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The effect of water treatment unit processes on cyanobacterial trichome integrity

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The effect of water treatment unit processes on cyanobacterial trichome integrity

Keywords: drinking water, water treatment plant, filtration, bacterial filaments, cyanotoxins, taste and odor

Abstract

Many toxic and/or noxious cyanobacteria appear in nature with a filamentous, stacked cell arrangement called trichomes. Although water treatment can be optimized to keep cyanobacterial cells intact and avoid the release of toxic and/or noxious compounds, many physical and chemical stresses encountered during the treatment process may result in trichome truncation, decreasing treatment efficiency by allowing single cells or short trichomes to reach the product water. This makes it possible for harmful/noxious compounds as well as organic matter to enter the distribution system. Investigations in a pilot and three full-scale water treatment plants were carried out in order to elucidate the degree of trichome truncation across different unit processes. It was found that genera (*Pseudanabaena*, *Planktolyngbya*) with short trichomes (<10-12 cells per trichome), are hardly affected by the unit processes (loss of one to four cells respectively), while genera (*Planktothrix*, *Geitlerinema, Dolichospermum*) with longer trichomes (30+ cells per trichome) suffer from high degrees of truncation (up to 63, 30, and 56 cells per trichome respectively). The presence of a rigid sheath and/or mucilaginous layer appears to offer some protection from truncation. It was observed that certain unit processes alter the sensitivity or resilience of trichomes to disruption by physical stress. Some genera (*Planktothrix*, *Geitlerinema*) were sensitive to pre-oxidation making them more susceptible to shear stress, while *Dolichospermum* sp. appears more robust after pre-oxidation. While the potential of toxicogenic genera breaking through into the product water is a real danger, in the current study no toxicogenic cyanobacteria were observed.

This work stresses the need for plant operators to study the incoming cyanobacterial composition in the raw water in order to adjust treatment parameters and thus limit the potential of toxic/noxious compound breakthrough.

1. Introduction

Cyanobacteria are photosynthetic prokaryotes that can be found worldwide. Climate change and human activities such as discharge of nutrients in water bodies may cause the occurrence of cyanobacterial blooms in most climatic zones (Elliott et al., 2006; Wiedner et al., 2007; Hall et al., 2013; Visser et al., 2016). Cyanobacteria are capable of producing a range of harmful (toxins) and nuisance (taste and odor, T&O) compounds that are a concern for water utilities worldwide. Exposure to cyanotoxins can be potentially dangerous not only to humans, but also to terrestrial and aquatic livestock and wild animals (Carmichael et al., 2001; Carmichael & Boyer, 2016).

The conventional water treatment train (coagulation, flocculation, sedimentation or flotation, and filtration) is normally well suited to remove cyanobacteria and is commonly used in the developed world. On the other hand, in developing regions such as the Northeast of Brazil, direct filtration (coagulation followed by rapid filtration) and its variants (ascending, descending, and double rapid filtration) have become the preferred water treatment method due to their lower cost of construction and operation. This technology, however, can only be safely applied where raw water presents low turbidity (< 10 NTU) and low cyanobacterial count (< 10^4 cells mL^{-1}) (Al-Kathily, 2014; Lima & Capelo-Neto, 2015).

The challenge for any water treatment technology is to remove cyanobacteria without compromising their structure, thus retaining secondary metabolites (toxins and/or taste and odor compounds) inside the cells (Wert et al., 2014). It is,

therefore, in the interest of water utilities to remove cyanobacterial cells intact and to ensure that no viable cells or dissolved toxins (or T&O) reach the product water. At the core of a large number of studies (Chow et al., 1998; Chow et al., 1999; Drikas et al., 2001; Dugan & Williams, 2006; De Julio et al., 2010; Dreyfus et al., 2016) is the concern that cells will lyse and introduce dissolved metabolites into the treatment stream and ultimately into the product water. An aspect that has received little or no attention is the effect of various treatment unit processes on the integrity of the cyanobacterial trichome. A trichome is an arrangement of cells into a filament, sometimes encased in a rigid sheath or a mucilaginous layer, that offers an evolutionary advantage over single cell arrangements (protection from predation, presence of specialized cells for e.g. nitrogen fixation) (Rippka et al., 1979). If cyanobacterial trichomes are truncated by physical or chemical stress during the treatment process, cyanobacterial break-through may occur as smaller particles are more likely to be poorly retained by granular media filtration than larger ones. Furthermore, should cell break-through occur and intact single cells or extremely short trichomes reach the product water, cell lysis might occur at the disinfection step or during distribution. While it is possible that the residual disinfectant might oxidize released toxic metabolites, the situation would be different in the case of odorous compounds that have been shown to be resistant to most disinfectants (Lalezary et al., 1986; Glaze et al., 1990; Jung et al., 2004). The objective of this study was to determine cyanobacterial trichome integrity throughout the drinking water treatment train and to observe whether there are any species-specific effects. The effect of unit processes of three full-scale water treatment plants (WTPs) employing different treatment strategies (1. direct filtration, 2. direct double filtration, and 3. conventional treatment) were examined on trichome length for a variety of filamentous cyanobacteria present in the raw water. In addition, the effects of filtration on cyanobacterial trichome length were

investigated in a pilot plant under different conditions (filter media grain size, with and without pre-oxidation, and surface application rates).

2. Materials and Methods

2.1 Study sites and full scale water treatment plants

For this study three water treatment plants (WTP) in the state of Ceará, Brazil (Figure 1) were selected based on different treatment technologies employed. WTP1 and WTP2 receive raw water from the same reservoir and serve the city of Fortaleza; WTP1 is adjacent to the supplying reservoir and utilizes single descending filtration while WTP2 is some 22 km away receiving its raw water via a pipeline and employs double filtration, ascending followed by descending. WTP3 serves the city of Varjota in the Western region of Ceará (Figure 1) and utilizes conventional treatment. All the filters in the full-scale WTPs are gravity fed. The pilot plant is located at WTP1 and receives the same raw water. It utilizes pressurized modified double filtration, descending filtration followed by descending filtration, in order to reduce plant size and construction costs. The treatment technologies utilized in each WTP are summarized in Table 1. Schematics of the full-scale WTPs, including sampling points for the full scale studies indicated (Figure 2), as well as the operating parameters of each WTP are summarized below (Table 2).

Figure 1: Geographic location of the three WTPs investigated in this study within the state of Ceará - Brazil.

Table 1: Treatment technologies employed in the selected WTPs and the pilot plant.

Table 2: Full-scale WTPs operating parameters including flow rate, chemical dosage, dosage, and hydraulic loading rates of the filters.

Figure 2: Schematics of the full-scale water treatment plants (A) WTP1; (B) WTP2; (C) WTP3; and sampling points (red dots)

Dosages of pre-oxidant, polymer and coagulant are determined by the WTP operators, using a jar-test, daily or whenever there was a significant change in water quality. In the case of highly eutrophic waters, which is the case in Ceará, pre-oxidation is a fundamental part of turbidity and color removal despite its potential drawbacks (formation of harmful disinfection by-products and cell lysis) if not optimized. Pre-oxidation is vital for improving coagulation because it can alter the surface of algal and cyanobacterial cells and change the zeta potential which leads to improved coagulation as detailed in Henderson et al., 2008; Xie et al., 2016; and Lin et al., 2017.

2.2 Pilot plant

The experimental runs were performed using a pressurized, rapid, dual-filtration pilot unit, with variable head and constant flow (1 m^3 h^{-1}) applying the same raw water as used by WTP1. Due to the smaller scale of the pilot plant a pressurized system was selected to simulate the same amount of pressure (one to two metres of water column) as is present in the full scale plants in this study. It is comprised of a down-flow pre-filter (PF) connected in series to a down-flow filter (F) (Figure 3). To study the impact of the grain size on the integrity of cyanobacterial trichomes, two filters (F1 an F2) were built using different granular media sizes (Table 3). Each filter was operated separately from the other, in series with the pre-filter (PF). By manipulating a sequence of sphere valves the PF+F1 or PF+F2 configuration could be operated. Samples were taken at the inlet (raw water), PF effluent and filter effluent. All filters (PF, F1, F2) were constructed using PVC pipes

with 200 mm internal diameter and a total height of 1.5 m. Silica sand, sieved to a specified size (Table 3), with a sphericity coefficient of 0.8 and apparent density of 2.65 x 10^3 kg m^{-3} was used as filter medium.

Figure 3: Simplified schematics of the modified double filtration (MDF) pilot system.

Table 3: Modified double filtration (MDF) media characteristics.

Application of pre-oxidant (Cl_2) and different hydraulic loading rates were tested (Table 4) to observe the impact on the integrity of cyanobacterial trichomes. The optimal coagulant (polyaluminium chloride [PACl] 23% - $Al_2(OH)_{4,5}Cl_{1,5}$) and cationic polymer (diallyldimethylammonium polychloride - $(C_8H_{16}CIN)_n$) doses applied were defined prior to every run based on bench-scale (jar-test) experiments. Each experimental run lasted for approximately 8 hours with samples taken at intervals of 2 h after a 2 h filter maturation period.

Table 4: Chemical and hydraulic operating parameters used in the pilot plant investigations.

