steady state
248 value varied between 6.32 $x10^4$ and 78 $x10^4$ $cells.ml^{-1}$, higher than those experienced
249 previously with the fresh and rotted straw although the applied dose rate was the same.
250 However, all three systems produced significant levels of reduction with an overall
251 average inhibition level of 75, 75 and 60% for the fresh, rotten and fungi pre-treated
252 straw, respectively. Similar durations for inhibition were seen until the end of the
253 experiments during the current investigation, a maximum of 13 weeks in the case of the
254 fungi pre-treated straw.

255 No significant differences were observed between the conditions in the control
256 and straw chemostats during all trials. In the first trial pH, temperature, alkalinity and
257 DOC remained constant at 7.5 – 8, 20°C ± 2, 45 $mg.l^{-1}$ and 4-6 $mg.l^{-1}$, respectively.
258 During the second trial, equivalent values of 7.5 – 8, 20°C ± 2, 50 $mg.l^{-1}$ and 6 $mg.l^{-1}$
259 were noted. The only major difference was observed in DO which was 6 and 9 $mg.l^{-1}$ for
260 the first and second trial respectively, although both were above any potential oxygen
261 limitation effects.

262

263

264 **Analysis of straw characteristics during degradation**

265 Analysis of the degradation of straw through ADF and ergosterol analysis
266 revealed that the cellulose component was preferentially consumed by the
267 microorganisms that colonised the straw (Fig. 3). To illustrate, initial mass fractions of
268 cellulose, lignin and fibre of the straw were 0.4, 0.1 and 0.5 respectively. As the trial
269 progressed cellulose content decreased to a mass fraction of 0.25 by month 3 and
270 ultimately become non-detectable after month 5 (Fig. 3). The mass fraction of fibre
271 remained effectively constant across the trial such that the lignin mass fraction increased
272 commensurately with the decrease in cellulose, reaching a maximum value of around 0.5
273 by month 9. The initial preferential reduction in cellulose has previously been reported
274 with a decrease in mass fraction from 0.4 to 0.15 over 28 days (Ball *et al.*, 2001) which
275 compares to the decrease from 0.4 to 0.24 in the current case. Similar results have also
276 been reported during investigation of the degradation of cellulose-lignin systems in
277 agricultural contexts (Wessén and Berg, 1986).

278 Ergosterol analysis was used as a surrogate for fungal biomass (Fig 4). This
279 showed that biomass in the straw phase increased from less than 0.01 $mg.ml^{-1}$ before the
280 start of the experiment to 0.26 $mg.ml^{-1}$ after 9 months compared to a much lower increase
281 observed in the water phase (maximum concentration of 0.034 $mg.ml^{-1}$). The
282 concentration profile in the straw was indicative of exponential growth indicating that the
283 fungal community was not substrate-limited over the course of the trial. Conversion of
284 the data into a first order rate expression yields a rate constant of 0.38 $month^{-1}$ which

285 compares to reported growth rates for fungi in other situations of 0.2-2 d^{-1} indicating that
286 fungal growth was relatively slow in the studied environment.

287 **Discussion**

288 In the current study all three straw systems were effective at inhibiting
289 *Scenedesmus* resulting in similar levels of inhibition. The inhibition levels reported
290 confirm previous work which found 75% inhibition in a field trial (Barrett *et al.,* 1999)
291 while being higher than the >50% reported for a field trial of mixed algal species (Everall
292 and Less, 1996). The results presented here support postulated theories associated with
293 the mechanisms of algal inhibition from barley straw to the release of phenolic
294 compounds through the degradation of lignin (Pillinger *et al.*, 1993; Barrett, 1994;
295 Everall and Lees, 1996 & 1997; Waybridge *et al.*, 2008; Murray *et al.*, 2009).

296 Exact mechanisms for the release of inhibitory phenolic compounds by WRF are
297 unclear due to the complexity of the system but degradation of lignin by WRF is thought
298 to occur in order for the fungi to access nitrogen rather than for obtaining metabolic
299 energy (Schlegel, 1997). The observed decrease in the cellulose fraction of the straw was
300 believed to be an important precursory stage to lignin degradation as a result of the
301 release of H_2O_2 from cellulose breakdown (Schlegel, 1997). This is because in order for
302 lignin to be solubilised, and therefore available for metabolism, non-specific enzymes
303 such as lignin peroxidise and laccase are required (Thiruchelvam and Ramsay, 2007).
304 These enzymes require H_2O_2 to work effectively (Schlegel, 1997).

