BIOMANIPULATING DIATOM GROWTH - AMMONIA AND CYANOBACTERIA REDUCTION IN WASTEWATER TREATMENT

***Dr Simon JC Tannock (AlgaEnviro Pty Ltd), Wakib Khan, Sonja Toft (Queensland Urban Utilities), Andrew Stevenson, Kambez Akrami, William Smith (Logan City Council), Brett Vurlow (South Gippsland Water*)**

ABSTRACT

Management of ammonia and cyanobacteria in wastewater lagoons is historically problematic. Diatomix is a micronutrient solution, using nano-scale silica with ten micronutrients only bio-available to diatom algae. Dosing is determined by correcting the wastewater N: P micronutrient ratio to that of the Redfield Ratio.

Diatomix has been trialed over the last 2 years with several Australian Utility Companies in their wastewater treatment and storage lagoons. The addition of Diatomix in the wastewater treatment systems has reduced cyanobacteria cell count by 75% to 99.6% over 10 weeks to 19 weeks respectively. At one site, the average cyanobacteria cell count was 1,583,000 cells/mL prior to the trial starting and 197,000 cells/mL over the same period (April and May) the following year when treated with Diatomix. At a second site, the ammonia concentration averaged 20.6 mg/L prior to the treatment and eight weeks later it averaged 1.1 mg/L.

At the sites tested the average percentage of cyanobacteria cells in the Total cell count was initially 85 – 100% (cyanobacteria cells/Total cells * 100%). With treatment, this percentage reduced to between 22 and 99%. In the trial where there was minimal change in the percentage of cyanobacteria within the Total cell count, there was however a 78% reduction in the cyanobacterial cell count from 2,550,000 cells/mL to 566,000 cells/mL.

There is no sustained increase in diatom counts at any site and this is likely due to two main factors; Firstly, algal cell count sampling has been for suspended algae only, no benthic sampling. Secondly, diatom algae are heavily grazed by zooplankton and invertebrate populations. Over time as these grazing populations grow their feeding pressure on diatoms is likely to reduce cell counts. In regards to ammonia concentration, by optimizing the micronutrient to nitrogen and phosphorus ratio the diatom activity increases with higher uptake of ammonia.

KEYWORDS

Cyanobacteria, blue-green algae, diatoms, ammonia, nutrients, wastewater, lagoon

PRESENTER PROFILE

Dr Simon Tannock is a Biotechnologist with a PhD in Environmental Engineering in wastewater and a Master’s Degree in the culture of microalgae. Combining his knowledge of microalgae culture and wastewater treatment, Simon has developed a unique system of biomanipulating wastewater lagoon and water storage systems to manage nutrients and the algal /zooplankton /invertebrate populations for better treatment outcomes. Thus, taking a preventative approach rather than the customary reactive approach as is currently the norm.

1 INTRODUCTION

Managing wastewater treatment, effluent water storage and raw water storage systems, such as lagoons, dams and reservoirs, requires the monitoring and management of various parameters in these water systems to ensure adequate treatment, maintenance of water quality and suitability of the water for use over long periods of time. The biology and species present in these treatment and storage systems is important as this can greatly affect the health of the entire treatment or storage system.

The presence of high levels of cyanobacteria (commonly called Blue-green algae), macroalgae and macrophyte weeds (e.g. Water net, Hornwort, Salvinia) can all negatively impact the health and use of the water. Most commonly, it is the availability of nitrogen and phosphorus that affect the presence of unwanted plants/algae in these systems. Biomanipulation through micronutrient addition can be used to prevent or greatly reduce the presence of species that affect the degree of wastewater treatment in a lagoon system or disrupt the long-term health of a water storage system.

Diatomix is a nano-silica micronutrient mixture manufactured by Australian company, AlgaEnviro Pty Ltd. This proprietary product has ten of the micronutrients required for growth of diatom microalgae adsorbed onto amorphous silica structures that are in the nano-scale of 5 – 20 nm in diameter. As diatoms are one of the few algae that have a requirement to take up silica, the micronutrients in the silica structures are only bio-available to the diatoms, thus the beneficial and essential micronutrients taken up with the Diatomix silica only enhances diatom growth.

With a micronutrient boost, diatoms can successfully out-compete other algae for the main nutrients, nitrogen and phosphorus (N and P). The ensuing reduction in available inorganic nitrogen and phosphorus in the water column can reduce the growth and presence of blue-green algae and larger macrophyte water weeds. The biomanipulation of a biological system to reduce a biological problem requires less energy and has no known detrimental effects upon the environment in regards to improving water treatment and water quality.

The liquid product has successfully been used in Australia for more than two years with results to-date demonstrating that treatment has reduced blue-green algae populations, and ammonia levels have also been reduced by up to 99%. One aspect of the use of the product that has yet to be fully investigated is the effect that dosing has on the higher trophic levels of the ecosystem in the waterbody. Anecdotal evidence has been noted that the invertebrate populations and the vertebrates such as fish and turtles do well and increase in number and activity after longer periods of dosing treatment. Complete surveys of these populations have yet to be initiated as they are not of operational interest to the wastewater operator.

This paper presents the results of a range of case studies from different water treatment or water storage systems. The case studies have trialed the product in wastewater treatment lagoons, wastewater effluent storage and raw water storage dam. The case studies have run from ten weeks to 20 months.

Most sites had unique or differing approaches to the method or timing of dosing as well as differences in the water being treated (i.e. raw water, sewage treatment or treated effluent) and there have been a range of responses that have provided valuable insights into the use of Diatomix and the most notable trends are presented.

