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Mutual Flocculation of Algae and Clay: Evidence and Implications

Abstract. *Algae-clay aggregates were formed when algal and clay suspensions were mixed in the presence of an electrolyte. The maximum ratio of clay to algae in the aggregates was 1.7, 0.2, and 0.03 milligrams of clay per milligram of algae (wet weight) for* Anabaena, Chlamydomonas, *and* Chlorella *sp., respectively. The aggregates formed at Ca^{2+} concentrations higher than 5×10^{-4}*M *or Na^{+} concentrations higher than 2×10^{-2}*M. *The mutual flocculation and subsequent sedimentation have many practical and ecological implications for bodies of water.*

The two materials most commonly found suspended in most lakes and impoundments are algal cells and clay particles. Aggregation of these particles would lead to the formation and sedimentation of larger particles and thus to the clarification of the water. These interactions may profoundly affect the ecological structure of bodies of water in terms of the temporal and spatial distribution of organisms, the concentration of suspended inorganic particles and nutrients, nutrient cycling, substrate utilization by filter-feeding organisms, and other processes.

Considerable information exists on microbial or microbial-clay aggregation in soils, water treatment plants, dental plaque, and other systems (*1*). Such aggregation is attributed to the presence of extracellular polymers, especially polysaccharides. Algae excrete large amounts of polysaccharides and other polymers. The amounts released may represent from 15 to 60 percent of the photoassimilated carbon (*2*). Thus, an effective aggregation of algae and clay particles could be expected; however, little work on this topic has been published. The aggregation of *Anabaena* filaments, attributed to the excretion of mucilage, was demonstrated by Walsby (*3*). Altman (*4*) suggested that muddy fish ponds be clarified through the addition of fertilizers, barnyard manure, hay, or other organic materials. Kimmel and Lehman (*5*) observed that the introduction of high-turbidity runoff to experimental water columns resulted in the removal of algae from the columns; the effect was apparently associated with clay-particle flocculation and sedimentation. The addition of less turbid runoff water did not remove algae from the water columns. Avnimelech (*6*) suggested that algae-clay aggregation leads to the sedimentation of the suspended load in tributaries of the Jordan River. Zur (*7*) isolated a blue-green alga, *Phormidium* sp., from the Jordan. This alga, and its filtrate, has a marked flocculation activity toward clay.

We undertook to study the interaction of clay and algae by using several representative algal species, to establish methods for such a study, and to provide some quantitative data on this interaction. *Euglena gracilis*, *Anabaena* sp., *Chlamydomonas* sp., and *Chlorella* sp. were grown in Bold's medium (*8*) enriched with nutrient broth. Algae were separated from the medium before each experiment by centrifugation and redispersed in a 0.2 percent sucrose solution, isotonic to the growth medium yet devoid of electrolytes, or in 10^{-3}*M* $CaCl_2$. Wyoming bentonite saturated with Na^+, having a particle size smaller than 0.2 μm, was prepared by high-speed centrifugation.

We determined the algal concentrations by using in vivo fluorescence (*9*) calibrated against microscopic counting. We determined the clay concentrations by measuring the light scattering at 420 nm. Scanning electron micrographs were taken after fixation of the samples with 2 percent osmium tetroxide.

Chlorella-bentonite suspensions flocculated only above a critical concentration of $CaCl_2$ or NaCl (Fig. 1a). The flocculation value was 5×10^{-4}*M* for $CaCl_2$ and 2×10^{-2}*M* for NaCl. Suspensions of algae without clay were not flocculated by either NaCl or $CaCl_2$ within the concentration ranges studied.

