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HDC project number:	BOF 70
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The results and conclusions in this report are based on an investigation conducted over a two-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

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GROWER SUMMARY

Headline

Chlorine dioxide, a powerful biocide that is environmentally benign and easily generated and monitored on-site, has been tested for the first time in the UK for use in hot-water treatment. It has promise as a replacement for formalin, which can no longer be used.

Background

To manage stem nematode (*Ditylenchus dipsaci*) and base rot (basal or *Fusarium* rot; *Fusarium oxysporum f.sp. narcissi*) in daffodils, hot-water treatment (HWT) of the bulbs is essential. For decades, the HWT dip used for treating daffodil bulbs invariably contained formaldehyde (formalin), a biocide (disinfectant) that provided general disinfection of the bulbs, water and equipment, as well as apparently augmenting the kill of stem nematodes by the hot water (HW) itself. At the end of 2008 the use of formaldehyde in the EU was withdrawn from the agriculture/horticulture industry, without any alternative biocide being available. Effective alternative biocides for use in HWT have therefore been sought urgently. One candidate, an iodophore-type biocide, 'FAM 30', is being investigated in other HDC-funded projects (BOF 61 and extensions), and in the project reported here (BOF 70) another potential biocide, chlorine dioxide (ClO_2), used for example as 'Harvest Wash', is being investigated following successful trials in the USA.

Summary of the project and main conclusions

Testing was carried out over two days using typical HWT facilities at a commercial site. ClO_2 (as freshly mixed sodium chlorite and hydrochloric acid) was added to the HWT system gradually, until a concentration of 4 to 5ppm (4 to 5mg/L) was reached, when HWT was started. Throughout the 3h HWT period, ClO_2 concentration was monitored, making further additions as necessary to maintain the target concentration of 4 to 5 ppm. Treated bulb stocks of 'Mando' and 'Quirinus' were planted in the field along with other batches treated in HWT with 'FAM 30' and 'Bravo 500'.

On day 1 of testing, the target concentration of ClO_2 was reached after a adding atotal of 95.5L of ClO_2 to the tank. During the HWT run, the dip was monitored at appropriate intervals, and further additions of ClO_2 made as required. During treatment the ClO_2 concentrations fluctuated between 0.44 and 7.65ppm, ending at 4.72ppm. A total of 118.0L ClO_2 was added to the tank on day 1.

An additional 19.25L of ClO_2 was added at the start of the second day of testing in order to achieve the target concentration, and an additional 16.0L ClO_2 was added over the

subsequent HWT period. During this 3h HWT period, ClO_2 concentrations fluctuated between 0.35 and 9.26ppm, though ending lower than intended (0.08ppm).

The total amount of ClO_2 added – 118L on day 1 and 35L on day 2 in the 21,000L HWT system – was comparable with the 105L of commercial formalin or 168L of 'FAM 30' required for this size of system at the usual application rates.

Systems for monitoring ClO_2 concentration achieved in the tank were compared of ClO_2 and the 'ChlordioXense' meter was more reliable than 'dip-stick' systems that are also commercialy avaialble. The high usage of ClO_2 indicated that there was evidently an enormous initial bioload present in the HWT system, thought to be largely due to sediments that had accumulated in the holding tank, and this emphasises the need for a thorough cleaning of the tanks and associated equipment prior to the start of the bulb dipping season. However, once the initial bioload had been neutralised, any further bioload introduced into the tank should be easily controlled by maintaining an appropriate ClO_2 concentration.

No specific concerns about using ClO_2 in daffodil HWT were identified. Using ClO_2 resulted in an acidic, but not excessively acidic, dip that settled at about pH4, which is unlikely to damage subsequent daffodil growth.

The tests carried out in this project involved manual dosing of the chemicals needed to generate ClO_2 in solution and while time consuming this process did not create and unpleasant working environment, given the usual precautions. There are however commercially avaialble automatic monitoring and dosing systems which would be considered essential for routine use by growers which would minimise contact with the chemical reagants and significantly reduce time inputs.

Using ClO_2 could have many advantages for bulb growers – general effectiveness, ease of monitoring and regulating solutions, and absence of harmful end-products. The only challenger so far identified, an iodophore biocide ('FAM 30'), may have limited use unless the problem of the rapid loss of its active substance (iodine) in bulb dips can be remediated.

Financial benefits

Commercial daffodil growing depends on the availability of effective HWT regimes. Because the UK bulb industry lacks a tested biocide to use in place of formalin, the financial benefits of using a proven alternative – whether it be ClO_2 or 'FAM 30' – could equate to the survival of the whole industry unless alternative means of stem nematode or base rot management are found. Until more is known about ClO_2, particularly in terms of the relevant legislation, its

long-term effects on the crop, and its position *vis-à-vis* 'FAM 30', it would be premature to attempt to calculate financial benefit of its use.

Action points for growers

At this stage HWT with ClO_2 cannot be recommended to growers. The treated crop needs to be examined in spring 2011, to ensure there has been no obvious damage to the crop. Further, the legal status of ClO_2 formulations needs to be clarified with the appropriate authorities, which is ongoing. Recommended actions will be available in the final report for the extension to this work, project BOF 70a.

SCIENCE SECTION

Introduction

In order to manage stem nematode (*Ditylenchus dipsaci*) and base rot (basal or *Fusarium* rot; *Fusarium oxysporum f.sp. narcissi*) in daffodil bulbs, hot-water treatment (HWT) of the bulbs, prior to planting, is essential. For decades, the HWT dip used for treating daffodil bulbs has invariably contained formaldehyde (as 'commercial formalin'), a biocide (disinfectant) that provides general disinfection of the bulbs, water and equipment, as well as apparently augmenting the kill of stem nematodes by the hot water (HW) itself. At the end of 2008 the use of formaldehyde in the EU was withdrawn from the agriculture/horticulture industry, without any alternative biocide being available. Effective alternative biocides for use in HWT have therefore been sought urgently.

