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ACTION OF DIATOMS ON CLAY

BY

JOSEPH HARVEY RENFREW

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A

THESIS

submitted to the faculty of the

SCHOOL OF MINES AND METALLURGY OF THE UNIVERSITY OF MISSOURI

in partial fulfillment of the work required for the

Degree of

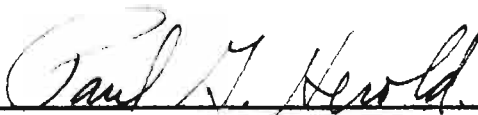
MASTER OF SCIENCE, IN CERAMIC ENGINEERING

Rolla, Missouri

1946

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Approved by



Professor of Ceramic Engineering

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In helping the writer to plan and conduct the enquiry along lines heretofore not attempted, and for his ever practical and helpful cooperation, Professor Paul G. Herold deserves special mention.

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## Introduction

With the recent trend toward the use of higher temperatures and more severe service conditions in the metallurgical and refractories industries there has developed a constantly greater demand for high-alumina clays, that is, clays in which the alumina content is considerably above that of pure kaolinite,  $\text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2 \cdot 2\text{H}_2\text{O}$ .

However, the known reserves of good diaspore are so small that ceramic engineers are already turning to basic refractories in order to have sufficient materials for work at high temperatures. Even the slightest reflection on the needs of the refractories industry will show that such substitutions are strictly limited, and possible only where the new material does not introduce further complications and difficulties.

Since 1891 it has been known that diatoms can decompose clay to obtain the silica for their frustules. Recent research in the Academy of Science of the USSR<sup>1</sup> has elucidated some of the finer points of this action of diatoms, but as the work was done in pure culture, and in minute quantities, there remains the question, can diatoms be induced to grow on a large enough scale so that man can use them to raise the alumina content of clays?

In the conviction that in the near future there will be a demand for high-alumina clays greater than the supply, this study was undertaken in order to learn whether an industrially practical process could be worked out, and if not, at least some further addition to knowledge could be made.

## Review of Literature

It has long been known that the frustules of diatoms are composed of opal, or hydrated silica. The earliest microscopists were very interested in the great beauty and seemingly endless variety of form presented by these siliceous algae, which they called by various names, such as Diatoms, Infusoria, Kieselalgen, Diatomaceae, and Bacillariae. The frustule contributes 30 to 55% of the dry weight of diatoms, and this is nearly pure silica.<sup>2</sup> Most diatomists have not been concerned with the source of this silica, and seem content with the theory that diatoms depend upon "dissolved silica."

Murray and Irvine, however, calculated that diatoms would be obliged to ingest from 250,000 to 300,000 times their weight of water in order to obtain the silica necessary for their skeletons.<sup>3</sup> The silica in true solution in natural waters can not, therefore, be considered as a source of silica for diatoms or other siliceous plants and animals. That diatoms extract their silica from clay was first demonstrated by Murray and Irvine.<sup>4</sup>

Vernadsky also proved that diatoms can decompose kaolinite, and definitely identified free hydrated alumina as a product of the reaction.<sup>5</sup>

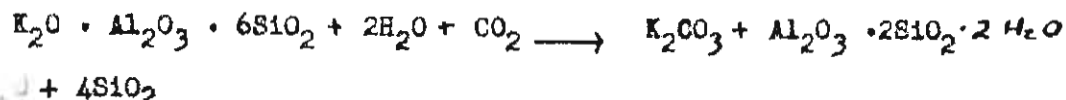
The decomposition of aluminosilicates requires a considerable amount of energy, according to methods customary in the ceramic industry. The following reaction might be considered typical:



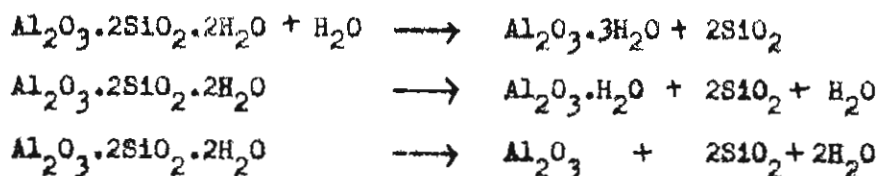
Nature has a slow but economical way of decomposing minerals. Heretofore, the formation of residual clays by the decomposition of feldspar has been considered primarily a process of chemical weathering, i.e. solution and removal of the potash, leaving silica



and hydrated aluminum silicate as the products:



Continued weathering leads to the formation of hydrated alumina, and finally to free alumina:



That this is not a complete explanation was shown by Coupin, who found diatoms were able to attack both orthoclase feldspar and kaolinite.<sup>6</sup> Quartz, glass, silica gel, and alkali silicates were unaffected by diatoms in his experiments, suggesting that the form in which silica occurs is important in the nutrition of diatoms.

These researches, mainly on salt-water diatoms, indicated that a more precise study of the mechanism of clay decomposition might yield valuable clues as to the nature of the reaction, and clear up the question of the influence of bacteria.

With these objects in mind, Vinogradov and Boichenko separated pure nacrite from kaolin and treated it with Nitzschia Palea W. Sm. and Navicula minuscula Grun in pure culture. They found both species of diatoms altered the crystalline structure of nacrite, though the resulting structure could not be deciphered. They showed the outer gelatinous, or pectinous sheath to be the means of contact by which diatoms attacked the clay mineral. When much pectinous slime was present, nacrite was destroyed.<sup>7</sup> Harvey considers this "slime" to be protoplasm, which "In the pennate diatoms..... streams out through holes from the interior of the cell, moves over the skeleton and

passes into the cell again through other holes; a fresh surface of protoplasm is more or less continuously being exposed to the water. The centric diatoms have many small pores in the skeleton exposing the protoplasm, and there is evidence (Schutt, 1899) that it extrudes to form a film on the exterior of the skeleton. The cell sap is acid in reaction; Dr. F. Gross (unpublished data) found a pH of about 4.5 by crushing diatoms in an unbuffered fluid with indicators. The cell contents, in common with other living cells, probably have a low oxidation-reduction potential.<sup>8</sup>

Diatoms, alone among algae, store their food in the form of an oil. The nature of this substance called by some authors "Diatomin" is not yet clearly established. However, Strain and Manning have discovered a type of carotene in the marine diatom Navicula Torquatum.<sup>9</sup> Also, Pool claims that Vitamin D is synthesized by diatoms.<sup>10</sup>

Hylland, referring to the hydrocarbons known to be present in dead diatoms, develops the theory that by the death of diatoms a large enough quantity of hydrocarbons could be developed to explain the origin of much of the world's petroleum.<sup>11</sup>

Card and Dun describe a deposit of mud four hundred miles in length and one hundred twenty miles wide, found at a depth (in the ocean) of between two hundred and four hundred feet, near Victoria Land in 70 degrees South Latitude. "This deposit is composed of diatom ooze, its thickness is unknown. In the Antarctic Regions the sea is often thick with Diatomaceae which also tinge it and the ice a dull yellow. Instances are on record of shallow estuarine harbours being choked by

their rapid accumulation."<sup>12</sup> Kew gives still better evidence in support of this theory:

"Accepting the theory of the organic origin of petroleum, he says, 'That the oil<sup>in</sup> the Los Angeles-Ventura region has been derived from the organic shales of the Eocene and Miocene is extremely probable.....At the time of the deposition of these formations the seas swarmed with countless numbers of minute organisms, which on dying dropped to the bottom.....Of these organisms the diatoms were the most numerous.' "<sup>13</sup>

The fatty drops thrown out by diatoms are not by themselves capable of decomposing nacrite.<sup>14</sup> Whether the formation of these oils is necessary to kaolin decomposition or merely incidental to diatom growth and death, remains to be clarified by future study.

Kofoed stressed the influence of floods, which first inhibit plankton growth in streams, then encourage it after a period of 10-20 days.<sup>15</sup> He also compared the barren Danube, (which Steuer found to be poor in organic materials, carbonates, and silica), with the Illinois River, which possessed an abundance of nutrients during the same period.<sup>16</sup>

King and Davidson found that low concentration of silica as sodium silicate could function as a limiting factor in the growth of diatoms; that up to about 100 mg. per liter growth was satisfactory, but that above these concentrations silicate was an inhibitory, even toxic factor.<sup>17</sup>

No study of diatoms would be complete without mention of diatomaceous earth. Also called kieselguhr and (incorrectly) infusorial earth, or tripoli, it is composed mainly of the remains of diatoms, along with silt, clay, and sand which are usually laid down simultaneously. Even the purest diatomaceous earth contains some alumina, probably as adventitious clay, but some clay appears to be inside the diatom shells,<sup>18</sup> or frustules.

Large deposits of diatomaceous earth, or diatomite, are worked in the United States of America and abroad. The chief uses of this earth are in the filtration of sugar and other organic chemicals, and as a support for various catalysts in the chemical industry. Its high porosity and permeability make it a valuable material for insulating brick, though its use for this purpose has declined due to competition of cheaper materials.

Weed describes diatom beds in process of formation near the Emerald Springs of the upper Geyser Basin, Yellowstone National Park.<sup>19</sup> Cooled brackish water flowing from hot springs into certain low-lying, swampy areas supported a great quantity of vegetation. Many species of diatoms were identified in the bottom ooze from this swamp, of which Denticula valida Ped. comprised the major portion. Analysis of certain geyser waters, shown in the following Table I, indicates a high per cent of silica, but whether all in solution or partly in suspension as clay and other aluminosilicates, the author does not specify.<sup>20</sup> He believed that algae play a major role in the deposition of siliceous sinter or "geyserite" from such waters.

Table I - Analyses of geyser waters<sup>20</sup>

(Constituents grouped in probable combination. Grammes per kilogram)

	Asta Spring	Splendid Geyser	Grand Geyser	Old Faithful Geyser	Great Geyser, Iceland	White Terrace Geyser, New Zealand
SiO <sub>2</sub> , Silica . . . . .	.1650	.2964	.3035	.3961	.5190	.6060
NaCl, sodium chloride . . . . .	.1320	.4940	.5643	.6393	.2379	1.6220
LiCl, lithium chloride . . . . .	.0048	.0140	.0218	.0340	.....	*.0950
KCl, Potassium chloride . . . . .	.0221	.0231	.0319	.0478	.....	.....
KBr, potassium bromide . . . . .	.....	.....	Trace	.0051	.....	.....
Na <sub>2</sub> SO <sub>4</sub> , sodium sulphate . . . . .	.0575	.0281	.0387	.0270	.1342	.....
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , sodium borate . . . . .	.....	.0335	.0350	.0213	.....	.....
Na <sub>2</sub> AsO <sub>2</sub> , sodium arseniate . . . . .	.....	.0025	.0014	.0027	.....	.....
Na <sub>2</sub> SiO <sub>3</sub> , sodium silicate . . . . .	.....	.....	.....	.0279	.....	+ .2290
Na <sub>2</sub> CO <sub>3</sub> , sodium carbonate . . . . .	.1463	.5286	.3209	.2088	.2567	.....
MgCO <sub>3</sub> , magnesium carbon- ate . . . . .	.0035	.0018	None	.0021	.....	Trace
CaCO <sub>3</sub> , lime carbonate . . . . .	.0295	.0075	.0070	.0038	.....	.025
FeCO <sub>3</sub> , iron carbonate . . . . .	.....	.0001	.....	Trace	.....	.....
Al <sub>2</sub> O <sub>3</sub> , Alumina . . . . .	.0112	.0051	.0061	.0017	.....	.005
H <sub>2</sub> S, hydrogen sulphide . . . . .	.....	.....	Trace	.0002	.....	.....
NH <sub>4</sub> Cl, ammonium chloride . . . . .	.....	.0002	.0012	Trace	.....	.....
CO <sub>2</sub> , carbonic acid . . . . .	.1045	.1989	.0587	.....	.....	.....
K <sub>2</sub> SO <sub>4</sub> , potassium sulphate . . . . .	.....	.....	.....	.....	.0180	.0750
MgSO <sub>4</sub> , magnesium sulphate . . . . .	.....	.....	.....	.....	.0091	.....
Na <sub>2</sub> S, sodium sulphide . . . . .	.....	.....	.....	.....	.0088	.....
Total . . . . .	.6764	1.6340	1.3905	1.3908	1.2305	<del>2.6570</del>
Specific gravity . . . . .	.....	1.00132	1.00108	1.00096	1.000205	1.00077

\* CaCl<sub>2</sub>+ Na<sub>2</sub>O

Allen, in checking the findings of Weed, confirmed the presence of many different species of diatoms in thermal waters, but never found living diatoms in waters where the temperature was higher than 40° C.<sup>21</sup>

Silica is deposited from alkaline thermal waters by purely inorganic processes also, a fact which confused Weed and other early writers on the role of algae as rock-formers. Also, diatomaceous earth, as usually mined, contains about 84.5% of SiO<sub>2</sub>, about what should be expected upon decomposition and removal of the organic substance of diatoms.

