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HYDROLYSIS OF CONDENSED PHOSPHATES—II: STERILE ENVIRONMENT

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Abstract—The kinetics of hydrolysis of pyrophosphate and tripolyphosphate in sterile lake water and algal culture media have been investigated. The rate of hydrolysis in sterile Lake Mendota water is approximately 1000 times faster than in distilled water. First order rate constants for the hydrolysis of pyrophosphate and tripolyphosphate in sterile Lake Mendota water at 25° C and pH 8.3 are 6.1×10^{-5} min^{-1} and 1.4×10^{-4} min^{-1}, respectively. The rates of hydrolysis in sterile lake water and algal culture media appear to be accelerated by calcium.

Résumé—La cinétique de l'hydrolyse du pyrophosphate et du tripolyphosphate a été étudiée dans le cas d'eaux stériles de provenance lacustre, ainsi qu'en milieu de culture d'algues. La vitesse d'hydrolyse dans l'eau sterile de lac Mendota est environ 1000 fois plus grande que dans l'eau distillée. Les premiéres constantes d'hydrolyse du pyrophosphate et du tripolyphosphate dans l'eau stérile du lac Mendota á 25° C et pour un pH 8.3 sont respectivement 6.1×10^{-5} min^{-1} et 1.4×10^{-4} min^{-1}. Ces vitesses d'hydrolyse dans l'eau de lac sterile et milieu de cultures d'algues semblent être augmentées par la présence de calcium.

Zusammenfassung—Die Kinetik der Hydrolyse von Pyrophosphat und Tripolyphosphat wurde in sterilem Seewasser und in Algenkulturen untersucht. In sterilem Wasser des Mendotasse ist die Hydrolysengeschwindigkeit etwa 1000 mal schneller als in destilliertem Wasser. Die Geschwindigkeitskonstanten erster Ordnung der Hydrolyse von Pyrophosphate und Tripolyphosphat betragen in sterilem Mendotaseewasser bei 25° C und pH 8.3 6.1×10^{-5} min^{-1} und 1.4×10^{-4} min^{-1}. In sterilem Seewasser und in Algenkulturen scheinen die Hydrolysegeschwindigkeiten durch Calcium beschleunigt zu werden.

1. INTRODUCTION

THIS paper is the second part of a study on the rates of hydrolysis of pyrophosphate, $P_2O_7^{4-}$ and tripolyphosphate $P_3O_{10}^{5-}$, in lake water and algal cultures. This paper covers the hydrolysis of these compounds in sterile Lake Mendota water, sterile modified Allen's and sterile modified Gorham's algal media.

2. PROCEDURE

The reagents, analytical methods and procedure have been described by CLESCERI and LEE (1965).

To insure that the hydrolysis of these compounds was a chemical reaction rather than a reaction induced by viable microbes, the lake water used in the experiments was sterilized by filtering through a sterile membrane filter (Millipore Filter HA, 0.45 μ pore size) into a sterile filter flask. This filter flask was used as the reaction vessel for these experiments. Aliquots of 50 ml were withdrawn aseptically for analysis. The

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algal media used in the hydrolysis studies were autoclaved in cotton plugged Erlenmeyer flasks which were the reaction vessels. Aseptic technique was used for withdrawal of the aliquot. Chloroform was added to the reaction vessels to retard microbial activity. The effect of chloroform on the rate of hydrolysis of condensed phosphates was determined. The data from this experiment are given in TABLE 1.

As seen in TABLE 1, the initial values of the orthophosphate content of the solutions containing chloroform were equivalent to the orthophosphate values of the solutions that did not contain chloroform. The final orthophosphate values of the solutions containing chloroform were equivalent to the orthophosphate values of the solutions that did not contain chloroform. Therefore, chloroform does not interfere with the analytical method or affect the rate of hydrolysis of pyrophosphate and tripolyphosphate in sterile, membrane filtered water.

