

FINAL REPORT

TO

NATIONAL AERONAUTICS AND  
SPACE ADMINISTRATION

ON

EFFECT OF WATER PROPERTIES  
IN THIXOTROPIC CLAY SYSTEMS  
ON BIOLOGICAL ACTIVITY

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Submitted by

Dr. Philip F. Low  
Department of Agronomy  
Purdue University  
West Lafayette, Indiana

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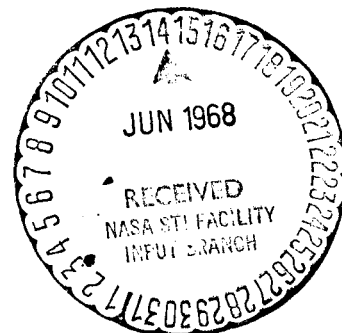
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The research conducted on the present project was designed to test the hypothesis that biological activity in a clay-water system is different when the system is in the sol state than when it is in the gel state. This hypothesis was based on observations by Droste-Hansen suggesting that subtle changes in water structure affect biological activity and on our own observations indicating that the structure of water in a clay-water system changes during the sol-gel transformation.

Our initial efforts were directed toward investigating further the properties of water in clay-water systems. It was found that the specific heat content of the water depends on the clay content of the system. Evidently, this is because the structure of the water changes as the proximity and arrangement of the particles change. Any change in water structure produces a corresponding change in the amount of heat consumed in order - disorder transitions. It was also found that the heat of compression of water in a clay system is different than that of an equal quantity of pure water. This difference is presumed to be the result of the afore-mentioned structural alteration in the water near the mineral surfaces.

The relative partial molar free energies of NaCl and water in the systems of interest were found to change with clay content. Crucial clay contents at which abrupt changes occurred were 0.5 percent and 4 percent by weight. The former clay content is the one at which slow gelation begins; it was identified by measurements of optical density. The latter clay content is the one at which rapid gelation occurs; it was identified by measurements of viscosity.

Turning our attention to testing the hypothesis on which the project was based, we placed lettuce seeds, which are known to be sensitive to the tension (relative partial molar free energy) of water, on the smooth surfaces of two clay-water systems. Both systems had been extruded into petri dishes through an 18-gauge hypodermic needle so that, initially, the particles within them were in parallel arrangement. However, subsequently, the petri dish containing one of them was rapped on the laboratory bench. Thus the particles in it were disarranged. The results were that the lettuce seeds germinated faster on the disturbed system. The undisturbed system was regarded as being in the gel state, whereas, the disturbed system was regarded as being in the sol state. Hence, the results supported the hypothesis.

The results summarized in the preceding paragraphs were presented in greater detail in earlier reports. However, those that follow have not been presented before. Therefore, they will be discussed at greater length.

In an effort to investigate further the properties of water in clay-water systems, the heats of gelation of suspensions containing 3, 4 and 6 percent Na-Wyoming bentonite were measured in a Calvet differential microcalorimeter. It was thought that, possibly, the structural reorganization of the water during gelation might contribute to the heat of gelation. However no heat of gelation was detectable regardless of whether the suspension was disturbed by rapping or stirring with a spatula. Hence, any heat exchanges involved in the gelation process must be extremely small.

It is known that light scattering and absorption by colloidal particles are dependent on the size of the particles. Therefore, in order to ascertain

the concentration of clay at which the individual particles began to form clusters, an optical study of suspensions of Na/Al- and Na-Wyoming bentonite was undertaken. First, using a Beckman DK-2 spectrophotometer, optical densities of suspensions at two clay concentrations were determined at different wavelengths. The results are shown in Figures 1 and 2. In these and subsequent figures, Na/Al-clay refers to Na-Wyoming bentonite containing a small amount of hydrous aluminum oxide impurity; Na-clay refers to Na-Wyoming bentonite without this impurity. Then the optical densities of suspensions at different clay concentrations were determined at a wavelength of 700 m $\mu$ . The results are shown in Figure 3. Since light absorption by clay particles is small in the visible range, and since light scattering increases with particle size, the results in these figures indicate that the hydrous oxide impurity increased the size of the scattering units. Further, in Figure 3, the plot for the Na/Al clay seems to curve over its entire length, whereas, the plot for the Na-clay is a straight line up to a clay concentration of about 0.25 percent and then curves thereafter. A reasonable interpretation is that clay particles clustered together at all concentrations in the Na/Al clay but did not begin to cluster until a clay concentration of 0.25 percent was reached in the Na-clay. The viscosity studies reported earlier support this interpretation.

If the structure of the water associated with particle surfaces changes with particle arrangement, there should be a corresponding change in the density of the water. Hence, the densities of droplets of suspensions having different measured clay concentrations were determined in density gradient tubes in a water bath at 25°C. These tubes were prepared by

filling glass cylinders with a mixture of xylene and bromobenzene in constantly varying proportions. They were water-saturated with KBr solution and calibrated with droplets of KCl solutions of known density. The results are shown in Figure 4.

