An improved method for detecting electrophoretic mobility of algae during the destabilisation process of flocculation: flocculant demand of different species and the impact of DOC

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ABSTRACT

The flocculant demand of five algal species was tested based on the electrophoretic mobility (EM) of the cells in a Zeta meter. The algae exhibited time-dependent behaviour in EM when observed immediately after destabilisation. The charge reversal could be delayed up to 5 min after flocculant addition. Flocculant experiments in synthetic and natural water demonstrated that the dissolved organic carbon (DOC) and pH values had a much more important influence on the coagulation process for the algal cells, than the interspecific differences of the algae.

Key words | charge density, DOC, drinking water treatment, electrophoretic mobility, flocculant demand, particle removal

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INTRODUCTION

The presence of algae in water reservoirs can cause a variety of problems during the treatment of drinking water, e.g. their effect on taste and odour, and the formation of toxic by-products by some algal species. In order to improve particle removal the natural overall negative charge on the particle surface has to be reduced to nearly neutral, so that the algal suspension is destabilised. When applying flocculation in the drinking water treatment process, charge neutralisation is achieved by the reaction of particle surfaces with hydroxocomplexes which are formed by the hydrolysation of metal (aluminium or iron) ions (Packam 1967; Gregory 1989). The dosage of metal salts must, however, not be so high that the isoelectric point (IP) is surpassed so that the particle surface charge becomes positive (Van Leeuwen *et al.* 1997). To observe the shift of the charge during flocculation experiments two different methods are commonly used:

1. The jar test for detecting indirectly the charge of a suspension (Bernhardt & Clasen 1994).
2. The observation of the mobility of single particles in an electric field established by the Zeta meter (Ives 1959; Bernhardt 1965; Tilton *et al.* 1972; Edzwald & Wingler 1990; Petrusevski 1996).

The overall problem of both methods is the time lag between flocculant addition and particle observation. With jar tests, time and energy input is needed for complete mixing and for the formation of flocs, while in the latter case a certain, but undefined time is required to transport the destabilised suspension into the observation cuvette of the Zeta meter.

It is widely accepted that the process of charge reversal on the surface of particles takes place within a few thousandths of a second after the flocculant addition, and that the growth of flocs starts immediately (Mhaisalkar *et al.* 1991). Therefore the electrophoretic mobility has already been disturbed by the formation of flocs and by reaction with ions on the boundary surfaces in the observation cuvette of the Zeta meter. To study the mechanism of destabilisation it is necessary to measure the resulting surface charge of the particles at once and without variation in energy input. To solve this problem, a modified Zeta meter measurement was developed. A destabilisation

chamber was directly connected to the Zeta meter cuvette, and the volume of the cuvette could be exchanged several times before the observation of particles began.

In the following application the electrophoretic mobility (EM) of very small algal cells was observed at varying flocculant dosages and pH values. These studies were undertaken in parallel in Australia and Germany. Algal cells with a cell dimension of less than 10 μm are known to cause problems in drinking water treatment. In addition to these small algal species, a large one, *Planktothrix rubescens*, was investigated, since this species is commonly found in mesotrophic to oligotrophic reservoirs. In contrast to earlier investigations, when several measurements of EM were averaged (Bernhardt & Clasen 1991; Van Leeuwen *et al.* 1997), the time dependence of the EM of algal cells was studied and will be discussed in context with the consequences for drinking water treatment. In addition the enormous effect of DOC on flocculant requirement for destabilisation will be demonstrated using various test waters of natural origin with the improved Zeta meter method.

MATERIAL AND METHODS

The electrophoretic mobility of algae was measured with a destabilisation unit connected to a Zeta meter with a modified observation cuvette, which enables continuous operation (Figure 1).

The destabilisation unit consists of a chamber of 25 ml volume with a central inlet for water and flocculant at the bottom and a radial outlet close to the top. The flocculant is introduced through a tube which is centrally arranged in the feed water tube. Because both tubes end at the bottom of the chamber a sudden widening of the inputs of feed and flocculant is achieved. This leads to a very intensive mixing which is increased by a 500 rpm stirrer. Setting the flow rate of the feed water to 150 ml/min, the residence time in the destabilisation chamber amounts to 20 sec. The destabilisation unit had been developed for continuously operating flocculation test apparatus (Clasen: unpublished data) but for the application described in this paper it was connected directly to the Zeta meter.

The Zeta meter is a particle electrophoresis apparatus (Rank Brothers Ltd, Cambridge, UK). The observation cuvette of this apparatus was modified to operate in flow mode. After 500 ml volume was collected at the outflow of the system (Figure 1), the flow was stopped by closing both clamps and the observation procedure of EM started. The water from the outlet was collected for various measurements, pH, concentration of algal cells and observations under the microscope.

Observation procedure

The flow from the destabilisation unit was interrupted by closing the clamps on both sides (Figure 1) to observe the EM of the algal cells to be measured. The applied potential between the electrodes was 100 V. In the German experiments at 18–20.9 °C the algal cells had to move a distance of one field of the optical grid in front of the video camera lens for each EM measurement, which amounts to 180 μm. Every third measurement the polarity of the direct current was reversed so that the particles moved in the opposite direction.

Temperature was controlled at 25 °C only for the Australian experiments. The EM was measured over a period of 2 min by picking particles at random. The procedure was done in triplicate and the results were averaged and given as the '2 min average' for the Australian experimental design.

To observe the time-dependent reaction of the algal cells, the EM of various algal cells or trichoms was observed over a period of 7 min in the German experiments. The EM of any single algal cell gives one point of the time-dependent curve, since the corresponding time after destabilisation was recorded. The time needed to determine one point varied between 2 sec and at least 120 sec depending on the velocity of EM. After 7 min in all experiments no more changes of the EM were observed, since all cells had reached equilibrium. To compare the results with the Australian experiments, the '2 min value' was estimated by interpolation on the basis of these time-dependent curves.