TABLE 1. EFFECT OF CHLOROFORM ON THE RATE OF HYDROLYSIS OF PYROPHOSPHATE AND TRIPOLYPHOSPHATE IN STERILE, MEMBRANE FILTERED WATER*

Pyrophosphate (mg P/l.)	Tripolyphosphate (mg P/l.)	$CHCl_3$ (5 ml/l.)	Initial ortho-phosphate (mg P/l.)	Final ortho-phosphate† (mg P/l.)
0.50	0	Yes	0.02	0.30
0	0.50	Yes	0.02	0.31
0.50		No	0.02	0.32
0	0.50	No	0.02	0.31

* Temperature: $25 \pm 2°$ C. Average values of triplicate results.
† Final orthophosphate values were recorded after 204 hr.

TABLE 2. TYPICAL ANALYSIS OF LAKE MENDOTA WATER*

Constituents	Concentration
	mg/l.
Hardness, total ($CaCO_3$)	176
Alkalinity, phenolphthalein ($CaCO_3$)	0
Alkalinity, total ($CaCO_3$)	142
Calcium (Ca)	30
Magnesium (Mg)	24
Iron, total (Fe)	0.1
Manganese, total (Mn)	0.05
Chlorides (Cl)	5
Sulfates (SO_4)	17
Orthophosphate (P)	0.04†
Ammonia-nitrogen (N)	0.4†
Nitrate-nitrogen (N)	0.08†
Organic-nitrogen (N)	0.6
Silica (SiO_2)	1‡
Chemical oxygen demand	20†
ABS (apparent)	0.02
pH	8.0

* Analysis by Wisconsin State Laboratory of Hygiene on Samples taken 15 April, 1964, with the exception of † and ‡.
† CLESCERI (1961).
‡ LEE (1962).

2.1. *Lake water*

An analysis of Lake Mendota water is given in TABLE 2. Typical data obtained for the pyrophosphate concentrations at various times during the hydrolysis of 0.05 mg P/l. pyrophosphate in lake water are given in FIG. 1, in which the logarithm of the pyrophosphate remaining is plotted versus time. The linearity of the plot indicates first-order kinetics. The rate constant for this reaction is 6.1×10^{-5} min^{-1}.

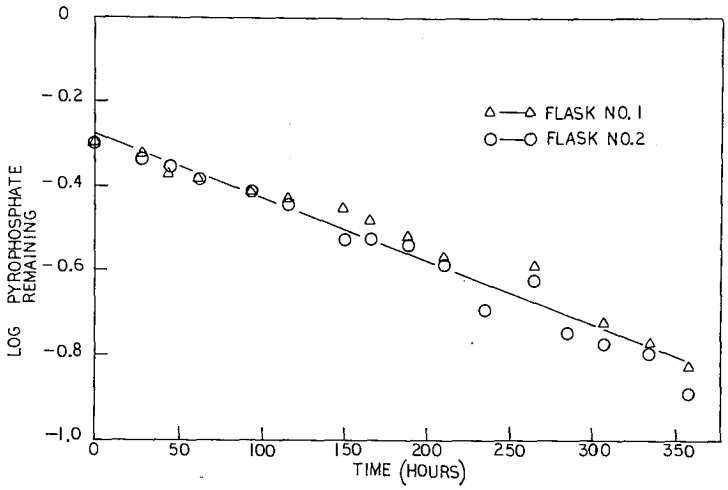

FIG. 1. Pyrophosphate hydrolysis in sterile Lake Mendota water. Temperature, 25° C; pH, 8.3; initial pyrophosphate concentration, 0.5 mg P/l.

The data in TABLE 3 are a list of first-order rate constants for the hydrolysis of pyrophosphate at various temperatures and hydrogen ion concentrations. These data are included to provide a comparison of the published rate constants with the rate