To obtain the apparent density of the clay and, from this, an idea of how the clay affects the density of the adjacent water, the results in Figure 4 were utilized in the following equation:

$$\frac{1}{\rho_c} = v_c = \frac{v - v_w^0 x_w}{x_c} \quad (1)$$

where  $\rho_c$  is the apparent density of the clay,  $v_c$  is its apparent specific volume,  $v$  is the specific volume of the suspension,  $v_w^0$  is the specific volume of pure water and  $x_w$  and  $x_c$  are the gram fractions of water and clay in suspension, respectively. The resulting curves of apparent density versus clay concentration are shown in Figure 5. Now, Na-Wyoming bentonite has a known density of 2.8 gm. per cm<sup>3</sup>. The Na/Al clay would have essentially the same density because the hydrous aluminum oxide impurity should have a density comparable to that of the clay itself and, in addition, this impurity is present in a very small amount. Previous experiments have shown that there are approximately 0.3 milliequivalents of Al<sup>+3</sup>, which would produce 0.0078 gm. of Al(OH)<sub>3</sub>, per gm. of clay. Hence it is evident that the low values of the apparent densities reported in these figures must be due to a decreased density of the water associated with the particles. Further, it is evident that the water surrounding the particles of Na/Al-clay must be less dense than that surrounding the particles of Na-clay. This is consistent with earlier observations

showing that the former clay has a greater effect on the vicinal water. Finally, attention is called to the fact that the apparent density of the Na/Al clay increases almost linearly with clay concentration, whereas, that of the Na-clay increases faster with clay concentration below a concentration of 0.25 percent than it does above. Recall that this concentration is the one at which clustering of the particles of Na-clay began. Evidently, therefore, particle arrangement affects the structure of the associated water.

To test the effect of the sol-gel transformation on microbial activity, a culture of Streptococcus faecalis (ATCC #8043) was obtained from American Type Culture Collection. This organism was chosen because it is anaerobic to microaerophilic, non-gas forming, tolerant to heat and cold and is insensitive to most concentrations of NaCl. Several chemical media at different concentrations in clay suspensions were tested as substrates and the one that gave maximum growth and, at the same time, interfered least with the physico-chemical properties of the clay, was: 0.1% glucose, 0.1% casamino acid, 0.01% yeast extract and 0.01% arginine. A stock solution at ten-times the concentration indicated above was autoclaved and stored under antiseptic conditions. One ml. of this stock solution was mixed with 9 ml. of the clay suspension to produce 10 ml. of final suspension at the desired clay concentration.

The viscosities of suspensions prepared as described above, and prepared without added chemical media, were measured with a Fann viscometer using the RL-B1 rotor-bob combination. Clay concentrations were 0.1, 0.5, 1.0, 2.0 and 3.0 percent by weight in the different suspensions. No

No differences in viscosity could be detected between suspensions with and without added nutrient medium. Further, x-ray and infra-red analysis of the clay in the presence and absence of the nutrient medium indicated that no adsorption of the medium by the clay occurred, i.e., the respective patterns were the same. Hence, it was concluded that the nutrient medium did not interfere with the physico-chemical properties of the clay.

To prepare the bacterial cells for inoculation, the lyophilized culture received from A.T.C.C. was mixed with 2 ml of tomatoe-juice broth and incubated at 37°C. After 48 hours, the cells were transferred to tomatoe-juice agar, incubated again at 37°C. for 24 hours and kept thereafter at 0°C. The resulting culture was transferred every week to fresh tomatoe-juice agar. Two to three days before the cells were used, two loopfuls of the culture were transferred to 10 ml. of tomatoe-juice broth and incubated at 37°C. for 24 hours. Following this, the inoculated broth was stored in the refrigerator for one or two days, i.e., until the suspension was to be inoculated. Then 0.5 ml of the broth was mixed with 10 ml of the clay suspension containing the nutrient medium.

Suspensions of Na-Wyoming bentonite containing the nutrient medium were prepared in pairs at different times. The clay concentrations of the respective pairs were: 0.5% versus 1.0%, 0.5% versus 2%, 1% versus 3%, 2% versus 5%, and 3% versus 5%. Both suspensions in a given pair were inoculated simultaneously. Immediately thereafter, they were injected through 20-gauge hypodermic needles into separate cells of the differential Calvet microcalorimeter. When placed in the calorimeter, these cells were connected electrically so that the signals they produced were in opposition. Therefore, the resulting thermogram represented the difference in heat produced within the samples. The results are reported in Table 1.

Table 1. Differences in heat produced by Streptococcus faecalis in suspensions of Na- and Na/Al-bentonite at different concentrations.

Clay concentration		Maximum displacement on recorder chart*	Duration of differential heat production	Difference in heat produced #
Sample 1	Sample 2			
(percent)	(percent)	(scale divisions)	(hours)	(calories)
Na-clay:				
0.5	1.0	0	0	0
0.5	2.0	0	0	0
1.0	3.0	0	0	0
3.0	6.0	18	14	0.41
4.0	6.0	6	20	0.18
4.0	6.0	9	15	0.19
Na/Al-clay:				
3.0	6.0	24	20	0.72

\* Chart span = 100 scale divisions on 30  $\mu$ v range.

