CHAPTER 8:

THE FLOCCULATION OF CYANOBACTERIA USING FLY ASH

1. Introduction

The removal of surface algae using flocculants has proven successful. Tenney *et al.* (1969) investigated the use of synthetic organic polyelectrolytes for the flocculation of algae. Algal cells form stable microbial suspensions, possess a chemically reactive cellular surface, and possess a net negative surface charge due to the ionisation of functional ionogenic groups. The stability of algal suspensions depends on the forces acting between the particles themselves as well as on the forces interacting between the particles and the water; hence algae can be classified as hydrophilic bio-colloids. Addition of a cationic synthetic organic polyelectrolyte induced algal flocculation, but anionic and non-ionic polymers were not effective. The mechanism of chemically induced flocculation was described in terms of a bridging phenomenon between the discreet algal cells and the linearly extended polymer chains, which formed a three-dimensional matrix capable of subsiding. The flocculation efficiency was directly related to the extent of polymer coverage of the active sites on the algal cell surface; the algal surface charge needed to be reduced to a level which allowed the extended polymer chains to bridge the separation distance established by electrostatic repulsion. Optimal algal flocculation occurred at 50% coverage of the algal surface. Flocculation was most effective at the low pH levels of 2 to 3 due to reduced electrostatic repulsion between the algal cells and improved polymeric bridging because of a greater extension of polymer chains. Flocculation efficiency was affected by the algal growth phase, with the least amount of flocculant required in the late log and early declining log phase. High molecular weight extracellular metabolites produced by algae accumulate rapidly during the late log phase. These polymeric molecules comprise of long chain polysaccharides, proteins and nucleic acids and are of sufficient length to form bridges between algal particles, hence enhancing flocculation. In later growth stages the accumulation of this material could have acted as a protective colloid.

The potential use of clays to control harmful algal blooms has been investigated in East Asia, Australia, the U.S.A. and Sweden. Minerals such as montmorillonite and montmorillonite-containing sediments such as phosphatic clay, kaolinite and yellow loess have been used effectively. Cell removal occurs through the flocculation of algal particles leading to the formation of larger aggregates which settle rapidly (Sengco & Anderson, 2004). Pan *et al.* (2006) investigated the algal removal abilities of 26 clays

and minerals, and found that sepiolite, talc, ferric oxide, and kaolinite were the most effective, with an 8h equilibrium removal efficiency >90% at a clay loading of $0.7 g.L^{-1}$. When the clay loading was reduced to $0.2 g.L^{-1}$, the removal efficiency for 25 of the materials decreased to below 60%, except for sepiolite which remained about 97%. The high efficiency for sepiolite to flocculate *Microcystis aeruginosa* cells in freshwaters was due to the mechanism of netting and bridging.

Divakaran & Pillai (2002) investigated the use of chitosan to flocculate three freshwater species of algae, and one brackish alga. Chitosan is obtained by the deacetylation of chitin and is a cationic polyelectrolyte, thus is expected to coagulate negatively charged suspended particles found in natural waters. The flocculation efficiency was sensitive to pH, with the optimal flocculation of the freshwater algae occurring at pH 7, which differs to the findings of Tenney *et al*. (1969). Microscopy showed that the cells were intact after flocculation, but stuck together in clumps. Culturing of the flocculated clumps showed growth as usual, indicating that the cells were alive, but the clumps remained settled and fresh cells took longer to surface. Zou *et al*. (2006) found that clay particles could be turned into highly efficient flocculants to remove *Microcystis aeruginosa* cells in freshwaters when they were modified by chitosan. As yet no assessment of the use of fly ash as a potential algal flocculant has been published.

Of the millions of metric tones of fly ash that are produced word-wide every year, only a portion (10-20%) is reused for productive purposes, primarily in cementitious (concrete and cement) products and in construction, such as highway road bases, grout mixes and for stabilising clay-based building materials (Iyer & Scott, 2001). The remaining amount of fly ash produced annually must either be disposed in controlled landfills or waste containment facilities, or stockpiled in slag heaps (Shackelford, 2000) all of which can be regarded as unsightly and environmentally undesirable. With competition for limited space and stricter regulations on surface and ground water discharge, water originating from fly ash disposal sites must be well managed. The long-term maintenance of ash disposal sites and the necessary water management involved can pose a significant financial burden. The development of other means of commercial exploitation of fly ash beyond the cement and construction industries is therefore a priority.

The use of fly ash in wastewater treatment has been studied extensively, and the results of laboratory tests showed that fly ash is a good sorbent for the removal of heavy metals (Ayala *et al*., 1998; Héquet *et al*., 2001). Estivinho *et al*., (2007) used fly ash to adsorb chlorophenols, which are highly toxic and mutagenic. They found that fly ash was a good alternative to activated carbon, with the reduced sorption capacity of fly ash when compared to activated carbon not being significant considering the lower costs of the fly ash.

Oguz (2005) assessed the use of Yatagan fly ash to remove phosphate, an essential macronutrient that spurs the growth of photosynthetic algae and cyanobacteria, from aqueous solutions. Fly ash was a highly successful adsorbant, with a phosphate removal efficiency in excess of 99%, and a phosphate adsorption capacity of $71.87mg.g^{-1}$. According to the X-ray spectra obtained, it was thought that there was an electrostatic attraction on the solid/liquid interface between the phosphate salts and the fly ash particles, which led to ion exchange and weak physical interactions. Agyei *et al*. (2002) examined the phosphate ion removal from solution using fly ash, slag and ordinary Portland cement (OPC). The rate and efficiency of phosphate adsorption increased in the order fly ash, slag, OPC, which was the same order as the increasing percentage CaO in the adsorbants. This led to the conclusion that the extent of phosphate removal was related to the percentage CaO or Ca^{2+} ions released into the solution via hydration and dissolution. This was confirmed by the research of Chen *et al*. (2006), who concluded that phosphate immobilization by fly ash was governed by the amount of Ca in the ash, especially CaO and $CaSO_4$. They also attributed a portion of the phosphate removal to the presence of Fe_2O_3. The greatest removal of phosphate occurred in alkaline conditions for high calcium fly ash.

The aims of this study were to evaluate the ability of various fly ash samples to flocculate algae, determine which sample was the most effective, and investigate the possible mechanism of flocculation. The fly ash samples were also tested for their ability to adsorb phosphate from aqueous solutions.

2. Material and Methods

2.1 Fly ash samples

The fly ash used in this study was provided by Eskom. Six samples from different power stations using coal from different mines (Table 1), with varying physical and chemical characteristics (Chapter 7), were tested for their ability to flocculate cyanobacteria.

Table 1: Fly ash samples

Sample Number	Power station	Coal Mine
1	Thutuka	Newdenmark
2	Arnot	Arnot Coal
3	Duvha	Middelburg mine BHP Biliton
4	Hendrina	Optimum
5	Kendal	Khutala
6	Matla	Matla Coal
7	Lethabo	Newvaal

2.2. Cyanobacteria samples

Water samples were taken from the eutrophied Hartbeespoort Dam during June and July 2007. The water had a high concentration of cyanobacteria at the time of sampling.