In HDC-funded Project BOF 61 the use of HWT in daffodil production was reviewed, and it was concluded that other postulated methods for controlling stem nematodes – such as microwave treatment, foliar-applied nematicides, or breeding for resistance – would be impractical, ineffective or too expensive to implement. The recommendation was, therefore, to investigate:

1. Other time/temperature regimes for HWT (e.g. hotter and (or) longer than the standard treatment of 3 hours at 44.4°C), to determine whether they would be effective in controlling stem nematode in the absence of formaldehyde
2. Other biocides or pesticides for addition to the HWT tank as alternatives to formaldehyde.

These options were investigated in a subsequent HDC project (BOF 61a). In laboratory experiments, the standard HWT regime was confirmed as being effective in controlling stem nematode, even in the absence of formaldehyde or another biocide. Candidate biocides were therefore tested for their effectiveness in controlling stem nematode during a 3-hour 'cold dip' treatment (immersion of bulbs in water at 18°C, in this case). An iodophore-type biocide, 'FAM 30', was found to be the most effective of those tested, and also did not apparently result in crop damage in small-scale tests. The other biocides tested were either less effective and (or) resulted in crop damage.

In HDC Projects BOF 61a and 61b the use of 'FAM 30' was tested on a field-scale and using commercial HWT equipment. Validation of this treatment on a field or commercial scale was necessary to test whether the results found in the laboratory could be applied at a practical, farm situation – for example, on the farm, soil contamination might affect results, or using mains (as opposed to deionised or distilled) water might result in other artefacts. 'FAM 30'

was tested at two rates in HWT, the maximum rate normally recommended by the manufacturer or supplier, and half of this rate. The treatments tested also included HWT with:

1. A chlorothalonil fungicide (as 'Bravo 500'), which had been identified in the same projects as an alternative to thiabendazole-based fungicides; also tested at full- and half-rates
2. Tank-mix 'FAM 30' plus 'Bravo 500' (in Project BOF 61b only)
3. Commercial formalin (as the standard for comparison, BOF 61a only)
4. Plain water (as a control).

In the first year of the BOF 61a field trial, 2009, crop growth in plots treated with 'FAM 30' (at either rate) or 'Bravo 500' (also at either rate) were as or more vigorous than those treated with commercial formalin. Similarly, in the first year of the BOF 61b field trial, 2010, crop growth was normal in all plots treated with 'FAM 30' and (or) 'Bravo 500'. In both cases, unsurprisingly, growth was poorer in control plots treated with plain water only. In the later trial, three replicates of the trial were lifted in summer 2010 to determine bulb yields, with three replicates being grown-on until 2011 for further assessments. In broad terms, the 'FAM 30' and 'Bravo 500' treatments were free of adverse effects on the crops (see the individual project reports for detailed results). However, until a full set of results is available, in 2011, the option of using 'FAM 30' in place of commercial formalin in daffodil HWT cannot be considered as advisable.

Despite identifying 'FAM 30' as a promising replacement for formaldehyde, and even if confirmed as such when the above trial has been completed, UK bulb growers would be unwise to depend again entirely on a single biocide for use in HWT. However, no other candidate performed as well as 'FAM 30' in the original tests (BOF 61a laboratory work). The other materials tested included chlorine dioxide (ClO_2) (as 'Harvest Wash') which, while not resulting in crop damage, failed to kill either base rot chlamydospores (when tested as a 3-hour HWT) or stem nematodes (when tested as a 3-hour dip at 18°C). In research conducted by Prof Gary Chastagner at the Puyallup Research & Extension Center of Washington State University, however, ClO_2 has been clearly demonstrated as an effective fungicide, at similar rates of use, against a number of pathogens, including the base rot fungus, when used in HWT, also without phytotoxic effects. ClO_2 has been used successfully by at least one major bulb grower in the Pacific North-West for several years, although details are lacking due to commercial considerations. The reason for this apparent discrepancy between research groups has not yet been established, but could be due to a number of factors, such as different formulations of the biocide, or different states or stages of the base rot spores used; it is also known of ClO_2 that the pH value and the concentrations of ions in the water may affect responses in some cases. Therefore, in 2010

it was proposed to the HDC BOF Panel that the feasibility of using ClO_2 in HWT should be re-examined, despite the earlier disappointing UK results. A literature review was appended to the BOF 70 project proposal, and is added in as appendix I in this report.

ClO_2 is a wide-spectrum biocide extensively used in fruit, vegetable and meat processing, in water treatment and in the health care sector. It has the advantages that it can be readily generated on-site from sodium chlorite and hydrochloric acid, and can be monitored and dosed using available and relatively straightforward technology. It is claimed to generate no environmentally or other harmful by-products, and to be relatively unaffected by organic matter in dip tanks.

In 2009, the first year UK growers carried out HWT without formalin, there were some reports of unpleasant smells emanating from HWT tanks. Presumably this was a result of the now-uncontrolled growth of micro-organisms in the absence of formalin, and it may become necessary to take steps to overcome this problem also.

Although it is known from research in the US that ClO_2 effectively controls a number of fungal plant pathogens, including the base rot fungus, no information appeared to be available on the effect of ClO_2 on nematodes. The HDC BOF Panel agreed that its effects on both stem nematode and base rot spores should be clarified, and the HDC funded the first stage of this proposal, which is reported here.

The legal position was clarified with the Chemical Regulations Directorate (CRD) and it was declared that there were no legal bars to developing this use. As a biocide, ClO_2 fell outside EU/UK pesticide legislation, though its use as a biocide in various situations (public and private hygiene situations, water treatment, food washing, ‘de-sliming’, etc.) is being reviewed by the EC under the Biocidal Products Directive (at which stage ClO_2 may pass or fail).

The commercial objective of Project BOF 70 was therefore to investigate ClO_2 as a practical biocide in bulb dipping, especially in HWT. ClO_2 appears to offer a number of advantages over other biocides, and a successful outcome could be of great use to the UK bulb industry. Provided that the dosing of ClO_2 in HWT tanks is found to be practical, and if the biocide is successful in controlling bacterial load without any adverse effects on the bulb crop, and it does not detract from the effects of HW and fungicides on the control of stem nematodes and the base rot pathogen, it would provide a tested and easily controlled technology for use in daffodil growing and a useful alternative to other biocides.