# Heat produced in sample 1 minus heat produced in sample 2.



Three observations are of special interest in this table. The first is that there was no difference in heat production between the samples in a pair until the clay concentration of the more concentrated one reached 6 percent. A Na-clay suspension gels at about 4 percent. Therefore, there was no difference in heat production unless the samples comprising the pair were in different states, i.e., one was in the sol state whereas the other was in the gel state. The second observation is that the bacteria produced more heat in the sol than in the gel. The third observation is that there was a greater difference in heat production between samples of Na/Al-clay than between corresponding samples of Na-clay. Now, as mentioned earlier, the properties of the water in a clay sol have been shown to be different than those in a clay gel. In particular, the water tension is higher in the latter. And the water properties are not affected as much by Na-clay as by Na/Al-clay. As regards the water tension, it is much higher in the Na/Al-clay. Therefore, these observations are in harmony with our hypothesis.

In another experiment, 10-ml samples of clay suspension containing about 4 percent clay and the nutrient medium were prepared in duplicate and inoculated simultaneously with the bacterium. Immediately afterwards they were injected through a 20-gauge hypodermic needle into separate cells of the differential Calvet microcalorimeter. During injection one of the cells was disturbed intermittantly by rapping it on the laboratory bench. The other was left undisturbed. As before, the cells were connected electrically within the calorimeter so that only the difference in heat produced in the two samples was recorded. A typical thermogram resulting from this procedure is illustrated in Figure 6. Another thermogram, produced by

placing 10 ml of the inoculated nutrient medium in one cell and the same quantity of distilled water in the other, is shown in Figure 7. The latter is included to illustrate characteristic thermogenesis by the organism itself. Experimental results for different pairs of suspensions are shown in Table 2. In all, about 10 pairs of samples were investigated, but integration of the area under the thermogram (to obtain the difference in heat production) was not accomplished in all cases. Therefore, not all the results are reported here. Nevertheless, in every case, the heat produced in the disturbed sample exceeded that in the undisturbed one.

Table 2. Differences in heat produced by Streptococcus faecalis in disturbed and undisturbed suspensions of Na- and Na/Al-bentonite at a concentration of 4 percent.

Clay type	Maximum displacement on recorder chart *	Duration of differential heat production	Difference in heat produced #
	(scale divisions)	(hours)	(calories)
Na-bentonite	15	15	0.34
	11	20	0.32
Na/Al-bentonite	13	4	0.08
	14	6	0.09
	14	14	0.25

\* Chart span = 100 scale divisions on 30  $\mu$ v range.

# Heat produced in disturbed sample minus heat produced in undisturbed sample.

A disturbance of a thixotropic clay gel converts it, or partially converts it, depending on the clay concentration, to a clay sol. In the present experiments, the conversion was essentially complete for the Na-clay because suspensions of this clay undergo rapid gelation at clay concentrations above 4 percent but remain sols for long periods of time at lower concentrations. In other words, the concentration of the Na-clay suspension was optimum for a disturbance to effect the gel-sol transition. On the other hand, the conversion of the Na/Al-clay gel to a sol must have been incomplete. However, in both systems, a disturbance increased the microbial activity, as indicated by thermogenesis. Among other effects, a disturbance reduces the water tension. Here again, the evidence supports the hypothesis.

In summary, this study was undertaken to study the properties of water in clay systems and to determine how a change in these properties, occasioned by a disturbance that rearranges the particles, affects biological activity. The results showed that the properties of water near the surfaces of clay particles are different than those of bulk water. Also, the results showed that particle arrangement influences these properties. And, in keeping with the hypothesis that the properties of water in clay systems affect biological activity, it was found that the germination of lettuce seeds and the thermogenesis of bacteria in these systems are affected by a disturbance that alters the properties of the water.