2.3. Flocculation experiments

2L beakers (surface area ≈ 133cm^2) were filled with 600ml of cyanobacteria-containing water from the Hartbeespoort Dam. The beakers were allowed to stand until all the cyanobacteria had floated to the surface to form a definitive layer. In order to determine which fly ash had the greatest flocculating ability, varying quantities of each fly ash were spread evenly over the surface using a sieve, and the beakers were allowed to

stand for 6h to allow the flocculated cyanobacteria to settle and form a layer on the bottom of the beaker. The top layer was carefully skimmed off, and the clear water pored off the bottom layer. The volumes and the chlorophyll-a concentrations of the top and bottom layers were measured to determine the flocculation efficiency. Chlorophyll-a was measured using the methanol extraction method (Lorenzen, 1967; Golterman & Clymo, 1970; Holm-hansen, 1978). 100ml of sample was filtered through a membrane filter (GF/C 0.45µm pore size, 47 mm diameter, Whatman) and the filter was placed into a 50ml Greiner tube, filled with 10ml methanol and wrapped in aluminium foil to avoid degradation by light. Following homogenisation, the tube was centrifuged for 10 min at 3200 rpm. The absorbance of the supernatant was measured at 665nm and 750nm against pure methanol (Spectronic® 20 Genesis™, Spectronic Instruments), using a 1cm cuvette. The following formula was then used to calculate the chlorophyll-a concentration:

$$\text{Chl a } (\mu g.l^{-1}) = (Abs_{665nm} - Abs_{750nm}) \times A \times Vm/V \times L$$

Where:
A = absorbance coefficient of Chl a in methanol (12.63)
Vm = volume of methanol used (mL)
V = volume of water filtered (L)
L = path length of cuvette (cm)

Once it was established which fly ash was the most effective, this ash was used to determine the amount of fly ash needed for optimal flocculation by investigating the effect of varying the amount of ash used and the thickness of the cyanobacterial layer. During the experiments, samples for re-growth experiments and electron microscopy were taken and the pH value was measured. Water samples to which no fly ash was added were used as negative controls.

2.4. Re-growth experiments

Samples from the bottom layer containing flocculated cyanobacteria and fly ash were taken at 6h and 36h after treatment. Modified Allen's BG-11 medium (Table 2), (Krüger & Eloff, 1977) was inoculated with these samples to test for re-growth of the

cyanobacteria. The cultures were grown in 250ml cotton plugged sterile Erlenmeyer flasks at ambient temperatures (24-26°C) with shaking to allow for aeration. Continuous lighting of 2000lux (Extech instruments Datalogging lightmeter model 401036) was provided by 18W cool white fluorescent lamps (Lohuis FT 18W/T8 1200LM) suspended above the flasks. The concentration of chlorophyll-a was measured immediately after inoculation and again after approximately two weeks. Growth media was inoculated with a sample of the floating layer from the control beaker (without addition of fly ash) as a positive control.

Table 2: Mineral composition of modified BG-11 medium

Component	Concentration
$NaNO_3$	$1.500 g.l^{-1}$
K_2HPO_4	$0.040 g.l^{-1}$
$MgSO_4.7H_2O$	$0.075 g.l^{-1}$
$CaCl_2.2H_2O$	$0.036 g.l^{-1}$
Na_2CO_3	$0.020 g.L^{-1}$
$FeSO_4$	$0.006 g.L^{-1}$
$EDTA.Na_2H_2O$	$0.001 g.L^{-1}$
Citric acid	$0.0112 g.L^{-1}$
Trace metal solution (Table 2.1)	$1 ml.l^{-1}$

Table 2.1: Trace metal solution for modified BG-11 media

Trace metal solution component	**Concentration ($g.l^{-1}$)**
H_3BO_3	2.8600
$MnCl_2.4H_2O$	1.8100
$ZnSO_4.7H_2O$	0.222
$Na_2MoO_4.5H_2O$	0.300
$CO(NO_3)_2.H_2O$	0.0494
$CuSO_4.5H_2O$	0.0790

2.5. Scanning electron microscopy (SEM) of flocculated cyanobacteria

SEM was performed on flocculated cyanobacterial samples as well as samples from the negative control. Samples were concentrated by centrifugation and, following removal of the supernatant, fixed in a solution of 2.5% gluteraldehyde in 0.075M sodium phosphate buffer (pH 7.4) overnight at 4°C. The samples were then rinsed 3 times in 0.075M sodium phosphate buffer (10min per rinse), centrifuging between each rinse. After rinsing with the buffer the material was fixed in 1% aqueous OsO_4 for 1.5h, then rinsed again in distilled water. The samples were dehydrated through an ethanol series (30%, 50%, 70%, 90%, 3x 100%; 10 min each), and were then dried to critical drying point. The dried samples were mounted on SEM slides, gold coated and viewed with a JOEL JSM-840 SEM. After the initial viewing, the samples mounted on the slides were covered with sticky tape, and the tape removed in an attempt to break up cell clusters so that the interior cyanobacteria could be seen. The samples were recoated and viewed again.

2.6. SEM of etched samples

In order to investigate the structure of the flocculated cyanobacterial colonies, samples were fixed and dehydrated as described for SEM, and were then embedded in epoxy resin (Quetol). The material was treated with half-concentrated resin for 1 h, followed by concentrated resin for 4h. The resin was allowed to polymerise for 48h at 60°C, and was then dried. Finally, the samples were sectioned with a diamond knife and contrasted with 4% aqueous uranyll acetate (15min) and lead citrate (5min). The resin blocks were then sectioned to produce smooth surfaces using a glass knife, and were then etched in NaOH dissolved in methanol (saturated solution) for 7min to remove the resin. Finally they were rinsed in methanol and dried. Slices of the resin blocks were then mounted on SEM slides, coated with gold and viewed using a Zeiss Ultra^{TM} 55.

2.7. Phosphate adsorption study

All glassware was prepared by rinsing once with 1M HCl and three times with distilled water to remove any residual phosphates from the glass surface. KH_2PO_4 was added to distilled water to make a 200mg.l^{-1} stock solution, and 50ml of this was added to 950ml

of distilled water in each beaker to give a final concentration of 10mg.l^{-1} PO_4-P (31.25mg.l^{-1} PO_4^{3-}). A 20:1 ratio of fly ash to PO_4^{3-} was used for treatment according to Oguz (2005), which was 625mg of ash per 1000ml. The samples were stirred continuously, and samples were taken at various time intervals after the addition of fly ash. 10ml was drawn up with a syringe and filtered through a 0.22µm filter disk into test tubes for PO_4-P testing. The phosphorus concentration of each sample was measured with the Spectroquant Phosphortest (PMB) 1.14848.001 (Merck), according to the manufacturer's instructions using the Photometer SQ118. The experiment was repeated with a 40:1 dosage of fly ash (1250mg ash per 1000ml), as well as with a higher initial PO_4-P concentration (20mg.l^{-1}) and 625mg of ash (10:1 treatment ratio).

3. Results

3.1. Flocculation experiments

The entire floating layer of cyanobacteria flocculated after the application of all 7 fly ash samples, but the fly-ash-cyanobacteria mixture separated into two layers after a few hours to form a floating top layer and a bottom layer. The top floating layer consisted of cyanobacteria and fly ash with low density, and the bottom layer of the fly ash particles more dense than water and the flocculated cyanobacteria. The results of flocculation with 5g (approximately 37.6mg.cm^{-2} surface area) of fly ash samples 1-7 48h after treatment are presented in Figure 1.