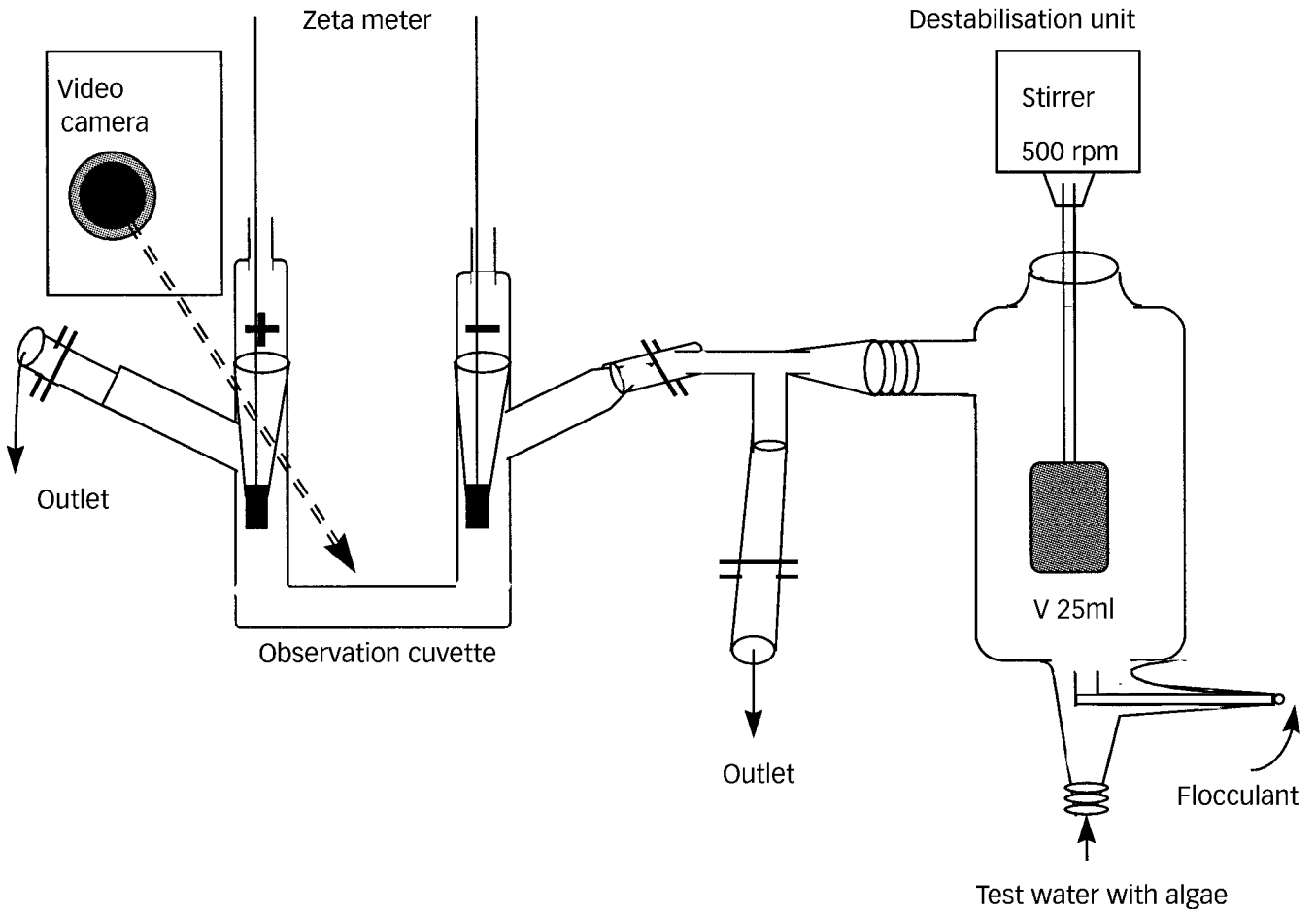

Figure 1 | A schematic diagram of the destabilisation unit and the modified observation cuvette of the Zeta meter.

Experiments with algal cells in test water

Four algal species (*Pseudanabaena* sp., *Microcystis aeruginosa*, *Chlorella* sp. and *Synechocystis minuscula*) were grown in batch cultures, but the blue-green alga *Planktothrix rubescens* was cultivated in a special reactor with pH control. If not noted explicitly elsewhere the experiments were carried out with the minute alga *Synechocystis minuscula* in a cell concentration of about 10^6 cells/ml, which corresponds to a surface area of 25 mm^2/ml (Table 1). Since the surface area of algal suspensions was expected to be the most important factor for the flocculant demand, other algal species were added in concentrations so that the same surface area was achieved. The surface area was calculated by using simple geometric solids such as spheres or cylinders. Because the maximum concentration of *Planktothrix* cultures is growth limited, the surface area in the test water was lower by at least a factor of 10 than the other algal species. To ensure that the physiological state of the algae in all experiments was comparable, algal cultures were used in the last third of the exponential growth phase. A synthetic medium (DEV 1998) was used as test water (Figure 2) for most experiments ($Ca(NO_3)_2 \cdot 4H_2O$: 141.2 mg/l; $CaCl_2 \cdot 2H_2O$: 191.2 mg/l; $MgSO_4 \cdot 2H_2O$: 320.4 mg/l; $NaHCO_3$: 172.0 mg/l; $KHCO_3$: 45.1mg/l and $CaCO_3$: 70.1mg/l), which stabilised the pH value much more effectively than the culture medium of algae or the natural

Table 1 | Cell shape, surface area and concentration of cells in the test water of the algal species investigated

Name of algal species	Cell shape	Average surface area of cell or trichom unit (μm^2)	Cell concentration in test water (cells/ml)	Average surface area in 1 ml (m^2)
Synechocystis	Sphere	25.00	1,000,000	25.0
Pseudanabaena	Coccoid	25.57	977,708	25.0
Chlorella	Sphere	100.71	250,000	25.0
Microcystis	Sphere	63.63	405,000	25.0
Planktothrix	Trichom colonies	17.83*	160,000	2.9

*Surface area is given for a trichom unit of 450 μm length.

waters filtered through a 0.22 μm filter. The latter was used for a few experiments to test the effect of DOC on flocculation:

1. In Australia (from South Para Reservoir, South Australia).
2. In Germany (test water with extracts of leaves from oak and gum trees).

For the Australian experiments the algal cultures were concentrated by centrifugation at 8,000 rpm for 10 min.