Materials and methods

HWT facilities and dispensing ClO_2

HWT of daffodil bulbs refers to immersion of the bulbs in HW to control stem nematode, base rot and other pests and pathogens. HWT is normally carried out in a purpose-built on-farm facilities, in the UK a treatment at 44.4°C for 3h being the standard one.

The HWT facility used in this project was at TH Charlton & Son Ltd, Hallgate Farm, Moulton, Spalding, Lincolnshire, which comprised of two front-loading treatment tanks and a common, overhead holding tank (Secker Welding Ltd), with a capacity as used (sufficient for using one treatment tank at a time, with some dip left in holding tank) of *ca* 21,000L and a load per tank of six wooden bulk-bins each containing *ca* 0.6t (a total of 3.6t of bulbs per tank).

In July 2010 site visits were made by Robert Holland (Tristel PLC), James Avery (Green-Tech Horticulture), the HDC Project Co-ordinator and the Project Leader to assess the most appropriate means of monitoring and providing the required in-tank concentrations of ClO_2. For other uses, ClO_2 concentration is routinely monitored via a redox sensor fitted in the tank, modulating the addition of ClO_2 to maintain the target level through a dispensing and mixing head connected to drums of sodium chlorite and hydrochloric acid. The usual system, however, seemed inappropriate for short-term testing in this situation: (a) the HWT facility comprised two treatment tanks and a common holding tank, complicating the arrangements required compared with a simple produce dip tank, and (b) the tanks were needed for the grower's regular HWT operations before and after the ClO_2 trial, meaning that any plumbing-in of equipment would delay the changeover between biocides and slow the grower's HWT programme. In the circumstances of this pilot study, it was deemed less disruptive to monitor in-tank ClO_2, and mix and dispense sodium chlorite and hydrochloric acid, manually. The sensing and mixing equipment is already a well-tried technology, so it was not critical to test that specific aspect at this time.

The HWT facility was used with ClO_2 on 5-6 August 2010. Prior to use, the previous dip was run to waste, and the system was flushed and then filled with fresh, mains water (running direct from the mains with no intermediate storage). The bulbs used were daffodil cv 'Mando' (5 August) and cv 'Quirinus' (6 August), both taken from the stocks at TH Charlton & Son Ltd, and, for comparative purposes, other batches of the same stocks received HWT with tank-mix iodophore biocide and chlorothalonil fungicide (as 'FAM 30' + 'Bravo 500') in the previous week ('Mando') or in the following week ('Quirinus'), with the intention of planting bulbs from the two treatments as separate, labelled blocks in the field.

Treatments and sampling

On 5 August 2010 the dip was brought to a temperature of *ca* 49°C and the target concentration of ClO_2, 4 to 5ppm (4 to 5mg/L), was built-up by the step-wise addition of mixed sodium chlorite and hydrochloric acid, using a 'ChlordioXense' spectrophotometer to measure actual ClO_2 levels and proceeding cautiously in order to avoid over-dosing. The amounts of ClO_2 added at each stage were based on the prior experience of Tristel PLC staff in other systems and a consideration of the bioload and other readings. No other chemicals (pesticides, adjuvants or anti-foam preparations) were added to the tanks.

It should be noted that this trials procedure was different to how a bulb grower would normally dose his bulb dipping tank, i.e. by adding the whole amount of biocide before the start of dipping; in this trial, ClO_2 was added gradually, in order to establish how much of the biocide was needed to neutralise the existing bioload in the system, and then to build-up to a suitable concentration with which to treat the bulbs.

The 3-hour HWT was started once the concentration of ClO_2 in the tank exceeded the target rate, by pumping the solution to the other treatment tank that was loaded with bulbs. The dip temperature was then just below the target treatment temperature of 44.4°C, which was then maintained during the treatment. Sampling and the addition of further ClO_2 continued, as appropriate, throughout the HWT period, following which the dip was pumped to the alternate tank for overnight storage and the treated bulbs were unloaded.

On 6 August this process was repeated with further bulbs, with the HWT period starting once the chlorine dioxide concentration in the tank was close to the target level.

At appropriate intervals over the 2-day test period, dip samples were taken to determine ClO_2 concentration, dip pH and bioload, using the methods detailed below. All dip samples were taken from near the top of the tank where there was good water flow, excluding any foam or floating debris.

Further details are given in Table 1 (see Appendix II).

Dip sampling

ClO_2 concentrations

Concentrations were measured by:

1. A portable spectrometer ('ChlordioXense'; Palintest Ltd, Gateshead, Tyne & Wear, UK),
2. ClO_2 'dip-sticks' ('Oxystix'; Bio-Cide International, Norman, OK 73072, USA), usually used on undiluted dip (referred to as BCI dip-sticks under Results),
3. ClO_2 'dip-sticks' (Industrial Test Systems, Rock Hill, SC 29730, USA), low-range tests usually used on dip diluted 100-fold to come on-scale (referred to as ITS dip-sticks).

The spectrometer measured ***actual*** ClO_2 levels in samples. Titration methods – on which the chemical reactions of the 'dip-sticks' are based – measure the sodium chlorite available to produce ClO_2, i.e. ***potential*** ClO_2 levels.

pH value

pH is important because pesticides may be inactivated or enhanced at different values of pH, while dips of very low (acidic) pH may be harmful to plants. The pH of dips was determined using a suite of narrow- and wide-range indicator papers ('Pehanon', Macherey-Nagel, Düren, Germany, and BDH, Poole, UK).