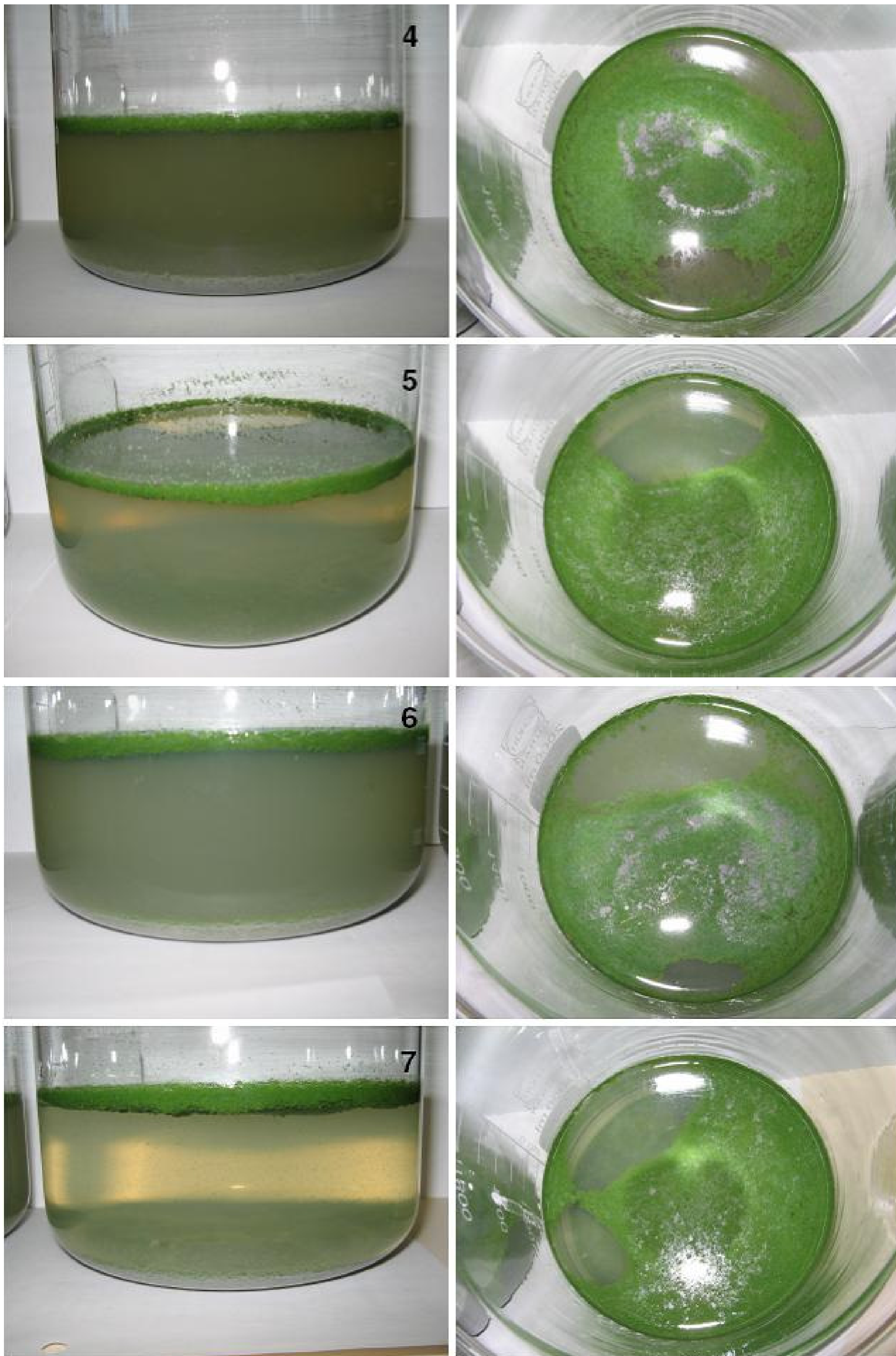

Figure 1: Results of flocculation tests 48h after addition of 5g of fly ash samples 1-7 ($37.6mg.cm^{-2}$). C is the negative control. The pictures on the left represent the view from above.

Water treated with fly ash samples 1, 2, 4 and 6 became turbid, darkened in colour and began to develop a strong odour within 6 hours after the treatment. Samples 3 and 5 only became turbid and darkened after 36h, and not to the same degree as samples 1, 4 and 6. Sample 7 became only slightly turbid and appeared to show an improvement after 60h. The water in the negative control remained clear with a floating cyanobacteria layer during the experimental period.

When the beakers were shaken after flocculation, some of the flocculated cyanobacteria floated to the surface again. This indicated that the attachment of the fly ash particles to the cyanobacteria was reversible in some cases.

The flocculation efficiency of the fly ash samples is presented in Table 3 and Figure 2. 5g, 6g and 8g of each fly ash were added to 600ml of water containing a similar amount of algae in order to determine which ash had the best flocculation efficiency. The treatment dosage was expressed in $mg.cm^{-2}$ surface area as well as $mg.cm^{-3}$ volume of algal layer.

Table 3: Cyanobacterial flocculation efficiency (%) after the addition of fly ash samples 1-7 at different dosages.

	5g $37.6mg.cm^{-2}$ $47mg.cm^{-3}$	**6g $45.1mg.cm^{-2}$ $50.1 mg.cm^{-3}$**	**8g $60.2 mg.cm^{-2}$ $57.3 mg.cm^{-3}$**	**Average**	**Standard Deviation**
Control	22.6	7.3	5.3	11.7	9.5
1	50.5	53.5	50.1	51.4	1.9
2	57.9	79	53.2	63.4	13.7
3	49.7	51.7	57.2	53	3.9
4	60.3	54.8	58.3	57.8	2.8
5	55.3	62.2	66.8	61.4	5.8
6	59.8	80.8	86.3	75.6	13.98
7	53.8	48.7	66.5	56.3	9.2

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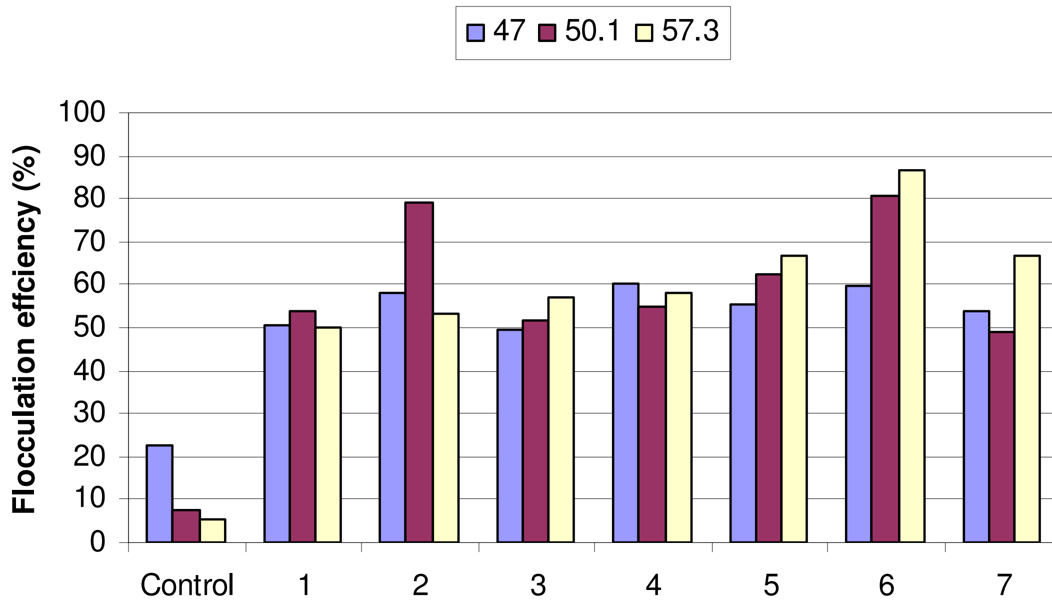


Figure 2: Flocculation efficiency of fly ash samples (1-7) at different dosages ($mg.cm^{-3}$)

Sample 6 (Matla) showed the highest average flocculation efficiency for the dosages tested, although it also had the highest standard deviation. This ash was chosen to investigate the optimal fly ash dosage for optimal flocculation by varying the fly ash amount as well as the thickness of the cyanobacterial layer. Figure 3 shows the flocculation efficiency of the ash compared with the dosage, at two different algal layer thicknesses (3mm and 9mm). Increasing the fly ash dosage only increased the flocculating efficiency to a certain point, after which further addition of fly ash had no effect. The flocculating efficiency was greater for the thinner layer of cyanobacteria when compared with the thicker layer at the same dosage. The maximum flocculation of the 3mm layer was 95%, whereas that of the 9mm layer was approximately 65%. The optimal amount of fly ash was between $40mg.cm^{-3}$ and $50mg.cm^{-3}$, depending on the thickness of the cyanobacterial layer.