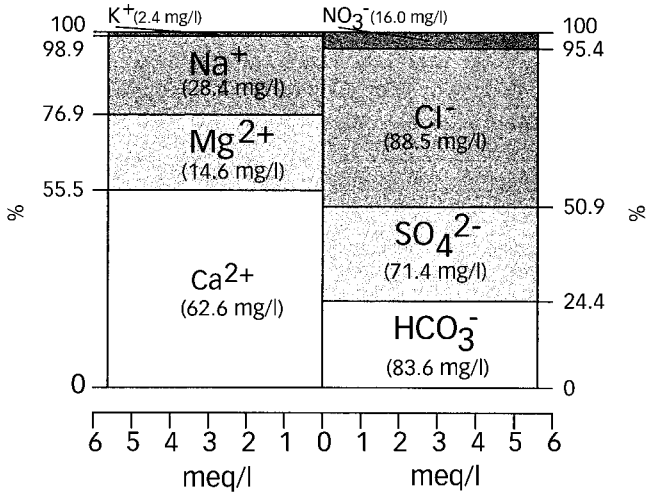

Figure 2 | Composition of ions in test water used for experiments.

The concentration of the stock algal culture of *Planktothrix* was too low, so the cells were concentrated by filtration, and rinsed gently from a filter without pressure, ensuring that the algal cells did not fully dry out. All concentrated algal cultures were diluted with the test water. The resulting concentrations of algal cells were measured by cell counts with an inverse microscope.

After gentle mixing with a magnetic stirrer, the pH value was adjusted by addition of a few drops of an acid (HCl) or alkali (NaOH) into the test water. The pH value was varied between 4.5, 5, 5.5, 6, 6.5 and 7 in the different experiments, but could not be controlled absolutely after flocculant addition. To compensate for the acidic effect of the flocculant addition on the pH, it was necessary to increase the pH of the test water to at least 10.2, which was measured in an empirical manner prior to the experiments.

Flocculant dosage

Aluminium sulphate was used as the flocculant in a stock solution of 12.4 g $Al_2(SO_4)_3 \cdot 18\ H_2O$ in 1 l distilled water. The flocculant dosage ranged over five values (1.0, 2.5, 5.0, 7.5, 10.0 mg/l Al) for the different experiments. The desired alum concentration was delivered by the selection of the appropriate pump speed.

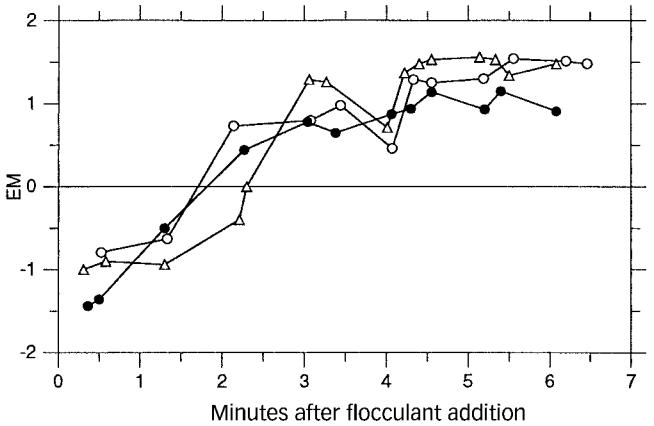

Figure 3 | EM of *Synechocystis* cells at different times after destabilisation in three replicates. Conditions of experiments were similar with 5 mg/l alum and pH values between 6.45 and 6.55.

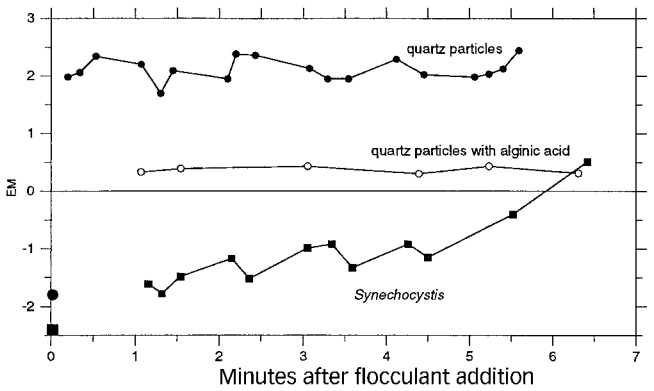

Figure 4 | EM of quartz particles with and without alginic acid in comparison with *Synechocystis* cells during a period of 7 min. All experiments were run at pH 6 with 1 mg/l alum dosage. Boxes at time zero give the initial charge of algae and quartz particles.

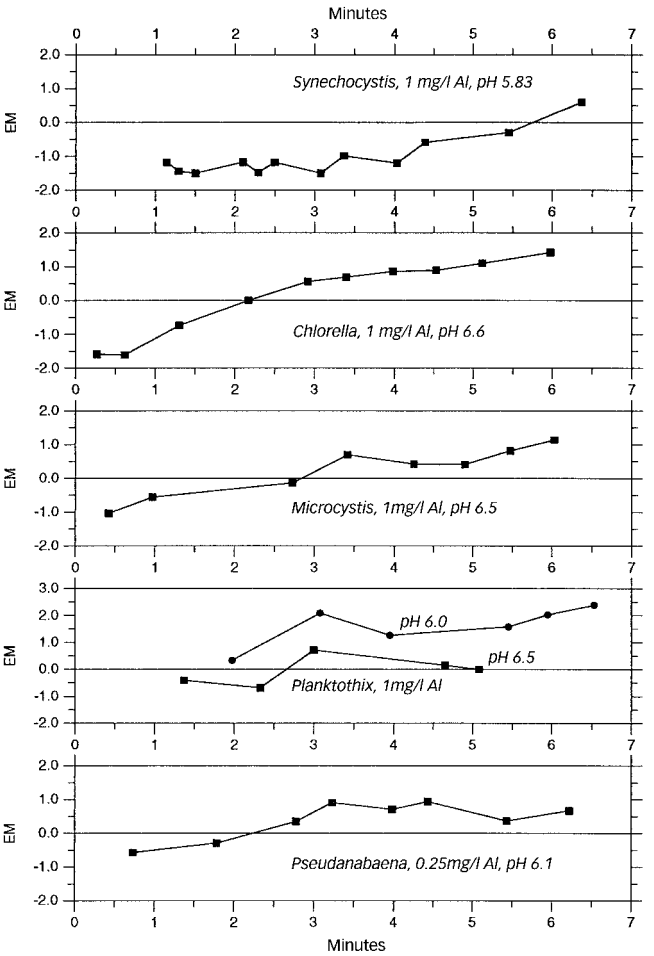

Figure 5 | EM of different algal species after addition of flocculant during a period of 7 min. From more than 20 experiments per species these curves were chosen, which demonstrate a charge reversal with the lowest concentration of alum.