2 DISCUSSION

2.1 CALCULATING THE REQUIRED DOSAGE

The volume of the product that should be dosed at a site is based on the Redfield-Brzezinski nutrient ratio (Brzezinski, 1985) and the findings of research in regards to the extended Redfield Ratio (Twining & Baines, 2004) that includes the ratio between nitrogen and iron. The Redfield-Brzezinski ratio is the atomic ratio of carbon, nitrogen, phosphorus and silica found in diatoms. The Redfield-Brzezinski nutrient ratio is stated to be C:Si:N:P = 106:15:16:1. With the addition of Fe into this ratio the complete ratio used for dosage calculation is C:Si:N:P:Fe = 106:15:16:1:0.001.

To calculate the volume of Diatomix required it is necessary to calculate the mass of nitrogen in the water to be treated. This mass is then converted into the molar mass of nitrogen. The necessary molar mass of iron is calculated from the above ratio. The volume of product to use is based on the mass of iron in the product. The remainder of the micronutrients in the product are based on a proprietary formulation and the respective ratios to the iron concentration in the product.

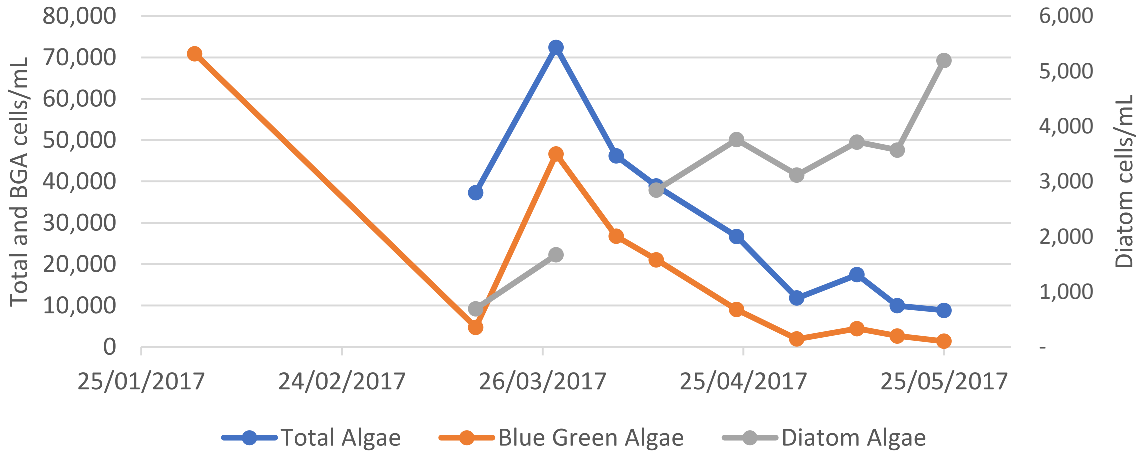
For example, to determine the standard dose of Diatomix, the mass of nitrogen – as ammonia and nitrate/nitrite in the water is calculated. The volume of the reservoir or lagoon is calculated using surface area of the waterbody multiplied by the depth, normally considered to be one metre. The one metre depth is assessed to be the extent of Photosynthetically Active Radiation (PAR) into the water, unless the water is known to be shallower or very turbid. The volume is then multiplied by the concentration of nitrogen to give the mass of nitrogen. For example, a concentration of 1 mg/L of N in a waterbody of one hectare is calculated to be 10 kg of nitrogen that needs to be treated.

In most instances, the water depth overall is greater than 1 metre so while the product dose may treat that first one metre of water, the water below also needs treating. The expectation is that with the correct dosage, there will be three cell doublings per week, and that the cell biomass will consume a given mass of nitrogen in that week. The dose of the product used per week is therefore based upon three times the mass of nitrogen in the water at any one moment in time. From the example above, the 10 kg of nitrogen per hectare is 30 kg per week to be dosed.

2.2 CASE STUDY 1 – RAW WATER STORAGE DAMS

Two raw water storage dams on a farm in New South Wales were treated with the product as a method to reduce and manage the cell counts of blue-green algae (cyanobacteria) populations in the storages. One of these dams was the supply of drinking water to the farm animals and the other was for process/ wash-down water on the farm. In both cases the farm needed to ensure low blue-green algae levels for the supply to be safe for both animal consumption and water used for equipment wash-down. Past events, where blue-green algae levels had been too high for safe water use resulted in using town supply water for long periods during summer. The associated costs of using town supply water were significant on operational costs.