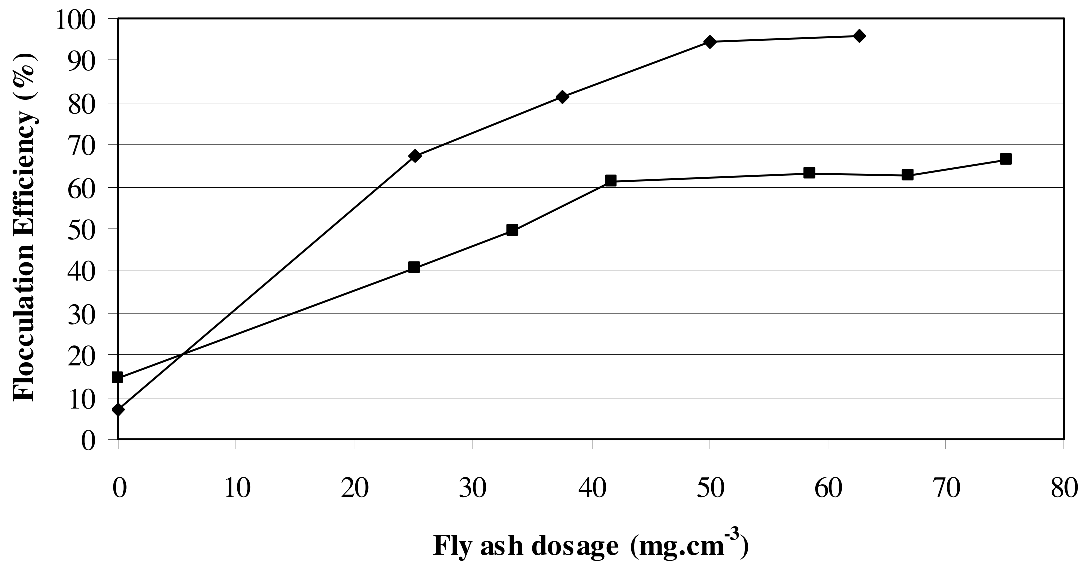


Figure 3: Flocculation efficiency of fly ash sample 6 at increasing concentration at two different cyanobacterial layer thicknesses (♦) 3mm thick (■) 9mm thick

3.2. Re-growth experiments

When BG-11 media was inoculated with cyanobacteria flocculated with fly ash samples 1, 2, 4 and 6, the media appeared pale green with few floating cells, whereas those inoculated with water treated with fly ash samples 3, 5 and 7 contained more floating cells the same colour as the control (Figure 4). The results of the re-growth experiments are shown in Table 4. After 6h, the cyanobacteria flocculated by all 7 ash samples were still alive as they showed growth in BG-11 media. However, after 36h, only cyanobacteria flocculated with fly ash samples 3, 5 and 7 were sufficiently viable to show growth.

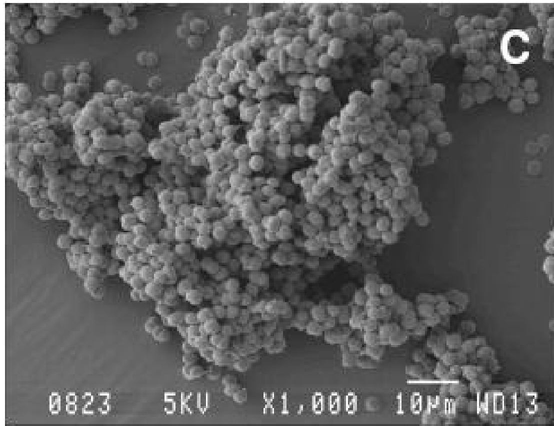
Figure 4: Cultures for re-growth experiment immediately after inoculation with cyanobacteria taken from the bottom of the beakers 36h after flocculation (a) inoculated with untreated cyanobacteria (Control); (b) inoculated with cyanobacteria treated with fly ash sample 5; (c) inoculated with cyanobacteria treated with fly ash 6.

Table 4: Re-growth of cyanobacterial samples taken 6h and 36h after flocculation with fly ash samples 1-7; (+) poor growth; + growth; □ no growth