RESULTS

Time-dependent behaviour of EM of algal cells after destabilisation

In contrast to earlier investigations (Van Leeuwen *et al.* 1997) the EM values of particles of one experiment were not averaged but were correlated to the experimental time after destabilisation (Figures 3, 4 & 5).

To test the accuracy of the results, the EM for several replicates with constant conditions was determined and the point of zero charge (PZC) was achieved within a comparable time after destabilisation. In Figure 3 the observed EM of three replicates during one time course of 7 min demonstrate that the results could be replicated. The EM of algal cells ranged between -1.44 and $+1.56$ during 7 min of measurement, and equilibrium was visible in the last 2 min. This could not be seen in the earlier experiments, since an average of EM values was calculated. For the chosen example (Figure 3) the averages would amount to 0.46, 0.83 and 0.74. This means that an average gives no realistic image of the maximal resulting charge of the algal cells after flocculant addition. Even when charge reversal is achieved for example after

Table 2 | Averages of initial charges of algal cells (n=20) and observed minima and maxima before test water input and after destabilisation (time zero of measurements)

	Initial EM at pH optimum	Range of EM without Al dose	Range of pH values in test water
Synechocystis	– 1.8	– 1.2– – 2.0	4.52–7.58
Pseudanabaena	– 2.0	– 1.9– – 3.3	5.00–7.12
Chlorella	– 1.8	– 1.2– – 2.2	5.41–7.08
Microcystis	– 0.9	– 0.6– – 2.1	5.59–7.94
Planktothrix	– 2.2	– 1.4– – 2.2	5.60–6.59

3 min (see Figure 3) there is still a variation between the EM of the different algal cells measured thereafter, which caused a standard deviation in all experiments in the range of ± 0.25 EM. This is an expected and well-known phenomenon (Ives 1959; Tilton *et al.* 1972).

A variation is pertinent also for the initial EM (Table 2). This refers not only to different algal species, but also to different cells of the same algal species. Under natural pH conditions within the range of 5.5 to 8, the EM of the same algal species shows a standard deviation of ± 0.6, and the deviation between the algal species (*Microcystis* and *Pseudanabaena*) is at least 1.7 EM units (Table 2).

Based on the experience with quartz particles the observed delay of destabilisation for algal cells was not expected. Quartz particles are destabilised immediately, even when they are covered with alginic acid to simulate the organic matter on cell wall surfaces (Figure 4). Under conditions with low flocculant dosage the cells of all algal species investigated need several minutes to achieve the isoelectric point (Figure 5).

Thus the EM of algal cells is time-dependent. This causes problems when measuring the EM (Figure 6), where algal cells approximate the point of zero charge and change their direction of motion during the period of observation. In this case time measurement was interrupted (Figure 6B).

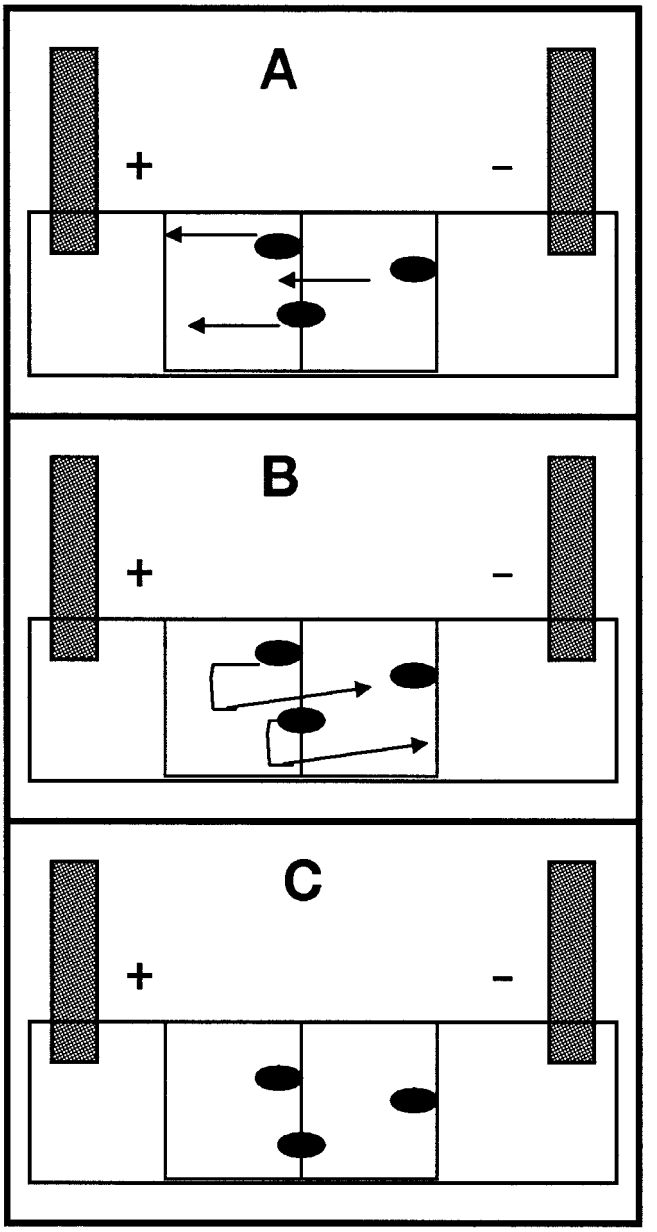

Figure 6 | Schematic demonstration of the three cases of algal cell behaviour in the observation chamber of the Zeta meter. (A) Algal cell is still intact and has its natural negative surface charge. (B) Algal cell is intact at the beginning of measurement, but it changes its charge as a result of destabilisation. In this case time measurement was interrupted and a new algal cell with the new direction near a line of the grid was chosen. (C) Algal cell changes its surface charge but only to the point of zero charge (PZC). The observation time was limited to 60 sec and as long as all other cells in the chamber behave in the same way.

