J. Aquat. Plant Manage. 31: 203-206

Control of *Microcystis aeruginosa* by Decomposing Barley Straw

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ABSTRACT

Growth of the blue-green alga *Microcystis aeruginosa* is inhibited by the presence of decomposing barley straw in laboratory culture to levels of 6% of that achieved in control experiments. The effect appears to be algistatic rather than algicidal. Final biomass in regrowth experiments is independent of previous treatment. Values for regrowth from control treatments ($2.96.10^6$ cells cm^{-3}) were not significantly different from values for regrowth of cells from the most inhibitory treatment ($2.67.10^6$ cells cm^{-3}). Cells inhibited by exposure to straw recovered, achieving the same growth rate as untreated cells when reinoculated into straw-free media. Growth inhibition of 95% can be achieved with 2.57 g straw (dry weight) m^{-3} water. These results are compared to the results of a survey in Great Britain and Ireland on the use of straw to control algae. Decomposing barley straw inhibits the growth of both filamentous and blue-green algal species in all types of water bodies so far assessed. Possible causes of the inhibitory effect are discussed.

Key words: algae, blue-green, growth inhibition.

INTRODUCTION

Problems associated with the development of large blooms of potentially toxin-producing cyanophyte algae have recently become a matter of public concern in the United Kingdom. The generally low rainfall in the U.K. during the past 4 yr has exacerbated algal problems, and the adoption of environmentally sound solutions to the growing number of algal problems in all areas of the water industry is now becoming more important.

Restrictions on the use of herbicides in potable water supplies and some environmentally sensitive areas have encouraged the use of alternative algal control strategies. The presence of decomposing barley straw in water can reduce the growth of a range of algal species under field (Welch *et al.* 1990) and laboratory conditions (Gibson *et al.* 1990). The mechanism by which growth inhibition is achieved is still largely unknown. However, the conditions necessary for the production of the inhibitory effect are now well established as a result of direct experimentation and many field observations. They are, primarily, the maintenance of aerobic conditions in the straw mass and development of a diverse microbial community leading to decomposition of the straw.

Previous observations, in a series of unreplicated field trials, have shown that decomposing barley straw can prevent the growth of *Microcystis aeruginosa* and other blue-green unicellular and green filamentous algal species (Barrett, pers. obs.). The work reported here demonstrates that the growth of *M. aeruginosa* in controlled laboratory conditions can be inhibited by the presence of decomposing barley straw or straw liquor (water in which straw was rotted) (see also Foundation for Water Research 1992).

MATERIALS AND METHODS

Barley (*Hordeum vulgare* var. Atem) straw was added to aged (2 weeks) dechlorinated tap water in fiberglass tanks in a glasshouse on 22 May 1991 at a rate of 1 kg straw m^{-3} water. The tanks were maintained at 20 ± 3C with natural daylight irradiance and continuous aeration provided by an aquarium pump. Samples were taken from the tanks between 78 and 92 days after the start of the aquatic decomposition process. Previous experiments have indicated that this is sufficient time for a significant algistatic effect to be produced (Gibson *et al.* 1990).

A culture of *Microcystis aeruginosa* Kützing emend Elenkin 1924 strain CCAP 1450/6 was obtained from the Cambridge Collection of Algae and Protozoa at the Institute of Freshwater Ecology in Ambleside, U.K. Jaworski's culture medium (JM) (Thompson *et al.* 1988) was prepared with sterile filtered straw liquor, and autoclaved if required. The inhibitory effect was different in autoclaved straw liquor JM. The control medium of JM in dechlorinated aged tap water was always autoclaved. An inoculum culture was prepared 3 days before the experiment to produce cells just before the onset of log-phase growth. This minimized the duration of the experiment, permitted the use of low inoculum cell densities, and made inhibitory effects easier to detect.

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To determine the effect of straw and straw liquor on the growth of *M. aeruginosa*, 5 cm (0.08 g wet weight) of barley straw was added to flasks containing 50 cm^3 sterile filtered or autoclaved straw liquor JM or tap water JM. The same weight of plastic straws was added to control experiments to provide a surface for colonization. Plastic straws do not affect the growth of *M. aeruginosa* (unpublished results). Flasks were inoculated with 7.10^4 cells cm^{-3} of an early log-phase culture, and incubated on an orbital shaker at 75 rpm at 20C and 150 µmol photons $m^{-2}s^{-1}$ for 72 hr. Cells were counted with a haemocytometer in triplicate samples from each flask and growth was expressed as a percentage of growth in control cultures.

