WHAT LIES

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about the author

After leaving University in 1991 with a degree in Chemistry & Management I have worked predominantly in the South East of England for four water treatment companies, eventually establishing my own Legionella Risk Assessment & Consultancy Business in 2007. The discussion and conclusions presented within this paper stem from personal experiences and knowledge gained whilst working in the industry over the past 20+ years. It is hoped that it will raise awareness of some key issues, enabling the industry to move forward with solutions to the problems identified.

BACKGROUND

GRP Tanks have been increasingly used for stored water over the past 50 years. However, their wide spread adoption in the UK coincided with the HSE ACOP & guidance on the prevention of Legionnaires Disease (HS(G)70 & L8).

The use of steel tanks for the storage of domestic potable water has been all but eliminated in new buildings. This is partly due to the legionella bacteria requiring iron as a nutrient, and partly due to the negative publicity derived from pictures of heavily corroded tanks, purported to have provided a perfect habitat for bacteria, including legionella.

GRP Tanks have been marketed as: -Maintenance free, corrosion resistant, inert/smooth anti-bacteria surfaces, providing excellent hygiene qualities.

Over the past 15 years, whilst carrying out inspections of CWST's, I have become increasingly aware of an issue with GRP Tanks. During their visual inspection it was routinely noted that small semi-spherical cysts appeared to be adhered to the side (& less often the base) of the tanks. These "cysts" varied in size from 1-2mm to 15mm in diameter, and the colour varied from cream to black.

Traditional sampling of these cysts by a laboratory for TVC analysis proved to be of little benefit, as the test is designed for a 1ml sample rather than a semi-solid. It was whilst speaking to a laboratory manager in 2007, that the idea of looking at the "cysts" under a microscope was first suggested. The resulting 9 seconds of video from a microscope camera proved that there was an issue which required further investigation (and also resulted in the replacement of the tank that was the source of the cyst).

Over the past 5 years, by using a 60 year old Watson microscope and fitting it with a 1.3 Mega pixel USB digital camera, allowing stills and video to be captured, I have built-up a library of higher organisms that have been isolated in cysts taken from GRP Tanks. The images to the right are, admittedly, rather amateur, nevertheless I believe that they convey the evidence found. (NB. Video footage is available on the envirosafeuk.co.uk website -the video truly brings to life the still photographs!)

SHOULD WE BE CONCERNED?

Whilst the microscope does allow us to see bacteria, due to its limited resolution and power, they only really appear as moving 'dots' (optical magnification of up to x230). What we do know is that nematode worms feed on bacteria and other organic matter, whilst also supporting the growth of bacteria within the worms. Therefore, the presence of nematode worms is a good indication of a well-established biofilm. Most nematode worms are thought to be harmless to humans, although some may be pathogens or may carry pathogens.

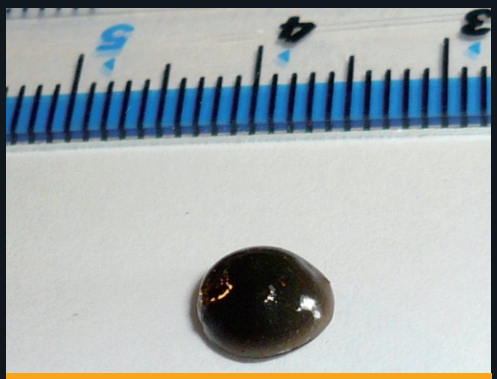
Work carried out to study the water quality of one site with an infected tank showed that the levels of bacteria, including pseudomonas aeruginosa, fluctuated with the ambient temperature. However, the bacteria levels in the down services quickly rose as the tank water temperature increased, even though the tank water rarely hit 20°C (only when the incoming mains water went above 20°C). The tank was replaced 2 years ago, and only the new tanks and new pipework were chlorinated (the site is an old people's home so chlorinating the down services is difficult). Since the new (large plastic) tanks have been installed, the water quality results have not shown the same "bloom" in bacteria levels during the summer months. The broad conclusion from these results is that the cysts/biofilm were very much alive during the winter, and were actively seeding the down services during the warmer months.

GRP TANK MANUFACTURE

Whilst removing the cysts from the sides of tanks for examination, it was noted that the cysts usually coincided with a raised "blister" area on the surface of the GRP. These blisters on the tank surface led us to believe that the cysts were actually growing out from the damaged surface of the tank.

GRP tanks are manufactured in 2 main ways:

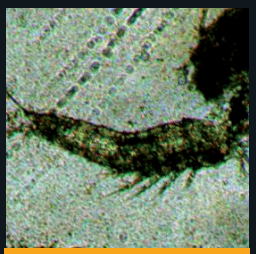
Cysts growing on right panel of a sectional tank, note the left panel is clear.