The hydraulic detention time of each filter was relatively small (approximately 8 minutes) compared to a conventional water treatment plant. Therefore, the raw water composition did not change significantly during sampling (data not shown), which allowed us to collect raw, PF and F samples within a short interval without compromising the results obtained.

2.3 Cell enumeration and toxin analysis

For cyanobacterial enumeration and identification, samples were collected every two hours in duplicate, stored in 1 L amber glass bottles and preserved with 1%

Lugol's iodine for pilot plant experiments. Full scale WTP samples were collected in duplicate at the specified sampling points (Figure 2) considering the retention time of the treatment process and stored as described. When non-fixed biological material had to be used for organism identification, samples were kept refrigerated (4 °C).

Cyanobacteria enumeration was carried out according to APHA (2012), CETESB (2012), and Lopes et al. (2015). In short: counting cells in filamentous cyanobacteria was performed by enumerating the cells in the first thirty trichomes, determining an average number of cells per trichome for each genus and then multiplying it by the number of trichomes counted. In the case of samples with very variable length trichomes, the number of cells per reticulum square was counted and multiplied by the number of reticules the trichomes occupied (CETESB, 2012).

Identification consisted of grouping organisms into specific taxonomic categories with the aid of identification keys (Komárek & Anagnostidis 1989; 1998; 2005; Anagnostidis & Komárek, 1990). Organisms were identified at their lowest taxonomical level possible (genus) under an optical microscope (Zeiss Axiovert A1) at different magnifications, depending on the size of the cell or structure analyzed. It was difficult to differentiate between *Cylindrospermopsis* sp. and *Raphidiopsis* sp. as both genera present with very similar structures, morphology, cell size, and specialized cells. Therefore, trichomes of these genera were reported as *Cylindrospermopsis*/*Raphidiopsis* sp. Toxin analysis was performed every day prior to each experiment by the state water company (CAGECE) in the raw water of the reservoir studied using the Abraxis® ELISA kit for microcystins (detection limit 0.09 μg L^{-1})., saxitoxins (detection limit 0.015 μg L^{-1}), and cylinderspermopsin (detection limit 0.5 μg L^{-1}). ELISA toxin analysis was performed according to the manufacturer's instructions for each kit.

3. Results

3.1 Influent cyanobacteria composition and overall WTP removal efficiency

On average 95% of the phytoplankton present in both reservoirs during the investigation were cyanobacteria (data not shown). This is common in the semi-arid Northeast of Brazil all year (Barros et al., 2017). A total of 14 cyanobacterial genera (data not shown) were encountered in the raw waters of the different WTPs, amongst them seven potentially cyanotoxin and T&O producing genera which were selected for in this investigation. Among the cyanobacterial genera encountered two presented with short trichomes of less than 12 cells per trichome (*Pseudanabaena* and *Planktolyngbya*), two with medium length trichomes of less than 30 cells per trichome (*Cylindrospermopsis*/*Raphidiopsis* and *Aphanizomenon*), and three with long trichomes with >30 cells per trichome (*Planktothrix*, *Geitlerinema*, *Dolichospermum*) (Table 5). Only the *Planktolyngbya* sp. possessed a rigid sheath. All of the trichomes except for *Dolichospermum* sp. were straight and unbranched, with *Dolichospermum* sp. presenting a helical trichome arrangement (Table 5).

The dominant genus was *Planktothrix* sp. (72 to 85% of total cyanobacterial biomass) in all investigations except from pilot plant run 3, where *Geitlerinema* sp. (69% of total cyanobacterial biomass) dominated. No toxic/noxious metabolites were detected during the experimental period, however, the presence of potential toxic/noxious compound-producers in itself can be considered a threat to public health and safety, as the triggers for toxin/noxious compound production and their biological function are not well understood.

Total cyanobacterial removal at every unit operation was monitored (Table 6). Even though the pilot plant, WTP1, and WTP2 received water from the same

reservoir, experiments were performed at different times and, therefore, cyanobacteria contents were different (Pilot plant = 9.24 x 10^5; WTP1 = 1.66 x 10^5; WTP2 = 0.58 x 10^5; WTP3 = 11.6 x 10^5 cells mL^{-1}). Overall cyanobacterial cell removal efficiencies were 94, 97, 98, and 97% (*n*=2, %RSD < 5%) for the pilot plant, WTP1, WTP2, and WTP3 respectively (Table 6). The unit process that removed most total cyanobacteria was the filtration step with 76, 96, 74, and 90% (n=2, %RSD < 5%) for the pilot plant, WTP1, WTP2, and WTP3 respectively.

Table 5: Selection of potential cyanotoxin and/or T&O producing cyanobacteria encountered in the raw waters from WTP1 - 3. Micrographs at 400x magnification under light microscope. Error = 1SD, *n*=30 PP = pilot plant, L = length, D = diameter.

Table 6: Total cyanobacteria count (including non-filamentous cyanobacteria) at each unit process in three full scale WTPs (WTP1: descending direct filtration; WTP2: direct double filtration (ascending/descending); WTP3: conventional treatment (coagulation/flocculation, sedimentation, filtration), and a pilot plant employing modified direct double filtration (descending/descending). n=2, %RSD < 5% for full scale plants; n=4, %RSD < 10% for the pilot plant.

3.2 Pilot plant

The use of the pilot plant allows the exploration of the effects of different conditions on cyanobacterial trichome integrity. The first two experiments (runs 1 and 2) compare the effect of the grain size in the pre-filter (PF) and two different grain sizes for the consecutive filter (Filter 1 = 1 mm; Filter 2 = 0.7 mm). The next two experiments (runs 2 and 3) explored the effect of the application of pre-oxidation (1 mg L^{-1} Cl_2) on the integrity of cyanobacterial trichomes. The last set of experiments (runs 3 and 4) investigate the effect of a decrease in the hydraulic loading rate (from 240 to 200 m^3 m^{-2} d^{-1}) on cyanobacterial trichomes in the presence of pre-oxidation (most closely resembling the conditions present in full scale WTPs).

3.2.1 Effect of grain size in pilot plant filters

The pre-filter (PF), which presented the largest grain size of all filters used (effective size = 2.40 mm), in most cases had little influence on the length of the cyanobacterial trichomes passing through. On average 3-5 cells per trichome were lost at this stage across the different genera (Figure 4). *Aphazinomenon* sp. was most affected (Run 2 in Figure 4) with a truncation of nearly 50%. Other genera significantly affected were *Planktothrix* sp in Runs 3 and 4 and *Geitlerinema* sp. in all runs ($p < 0.05$) (Figure 4).

Considering the complete filtration system, although trichome breakage was observed for most of the genera present, each genus reacted differently to the conditions applied in the pilot plant (15 to 73% of cell per trichome lost). The decrease of the filter average grain size from effective size 1.00 mm to effective size 0.72 mm appears to have increased the cell loss (i.e. the degree of truncation) in all genera, but *Planktothrix* sp., in a range from 4 to 136% (Figure 4, Table 7).

3.2.2 Effect of pre-oxidation in the pilot plant

The application of 1 mg L^{-1} of Cl_2, used as a pre-oxidant to enhance coagulation, had a marked effect on *Planktothrix sp.* with a higher degree of cell loss (49%) per trichome in this experiment compared to the experiment with the same grain size but without pre-oxidation (26%) (Runs 2 and 3 in Table 7). On the other hand, *Dolichospermum* sp*.* and *Aphanizomenon* sp*.* showed a decrease in cell loss compared to the experiment with the same grain size and no pre-oxidation (Runs 2 and 3 in Figure 4). Chemical stress appears to induce susceptibility to physical stress for the *Planktothrix* genus (increased truncation by 226% - runs 2 and 3 in Table 7 and Figure 4. *Planktothrix* sp., the dominant genus in most of the experiments, appears more sensitive towards chemical stress than to physical stress, or more precisely, chemical stress appears to induce a susceptibility to

physical stress. *Geitlerinema* sp. appears less sensitive to chemical stress (40% truncation) than to physical stress (66% truncation; Runs 2 and 3 Figure 4).

3.2.3 Effect of hydraulic loading rate on trichome intergrity in the pilot plant

The decrease in the hydraulic loading rate from 240 to 200 m d^{-1} decreases shear stress on cyanobacteria maintaining inter-granular space (i.e. grain size in the filter) constant (Runs 3 and 4 in Table 7 and Figure 4). This change caused the least trichome breakage (16%) in *Geitlerinema* sp., similar to what occurred in the first experiment (lower shear stress than in the second experiment, Run 1 versus Run 2). *Pseudanabaena* sp. suffered 28% and 47% trichome truncation in the third and fourth experiment (decrease of hydraulic loading rate, Runs 3 and 4), respectively. However, when considering the actual number of cells lost per trichome, it is apparent that while per cent losses are approximately a quarter and half of the cells per trichome, the trichome was only shortened by two and four cells respectively. *Planktothrix* sp. and *Dolichospermum* sp. appear markedly susceptible to trichome breakage under the conditions present in the experiment with the decreased loading rate as compared to the experiments with 1.00 and 0.72 mm effective grain size (Run 1 versus Run 2). There appears to be no additional effect of the application rate decrease on *Cylindrospermopsis*/*Raphidiopsis* trichome breakage.