To determine cell viability after exposure to straw or straw liquor, aliquots containing an equal number of cells were removed from each treatment. The samples were centrifuged for 2 min and the pellet was resuspended in 10 cm^3 distilled water JM, and the suspension re-centrifuged. The pellet was resuspended in 3 cm^3 distilled water JM, and 1 cm^3 was inoculated into each of three lots of 50 cm^3 in 250 cm^3 conical flasks. The cultures were grown for 72 hr as described above and the number of cells in each flask was counted.

To determine if the inhibitory effect caused by decomposing barley straw exhibited dose response characteristics, straw, which had been rotting for 85 days, was washed five times in distilled water and cut into 1-mm and 1-cm pieces. Straw pieces (not autoclaved) were put into 50 cm^3 autoclaved straw liquor culture medium in conical flasks. There were three replicates of each of the following application rates; 1, 2, 3, 4 and 5 mm and 1, 2, 3, 4, 5, 10 and 25 cm. Control flasks did not have any added straw. The flasks were inoculated with a culture of *M. aeruginosa* growing logarithmically, and cells were counted in all flasks after 72 hr. Growth was expressed as a percentage of control values. A dose response effect was not observed when straw liquor JM was diluted with distilled water in the absence of straw pieces.

To assess the effects of decomposing straw on the growth of blue-green algae under field conditions, a survey was carried out among people who had contacted the authors for advice on the application of straw. Blue-green algae occurred in 47% of the sites surveyed. An arbitrary score was assigned for algal control based on the personal opinions of the site managers or, in some cases, on the basis of cell counts. Data on factors such as area of water body, geographical location, nutrient loading, use, extent of weed problem, straw application rate, duration of control and any associated benefits or problems were collected.

RESULTS AND DISCUSSION

Decomposing barley straw in combination with all the types of culture medium tested inhibited the growth of *M. aeruginosa* (Table 1). The inhibitory effect was enhanced when straw liquor and barley straw were used in combination. Straw liquor and straw in tap water culture medium both produced an effect but to a lesser extent. The inhibitory effect was lost when straw liquor was autoclaved. Loss of inhibition was also noted by Gibson *et al.* (1990) when straw was autoclaved. Stimulation of growth by 93% was observed in treatments of autoclaved straw liquor with plastic straws. This effect has been observed in other unpublished experiments. The compounds released by decomposing straw are a complex mixture of stimulatory and inhibitory factors, and it may be that autoclaving selectively destroys the inhibitory component(s) of the liquor. There was less inhibition in filter-sterilized straw liquor without straw, which may suggest the inhibitory substance is continually produced by the decomposing straw or that it does not have a long persistence time in aqueous solution. This hypothesis is supported by comparing the data for autoclaved straw liquor/barley straw with those for autoclaved straw liquor/plastic straws (Table 1). There is an inhibitory effect with barley straw which is not evident with plastic straws.

TABLE 1. GROWTH OF *M. aeruginosa* IN JAWORSKI'S MEDIUM CONTAINING BARLEY STRAW OR PLASTIC STRAWS EXPRESSED AS PERCENT OF CONTROL VALUES. NUMBERS IN PARENTHESES ARE STANDARD ERRORS OF THE MEAN OF THREE REPLICATES.

Treatment	Growth	
Barley straw/Tap water	30.9	(3.2)
Barley straw/Autoclaved straw liquor	6.0	(1.3)
Barley straw/Sterile filtered straw liquor	11.6	(4.7)
Plastic straw/Autoclaved straw liquor	193.0	(12.6)
Plastic straw/Sterile filtered straw liquor	67.3	(4.7)
Plastic straw/Tap water	100.0	(12.7)

When cells were removed from the treatments and reinoculated into fresh culture media at the same inoculation density, the biomass achieved at the end of 72 hr was the same for each sample (Table 2). This indicates that cells remain viable after exposure to straw in the conditions used in these experiments, and that the effect of decomposing straw is algistatic rather than algicidal.