	6h	**36h**
Control	+	+
1	+	□
2	+	□
3	+	+
4	+	(+)
5	+	+
6	+	□
7	+	+

3.3. SEM of flocculated cyanobacteria

SEM was used to observe the binding of the fly ash particles to the flocculated cyanobacterial cells (Figure 5). The floating cyanobacteria sampled from the untreated control were in large clusters, with cells in various stages of cell division. Extracellular polymers were visible on the cluster surfaces (Figure 5C). Cells sampled from the bottom of the untreated control that had sunk of their own accord (not shown) had a larger amount of extracellular material than the floating cells. Clusters were also observed in the SEM pictures of the flocculated cyanobacteria, but the cluster surfaces were composed mainly of fly ash. A few cyanobacteria were visible on the surfaces, distinguished by their surface properties and by the fact that some of the cells were in the process of dividing. The amount of cyanobacteria visible in the pictures was much less than expected, considering the volume ratio of fly ash to cyanobacteria.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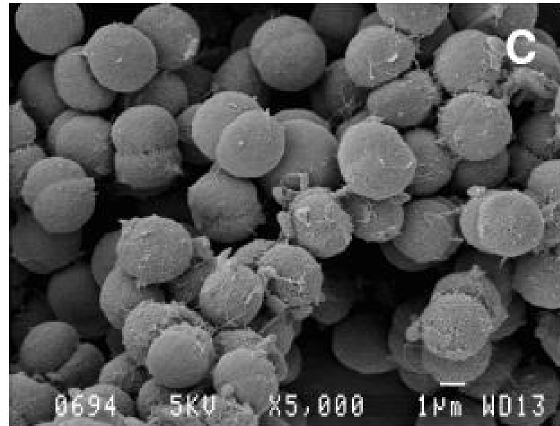
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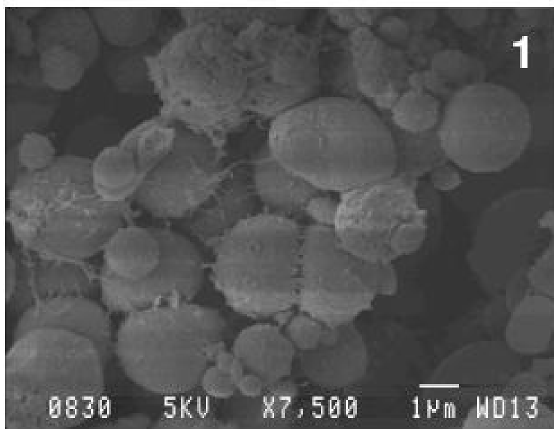
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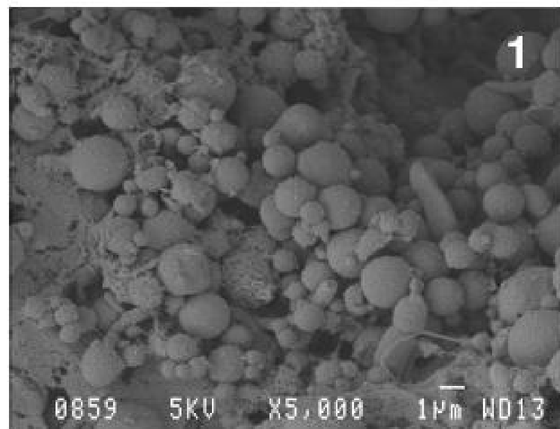
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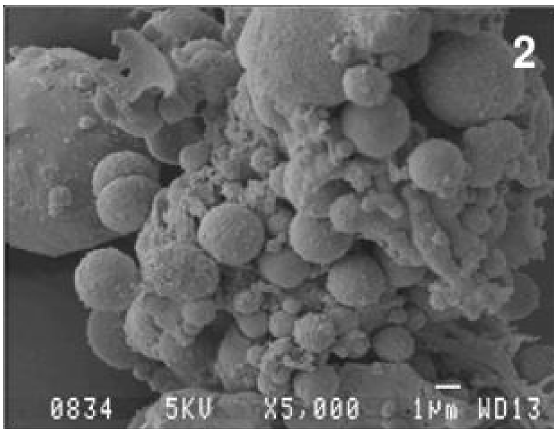
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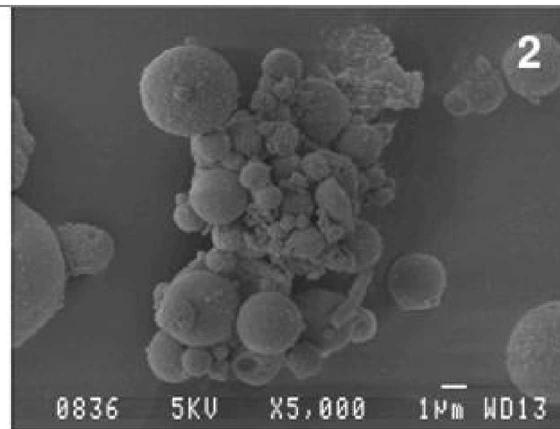
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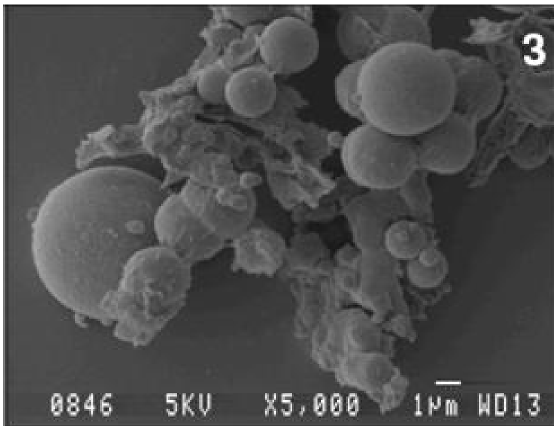
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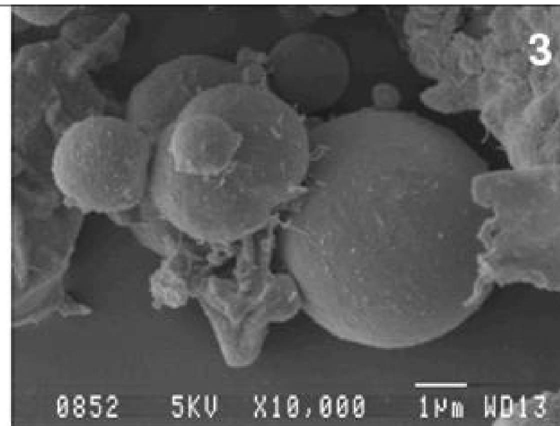
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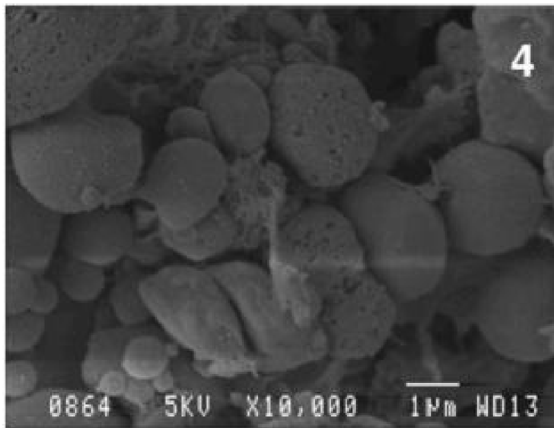
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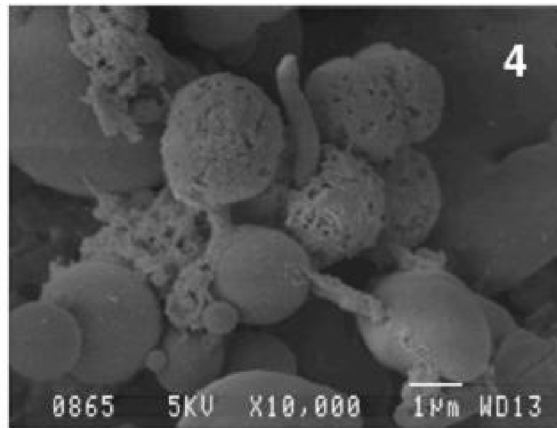
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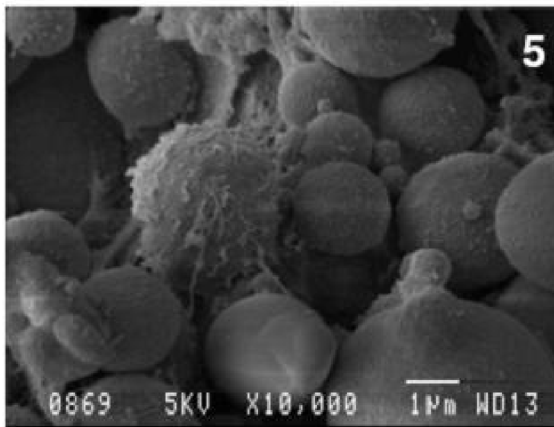
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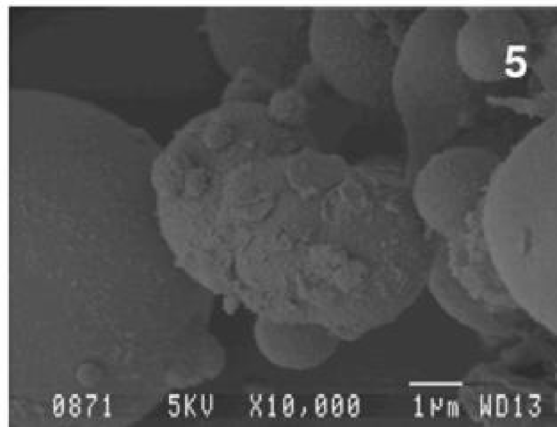
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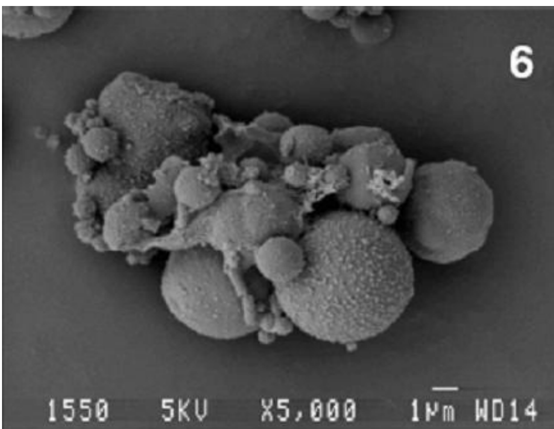
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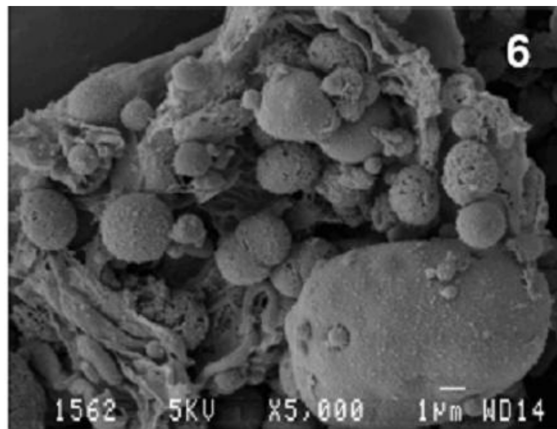
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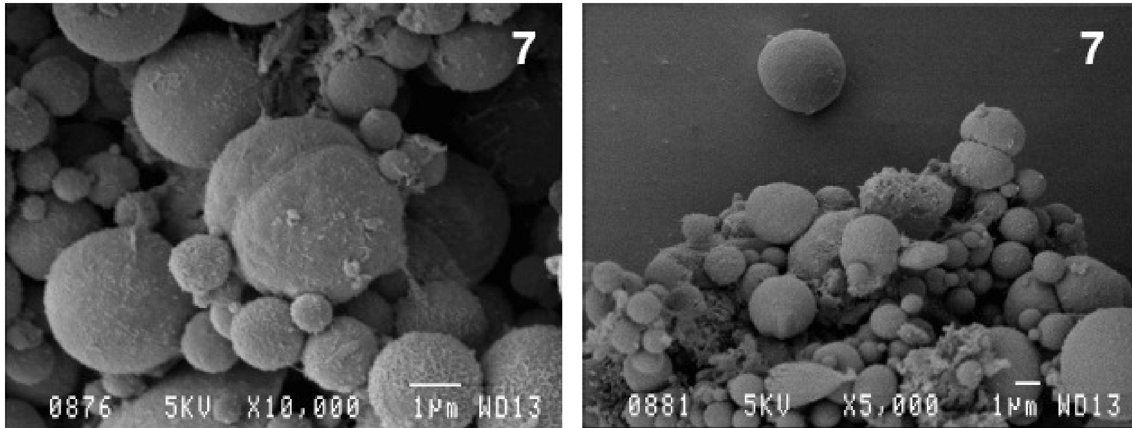