TABLE 3. RATE CONSTANTS FOR THE HYDROLYSIS OF PYROPHOSPHATE

Temperature (° C)	Hydrogen ion concentration (moles/l)	Medium	k (min^{-1})	References
25	5×10^{-9}	Sterile Lake Mendota water	6.1×10^{-5}	This paper
39.8	1.273×10^{-1}	Distilled water	9×10^{-5}	FRIESS (1952)
60	10^{-1}	Distilled water	1.1×10^{-3}	VAN WAZER *et al.* (1955)
60	10^{-4}	Distilled water	1.2×10^{-4}	VAN WAZER *et al.* (1955)
65.5	1×10^{-2}	Distilled water	5.4×10^{-4}	CROWTHER and WESTMAN (1954)
65.5	1×10^{-3}	Distilled water	3.3×10^{-4}	CROWTHER and WESTMAN (1954)
65.5	1×10^{-5}	Distilled water	2.9×10^{-4}	SMITH (1959)
65.5	1×10^{-5}	Distilled water	2.8×10^{-4}	GREEN (1950)
65.5	1×10^{-6}	Distilled water	1.3×10^{-4}	CROWTHER and WESTMAN (1954)
65.5	1×10^{-7}	Distilled water	7.0×10^{-5}	GREEN (1950)
65.5	5×10^{-10}	Distilled water	1.0×10^{-5}	CROWTHER and WESTMAN (1954)
65.5	1.25×10^{-11}	Distilled water	4.8×10^{-6}	CROWTHER and WESTMAN (1954)
87.8	1×10^{-5}	Distilled water	3.6×10^{-3}	GREEN (1950)
87.8	1×10^{-7}	Distilled water	9.9×10^{-4}	GREEN (1950)
87.8	1×10^{-9}	Distilled water	8.1×10^{-5}	GREEN (1950)

constant obtained for the hydrolysis of pyrophosphate in sterile lake water reported in this investigation.

Since no data have been published for the rate of hydrolysis of pyrophosphate at 25° C, rate constants at temperatures other than 25° C were transformed to rate constants at 25° C by using Arrhenius' equation:

$$k = Ae^{-E/RT}.$$

An activation energy of 25 kcal/mole of P–O–P linkages was used for these calculations. These data are presented in TABLE 4.

TABLE 4. RATE CONSTANTS FOR THE HYDROLYSIS OF PYROPHOSPHATE AT 25° C

Hydrogen ion concentration (moles/l.)	Medium	k (min^{-1})	References
1×10^{-7}	Distilled water	4.5×10^{-7}	GREEN (1950) (65.5° C)
1×10^{-7}	Distilled water	6.4×10^{-7}	GREEN (1950) (87.8° C)
1×10^{-9}	Distilled water	5.2×10^{-8}	GREEN (1950) (87.8° C)
5×10^{-10}	Distilled water	6.5×10^{-8}	CROWTHER and WESTMAN (1954) (65.5° C)
5×10^{-9}	Sterile Lake Mendota water	6.1×10^{-5}	This paper

Distilled water was the medium used in all the hydrolysis experiments that were selected to be compared to the hydrolysis of pyrophosphate in sterile Lake Mendota water. Also, the hydrogen ion concentration in the experiments selected for comparison was similar to the hydrogeni on concentration of the lake water experiment. The rate of hydrolysis was the most rapid in the sterile lake water; in fact, it was approximately 100–1000 times greater than in distilled water.

By using membrane filtration as a method for sterilizing lake water, the possibility exists that enzymes (phosphatases) may be some of the dissolved substances. Thus, the increased rate of hydrolysis in lake water may be due to the presence of phosphatases as well as ionic environment.

The hydrolysis rate of tripolyphosphate in sterile lake water was evaluated by determining orthophosphate at various times. Since no simple method, such as plotting the data, can be employed to determine a rate constant for the hydrolysis of tripolyphosphate, an equation was developed which can be utilized to calculate the rate constant. The derivation of this equation is based on the assumption that the hydrolysis of pyrophosphate and tripolyphosphate are first-order independent reactions (SMITH, 1959).