Algal suspensions were mixed with

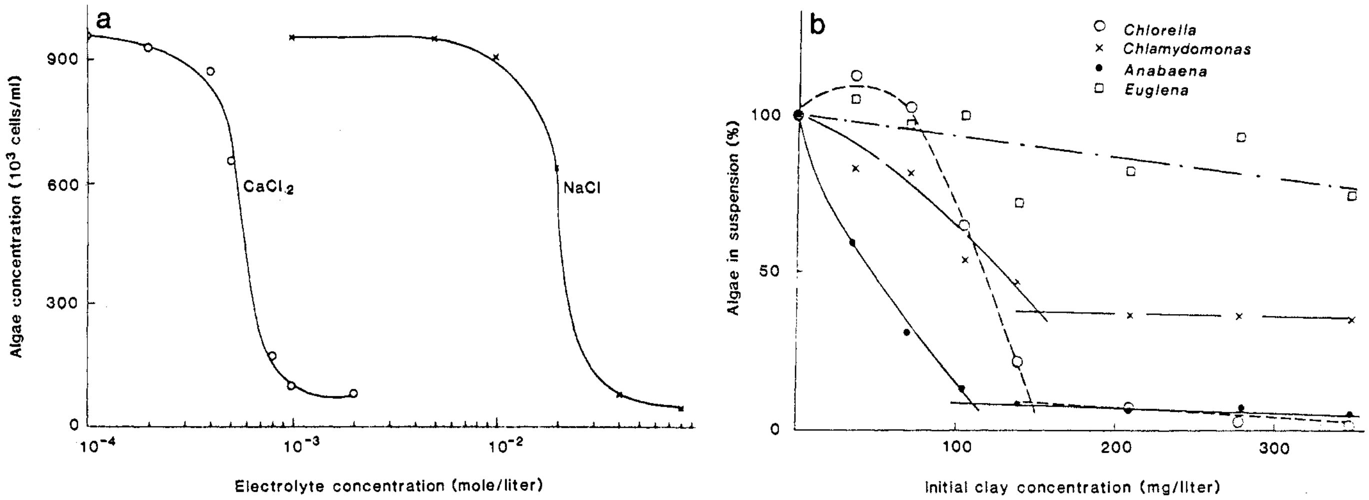

Fig. 1. (a) Flocculation of bentonite clay and *Chlorella* suspensions as a function of NaCl and $CaCl_2$ concentrations. (b) Algal concentration in the top 6 cm of the suspension, after 120 minutes of sedimentation time, as a function of the initial clay concentration.

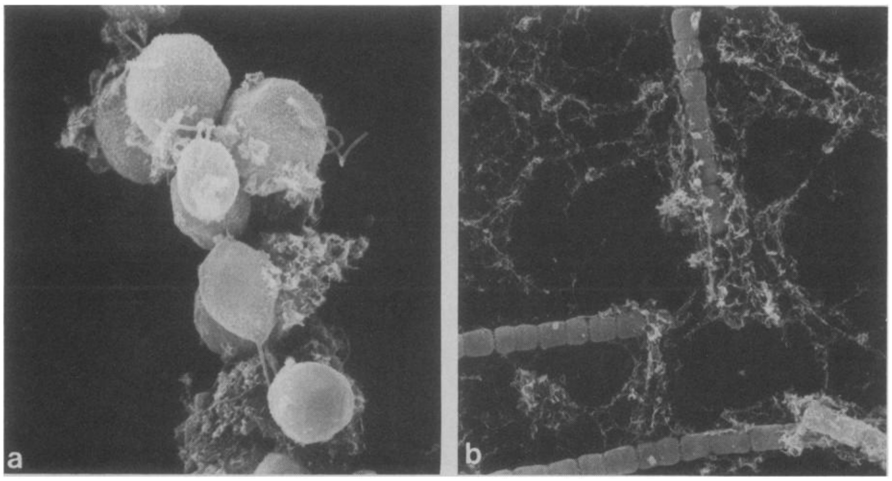

Fig. 2. (a) Scanning electron micrograph of a clay-*Chlamydomonas* cluster (×1470). (b) Scanning electron micrograph of a clay-*Anabaena* cluster (×1200).

varying amounts of clay and adjusted to $5 \times 10^{-4}M$ with respect to $CaCl_2$. Relative algal concentrations in the top 6 cm layer of the suspension after 120 minutes of sedimentation time are shown in Fig. 1b.

The most pronounced effect occurred when clay was added to the *Anabaena* suspension. Flocculation reduced the algal density to almost one-half with the lowest clay addition (34.8 mg/liter). Less than 10 percent of the algae present in the control treatment (fewer than 5×10^3 organisms per millimeter) was found in the suspension when the clay addition was above 110 mg/liter. A small reduction could be detected with the addition of clay beyond this concentration. Flocculation by *Chlorella* occurred only at higher clay additions, and stable low densities (about 14×10^3 cells per milliliter) were achieved with clay concentrations above 200 mg/liter. *Chlamydomonas* suspensions were readily flocculated, yet the residual algal densities were high even with high clay additions (about 95×10^3 cells per milliliter). Very slight flocculation of *Euglena* could be detected with the addition of clay.