Figure 5: Scanning electron microscopy of the flocculated cyanobacteria and fly ash at various magnifications. There are two examples for each ash treatment, as well as for the untreated control (C).

Because it seemed likely that the cyanobacterial cell clusters were encapsulated by the fly ash particles, sticky tape was fixed to and removed from the mounted SEM slides in an attempt to pull the clusters apart to remove the outer fly ash layer and reveal the cyanobacteria cells. Figure 6 presents the results of the slides from fly ash 6 and fly ash 7. These pictures show that the cyanobacterial clusters were indeed surrounded by the fly ash particles, as more cyanobacteria were visible in the centre of each cluster. It was observed in these pictures that the cyanobacterial cells appeared healthier in the sample flocculated with fly ash 7 than that flocculated with fly ash 6.

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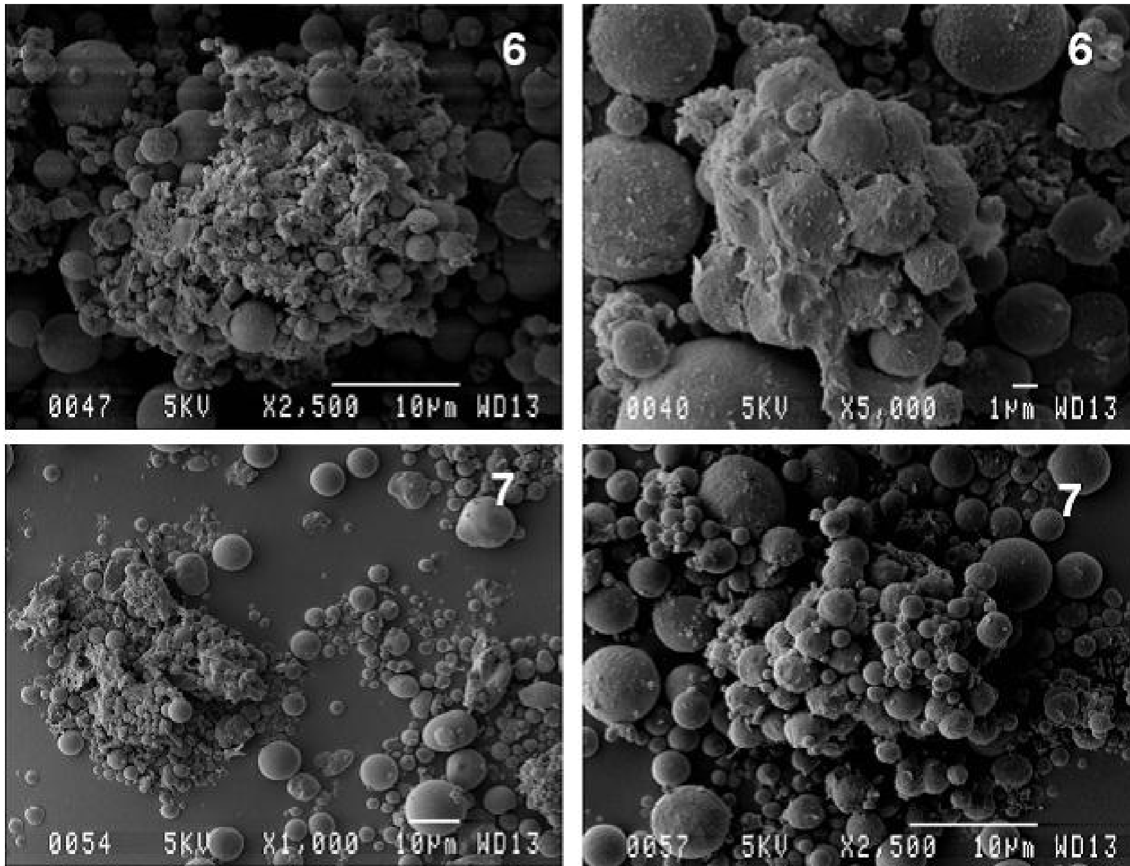

Figure 6: SEM pictures of flocculated cyanobacterial clusters from samples 6 and 7 broken up with tape

To further confirm the assumption that the fly ash particles enclosed the cyanobacterial clusters, new samples were embedded in resin which was cut to produce a smooth surface, and the resin was then etched away. When viewed under SEM, cell colonies in the untreated control displayed smooth edges, indicating that they were encapsulated in a layer of extracellular polymers (Figure 7: C1 and C2). In the pictures of the flocculated cyanobacterial cell clusters, spherical fly ash particles were visible on the edges as well as many broken pieces that appeared to be fly ash particles damaged during sample preparation (Figure 7: 6a and 6b).