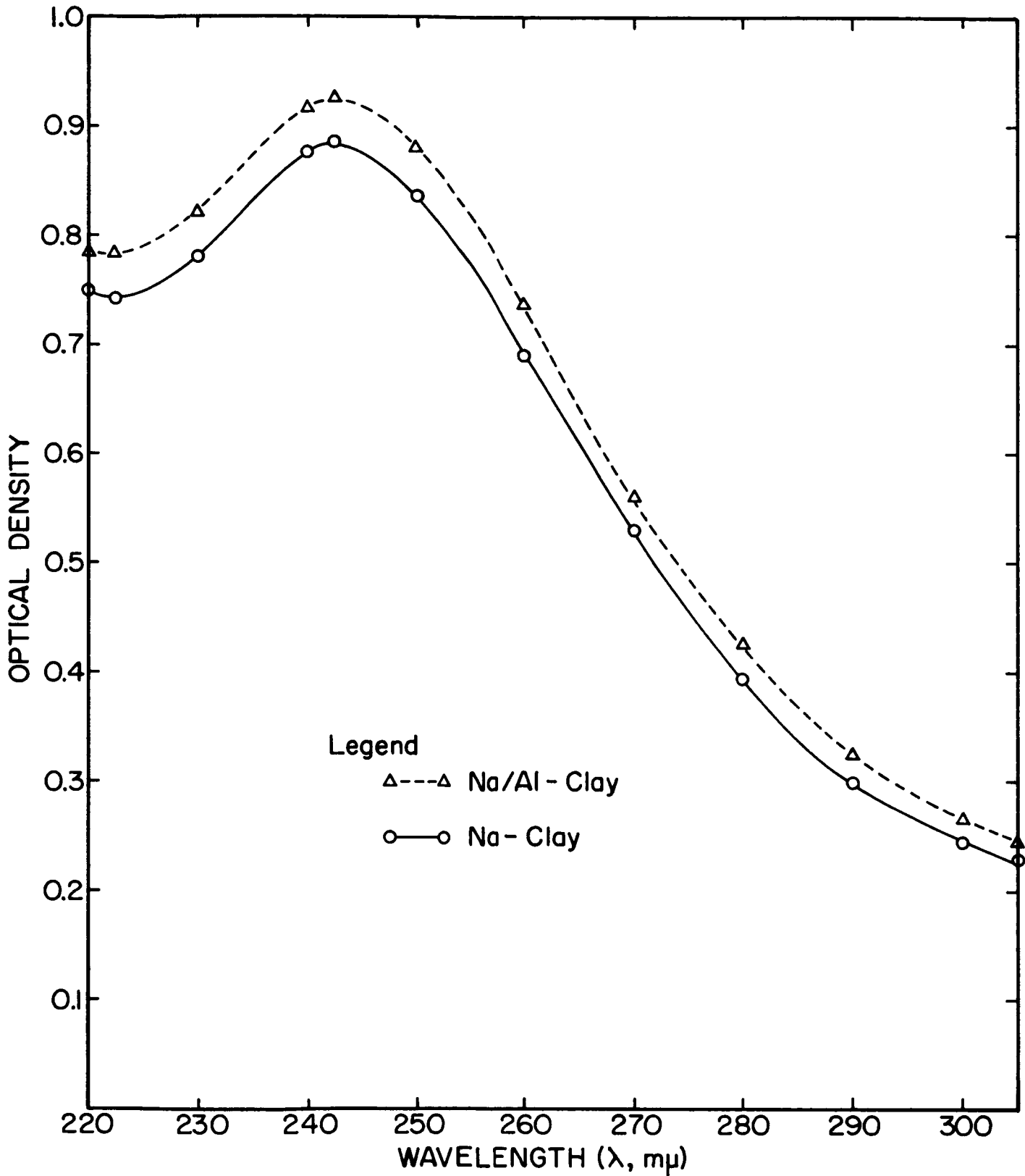


Fig. 1. The optical densities of Na/Al- and Na-clay at wavelengths in the range of 220 to 305 mμ.