Three of the four patterns of algal flocculation in Fig. 1b are biphasic. The first phase, which is associated with the lower clay concentrations, indicates that an increased clay concentration sharply increases the degree of flocculation and asedimentation. Thereafter, at the second phase, any increase in the clay concentration leads to a very mild, if any, increased flocculation. The transition between these regions is quite sharp. The transition point reflects the maximum ratio of clay to algae possible in the floccules. Any addition of clay beyond this point has no significant effect on the algae-clay floccules. The amounts of algae and clay sedimented at the saturation point can be obtained by graphical interpolation, and the ratios of clay to algae can be calculated. Those ratios were 5×10^{-5}, 8×10^{-7}, and 1×10^{-7} mg of clay per algal unit, or, calculated on the basis of the average dimensions, 1.7, 0.2, and 0.03 mg of clay per milligram of algae (wet weight) for *Anabaena*, *Chlamydomonas*, and *Chlorella*, respectively. Additional experiments with three initial densities of *Chlorella* (5.66×10^5, 1.114×10^6, and 2.003×10^6 cells per milliliter) yielded a saturation ratio of clay to algae, averaged for all the initial algae densities used, of $1.15 (\pm 0.15) \times 10^{-7}$ mg of clay per cell.

Visual and microscopic observations were conducted in parallel with the experiments described above. Floccules or clusters could be observed macroscopically about 30 minutes after the clay and algae were mixed. Sedimentation was completed after 1 to 3 hours, leading to the formation of a voluminous gel-like sediment. Sediment mixing, even after prolonged periods of incubation (up to a few weeks at room temperature), resulted in the same flocculation and sedimentation pattern.

Scanning electron microscopy (Fig. 2, a and b) revealed very distinct flocculation or clustering of algae and clay. The high affinity of the clay for the *Anabaena* cells is obvious (Fig. 2b). In this case it seems that the clay is adsorbed and is forming a network structure around the perimeters of the *Anabaena* cells or their exudate of flocculating agents. It seems that more clay is adsorbed at the crosswalls of this filamentous alga.

Our data indicate that the mutual aggregation of algae and clay particles is a rather widespread phenomenon. Of the four algal species tested, representing different physiological and taxonomic groups, three, *Anabaena, Chlorella*, and *Chlamydomonas*, had a definite tendency to form clusters with clay. One species, *Euglena gracilis*, showed only a slight tendency for flocculation, probably because of its vigorous motility. However, *Chlamydomonas*, a motile alga, formed clusters and sedimented readily with the clay.

The efficiency of algae-clay flocculation is indicated by the concentration of algae remaining in suspension at high clay concentration or by the ratio of clay to algae in clusters saturated with respect to clay. The variable flocculating potential of different algae probably depends on the composition and properties of the cell wall, on the extent and type of excretions, physiological conditions, age, and other factors. Observations by scanning electron microscopy suggest that the high affinity between the clay and *Anabaena* was due to an extracellular flocculating agent. The preferential adsorption on the crosswalls of the *Anabaena* filaments is in agreement with the report that mucilage is secreted at that location (*3*). Further study is needed to elucidate these aspects.

Microbial and microbial-clay flocculations are affected by the presence of electrolytes. Some investigators have suggested that polyvalent cations are essential if flocculation is to occur, assuming that these ions form complex bridges between two negatively charged particles (*10*).

Others have shown that monovalent cations at high concentrations would replace polyvalent ions (*11*). Their explanation, based on the electrical double-layer theory, is that the monovalent cations reduce the electrostatic repulsion between the negatively charged clay particles and organic polymers, enabling the particles to approach closely enough to each other to form bonds (hydrogen or van der Waals bonds). The increase in the flocculation value from $5 \times 10^{-4}M$ for $CaCl_2$ to $2 \times 10^{-2}M$ for NaCl (a 40-fold increase) is close to the 64-fold increase predicted on the basis of the electrical double-layer theory (*12*). The electrolytes appeared to diminish the electrostatic repulsion rather than form a bridge between the algae and clay particles. Regardless of the mechanism by which electrolytes induce flocculation, it seems that in freshwater systems, which usually contain NaCl at lower than its flocculation value, divalent cations are essential for algae-clay flocculation.

The mutual flocculation of algae and clay has many important ecological and practical implications in lakes and other bodies of water. Some of those implications are discussed below.

An immediate outcome of algal-clay flocculation is the formation of larger particles having a higher sedimentation velocity. Thus, flocculation leads to the removal of suspended inorganic particles and algae from the water and to their accumulation at the bottom of water bodies. Sedimentation of algae leads to a downward displacement of nutrients they had taken up from the euphotic zone of the water column.