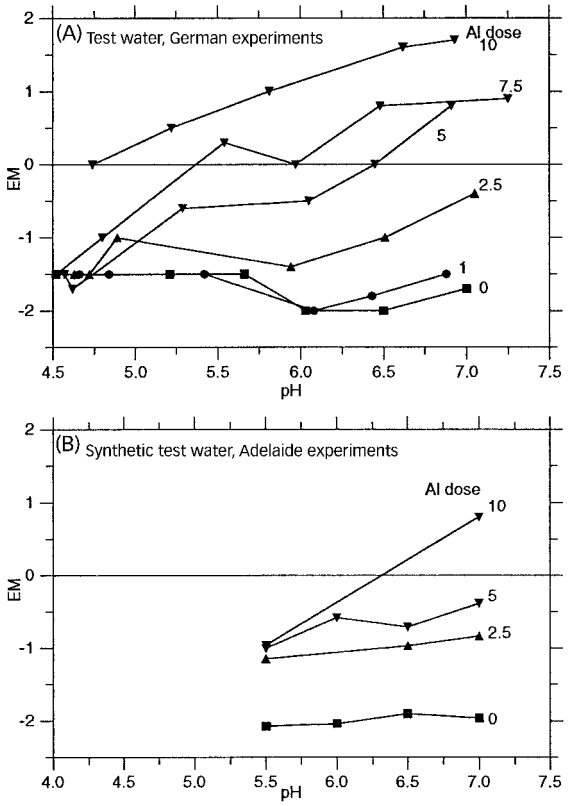

Figure 7 | EM of *Synechocystis* cells is shown with increasing pH values (4.5, 5, 5.5, 6, 6.5, 7) and alum concentrations (1, 2.5, 5, 7.5, 10 mg/l) in test water. (A) German experiments: EM is given as 2 min values interpolated from time-dependent curves; (B) Australian experiments: EM is given as 2 min averages (n>17 measurements).

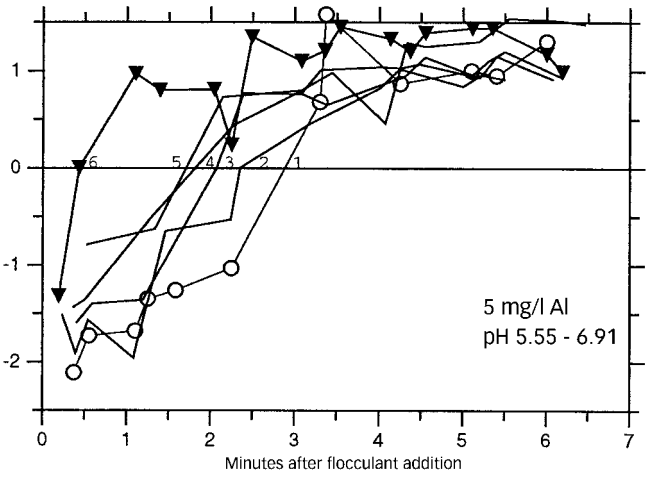

Figure 8 | EM of *Synechocystis* cells under increasing pH conditions (from No. 1 to No. 6) during a period of 7 min after destabilisation with 5 mg/l alum.

The two minutes value

When using the 2 min value to characterise the EM of algal cells under given conditions only a part of the total reaction of the cells is considered. But in most cases, when the given Al dosage under the selected pH was high enough to effect a charge reversal within 7 min, the 2 min value had increased in comparison with the initial charge. For example, in an experimental series with the alga *Synechocystis* comprising 36 experiments, in 15 cases the charge reversal began within the first 2 min (Figure 7A). Only in 4 cases when the change of charge was not visible during the first 2 min, did it change within 7 min (corresponding curves not shown). In all other cases no charge reversal was achieved within 7 min, since the flocculant addition was too low. Therefore the 2 min values of EM can still be used as a parameter to characterise the effect of different experimental conditions. Since in Figure 7B the 2 min averages are given for Australian experiments, the initial charge of the algal cells is included in the averages, because of the time lag of charge reversal. For this reason most EM values are lower than the 2 min values of Figure 7A under the same conditions.

However, in both experimental series the effect of increasing pH values (Figure 7) on the EM of *Synechocystis* cells was very strong as it increases the charge reversal of algae and shortens the time lag of reversal at the same alum dosage.

To demonstrate the effect of pH on the EM of algae in more detail, 6 replicates with increasing pH values over a very small range are given in Figure 8. Minor increases in pH effect a faster occurrence of the isoelectric point; it is achieved in a much shorter time at pH 6.91 than at pH 5.55.

The destabilisation of *Synechocystis* cells presented by the 2 min values (Figure 7) also showed strong dependence on the alum dosage. At pH values above 5.5 a higher alum dosage effects an increase of the EM of algae, whereas the effect below pH 5.5 and at low alum dosage was not significant.

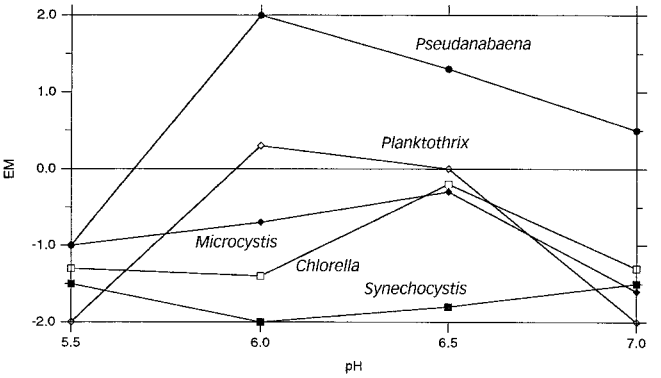

Figure 9 | 2 min values of EM for five algal species with increasing pH and an alum dosage of 1 mg/l in test water (pH values are optimised).

Characteristics of different algal species

Algal species with small size and comparable shape (Table 1) were chosen to investigate the effect of different pH conditions and flocculant concentrations. The initial charge density without Al dosage and at optimum pH (6–6.5) differed between the algal species, but was below −0.9 EM in all cases. *Microcystis* cells were charged much more negatively in Australian than in German experiments (2.1 EM vs 0.9 EM), which could be an effect of the higher temperature during the Australian experiments.

Most algal species investigated have a flocculant demand of at least 1 mg/l alum for inducing their charge reversal during 7 min (see Figure 5) except for *Pseudanabaena*, which was already destabilised with an alum dosage of 0.25 mg/l Al. The pH optimum ranges between 6.1 and 6.6 for the five algal species.