Whether diatomites higher in silica have been secondarily enriched by silica-bearing solutions is a matter to be determined by further study. Also, little attention seems to have been given to the effect of suspended clay on the diatoms and other plankton in diatom beds, either at present in process of formation, or in deposits from past geologic eras.

Table II - Chemical Composition of Diatoms (Dry Weight Basis)

Organisms and Authority	N	Crude Protein Nx6.25	Ether Extract	Pentos-ans	Carbo-hydrates	Crude Fiber	N-free Extract	Organic Matter	Al <sub>2</sub> O <sub>3</sub>	H <sub>2</sub> O	Ash	SiO <sub>2</sub>
Diatoms, chiefly Chaetoceras <sup>22</sup>		Albumin 10-11.5%	2.5		21.5						64.5 to 66.0	50.0 to 58.5
do <sup>23</sup>	1.56	N crude protein 9.75	2.80								65.2	54.5
Asterionella <sup>23</sup>	2.20	13.75									57.52	49.48
Diatom-acea <sup>23</sup>	3.66	22.87	13.60	2.87		1.43	22.60				39.50	30.78
Diatoms, chiefly Rhyzolenia, Chaetoceros, and Thalassothrix <sup>24</sup>								16.75	1.38	4.87		1.00* 76.00

\*Silica Soluble in Acid

Table III - Analyses of Diatomaceous Earths of the  
United States of America<sup>25</sup>

Location and Original Reference	Per Centage Composition							
	Water	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	CaO	MgO	Miscellaneous	Total
Herkimer Co., N.Y. <sup>a,d</sup>	12.12	86.51	0.45	0.37	0.12	0.42	—	99.99
Richmond, Va. <sup>e</sup>	8.37	75.86	9.88	2.92	0.29	0.69	0.94	98.95
Wilmington Wharf, Va. <sup>e</sup>	3.40 <sup>b</sup>	82.85	6.76	2.34	0.35	1.06	3.15 <sup>c</sup>	99.91
Pope's Creek, Md. <sup>f</sup>	3.47	81.53	3.43	3.33	2.61	5.63	—	100.00
Clermont, Fla. <sup>g</sup>	—	98.43	—	—	—	—	—	—
Ellensburg, Wash. <sup>h</sup>	5.98	84.91	1.75	2.04	0.11	0.19	4.94	99.92
Fossil Hill, Nev. <sup>i</sup>	5.99	86.90	4.09	1.26	0.14	0.51	1.18	100.07
Lompoc, Calif. <sup>j</sup>	5.00	89.30	4.00	0.70	0.40	0.40	0.20	100.00
Monterey, Calif. <sup>i,k</sup>	4.89	86.89	2.32	1.28	0.43	Trace	3.58	99.39
Pit River, Calif. <sup>l</sup>	—	96.02	1.03	0.62	0.12	—	—	97.79

a. Water-settled to remove clay prior to analysis

b. Includes "loss on ignition"

c. Includes "Soda, 0.99 per cent; Potash 1.07 per cent; Titanium oxide 1.09 per cent"

d. N. Y. State Museum, Ann. Rept. 57, 1, Sec. 1, 171

e. Watson, Mineral Resources Va., 1907, 218

f. Calif. State Mining Bur. Bull. 38: 289 (1906)

g. C. Lindley Wood, Private Communication

h. S. A. Milton, Private Communication

i. Arnold and Anderson, U. S. Geol. Survey Bull. 315, 445; from Calif. State Mining Bur. Bull. 38:28 (1906)

j. Thatcher, U. S. Patent 1,477,394 (1923)

k. Lawson and Pasada, Univ. Calif. Dept. Geol. Bull. 1:25

l. Root, Calif. Bur. Mines 22, No. 2 (1926)



Table IV - Analyses of Various Foreign Diatomaceous Earths

Location and Reference	Water	Water + Volatiles	Organic Matter + Combined Water	Organic Matter + CO <sub>2</sub>	SiO <sub>2</sub>	TiO <sub>2</sub>	Fe <sub>2</sub> O <sub>3</sub>	Al <sub>2</sub> O <sub>3</sub>	CaO	MgO	Alkalis		Misc. Total
											K <sub>2</sub> O	Na <sub>2</sub> O	
<b>Unterluss,<sup>26</sup></b>													
<b>Hannover, Germany</b>													
White . . . . .	3.49			3.58	89.17	0.12	0.35	1.89	Trace	0.22	0.39	0.69	99.90
Gray . . . . .	5.26			8.43	81.25	0.15	1.34	1.82	0.18	0.20	0.69	0.68	100.00
Green . . . . .	4.83			16.17	71.22	0.14	2.22	4.09	Trace	Trace	0.79	0.54	100.00
<b>Altenschlirf,<sup>26</sup></b>													
<b>Hesse . . . . .</b>													
	3.50			1.03	90.80	0.50	2.68	0.97	*	0.30	0.41	0.53	100.72
<b>Auxillae, Auvergne, France<sup>26</sup></b>													
White . . . . .	2.33			1.74	91.61	0.10	2.22	1.53	0.18	0.29	Trace	Trace	100.00
Green . . . . .	3.00			4.17	88.56	0.18	0.73	2.16	0.26	0.02	0.47	0.44	100.02
<b>Toome, Ireland<sup>27</sup></b>	7.35	—	7.20	—	74.90	—	1.90	6.68	1.01	0.72	0.24		100.00
<b>Gmund, Austria<sup>28</sup></b>													
No. 1 . . . . .					94.02		1.95	0.86	2.00	0.54			0.63
No. 2 . . . . .					84.94		2.62	6.06	2.96	1.04			2.38
<b>Algeria<sup>29</sup></b>													
No. 1 . . . . .		9.14			89.76		0.20	0.80	0.10	Trace			—
No. 2 . . . . .		3.50			84.20		1.00	4.00	0.51	0.21			1.67*
No. 3 A A . . . . .		7.40			74.00		1.06	1.99	5.00	1.58			8.97

\* Analysis evidently incomplete

As silica is only a portion of the food requirements of diatoms, and other factors also are important, an understanding of the general conditions of diatom growth is necessary if optimum growth is to be obtained in the laboratory. A diatom cell resembles a pill box, having two halves, called valves, which fit one over the other. Growth is by cell division. Before the division takes place a new half to match each valve is developed inside the already existing half; hence it is slightly smaller than the mother cell. One of the daughter cells is therefore exactly like the mother cell, but slightly smaller. The process is said to continue until further decrease in size is impossible, at which<sup>stage</sup> an auxospore is formed by conjugation. The latter is of large size, and will on multiplication give a new large cell resembling the mother cell in every detail.<sup>30</sup>

More than ten thousand varieties are known.<sup>31</sup> All known geometric shapes are represented, and many are of extraordinary beauty and delicacy. The marine types are usually thick-walled and circular, triangular, or square in shape, whereas fresh water types are thin, long, and diaphanous. In size they vary from the very tiny Cyclotella, less than ten microns in diameter, to such large forms as Surirella and Navicula, which sometimes occur as long as one millimeter.<sup>32</sup>

Diatomaceous earth contains diatoms from several geologic epochs, at least as old as the Jurassic.<sup>33</sup> As some diatoms have become extinct, the presence of their fossils in certain strata gives an indication of the geologic age of the stratum or formation.<sup>34</sup>

Analogous to their use by geologists is the use of diatoms to identify certain typical conditions of salinity, temperature, currents, etc. in the oceans.<sup>35</sup> Both oceanographers and sanitarians have studied diatoms and other phytoplankton for their important function as food for fish

and lower aquatic life. From these standpoints considerable work has been done, and it will now be discussed briefly.

Temperature was at first thought to be a primary factor influencing diatom growth, but as data began to accumulate it became clear that other factors were of greater significance. The scanty information available is given in Table V below:

Table V - Relation of Temperature to Microscopic Growths<sup>36</sup>

Number of occurrences of growths of 1000 standard units or over - Croton Reservoirs, 1921  
After Hale

Organism	Temperature, Degrees F.				
	30-40	40-50	50-60	60-70	70-80
<b>Diatomaceae;</b>					
Synedra	3	0	5	2	1
Fragilaria	1	0	0	0	0
Melosira	1	5	4	1	1
Asterionella	1	8	7	1	0
Tabellaria	<u>0</u>	<u>2</u>	<u>0</u>	<u>0</u>	<u>0</u>
Total	6	15	16	4	2
<b>Cyanophyceae;</b>					
Aphanizomenon	5*	10	4	7	4
Anabaena	0	5*	7*	9	12
Coelosphaerium	0	0	0	0	1
Mixture	<u>2*</u>	<u>2*</u>	<u>4</u>	<u>5</u>	<u>13</u>
Total	7	17	15	21	30

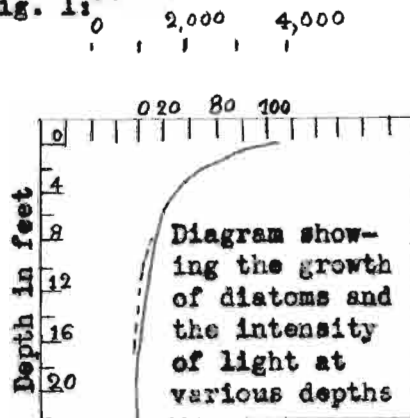
\*Persistence, not start of growth

It will be seen from the above table that the optimum temperature for growth of the five diatom species studied is in the neighborhood of 4.5 to 16 degrees Centigrade, and that with rising temperatures diatoms give way to the blue-green algae.<sup>36</sup>

Diatoms and many other algae are said to thrive best in somewhat subdued light, but the optima are not the same for all species. In fact, for Biddulphia mobiliensis it is about 1600 luxes, whereas certain arctic species will grow under the ice in March. But high illumination does not necessarily mean high rates of photosynthesis, for Schreiber found that rate of multiplication was proportional to light intensity only from 200 to 400 luxes.<sup>37</sup>

When subjected to very bright light, diatoms tend to group together their otherwise scattered chromatophores into knots. This condition was described by Schimper, and is known as a systrophe.<sup>38</sup> In addition to systrophism, many individuals will combine into thick, felted groups, thus acquiring additional protection. These felted groups (flocs) consist of several different genera and species, and necessarily bear no relation to the true colonial species such as Fragillaria.

Fresh water diatoms appear to be very sensitive to light. According to Whipple they can not grow in darkness nor in bright sunlight. The close correspondence between light intensity and diatom growth is shown by Fig. 1:<sup>39</sup>



Number per c.c.

Intensity of light

Lake Cochituate water located in Lake Cochituate Nov. 29, 1895. Examined Dec. 9, 1895. Temperature 40° - 44° Color 0.33. The diatoms were chiefly Asterionella and Melosira. The intensity of light at different depths was calculated on the assumption that a layer of water one foot in depth absorbs 25% of the light falling upon it.