Table 7: Total absolute and per cent cell loss per trichome of investigated cyanobacterial species during modified double filtration in the pilot plant. Loss represents the difference between cells per trichome in the raw water to cells per trichome determined in the final filter effluent. Run 1) F2 as secondary filter with no addition of Cl_2 and with 240 m^3 m^{-2} d^{-1} hydraulic loading rate.; Run 2) F1 as secondary filter with no addition of Cl_2 and with 240 m^3 m^{-2} d^{-1} hydraulic loading rate.; Run 3) F2 as secondary filter with the addition of 1 mg L^{-1} Cl_2 and with 240 m^3 m^{-2} d^{-1} hydraulic loading rate; Run 4) F2 as secondary filter with the addition of 1 mg L^{-1} Cl_2 and with 200 m^3 m^{-2} d^{-1} hydraulic loading rate. n=4; %RSD < 10%

Figure 4: Changes of trichome length during filtration in a modified direct double filtration pilot plant. Samples taken of influent raw water, pre-filter effluent, and secondary filter effluent every two hours for 8h. Run 1: no pre-oxidation, hydraulic loading rate of 240 m^3 m^{-2} d^{-1} and filter 1 (F1 – 1 mm grain size) as secondary filter; Run 2: no pre-oxidation, hydraulic loading rate of 240 m^3 m^{-2} d^{-1}, and filter 2 (F2 – 0.7 mm grain size) as secondary filter; Run 3: pre-oxidation with Cl_2 (1 mg L^{-1}), hydraulic loading rate of 240 m^3 m^{-2} d^{-1} and F2 as secondary filter; Run 4: pre-oxidation with Cl_2 (1 mg L^{-1}), hydraulic loading rate of 200 m^3 m^{-2} d^{-1}, and F2 as secondary filter. No label for a genus represents total removal of that genus at the preceding unit process. *n*=4, %RSD < 10%.

3.3 Full scale water treatment plants

3.3.1 WTP1

WTP1 employs descending direct filtration with effective grain size of 0.72 mm (the same as Filter 2 in the pilot plant) and used the same water as the pilot plant study but with a time lag between sampling. Samples were taken from the raw water, coagulated/pre-oxidized water and filtered water. *Planktothrix* sp. appears most susceptible to pre-oxidation (in this case with chlorine dioxide - ClO_2). None of the other genera evaluated suffered as much trichome truncation as *Planktothrix* sp. (WTP1 in Table 8 and Figure 5). *Planktolyngbya* sp. lost half of its cells, shortening the trichome to 5 cells per trichome. Remarkably, *Pseudanabaena sp.* did not suffer any truncation. *Cylindrospermopsis/Rhaphidiopsis* sp. trichomes were truncated to a similar amount as observed at the pilot scale (between 25 to 30%).

3.3.2 WTP2

WTP2 receives its water from the same reservoir as WTP1. Therefore, the phytoplankton composition was the same as for WTP1. The plant applies direct double filtration, where the pre-filter (effective grain size 2.27 mm) is operated as an ascending filter and the second filter (effective grain size 0.86 mm) is operated as a descending filter (Table 8 and Figure 5). *Planktothrix* sp. was the only genus markedly impacted by pre-oxidation (Cl_2), confirming its susceptibility to chlorine

observed in the pilot plant experiments (WTP2 in Figure 4 and Figure 5). The pre-filtration step did not have a marked impact on the truncation of the cyanobacterial trichomes, except for *Planktothrix* sp. The observations made in WTP1 hold true after the pre-filtration step in WTP2, which makes sense if one considers the pre-filtration as having no impact on trichome length, then the process applied at WTP2 is essentially the same as WTP1.

3.3.3 WTP3

WTP3 has a different water source than WTPs 1 and 2. Here, also, it can be observed that *Planktothrix* sp. suffers truncation between the raw water and coagulated samples; however, *Planktothrix* sp. suffers less trichome truncation (30%) in the filtration step than in the other WTPs (WTP1: 53%; WTP2: 55%). *Pseudanabaena* sp. and *Planktolyngbya* sp., present in the raw water with short trichomes (< 15 cells), appear to be little affected by the different unit processes (WTP 3 in Table 8). *Geitlerinema* sp., which presented trichomes of medium length (25 cell) suffered most trichome truncation during the filtration step as observed in the other two WTPs (Figure 5) and in the pilot plant (Run 3 in Figure 4 and Table 7). *Cylindrospermopsis/Rhaphidiopsis* sp. trichomes were truncated to a similar degree (29%) as observed at the pilot scale and at the other WTPs (all Runs in tables 6 and 7, as well as Table 8 and Figure 5). Overall, this WTP seems to have had the least effect on trichome truncation as compared to WTP1, WTP2 and the pilot plant experiments with oxidation (Runs 3 and 4).

Table 8: Total absolute and per cent trichome loss of investigated cyanobacterial genera caused by different treatment technologies. Loss represents the difference between cells per trichome in the raw water to cells per trichome determined in the final filter effluent. WTP1 ascending direct filtration; WTP2 direct double filtration (ascending /descending); WTP3 conventional treatment. *n*=4; %RSD < 10%; * nd = not detected

Figure 5: Cyanobacteria trichome cell loss during different unit processes applied by three full scale WTPs. A value of zero represents complete removal of that genus after that unit process. *n*=2; %RSD < 10%.

3.3.4 Comparison of unit processes across full scale WTPs

Across all treatment technologies, *Geitlerinema* sp. and *Planktothrix* sp. trichomes were the most susceptible to breakage (average trichome truncation across all WTPs 71% and 67% respectively). As observed in the pilot plant, *Geitlerinema* sp. appeared most susceptible to shear stress in the filters while *Planktothrix* sp. was most susceptible to pre-oxidation at the coagulation step (Figure 5). Both operations were applied in all WTPs. *Pseudanabaena sp.*, with shorter trichomes (8±2 cells), was less susceptible to truncation across the different unit processes, as also observed in the pilot plant investigations. The average trichome length of *Cylindrospermopsis/Rhaphidiopsis* sp. was shorter in the full-scale investigation than observed during the pilot plant trials (13 compared to 29 cells per trichome). The trichomes here proved more resilient, losing no more than a third of their length regardless of the unit operation.

4. Discussion

Although one of the tenets of water treatment regarding cyanobacteria is that none of the unit processes applied should be harmful to the integrity of the cell (Chow et al., 1999), to our knowledge no research has been directed at the integrity of cyanobacterial trichomes. A total of seven potential cyanotoxin/T&O producing filamentous cyanobacterial genera were evaluated in the incoming water of three full scale WTPs and a modified direct double filtration pilot plant (Table 5). It has been demonstrated in this investigation that cyanobacterial trichomes suffer truncation along the treatment train independent of the treatment technology

applied. Breakthrough of cyanobacterial cells reaching the product water, either as single cells or smaller trichomes has been observed. This can be problematic when certain toxin/noxious compounds producing genera reach the product water (Hisbergues et al., 2003; Somdee et al., 2013; Lopes et al., 2015; Suurnäkki et al., 2015).

4.1 Effect of morphology on trichome truncation and removal efficiency

Zamyadi et al. (2013) have proposed that the removal efficiency of different water treatment processes was species/genus dependent. While in their study the observed cyanobacteria where morphologically different (coccoidal colonial, coccoidal filamentous, and cylindrical filamentous) this was not the case in the current study where all genera where cylindrical filamentous with the exception of *Dolichospermum* sp. (coccoidal filamentous) in the pilot plant studies.

Morphological differences of the trichomes (number of constituent cells, presence/absence of a rigid sheath and or a mucilaginous layer), however, appears to have influenced trichome truncation. *Pseudanabaena* sp. suffered little trichome truncation and achieved breakthrough in all WTPs, its short trichome length and potential for developing a mucilaginous layer most likely protected it from truncation (Table 8, Figure 5). On the other hand, in the full-scale studies *Aphazinomenon* sp. suffered some truncation but was completely retained by the descending filter in WTP1 not achieving breakthrough. In WTP2 *Aphazinomenon* sp. suffered more marked truncation (52%) and achieved breakthrough. This indicates that not only trichome truncation is species/genus dependent, but is also removal efficiency. Similar observations have been made by several other studies concerning different unit processes in full scale WTPs (Ewerts et al., 2013; Shang et al., 2018; Dugan and Williams, 2006; Joh et al., 2011; Mohamed et al., 2015). These studies, however, mostly compared morphologically different cyanobacteria

(colonial versus filamentous), usually concluding that colonial cyanobacteria (usually coccoids) are more readily removed by the conventional treatment train than filamentous cyanobacteria. This indicates that the normally applied charge neutralization coagulation is efficient at removing coccoidal, colonial cyanobacteria, but not at removing filamentous genera. The ineffectiveness of charge neutralization can be linked to the trichome length. Beyond a certain number of cells (4-5), cyanobacterial cells can no longer be considered colloidal particles (Elimelech et al., 1995) and charge neutralization becomes ineffective (Pieterse and Cloot, 1997); especially with the long trichomed cyanobacterial genera like *Dolichospermum*, *Planktothrix*, *Cylindrospermopsis* present in the current investigation. This phenomenon was most markedly observed in the sedimentation step in WTP3, which did not remove a large portion of cyanobacterial biomass using charge neutralization/coagulation. Therefore, another mode of coagulation/destabilization, like sweep coagulation, should be aimed for (Henderson et al., 2008; Mohammed, 2015). Sweep coagulation, while also to a degree causing charge neutralization, works on the basis of electrostatic attraction (negatively charged cyanobacterial cell/trichome surface is attracted by positively charged large aggregates of aluminium hydroxide formed when the coagulant is added to water; Ghernaout and Ghernaout, 2012). This sweep floc formation is more beneficial with longer trichomes that can no longer be considered colloidal. In order to improve the coagulation-flocculation process, the use of commercial polymers has shown potential. When using polymers, Yap et al. (2014) observed cyanobacterial removal in excess of 90% with a maximum of 99%. However, no impact of polymer on cyanobacteria cell or trichome has, yet, been reported in the literature and, therefore, it has not been considered as an important chemical for trichome truncation.