The 2 min values of EM under different pH conditions, but under the same alum dosage of 1 mg/l (Figure 9) varied with the different species. The coccoid alga *Pseudanabaena* and the trichom forming alga *Planktothrix* had a positive EM at optimum pH, whereas a complete charge reversal could not be seen for the spherical algal species because of their time lag for destabilisation. However, a flocculant dosage of 1 mg/l Al was sufficient for charge reversal at optimum pH (pH 6.5) during a period of 7 min even with the trichom forming alga (see Figure 5).

In Australian experiments the amount of alum required to neutralise the surface charge was much higher for *Synechocystis* cells (Figure 10) compared with *Microcystis* cells (not shown) under the same flocculant conditions, which corresponds with the results of the German experiments presented in Figure 9.

Influence of DOC on EM of algae

The presence of natural DOC in the test solution was found to have a major impact on the flocculation of algal cells and this can be seen from experiments examining their electrophoretic mobility. The flocculation of *Synechocystis* cells was initially monitored using electrophoretic mobility in a synthetic water without DOC present. The 2 min values of electrophoretic mobility are shown for varying alum dosages and pH values (Figure 7). Charge destabilisation could be achieved using alum doses between 7.5 and 10 mg/l. Increasing alum doses result in charge neutralisation occurring also at lower pH values. For test waters without DOC the optimal charge neutralisation was obviously at a higher pH, since destabilisation occurred with an addition of 10 mg/l alum at pH 5, 7.5 mg/l alum at pH 6, while at pH 7 an alum dose of between 3 and 5 mg/l would have been sufficient.

In the presence of natural DOC in reservoir waters (9.6 mg/l C), and leaf extracts in test water (Figure 10A, B, C—10 mg/l) it was possible to destabilise *Synechocystis* cells at low pH values, but it caused an impairment of flocculation near neutral pH conditions (e.g. Figures 7 and 10). In the presence of DOC and very low flocculation pH (5.0), an alum dosage of 5 mg/l effected charge reversal, but not in synthetic media without DOC (Figure 7). Under increasing flocculation pH this effect decreases and reverses in neutral media, when destabilisation of *Synechocystis* was not achieved even under very high alum dosages (Figure 10).

DISCUSSION

'Time lag effect' of the destabilisation of algal cells

We found that the algal cells were not destabilised immediately as quartz particles were, but needed several minutes before charge reversal was achieved (Figure 4). This is

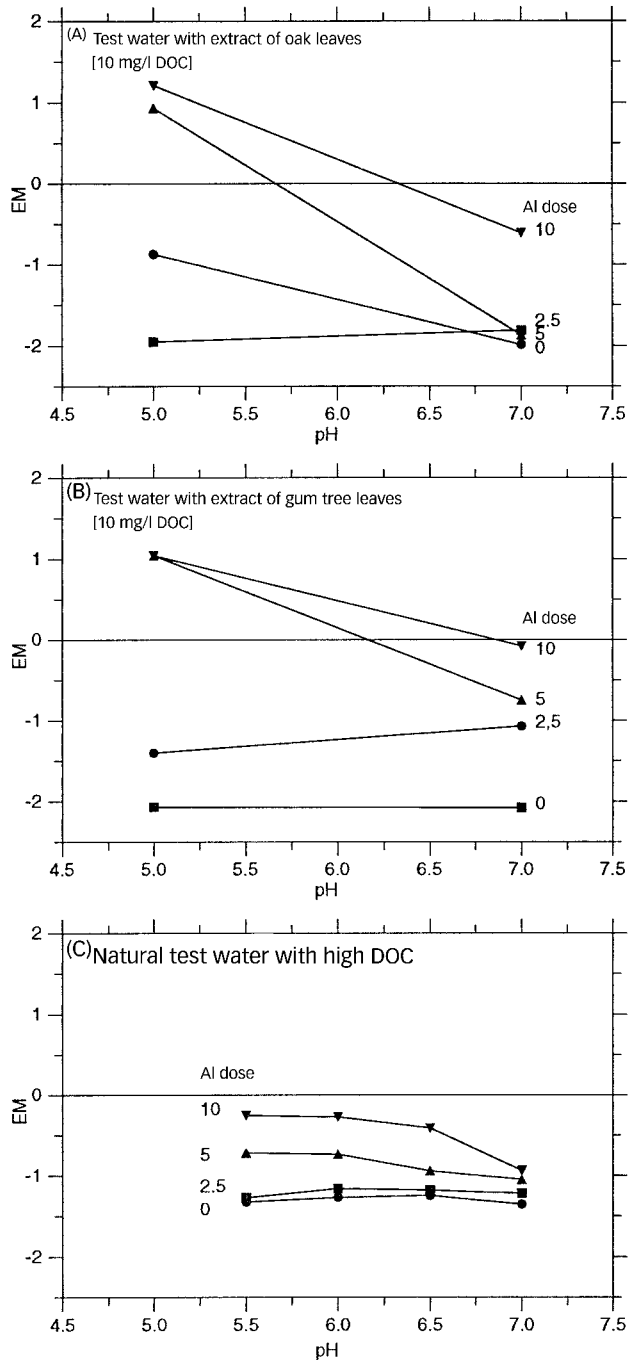

Figure 10 | EM of *Synechocystis* cells in waters with high concentrations of DOC with increasing pH and alum dosage (0, 2.5, 5, 10 mg/l).

in disagreement with the expectations from earlier investigations (Tilton *et al.* 1972; Van Leeuwen *et al.* 1997). One reason why this delay was not discovered before was the uncontrolled time delay between the start of the destabilisation procedure and the beginning of observation in the Zeta meter in earlier investigations, since time-dependent behaviour was not expected. The modified method for detecting EM conducted in this paper reduces and controls the time between destabilisation and particle observation. This is achieved by connecting the destabilisation chamber to the observation cuvette and by exchanging the volume of the latter several times by the continuous throughput of destabilised water until an equilibrium for reaction velocity and for boundary effects was observed.

In addition, the much higher flocculant dosage (above 5 mg/l alum) used in earlier investigations shortened the time delay of destabilisation (Figure 11) and the charge reversal of algal cells occurred in a few seconds. Only with the extended observation time did it become obvious that algal cells could be destabilised with a dosage of only 1 mg/l alum (Figure 5), when flocculation pH is chosen in the optimum range of 6 to 6.5.