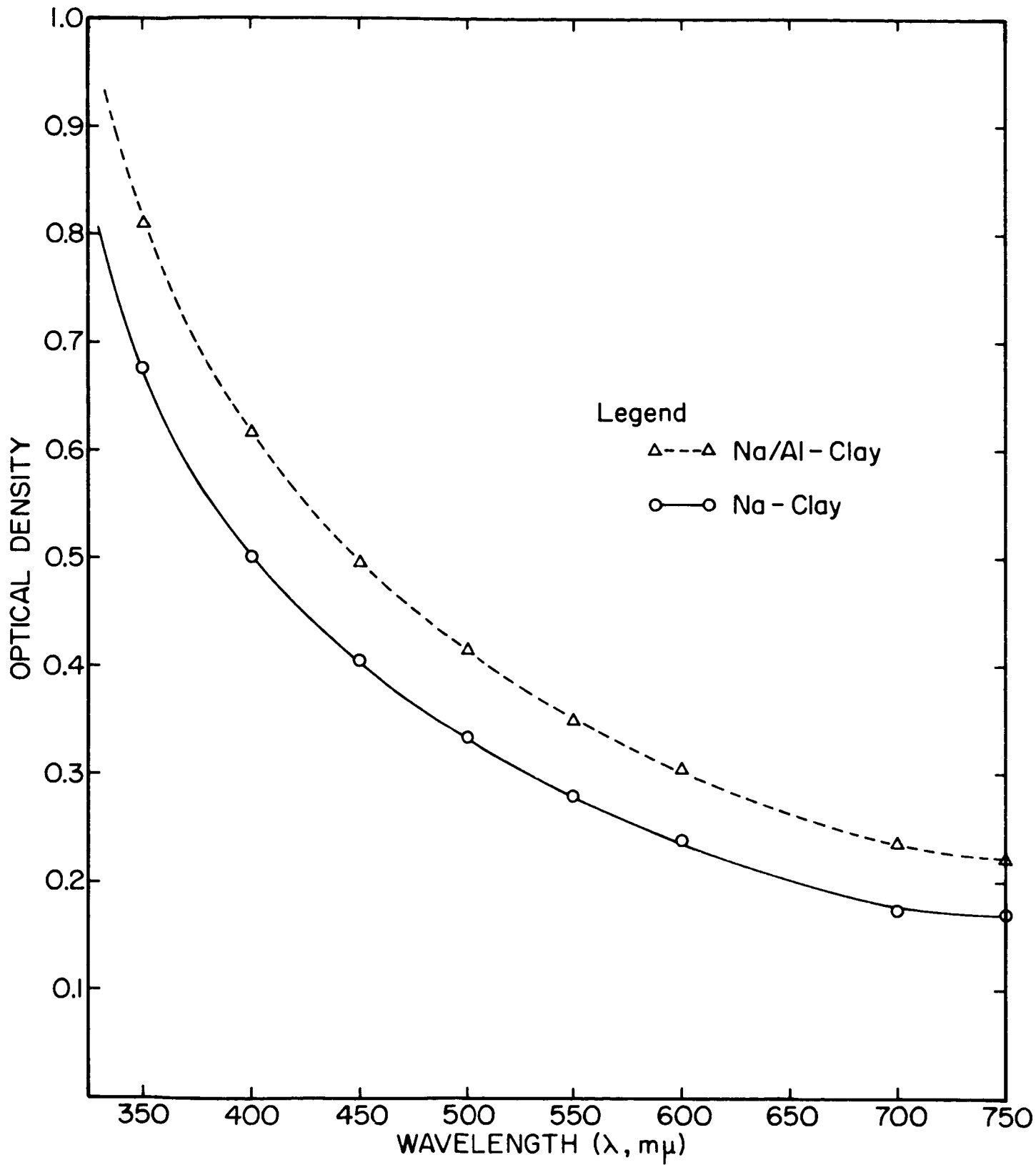


Fig. 2. The optical densities of Na/Al- and Na-clay at wavelengths in the range of 325 to 750 mμ.

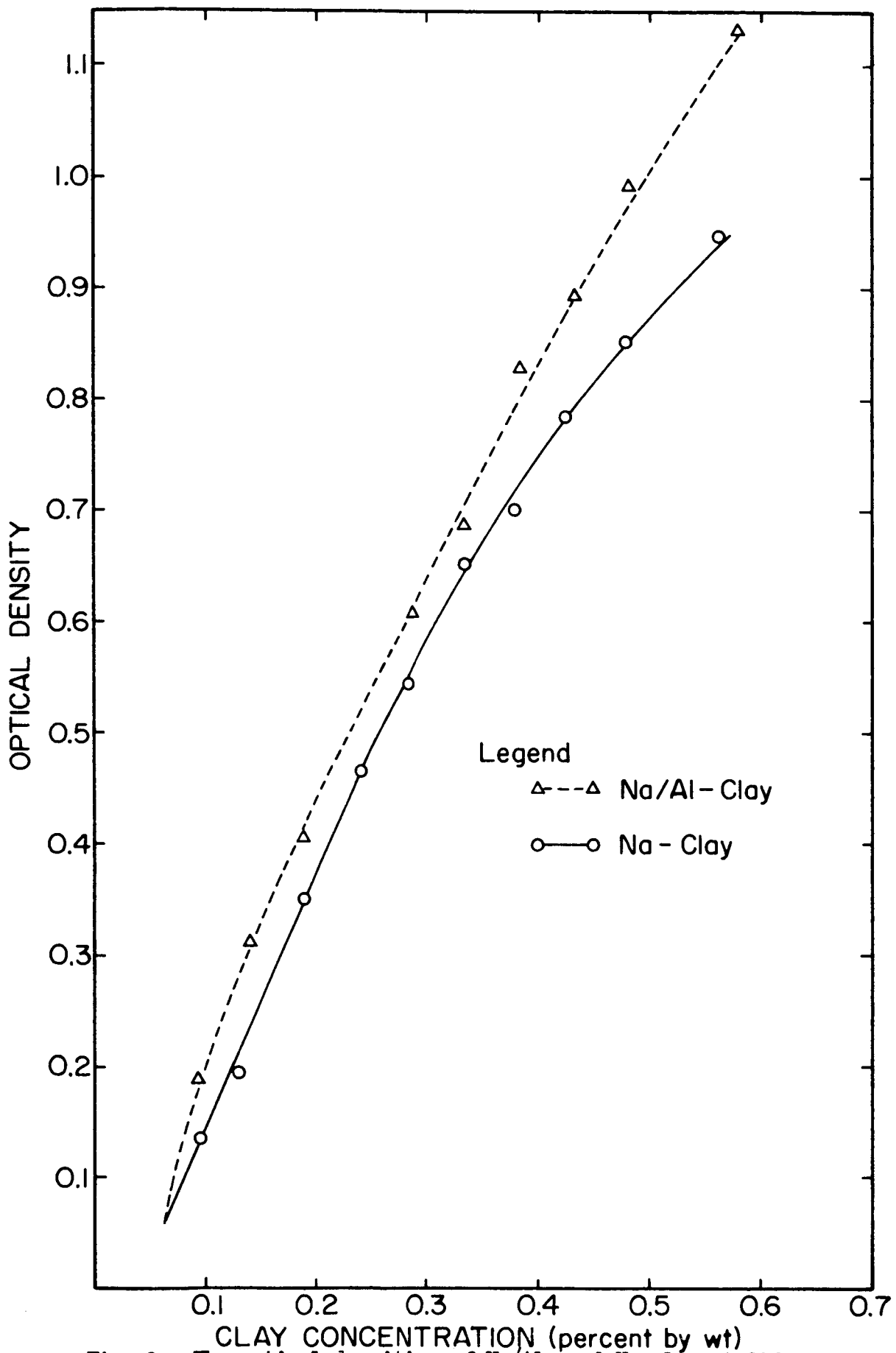


Fig. 3. The optical densities of Na/Al- and Na-clay at different clay concentrations (wavelength = 700 m $\mu$  ).