Bioload

Bioload was measured in two ways:

1. Bacterial pollution was evaluated using 'Petrifilm' (3M Microbiology Products, St Paul, MN, USA). The 'film' consists of a paper containing a nutrient medium (specific to aerobic bacteria) and a transparent plastic cover. Having peeled back the cover sheet, a pipette is used to place a 1ml-sample of the well-mixed dip sample as a single drop in the centre of the 'Petrifilm'. The cover sheet is then gently replaced and the sample evenly spread across the film by pressure from a plastic 'spreader'. The unit is left at room temperature for three days, after which bacterial growth on the film is assessed according to a visual scale provided by the manufacturer, ranging from 0 (not polluted) to 5 (very strongly polluted). No bacterial growth was scored 0, increasing numbers of distinct bacterial colonies were scored 1 to 4, and total coverage of the film by colonies was scored 5. At a score of 1 it was easy to count the bacterial colonies.
2. Bioload generally was determined using the 'Clean-Trace NG Luminometer' system (Biotrace International PLC, Bridgend, UK). This is based on a luminescent reaction between a reagent on a 'measuring pen' and adenosine triphosphate (ATP, produced by all living cells) in the sample. Essentially, the dip was sampled by dipping in the 'pen' (similar to that used forensically in swabs), replacing the pen in its holder (which mixes sample and reagents) and inserting it into a luminometer which gives a reading in 'relative light units' (RLU) within 30sec. The scale suggested by the manufacturer runs from 0 to 500RLU, meaning not or only slightly polluted, to >3,000RLU, meaning strongly polluted. Tests of fresh mains water, included as checks at the start and end of testing, typically gave values <20RLU.

Results

The progress of the treatments and observations is detailed in Table 1 (see Appendix II), and the main results are summarised in Figure 1.

Table 1 shows that a lengthy period was needed on the first day of testing to reach the target ***ClO_2 concentration*** of 4 to 5ppm without risk of over-dosing, due to the unexpected and exceptionally large bioload initially present in the tanks and HWT system. Such a large bioload was outside the experience of the Tristel staff involved in the project, much of whose work involves projects in manufacturing and medical facilities: nevertheless, the target concentration of ClO_2 was reached (and exceeded, 7ppm) after a total of 95.5L of ClO_2 had been added. This operation would be achieved much more speedily and precisely once the necessary experience has been gained or if the usual automated monitoring and dosing methods had been used.

During the first of the two HWT runs, the dip was monitored at appropriate intervals, and further additions of ClO_2 made as judged necessary. Initially the ClO_2 level fell rapidly, probably as a consequence of the dip contacting fresh bioload when pumped to the other treatment tank. During treatment the ClO_2 concentrations fluctuated widely between 0.44 and 7.65ppm, ending close to the target concentration at 4.72ppm. Over the 3h period, an additional 22.5L of ClO_2 was added, making a total of 118.0L ClO_2. This is illustrated in Figure 1, where the red triangles show the total amount of ClO_2 added, and the black lines/triangles shown the actual ClO_2 concentrations in the tank.

On the second day of testing, an additional 19.25L of ClO_2 was added in order to approximate to the target concentration (4ppp ClO_2), and an additional 16.0L ClO_2 was added over the HWT period. During the 3h period, the ClO_2 concentrations fluctuated between 0.35 and 9.26ppm, though ending lower than intended (0.08ppm) and indicating the need to continue monitoring ClO_2 concentrations throughout HWT.

While the amount of ClO_2 added may seem large – 118L on day 1, plus 35L on day 2 – this was starting with plain water. Conventionally, the full rate of biocide would be added to the HWT tank at start-up (followed by regular topping-up). For a capacity of comparable size to the system as used here - 21,000L - initial additions of 105L of commercial formalin, or of 168L ‘FAM 30’, would be required, at the usual rates. Hence the quantities of the three biocides required are of the same order.

The ‘ChlordioXense’ meter was easy to use in the field situation, is relatively inexpensive, and gives the actual levels of ClO_2. The two types of ‘dip-sticks’ used, however, indicate only the potential concentration of ClO_2; there did not seem to be any correspondence between the different measurement methods, and the ‘dip-sticks’ used seemed to be little value in this situation.