This pH range was predicted to be the optimal for formation of hydroxo metal complexes, as a product of hydrolysis reactions of alum flocculation, by Amirtharajah & Mills (1982) who summarised investigations carried out before 1970. Recently, Mhaisalkar *et al.* (1991) pointed out that a rapid mix is important to ensure that hydroxo metal complexes get in contact with the particles. Rapid mixing was conducted in our study by use of the destabilisation unit (Figure 1).

Using a continuously operating test apparatus for flocculation (unpublished data) alongside our investigations with the Zeta meter, it could be shown that the physical and chemical parameters of our experiments greatly improved the flocculation and filtration of algal particles. A dose of 1 mg/l alum at pH 6 was sufficient to remove *Synechocystis* cells tested by the apparatus for flocculation, although a change of EM by cells could not be seen before 6 min after destabilisation. Higher alum dosages could not improve the elimination rate (98%) by flocculation and filtration, but decreased the time needed to achieve charge reversal of algal cells observed in the Zeta meter.

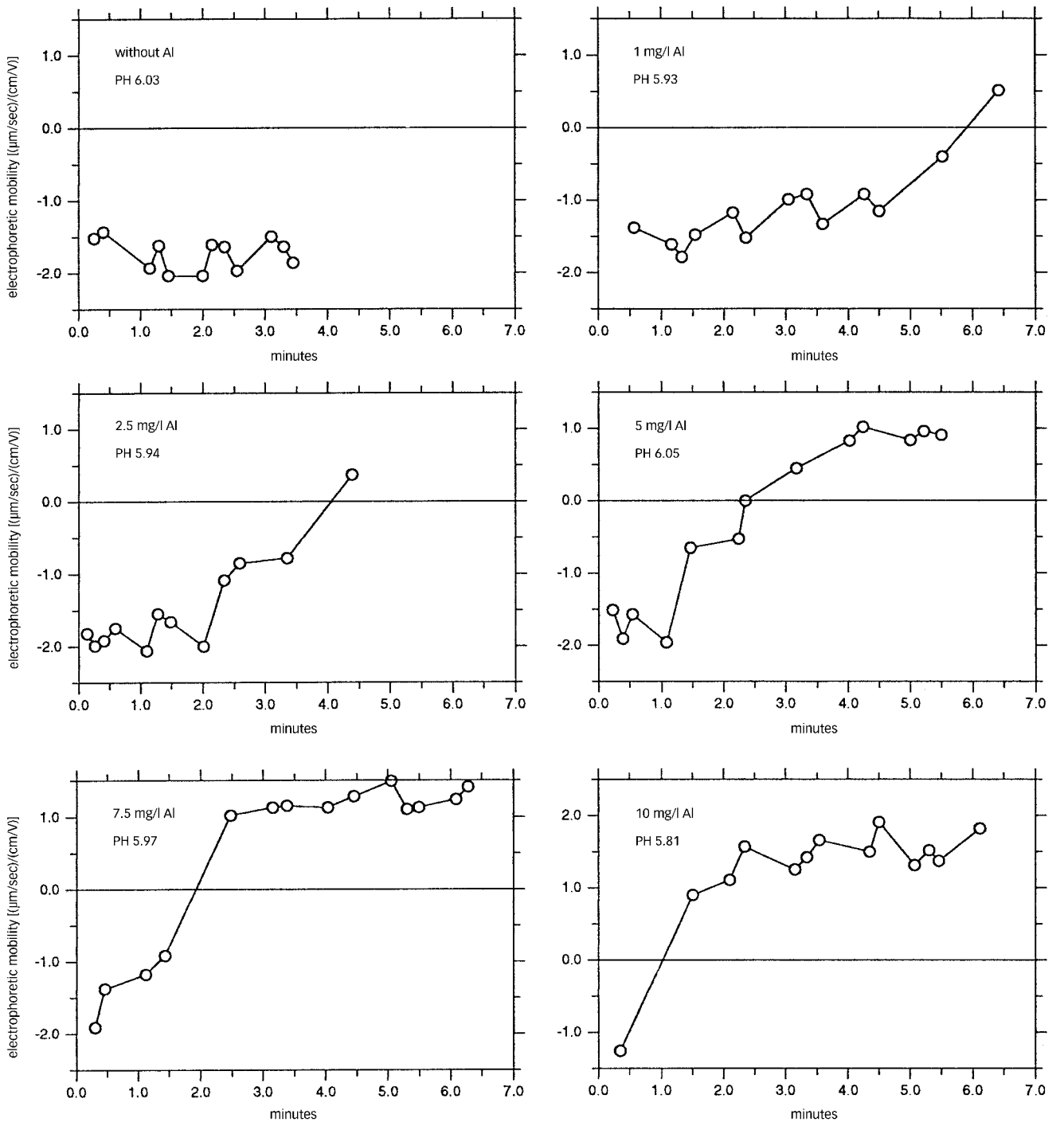

Figure 11 | EM of *Synechocystis* cells at pH 6 during a period of 7 min and increasing alum dosage (0, 1, 2.5, 5, 7.5 and 10 mg/l).

Since the onset of algal flocculation does not occur immediately after destabilisation during the process of flocculation, and needs more time than other particles do, it is of concern when designing new water treatment facilities.

The reason for the 'time lag effect' of algal cells cannot be explained by the presence of organic matter on the cell surface (Hoyer *et al.* 1985; Lüsse 1993). Simulating such organic matter on quartz particles with alginic acid showed increased flocculant demand for destabilisation, but alginic acid could not induce a time-dependent behaviour of the quartz particles (Figure 4).

The surface structures of algal cells influence destabilisation in a similar way: probably as a result of a kinetic effect the flocculant demand of algal cells is much higher than that of quartz particles in a comparable concentration. The surface structure of algal cells is a multilayer system with various interactions with the surrounding medium (Hoyer *et al.* 1985), whilst the ratio of surface area to particle volume can be expected to be much higher for quartz particles than for algal cells. The specific properties of algal cells induce a higher demand of algae for flocculant compared with quartz particles, but they are not expected to cause the observed delay of the destabilisation process.