Cyanobacterial trichome length could also play a role in removal where longer trichomes are more readily retained than shorter trichomes. Shorter trichomes are more readily advectively transported through the inter-grainal space in the filters. The effect of trichome length was observed in the cases where double filtration is employed (pilot plant and WTP2). Pre-filtration was not very effective in removing a large portion of the incoming cyanobacterial biomass. This is particularly noticeable when comparing WTPs 1 and 2, which use the same raw water. The presence of the ascending pre-filter at WTP2 does not markedly improve cyanobacterial removal compared to WTP1 (without a pre-filter). This is most likely due to the larger inter-grainal space, allowing most cyanobacterial trichomes to pass through without being retained. A similar observation was made by Silva et al. (2012). Pre-filtration is employed to decrease turbidity (caused by biotic and abiotic particles) in the water and in the current study decreased turbidity between 30 and 50 % (data not shown). In both the pilot plant and full-scale experiments species with shorter trichomes such as Planktolygnbya and Pseudanabaena sp. suffered little trichome breakage (1 to 2 cells per trichome) and were less likely to be retained by the filtration step than longer trichomed genera. This observation is supported by the findings of Kloep and Röske (2004) in their column filtration study with four green algae. They found that advective interstitial transport through granular medium depended primarily on algal size and morphotype. Further, Pazouki et al. (2016) have demonstrated that species with a lower biovolume are more readily advectively transported through a granular filter medium than species with larger biovolume. A similar observation was made by Joh et al. (2011) who found that cell and colony morphology influenced filtration efficiency. Although trichome integrity was not determined, Dugan and Williams (2006) found that unicellular *Microcystis aeruginosa* presented consistently higher breakthrough than the filamentous *Dolichospermum flosaque* after passing

through a dual media filter (sand 0.44 mm grain size and anthracite 1.0 mm grain size). The grain size of anthracite layer resembles the media grain size in the F1 filter of the pilot plant. Dugan and Williams' (2006) study confirms the findings of the current investigation as it could be proposed that unicellular *M. aeruginosa* could be compared to a severely truncated (or naturally short) trichome. A unicellular organism similar to a short trichome may pass readily through granular media, as also demonstrated by Kloep and Röske (2004) when applying abiotic microspheres and unicellular spherical *Chlorella* sp. to their column experiments. Another factor affecting trichome truncation is the presence of a rigid sheath or mucilaginous layer. The rigid sheath should offer some protection against physical and chemical stressors. *Planktolyngbya* possesses typically a rigid sheath (Komárek, 1992) and presented the lowest cell loss per trichome in the full-scale WPTs observations (Table 8). Further, Moisander et al. (2002) have demonstrated that planktonic *Dolichospermum* sp. are more susceptible to shear stress than other cyanobacterial genera and concluded that the ability to withstand shear stress is genus and species specific and based on the individual morphology.

4.2 Effects of chemical stress on trichome truncation

In two of the pilot plant trials 1 mg L^{-1} Cl_2 was added as a pre-oxidant (Runs 3 and 4 in Figure 5 and Table 7). Application of a pre-oxidant is a common practice in waters heavily affected by cyanobacteria (Lin et al., 2015). Shen et al. (2011) have found that in the presence of algal concentration greater than 10^4 cells mL^{-1} (constantly encountered in the reservoirs studied here) pre-chlorination is necessary to achieve algal destabilization (i.e. charge neutralization) and bring about effective removal, although it could induce cell lysis and toxin release if not adjusted carefully to a dose that achieves algal destabilization without inducing cell lysis. The pre-oxidant dose is dependent on evaluating incoming cyanobacterial

cell concentration and community composition as well as water quality (Henderson et al., 2008).

When comparing Run 2 (without Cl_2) to Run 3 (with Cl_2) in Table 7, the application of the pre-oxidant appears to have no effect on trichome truncation, except in the case of *Planktothrix* sp. The pre-oxidant appears to have severely sensitized *Planktothrix* trichomes to the point where they become sensitive to shear stress (discussed in section 4.3). Cell damage of bacteria by chlorine is not fully understood and the underlying mechanisms are yet to be elucidated (Ofori et al., 2017). However, it could be proposed that the surface morphology of the cells/trichomes is altered in a way that weakens the trichome thus making it more susceptible to physical stressors.

Dolichospermum sp. displays less truncation in the presence of the pre-oxidant (Run 2 versus Run 3 in Table 7). This might be due to the fact that the presence of the pre-oxidant caused a change in trichome configuration or to the formation of micro-flocs that protect the normally rather fragile trichomes of *Dolichospermum* sp. Ho et al. (2012), after direct filtration and subsequent backwashing of the filters, observed that *Dolichospermum circinalis* cells were surprisingly robust. The authors concluded that the cyanobacterial cells were protected within the flocs and were able to withstand the applied physical stress.

Neither *Cylindrospermopsis/Raphidiopsis* sp. nor *Pseudanabaena* sp. appear affected by the pre-oxidation step. As in the previous two runs (1 and 2) *Cylindrospermopsis/Raphidiopsis* sp. suffers trichome truncation of approximately a quarter to a third of its original length when chlorine is applied (Runs 3 and 4), displaying no increased sensitivity under pre-oxidation conditions in regards to trichome breakage. *Pseudanabaena* sp. loses two cells of its relatively short trichome (Table 7), which seems to confirm the observation that a short trichome

makes truncation across different unit processes less severe, unless the genus is susceptible to pre-oxidation.

The trends observed in the pilot plant experiments were also observed in full-scale. *Planktothrix* sp *and Geitlerinema* sp appear particularly sensitive to the addition of the treatment chemicals, most likely to the pre-oxidant (Figure 5 and Table 8). Truncation of Planktothrix sp and Geitlerinema sp trichomes were increased compared to the pilot plant, which could be due to the fact that a stronger oxidant was used in WTP1 (ClO_2). Trichome truncation of 73% and 100%, respectively, could be observed (Table 8). Similar to the pilot plant experiments, *Cylindrospermopsis/Raphidiopsis* sp. loses very few cells during pre-oxidation. Unlike in the pilot plant, *Geitlerinema* sp. trichomes were hardly truncated. Extensive truncation, however, could be observed after filtration, which was not the case in the pilot plant, potentially indicating that the pre-oxidant weakened the trichome to make them more susceptible to breakage in the filtration step as discussed elsewhere in this paper (Mohammed, 2015; Lin et al., 2017; Sheng et al., 2018). The longer retention time in the full-scale filters compared to the pilot plant might account for this difference in susceptibility. *Planktolyngbya* sp. was present in the raw water for the full-scale trials and appears affected by the application of the pre-oxidant, as the only cell loss could be observed after this step. *Planktolyngbya* is the only genus in the current study that presents a rigid sheath, this, however, did not offer protection from the chemical stress of the pre-oxidation. Wert et al. (2013) have found that the potential protection offered by an outer sheath is species dependent. This might explain why different genera of cyanobacteria in this study are affected differently by pre-oxidation. According to the observations of the pilot scale experiments, *Planktolyngbya sp.* should be doubly protected because it is short-trichomed and it is known to have a rigid sheath (Table 4; Komárková-Legnerová and Cronberg, 1992). Considering that the

trichome truncation occurred in the coagulation/preoxidation step, it becomes apparent that neither a rigid sheath, nor a short trichome length afforded protection from chemical stress. The difference in this case, however, is that the preoxidant used at this WTP was chlorine dioxide (ClO_2; E° in water at 25°C and pH 8 [pH of the raw water] = 0.91 V), a much stronger oxidant than chlorine (Cl_2; E° in water at 25°C and pH 8 [pH of the raw water] = 0.77 V) used in the pilot plant (James et al., 2004).

4.3 Effects of physical stress on trichome truncation

In the pilot plant and full scale WTPs grain size smaller than 2.4 mm (pre-filter) influences trichome truncation with different intensities across different genera, probably due to the shear stress between cyanobacterial trichomes and media grains. Genera with shorter trichomes like *Pseudanabaena* appear less sensitive to trichome truncation caused by shear stress, while genera with longer trichomes appear more sensitive (e.g. *Dolichospermum*). *Cylindrospermopsis*/*Raphidiopsis* sp., independent of the conditions, is truncated approximately by one third of its original length. *Planktothrix* sp., normally insensitive to physical stress (15 and 26%, Runs 1 and 2, Table 7, Figure 4), appears more fragile after the application of chemical stress (49 and 47%, Runs 3 and 4, Table 7, Figure 4).