305 Although the inhibition achieved for each of the systems was similar, the onset of
306 inhibition and the kinetics of inhibition were very different for each system investigated.
307 The observation is in agreement with previous findings which have indicated that up to

308 12 weeks are required after the addition of fresh barley straw before it is seen to be
309 effective suggesting that key transformations must occur within the straw before it
310 becomes effective at inhibiting algal growth (Gibson *et al.,* 1990; Pillinger *et al.,* 1994;
311 Terlizzi *et al.,* 2002). Explanations for the differences observed in the lag phase for the 3
312 systems are consistent with typical biological systems. A population must firstly colonise
313 the barley straw before the population grows and significant degradation of the straw
314 takes place. The differences in the lag phases represent the differences in the colonisation
315 stages of the straw added to the water before lignin degradation takes place.

316 The differences in the rates of inhibition for different straw systems was less clear
317 although it was likely to reflect different conditions linked to nutrient availability for the
318 colonising microorganisms. Lignin degradation occurs when nitrogen is limiting (Kirk
319 and Farrell, 1987). When fresh straw is added into a reservoir, other soluble nitrogen
320 sources are usually readily available, particularly in reservoirs where algal blooms are
321 likely. Therefore in-reservoir colonisation and breakdown of straw by WRF is likely to be
322 slower due to these alternative nitrogen sources. This helps explain the difference in the
323 lower inhibition rates for reservoir rotted straw than for fungi-augmented straw in these
324 trials. A similar observation to this has been made for studies on fungi growth on copper-
325 treated wood where control of nutrients has been shown to greatly enhance fungal
326 activity and kinetics (Humar and Pohleven, 2005). Consequently, controlled pre-
327 treatment by fungal augmentation in a reactor provides ideal conditions for maximising
328 fungal activity for lignin degradation and hence release of phenolic compounds required
329 to inhibit algal growth. The ergosterol analysis showed that the fungi population grew by
330 440% after three months. Similar findings have been reported using ergosterol analysis

331 on the fungi population grown on wheat straw where exponential growth was observed in
332 terms of an initial lag phase of 21 days and an increase in fungal mass of 300% after 84
333 days (Robertson *et al.,* 2008). In both cases significant fungal growth coincides with a
334 significant decrease in the cellulose mass fraction and the onset of lignin degradation.
335 The maximum concentration of 260 $\mu g_{ergosterol}.g^{-1}$ detected in the current study was
336 considerably lower than the 1398 $\mu g_{ergosterol}.g^{-1}$ reported after 35 days under enriched
337 conditions for *P. chrysosporium* but higher than those reported when using just wood
338 block at 23 $\mu g_{ergosterol}.g^{-1}$ (Niemenmaa et al., 2008). Fungal growth was therefore
339 restricted on barley straw (but not by as much as on wood) which further explains the lag
340 phase and the prolonged operation observed in this work.

341 Comparison with previous investigations confirm the applied dose of 0.25 $kg.m^{-3}$
342 used in the current study to be appropriate when applied as either extract (Ball *et al.*,
343 2001) or whole straw (Murray *et al.*, 2009). In contrast, doses of an extract equivalent to
344 7.2 $kg.m^{-3}$ in laboratory trials have been reported to be ineffective at controlling the
345 growth of *Scenedesmus* but effective against *Synura* and *Microcystis* which were
346 significantly inhibited within a few weeks (Ferrier *et al.*, 2005). Comparing the findings
347 between the current study and those previously reported suggest that the kinetics of algal
348 inhibition by barley straw to be species specific, and perhaps strain specific for any given
349 algal species. Such a finding was not unexpected as the characteristics of different algae
350 are known to vary widely in terms of their physiology (Andersen, 2005) and the organic
351 matter that surrounds the alga surface (Henderson *et al.*, 2008c). As the inhibition
352 mechanism for barley straw requires the adsorption of the toxin onto the algal surface,
353 both the inherent toxicity of the cell and the transfer of the toxin through the algal organic