Cyst on a microscope slide

Human hair over Nematode x100

Nematode x100



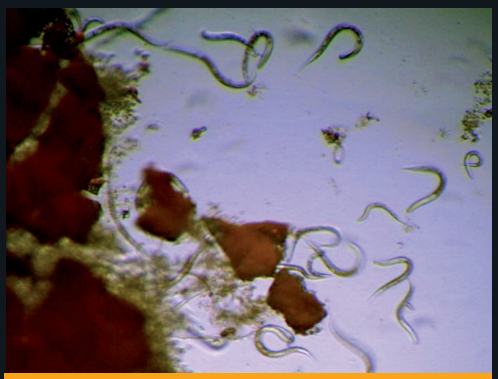
Unidentified larvae x100

Ciliate x400

BENEATH

einforced Plastic (GRP) Cold Water Storage Tanks (CWST) and the efficacy of current cleaning practices

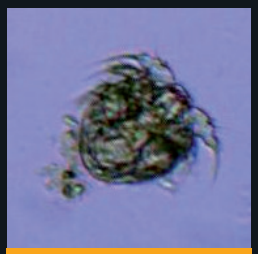
Close-up of a cyst on the surface of a GRP Tank



Nematode worms x40

Rotifer x200

Ameboe x400



Unidentified Mite x400

Water Bear x400

-Sectional tanks are usually manufactured utilising the "hot-pressed" method, whilst one-piece tanks are usually manufactured by hand laminating, with sheets of fibre glass being layered over a mould.

Both manufacturing methods utilise layers of fibre-glass and resin. The coating that comes in contact with the water is called the gel coat, and this utilises a very fine glass mesh to give a smooth appearance.

The visible blistering on the surface of the GRP surface is also known as osmosis, and this phenomenon has been well documented, with regard to fibreglass boats and yachts, for many years.

OSMOSIS

The Gel coat on a GRP/fibreglass tank is not completely perfect/homogenous so, as a result, water molecules can pass through the surface of the tank and ultimately through the whole tank. This "leaking" process happens so slowly as not to be noticeable. However, when the water molecules encounter air gaps, unreacted resin or other soluble material within the GRP matrix (such as the emulsion binder that is used in the manufacture of the fibreglass matting), the result is a build-up of pressure within the GRP matrix. This pressure build-up within the tanks structure manifests itself as a surface blister.

PHANTOM OSMOSIS

A second pathway to the formation of surface blisters is believed to be due to poor manufacturing techniques. During the layering of the fibreglass, it is vitally important to squeeze out any air trapped between the layers. This is more common in hand laminated one-piece tanks, where applying an even pressure around corners and over large areas is more difficult. The trapped air can rapidly track towards the surface of the tank wall from within the matrix during the curing process and result in surface bubbling, even before the tank has left the factory or relatively quickly thereafter.

Where sectional tanks have been found to be suffering from Osmosis, it is not uncommon to find perfectly sound sections next to very poor sections and, more often than not, the poor sections are "specials" rather than the basic panel.

THE TANK MANUFACTURERS' VIEW

Once the problem with Osmosis had been identified, we did speak to a number of tank manufacturers. Some were in denial, some claimed that only the low cost manufacturers (who used substandard raw materials and techniques) suffered from this problem and some blamed the sites. The last opinion was based on the belief that sites were allowing hot water into tanks (from calorifier vents or faulty check valves). Excessively hot water can force any trapped air to the surface, resulting in damage to the tank coating. This may actually be the case in a few instances. However, it cannot explain the instances where osmosis has been noted in tanks not connected in any way to a heat source!

THE PERFECT HABITAT

In relation to the size of a water molecule, bacteria are huge (approximately 100,000 times larger), therefore bacteria cannot normally penetrate a GRP Gel coating. However, as the osmotic pressure builds up inside the blister, the Gel coating is stretched and finally cracks. These cracks provide the perfect habitat for the establishment of a biofilm.

The progression in the life cycle of a biofilm has been studied previously. By the time the biofilm is visible, growing out of the blister on the side of the tank, the biofilm has usually progressed to the point where higher life forms, such as nematode worms, are already living within the biofilm (live nematode worms have been found in over 90% of the cysts that have been microscopically examined).

TANK INSPECTIONS

HSC L8 specifies that cold water storage tanks are visually inspected on an annual basis. It is our experience that routine tank inspections carried out by site operatives or subcontracted water hygiene "specialists" do not identify the presence of cysts growing out of the tank's walls. The cysts are either ignored as insignificant, or totally missed, especially the lighter coloured cysts, which can blend in with the colour of the GRP. This is not totally surprising as there is a lack of knowledge within the industry, which is one of the main reasons for writing this paper.