Fig. 1 - Relation of Light Intensity to Diatom Growth

Variation in the pH of the nutrient solution has a very marked influence on diatom growth. Bachrach and Lucciardi, working with marine forms of Naviculae and Nitzschia in sea water enriched with urea, altered the pH of cultures by addition of acid or alkali, and exposed them to north light. Diatom counts were made at the beginning, and after four to five days. The optimum rate of increase appeared to be between initial pH values of 7.3 to 8.2.<sup>40</sup>

Table VI - Influence of pH on Diatom Growth <sup>41</sup>

Résultats au bout de cinq jours:

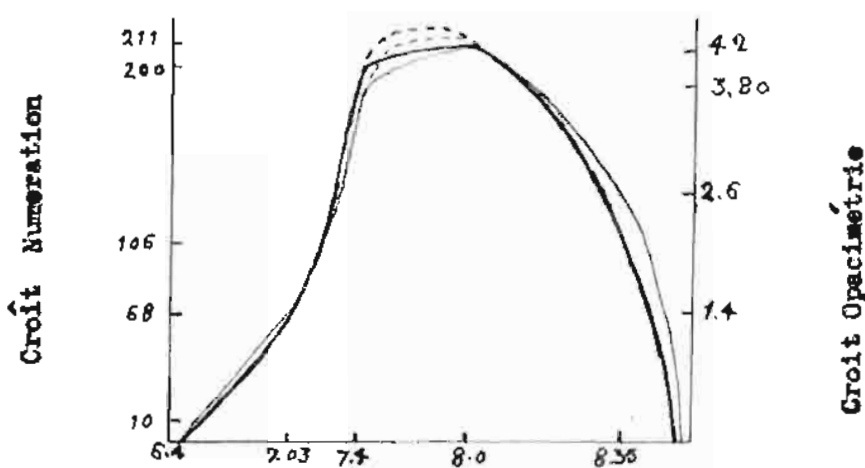
pH primitifs	pH moyens des cultures	pH moyens des témoins	Croît Nombre de Diatomées au mm <sup>3</sup>	Tableau II Croît Opaci- métrie
9.7	8.27	8.27	32	1
9.3	8.67	8.4	143	4
8.2	8.75	8.37	171	5.75
7.2	8.45	8.26	205	8
6.6	8.32	8.25	86	2
5.4	8.15	8.3	162	3
4.6	7.43	6.68	11	0
3.6	3.7	3.7	0	0

Table VI (continued)

II - Experience en tubes bouchés au liège et paraffines

pH initial	pH final cultures (moyennes)	pH final témoins (moyennes)	Croît Numération par rectangle et au mm <sup>3</sup>	Tableau III Croît Opacimétrie
9.7	9.17	9.15	0 (precipité)	0
8.9	9.16	8.86	1.06	106
8.2	9.16	8.0	2.11	211
7.3	8.8	7.4	2.01	201
6.7	8.35	7.03	0.68	68
5.8	6.4	6.4	0	0

Resultats obtenus quatre jours apres l'ensemencement.



Influence du pH sur le croît. (Tableau III) Experiences en tubes bouchés et paraffines. (En pointillé, courbes interpolées entre le points expérimentaux correspondant aux pH 7.4 et 8.0)

Fig. 2 - Influence of pH on Diatom Growth 41

In the culture of Nitzschia Closterium, Stanbury concluded that the wave length of light is not nearly so important as the total amount of energy absorbed.<sup>42</sup>

Dissolved nutrient salts play a vital role in influencing diatom growth. Liebig's law of the minimum states that growth is limited by that factor which is present in minimal quantity. Brandt appears to have been the first to show that low concentrations of nitrogen and phosphorous might be limiting factors for diatoms.<sup>43</sup>

Diatoms utilize nitrogen and phosphorus at a ratio of about 15 atoms of nitrogen to one of phosphorus, with a deviation called the "anomaly of the Nitrate-Phosphate ratio." Ketchum has shown that two synthetic processes appear to be at work: one forming a compound containing both nitrogen and phosphorus, and the other forming a phosphorus compound having no nitrogen.<sup>44</sup> The present writer suggests that this dual function of phosphorus in diatom metabolism may be responsible for the anomaly.

The fundamental role of phosphates in phytoplankton nutrition in the sea is demonstrated by the almost exact coincidence of areas high in plankton production with areas of high phosphate concentration. For example, in the South Atlantic where  $P_2O_5$  is highest, (ca. .01 mg. per liter), the maximum number of individuals is found (ca. 100,000 per liter).<sup>45</sup>

Harvey found that phosphates were not only directly necessary for formation of phosphorus-bearing organic compounds, but were also instrumental in increasing the rate of photosynthesis.<sup>46</sup> He also found that nitrate-nitrogen and ammonium-nitrogen were equally effective as nitrogen foods for diatoms; and that during the vernal outbursts of

of growth in the sea, the diatoms consumed two and one half times as much nitrate-nitrogen as phosphorus (as  $P_2O_5$ ). However, in his experiments, ten times as much nitrate-nitrogen was required to bring about the same amount of carbon fixation as was necessary under natural conditions.<sup>47</sup>

ZoBell compared the value of nitrogen as nitrate, nitrite, and ammonium-nitrogen in the nutrition of Nitzschia Closterium, Nitzschia bilobata, Navicula sp., and Chlorella sp. He concluded that diatoms extracellularly reduce nitrate to nitrite.<sup>48</sup> Beckwith demonstrated the same in the case of Chlorella, adding that this diatom assimilates nitrogen in the form of nitrite.<sup>49</sup> Schreiber<sup>50</sup> and Braarud and Fjyn<sup>51</sup> found certain phytoplankton utilized nitrogen more efficiently as ammonium than as nitrate. Bond found certain other phytoplankton responded well to ammonium nitrogen, though apparently no comparison with nitrate was made.<sup>52</sup>

Table VII - Effect of Various Nitrogen Concentrations on the Growth of Nitzschia Closterium<sup>53</sup>

Form of nitrogen	millimols		millimols in sea water	
	Optimum	Inhibitory	average	range
Ammonium	0.01 - 0.05	4	0.001-0.005	0-0.02
Nitrite	0.05 - 5.00	25	0.0001	0-0.001
Nitrate	0.10 -40.0	300	0.002-0.01	0-0.05

As in higher plants, carbon dioxide is a fundamental requirement for diatom growth. Marine diatoms can obtain their carbon dioxide readily either from solution or by hydrolysis of bicarbonates.<sup>54</sup> Eddy claims that  $CO_2$  is a very definite factor influencing diatom



growth in fresh water. He divided a culture of diatoms, mainly Synedra, into four pairs of tests. To the first pair he added CO<sub>2</sub>; to a second, air, and into a third he allowed water to drip, causing considerable aeration. The fourth pair of cultures was allowed to remain undisturbed as a control. The CO<sub>2</sub>-fed cultures showed a greater increase than the air-cultures, but less than the controls.<sup>55</sup>

Gran and Harvey state that lack of iron may at times function as a factor limiting diatom growth. During periods of heavy plankton growth the quantity of iron in sea water has been shown to diminish, probably due to withdrawal by diatoms.<sup>56</sup>

Diatoms will absorb both phosphate and iron on their surfaces, utilizing only a minute portion of the iron inside the cell. One milligram of Fe gave the same growth response as 175 mg. of phosphorus, yet several times more iron than phosphorus is found in and on diatoms in nature.<sup>57</sup>

In diatom research considerable use has been made of a culture medium known as "Erdschreiber", a solution prepared from sterilized sea water enriched with nitrates, phosphates, and soil extract. Harvey found that a subculture of the centric diatom Ditylum brightwellii Ehrb. when transferred to natural sea water enriched with nitrates, phosphates, silicates, and iron, failed for some reason to show satisfactory growth. Allen (1914) observed similar results when the diatom Thalassiosira gravis was transferred to an artificial sea water prepared from distilled water and pure chemicals, but when a very small quantity of natural sea water or of a boiling water extract of Ulva was added, a rich growth of this diatom occurred. As a result of much research he concluded that natural sea water contained a

substance or substances of an organic nature necessary for the growth of Thalassiosira gravida.<sup>58</sup>

Further research showed that there were two substances possessing growth promoting activity, an "A" accessory which is adsorbed on charcoal and insoluble in aqueous butyl alcohol, and an "N" accessory which is not adsorbed on charcoal and dissolves in aqueous butyl alcohol.

Several organic compounds, such as aneurin (Vitamin B<sub>12</sub>), biotin, cystine, glutathione, and methionine have been shown to possess "A" activity.

It will be noted that all of these compounds contain divalent sulfur, in the group  $-S-CH_2-CH(NH_2)COOH$ .<sup>59</sup>

The nature of the "N" accessory required by diatoms has not yet been clearly determined. However, Harvey gives a method of concentration which may prove useful to future investigators.<sup>60</sup>

Manganese and other elements appear to be necessary to diatom metabolism; they need be present only as minute traces, yet their absence may prove a limiting factor.<sup>61</sup>

### Method of Approach

First of all it was decided not to use salt water species of diatoms, as the high concentrations of alkalis and alkaline earths present in the cultures would very probably render the clays unsuitable for ceramic uses. Even if very satisfactory improvement in the alumina content were so obtained, the subsequent purification required would be likely to add too much to the cost.

Fresh water diatoms were therefore decided upon, and the problem of an adequate source of live diatoms was tackled. No live diatoms were obtainable from biological supply houses, and at that stage of the research it was not known what species would be suitable. Whether to work with pure cultures of diatoms, i. e. cultures containing only one species of diatoms, and free from bacteria, or to develop and use "persistent cultures" was then decided. The latter are cultures developed in the laboratory from natural sources by enrichment of natural cultures with the various chemical substances needed for healthy growth. This in itself proved to be an important problem, as there appears to be no present knowledge of the nutrition requirements of fresh water diatoms, in spite of the abundant references to them in sanitary and general biological literature.

For the purposes of clarity, then, collections of diatoms from ponds and streams, and their concentrates will be termed "natural cultures." If upon addition of suitable nutrients a viable culture is obtained, it will be called a "persistent culture."

### Preparation of Diatom Cultures

For the first series of experiments scum and green algae, etc. were taken from a stagnant pond in a nearby town. Cultures from this source were named "J", for the initial letter of the name of the source. To vary the possible species a sample of bottom debris and shoreline mud were obtained from a pond in Rolla, Missouri. These cultures were named "R", according to the same system. Natural cultures from both sources were stirred and macerated for several hours to free the diatoms from algae and higher plants with which they are usually associated. An ordinary steel razor blade, attached to a simple clamp (of brass) was used for the cutter. At the end of the maceration period the brass portion of the jig was observed to be highly polished, and in contrast, the steel of the blade was stained reddish brown to black. Iron and sulphur bacteria may have been responsible, but the matter was not investigated.

The first attempt to filter through 100 mesh, through 200 mesh, and through a coarse Mandler porcelain filter proved unsatisfactory. No diatoms would pass the latter, and examination of the 100 mesh and 200 mesh portions microscopically showed poor separation. Each natural culture was then filtered through 200 mesh, through 270 mesh, and through 325 mesh. The resulting portions were placed in cylindrical glass battery jars, and transferred to a south window. Small portions of a thick meat soup were added to provide nitrogenous food. All cultures so fed appeared to grow well in comparison to those which were left unfed.