Only *Planktothrix* sp. and *Geitlerinema* sp. appear affected by the pre-filtration step (Figure 4). Both occurred with relatively long trichomes (*Geitlerinema* sp. 31 ± 7, *Planktothrix* sp. 41 ± 18 cells per trichome; Table 5) which might make them more susceptible to increased shear stress due to the decrease in grain size.

The reason why *Dolichospermum* sp. and *Geitlerinema* sp. appear to be most susceptible to trichome breakage (63-73% and 33-62%, respectively, Runs 1 and 2 in Table 7) could be that neither species of either genus observed here displayed a rigid sheath and only a slight mucilaginous binding layer (Table 4; Komárek &

Anagnostidis, 1998; 2005; Wacklin et al., 2009) and the relatively long trichomes. The fact that *Dolichospermum* sp. so readily suffered from trichome breakage presents cause for concern for water utilities, as many *Dolichospermum* species are reported to be producers of harmful and nuisance secondary metabolites like saxitoxins and taste and odor compounds e.g. geosmin and 2-methylisoborneol (Izaguirre & Taylor, 2007; Tsao et al., 2014; D'Agostino et al., 2014).

In contrast, *Cylindrospermopsis*/*Raphidiopsis* sp. appears robust as it is only truncated by 25-26% (Runs 1 and 2 in Table 7) of its original average length. Neither genera *Raphidiopsis* or *Cynlindrospermopsis* are known to have rigid sheaths or envelopes (Komárek & Anagnostidis, 1989; Komárek & Komáková, 2003), hinting at the possibility of another factor or factors, yet unidentified. As in the case of *Dolichospermum* sp., the fact that *Cylindrospermopsis* sp. trichomes break, even to a smaller extent, is cause for concern for water authorities as strains of *Cylindrospermopsis* are capable of producing two groups of cyanotoxins, saxitoxins and cylindrospermopsins (Fastner et al., 2003; Hoff-Risseti et al., 2013).

Pseudanabaena sp. is usually found in short trichomes (Komárek et al., 2014) which might present sufficient protection against shear forces present in granular media, which is confirmed by the low amount of cells lost (1-3 Runs 1 and 2 in Table 7).

For *Aphanizomenon* sp., more cells were lost with decreasing granular media particle size (8 cells per trichome [Run 2] compared to 3 cells per trichome [Run 1], Table 7), most likely due to the increase in shear stress. Wang and Lan (2018) report that *Aphanizomenon flos-aquae* is extremely sensitive (lethally damaged) to shear stress by stir bar mixing. This effect was not observed in this study.

Decreasing the hydraulic loading rate of the filters while maintaining a pre-oxidant dose of 1 mg L^{-1} Cl_2 resulted in no further trichome truncation for either *Planktothrix* sp. or *Cylindrospermopsis / Raphidiopsis* sp (Figure 4, Runs 3 and 4 in Table 7). This is expected as decreasing the hydraulic loading rate would decrease the physical stress exerted on the cyanobacteria. *Geitlerinema* sp. truncation decreased from 67% to 16%, as would be expected due to the decrease in shear stress resulting from lower interstitial velocity (Figure 4, Runs 3 and 4 in Table 7).

The trichome truncation in *Pseudanabaena* sp., *Dolichospermum* sp., and *Aphanizomenon* sp. increased significantly ($p < 0.05$) (Runs 3 and 4 in Table 7). Whether the pre-oxidant had more time to affect the trichome due to the lower loading rate, indicating that the hypothesis of potential protective effect of micro-floc formation might be species specific, or that another mechanism is at work, suggests there is an effect of the pre-oxidant that could not be isolated with the current experimental design.

Sedimentation, as might be expected, had very little effect on trichome integrity (Figure 5). There are no major shear stresses working during sedimentation. Chemical stress can be present due to residuals from the treatment chemical addition step. Fan et al. (2016) have found that cyanobacteria with colonial arrangement and a cover of mucilaginous material protected the cyanobacteria for longer from Cl_2 than the single cells were. While in this study only *Pseudanabaena*, *Planktolyngbya*, and *Dolichospermum* are reported to have either a sheath of mucilaginous envelope, the cell arrangement into trichomes may offer more protection from oxidation than single cells would have. Further, this might explain the fact that trichome truncation is not observed after pre-oxidant addition, but in the consecutive steps. A certain exposure time is necessary before the effects of the oxidation can be observed. The hydraulic contact time is approximately 10 and

15 minutes during the pre-filtration step in WTP2 and the flocculation step in WTP3, respectively (Figure 2). Only *Planktothrix* sp. shows marked trichome truncation during this unit process. As could be observed in the pilot plant investigations as well as the full scale WTPs, *Planktothrix* sp. displays an apparent sensitivity towards the chemical stress posed by the pre-oxidant.

As was observed in the pilot scale studies, pre-filtration had little effect on trichome integrity with the notable exception of *Planktothrix* sp. (Figure 5, Table 8). However, it could be proposed based on species-specificity of the sensitivity towards pre-oxidation observed by Wert et al. (2013) that pre-oxidation with ClO_2 has weakened the *Planktothrix* sp. trichomes to make them more susceptible to shear stress. Unlike in the pilot plant study, *Aphanizomenon* sp. did not appear to be protected from truncation after the addition of the treatment chemicals, which might be due to the change of the dose and type of the pre-oxidant.

Across all three full scale WTPs, descending filtration is the final unit process before disinfection. This unit process is by far the most destructive in the full scale plants. However, the destructive potential posed by the physical shear stress has to be put into perspective based on the preceding unit processes and the potential residual effects. This is particularly true for the addition of the treatment chemicals that alter the surface of the cyanobacterial trichome, either by destabilization through charge neutralization or similar means (Lin et al., 2015). *Planktothrix* sp. continued to lose cells in all WTPs investigated. Filtration usually applies shear stress and pressure, which will be the mode of trichome truncation for *Planktothrix* sp. The results from the first two experiments of the pilot plant trials show that *Planktothrix* is not normally susceptible to shear stress, however, upon the addition of a pre-oxidant, a weakening occurs that makes it very susceptible.

Geitlerinema sp. and *Aphanizomenon* sp. are the only genera completely removed from the filter effluent, and only in WTP1. *Geitlerinema* sp. showed severe

truncation in WTP2 and some truncation in WTP3 after the filtration step. This is noteworthy as the pre-oxidant (Cl_2) concentration applied was higher in WTP3 (5 as opposed to 3 mg L^{-1}), as well as the coagulant dose applied (10 as opposed to 5 mg L^{-1}). The increased pre-oxidant dose in WTP3 might have more successfully destabilized the cyanobacteria and in combination with the increased coagulant dose might have shifted the mode of coagulation from charge neutralization to sweep coagulation. Changing the mode of coagulation might have led to either protection of the cyanobacterial trichomes in the case of *Geitlerinema* sp. and *Planktothrix* sp. in WTP3 compared to the severe truncation observed for both genera in WTP2 (Figure 5).

5. Conclusion

Different unit processes were investigated in order to determine at what stage of the treatment process trichome truncation occurs, to what degree, and whether intact cyanobacterial cells reach the product water. The major findings are as follows:

- For most cyanobacterial genera the filtration step was most damaging, most likely due to shear stress;
- Sensitivity to pre-oxidation would increase with increasing hydraulic retention time (and therefore contact time), oxidant type, and dose.
- Sensitivity to filtration is enhanced by preceding chemical stress in the form of pre-oxidation. *Planktothrix* sp. appeared particularly susceptible to this;
- It was observed that genera with short trichomes (< 10-12 cells per trichome) survived the treatment process well and reached the product water with little cell loss;
- Due to different characteristics of the trichome surface, pre-oxidation might have caused a protective effect by changing the charge on the surface or

the physical orientation of the trichomes. This was especially so for *Dolichospermum* sp. where inclusion in micro-flocs was proposed as a protective phenomenon.

To determine if a rigid sheath and/or the presence of a mucilaginous layer does indeed offer protection to the various unit processes more research needs to be conducted. Although no toxicogenic cyanobacteria were detected in this study, the fact that cyanobacterial cells reached the product water indicates that the treatment technologies applied in the WTPs assessed in this study need to be re-evaluated. Monitoring of the incoming cyanobacterial composition in the raw water is crucial for the adaptation of treatment parameters that minimize not only cell lysis, but also maintain trichome integrity. Despite its advantages for coagulation the application of pre-oxidation needs to be carefully evaluated based on cyanobacterial composition. Furthermore, the type of coagulation applied could be optimized based on the major morphology of the cyanobacteria, switching from charge neutralization coagulation to sweep coagulation when filamentous cyanobacteria are present. However, adjusting the type of coagulation, carries financial implications, which have to be weighed against the advantages posed by increased removal.

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Table 1: Treatment technologies employed in the selected WTPs and the pilot plant.