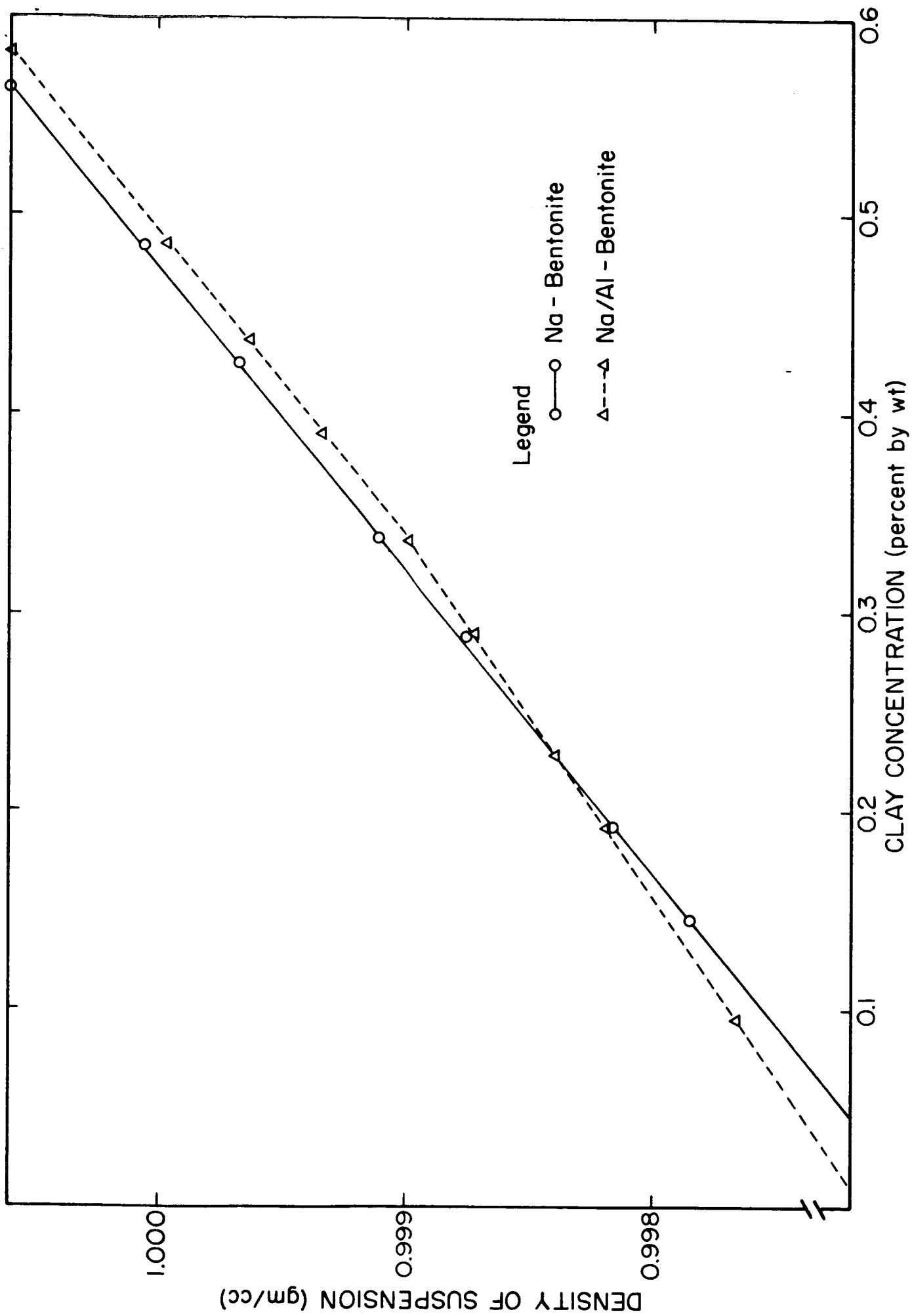


Fig. 4. The densities of suspensions of Na/Al and Na-clay at different clay concentrations.