TABLE 2. GROWTH OF *M. aeruginosa* AFTER EXPOSURE TO DECOMPOSING BARLEY STRAW OR STRAW LIQUOR AND TRANSFER TO FRESH MEDIUM. GROWTH MEASURED AFTER 72 HR AND EXPRESSED AS CELL NUMBERS (10^6 cm^{-3}). NUMBERS IN PARENTHESES ARE STANDARD ERRORS OF THE MEAN OF THREE REPLICATES.

Treatment	Growth	
Barley straw/Autoclaved straw liquor	2.67	(0.30)
Barley straw/Sterile filtered straw liquor	2.31	(0.12)
Plastic straw/Autoclaved straw liquor	1.96	(0.44)
Plastic straw/Sterile filtered straw liquor	2.10	(0.12)
Plastic straw/Tap water (control)	2.96	(0.75)

The degree of growth inhibition of *M. aeruginosa* was dependent on the amount of straw in the flask (Figure 1). At the lowest dose tested, growth was only 5% of that in control flasks. This dose of 2.57 g m^{-3} corresponds well with the minimum effective application rates used in field conditions of three 20-kg bales per hectare.

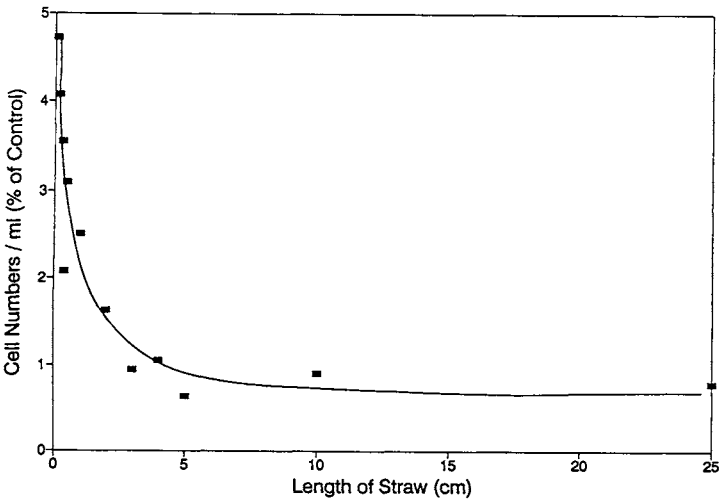

Figure 1. Relationship between length of 92-day-old decomposing barley straw in sterile filtered straw liquor and growth of *M. aeruginosa* expressed as a percentage of growth in control experiments. n = 1. The curve fitted by regression in Lotus Freelance has the equation $Y = 1.97 X^{-0.39}$, $r^2 = 0.87$.

Results from a survey of field sites in which straw has been applied to control blue-green and green filamentous algae, or a mixture of both, are given in Table 3. The survey included a range of types of water body in all areas of the U.K. and Eire. These sites were all static waters and consisted of a mixture of ponds, lakes and reservoirs. Blue-green algae were the main cause of the algal problem in 47% of the sites surveyed. The data were supplied by the site owners or managers, and were based on various assessment techniques of the amount of algal growth before and after the application of straw. Some assessments were made visually and others were based on cell counts. These results are from unreplicated field trials and, as such, information derived from them can only be used to indicate trends. However, the information gained shows that algal control was achieved to some extent in all types of water body but was better in the smaller ponds. This may be an artifact that results from the difficulties of applying straw to large water bodies where inadequate distribution of the algistatic factors could have occurred.

TABLE 3. RESULTS OF A SURVEY ON THE USE OF BARLEY STRAW TO CONTROL ALGAL GROWTH IN GREAT BRITAIN AND IRELAND. VALUES GIVEN ARE THE MEANS OF AN ASSESSMENT OF ALGAL GROWTH CARRIED OUT BY THE OWNER/MANAGER BASED ON A SCALE OF 0 TO 9 (0 = no control, 9 = no algal growth). THERE HAS BEEN NO INDEPENDENT ASSESSMENT OF THE SCORES GIVEN IN THIS TABLE. NUMBER OF SITES ASSESSED IN PARENTHESES.