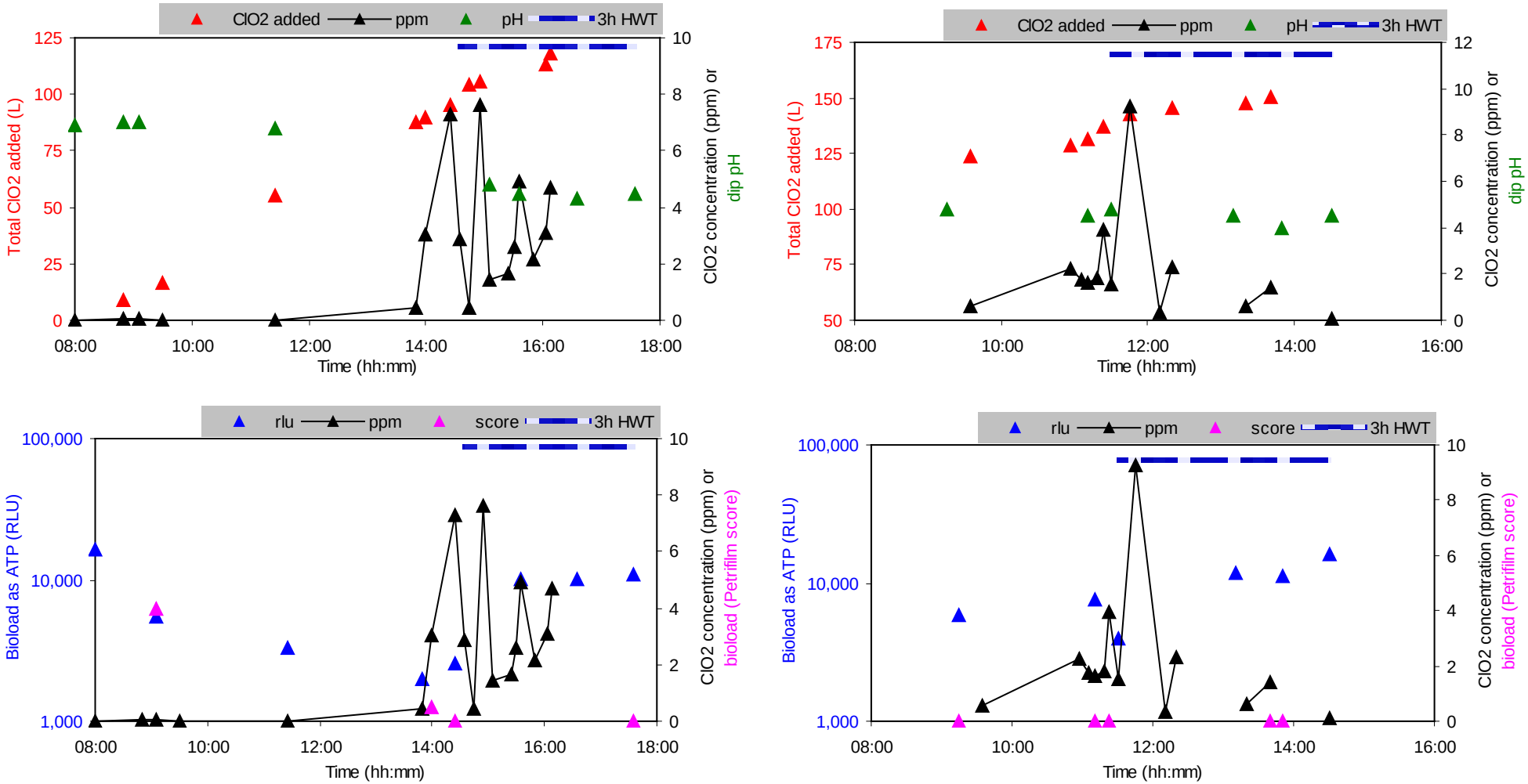
The bacterial ***bioload*** was estimated at intervals through the operation using the 'Petrifilm' system. Near the start of the test, this showed a 'highly polluted' dip (score 4.0); 5h later a very low score of 0.5 was indicated, and thereafter all samples were entirely free of bacterial bioload. This is illustrated in Figure 1, where the pink triangles show the 'Petrifilm' scores; the blue triangles show bioload measured as ATP (see also Table 1).

Measuring bioload as ATP, very high levels were found in the untreated water taken from the treatment tank (16,000RLU), and even higher levels in water remaining in the header tank (25,000RLU, not shown in Table 1). While adding ClO_2 prior to HWT, ATP levels fell to around 2,000RLU prior to the start of HWT, during which higher levels of about 10,000RLU were recorded, perhaps not surprising in the light of the large amounts of soil and potentially diseased bulbs being treated. On day 2 ATP levels were higher, about 4,000RLU near the start of HWT and then rising, reaching 16,000RLU by the end of treatment.

The ***pH*** of mains water at the test site was *ca* 7.0, and during the work the pH of the dip fell, with a low point of 4.0, due to the acidic components. This is not an acidity that would be expected to result in damage to daffodil bulbs, and would also be compatible with the use of some fungicides. This is illustrated in Figure 1, where the green triangles show pH (see also Table 1).

From the viewpoint of ***operator safety***, no hazards were noted other than the obvious ones of needing care and protective gloves when making up the two reagents for adding to the tank (which would be unnecessary where monitoring and dosing equipment were being used). The characteristic odour of ClO_2 was noticeable near the top of the tank to both experienced and inexperienced operatives, but not until a relatively high concentration (7ppm) had been reached. The smell was not considered unpleasant or irritant.

Figure 1. Data for HWT using ClO_2 with day 1 on left, day 2 on right; the actual periods of HWT are shown by the blue bar. Top figures: total ClO_2 added, ClO_2 concentration in dip, and dip pH. Bottom figures: ClO_2 concentration in dip (repeated from the top figures), and bioload as ATP concentration and 'Petrifilm' score



Discussion

Although the work described here was only the first step in evaluating ClO_2 for use in the UK bulb industry, it yielded a number of useful conclusions.

1. There was evidently an enormous initial bioload present in the HWT system, thought to be largely due to sediments that had accumulated in the holding (or slave) tank which, not unusually, presented difficult access for cleaning purposes. This emphasises the need for a thorough cleaning of the tanks and associated equipment prior to the start of the bulb dipping season. However, once the initial bioload had been counteracted, any further bioload introduced into the tank should be completely controlled by maintaining an appropriate ClO_2 concentration. This factor should be taken into account in the design of HWT systems. Possibly, the formalin previously used in HWT tanks was powerful enough to deal with such bioloads.
2. To our colleagues from Tristel PLC, the 3-hour treatment time and 5ppm concentration used here and in US trials, seemed excessively long and high compared with the ClO_2 treatments used in other applications. It was suggested that, providing any initial bioload had been dealt with, the operating concentration could perhaps be reduced; alternatively, the length of exposure to ClO_2 could be reduced, either by maintaining the required concentration only for a short part of the 3-hour HWT period, or by giving the ClO_2 treatment as a separate, short post-HWT dip (though this would be inconvenient for growers). It is important to measure actual, rather than potential, ClO_2 concentrations.
3. No other concerns about using ClO_2 for daffodil HWT were identified. The use of ClO_2 resulted in an acidic, but not excessively acidic, dip that settled at about pH4, insufficiently acid to damage daffodil growth (see report of Project BOF 43). ClO_2 was not unpleasant to work with, given the usual precautions, but, given the fluctuations in ClO_2 concentrations observed, an automatic monitoring and dosing facility would be essential.