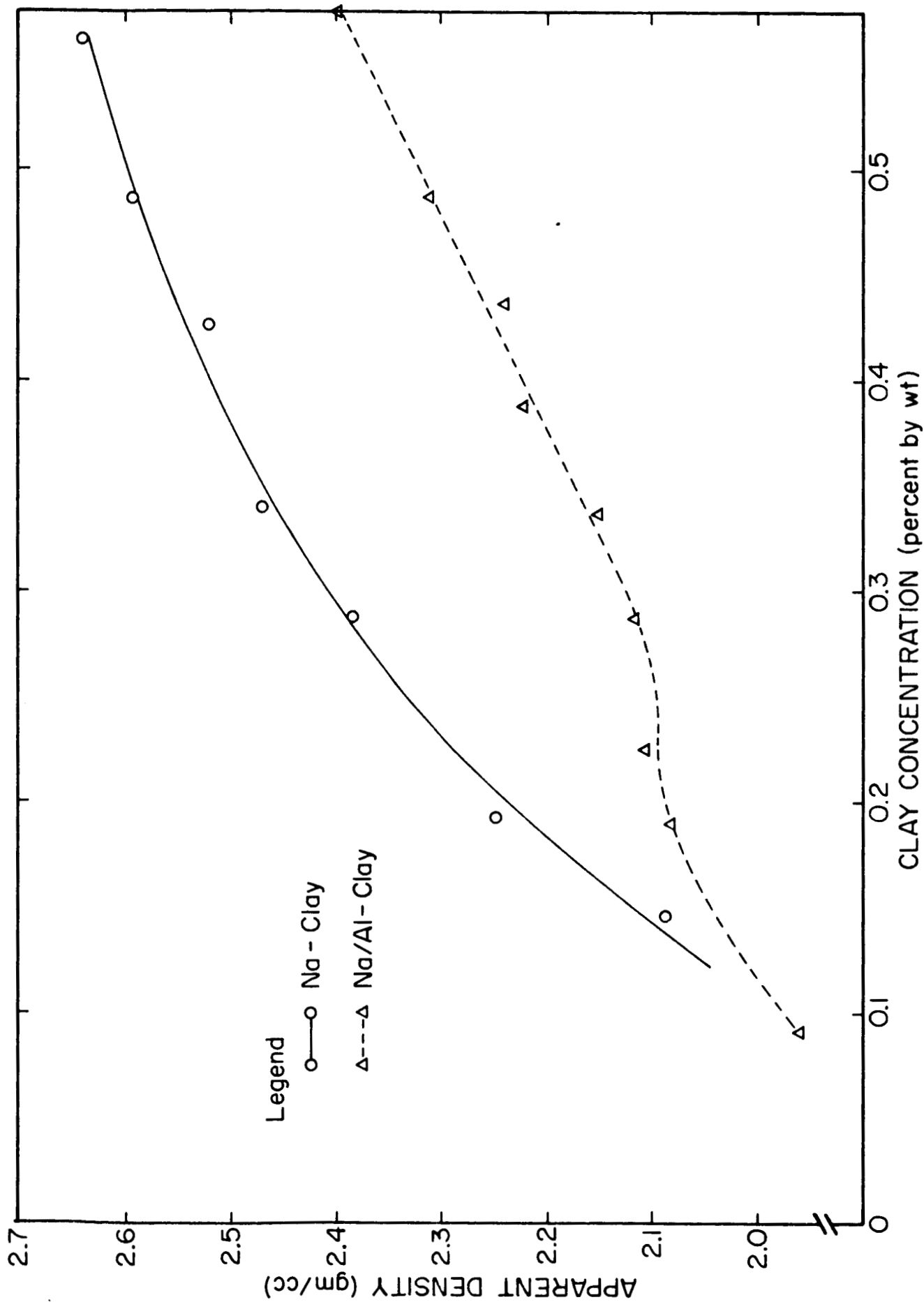


Fig. 5. The apparent densities of Na/Al- and Na-clay at different clay concentrations.