354 matter (AOM) layer will be important. Consequently, appropriateness of barley straw in
355 inhibiting different algal species is not expected to follow traditional biological
356 classification based on differences in pigmentation and cell complexity arising as a result
357 of evolution (Bellinger, 1992). Instead, species from all phyla are expected to be
358 susceptible. A parallel can be made with other forms of treatment where effectiveness
359 mirrors specific chemical and physical properties rather than biological classification. For
360 instance, the use of low energy ultrasound has been shown to be effective only for
361 filamentous algae irrespective of phyla (Purcell *et al.*, 2009). Similarly, optimum
362 conditions for coagulation and clarification of algae have been shown to relate to the
363 charge density of the algae and AOM as well as shape factors that cross all phyla
364 (Henderson *et al*., 2008c). As a result, barley straw appears to fit this pattern such that
365 generalisations cannot be made regarding susceptibility based on biological classification.
366 The key practical implication of these results relates to how responsive the system
367 to be treated needs to be. Current approaches to application of barley straw require long
368 term planning to ensure that the straw is inhibitory during the exponential growth phase
369 of the algal bloom. Having a stock of straw pre-treated by WRF provides a solution that
370 can be more responsive and as such can be applied in situations where blooms are
371 unexpected in terms of location or time. The need to establish and maintain WRF
372 populations remains the critical step and, while pre-treatment reduces the time required
373 for inhibition, the net benefits of developing pre-treatment reactors remains uncertain.
374 Further work is required to optimise the pre-treatment stage to minimise its duration as
375 well as maximise the inhibitory impacts of adding the straw.
376

377 **Conclusions**

378 The principle of utilising an engineered pre-treatment to enhance the impact of
379 barley straw has been demonstrated. The enhancement is seen in terms of both a
380 reduction in the lag time for inhibition of bloom-forming alga and the rate of inhibition.
381 The consequence of this is that pre-treatment enables barley straw to be utilised in a more
382 responsive way to manage unpredicted algal bloom outbreaks.

383

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387

388 **References**

389 Andersen, R. A. (2005) Microbiology: Algae and the vitamin mosaic, *Nature*, **439**, 33-35.

390 Ball, A. S., Williams, M., Vincent, D. and Robinson, J. (2001) Algal growth control by a
391 barley straw extract. *Bioresource Technology*, **77**, 177-181.

392 Barrett, P. R. F. (1994). Field and laboratory experements on the effects of barley straw
393 on algae. *BCPC monograph no59. Comparing glasshouse and pesticide*
394 *preformance II.* London.

395 Bellinger, E. G. (1992) A Key to Common Algae: Freshwater, Estuarine and Some
396 Coastal Species. The Institution of Water and Environmental Management,
397 London.

398 Demirbas, A. (2005) Effect of lignin content on aqueous liquefaction products of
399 biomass. *Energy Conservation and Management,* **41**, 1061-1067.

400 Everall, N. C. and Lees. D. R. (1996) The use of barley straw to control general and blue
401 green algal growth in a Derbyshire reservoir. *Water Research,* **30**, 269-276.
402 Everall, N. C. and Lees. D. R. (1997) The identification and significance of chemicals
403 released from decomposing barley straw during reservoir algal control. *Water*
404 *Research*, **31**, 614-620.
405 Ferrier, M. D., Butler, B. R., Terlizzi, D. E. and Lacouture, R. V. (2005) The effects of
406 barley straw (*Hordeum vulgare*) on the growth of freshwater algae. *Bioresource*
407 *Technology,* **96**, 1788-1795.
408 Garrote, G., Domínguez, H. and Parajó, J. C. (1999) Hydrothermal processing of
409 lignocellulosic materials. *Holz als Roh- und Werkstoff*, **57,** 191–202.
410 Gibson, M. T., Welch, I. M., Barrett, P. R. F. and Ridge, I. (1990) Barley straw as an
411 inhibitor of algal growth II: laboratory studies. *Journal of Applied Phycology*, **2,**
412 241–48.
413 Henderson, R. K., Chips, M., Cornwell, N., Hitchins, P., Holden, B., Hurley, S., Parsons,
414 S. A., Wetherill, A. and Jefferson, B. (2008a) Experiences of algae in UK waters:
415 a treatment perspective. *Water and Environment Journal*, **22**, 184-188.
416 Henderson, R. K., Parsons, S. A. and Jefferson, B. (2008b) The impact of variable algal
417 functionality on treatment. *Water Research,* **42**, 1827-1845.
418 Henderson, R. K., Baker, A., Parsons, S. A. and Jefferson, B. (2008c) Characterisation of
419 algogenic organic matter extracted from cyanobacteria, green algae and diatoms.
420 *Water Research*, **4**2, 3435-3445.