The next question was, out of the immense number of varieties of diatoms, and the wide difference of growth conditions, such as tropical heat, Arctic cold, fresh or brackish water, strong or dim light, limnetic or lacustrine conditions, how to find within reasonable time the right species for the job in hand, that is, the decomposition of clay. Development of pure strains would be time-consuming and expensive, as the writer was thinking in terms of an economic process, e. g. cheaper high-alumina clays. Persistent cultures, in which one species was dominant, were thought to be superior, but their development entailed almost as much time. Therefore, the writer hit upon the idea of reversing the approach completely: choose the conditions deemed practical from an economic standpoint, and subject a heterogeneous mixture of diatoms to the treatment. The variety which survives ought to be the right one for the task.

The action of diatoms on clay, or simply, the reaction, might proceed in a number of different possible ways. Under moist, semi-solid conditions, Coupin and Vinogradov report that one to two months are required for any substantial change in the physical and chemical appearance of the treated clay. On the other hand, it might be induced to proceed more rapidly in higher dilutions.

As King and Davidson had worked with water-cultures, using sodium silicate as the siliceous nutrient, it was decided to try using rather strong clay slips, in which the actual clay content was fairly high, yet not so high as to impede easy stirring. The latter authors had pointed out that in concentrations above 100 mg. per liter, silicate showed inhibitory, even toxic effects, but the writer thought the very low

solubility of clays, and their easy colloidal dispersion, might make it possible to conduct the reaction at a moderate rate, under conditions not too inimical to diatom growth.

For the clay, a common brick clay of Pleistocene age was chosen. This was done because practically all the inorganic nutrients could be provided from the clay itself, and if the results proved satisfactory, the same reaction could be done with a pure clay, employing suitable enrichments, if necessary.

### Investigations

The first experiment was tried in bright sunlight, using urea as the source of nitrogen. 400 <sup>ml</sup> cc. beakers were used, each containing 10 grams of the common brick clay, 200 <sup>ml</sup> cc. of distilled water, inseminated with 2.5 ml. of culture. 100 <sup>ml</sup> cc. of urea solution were added in amounts according to the following geometric scale:

Table VIII - Tolerance to Urea, in strong sunlight

Sample Number	N-Content, Grams	Urea Used
J 1	26.2	56.0 g. crystalline urea
J 2	6.56	14.02 " "
J 3	1.64	3.50 " "
J 4	.41	.875 " "
J 5	.1024	.2185 " "
J 6	.0256	100.0 ml. of sol'n cont'ng 546 mg./L.
J 7	.0064	25.0 " " " " " " "
J 8	.0016	6.25 ml. of above solution
J 9	.0004	1.56 " " " "
J 10	.0001	.39 " " " "
R 11 - R 20	(same amounts as in the J cultures)	

The tests were exposed to strong sunlight in August, with daily turning to equalize illumination. After 17 days they were removed to the laboratory for comparison with the natural cultures. The urea tests showed as yet no signs of visible growth, whereas all the natural cultures showed vigorous growth. The pH of all tests was determined, using a Beckmann Meter with one glass and one calomel electrode, with results as shown in Table IX:

Table IX - pH of Natural Cultures Compared with Urea Tests

J. Tests	R. Tests	Natural Cultures
1. 8.30	11. 8.18	J (-270) 9.00
2. 8.00	12. 8.28	
3. 8.11	13. 8.33	J (-325) <sub>1</sub> 9.30
4. 8.22	14. 8.30	
5. 8.00	15. 8.30	J (-325) <sub>2</sub> 8.88
6. 7.80	16. 8.00	
7. 7.77	17. 8.09	
8. 7.70	18. 8.10	R Mud <sub>1</sub> 9.02
9. 7.70	19. 8.11	
10. 8.18	20. 8.00	R Mud <sub>2</sub> 8.42
		R Liquid 8.60

A crust had accumulated on the sides of the battery jars housing the natural cultures. Scrapings were analyzed, and found to consist of 15.90% total solids on the moist basis. On the dry basis, the ignition loss was 42.7%; the ash was composed of silica 44.8%, other constituents 55.2%.

Attempts were made to count the number of diatoms per cubic centimeter in the natural cultures and in the tests. Diatom counts of the natural cultures varied from 800,000 cells per ml. to 2.6 million cells per ml. The wide variation between successive counts on the same sample suggested that an ordinary microscope slide would not give accuracy sufficient to warrant the drawing of any conclusions.

The urea tests were stirred vigorously with a pipette, and 1 ml. withdrawn, diluted to 100 ml., and the resulting liquid tested for diatom content. Very few diatoms were observed, indicating that growth was scanty, or that it was concealed by the suspended clay. Without other disturbance, the urea tests were left in the laboratory upon a table exposed to light from an east window and a south window. Evaporation losses were made up with distilled water. Little, if any, direct sunlight reached the tests during this period.

After 43 days in the laboratory, a green and brown color at the clay-water interface indicated diatom growth, especially in the tests where the urea concentration was low. Tests J-8, 9, and 10 and R-19 and 20 showed marked green coloration. Slight green coloration was also observed in tests R-16, 17, and 18. A slight brown coloration was found in tests J-6, 8, and 10, R-17 and 20. The brown coloration was marked in tests J-9 and R-18. In a few tests the brown coloration had developed to a felted mass which trapped gas bubbles.

On the 64th day growth appeared to have reached a maximum. pH was determined and appearance noted as shown in Table I:



Table I - Tolerance to Urea, in subdued light

Sample No.	Zero *	Color	Slight Brown (a) Marked (b)	Film	Remarks	pH
1	x				Urea crystals	7.6
2	x				" "	8.2
3	x					8.03
4	x					7.08
5	x					7.15
6	x					7.23
7			x (b)			7.73
8				x	Trapped bubbles	8.00
9				x	" "	8.26
10			(b) { x x	x	x	7.93
11	x				Urea crystals	7.65
12	x				" "	8.2
13			x (a)			7.98
14	x					7.13
15	x					7.35
16				x	Trapped bubbles	7.94
17				x	x	8.16
18				x	x	8.09
19			(b) { x x	x	x	8.23
20			x (b) (b)			8.18

\* Zero color indicates no change from original color of clay, a tan to cream.

In all cultures which developed the brown, felted mass of film and trapped gas bubbles, examination under the microscope showed an immense mass of diatoms, practically all being a variety of Naviculae. They were found to be about 4.9 microns in diameter and 48.9 microns in length. A few Nitzschia and sigmoid types, both larger varieties, were present.

Before a comparison between raw and treated clays could be drawn, some method had to be devised for making a fairly clean separation of diatoms from clay. Live diatoms have a specific gravity slightly greater than 1.0,

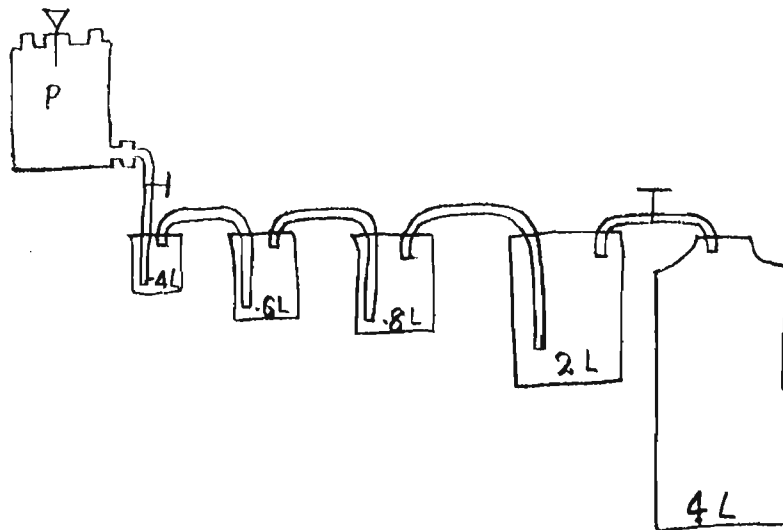
but due to their minute size, most methods of sedimentation give sludges high in diatoms. A complicating factor is that many are actually firmly attached to clay particles of size larger than themselves, or have on their surface adsorbed clay particles.

#### Separation of Diatoms from Clay

The Sharples Super-Centrifuge was used in the attempt to separate diatoms from clay, without substantial result. At 50,000 R. P. M. 12.5% of the diatoms passed over in the water phase, 87.5% remaining in the clay. At 43,000 R. P. M. the proportions were found to be 28.6% and 71.4% respectively, suggesting that considerably lower speeds should be used. Here it should be noted that in the pure diatom-containing liquid obtained at 50,000 R. P. M. the diatoms showed a marked tendency to attack the glass, forming a thin, adherent crust in only 24 hours. Beakers containing clay and diatoms which had been standing almost three months showed no such crust, indicating that diatoms appear to prefer clay, but in its absence will attack glass. Natural cultures which contained little or no initial clay also developed a similar though weaker crust.

From 43,000 R. P. M. down to 24,000 R. P. M. there appeared to be about 20 to 25% separation of diatoms from clay, with increasing contamination at lower speeds. It was therefore, decided to try classification in slowly moving water. A simple elutriator was devised, as shown in Fig. 3:

Fig. 3 - Elutriator for Separating Diatoms from Clay



Diatom-clay suspension from the urea run was fed to the feed-tank, P, and repeatedly washed with fresh distilled water or clear effluent from the jar E. Some idea of the efficiency of elutriation can be obtained by an examination of Table XI, Analysis of Elutriated Diatom-clay suspensions:

Table XI - Analysis of Elutriated Diatom-clay Suspensions

Portion	Dry Wt.	Ign. Loss %	SiO <sub>2</sub> %	TiO <sub>2</sub> %	Fe <sub>2</sub> O <sub>3</sub> %	Al <sub>2</sub> O <sub>3</sub> %	Note: This and subsequent analyses done by A.S.T.M. methods. TiO <sub>2</sub> colorimetrically; Fe <sub>2</sub> O <sub>3</sub> and TiO <sub>2</sub> by the Cupferron method of Baudisch and King. <sup>62</sup>
P	5.292	8.53	61.90	.45	1.53	17.02	
A	1.452	9.25	59.5	.52	2.60	3.08	
B	.8479	15.40	50.8	.63	6.60	18.72	
C	.6862	13.20	34.6	.56	7.38	25.5	
D	.8179	23.65	37.4	.44	6.75	26.01	
E	.2355	40.65	31.65	.43	4.14	.96	

The suspended matter in E proved on settling to be a fine yellow flocculant precipitate. On ignition it gave a faint odor of burning nitrogen compounds. To learn what effect the elutriator might have on the purity of the clay itself, an equal weight of raw, dried, clay was passed through the elutriator, with results as shown in Table XII:

Table XII - Effect of Elutriation on Common Brick Clay

Portion	Dry weight	Ignition loss	SiO <sub>2</sub>	TiO <sub>2</sub>	Fe <sub>2</sub> O <sub>3</sub>	Al <sub>2</sub> O <sub>3</sub>
P	7.0068	7.92%	61.66	1.05	1.70	1.51
A	.8649	8.26	57.9	1.22	6.88	12.47
B	.3070	1.79	56.5	1.79	3.80	18.06
C	.2353	13.50	47.5	2.02	9.05	18.73
D	.5246	42.75*	27.3	.67	5.29	12.34
E	.2391	16.1	38.8	.80	11.63	16.39

\* Probably represents sample incompletely dried.

As test number 16 appeared to have the best growth, it was passed through the elutriator. Liquids from the jar E were repeatedly discarded and settled clay returned to the beaker A. Fresh distilled water or effluents from E after settling were used for the elutriation. Comparison of treated clay with starting clay showed little, if any change in the chemical composition, as shown in Table XIII:

Table XIII - Comparison of Treated Clay with Starting Clay, Urea Run

Constituent	Starting Clay	Treated Clay
Ignition Loss	8.55	8.34
Silica	59.70	60.47
Titania	1.19	1.20
Ferric Oxide	5.21	6.17
Alumina	16.20	10.63
Not determined (mostly alkaline earths and alkali metal oxides)	<u>9.15</u>	<u>13.19</u>
	100.00	100.00

It is apparent that if any change had been accomplished, it was outweighed by errors of manipulation or analysis, or both.