The reactions for which this equation has been derived are as follows:

$$Me_5P_3O_{10} + H_2O \xrightarrow{k_1} Me_3HP_2O_7 + Me_2HPO_4,$$
$$Me_3HP_2O_7 + H_2O \xrightarrow{k_2} Me_2HPO_4 + MeH_2PO_4,$$

where, Me is a symbol signifying a metal, and k_1 and k_2 are the first-order rate constants for the hydrolysis of tripolyphosphate and pyrophosphate respectively. The equation is:

$$C = -A_0\left[e^{-k_1 t} + \frac{2}{(k_2-k_1)}\left(k_2 e^{-k_1 t} + ck_1 e^{-k_2 t}\right)\right]$$

$$+A_0\left[1 + \frac{2}{(k_2-k_1)}\left(k_2+ck_1\right)\right]+C_0,$$

$$\text{where, } c = \frac{B_0(k_2-k_1)}{A_0 k_1} - 1.$$

The terms of the equation are:

k_1 = the first-order rate constant for the hydrolysis of tripolyphosphate,
k_2 = the first-order rate constant for the hydrolysis of pyrophosphate,
C = the orthophosphate concentration in moles/l. at any time,
t = the time in minutes at which a value for C is recorded,
A_0 = the initial tripolyphosphate concentration in moles/l.,
B_0 = the initial pyrophosphate concentration in moles/l., and
C_0 = the initial orthophosphate concentration in moles/l.

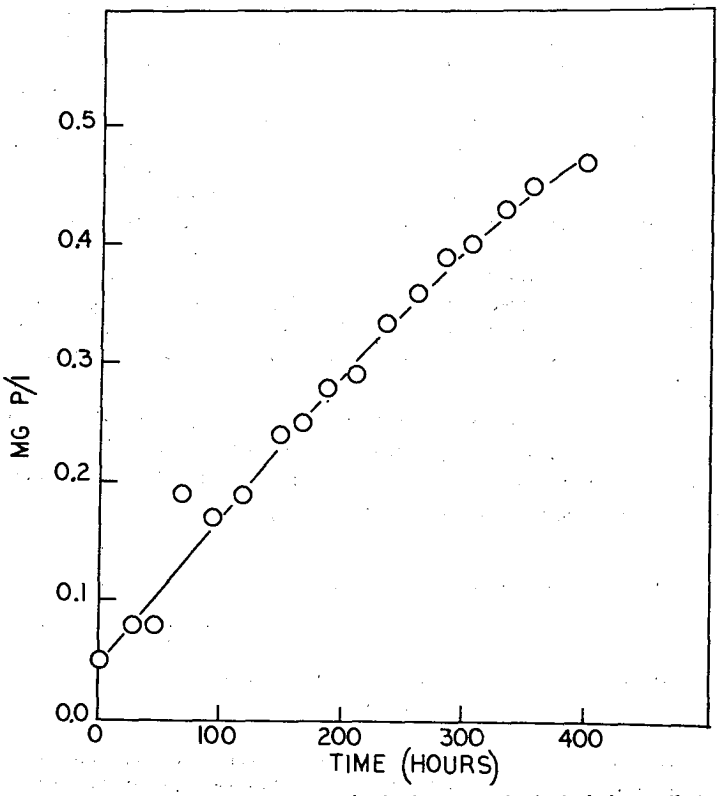

FIG. 2. Appearance of orthophosphate from tripolyphosphate hydrolysis in sterile Lake Mendota water. Temperature, 25° C; pH, 8.3; initial tripolyphosphate concentration, 0.5 mg P/l.

FIGURE 2 contains typical data for the appearance of orthophosphate from the hydrolysis of tripolyphosphate in sterile lake water. These equations were programmed to solve for k_1 on the IMB 1620 Computer using the measured values of

orthophosphate, the initial concentrations of pyrophosphate and tripolyphosphate and the rate constant for hydrolysis of pyrophosphate obtained in this study under identical conditions. A first-order rate constant of 1.4×10^{-4} min^{-1} is obtained for the hydrolysis of tripolyphosphate in sterile lake water.