At this stage HWT with ClO_2 cannot be recommended to growers, as we simply do not know enough about it. First, the treated crop needs to be examined in spring 2011, to ensure no obvious damage has been caused to the crop. Secondly, to ensure an understanding of the mechanisms involved, laboratory-based assays of the effect of ClO_2 on the base rot pathogen (chlamydospores and conidia), stem nematode (active and wool-stage nematodes) and typical bioload organisms should be evaluated. Thirdly, a longer-term assessment should be made, using fully monitored and dosed ClO_2 over an HWT programme lasting several weeks; preferably, measurements of any effects of the treatment on bulb and flower yields and quality should be incorporated. Finally, the legal status of ClO_2 formulations needs to be clarified, whether regarded as a biocide for disinfection of facilities and water (clearly very necessary) or re-classified as a pesticide (for pest and pathogen control). Currently, Tristel PLC is supporting ‘sodium chlorite for the generation *in situ* of

chlorine dioxide' under the EU Biocidal Products Review for the relevant 'product type' (PT2), and this needs to be supported by the HDC and followed through to ensure the method becomes available to growers.

Using ClO_2 could have many advantages for bulb growers – general effectiveness, ease of monitoring and regulating solutions, and absence of harmful end-products. The only challenger so far identified, an iodophore biocide ('FAM 30'), may have limited use unless the problem of the rapid loss of its active substance (iodine) in bulb dips can be remediated.

Acknowledgements

The HDC and the author are grateful to Tom Charlton of TH Charlton & Son Ltd for providing ready access to his HWT facilities along with the bulbs for treatment. We thank Rob Holland and colleagues (Tristel PLC) for providing equipment and reagents and for carrying out the treatments, and James Avery (Green-Tech Horticulture) for his facilitation of the project. The author thanks Prof Gary Chastagner (Washington State University) for sharing the results of his investigations and for helpful discussions.

Appendix I – Background information

Narcissus: Chlorine dioxide - a potential disinfectant for use in hot-water treatment and other bulb dips

Introduction

Chlorine dioxide (ClO_2, here abbreviated to CD) is a general disinfectant that is being increasingly used instead of chlorine to treat drinking water and sanitise meat, vegetable and fruit processing facilities. The brief review in this introduction has been condensed from that of Chastagner & Riley (2005).[1]

- CD has greater biocidal activity than sodium hypochlorite, iodine, quaternary ammonium compounds, glutaraldehyde and phenol.
- Compared with other hypochlorites, CD is less affected by pH, less reactive to organic or inorganic materials, removes phenolic tastes and odours, and produces fewer (or no) toxic or carcinogenic by-products.
- CD can be used to sanitise tanks used for aseptic juice storage.
- Direct application of CD post-harvest to peppers killed *Escherichia coli* on the fruit surface, and low concentrations applied to inert surfaces killed a number of plant pathogens on them.
- Studies have shown that water-based application of CD has the potential to control the spread of pathogen inocula, while direct application of CD controlled inocula on leaf surfaces, with several plant species being uninjured by multiple exposures to CD (up to $20mg/L^{-1}$).
- Exposing tulip bulbs and cut-flowers to CD ($5\text{-}20mg/L^{-1}$) significantly reduced the development of *Penicillium* blue mould on the bulbs and of *Botrytis* on the flowers.
- The activity of CD was reduced less quickly by the presence of organic matter in dump tanks than was plain chlorine.

Although the concentrations, exposure periods and phytotoxicity need to be examined further, controlled-release CD technology could have an application in bulb and cut-flower storage. Further, Chastagner & Riley (2005) suggested that, since CD is very active in oxidising organic compounds, it may have an application in treating ethylene-related problems in sensitive bulbs and cut-flowers.

The HWT of narcissus in the USA – and the role of CD

Since the 1930s formaldehyde has been considered essential in the production of narcissus bulbs.[2] As 'commercial formalin' (a saturated aqueous solution of formaldehyde) it has been routinely added to pest- and disease-controlling bulb dips either at ambient temperatures or as part of HWT. In the case of HWT usage, formaldehyde is a control for stem nematodes (*Ditylenchus dipsaci*) and also gives incidental control of other pests and some control of fungal pathogens, primarily base rot (*Fusarium oxysporum f.sp. narcissi*). This, at least, has been the scenario for using formaldehyde in UK and Dutch bulb-growing. However, in the USA formaldehyde appears to have been considered primarily a control for base rot, "although the addition of formaldehyde gives some slight improvement in nematode kill" (Chastagner & Riley, 2002[3]). In the USA, restrictions on the use of formaldehyde by bulb growers in about 2000, have been estimated by the Washington State Department of Agriculture to have resulted in a loss of $16m by the State's growers (2002 figures), though some growers received 'third-party registrations' that allowed them to continue using formaldehyde for this particular purpose. Likely alternative disinfectants, including CD, have therefore been investigated by Prof. Gary Chastagner at Washington State University's Research & Extension Center at Puyallup.

Chastagner & Riley (2002) evaluated the effect of CD on a high concentration of base rot inoculum in HWT at 43-44°C. In a control (plain water) tank, the initial inoculum level was

reduced by 68, 75, 94 and 99.8% after ½, 1, 1½ and 2 hours, respectively. **In the tank with CD (at 2.5 mg/L^{-1}) no viable inoculum was detected after 5 minutes.** Discs of bulb scale were also placed in the tanks, and the numbers of discs subsequently developing infections were determined. In this experiment all discs from the control treatment became infected, while none of those treated with CD did. Other tests, using lower levels of inoculum, showed that lower CD concentrations (1.0 or 1.5 mg/L^{-1}) also resulted in no viable inoculum being recovered after a few minutes' treatment.

Chastagner & Riley (2002) also carried out field trials. Bulbs of narcissus 'Dutch Master' received HWT for 4 hours at 43-44°C, in 0.5% formaldehyde or in CD at 5 or 10 mg/L^{-1}. There were controls that received HWT in plain water only, or were entirely untreated. Bulbs treated in plain water died-back prematurely and showed reduced yields and very high levels of base rot. Treatment with either formaldehyde or CD (at either concentration) resulted in delayed die-back, increased yields and lower base rot levels, compared with the plain water control. The lower concentration of CD was as effective as the standard concentration of formaldehyde.