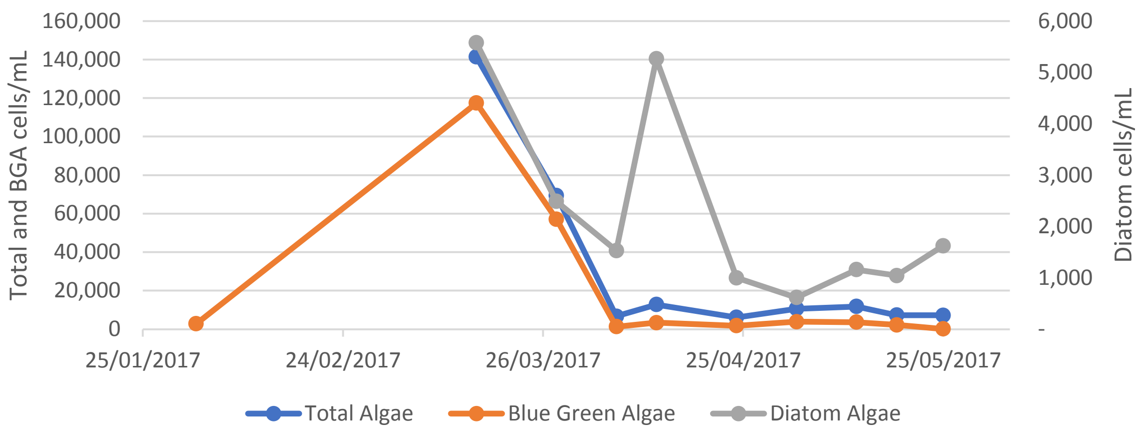
Figure 1 - Dam 1 – Raw water storage treated with Diatomix to reduce BGA cell counts. This 3.35 Ha dam required 0.6 litres of Diatomix per week.



The blue-green algae (BGA) cell counts were variable to begin with and in Dam 1 these went from 70,900 BGA cells/mL down to 4,700 BGA cells/mL and then returned to 46,600 BGA cells/mL two days after dosing had started (Figure 1). Once the dosing had begun in Dam 1, the reduction in cell count of blue-green algae was 42% in the first six days, and 96% from the start of dosing until the cell counts began to stabilise at an average of 2,500 BGA cells/mL. In the same time frame from the start of dosing there was a 240% increase in the diatom cell count and by the end of the sample period this had increased by 750% to 5,200 diatom cells/mL. The average cell count of the final three weeks of sampling for diatoms was 3,900 cells/mL, which was 50% higher than the BGA cell count average over the same period.

The second dam on this property had similar results in that the high initial BGA cell count reduced once the product dosing had begun. Dam 2 BGA cell counts ranged from 2,700 BGA cells/mL to 117,000 BGA cells/mL (Figure 2). The dosing of the product started two days before the second algae sample was taken. Following the dosing, the BGA cell counts reduced by 45% in the first 12 days between samples and by 99% within three weeks. From this point the BGA cell count remained below 3,900 BGA cells/mL with an average of 2,300 cells/mL. The diatom cell count was initially high after the first dose of the product, at 5,600 diatom cells/mL, and then also reduced by 72% after three weeks, before then spiking again to 5,300 cells/mL. The average cell count after the second spike was 1,800 cells/ml during the final month of sampling. This was 79% of the average cell count of the BGA cells.

Figure 2 - Dam 2 – Raw water storage treated with Diatomix to reduce BGA cell counts.



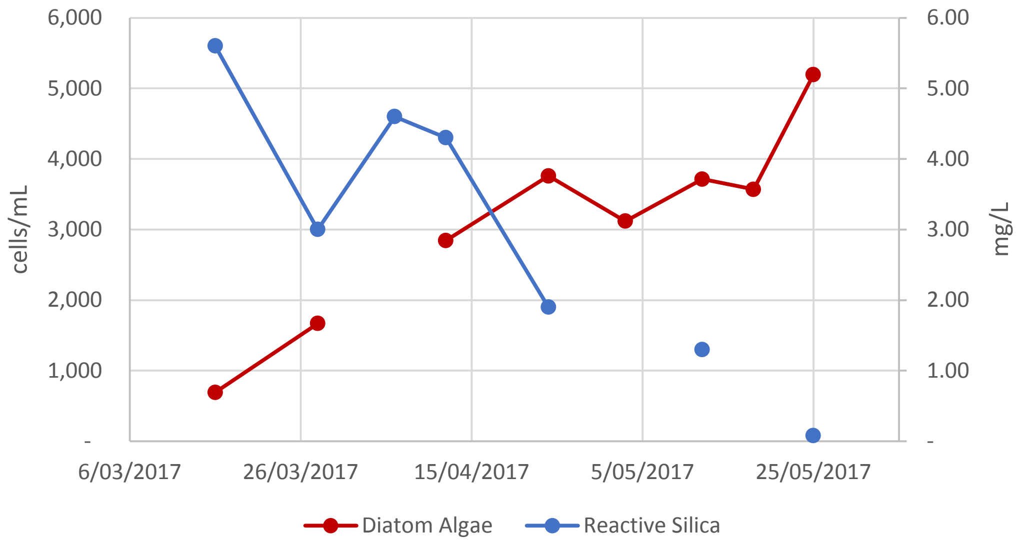
One of the disadvantages of monitoring case studies or trials with commercial clients is that the parameters monitored is directly related to the budget of the industry partner or client. In most instances, the sampling undertaken is performed to the extent that it informs the site manager as to what is occurring in regards to the parameters that are of interest for operational purposes.

These data sets do not always support or elucidate the full story of what is changing within a system when the product is dosed. This is true of most of the data presented here. The data on diatom cell counts usually demonstrates that there are changes to these populations during dosing of the product, but they do not necessarily support a conclusion that increased suspended diatom cells were the factor affecting the change in BGA cell count.

One other parameter that is usually cheap to monitor and has proven to be useful in assessing diatom activity in these systems is the concentration of reactive silica. As diatoms require silica on a nearly 1:1 atomic ratio to nitrogen, as shown above in the Redfield-Brzezinski nutrient ratio, monitoring of the reactive silica will demonstrate diatom activity. Where the concentration goes down, the inference is that there is diatom algae growth from species that are not seen in the suspended algae counts, i.e. the benthic and periphyton species growing in biofilms and upon the benthic layers in areas that are sufficiently shallow to receive light. These changes are usually transitory due to the equilibrium between the colloidal 'total silica' and reactive silica, where a reduction in reactive silica will be compensated by the solubilization of some of the polymeric total silica present in the water (Belton et al., 2012).