#### Promotion of Rapid Growth

In the hope that the growth of diatoms could be speeded up with greater feeding of nitrogenous foods, agitation, and higher temperatures many experiments were tried, both in north light, and under a common mercury-vapor lamp, but all such attempts ended in failure. Briefly, they may be outlined as follows:

Weak intensity artificial light with fifteen liters of culture plus 1 kg. of clay, and 20 g ammonium carbonate; stirred very slowly. Visible growths did not appear, and microscopic counts could not be relied upon due to the high errors of sampling and estimation.

Mercury vapor lamp = 30 foot-candles, 100 g clay, plus 1.96  $H_2O$ , temperature controlled at 38 degrees C., fed with 10 ml. each of the following nutrient solutions:

Stock potassium nitrate, strength	21.6 g. per liter
" phosphoric acid	.950 g " "
" urea	.140 g " "
Growth promoting substances,	20 mg. per liter (Alpha-alanine, l-cystine, and commercial vitamin B <sub>1</sub> were used for this purpose)

Tests were run on the same clay in grain sizes obtained from the hammer mill, and from the ball mill. Equal quantities of diatom culture were placed in all jars, and the whole placed in the constant-temperature bath. pH of all cultures was adjusted to 8.0, and kept close to that figure by daily doses of carbon dioxide. After three weeks a heavy oil film had formed on all tests, and there were no diatoms present. Apparently those added had died and gone into solution.

With similar amounts of nutrients a cold weather experiment was run in a large open wooden tank on the roof. Atmospheric temperatures varied somewhat, but due to ice formation, the water above the clay layer remained about 4 degrees centigrade for the five weeks observed. Green algae appeared to flourish under these conditions, for they grew so rapidly that they soon covered the entire clay surface. Microscopic examination showed many green algae, along with many protozoa and metazoa. Only a few live diatoms, sp. Navicula survived.

To determine whether the iron content of the common brick clay had been the inhibiting factor causing the failures, a series of tests was run with washed Georgia Kaolin, both pure, and with various amounts of iron added. The latter was added as ferric chloride, precipitated with ammonia, and pH adjusted to 8.0. Seven samples were exposed to north light at 20-25 degrees C., five to north light at 13-18 degrees C., and four to strong mercury vapor lamp light, turned constantly on the blunger used for preparing slips. From time to time the machine was stopped and the culture tested for appearance of bubbles. There appeared to be no gas formation, or some process by which it was absorbed rapidly, for at each examination a rush of air into the jars showed the presence of gases in the jar at reduced pressure. In eight days all cultures were dead or dormant. The cause of the failures had yet to be explained.

Four kg. of Florida Kaolin were placed in the constant temperature bath with 400 liters of tap water, fed with .433 g  $KNO_3$ , .043 ml. of 85%  $H_3PO_4$ , two grams alpha-alanine, two grams l-cystine, and the temperature controlled at 25 degrees C. for five days. Carbon dioxide was bubbled in at intervals to keep the pH between 7.0 and 8.0. No growth could be observed, and few of the starting cultures survived.

The above experiment was repeated with Georgia Kaolin, using a fresh sample of diatoms from a stream passing through the school golf course. An unauthorized person switched on both heaters sometime during the second day, for the temperature was then found to be 35 degrees C. No live diatoms were found either in the main tank or in the 15 liter glass tank run simultaneously. Because of the previous failure with Georgia Kaolin in north light as well as in artificial light, it was not deemed necessary to repeat the experiment.

On the theory that a lack of "trace elements" was limiting growth, 1.0 kg. of coal ash was ground in a ball mill, washed with water, extracted with boiling HCl, filtered, and the filtrate neutralized. The resultant suspension was divided into an iron-bearing portion and one from which the iron was removed by ether extraction. The two portions were then evaporated to dryness and ground in an agate mortar. These extracts appeared to lend neither advantage nor disadvantage, indicating that the cause of poor growth must be sought elsewhere.

One factor was common to all the experiments tried up to this time: a high proportion of clay to water in the test runs. As it was suspected that the clay was itself the inhibiting factor in the concentrations used, experiments were then planned using very low concentrations of clay.

Natural cultures were taken from the above-mentioned stream in such a manner as to keep the proportion of clay, silt, and higher plants and animals to a minimum, and no separations nor concentrations were attempted. Two liter samples of stream water were collected from places in the stream where the surface flow was impeded for some cause, such as submerged logs, wide channel flowing over patches of grass, etc. In this manner cultures were

obtained which contained somewhere in the neighborhood of 200 to 500 cells per cubic millimeter. Counts on the samples were made with a Spencer Bright-line Haemocytometer, in sodium light using Leitz 3x ocular and 45x objective, with estimates as follows:

Table XIV - Diatom Counts on Natural Cultures

Date Gathered: March 12, 1946

Jar No. 1	180 cells per mm. <sup>3</sup>
" " 2	780 " " "
" " 3	575 " " " (average of two determinations)

A preliminary run indicated that above 1.0 g. pure clay per liter, growth was slowed down; for the first experiment of this series concentrations were therefore, chosen from 0.0 g. to .80 g. clay per liter. For reasons which will now be explained this series will be known as the first, second, and third Turbidity Runs.

#### Turbidity Runs

If diatoms can attack clay micelles and assimilate the silica, freeing the alumina, then the result should be a continuous lowering of the turbidity caused by suspended clay. Turbidity caused by the diatoms themselves should not be confused with turbidity due to the colloidal clay; hence a means had to be devised to separate the effects of the two factors. Actively growing diatoms rise to the surface of the culture due to buoyancy from the oxygen formed by the photosynthetic reaction. Vigorous stirring sets these bubbles free. The flocs, now become heavier than the culture medium, begin to sink, rapidly at first. After allowing three minutes for the diatom flocs to settle, the writer found it practicable to take 25 cc.



samples of the culture solution from a point 5 cm. under the surface. Samples were then taken to the laboratory where they were stirred vigorously and turbidity read on a Klett-Summerson Colorimeter. Numbers so obtained were not standardized in terms of turbidity standards used in sanitary analysis, but they nevertheless indicated the trend of the reaction.

Five jars were marked at the 1.9 liter mark, and filled to the mark with culture solution prepared in the following manner: All samples were bulked and the diatom-rich sediments separated by decantation. An equal portion of supernatant liquid was then added to each jar, followed by an equal quantity of diatom sediments.

No chemicals were added, samples were not filtered, and no water was added. The small amount of adventitious sand was discarded. Jars were then numbered as follows, and fed with their respective weights of Georgia Kaolin:

Table XV - First Turbidity Run

Number	Purpose	Georgia Kaolin, g.	Ga. Kaolin, g./L.	3 Minute Turbidity Reading		Change
				Initial T. pure	Initial T. after add. of clay	
6	Control	0.0	0.0	10	—	—
7		0.38	0.200	10	59	49
8		0.88	0.463	10	68	58
9		1.14	0.600	2	107	105
10		1.52	0.800	5	132	127

Jars were then placed in north light, open to half the sky. (The south light was excluded by a high wall.)

Table XVI - Macroscopic Evidence of Photosynthesis, First Turbidity Run

Days	0	1	2	3	4	5	6	Remarks
Test No.	20.0	14.5	7 <sup>o</sup>	9.5	15	—	—	Temperature, ° C.
6.	++	+++	+ ++	++	6+		nil	Allowed to stand in laboratory, 5th day. Plus signs indicate bubble formation.
7.	++	++	+ +	+	3+		+	
8.	++	++	+ ++	++	4+		+	
9.	++	+	- +	+	++		++	
10.	++	+	- ++	+	++		++	

The greater the amount of clay added, the whiter were the diatom flocs. Many very prominent. Test was stopped at end of the fourth day, and all tests checked under the microscope. All had live diatoms in numbers too large to count. Color varied from dark brown (no. 6) to almost white (no. 10). For curve showing reduction of three-minute turbidity with time, see Fig. 4.

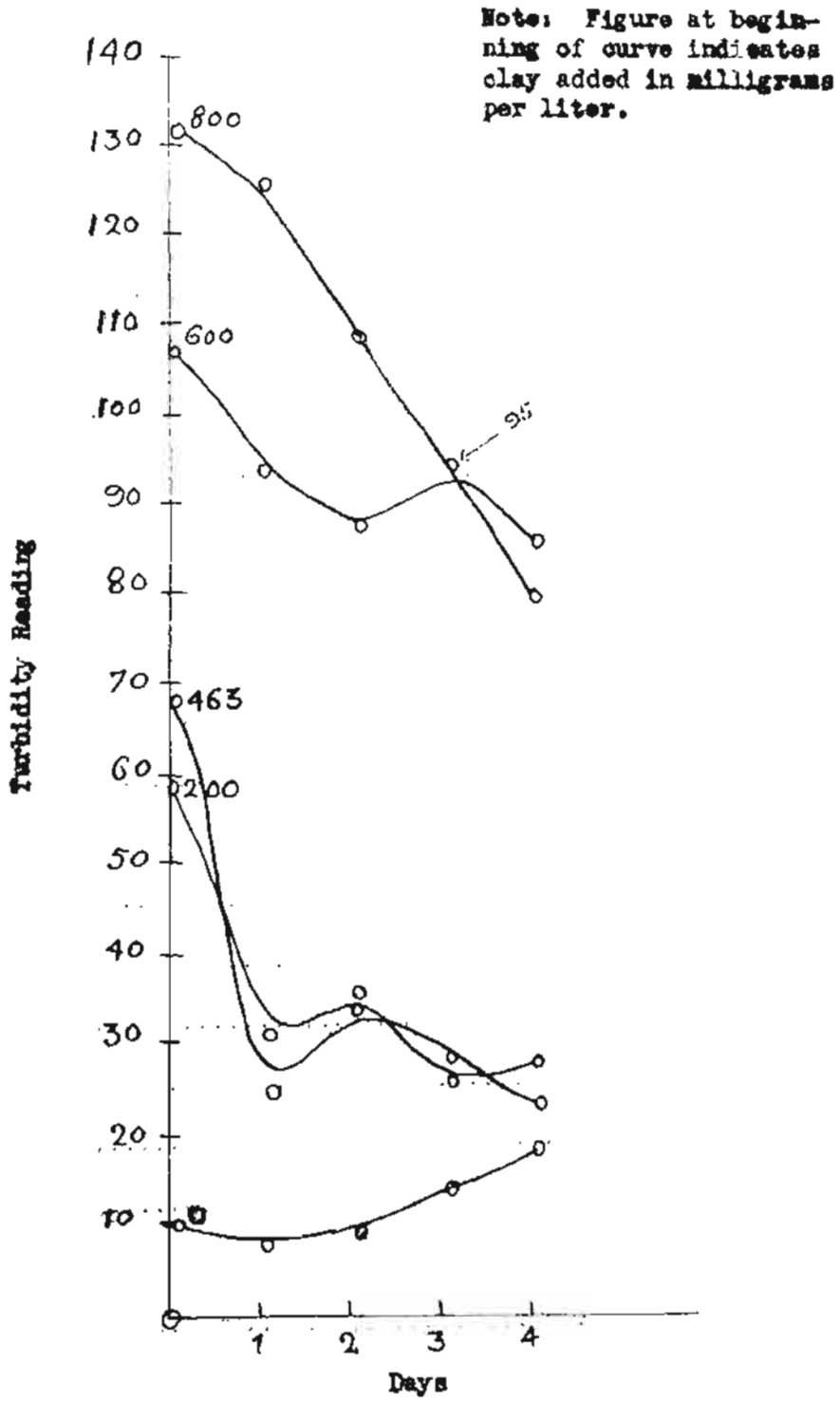


Fig. 4 Effect of Diatoms on Turbidity, First Turbidity Run.