Chastagner & Riley (2005) showed that CD has a higher biocidal activity against several micro-organisms, on a concentration basis, than sodium hypochlorite, iodine, quaternary ammonium compounds, glutaraldehyde and phenol. Trials showed that CD (5-10mg/L^{-1}) effectively controlled *Fusarium* inocula levels during daffodil HWT, reducing the spread of base rot. The bulbs showed no adverse effect of this treatment. Inocula of *Alternaria alternata*, *Botrytis cinerea*, *Fusarium oxysporum f.sp. narcissi*, *Penicillium corymbiferum* and *Rhodococcus fascians* were exposed to various concentrations of CD (0 and 5 to 1000mg/L^{-1}) for 1 hour at 20°C. No spores germinated following treatment at 25 mg/L^{-1} and germination was largely inhibited even at 5 mg/L^{-1}. These results are summarised in the table below, but note the rider that *in this experiment* the percentage germination of base rot spores was low.

Percentage reduction in spore germination after 1-hour CD treatments at 20°C. In the case of *Fusarium*, although the treatments were successful, the percentage of spores germinating in controls was low (34 and 2% for micro- and macro-spores, respectively), so *these* results need to be confirmed.						
CD concentration (mg/L^{-1})	*Alternaria*	*Botrytis*	*Fusarium*		*Penicillium*	*Rhodococcus*
			micro	*macro*		
5	90	97	100	100	93	79
25	100	100	100	100	100	100

Chastagner & DeBauw (2008)[4] reported that "chlorine dioxide is an effective replacement for formaldehyde" in HWT for the management of base rot. In one experiment a stock of daffodil bulbs treated in plain water had 98 bulbs with base rot, while non-HWT bulbs and bulbs HWT in formaldehyde (0.5%a.i.) or CD (at 5 or $10mg/L^{-1}$) had 12 to 18 bulbs affected.

Copes *et al.* (2004)[5] studied the activity of CD in solutions of different ions and pH values against micro- and macro-conidia of *Fusarium oxysporum* f.sp. *narcissi* (and conidia and aleuriospores of *Thielaviopsis basicola*). CD had a similar effect on both propagules of both species, and there were interactions among the divalent metal ion solution, nitrogen and hard water solution and pH treatments. A higher concentration of CD was required at pH 8 than at pH 5 to achieve a 50% lethal dose (LD_{50}). The addition of the divalent metal ion solution required an increase in CD concentration to maintain the LD_{50}. When combined with the nitrogen and hard water solution, the divalent metal ion solution placed a higher demand on CD at pH 5 and a lower demand on CD at pH 8, requiring an increase and decrease in a CD concentration, respectively, to achieve the LD_{50}. The CD doses resulting in 50% mortality ranged from 0.5 to 7.0 mg L^{-1} for *F. oxysporum* conidia.

Unfortunately, the effect of CD on stem nematode does not appear to have been published. However, at least one Washington State grower has routinely used CD for over 5 years with apparent success. A literature search on CAB Abstracts covering 1973 to week 51 of 2009 found:

- No references combining CD and stem nematode or *Ditylenchus dipsaci*
- Three references (all cited above) combining CD and narciss*/daffodil$
- Five references combining CD and *Fusarium*, describing the effects of CD on the control of *Fusarium* species pathogenic on other crops; one of these[6] described the inconsistent effects of CD in controlling *Fusarium* dry rot of potato, due to different methods of activating and diluting the reagent solutions.

Commercial equipment for CD application

In the USA, several companies market automated CD-generating systems for agricultural uses, usually generating CD on site. The technology is not regarded as particularly specialised (Chastagner & Riley, 2002, 2005). In the research at Puyallup the 'Fresh-Pak' CD system (CH_2O International Inc, Olympia, WA) was employed. This produces CD by mixing sodium chlorite with hydrochloric acid via sensors and computer-controlled mixing. A quick survey of the internet found UK firms dealing with chlorine dioxide. The concentrations of CD can be measured using a standard kit, this depending on whether free chlorine or chlorine dioxide is present.

HDC-funded projects

Following a literature review (Lole, 2006)[7], Lole (2007)[8] included CD amongst several candidate disinfectants tested for the control of the 'wool' stage of stem nematode and of the chlamydospores of base rot fungus under HWT conditions (3 hours at 44.4°C). Both nematode wool and base rot chlamydospores were used as fresh material collected from infested daffodil bulbs, either following air-drying for a few weeks (wool) or culturing on Nash Medium and then potato dextrose agar medium (chlamydospores). CD was used as 'Harvest Wash' at 50mg $a.i.L^{-1}$ (0.25ml 'Harvest Wash' in 100ml water, plus 0.025g activator), and the control was plain tap water.

It was found that HWT alone was sufficient to kill all *nematodes*, irrespective of whether a disinfectant was added. The test was repeated at room temperature (*ca*. 18°C), when mean nematode survival with CD was 27%, similar to the result in the plain water controls, while treatment with either hydrogen cyanamide (as 'Cultamide') or an iodophore disinfectant ('FAM 30') resulted in total kill. These results with iodophore disinfectant confirmed earlier results (Lole, 1990).[9] Consequently only 'FAM 30' was taken on to further trials against nematodes.

The test against *base rot* chlamydospores at 44.4°C showed that plain HWT had no effect on chlamydospores survival. HWT with CD was also ineffective. However, adding hydrogen cyanamide, iodophore disinfectant or chlorothalonil (as 'Bravo 500') each gave total control of spores. As a result of these tests, CD was also eliminated from further trial with base rot in this project.

The phytotoxicity of these treatments on daffodil bulbs was also recorded in a small-scale, one-year-down trial using the same biocide concentrations and HWT temperatures as described above (Lole, 2008).[10] Some of the tested materials, including hydrogen cyanamide, produced a variety of phytotoxic symptoms, whereas there were no adverse symptoms in bulbs treated in plain water, CD, iodophore disinfectant or chlorothalonil.