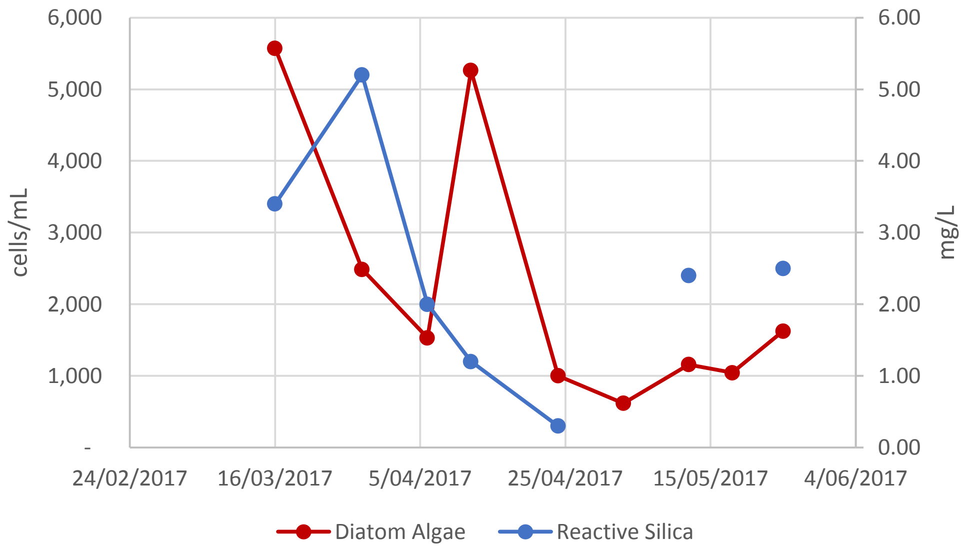
This effect is presented in Figure 3, where the Dam 1 diatom cell count is compared to the concentration of reactive silica. As the cell count increases the reactive silica concentration decreases. The diatom cell count increased from 700 cells/mL up to 5,200 cells/mL during the test period, but this is incongruous to account for a reduction of reactive silica to the extent of a 6.1 mg/L. The hypothesis being that the other diatom algae in the benthos and periphyton zones account for much of this silica reduction, but are not represented in the suspended algae cell count. No literature was found for the weight of individual diatom cells or for a comparison of diatom cell density to dry weight.

Figure 3 - A comparison of the diatom cell count and the reactive silica concentration during the dosing of the product in Dam 1. The extent of the mass reduction in silica is not consistent with the biomass of the diatom cell count.



A similar pattern was observed in the Dam 2 samples, but not as clearly (Figure 4). A reduction of 4.9 mg/L of silica was observed during the period that diatoms were blooming up to a cell count of 5,300 cells/mL. As with Dam 1, the mass of silica reduction seems inconsistent with the cell count of diatoms present.

Figure 4 - The diatom cell count and the reactive silica concentration during the dosing of Diatomix in Dam 2.



The data supports that the dosing of the product is associated with a transitory reduction in the concentration of reactive silica, as well as a reduction in the cell count of blue-green algae. Due to the seasonality and length of sampling no conclusion can be drawn in on the long-term reduction of BGA cell populations, but the data supports that in the period of dosing the BGA cell counts were reduced and stabilized at lower levels.

2.3 CASE STUDY 2 – WASTEWATER EFFLUENT STORAGE LAGOON

The presence of blue-green algae in wastewater treatment and wastewater effluent storage is common. The Total and BGA cell counts can increase to a point where management of the lagoon becomes problematic and use or release of treated effluent can be restricted.

In a wastewater effluent storage lagoon that had an anecdotal history of high BGA counts, the managing council trialed the product to assess suitability for reducing and control of long-term summer blooms. There was a lack of historic data suitable for assessment, but a site visit six weeks prior to the start of the case study showed that the bloom present was substantial (Photograph 1). The council started a monitoring program of Total and BGA cells at the beginning of the study with the initial cell count on Day 1 of dosing being 2,770,000 Total cells/mL with 99.2% of those cells being BGA cells.