TANK CLEANING

When GRP tanks are cleaned, the cleaning operatives focus their attention on the floor of the tank, removing the suspended solids that have entered the system from the supply. Unfortunately, the walls of the GRP tanks are not always washed down, so cysts remain untouched and continue to grow.

During a discussion with one cleaning company concerning the presence of numerous visible cysts on the walls of a tank they were cleaning, we were informed how the cleaning operatives had tried to remove the cysts, removing the blistered gel coat entirely in places, leaving a rough surface. However, 2 weeks later the cysts were back and already full of nematodes, suggesting that the higher life forms were able to "shelter" in the blisters during the clean.

TANK REFURBISHMENT.

Once we had established that cleaning the tanks, in line with HSC L8, was ineffective, we considered tank relining using a polyurethane coating. It was hoped that the surface preparation (grinding off the gel coat) and painting would reseal the damaged tanks surface. This process was carried out on two large one-piece tanks (2.5 & 5m3). During the annual tank inspection of both of these tanks, following the refurbishment, it was noted that whilst the number of cysts had dramatically reduced, cysts where still found. They had obviously penetrated the new polyurethane coating, possibly from the tank structure outwards.

L8 CHLORINATIONS

The conclusion drawn from the above results was that the biofilm was surviving within the GRP matrix of the tank wall.

We were then asked to carry out an emergency chlorination on a small one-piece tank that had high pseudomonas levels. The tank in question held only 300 litres of water and acted as a break tank, supplying a hospital endoscopy cleaning machine. In the past 6 years, this tank had been replaced 3 times (one tank only lasted a day due to poor manufacture, the second tank less than a year, due to osmosis – both replaced FOC under warranty by the supplier). The third tank had been flagged up during the site's last tank inspection as showing the signs of osmosis, and active cysts. It had been cleaned and chlorinated by the site's main water hygiene contractors 2 months earlier.

On arrival to site, the tank was inspected and the tank was found to be very clean, apart from several cysts which were noted growing out of the sides of the tank. The cysts were knocked off the surface of the tank in the belief that this would allow the chlorine to penetrate any surface cracks. The tank was then chlorinated to >100mg/l for 2 hours (double the L8 guidelines for both chlorine level and contact time).

The cysts that had been knocked off the tank walls had sunk to the bottom of the tank, so whilst draining down the tank, the cysts were collected (along with some of the chlorination solution) in a sterile sample bottle, for microscopic examination, which took place six hours after the sample was taken. Under the microscope, it was clear that the nematode worms in the cysts seem to have been completely unaffected by the chlorine and were very much alive (after 8 hours in chlorinated water).

The chlorine solution strength was checked and maintained at or slightly above 100mg/l and the cysts microscopically examined every 6 -10 hours until all of the nematode worms had died. This process took 36 hours before it was recorded that all the nematode worms were no longer moving. The only conclusion that can be drawn from these observations is that the standard L8 chlorination protocol is flawed.

OXIDISING BIOCIDES VERSUS THE NEMATODE

There are numerous papers that have been written on the study of the effectiveness of various oxidising agents versus bacteria. These trials usually utilise expensive laboratory equipment but, from the work we had already carried out, we devised a somewhat lower technology approach. It could be argued that this approach is more meaningful than many of its high-tech predecessors, as the results are visual and conclusive. In our opinion, there is little benefit in knowing that the bacterial levels have reduced by a factor of log 5, when there are higher life forms still swimming around with the ability to re-infect the system.

The results shown in Table 1 below should be taken as preliminary observations. At least two more complete set of tests need to be carried out to confirm the validity of the results. The results for Sodium Hypochlorite are in line with our previous findings.

The Hydrogen Peroxide test strips initially used to make up the stock solutions proved to be inaccurate and, as a result, the Peroxysil 50 solution was found to be over 1000mg/l. This was diluted and the test restarted, however, the solution was still found to be double the intended strength when rechecked. Similarly, the strength of both the bromine & chlorine dioxide solutions are expressed as free chlorine, however these solutions were only ever used as a rough comparison as their field use at this strength would be difficult.

It is therefore the buyer's responsibility to push manufacturers to offer extended warranties.

The cysts used in this test varied in size from 2 -5mm diameter semi spheres and they were all taken from the same tank. A stock solution volume of 20ml was added to a sterile specimen bottle along with 3-4 cysts. At each time interval, a cyst or part of a cyst was examined under the microscope. This process required the sample to be flattened under a cover plate, breaking any whole cysts open. After examination, the remains of the cyst were washed back into the specimen bottle, to allow comparison of the agent against whole cysts and smashed cysts (to test biofilm penetration).

Hypochlorite (active ingredient being Hypochlorous Acid), which is in line with previous research.

TEST CONCLUSIONS SO FAR

Taking into account the short-comings in the test methodology, the first conclusion is that all of the products failed in the time frame that is suggested by L8. Biofilm penetration is particularly poor for Sodium Hypochlorite (active ingredient being Hypochlorous Acid), which is in line with previous research.