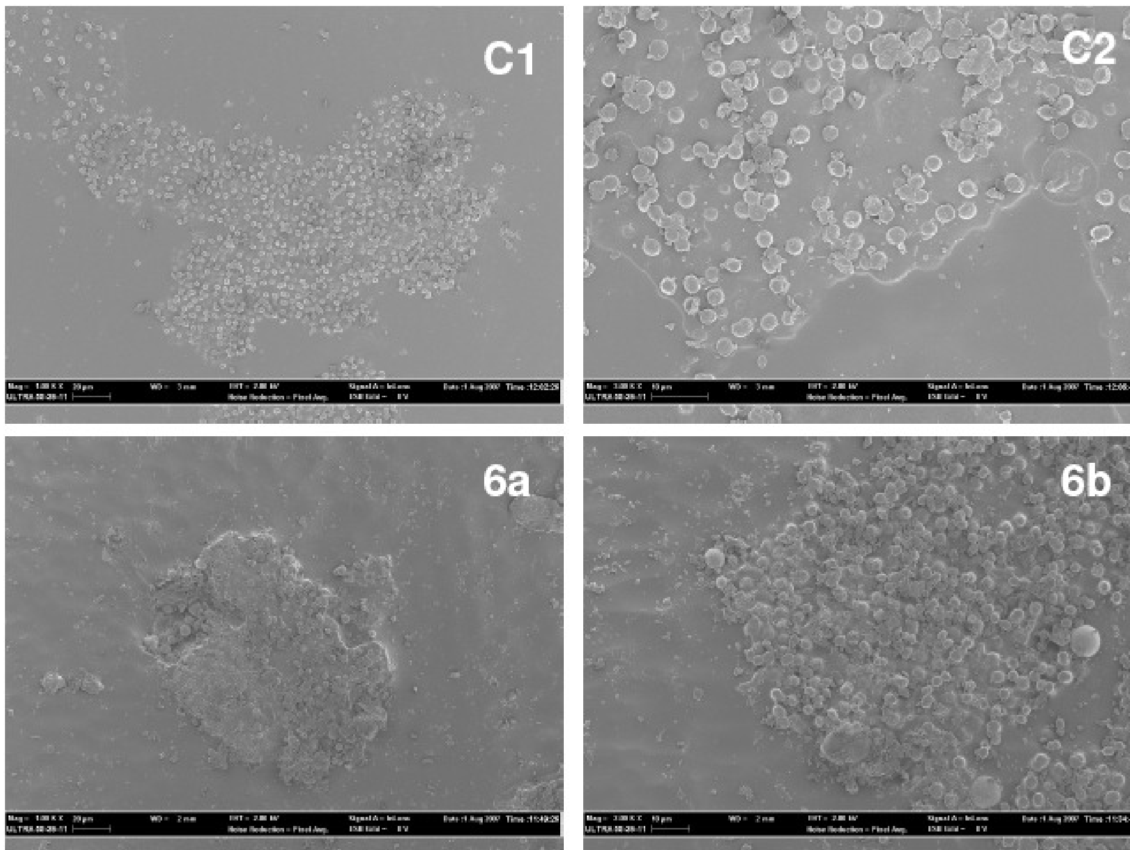

Figure 7: Scanning electron microscopy of the untreated control (C), and cyanobacterial colonies flocculated by fly ash sample 6 (6a and 6b). Samples were prepared by etching, and the magnification is as follows: C1: 1000x; C2: 3000x; 6a:1000x; 6b:2000x

3.4. Phosphate adsorption study

A 20:1 ratio of fly ash to PO_4^{3-} was used for treatment according to Oguz (2005), which was 625mg of ash per 1000ml water with a final concentration of $10mg.l^{-1}$ PO_4-P. The PO_4-P concentration was measured at various time intervals after the addition of the ash, but after 6h of continuous stirring there was no reduction in the PO_4-P. The experiment was then repeated with a 40:1 dosage of fly ash (1250mg ash per 1000ml water with a final concentration of $10mg.l^{-1}$ PO_4-P), and again no adsorption was apparent. Finally, in an attempt to increase the adsorption capacity of the ash, 625mg of the fly ash samples were added to a solution with a higher initial PO_4-P concentration of $20mg.l^{-1}$ (10:1 treatment ratio), and once again the PO_4-P concentration remained constant. These results were unexpected.

4. Discussion

All of the ash samples tested were able to flocculate the cyanobacteria to some degree, although sample 6 (Matla) had the greatest flocculation efficiency. The flocculation efficiency of this ash increased in a linear fashion with the amount of fly ash applied up to a point of maximum flocculation, after which further addition had no effect. For the Matla ash, the optimum amount of ash for maximum flocculating efficiency was approximately 45mg ash per 1cm^3 cyanobacterial biomass. This translates to 45g of fly ash per m^2 of surface area and 1mm thickness of the cyanobacterial layer.

Matla fly ash had the greatest percentage of small particles below 1µm (Chapter 7). According to the results from the XRD and XRF, there did not seem to be a significant difference between the samples in terms of their chemical properties. Thus, the flocculating efficiency is most likely directly related to the particle size, with the ash samples with the smallest particles being the most effective.

When the fly ash samples were added to water from the Hartbeespoort Dam (Chapter 7) there was a smaller increase in pH than when the ashes were added to distilled water, indicating that the dam water had a buffering effect.

The portion of the fly ash that was less dense than water remained floating on the surface, which would not be desirable in the treatment of a natural water body. This portion of the ash played no role in the flocculation of the cyanobacteria. In order to solve this problem, fly ash could be separated into two phases; that which is more dense than water and that which is less dense by first floating off the less dense phase and removing it (Kruger, 1996). The dense ash could then be filtered out and dried, and this phase used as a cyanobacterial flocculant.

The attachment of fly ash to some of the cyanobacteria was reversible when the beakers were shaken. This may pose a problem in a naturally occurring water body, as the normal mixing due to wind and fish activity could also cause detachment. In this case the cyanobacteria would not be retained at the bottom of the lake long enough to be killed by a lack of light or by the fly ash itself.

As can be seen by the SEM pictures, the cyanobacteria (*Microcystis aeruginosa*) form large colonies of cells. These colonies are enveloped in extracellular polymers, forming a protective layer. The mechanism of flocculation seemed to be related to this slime layer, as the fly ash particles appeared to stick to the outer surface of the colonies. Once sufficient fly ash had become attached to the outer surface of the colony it became too dense to remain floating, sinking to the bottom. The cyanobacteria may be able to overcome this by producing more gas vacuoles to increase buoyancy (Oliver, 1994) but the density of the fly ash may be too great to overcome. This appears to have been the case, as the cyanobacteria did not return to the surface, even after 48h. Vigorous shaking did release some cell colonies to the surface; these may have had less fly ash particles attached to them.

