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

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

Key Words: Algal control, Barely straw, Chemostat, *Scenedesmus*

INTRODUCTION

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

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

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

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

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

MATERIALS AND METHODS

Algae preparation

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

Fungi preparation

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

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

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

Chemostat preparation

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

could grow consistently in just tap water. The tanks were kept mixed with aerating aquarium pumps (Maidenhead Aquatics, Woburn Sands, UK). The algae cultures were allowed one month to acclimatise and then blank data was collected for three weeks before straw was added. In total, two tanks were prepared for each of the straw conditions tested, which were: i) fresh barley straw; ii) pre-rotted rotted barley straw and iii) fungal pre-treated barley straw. Four further tanks were kept as controls. A chemostat was also prepared to analyse fungal metabolites (acid digestible fibre (ADF) and ergosterol) and their impact on algae growth. This was prepared and maintained identically to the fresh straw chemostats.

Straw preparation

Three sources of barley straw were used:

(i) Fresh straw (obtained from a local pet shop).

(ii) Four month old rotted straw (obtained from a surface water reservoir in the Yorkshire region).

(iii) Fungal-treated straw.

Fungal-treated straw was prepared by placing 25 g of fresh straw into a 1.5 l acid washed beaker sealed with tin foil. The straw was then autoclaved at 121 °C and 50 atm for 15 mins (PriorClave, London, UK). Once autoclaved the straw was stored in darkness overnight in an ethanol-cleaned sealed environment chamber (Sanyo MLR-450H, Sanyo, Japan) at 100% humidity. The cooled straw was opened under aseptic conditions inside the laminar flow cabinet. Three 1 cm^2 squares of *P. chrysosporium*, *P. ostreatus* or *T. versicolor* were placed at the bottom, middle and top of the straw. Once the fungi were 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

ammonium bromide (ACROS Chemicals, Geel, Belgium) in a 250 ml Erlenmeyer flask. The top of the flask was plugged with an inverted 50 ml volumetric flask to prevent evaporation. Samples were simmered for 1 hour, followed by immediate transfer of the straw to a no. 2 porosity sinter (W2). The straw was then rinsed with 50 ml aliquots of boiling water followed by acetone until no more colour was removed. The sample was filtered through the sinter under vacuum. Once rinsed the samples were dried at 105°C for 2 h, then weighed once cooled (W3). Once weighed the sinter was half-filled with cooled (15°C) 72% H_2SO_4 and stirred with a glass rod. The acid was allowed to drain away under gravity and continuously topped up with fresh acid for 3 h. After 3 h the acid was filtered off under vacuum and the sample rinsed with boiling water followed by acetone until colour was no longer removed. The sinter was then dried at 105°C for 2 h and weighed (W4) followed by 2 h at 550°C and then re-weighed (W5).

The weights were then used to calculate the following:

% Acid digestible fibre = (W3 – W2) x 100 / W1

% Lignin = (W4 – W5) x 100 / W1

% Cellulose = (W3 – W4) x 100 / W1

Ergosterol

The fungal population was quantified using ergosterol analysis according to the rapid ultrasonication method described by Ruzicka *et al.* (1995). Samples were taken at 3, 6 and 9 months from both the bulk water in the chemostat and from water extracted from within the straw bundle. Ergosterol was quantified by taking the filtrates from these samples through a 0.7 µm filter paper and adding it to a 50 ml polyethylene tube. A 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.$week^{-1}$
229 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

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

Discussion

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

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

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

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

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

matter (AOM) layer will be important. Consequently, appropriateness of barley straw in inhibiting different algal species is not expected to follow traditional biological classification based on differences in pigmentation and cell complexity arising as a result of evolution (Bellinger, 1992). Instead, species from all phyla are expected to be susceptible. A parallel can be made with other forms of treatment where effectiveness mirrors specific chemical and physical properties rather than biological classification. For instance, the use of low energy ultrasound has been shown to be effective only for filamentous algae irrespective of phyla (Purcell *et al.*, 2009). Similarly, optimum conditions for coagulation and clarification of algae have been shown to relate to the charge density of the algae and AOM as well as shape factors that cross all phyla (Henderson *et al*., 2008c). As a result, barley straw appears to fit this pattern such that generalisations cannot be made regarding susceptibility based on biological classification.

The key practical implication of these results relates to how responsive the system to be treated needs to be. Current approaches to application of barley straw require long term planning to ensure that the straw is inhibitory during the exponential growth phase of the algal bloom. Having a stock of straw pre-treated by WRF provides a solution that can be more responsive and as such can be applied in situations where blooms are unexpected in terms of location or time. The need to establish and maintain WRF populations remains the critical step and, while pre-treatment reduces the time required for inhibition, the net benefits of developing pre-treatment reactors remains uncertain. Further work is required to optimise the pre-treatment stage to minimise its duration as well as maximise the inhibitory impacts of adding the straw.

Conclusions

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

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

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