More likely, another characteristic of algae is important in the delay of destabilisation: living algal cells influencing their charge loading actively by the transport of ions across their cell wall in order to prevent an osmotic gradient to their surrounding medium. The active transport of ions across the cell membrane could effect the time lag between addition of flocculant and destabilisation of the algal cells. As long as the physiological state of the cell remains intact, it is able to handle the cations in its surrounding media and the cell re-establishes its initial negative charge. Pieterse & Cloot (1997) stated that algal cells have many properties which distinguish their behaviour during flocculation processes from that of inorganic colloidal particles.

In accordance with our hypothesis the time lag between flocculant addition and the attainment of the isoelectric point decreased with increasing alum dosage (Figure 11). That observation can be explained by the disturbance to the repair mechanism of algal cells at high doses of flocculant which led to immediate destabilisation. Other physiological perturbations have comparable effects on the Zeta potential of algal cells, such as unusually high (>8) or low (<4.5) pH values (Ives 1959; Foess & Borchardt 1969), or the destruction of algal cells by copper sulphate, iodine or ozone, which all lower the initial negative electric charge of the algal particles (Ives 1959), and facilitate the destabilisation process.

Ratio of flocculation demand to surface area and initial surface charge of algae

Another interesting result of our investigations is the fact that the requirement of flocculant for algal destabilisation is not absolutely dependent on the surface area, since comparable surface areas of algal particles required different amounts of flocculant for achieving charge reversal (Figure 9). According to Bernhardt & Clasen (1991), there is still a stoichiometric relationship between the total surface area of the algal cells and the chemical dosage required for the flocculation process given for one algal species (e.g. *Synechocystis*). Nevertheless, the flocculant demand ascertained for charge neutralisation of a given surface area of one species cannot easily be transferred to other algal species, not even to those with similar cell shape, size and the same taxonomic membership. This is well documented by the much lower flocculant demand (by a factor of 4) of the blue-green *Pseudoanabaena* in comparison with *Synechocystis* (Figure 7). This observation may be explained by the differences between the functional groups of the peripheral cell wall of the two blue-greens, which determines the stability of the algal cells (Bernhardt 1965).

On the other hand it was expected that the green algae (e.g. *Chlorella*) would have a higher flocculant demand than blue-green algae do because of a more complex cell surface with a compact layer of extracellular organic matter on green algal cells (Kunikane *et al.* 1986). However, our results demonstrate a similar flocculant demand to achieve charge reversal for *Chlorella* and blue-greens under optimal flocculation conditions. Additionally the optimum flocculant concentration is not directly related solely to the initial surface charge of the algae (see Table 2).

Influence of DOC on EM of algae

An enormous effect of DOC on the flocculant demand is apparent and is illustrated by our experiments with and without DOC (Figures 7 and 10). Without DOC present, increasing alum doses resulted in charge reversal at all pH levels, but the optimal destabilisation was at a higher pH.

However the interesting observation is that in the test waters to which natural DOC extracts had been added (Figure 10), charge neutralisation was hindered at high pH and therefore this made flocculation appear to occur preferentially at a lower pH. At pH 5, between 3 and 5 mg/l alum would suffice for charge neutralisation whereas at pH 7 an alum dose above 10 mg/l would be required. From this observation, it appears that the surface properties of the algal cell are affected in the presence of DOC. It is widely thought that DOC covers inorganic colloidal particles and changes their electrophoretic mobility. This fact is also well demonstrated by our experiment with alginic acid on quartz particles (Figure 4).

It is quite possible that DOC also coats onto the surface of the algal cell causing the algal cell to behave in a similar manner as the DOC. It is well documented that DOC (and colour) coagulates more effectively at a lower pH and is less effective at a higher pH. This therefore results in lower alum doses being required to neutralise the algal cells in the presence of DOC, at the lower pH. Conversely this also implies an impairment of flocculation of the algal cells at the higher pH, as the reaction of alum with the DOC at the higher pH requires significantly higher alum doses. The process is therefore an increased demand at all pH concentrations to counteract the increased DOC, but a considerably higher demand at the higher pH because of the poorer coagulation of the DOC.

These results are congruent with investigations by a charge titration unit (Bernhardt *et al.* 1985; Bernhardt & Clasen 1994) which shows the major effect on flocculation by dissolved natural organic matter at pH 7. Earlier investigations by Rebhun (1990) demonstrated the negative influence of humic acid on the stability of flocs in flocculation experiments with clay dispersion and alum.

SUMMARY AND CONCLUSIONS

An improved method for detecting electrophoretic mobility of algae was developed in order to determine the flocculant demand of different algal species and the impact of DOC on the destabilisation process of flocculation. The modified method for detecting EM, introduced in this paper, enables control of the time protocol, improves the experimental conditions by reducing boundary effects because of its flow mode, and produces results with good repeatability (Figure 3). These modified Zeta meter measurements give new insights into the time-dependent behaviour of algal cells during destabilisation and the flocculant demand of different algal species. The time-dependent behaviour was not obvious in earlier investigations since the measurement procedure was not time-controlled in respect to the time between destabilisation and the beginning of observation, and to the total observation period.

The algal species had different initial negative surface charges, which showed no correlation to the flocculant demand needed to achieve their charge neutralisation. Even when comparable cell-shaped blue-green species with similar cell surface areas were dosed, the flocculant demand differed considerably: *Pseudanabaena* was destabilised with 0.25 mg/l alum under almost all pH regimes after 2 min, but *Synechocystis* cells required 1 mg/l.

It was demonstrated that rapid mixing, combined with an optimal flocculation pH range between 6 and 6.5, gives the best results for flocculation: an alum dose of 1 mg/l is sufficient to destabilise a highly concentrated algal suspension e.g. of more than 1 million cells per milliliter. This is a rather low flocculant demand and is seldom sufficient for natural raw waters of reservoirs.

The reason for the discrepancy between theoretical flocculant demand exhibited by algal particles, and practical experience with natural raw water, is found in the simultaneous occurrence of inorganic particles and DOC.

The experiments demonstrate the enormous effect of DOC on the flocculation of algae. The variation of flocculant demand between different small algal species is much lower than the effect of an addition of 1 mg/l DOC. Therefore the removal of DOC is one of the key processes,

if not the single most important process, affecting flocculation in water. In future, most effort has to be made to reduce the impairment of flocculation by DOC. Treatment processes have to be developed which eliminate those substances just before particle flocculation begins.

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