Bromine could be generated from sodium hypochlorite and sodium bromide, but effluent discharge may be an issue. Further tests would be needed to prove its effectiveness, and its safe use at high concentrations would also need to be assessed.

Chlorine dioxide appears to have good biofilm penetration (in line with previous research) but at this concentration safety would be an issue, due to evolved chlorine dioxide gas.

B&V's new Abulox product is a stabilised form of Hypochlorous Acid, but the test results are not promising for this application -roughly in line with standard sodium hypochlorite generated Hypochlorous Acid.

Aquatreat's Peroxysil 50 stabilised Hydrogen Peroxide benefitted from being used at double the recommended strength. The bubbles that formed around the cysts (visible to the naked eye) were a clear indication that the product was reacting with the biofilm.

Huwa San TR50 was actually dosed at half the recommended level for domestic systems (100mg/l rather than 200mg/l) and seemed to lose its strength quickly, possibly due to the product reacting with the biofilm -further validation tests are required.

WHAT'S NEXT?

We have recently carried out a field trial using Peroxysil 50 on 4 tanks at 1000mg/l. This is the dosage rate recommended for cleaning cooling towers, and as we are dealing with a biofilm, this seems to be the next logical step (it was also the strength used by mistake in the laboratory trial, the results of which looked promising). The Hydrogen Peroxide products also benefit from being safe to use at these elevated concentartions. The contact time we used was in excess of 16 hours (overnight), which is somewhat longer than the manufacturers' recommendations for cooling towers.

The aim of this field trial was to determine if we could stop regrowth of the cysts following the clean. The tank surfaces were scrubbed clean, removing all the cysts from the surface of the tank, and then cleaned with an aquavac to remove as much biofilm as possible. This allowed the biocide instant access to the biofilm within the tank matrix, which would normally be protected by the external cyst. The effectiveness of this technique will take up to 6 months to evaluate and a further 6 months or more to establish a validated result, however, after just 2 months, one of the tanks with poor flow and therefore high temperatures (25°C) is already showing significant regrowth. The other 3 tanks currently look clear of any cysts.

THE CONCLUSIONS SO FAR

Anyone considering the installation of a new GRP tank should be aware that they are not all the same. It is unclear why suppliers don't offer extended warranties on the performance of their tanks. Longer 5 and 10 year guarantees were alluded to in the past, however, as costs have been squeezed these warranties have dropped to the standard 1 year, with caveats that make many of these warranties unenforceable.
It is therefore the buyer's responsibility to push manufacturers to offer extended warranties.

Landlords/Facilities Managers that find they have tanks with osmosis and cysts need to employ a suitably qualified person to carry out a risk assessment, taking into account the use of the tank water and the degree of damage to the tank, in order to instigate a suitable action plan (monitor, clean, refurbish or replace).

SUPPLEMENTARY DOSING

One solution that has not been discussed above is the use of a low level auxiliary biocide to continuously dose the system and inhibit the regrowth of a cyst after cleaning. Our current limited experience of this as a solution is inconclusive:

- On a large hospital site where the main supply is supplemented with chlorine dioxide the results are not promising. However, the worst tanks are those fed with softened water, where the softening process is known to reduce the chlorine dioxide reserves, or where the tank's turnover is low resulting in a loss of the reserve.
- A small tank with a continuous recirculation and redox controlled addition of chlorine dioxide has proven to have no visible regrowth.

The possible benefits of auxiliary dosing need to be weighed against the risks associated with using chemicals and any on-going maintenance requirements.

> "Anyone considering the installation of a new GRP tank should be aware that they are not all the same."

TABLE 1

	Time taken until dead Nematode worms are first noted	Time taken until all Nematode worms are dead
Sodium Hypochlorite @100mg/l	28 Hours	78 Hours +
Bromine (from BCDMH) @100mg/l as Cl	3 Hours	20 Hours
Ashland Generox 100 stabilised ClO2 @100mg/l as Cl	3 hours	20 Hours
B&V Abulox Hypochlorous acid solution @100mg/l	20 Hours	78 Hours
Aquatreat Peroxysil 50 Stabilised hydrogen peroxide solution @200mg/l	1 Hour	8 Hours
Huwa San TR50 Stabilised hydrogen peroxide solution 100mg/l	1 Hour	78 Hours

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Acknowledgements

Stansted Laboratories

Mercian Laboratory

Eurofin Laboratories

Dr G D Fowler – Imperial College, London.

Aquatreat Chemical Products Ltd for the supply of test quantities of both Huwa Sam TR50 and Peroxysil 50.

B&V Water Treatment for the supply of test quantities of Abulox.

Ashland Water Technologies for supply of test quantities of Generox100.