WTP	Pre-oxidation	Coagulation	Flocculation	Sedi-mentation	descending filtration	ascending filtration	Disinfection
1	✓	✓	✗	✗	✓	✗	✓
2	✓	✓	✗	✗	✓	✓	✓
3	✓	✓	✓	✓	✓	✗	✓
Pilot Plant	✓	✓	✗	✗	✓*	✗	✗

*The pilot plant is built to perform dual descending filtration using different grain sizes

Table 2: Full-scale WTPs operating parameters including flow rate, chemical dosage, dosage, and hydraulic loading rates of the filters.

WTP	1	2	3
Flow rate ($m^3\,sec^{-1}$)	8.5	1	0.02
Pre-oxidant	ClO_2	Cl_2	Cl_2
Pre-oxidant dose (mg L^{-1})	1	3	5
Coagulant (Polyaluminium chloride – PACl) (mg L^{-1})	7	5	10
Polymer (diallyldimethylammonium polychloride) dose (mg L^{-1})	0.8	0.8	1
Filters hydraulic loading rates (m d^{-1})	240	120/180*	240
Filter Effective grain size (mm)	0.72	2.27/0.86*	0.72
Filter run times (h)	8	24/12*	8

*first value for pre-filter (ascending), second value for second filter (descending)

Table 3: Modified double filtration (MDF) media characteristics.

Parameter	Pre-Filter (PF)		Filter 1 (F1)	Filter 2 (F2)
Number of Layers	2		1	1
Smallest grain (mm)	2.00	3.36	0.84	0.52
Largest grain (mm)	3.36	4.90	1.41	1.68
Media height (m)	0.40	0.10	0.80	0.80
Effective Size (mm)	2.40	3.60	1.00	0.72
Coefficient of uniformity	1.2	1.1	1.2	1.6

Table 4: Chemical and hydraulic operating parameters used in the pilot plant investigations. Coagulant used was poly aluminum chloride, polymer was diallyldimethylammonium polychloride, and pre-oxidant was chlorine (Cl_2).

Run	System	Dosages			Hydraulic loading rate ($m^3 m^{-2} d^{-1}$)
		Coagulant ($mg\ L^{-1}$)	Polymer ($mg\ L^{-1}$)	Pre-oxidant ($mg\ L^{-1}$)	
1	PF + F1	5.5	1.6	0	240
2	PF + F2	4.7	1.2	0	240
3	PF + F2	7.5	1.5	1.0	240
4	PF + F2	4.0	0.8	1.0	200

Table 5: Selection of potential cyanotoxin and/or T&O producing cyanobacteria encountered in the raw waters from WTP1 - 3. Micrographs at 400x magnification under light microscope. Error = 1SD, *n*=30 PP = pilot plant, L = length, D = diameter.

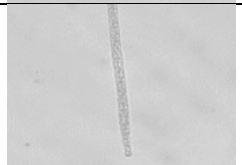
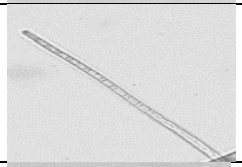
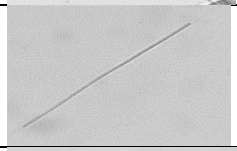
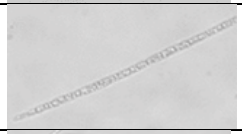
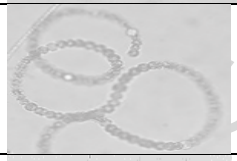
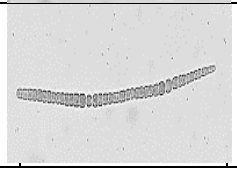
Organism	Micrograph	Average cells per trichome	Cell size (µm)	Sheath	Mucilaginous layer	Secondary metabolites	Occurrence	References
Pseudanabaena sp.		8 ± 2	L: 4±0.1 D: 2±1	-	some species	anatoxin-a, 2-methylisoborneol	PP, WTP1, WTP2, WTP3	Gorham et al., 1982; Izzaguire and Taylor, 1998; Komárek 1992
Planktothrix sp.		41 ± 18	L: 4 D: 4±1	-	-	microcystins, saxitoxins, cylindrospermopsin, anatoxin	PP, WTP1, WTP2, WTP3	Sivonen et al., 1989; Komárek 1992
Geitlerinema sp.		31 ± 7	L: 3 D: 1	-	-	unidentified toxin	PP, WTP1, WTP2, WTP3	Bernard et al., 2011; Komárek & Anagnostidis 2005
Planktolyngbya sp.		11 ± 3	L: 4 D: 1	rigid sheath	-	microcystins	WTP1, WTP2, WTP3	Brand and Villena, 2002; Komárek 1992
Cylindrospermopsis / *Rhaphidiopsis* sp.		22 ± 9	L: 5 D: 3±1	-	-	cylindrospermopsin, saxitoxins	PP, WTP1, WTP2, WTP3	Sivonen et al., 1989; Komárek & Komárková 2003
Dolichospermum sp.		49 ± 10	D: 4±1	-	+	saxitoxins, 2-methylisoborneol, geosmin, anatoxin-a	PP	Sivonen et al., 1989; Tsao et al., 2014; Wacklin et al. 2009
Aphazinomenon sp.		17 ± 6	L: 4 D: 4±1	-	some species	saxitoxins, cylindrospermopsin	PP, WTP1, WTP2, WTP3	Pereira et al., 2001; Spoof et al., 2006; Komárek and Kováčik, 1989

Table 6: Total cyanobacteria count (including non-filamentous cyanobacteria) at each unit process in three full scale WTPs (WTP1: descending direct filtration; WTP2: direct double filtration (ascending/descending); WTP3: conventional treatment (coagulation/flocculation, sedimentation, filtration), and a pilot plant employing modified direct double filtration (descending/descending). n=2, %RSD < 5% for full scale plants, n=4, %RSD < 10% for the pilot plant.

Plant	Raw Water cells mL^{-1}	Sedimented cells mL^{-1}	Pre-filtered cells mL^{-1}	Filtered cells mL^{-1}	Total Removal (%)
WTP1	$1.66x10^5$	n/a	n/a	$0.062x10^5$	96
WTP2	$0.58x10^5$	n/a	$0.074x10^5$	$0.019x10^5$	97
WTP3	$11.60x10^5$	$3.39x10^5$	n/a	$0.324x10^5$	97
Pilot plant	$9.24x10^5$	n/a	$2.49x10^5$	$0.579x10^5$	94

Table 7: Total absolute and per cent cell loss per trichome of investigated cyanobacterial species during modified double filtration in the pilot plant. Loss represents the difference between cells per trichome in the raw water to cells per trichome determined in the final filter effluent. Run 1) F2 as secondary filter with no addition of Cl_2 and with 240 $m^3\ m^{-2}\ d^{-1}$ hydraulic loading rate.; Run 2) F1 as secondary filter with no addition of Cl_2 and with 240 $m^3\ m^{-2}\ d^{-1}$ hydraulic loading rate.; Run 3) F2 as secondary filter with the addition of 1 mg L^{-1} Cl_2 and with 240 $m^3\ m^{-2}\ d^{-1}$ hydraulic loading rate; Run 4) F2 as secondary filter with the addition of 1 mg L^{-1} Cl_2 and with 200 $m^3\ m^{-2}\ d^{-1}$ hydraulic loading rate. *n*=4; %RSD < 10 %

Genus	**Total cell loss per trichome**							
	Run 1		**Run 2**		**Run 3**		**Run 4**	
	Cells lost	**% loss**	**Cells lost**	**% loss**	**Cells lost**	**% loss**	**Cells lost**	**% loss**
Pseudanabaena sp.	1	19	3	45	2	28	4	47
Planktothrix sp.	6	26	3	15	21	49	15	47
Geitlerinema sp.	9	33	13	62	24	67	6	16
Cylindrospermopsis / Raphidiopsis sp.	8	25	8	26	5	22	8	29
Dolichospermum sp.	22	63	39	73	19	38	56	98
Aphanizomenon sp.	3	39	8	46	0	0	10	49

Table 8: Total absolute and per cent trichome loss of investigated cyanobacterial genera caused by different treatment technologies. Loss represents the difference between cells per trichome in the raw water to cells per trichome determined in the final filter effluent. WTP1 ascending direct filtration; WTP2 direct double filtration (ascending /descending); WTP3 conventional treatment. *n*=4; %RSD < 10 %; * nd = not detected

Genus	Total cell loss per trichome					
	WTP1		WTP2		WTP3	
	Cells lost	% loss	Cells lost	% loss	Cells lost	% loss
Pseudanabaena sp.	0	0	4	44	4	40
Planktothrix sp.	35	73	63	88	22	45
Geitlerinema sp.	30	100	30	79	9	35
Planktolyngbya sp.	5	50	4	36	1	11
Cylindrospermopsis / Raphidiopsis sp.	4	36	5	33	4	29
Aphanizomenon sp.	17	100	12	52	nd	nd

Highlights

- Environmental, pilot and full-scale treatment plant water samples were examined under a light microscope to evaluate trichome integrity;
- Treatment train breaks cyanobacterial trichomes leading to smaller trichomes and single cells;
- Morphological factors affect trichome truncation resulting in single cells in product water;
- Filtration step was the most damaging unit process for most cyanobacterial genera;
- Trichome integrity should be considered when designing and operating treatment processes.