421 Hou, H., Zhoua, J., Wang, J., Du, C., Yana, B. (2003) Enhancement of laccase production
422 by *Pleurotus ostreatus* and its use for the decolorization of anthraquinone dye.
423 *Process Biochemistry*, **39**, 1415-1419.

424 Humar, M. and Pohleven, F. (2005) Influence of nitrogen supplement on tehgrowth of
425 wood decay fungi and decay of wood. *International Biodeterioration and*
426 *Biodegradation*, **56**, 34-39.

427 Kirk, T. K. and Farell, R. L. (1987) Enzymatic "combustion": the microbial degradation
428 of lignin. *Annual Review of Microbiology*, **41**, 465-505.

429 Mohammed, M. and Markert, B. (2006) Toxicity of heavy metals on *Scenedesmus*
430 *quadricauda* (Turp.) de brebisson in batch cultures. *Environmental Science and*
431 *Pollution Research*, **13**, 98-104.

432 Murray, D., Jefferson, B. and Parsons, S. A. (submitted) Inhibition of three algae species
433 using chemicals released from barley straw. *Environmental Technology*.

434 Niemenmaa, O., Galkin, S., Hatakka, A. (2008) Ergosterol contents of some wood-rotting
435 basidiomycete fungi grown in liquid and solid culture conditions. *International*
436 *Biodeterioration and Biodegradation*, **62** (2), pp. 125-134

437 Pillinger, J. M., Cooper, J. A., Ridges, I. and Barrett, P. R. F. (1992) Barley straw as an
438 inhibiter of algal growth III: the role of fungal decomposition. *Journal of Applied*
439 *Phycology*, **4**, 353–355.

440 Pillinger, J. M., Gilmour, I. and Ridge, I. (1995) Comparison of anti algal activity of
441 brown rotted and white rotted wood and in situ analysis of lignin. *Journal of*
442 *Chemical Ecology,* **21**, 1113-1125.

443 Purcell, D., Parsons, S. A. and Jefferson, B. (2008) Singing algae to sleep: Understanding
444 the use of Ultrasound for Algal Control. IN: *Proceedings of the WQTC*, 16-20th
445 November 2008, Cincinnati, USA.
446 Ruzicka, S., Norman, M. D. P. and Harris, J. A. (1995) Rapid ultrasonication method to
447 determine ergosterol concentration in soil. *Soil Biology and Biochemistry*, **27**,
448 1215-1217.
449 Robertson, S. A., Mason, S. L., Hack, E., Abbott, G. D., (2008) A comparison of lignin
450 oxidation, enzymatic activity and fungal growth during white-rot decay of wheat
451 straw. *Organic Geochemistry,* **39**, 945–951
452 Rogalinski, T., Ingram, T. and Brunner, G. (2008) Hydrolysis of lignocellulosic biomass
453 in water under elevated temperatures and pressures. *Journal of Supercritical*
454 *Fluids*, **47,** 54 – 63.
455 Schlegel, H. G. (1997) General microbiology, Cambridge University Press, UK.
456 Thiruchelvan, A. T. and Ramsay, J. A. (2007) Growth and laccase production kinetics of
457 Trametes vericolor in a stirred tank reactor. *Applied Microbiology and*
458 *Biotechnology*, **74**, 547-554.
459 Terlizzi, D. E., Ferrier, M. D., Armbrester, E. A. and Anlauf, K. A. (2002) Inhibition of
460 dinoflagellate growth by extracts of barley straw (*Hordeum vulgare*), *Journal of*
461 *Applied Phycology,* **14**, 275–280.
462 Waybright, T. J., Terlizzi, D. E. and Ferrier, M. D. (2008) Chemical characterization of
463 the aequious algistatic fraction of barley straw (*Hordeum vulgare*) inhibiting
464 *Microcystis aeruginosa*. *Journal of Applied Phycology*, In press.

465 Welch, I, M., Barrett, P, R, F., Ridge, I (1990) Barley straw as an algal inhibitor of algal
466 growth I: studies in the chesterfield canal. *Journal of Applied Phycology* **2**, 241-
467 248

468 Wessén, B. and Berg, B. (1986) Long term decomposition of barley straw: Chemical
469 changes and ingrowth of fungal mycelium. *Soil Biology and Biochemistry*, **18**, 53-
470 59.