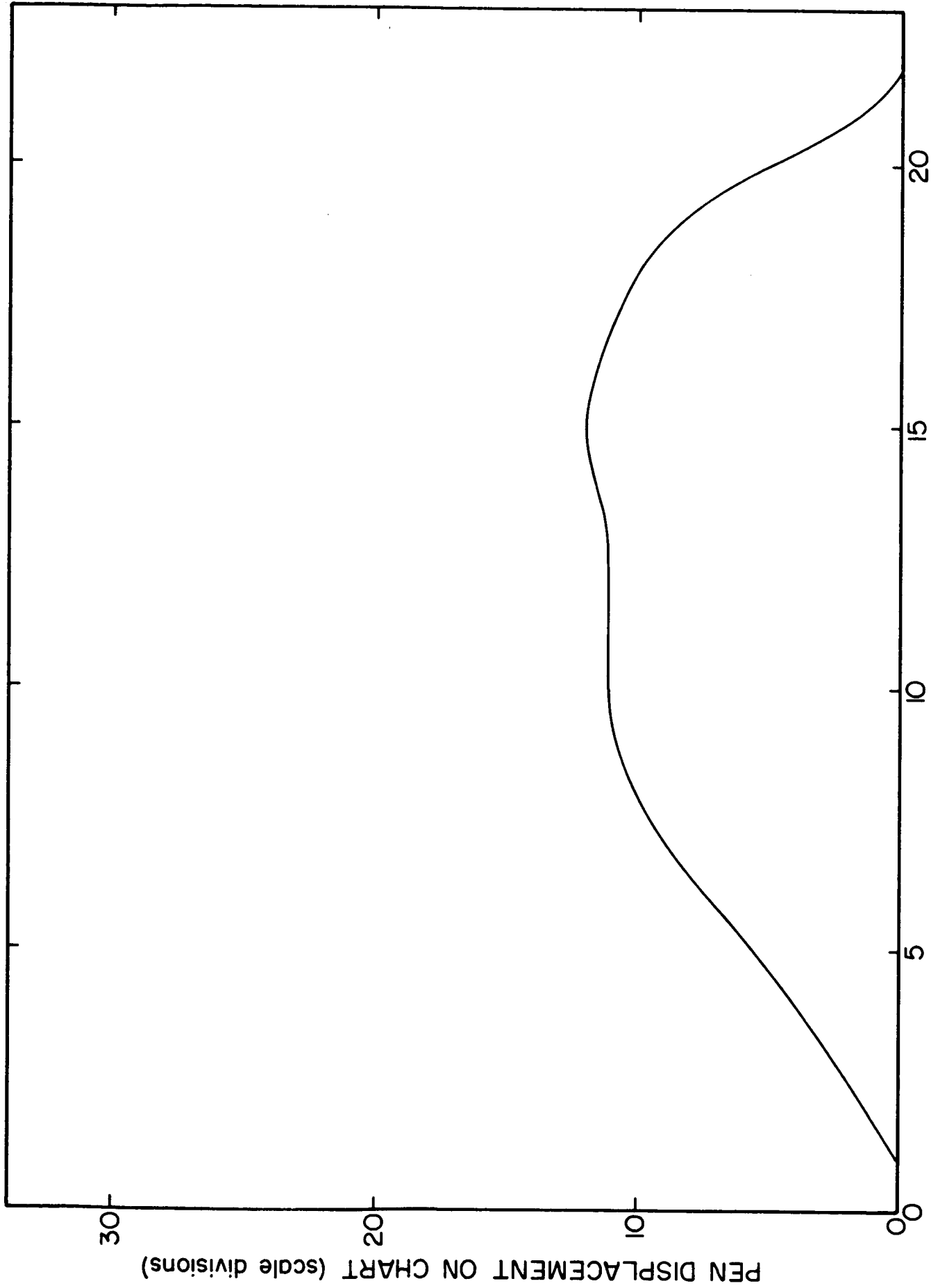


Fig. 6. Typical potentiometric record of the difference in heat produced by Streptococcus faecalis in disturbed versus undisturbed clay systems.

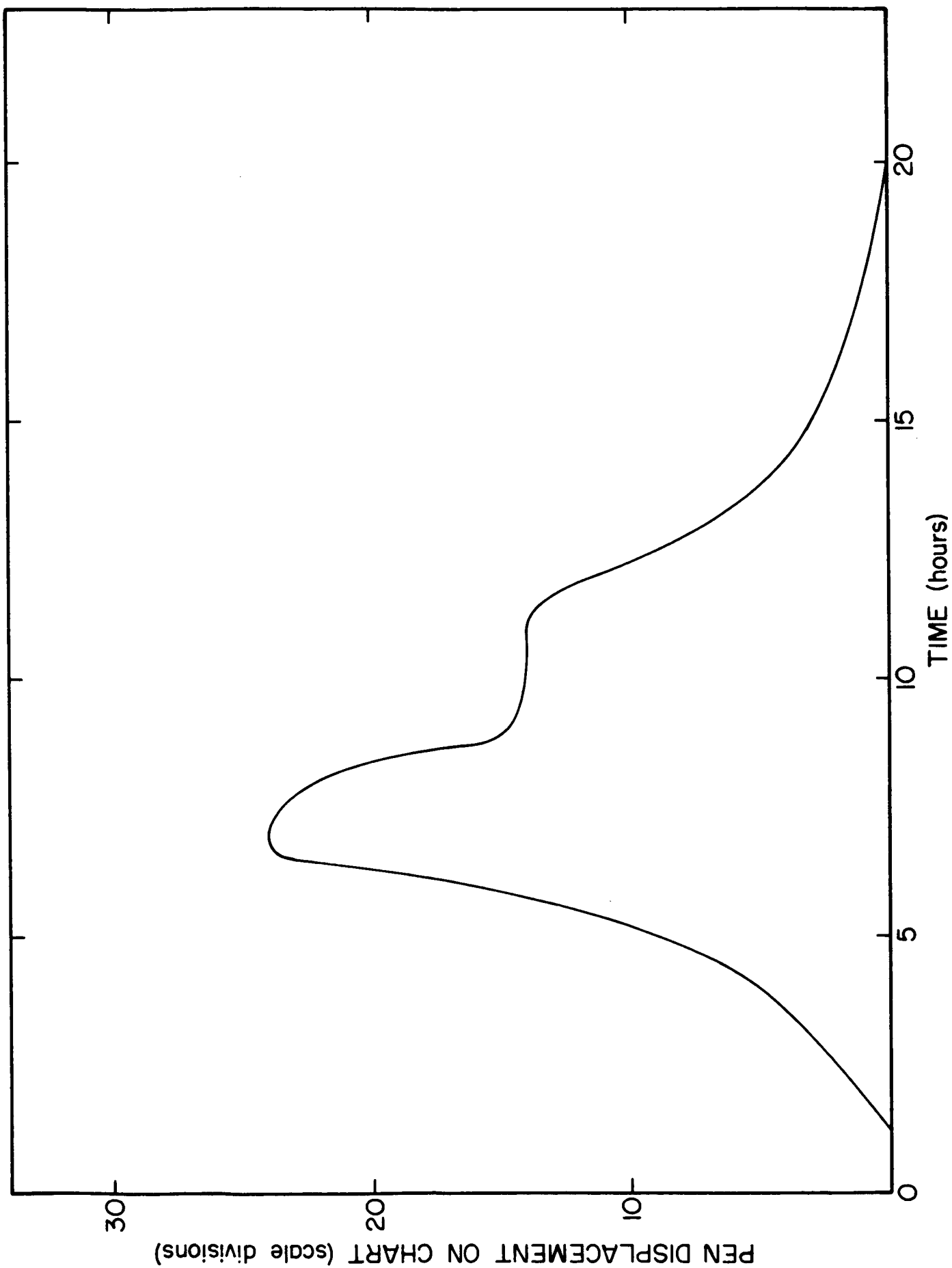


Fig. 7. Typical potentiometric record of the heat produced by Streptococcus faecalis in nutrient medium.