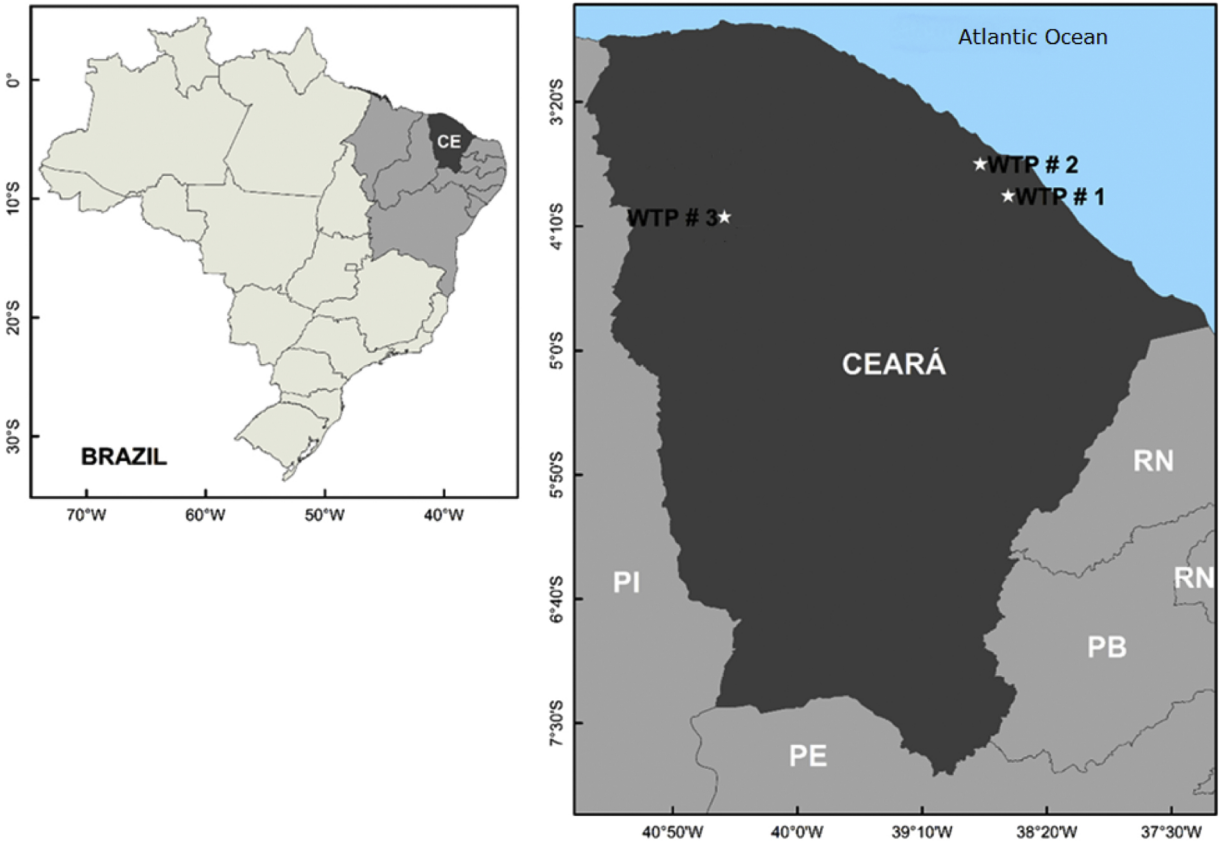

Atlantic Ocean
CE
BRAZIL
0°
10°S
20°S
30°S
70°W
60°W
50°W
40°W
WTP # 2
WTP # 1
WTP # 3
CEARÁ
RN
PI
RN
PB
PE
3°20'S
4°10'S
5°0'S
5°50'S
6°40'S
7°30'S
40°50'W
40°0'W
39°10'W
38°20'W
37°30'W

Figure 1

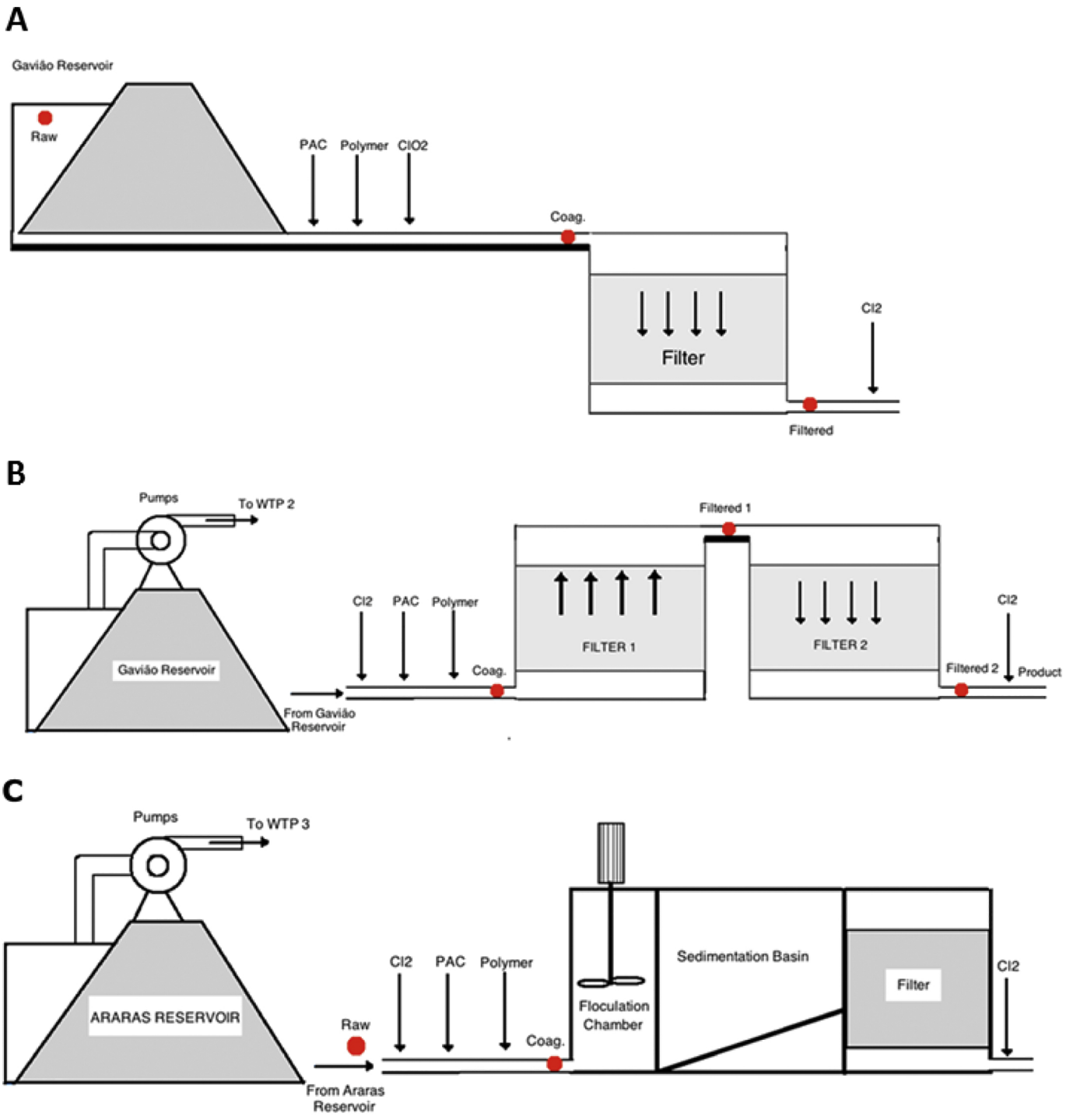

A
Gavião Reservoir
Raw
PAC
Polymer
ClO2
Coag.
Filter
Cl2
Filtered
B
Pumps
To WTP 2
Gavião Reservoir
From Gavião Reservoir
Cl2
PAC
Polymer
Coag.
Filtered 1
FILTER 1
FILTER 2
Cl2
Filtered 2
Product
C
Pumps
To WTP 3
ARARAS RESERVOIR
From Araras Reservoir
Raw
Cl2
PAC
Polymer
Coag.
Floculation Chamber
Sedimentation Basin
Filter
Cl2