A larger field trial was set up in 2008, using a stock of narcissus 'Golden Harvest' bulbs that showed a high level of base rot symptoms and were also infested by adding 'Dutch Master' bulbs that had well-developed stem nematode infections (Lole & Hanks, 2009).[11] Using commercial-type HWT equipment, the bulbs were given HWT (3 hours at 44.4°C) with plain water, standard-rate formalin, full- or half-rate iodophore disinfectant 'FAM 30' or full- or half-rate chlorothalonil as 'Bravo 500'. In the first growing season, in the control plots (HWT in plain water) there was poor bulb survival and shoot emergence. However, the bulb plots treated with formalin or either rate of iodophore disinfectant or chlorothalonil all grew and flowered normally, with similar flower yields and leaf growth. This trial will be fully assessed after the second growing season, in 2010.

A second field trial was set up in 2009 (BOF 61b). In this, stocks of eight narcissus cultivars were HWT (3 hours at 44.4°C) with plain water (control), half- or full-rate iodophore disinfectant, half- or full-rate chlorothalonil, or half-rate iodophore disinfectant *plus* half-rate chlorothalonil. This trial will be the subject of an HDC Open Day in spring 2010. Three replicate blocks will be lifted and bulb yield and quality assessed in summer 2010, and the remaining three replicates in 2011.

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Appendix II (Table 1)

Table 1
Narrative of ClO_2 dosing, HWT operations and dip readings
(periods of HWT shown by shading)

Date	*Time*	*Operations*	*Dip pH value*	*Dip bioload*		*Dip ClO_2 (ppm)*		
				ATP (RLU)	*Bacteria (score)*	Chlordio -Xense*	*BCI stick**	*ITS stick**
05 Aug	08:00	Plain water in RH tank sampled	6.9	16,241	-	0	-	0
	08:50	8.8L ClO_2 had been added, tank sampled	7.0	-	-	0.03	10	0
	09:05	Tank sampled	7.0	5,484	4.0	0.02	-	-
	09:30	A total of 16.5L ClO_2 had been added	-	-	-	0.03	-	-
	11:25	A total of 55.0L ClO_2 had been added, tank sampled	6.8	3,295	-	0.02	-	0
	13:50	A total of 88.0L ClO_2 had been added, tank sampled	-	1,973	-	0.42	-	-
	14:00	A total of 90.0L ClO_2 had been added, tank sampled	-	-	0.5	3.03	-	-
	14:25	A total of 95.5L ClO_2 had been added, tank sampled	-	2,581	0	7.27	-	-
	14:30	LH tank had been loaded with bulbs, dip pumped from RH to LH tank	-	-	-	-	-	-
	14:35	3h HWT started in LH tank	-	-	-	2.88	-	>1.6
	14:45	A total of 104.5L ClO_2 had been added, tank sampled	-	-	-	0.44	-	-
	14:55	A total of 106.0L ClO_2 had been added, tank sampled	-	-	-	7.65	-	15
	15:05	Tank sampled (this is the first point at which the characteristic smell of ClO_2 could be detected close to the tank)	4.8	-	-	1.45	-	-
	15:25	Tank sampled	-	-	-	1.65	-	-
	15:30	Tank sampled	-	-	-	2.61	-	-
	15:35	Tank sampled	4.5	10,207	-	4.89	-	6
	15:50	Tank sampled	-	-	-	2.14	-	-
	16:03	A total of 113.5L ClO_2 had been added, tank sampled	-	-	-	3.11	-	-
	16:08	A total of 118.0L ClO_2 had been added, tank sampled (last dose)	-	-	-	4.72	-	-
	16:35	Tank sampled	4.3	10,159	-	-	-	7
	17:35	Tank sampled; end of 3h HWT, dip pumped to RH tank and bulbs unloaded	4.5	10,939	0	-	-	4

Date	Time	Operations	Dip pH value	Dip bioload		Dip ClO_2 (ppm)		
				ATP (RLU)	Bacteria (score)	Chlordio -Xense*	BCI stick*	ITS stick*
06 Aug	09:00	Previously treated water had been pumped from RH tank to LH tank and 1,400L untreated water top-up added from holding tank	-	-	-	-	-	-
	09:15	Tank sampled	4.8	5,935	0	-	-	<0.1
	09:35	A further dose of 5.5L ClO_2 had been added, tank sampled	-	-	-	0.58	-	-
	10:57	A total further dose of 11.0L ClO_2 had been added, tank sampled	-	-	-	2.24	-	-
	11:05	Tank sampled	-	-	-	1.75	-	-
	11:11	A total further dose of 13.75L ClO_2 had been added, tank sampled	4.5	7,619	0	1.63	-	0.15
	11:18	Tank sampled	-	-	-	1.79	-	-
	11:23	A total further dose of 19.25L ClO_2 had been added, tank sampled, pumped to RH tank which had been loaded with bulbs	-	-	0	3.93	-	-
	11:30	RH tank now full, 3h HWT started	4.8	3,962	-	1.55	-	0.6
	11:45	A total further dose of 24.75L ClO_2 had been added, tank sampled	-	-	-	9.26	-	-
	12:10	Tank sampled	-	-	-	0.35	-	-
	12:20	A total further dose of 27.5L ClO_2 had been added, tank sampled	-	-	-	2.30	-	-
	13:10	Tank sampled	4.5	11,733	-	-	-	<0.1
	13:20	A total further dose of 29.75L ClO_2 had been added, tank sampled	-	-	-	0.61	-	-
	13:40	A total further dose of 35.25L ClO_2 had been added (last dose), tank sampled	-	-	-	1.44	-	-
	13:50	Tank sampled	4.0	11,301	0	-	-	<0.1
	14:30	Tank sampled, end of 3h HWT	4.5	16,234	0	0.08	-	<0.1

* The spectrometer (ChlrordioXense) measured ***actual*** ClO_2 levels in samples. Titration methods – on which the chemical reactions of the 'dip-sticks' are based – measure the sodium chlorite available to produce ClO_2, i.e. ***potential*** ClO_2 levels.

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Gordon Hanks, 04 January 2010