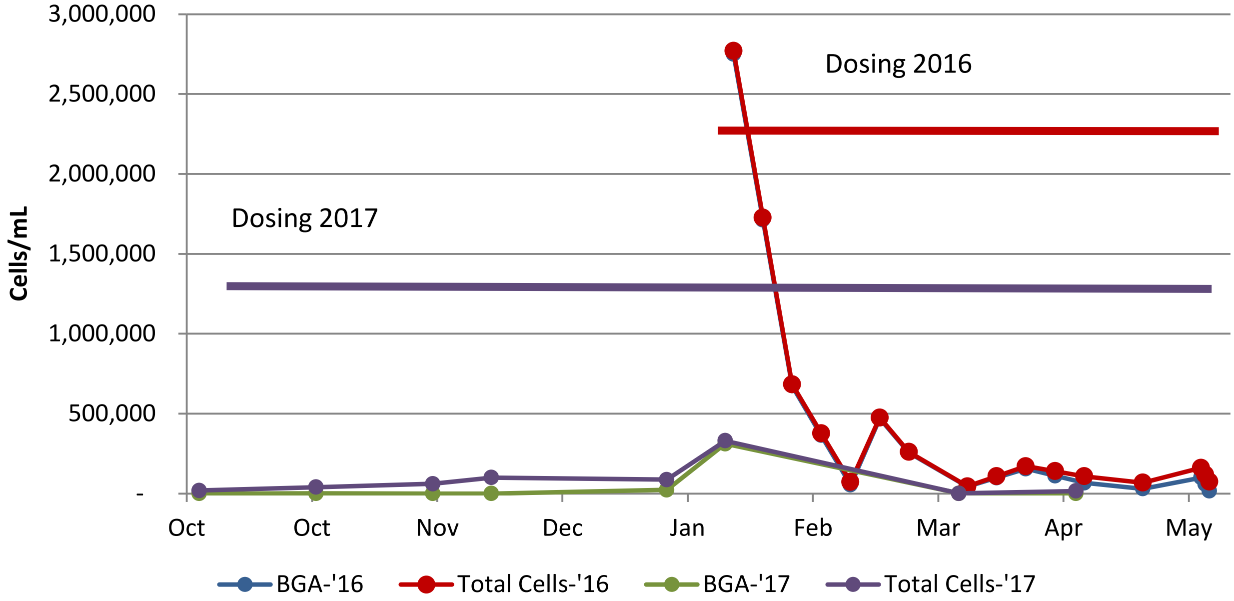
Photograph 1 – A Wastewater Effluent Storage Lagoon in SE Queensland, prior to dosing of the product, Diatomix.

The Total cell count began to reduce quickly once the product was dosed. Arguably this could have been caused by the normal ‘crash’ of an intense bloom, but in this instance the crash was not followed up by a BGA recovery and a subsequent high bloom (Figure 5). The ‘bloom, crash, bloom’ behaviour that is common to wastewater storage systems where BGA is a problem is not observed once dosing starts.

It is worth explaining that because the Total cell count and the BGA count are almost the same values, the BGA data points are more difficult to identify as they are positioned behind the Total cell count markers in Figure 5.

Following the success of the 2016 dosing regimen, the council chose to restart dosing in late winter (October – 2016) to ensure a healthy diatom population was present prior to BGA populations increasing their activity in spring as temperatures increased (e.g. October - November). Dosing of the product was achieved with the use of a proprietary dosing unit supplied by the product manufacturer, AlgaEnviro. The consistent and proportional dosing of the product has been important in achieving the most consistent control of the BGA populations.

Figure 5 – The treatment of a wastewater storage lagoon over two seasons. Dosing started in February of 2016 until June. Following a successful reduction of BGA, dosing started again in October, 2016 prior to the usual spring bloom.



The monthly blue-green algae samples taken from the lagoon between October and December 2016 were 50, 575, 0 and 0 BGA cells/mL. In very early January, 2017, the dosing unit was offline for two weeks. The BGA cell counts for the next two months were considerably higher than the earlier values, at 22,600 and 311,000 BGA cells/mL in January and February respectively. Despite the short interruption in dosing and the associated BGA bloom, the higher of these two BGA counts was still only 11% of the maximum bloom seen in the year prior at the same time of year. The April and May values were 900 and 0 BGA cells/mL respectively, but these results are likely to have been influenced to some extent by the effects of post-Cyclone Debbie storms that crossed this area of QLD with some flooding into the lagoon.

During the 2016 – 2017 dosing period the site staff took photographs to record the differences seen in the lagoon prior to treatment and during dosing. The framing of the 'before' and 'during treatment' images was not kept the same, but the pictures do emphasise the difference that can be attained with correct dosage of the product (Photograph 2).

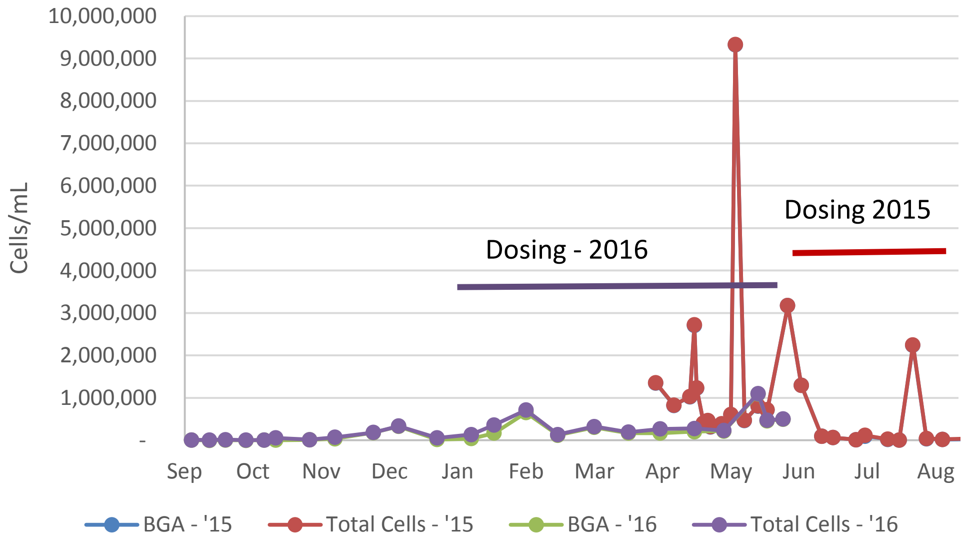
Photograph 2 – February 2016, before treatment of a wastewater effluent storage lagoon commenced and November 2016, after nine weeks of dosing with Diatomix

2.4 CASE STUDY 3 – WASTEWATER TREATMENT LAGOON

Use of the product in a wastewater treatment lagoon to reduce the presence of BGA was tested during the autumn and winter of 2015. Prior to this period the BGA cell counts made up 99% of the Total cell count and the average BGA cell count was 1,5200,000 cells/mL. The cell counts monitored from April of 2015 demonstrated the common bloom, crash, bloom behavior mentioned above. With the dosing starting in June 2015, the cell count dropped immediately and remained low for the next four months, with the exception of one bloom in August.