### Second Turbidity Run

In order to secure earlier response of diatoms to clay treatment it was decided to try again the transition to work with persistent cultures, though gradually. Therefore, diatoms from the preliminary turbidity run were used as stock culture. To them was added 2.5 liters of water in which dead leaves and other residues from previous gatherings had been standing for about three months. The extract was not sterilized, as it was wanted for its growth promoting properties, which are to a certain extent destroyed by boiling. As it was supporting a vigorous growth of grasses and some clover, it was thought to be capable of reviving a dormant diatom culture, as was indeed the case.

500 cc. of the above plant extract were added to each of five jars. Diatom liquid and concentrates were added as before, in equal quantities, in such manner as to obtain approximately the same number of diatoms per culture. The cultures so obtained were found under the microscope to be much more active than they were at the close of the preliminary turbidity run. Georgia Kaolin was then added as follows:

Table XVII - Second Turbidity Run: Clay Added

Number	Clay added, mg./L.	3 Minute Turbidity		
		pure	with clay	difference
11 (control)	000	64	—	—
12	200	64	107	43
13	400	64	156	92
14	600	74	203	129
15	800	70	231	161

Table XVIII - Second Turbidity Run; Macroscopic Evidence of Photosynthesis

No.	Date	0	1	2	3	4	5	6	Remarks
		14.5	7	9.5	15	19	21	18	
11(control)		nil	nil	5+	6+	4+	5+	5+	Temperature, °C. Fifth day: flocs becoming felted.
12		nil	nil	4+	5+	3+	4+	4+	
13		nil	nil	3+	4+	3+	4+	3+	See Fig. 6 for curves showing reduction of turbidity v. time
14		nil	nil	++	3+	++	++	3+	
15		nil	nil	+	+	++	++	++	

On the seventh day, diatoms from test number 15 were checked under the microscope and found to be moderately active. On the eleventh day diatoms from the same test were again examined, and found viable, but there was no visible bubble formation. All diatom cultures and tests were combined into a 10 liter jar, filling it to about the 8 liter level. The contents were enriched with .4 ml. of stock phosphoric acid solution (4.3 ml. of 85% acid diluted to 1.0L.), and allowed to stand in an open north window.

After 24 hours the diatoms had become very active, indicating that their dormant state had been due primarily to lack of phosphates. To check whether nitrogenous foods were needed, four portions of 1.5 liters were withdrawn. The first was left alone, while to the second 4.3 mg of  $KNO_3$  were added, to the third were added 4.3 mg. of  $MnO_3$  plus .45 mg. of

Note: Figure at beginning of curve indicates clay added in milligrams per liter.

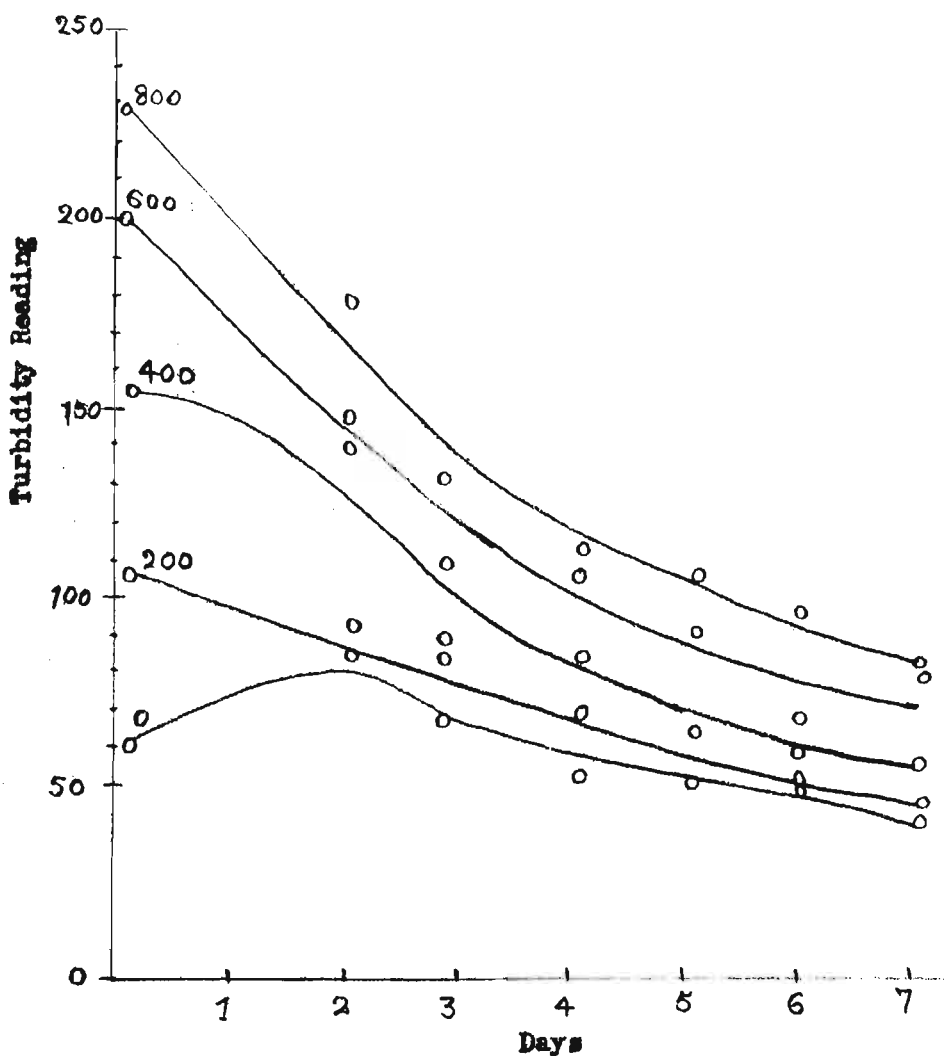


Fig. 5 Reduction of Turbidity by Enriched Diatom Cultures, Second Turbidity Run.

urea, and to the fourth was added only .45 mg. of urea. After 24 hours the first had less bubbles than the remaining culture in the 10 L. jar, the second showed more bubble formation than the first, the third was still better, and the fourth was about the same as the third. As a subsequent attempt to enrich cultures with potassium nitrate ended in failure, it was concluded that no such additions were required for that persistent culture.

The dominant diatom in the culture at this stage was probably Nitzschia acicularis. This diatom or diatoms associated with it seem to have a strong attraction for an unidentified small black fly, for large numbers of them were found drowned in the stream and in cultures on the roof of the laboratory. It is quite possible that decomposition of bodies of insects and other forms of life yields adequate supplies of nitrogenous food, for adequate growth was obtained without addition of nitrogen in any form; it was only necessary to continue adding small quantities of phosphoric acid from time to time.

#### Third Turbidity Run

To make sure that the possible range of favorable growing conditions was adequately covered, turbidity was determined on tests in which the clay concentration varied from .5 g. per liter to 64.0 g. per liter. To save time, readings were taken at the end of two minutes' settling time. A blank was run on 2 g. of clay suspended in a liter of water. Initial turbidity on three cultures, before addition of clay, was determined as before. The average, 48, was assumed to be true of all tests, as the number of diatoms was approximately equal. For results, see Table XIX;

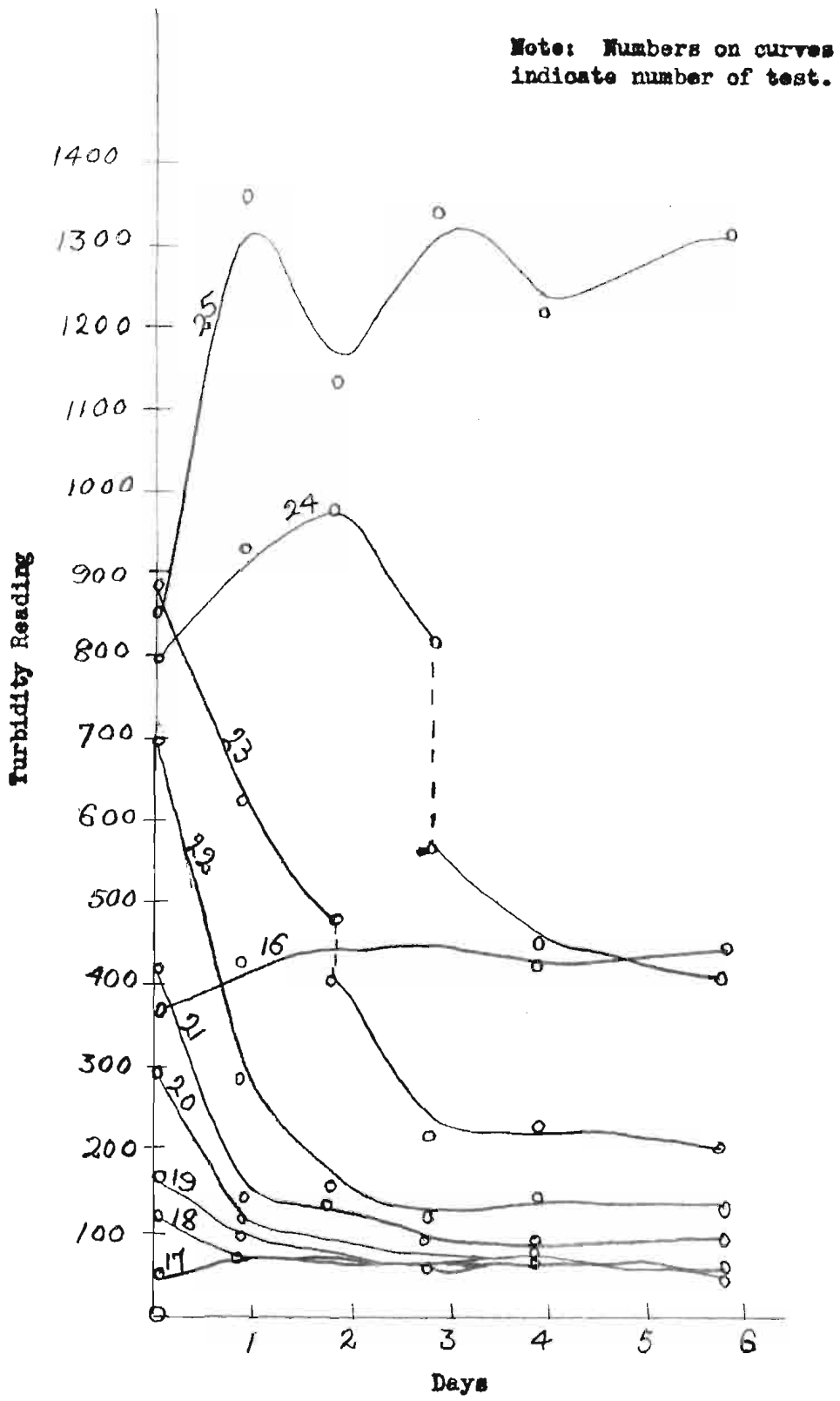


Fig. 6 Reduction of Turbidity by Enriched Diatom Cultures, Third Turbidity Run.



Table XIX - Third Turbidity Run

A. Turbidity Readings

No.	Clay g/L.	0	1	2	3	4	5	6	7da.	Remarks
16	2	370	427	440	445	430	*	445	445	*No rdg. taken;
17	nil	48	78	69	53	66		56	59	Blank; no diatoms
18	.5	123	75	77	54	66		51	75	Control
19	1.0	175	93	72	62	68		48	76	
20	2	305	112	90	66	77		57	102	
21	4	435	140	137	82	84		92	143	
22	8	2 x345	288	157	120	143		136	270	
23	16	5 x177	2x310	405	220	230		204	380	2nd da. diluted 5 ml to 10 ml-rdg = 468
24	32	5x 158	2x 465	1x680 2x490	1x570 2x405	445		415	560	
25	64	5x 165	2x 680	1x780 2x565	** 2x670	850 2x620		** 2x660	2x530	** above 900 For curves, see Fig. 7.