TABLE 5. RATE CONSTANTS FOR THE HYDROLYSIS OF TRIPOLYPHOSPHATE AT 25° C

Temperature (° C)	Hydrogen ion concentration (moles/l.)	Medium	k (min^{-1})	References
25	5×10^{-9}	Sterile Lake Mendota water	1.4×10^{-4}	This paper
42	1.273×10^{-1}	Distilled water	7.9×10^{-4}	FREISS (1952)
44.5	1.273×10^{-1}	Distilled water	9.6×10^{-4}	FRIESS (1952)
49	1.273×10^{-1}	Distilled water	2.2×10^{-3}	FRIESS (1952)
60	10^{-4}	Distilled water	4.0×10^{-4}	VAN WAZER *et al.* (1955)
60	10^{-7}	Distilled water	7.5×10^{-5}	VAN WAZER *et al.* (1955)
60	10^{-10}	Distilled water	2.1×10^{-5}	VAN WAZER *et al.* (1955)
65.5	4×10^{-2}	Distilled water	8.6×10^{-3}	SMITH (1959)
65.5	1.0×10^{-2}	Distilled water	2.2×10^{-3}	CROWTHER and WESTMAN (1954)
65.5	1.0×10^{-3}	Distilled water	1.6×10^{-3}	SMITH (1959)
65.5	1.0×10^{-3}	Distilled water	9.4×10^{-4}	CROWTHER and WESTMAN (1954)
65.5	1.0×10^{-5}	Distilled water	6.7×10^{-4}	SMITH (1959)
65.5	1.0×10^{-5}	Distilled water	2.9×10^{-4}	SMITH (1959)
65.5	5×10^{-10}	Distilled water	1.5×10^{-5}	CROWTHER and WESTMAN (1954)
82	10^{-8}	Distilled water	6.3×10^{-4}	QUIMBY *et al.* (1954)
90	10^{-4}	Distilled water	1.3×10^{-2}	VAN WAZER *et al.* (1955)
90	10^{-7}	Distilled water	2.5×10^{-3}	VAN WAZER *et al.* (1955)
90	10^{-10}	Distilled water	3.6×10^{-4}	VAN WAZER *et al.* (1955)

TABLE 5 lists first-order rate constants for the hydrolysis of tripolyphosphate at various temperatures and hydrogen ion concentrations. These data are included to allow a comparison of the published rate constants with the rate constant for the hydrolysis of tripolyphosphate in sterile lake water.

TABLE 6. RATE CONSTANTS FOR THE HYDROLYSIS OF TRIPOLYPHOSPHATE AT 25° C

Hydrogen ion concentration (moles/l.)	Medium	k (min^{-1})	References
10^{-7}	Distilled water	9.1×10^{-7}	VAN WAZER *et al.* (1955) (60°)
10^{-7}	Distilled water	1.3×10^{-6}	VAN WAZER *et al.* (1955) (90°)
10^{-8}	Distilled water	7.1×10^{-7}	QUIMBY *et al.* (1954) (82°)
5×10^{-10}	Distilled water	9.7×10^{-8}	CROWTHER and WESTMAN (1954) (65.5°)
10^{-10}	Distilled water	2.5×10^{-7}	VAN WAZER *et al.* (1955) (60°)
10^{-10}	Distilled water	1.8×10^{-7}	VAN WAZER *et al.* (1955) (90°)
5×10^{-9}	Sterile Lake Mendota water	1.4×10^{-4}	This paper

Since no comparable data have been published for the hydrolysis rate constant at 25° C, some rate constants were selected from TABLE 5 and were transformed to rate constants at 25° C by using the Arrhenius equation. These data have been included in TABLE 6 with the rate constant for the hydrolysis in sterile lake water. As seen in TABLE 6, the rate of hydrolysis of tripolyphosphate in sterile lake water was approximately 100–1000 times greater than the rate in distilled water. This difference in rate of hydrolysis can be attributed to the dissolved substances in the sterile lake water.

The rate of hydrolysis of tripolyphosphate in sterile lake water (1.4×10^{-4} min^{-1}) is approximately 2.5 times greater than the rate of hydrolysis of pyrophosphate in sterile lake water (6.1×10^{-5} min^{-1}).

2.2. *Modified Allen's and modified Gorham's algal medium*

The extent of hydrolysis of pyrophosphate and tripolyphosphate was determined in sterile modified Allen's and modified Gorham's algal media. The composition of these media are presented in TABLE 7.