There will have been some influence from lower winter temperatures; traditionally winter demonstrates lower Total cell counts and notably lower BGA cell counts. The bloom in August was as a result of a large rain event where the increase in stormwater flow into the lagoon disturbed the sludge layer resulting in a release of nutrients, with the subsequent bloom ensuing. If the low cell counts were due solely to the winter temperatures and were not influenced by the product dosing then the August bloom would be expected to be have a very low BGA count or that the percentage of BGA cells within the Total cell count would be low. In fact, 99.6% of this August bloom consisted of BGA cells (Figure 6) which suggests that temperature was not the sole factor influencing the reduced BGA cell count.

Figure 6 – Use of the product, Diatomix, to manage and reduce Blue-green algae cell counts over the 2015 and 2016 seasons in a wastewater treatment lagoon.



During the following spring/summer period (October 2015 – May 2016) the lagoon was monitored for signs of BGA cell growth, and when the first increase was observed, in December 2015, the dosing was recommenced in January, 2016. An initial small bloom of BGA was observed in February and then cell numbers settled to low levels through much of the summer/autumn seasons of 2016. The small bloom in May, 2016, was also associated with a large rain event, but the cell counts quickly reduced again. Site operators at this lagoon also recorded changes over time in this lagoon. The 'before' and 'during treatment' pictures are presented in Photograph 3.

Photograph 3 – A wastewater treatment lagoon before (left) dosing with the product and during treatment (right) in 2015 and 2016.

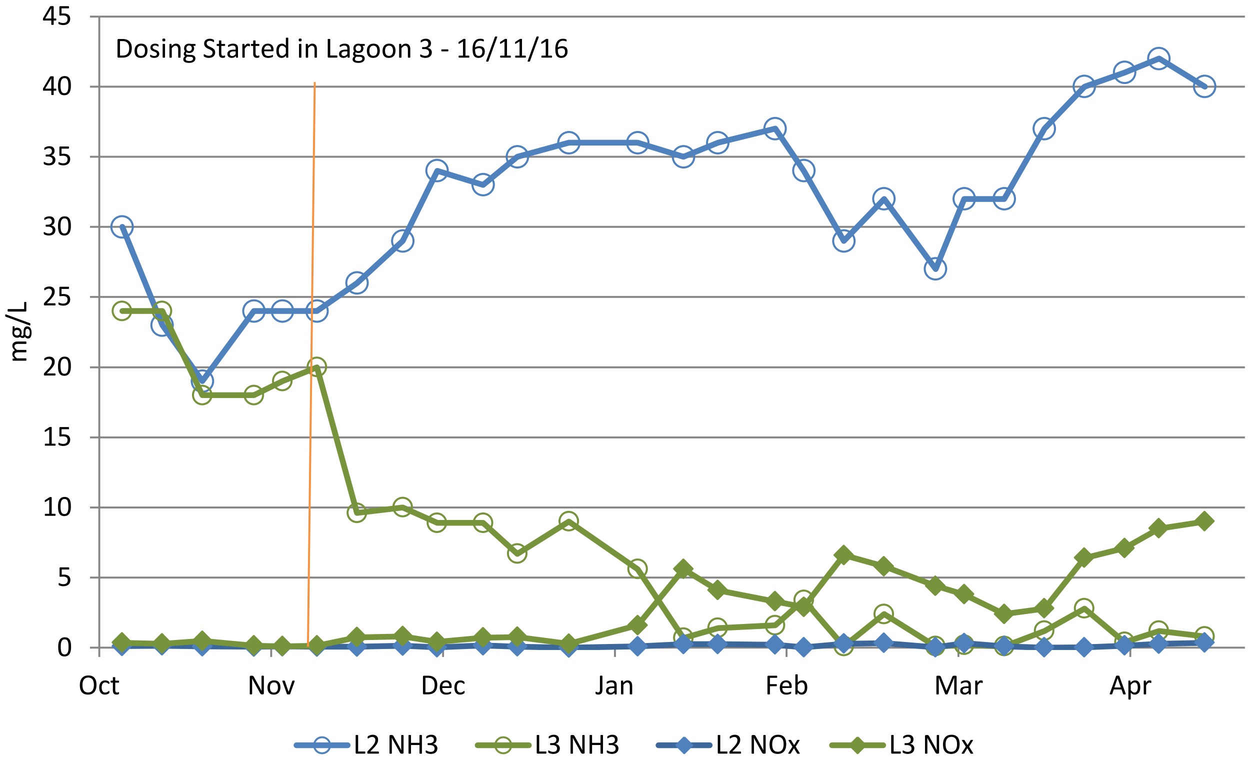
2.5 CASE STUDY 4 – AMMONIA REDUCTION IN A WASTEWATER TREATMENT LAGOON

There are on-going discussions in literature regarding the uptake of ammonia/ ammonium versus nitrate and whether the uptake of one inhibits the other and also whether diatoms, cyanobacteria and other algae have a preference for either ammonia or nitrate (Dortch, 1990; Domingues et al., 2011). Diatoms have been recorded to have a preference for nitrate over ammonia, and did not respond to ammonium supplementation (Domingues et al., 2011). This would suggest that a high ammonia concentration would be unaffected by biomanipulation of the nitrogen to micronutrient ratio as occurs when the product is dosed.