The sedimentation of algae with clay may be a selective mechanism leading to the preferential accumulation of flocculating algae in the sediment and the preferential survival of nonflocculating algae in the water. This selectivity may be due to taxonomic or physiological differences among the algae. As for bacteria, the flocculation tendency is higher during the declining growth and death phases or when the culture is under stress (*1*). Thus it is possible that the algal-clay flocculation is a population control mechanism leading to selective flocculation and sedimentation of the older and less active algal cells.

Another interesting implication relates to the harvesting of algae by filter feeders. These organisms can consume only particles larger than the openings in their filters. Thus, for example, the silver carp, which can utilize only particles larger than 20 to 50 μm (*13*), cannot feed on the cells of the algae that we used in this work but might well be able to harvest the clusters they form with clay.

Most researchers believe that algal growth in turbid water is limited because of the absorption and scattering of light by the clay particles. Possibly, flocculation and sedimentation of algae are other processes that limit algal growth.

In addition to the interesting ecological implications, the mechanism studied here may provide the means for managing lakes and other bodies of water. It is possible that turbid water could be clarified through the enhancement of algal growth or that algal-rich water could be clarified through the addition of clay to the water.

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Influence of Cholesterol on Water Penetration into Bilayers

Abstract. *X-ray diffraction and capacitance measurements have been used to calculate the depth to which water penetrates in fully hydrated bacterial phosphatidylethanolamine bilayers in the presence and absence of cholesterol. The data indicate that cholesterol decreases the depth of water penetration by about 2.5 angstroms.*

The depth to which water penetrates bilayer membranes and the role cholesterol plays in modulating this penetration are of interest for several reasons. (i) This information is vital for calculations of the total hydrophobic contribution to the free energy of bilayer formation (*1*). (ii) A better interpretation of high- and low-frequency membrane capacitance as well as reflectance measurements could be made if interfacial boundaries were more precisely defined (*2, 3*). (iii) The energetics of molecular adsorption to and transport through bilayers involves the displacement of interfacial water (*4*). (iv) The depth to which water penetrates micelles is a controversial issue (*5, 6*), to which the data obtained from these measurements on bilayers are relevant. (v) This information may be correlated with the potential energy profile across bilayer membranes (*7*). (vi) Some of the biochemical and physiological effects that occur when cholesterol is added to or removed from membranes (*8–10*) may be related to the influence of cholesterol on water penetration.

Data that can be related to the penetration of water into bilayers have been obtained by several techniques, including electron spin resonance, capacitance, and neutron diffraction measurements. Griffith *et al.* (*11*) used electron spin resonance to determine the polarity profile across liver microsomal and myelin membranes. From this profile, it was inferred that water penetrates roughly one-third of the distance from each surface into the bilayer. However, the influence of the bulky nitroxide spin label on this measurement is not known (*12*). The dielectric thickness for a variety of planar bilayers has been obtained by capacitance measurements (*13, 14*). In these capacitance experiments, care must be taken to use "solventless" planar bilayers to make structural comparisons with solventless multilamellar vesicles (*15*). Neutron diffraction experiments provide direct information on the depth of water penetration in phospholipid bilayers. By this technique, water penetration has been measured for partially hydrated samples of gel-state dipalmitoyl phosphatidylcholine (*16*) and liquid crystalline dimyristoyl phosphatidylcholine : cholesterol (2:1) and egg lecithin : cholesterol (2:1) multilayers (*17*). It was shown that water penetrates to the lecithin carbonyl group in dimyristoyl phosphatidylcholine : cholesterol (2:1) bilayers (*17*). The effect of cholesterol on the depth of water penetration into fully hydrated liquid crystalline bilayers has not previously been measured.

We combined x-ray diffraction data from fully hydrated bacterial phosphatidylethanolamine (BPE) bilayers, in the presence and absence of cholesterol, with capacitance data from solventless planar bilayers made from the same lipids under identical ionic conditions. The results show that cholesterol may decrease the penetration of water into the bilayer by about 2.5 Å, displacing water from the deeper carbonyl group in BPE.

For x-ray diffraction experiments, hydrated lipid samples were made as follows. A chloroform solution of the appropriate lipid was evaporated to dryness and then excess (≥ 70 percent by weight) 0.1*M* NaCl was added to the

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