B. Bubble Formations: Judged by Floating Floccs

Day	0	1	2	3	4	5	6	7da.	Remarks
Temperature, °C	19	21	18	19	18		24.5		
Test No.									
16	nil	nil	nil	nil	nil	nil	nil	nil	
17	nil	8+	8+	8+	8+	8+	8+	8+	
18	nil	7+	8+	8+	8+	8+	8+	8+	
19	nil	6+	6+	8+	8+	8+	8+	8+	
20	nil	5+	5+	8+	8+	8+	8+	8+	see pH below
21	nil	4+	4+	6+	6+	6+	6+	6+	
22	nil	3+	3+	4+	4+	4+	4+	5+	
23	nil	++	++	3+	3+	4+	4+	4+	
24	nil	+	+	++	++	3+	3+	3+	
25	nil	tr.	nil	+	+	++	++	++	

pH of test no. 20 at beginning of run was found to be 8.3; on the sixth day it had fallen to 7.4

pH of stream water as received at laboratory: 8.5

pH of " " " " plus equal quantity of plant extract: 8.6



Table XIX - (continued)

Eighth day (cont)

- No. 17 Most diatoms, including Nitzschia, inactive; a few active Naviculae.  
 " 21 Many active diatoms; flocs heavier, i.e., more dense. Nitzschia  
 and Naviculae both active.  
 " 24 Nitzschia active.

## Results of Third Turbidity Run

Tests numbers 17, 21, and 24 were elutriated as in the Urea Run, with the difference that settled fractions were repeatedly returned to the beaker A, and diatom-rich sediments from jar E were discarded. As test number 17 was mostly diatoms, it was, of course, reserved for analysis; only adventitious sand was discarded. Comparison of control with starting clay and the two treated clays is given in Table XX:

Table XX - Analyses, Third Turbidity Run

Constituent	Diatom Culture Test No. 17	Diatom Culture+ 4g. clay/L Test No. 21	Diatom Culture+ 32g. /L Test No. 24	Washed Georgia Kaolin
Ignition loss	14.36	14.29	13.81	13.73
Silica	67.15	49.57	44.92	44.70
Titania	.83	1.57	1.52	1.50
Ferric Oxide	3.37	1.63	.63	1.02
Alumina	10.70	32.40	35.00	39.05*
Others	<u>3.59</u>	<u>.54</u>	<u>4.12</u>	<u>        </u>
	100.00	100.00	100.00	100.00

\* By difference; was 40.48% as determined.

## Large Area Run

In order to determine whether the reaction would be hastened by exposing a large area to the light of the open sky, at high dilutions, 176 g. of clay were added to a culture solution prepared as will be explained later, and added to the tank placed on the roof in such a position that no shadows fell on it at any time of the day. The area of the tank was 17,600 cm<sup>2</sup>, which gave a distribution of 0.01 g. of clay per cm<sup>2</sup>.

Log of Run

0 Days - Diatoms collected from golf course stream. Added all persistent cultures on hand which showed active bubble formation. Liquids, nutrients added as follows:

Tap water: 20 L.

Persistent cultures:

Supernatant liquids: 9 L.

Diatom concentrates: 200 cc.

Fresh cultures (today's gathering)

Supernatant liquid: 4.5 L.

Diatom concentrate: 150 cc.

Mixed s. l.

Mixed d. concentrates.

Volume of this made up to 1.0 L.

Diatom count:  $22.5 \times 10^9$

Enrichment:

$P_2O_5$  enrichment of .26 mg = 260 $\gamma$  / L. was previously successful.

Hence for 35 L., must use  $35 \times .25$  mg. = 8.75 mg. For this, used 17.5 of stock phosphoric acid, which has .5 mg. / ml.

Enriched culture plus clay plus d. cultures added to liquids in tank.

First Day - Some bubble formation over entire area of bottom.

Second Day - Bubbles larger than yesterday. Cloudy

Third Day - Cloudy. T. at 4:00 p.m. was 13 degrees C.

Fourth Day - Bubbles still larger - no large flocs in evidence yet. T.

20 degrees C. Small floating material examined under microscope: Heavy growth of Asterionella sp.

Added 20 L. water from tap.

10 cc. stock phosphoric acid (same strength as above)

1 g. of Fe<sup>-</sup> bearing trace element solids

**Eighth Day -** Bubbles were absent from fourth day until today. Weather cold and rainy during the interval. A few bubbles now forming over the entire area of tank.

**Eleventh Day -** Large number of bubbles, but not much floating material.

T. 18 degrees C. Weather has been mostly cold and rainy since the eighth day.

**Fifteenth Day -** Thicker portions of the clay layer show felting and large quantity of trapped bubbles.

As this experiment was run simultaneously with the last trickling filter run, from which reportable results ensued, under conditions of more precise control, it was not thought practicable to make chemical analyses. It will be seen that comparison with the Urea Run is very favorable. The results which took two months in the laboratory were reproduced on the roof, (where the intensity of illumination was very much greater, in spite of the cloudy weather) at low temperatures, in a period of only two weeks.

#### Trickling Filter Runs

Suppose diatoms are capable only of effecting a partial disintegration of the kaolinite molecule. In that event, clays treated by them should have their molecules degraded in size by the loss of the silica tetrahedrons,

and the loss should show in a higher proportion of alumina to silica in the remaining clay.

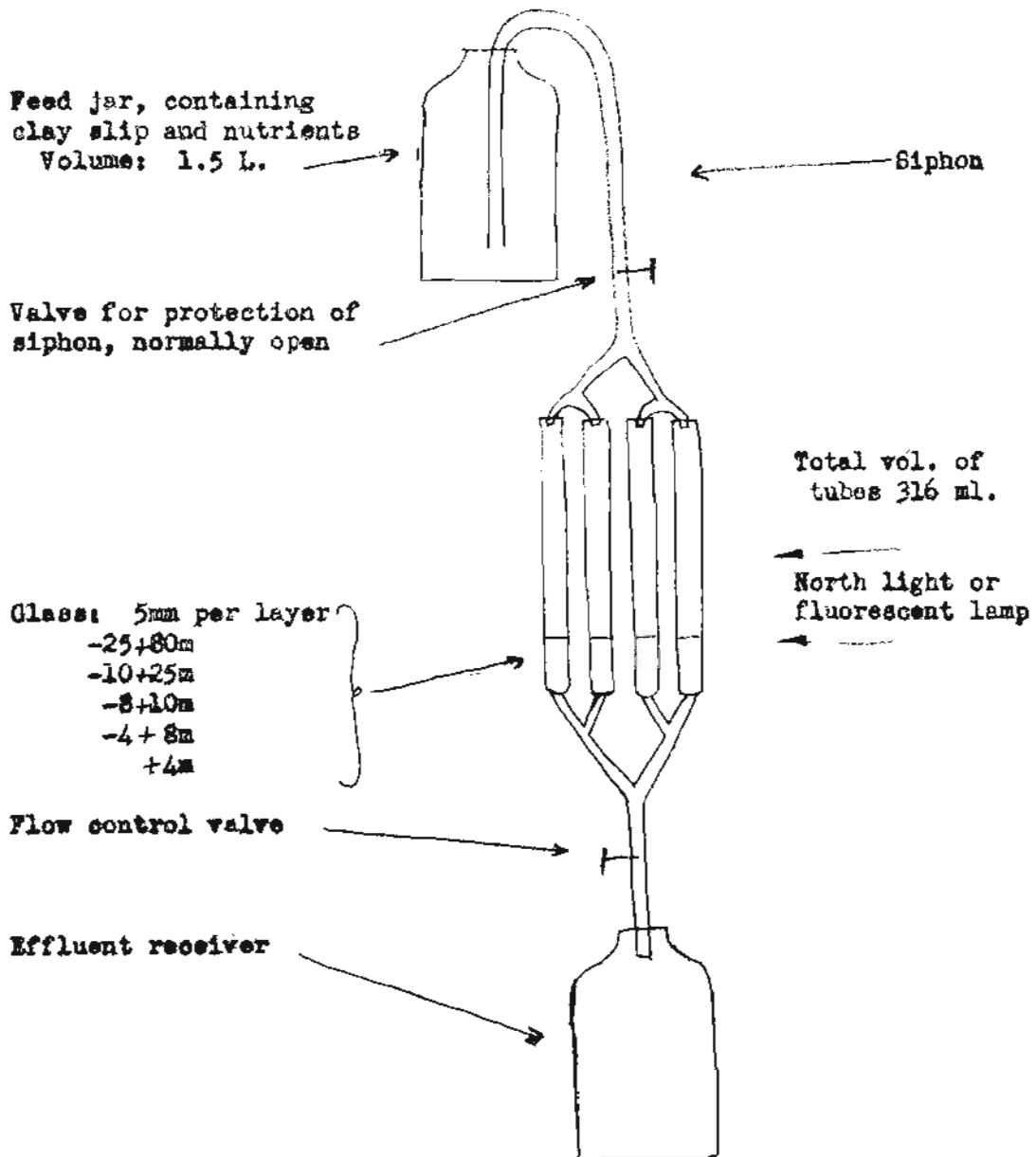
However, if diatoms can disintegrate the molecule completely, all silica would be assimilated and only hydrates of alumina would remain, in solution or colloidal suspension. Any clay which remains would therefore be unreacted clay, i. e., its chemical composition would not be substantially altered—it would be merely reduced in quantity.

With these considerations in mind, it was decided to set up a trickling filter so that a clean separation of liquid and solid reaction products could be made. The first trickling filter consisted of a glass aspirator bottle fitted with top and bottom stopcocks, and filled with glass beads. Diatom concentrates were to be supported on the beads, and clay slip plus nutrients introduced from above. As the beads were not properly sized, diatom flocs passed out of the filter into the effluent receiver, and the first run was abandoned.

Four pieces of half-inch glass tubing were assembled in parallel to give a large surface for illumination. As before, clay slip was to be introduced at the top, and clear effluent was to be drawn off at the bottom. After several attempts it was found that the top layer of the filter would permit flow, yet retain the visible diatom flocs, when composed of powdered glass -25 mesh plus 80 mesh.

The second trickling filter run was tried with the apparatus just described, but failed for the same reason as the first.

Fig. 7 - Apparatus, Tricking Filter Runs II & III



### Third Trickling Filter Run

As this run failed for reasons which will be explained presently, no formal attempt will be made to present data, but the log of the experiment will be given, in chronological order:

Zero Day - 2:30 p.m. Fresh sample taken from stream -- pH = 7.7

11:30 p.m. Enriched 1.0 liters of this culture with .5 L plant extract and .03 ml. stock phosphoric acid = 15 gamma = .015 mg P<sub>2</sub>O<sub>5</sub>  
Clay: 3 g. added = 2 g./L

Light: Open N. window. Intensity = 110 ft. candles at 10:45 a.m.  
Diatoms stay on top of filter bed. Flow limited by diatom matting, not by glass grain size.

First Day - A.M. Two hours required for slip to run through the diatom flocs. <sup>p.m. Repeated.</sup> These two runs gave an effluent which had no settled solids after 18 hours standing. Apparently a true colloid. Closed flow control valve, let stand overnight with tubes full of culture. So far no bubble formation.

Second Day - 10:00 a.m. T = 17° C. All tubes now show active bubble formation, including floating flocs. Weather cloudy.

12:00 noon. Effluent started through a third time. Light intensity 205 ft. candles. T. = 23° C. Bubble formation very active.

4:00 p.m. Clay had flocculated in feed jar; decanted supernatant liquid, added clay flocs to tubes and replaced liquid in feed jar. Flow started again. Small portion of flocculated clay examined; many diatoms present; most were rather inactive. Light intensity 215 ft. candles. Sky beginning to clear.