At a wastewater treatment lagoon system that consisted of three lagoons in series, the ammonia concentration was consistently high and problematic in regards to achieving effluent treatment goals. The concentration of blue-green algae was also problematic.

The product was dosed into the third lagoon and the concentrations of ammonia-N and Nitrate + Nitrite-N (NO_x - N) were monitored in lagoon two (L2) and lagoon three (L3) prior to and during the dosing of the product.

Figure 7 – The concentration of Nitrogen as ammonia and NO_x in two wastewater treatment lagoons in series. Dosing of Diatomix immediately started the reduction of ammonia.



With the commencement of dosing the product, the ammonia concentration began to reduce. With an initial average of 20.5 mg/L of NH_3-N in Lagoon 3 the concentration reduced at a rate of 0.3 mg/L NH_3-N/day until it stabilised in mid January to an average of 1.2 mg/L of NH_3-N for the rest of the initial test period. There was an increase in NO_x-N that appeared after the majority of NH_3-N had been consumed. The average concentration of NO_x-N increased from a pre-dosing average of 0.25 mg/L NO_x-N to an average of 5.1 mg/L NO_x-N. The NO_x-N increasedat a rate of 0.08 mg/L/day which only accounts for 28% of the ammonia that was reduced (Figure 7). The average concentration of ammonia in Lagoon 2 increased during the period of the study. As the effluent from Lagoon 2 was entering Lagoon 3, this ammonia was also taken up by the algal assemblages or oxidised to nitrate.

One of the notable differences in this case study was that the lagoon was a rock lined (rip-rap) waterbody rather than one with a natural base with vegetated shoreline. Case studies to date (data not shown) where the waterbody has a rip-rap or a plastic liner have had less of a reduction of the blue-green algae populations than the systems with a natural base with vegetation along the shore. The current hypothesis for this outcome is that the natural vegetation along the shore line, much of which occurs up to 1 m in depth, increases the habitat and surface area for benthic and periphyton diatom algae to grow.

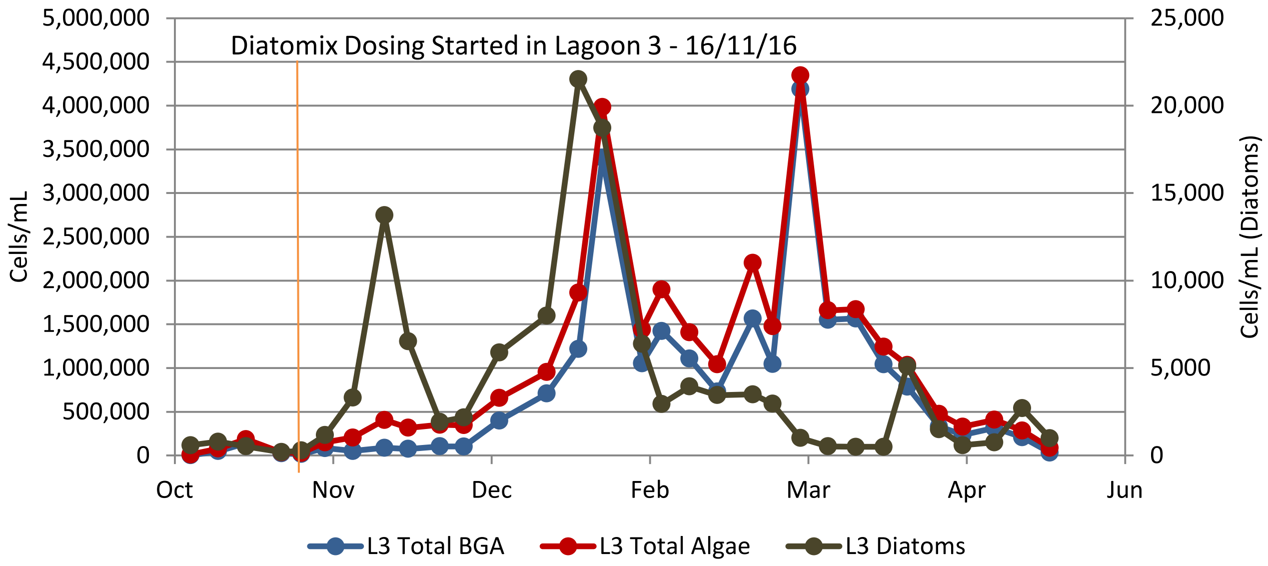
Noted earlier is that there has been anecdotal evidence of much increased invertebrate and vertebrate activity in these systems. While the changes in the extant ecology have not been formally recorded in these studies it makes sense to expect an increase in higher trophic levels of the food chain when diatoms, the feed source at the bottom of the food chain are growing more consistently due to the micronutrient supplementation from the product. With a more natural shoreline and underwater structures (leaves, twigs, branches) the higher trophic levels of gastropods (snails), invertebrates (e.g. insects) and small fish will also have a more suitable habitat for growth, breeding and protection than is present with a plastic liner or rock liner.