The re-growth experiments indicated that four of the seven fly ash samples (1: Tutuka; 2: Arnot; 5: Kendal and 6: Matla) caused cyanobacterial cell mortality within 36h of flocculation. Samples from these flocculation tests did not show re-growth in cyanobacterial growth media. However, the remaining samples (3: Duvha; 4: Hendrina; and 7: Lethabo) showed growth comparable to the media inoculated with the untreated control. The same samples that did not re-grow had a greater degree of turbidity, colouring and odour than the samples that were capable of growth. Furthermore, when examined with SEM, many of the cyanobacterial cells flocculated with fly ash sample 6 appeared to have damaged cell walls, when those flocculated with sample 7 (which showed re-growth) appeared to be healthy (smooth surfaces in various stages of cell division) and comparable to the control. It is possible that fly ash samples 3, 4 and 7 were capable of causing cyanobacterial cell mortality, but required more time than the 36h of the experiment.

One possible explanation for the killing effect seen with some ashes was the potential leaching of elements toxic to cyanobacteria. The pH of the water for the flocculation tests was above pH 7, therefore the results obtained for leaching in distilled water (Chapter 7) were expected to be similar to the leaching in the flocculation tests. Of the toxic elements (As, B, Cr, Hg, Ni, Pb and Zn), only B, Cr and Zn were present in solution, all others were below the detection limit of 0.01ppm. B was below 0.2ppm for all samples except for sample 5 (Kendal) at 1.09ppm and sample 7 (Lethabo) at 3.11ppm. The Cr concentration was the highest for samples 1 (Tutuka), 2 (Arnot), and 6

(Matla) at 0.24ppm, 0.12ppm and 0.27ppm respectively. None of the samples showed a Zn concentration above 0.1ppm in solution. These results correlate partially to the mortality results, in that samples 1, 2, and 6 leached the highest amount of Cr, and these did not show growth. However, the Cr concentration was low in sample 5, and this sample did not show growth either. The B concentration was high in this sample, but was higher in sample 7, which showed healthy re-growth. Palumbo *et al.* (2007) investigated the toxicity of several fly ash leachates using the Microtox© system, which is a standard biosensor based measurement technique for toxicity testing of water and soil. The method makes use of the luminescent bacterium *Vibrio fischeri* NRRL-11177. The luminescent bacteria were added to the leachates and the toxicity was measured by the decreased luminescence compared to a negative control. Of 8 leachates tested, which were leached at various pHs and contained both B and Cr, only one highly alkaline (12.4) leachate exhibited toxicity. This may also have been caused by the high pH, as the toxic effect was reduced when the leachate was neutralised. Therefore, although it is possible that the high concentration of Cr in the samples that did not show re-growth may have caused cell morbidity, it is unlikely when comparing the results from this study with those of Palumbo *et al.* (2007). However, cyanobacteria may be more sensitive to a high Cr concentration than *Vibrio fischeri.*

When the fly ash samples were leached in water at pH 2 (Chapter 7), more metals were leached than in distilled water, and a higher concentration of toxic elements was leached. However, the percentage of each toxic element that was leached from the fly ash samples was below 3% for all the elements. No Hg or Pb was leached, even at this low pH.

The concentrations of toxic metals leached in distilled water (Chapter 7) were above the DWAF target water quality range (TWQR) for human consumption as well as aquatic ecosystems. In acid water the concentrations of Al, As, Ca, Cr, Cu, Pb, Mg, Mn, Se and Zn greatly exceeded the TWQR for aquatic ecosystems. The amount of fly ash used in the leaching experiments was 50g per 1000ml (5% wt/vol). However, when 6g of the Matla fly ash was added to 600ml of water containing cyanobacteria, 81% of the cyanobacteria were flocculated. This translates to a 1% leaching solution, and the concentrations of toxic elements in the water can be expected to be less than those leached at high concentrations of ash.

Therefore, fly ash can potentially be used to flocculate cyanobacteria from a natural water body. The amount needed to achieve sufficient flocculation will have a negligible effect on the water chemistry because the elements leached will be highly diluted. The pH values of the sediment are seldom below pH 2, and the fly ash itself would have a neutralising effect on acidic sediments. A sediment pH of 2 is a "worst case scenario", and at the low relative dosages of fly ash needed for flocculation it is unlikely that the DWAF TWQRs would be exceeded.

When Agyei *et al*. (2002) and Chen *et al*. (2006) examined phosphate ion removal from solution using fly ash; they concluded that the extent of phosphate removal was related to the percentage CaO or Ca^{2+} ions in the ash. Oguz *et al*. (2005) used a 20:1 ratio of Yatagan fly ash (11.57% CaO) to PO_4^{3-}, and found that the phosphate removal efficiency was 99%, and the phosphate adsorption capacity 71.87mg.g^{-1}. The ash used by Agyei *et al*. (2002) consisted of 4.1% CaO, and more than 85% of the PO_4^{3-} was adsorbed from solution at a dosage ratio of 25:1. The fly ash samples used in this study had CaO concentrations which ranged from 3.41% to 6.9%. Although these concentrations were less than half the amount of CaO found in the Yatagan fly ash used by Oguz *et al*. (2005), no PO_4^{3-} was adsorbed by any of the fly ash samples tested, even at a treatment ratio of 40:1. This was unexpected, especially for samples 1, 2 and 6 which had CaO concentrations above 6.5%. Furthermore, the CaO concentrations of the ash samples were comparable to that of the ash used by Agyei *et al*. (2002), which consisted of 4.1% CaO. Even when the treatment ratio was more than double that used by Agyei *et al*. (2002), no adsorption was observed. Chen *et al*. (2006) also attributed a portion of the phosphate removal to the presence of Fe_2O_3. The ash samples 1-7 had a high Fe_2O_3 content ranging from 3.28% to 5.15%. It was not clear why the fly ash samples tested were not capable of adsorbing PO_4^{3-} from solution.

Activated carbon is often used to remove the toxins produced by cyanobacteria, as well as taste and odour compounds such as geosmin (Cook & Newcombe, 2004). Fly ash is capable of adsorbing toxic compounds, and so has potential for use in water treatment as well as in natural water bodies where the toxin level is above the recommended health standards as a result of a severe algal bloom.

5. Conclusion

Fly ash was generally an effective flocculant of cyanobacteria. Fly ash with a large amount of small particles was the most effective; in this study the ash from the Matla power station had the highest flocculation efficiency. The optimal dosage of Matla fly ash was 45g per m^2 of surface area and 1mm algal layer. The mechanism of flocculation appeared to involve the binding of the fly ash to the extracellular polymers on the surface of the cyanobacterial cell colonies, causing them to become too dense to remain afloat. Only the fly ash particles that were more dense than water were involved in the flocculation process, as the less dense particles remained floating on the surface. Fly ash added to water from the Hartbeespoort dam had a smaller pH increase than in distilled water. Four out of the seven fly ash samples tested caused cyanobacterial cell death after 36h. This was possibly related to the leaching of toxic elements, although only a small percentage of the total amount of trace elements were leached into solution, even at the low pH value of 2. This implies that the addition of fly ash to natural water bodies may not be hazardous, especially considering the added benefits of toxin removal from the water. None of the fly ash samples tested were capable of adsorbing phosphate from solution, despite the fact that the percentage of CaO in the samples was camparable to other ashes that showed a high phosphate adsorption efficiency

The results of this study cannot simply be extrapolated to a large scale treatment of a natural system. Future research questions should include the following:

- What causes cyanobacterial cell death, and would this affect other aquatic organisms?
- Would the concentrations of toxic elements leached into solution in a natural water body be high enough to affect other organisms (ie. be above the DWAF TWQR)?
- How would the natural mixing of a water body affect the permanence of cyanobacterial flocculation?

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