Third Day - 3:00 p.m. Sampled brown scum on surface of feed jar;

Many protozoa and a few metazoa; trace only of diatoms. Odor of feed and receiver jars decidedly similar to that of sour cottage cheese or lactic acid.  $T = 23^{\circ}\text{C}$ .

6:30 p.m.  $T = 23^{\circ}\text{C}$ . About 5 cm of each tube has been occupied by oxygen; flow very slow. Bubbles still forming.

Fourth Day - A.M.  $T = 18^{\circ}\text{C}$ . Bubble formation active; still much unreacted clay.

P.M.  $T = 20^{\circ}\text{C}$ . Stirred to mix all clay with diatoms. Very many bubbles in d. layer. Flow very slow.

Sixth Day - Many trapped bubbles released on vibration induced by striking apparatus with hand.

Seventh Day - Ditto.

13th Day - Sampled a diatom floc from one tube. Had an immense number of live d., mainly Nitzschia acicularis. All d. showed chromoplasts, but did not move. Inactive state? Bubble formation slow; no liquid passing into receiver.

15th Day - Removed from window and kept in dim light. Bubble formation still very slow, but proved by rising and sinking flocs. Odor of all 4 tubes now resembles fish oil. Sampled tube having strongest odor. All d. under the microscope showed bright yellow-green spots, but did not move. Experiment abandoned.

#### Fourth Trickling Filter Run

In order to give the diatoms a greater quantity of nutrient solution at any one time, and to permit better flow through the filter, a new filter was prepared in the neck of an inverted bell jar. As before, the filter bed was composed of glass, with particle sizes as shown in Fig. 8:

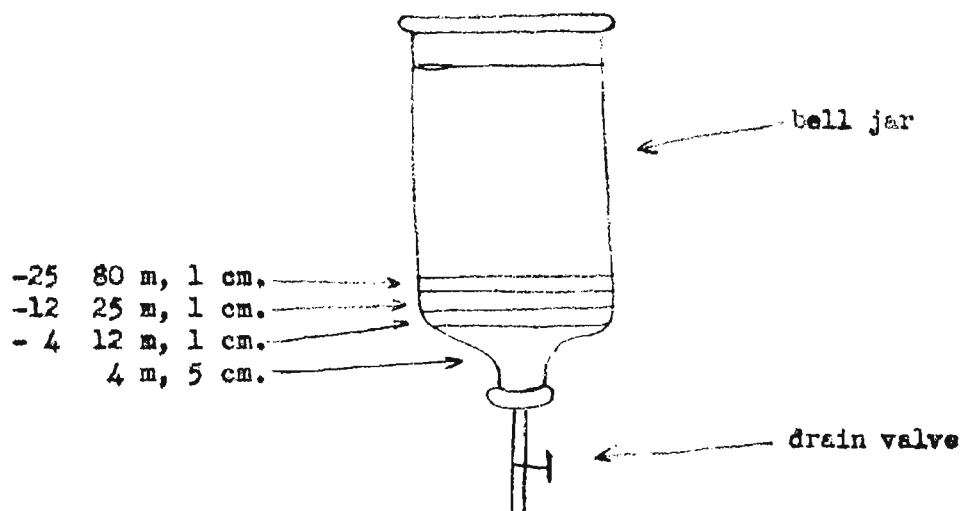


Fig. 8 - Diatom Filter Number 4

To be strictly accurate, this run should have been named otherwise, for the filter is no longer of the trickling type. As will be seen from the log, this apparatus met all the requirements of the research undertaken:

Zero Day - 2.5 liters from laboratory stock culture taken for culture liquid. Had, in bottom, a few active d. and many dormant d. Bubbled CO<sub>2</sub> into liquid for fifteen minutes. Let stand overnight.

First Day - Checked pH of liquid: 6.55. Added trace of Na<sub>2</sub>CO<sub>3</sub> -- pH to 8.9. More CO<sub>2</sub> added to pH of 7.7.

Counted diatoms from gathering four days old. Strength of diatom concentrate about  $145 \times 10^6$  diatoms per cc. Total number added ca  $4.5 \times 10^9$ .

Clay: Washed Georgia Kaolin, through 80 m., 8 g. per liter = 20 g.

Culture: 31 ml. of diatom concentrate.

Enrichment: .1 ml. of stock phosphoric acid (containing 500 gamma

P<sub>2</sub>O<sub>5</sub> sol. Placed on roof, open to bright sunlight at all times of the day.

Third Day - First sign of bubble formation.

Fourth Day - Entire surface of clay covered by bubbles. Color of surface greenish yellow. Some tiny flocs beginning to rise to surface. Temperature 9° C.

Fifth Day - Very rapid bubble formation: Many flocs on surface of liquid. Entire clay layer has large quantity of bubbles which escape on stirring slightly, or jarring the apparatus slightly. T. = 18°C.

Sixth Day - T = 18° C. pH = 8.75. Observed flocs from surface under the microscope. Very great number: not counted, as reaction does not appear to be complete. Drew off 600 cc. Added CO<sub>2</sub> to the 600 cc. portion and returned same to jar. pH now 7.8. Three hours later, bubble formation found to be very active. Sky partly cloudy; Intensity = 860 ft. candles.

Seventh Day-9 a.m. partly cloudy, T. = 18.5° C.

12:00 noon slightly cloudy, T. = 26° C. Intensity through clouds ca 2,000 foot-candles; In open sky, much greater. Entire d. layer active. Stirring liberates large number of bubbles at every point on the diatom-clay layer. Several black flies drowned on surface of supernatant liquid.

Eighth Day- 9:30 a.m. Very dark; has been raining steadily since about

5:00 a.m. Covered top of jar against rain by means of cellophane. T. = 9° C. No bubbles in evidence.

2:30 p.m. T = 13.5° C. Bubble formation active; as rain had ceased, removed the cellophane cover.

Ninth Day - 10:00 a.m. T = 13° C. moderate bubble formation. Almost clear sky.

3:30 p.m. T = 19° C. Very active bubble formation; sky clear, Intensity over 3,000 ft. candles. pH = 8.4.

Tenth Day - 3:00 p.m. Active bubble formation. Sky clear, T = 26° C. pH=8.85.

Twelvth Day - 10:00 a.m. Sky clear. pH = 8.83. T = 31° C. Bubble formation only moderate.

3:30 p.m. pH = 8.60. T = 32° C. Sky clear, bubbles only moderate. Stopped the run. Drew off supernatant liquid for analysis. Removed diatom clay with some glass fines. Freed by several decantations, discarding clean glass. Once free from glass fines, volume made up to 1.0 liter for diatom count. Strength was 6.7 million cells per cc., indicating a total number of  $6.7 \times 10^9$ . This is an increase of  $2.2 \times 10^9$  in twelve days. The clay was separated from diatoms by elutriation, as described earlier in this paper.

Table XXI - Analyses, Trickling Filter Run No. 4

A. Before Treatment

Constituent	Diatom concentrate %	Supernatant liquid mg./L.	Washed Georgia Kaolin %
Ignition loss	18.7	189	13.73
Silica	56.85	26	44.70
Titania	1.09	8.0	1.50
Ferric oxide	4.36	19.5	1.02
Alumina	1.75	10.0	39.05*
Others	<u>17.25</u>	Total	<u>100.00</u>
	100.00	solids 517.0	
Sp. Gravity	1.069		*By difference

B. After Treatment

Constituent	Treated clay (washed)	Diatom residues (washings)	Supernatant Liquid mg./L.
Ignition loss	13.51	14.30	177.0
Silica	48.25	45.21	5.7
Titania	1.48	1.05	1.25
Ferric oxide	.70	.78	.75
Alumina	36.42	36.97	13.00
Others	<u>- -</u>	<u>1.69</u>	Total
	100.36	100.00	solids 966.7

In the treated clay as compared with the washed Georgia Kaolin, very little qualitative change has been made, except perhaps the addition of a little silica and some decrease in ferric oxide.

However, if comparison is made between the supernatant liquid before and after, it will be noted that the diatoms effected a reduction of silica from 26 mg./l. to 5.7 mg./l., and an increase of alumina from 10.0 mg./l. to 13 mg./l.

These changes in composition are obviously of no importance commercially due to the slowness of the reaction and the very dilute nature of the culture medium required. But viewed from a geologic standpoint, the reaction is of the greatest importance in interpreting the processes of chemical weathering. The supernatant liquid after diatom treatment contains, it is thought, most of the alumina freed by the decomposition of the kaolinite molecule. It will be noticed that the proportion of alumina to silica in the liquid is much higher than that demanded by the formula for pure kaolinite.

Assuming for the moment that all silica remaining in the liquid is combined as clay, the 5.7 mg. will account for 4.83 mg. of alumina, leaving 8.17 mg of alumina to form bauxite, gibbsite, or diaspora. The writer is therefore of the opinion that the action of diatoms on clay and feldspar must be considered as one of the biological factors in weathering and redeposition of clays.

#### Conclusions

1. Diatoms attack the aluminosilicate linkages in clay and feldspar, assimilating the silica, and freeing hydrated alumina to the surrounding medium.
2. The reaction is slow, even under optimum conditions as known at present.
3. The lower the clay concentration, the more rapidly are diatoms able to utilize the silica.
4. Diatoms will adsorb on their frustules much more clay than they may need.

5. Increasing the clay concentrations inhibits diatom growth directly by reduction of light intensity, and

6. indirectly by adsorption on the diatoms, impeding their access to other nutrient substances.

7. Formation of oil-films on streams in the autumn and winter months is due largely to the decomposition of diatoms and other algae.

8. Formation of oil films on persistent cultures is invariably associated with stagnation and death of the culture.

9. The high quantity of suspended clay and silt in autumn floods, combined with falling temperatures, kills or renders dormant enormous numbers of diatoms, perhaps explaining the organic origin of petroleum without the need to postulate great geologic catastrophes.

10. Fresh water diatoms can be cultured in the laboratory for long periods without nitrogenous enrichment, provided minimal quantities of phosphates are added from time to time.

11. If in the future this action of diatoms on clay is to be used industrially, a satisfactory means must be worked out whereby diatoms can be grown in stock cultures of several millions of cells per cubic centimeter, and then transferred without shock to treatment tanks of the optimum dilution for the result desired. The writer is not satisfied that this can be done with present knowledge.

12. Considerable study will have to be made of fresh water diatoms before their nutrition requirements can be stated with reasonable accuracy.

13. Growth of diatoms in a suspension of clay tends to coagulate the clay into larger and larger particles.

14. The growth-controlling factors affecting diatoms are interrelated

and interdependent. The achievement of successful growth depends upon the right combination of several factors.

15. Bacteria do not appear of themselves to interfere with the action of diatoms in decomposing the kaolinite molecule.

16. Carbon dioxide may be used in small quantities to aid growth of fresh water diatoms, provided the pH is kept on the alkaline side, and not too far from 8.2.

17. Diatoms attach themselves to clay micelles and crystals by means of a sheath of protoplasm or pectinous substances. This bond may be partially broken when diatoms are transferred to distilled water.

#### Summary

Diatoms attack clays under natural conditions, at a rate which is measurable, though very slow. The silica which diatoms absolutely require for their frustules is nevertheless an inhibiting factor when present as clay, in concentrations in the neighborhood of twenty grams per liter. The inhibiting influence of clay on diatom growth is roughly proportional to the amount of clay added. Under optimum conditions in nature, diatoms are able to free enough alumina to account for the formation of high-alumina clays such as diaspore, and the minerals Gibbsite and Boehmite.



FOOTNOTES

1. Vinogradov, A. P., and Boichenko, E. A., "Decomposition of Kaolin by Diatoms" C.R. (Doklady) de l'Acad. des Sci. de l'URSS, XXXVII, 4, (1942).
2. Whipple, G. C., "The Microscopy of Drinking Water", 4th Ed., 186-187, (1927).
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