In this study, the reduction of blue-green algae in the third lagoon was not as significant as that observed in other studies (cf. case study 2 had a 99.6% BGA reduction). There was a significant change in diatom algae with a 1,280% increase in the diatom cell count average after dosing commenced, up to the end of February, and a 290% increase of the average cell count between the Feb to May period, versus the pre-dosing value.

The anticipated stabilization and ongoing reduction of the blue-green algae cell count did not occur for the entire period of dosing (Figure 8). Blue-green algae cell counts remained relatively low (< 104,000 BGA cells/mL and below an average of 42% of the Total Cell Count) up until mid-December. The dosing unit at the site had a faulty battery and, as with Case Study 2, when the dosing ceased, it would appear that the competitive pressure from the diatom growth was reduced and the blue-green algae population subsequently increased, up to 3,405,000 BGA cells/mL within one month of the fault. The issue with the doser was not detected until late December when manual dosing was restarted. The spike in diatom numbers from late December is in line with the renewed manual dosing of the product and through January the diatom cell count increased and is considered to have had a positive effect on reducing the BGA cell counts back down.

The on-going stability of this BGA reduction was not as great as that seen in other case studies and this has been considered to be partly associated with the lack of higher trophic feeding upon the diatom populations. Without consistent grazing and removal of diatoms cells the biofilms are perhaps not suited to on-going exponential growth of these cells and therefore it is possible that the populations could move into a stationary phase, where higher populations, growing more slowly are present. This aspect does require further investigation to properly justify this viewpoint.

Figure 8 – Total, BGA and Diatom cell counts from a wastewater treatment lagoon treated with Diatomix. The rock lining of the lagoon may have influenced the ecology within the lagoon system.



Taking into consideration the data in this particular case study, as well as the data from studies in other waterbodies with man-made liners, the lack of photic zone habitat for diatoms and higher trophic levels may influence the desired outcomes. Methods to address this are currently being investigated. A floating biofilm system, similar to a greenshell mussel spat catching set-up, with the use of the 'Christmas tree' rope, which has a very high surface area will be trialed to see if creating high surface area environments in the photic zone will support more periphyton diatom growth. These floating systems will also have habitat suited to the higher trophic organisms that feed upon diatoms and take up the nitrogen and phosphorus within the diatom biomass. The use of such systems will be a low-cost, second step to improving the long-term stability of lagoons that rely on microalgae growth for treatment.

3 CONCLUSIONS

The use of a proprietary product, Diatomix, manufactured in Australia by AlgaEnviro Pty Ltd was trialed to assess its suitability for biomanipulation of algal populations in stored raw water or water treatment systems. The main area of interest in regards to these trials was the efficacy of such a treatment to reduce the presence of Cyanobacteria, or blue-green algae, as well as the reduction in ammonia and nitrate levels.

The product is a liquid fertilizer that contains nano-scale amorphous silica with ten other elements that are commonly considered to be necessary for plant or algae growth. These micronutrients - iron, potassium, calcium, manganese, magnesium, copper, boron, molybdenum, zinc and cobalt, are adsorbed to the silica structure and therefore are only bioavailable to diatom algae, as this class of algae utilizes the silica added to the water.

The extent or duration of trials and the data collected during these periods is not necessarily suitable for an in-depth analysis of the complete effect upon the ecosystem. Nor do these trials address or monitor all the pathways by which nitrogen may be removed. As such this data is unsuited to a rigorous analysis, as may be desired by more academic readers. The companies trialing the product worked within set budgets, with the parameters monitored being those of interest to them for treatment outcomes, more so than for scientific enlightenment. Similarly, the duration and intensity of monitoring was generally at a level suited to determine efficacy in operational use. There is still a lot of room for more in-depth investigation to understand the effects, benefits and any possible but unlikely detrimental outcomes to the dosing of the product.

The data presented has demonstrated that dosing of the product, Diatomix, does reduce the blue-green algae cell counts, and in some systems this can be to a level greater than 90%, and for sustained periods of time. Achieving this outcome throughout the complete spring to autumn period, which is when BGA can be problematic, is very unusual and highlights the significance of these outcomes.

ACKNOWLEDGEMENTS

AlgaEnviro Pty Ltd wishes to acknowledge the assistance of its clients in the collection and provision of the data that has made this paper possible.

REFERENCES

Belton, D.J., Deschaume, O., Perry, C.C. (2012) ‘An overview of the fundamentals of the chemistry of silica with relevance to biosilicification and technological advances’ *The FEBS Journal*, 279, 10, 1710 – 1720.

Brzezinski, M. (1985) ‘THE Si:C:N RATIO OF MARINE DIATOMS: INTERSPECIFIC VARIABILITY AND THE EFFECT OF SOME ENVIRONMENTAL VARIABLES’ *Journal of Phycology*, 21, 347 – 357.

Domingues, R.B., Barbosa, A.B., Sommer, U., Galvão, H.M. (2011) ‘Ammonium, nitrate and phytoplankton interactions in a freshwater tidal estuarine zone: potential effects of cultural eutrophication’ *Aquatic Sciences* 73, 3, 331 – 343.

Dortch, Q. (1990) ‘The interaction between ammonium and nitrate uptake in phytoplankton’ *Marine Ecology Progress Series*, 61, 183 – 201.

Twining, B.S., Baines, S.B. (2004), ‘Element stoichiometries of individual plankton cells collected during the Southern Ocean Iron Experiment (SOFeX)’ *Limnology and Oceanography*, 49, 6, 2115 – 2128.