Figure 2

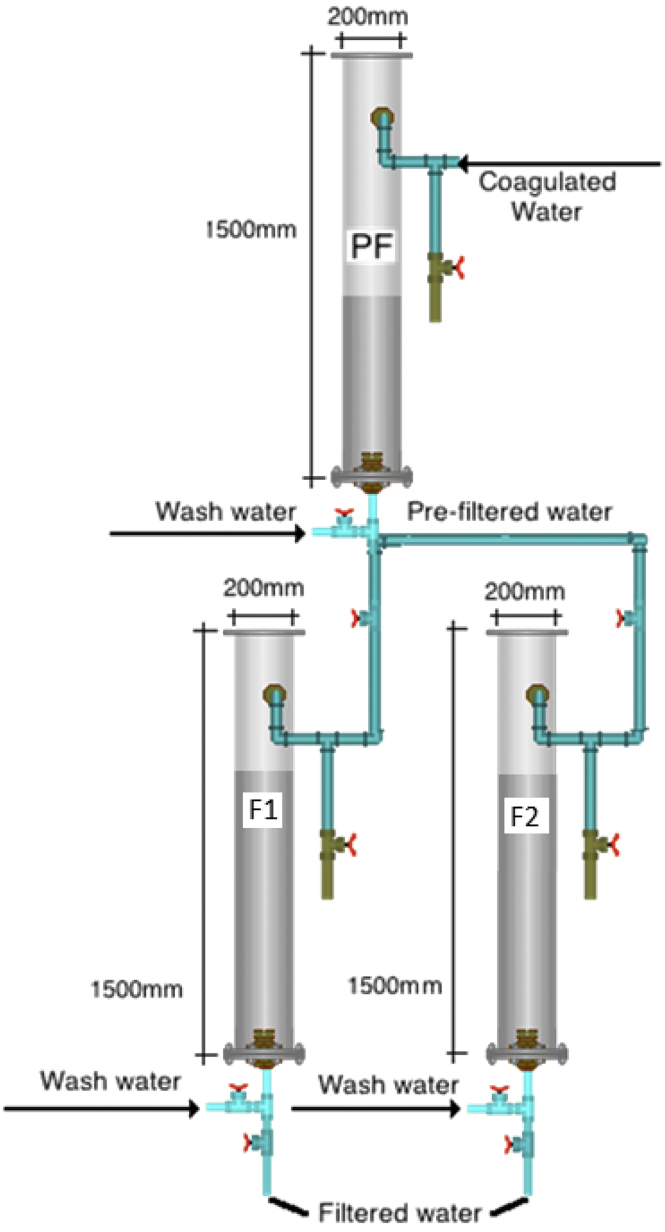

200mm
1500mm
PF
Coagulated Water
Wash water
Pre-filtered water
200mm
200mm
F1
F2
1500mm
1500mm
Wash water
Wash water
Filtered water

Figure 3

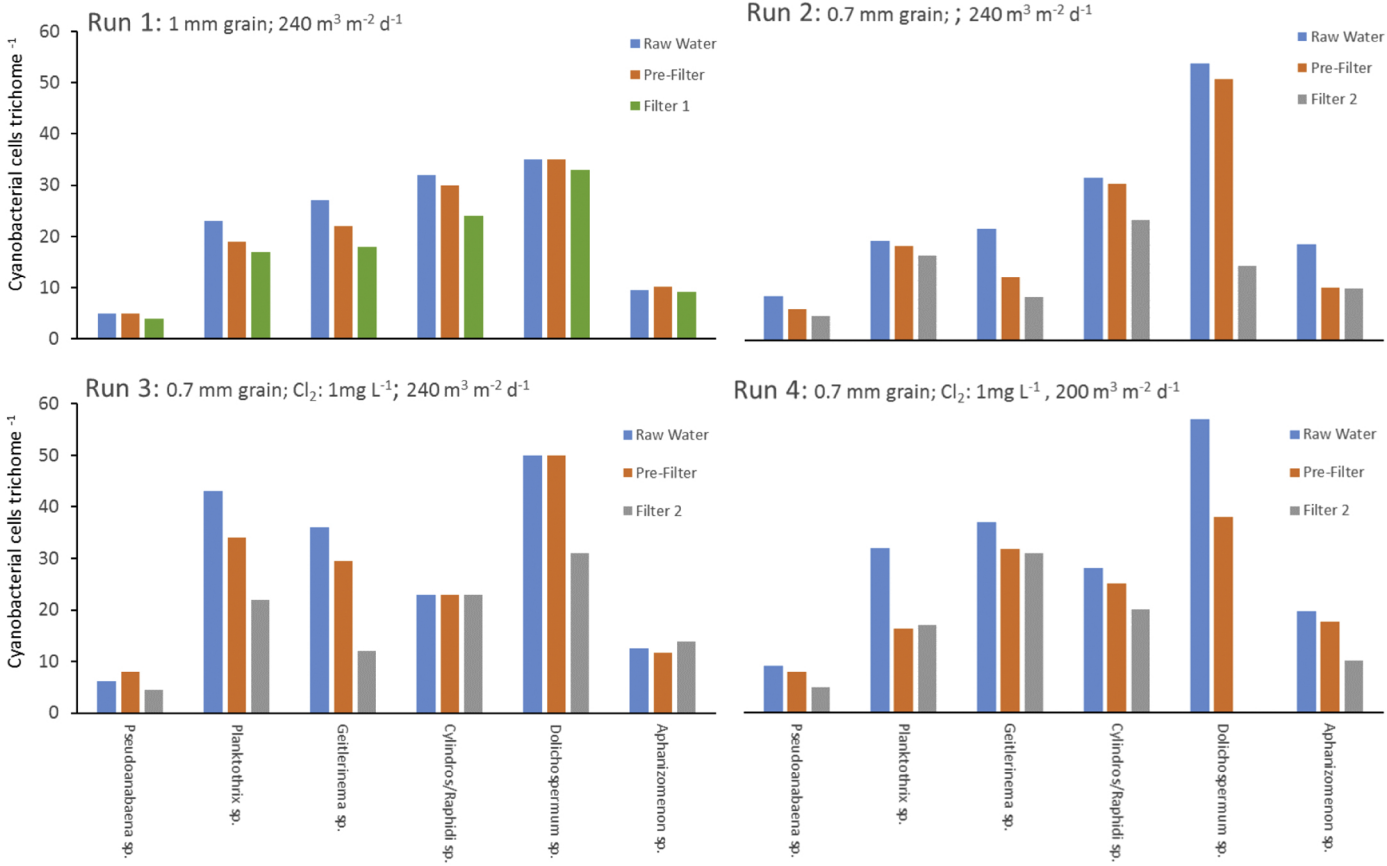
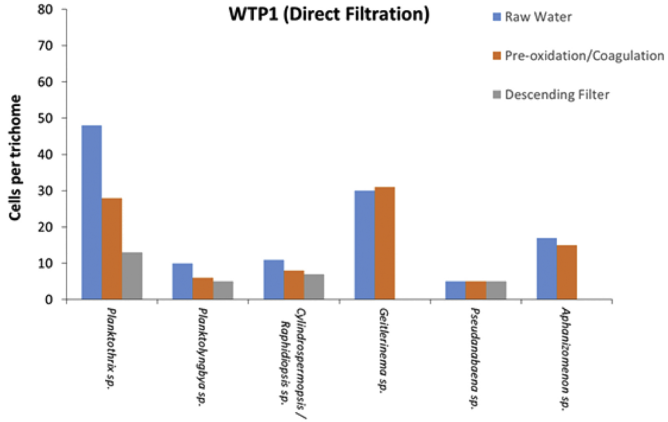
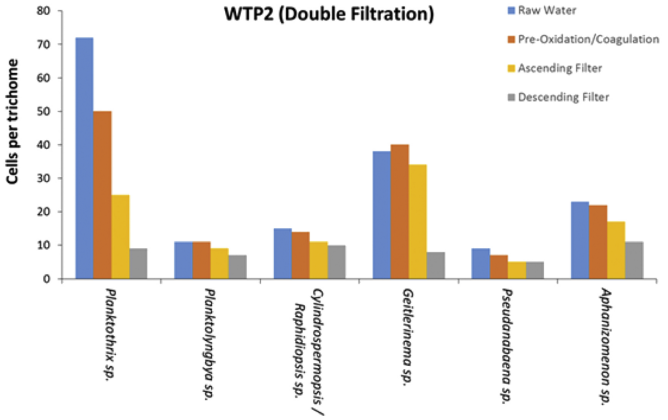

Run 1: 1 mm grain; 240 m3 m-2 d-1
Raw Water
Pre-Filter
Filter 1
Cyanobacterial cells trichome -1
Run 2: 0.7 mm grain; ; 240 m3 m-2 d-1
Raw Water
Pre-Filter
Filter 2
Run 3: 0.7 mm grain; Cl2: 1mg L-1; 240 m3 m-2 d-1
Raw Water
Pre-Filter
Filter 2
Cyanobacterial cells trichome -1
Run 4: 0.7 mm grain; Cl2: 1mg L-1 , 200 m3 m-2 d-1
Raw Water
Pre-Filter
Filter 2
Pseudoanabaena sp.
Planktothrix sp.
Geitlerinema sp.
Cylindros/Raphidi sp.
Dolichospermum sp.
Aphanizomenon sp.

Figure 4


WTP1 (Direct Filtration)
Raw Water
Pre-oxidation/Coagulation
Descending Filter
Cells per trichome
80
70
60
50
40
30
20
10
0
Planktothrix sp.
Planktolyngbya sp.
Cylindrospermopsis / Raphidiopsis sp.
Geitlerinema sp.
Pseudanabaena sp.
Aphanizomenon sp.


WTP2 (Double Filtration)
Raw Water
Pre-Oxidation/Coagulation
Ascending Filter
Descending Filter
Cells per trichome
80
70
60
50
40
30
20
10
0
Planktothrix sp.
Planktolyngbya sp.
Cylindrospermopsis / Raphidiopsis sp.
Geitlerinema sp.
Pseudanabaena sp.
Aphanizomenon sp.


WTP3 (Conventional Treatment)
Raw Water
80

Figure 5