Type of water body	Mean score	(SE)
Pond (8)	6.75	(0.88)
Drainage ditch (6)	6.17	(0.87)
Lake (15)	6.00	(0.83)
Canal (3)	5.67	(2.85)
Reservoir(4)	3.75	(1.89)
Saltwater pond (1)	9	

The results of laboratory experiments reported here, and by Gibson *et al.* (1990), strongly suggest that the inhibitory effect of straw on algal growth is caused by the release of a chemical during aerobic microbial decomposition of the straw. This chemical, or mixture of chemicals, so far unidentified, is algistatic rather than algicidal. This has implications for control of *M. aeruginosa* and other algae in the field. To achieve long-term control, the straw should remain in the water continuously during the period when algal growth might occur, and should be replaced before it has totally decomposed to keep sufficient concentrations of the algistatic factors in the water.

The response of blue-green algae to straw appears to have different characteristics than those of green filamentous algae. Although the effect demonstrated here is algistatic, blue-green algae appear to have a much shorter survival time than filamentous green algae when exposed to the algistatic factor. When straw was added to a sewage works settlement pond in Australia, containing a bloom of *Anabaena sp.*, the algal population decreased to near zero within a week (M. Hindmarsh, pers. comm.). Similar observations have been made by the authors in a 6-ha lake containing a bloom of *Oscillatoria agardhii*. Before the addition of straw the number of filaments was 10,000 cm^{-3}; 3 weeks after the addition

of straw as anchored bales to the lake, *O. agardhii* was undetectable. In contrast, at least 2 months decomposition was required before control of the filamentous green alga *Cladophora glomerata* became significant (Welch *et al.* 1990).

There may be several reasons for the inhibition of algal growth caused by decomposing straw, which include the production of antibiotics by the fungal flora and the release of straw cell wall components modified during microbial decomposition. The production of antibiotics by soil microorganisms in association with wheat straw residues (McCalla and Norstadt 1974, Wright 1956) has not been examined in aquatic situations with barley straw. However, a dense population of a very wide range of microorganisms (bacteria, fungi, actinomycetes) is associated with decomposing barley straw (Pillinger, pers. comm.) and the possibility of *in situ* antibiotic production cannot be ruled out.

The release of phenolic compounds such as ferulic acid and *p*-coumaric acid from decomposition of straw cell walls, and other aromatic compounds from the incomplete decomposition of lignin may also contribute to the effect. However, assuming that 1% of the carbon in the straw is released as *p*-coumaric acid, the concentration achieved at the dose rates used here would be 2.3 ng ml^{-1}. This is lower than the concentrations reported to reduce algal growth in laboratory conditions (Dedonder and Van Sumere 1971).

The complexity of the mixture released suggests that the cause of the inhibition is not due to any single chemical and that the observed effect may be produced by synergistic interaction of all inhibitory components of the system. This is supported by Rice (1984) and although allelopathy is not a strictly correct description of this situation, some of the chemicals possibly responsible for the inhibition of growth are produced by algae as allelochemicals, notably fatty acids and phenolic acids.

The use of decomposing barley straw to inhibit the growth of algae is increasing as the technique becomes more widely known. The presence of decomposing straw in water can help to prevent the development of blue-green algal blooms in most situations by preventing the rapid increase in population numbers. The survey data reported here and other current laboratory and field experiments provide evidence for the control of both blue-green and filamentous green algae with barley straw under natural environmental conditions.

There do not appear to be any limits imposed by the type of water body on the use of straw to reduce algal problems, and the technique could have a wide application. The conditions which encourage the development of algal blooms, such as high temperatures, also encourage the decomposition of the straw, and a close relationship between production of an anti-algal effect and development of blooms can be envisaged. Work is continuing into the identification of the active principles involved in the inhibition, what aspect of algal metabolism is specifically inhibited, and development and optimization of the method of application to different types of water body.

ACKNOWLEDGMENTS

The Aquatic Weeds Research Unit acknowledges receipt of financial assistance from the Foundation for Water Research (Allen House, The Listons, Liston Road, Marlow, Bucks, SL17 1FD, England, U.K.) while carrying out this work. We would like to thank Sophie Broomfield and James Lambden for their excellent technical assistance.

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