TABLE 7. CONCENTRATION OF COMPOUNDS USED IN MEDIA

Compound	Modified Gorham's (mg/l.)	Modified Allen's (mg/l.)
NH_4Cl	—	50
$NaNO_3$	496	1000
Phosphate	As desired	As desired
Ferric citrate	6	—
$FeCl_3$	—	3
$MgSO_4 \cdot 7H_2O$	75	513
$CaCl_2 \cdot 2H_2O$	36	66
$Na_2SiO_3 \cdot 9H_2O$	58	—
Citric acid	6	—
Na_2–EDTA	1.2	—
Na_2CO_3	500	500

The experimental techniques and calculation procedures were the same as described above for the hydrolysis of pyrophosphate and tripolyphosphate in sterile lake water. TABLE 8 summarizes the results of these investigations.

TABLE 8. FIRST-ORDER RATE CONSTANTS FOR PYROPHOSPHATE AND TRIPOLYPHOSPHATE HYDROLYSIS IN STERILE ENVIRONMENT

Temperature (° C)	Medium	pH	Pyrophosphate (k, min^{-1})	Tripolyphosphate (k, min^{-1})
25	Distilled water	9.3	6.5×10^{-8}	9.7×10^{-8} (calc.)
25	Sterile Lake Mendota water	8.3	6.1×10^{-5}	1.4×10^{-4}
23	Sterile modified Allen's medium	8.3	7.4×10^{-6}	5.5×10^{-5}
23	Sterile modified Gorham's medium	8.3	6.1×10^{-6}	1.9×10^{-5}

Examination of TABLE 8 shows that both pyrophosphate and tripolyphosphate hydrolyzed about 1000 times more rapidly in sterile lake water as compared to distilled water. Both compounds hydrolyzed about 5–10 times slower in either algal media than sterile lake water. Depending on the medium studied, tripolyphosphate hydrolyzed approximately 5–10 times faster than pyrophosphate.

3. DISCUSSION

This study has shown that (in sterile lake water and algal culture media), the rate of hydrolysis of pyrophosphate and tripolyphosphate is several orders of magnitude faster than has been found in distilled water at similar pH and temperature. The increased rates of hydrolysis can possibly be attributed to dissolved substances in solution. GREEN (1950) and HUFFMAN and FLEMING (1960) found that calcium ion increased the rate of hydrolysis of condensed phosphates. In the present paper, summarized in TABLE 9, a correlation is noted between calcium ion concentrations and rate constants for the hydrolysis of pyrophosphate and tripolyphosphate in sterile lake water, sterile modified Allen's medium and sterile modified Gorham's medium.

TABLE 9. RATE CONSTANTS FOR THE HYDROLYSIS OF PYROPHOSPHATE AND TRIPOLYPHOSPHATE AT 25° C

Sterile medium	Ca (mg/l.)	Pyrophosphate	Tripolyphosphate rate constant
Lake water	30	$6.1 \times 10^{-5} min^{-1}$	$1.4 \times 10^{-4} min^{-1}$
Modified Allen's	18	$7.4 \times 10^{-6} min^{-1}$	$5.5 \times 10^{-5} min^{-1}$
Modified Gorham's	10	$6.1 \times 10^{-6} min^{-1}$	$1.9 \times 10^{-5} min^{-1}$

The data in TABLE 9 show that regardless of the medium employed, pyrophosphate is more stable than tripolyphosphate under the test conditions. However, the hydrolysis of pyrophosphate and tripolyphosphate is the most rapid in sterile lake water, which has the highest concentration of calcium ion. The intermediate rate of hydrolysis of of pyrophosphate and tripolyphosphate occurs in sterile modified Allen's medium which has the next highest calcium ion concentration.

In modified Gorham's medium, the rate of hydrolysis of pyrophosphate and tripolyphosphate is the slowest, and the calcium ion is the least of three media. The relationship of higher calcium ion concentration and increased rate of hydrolysis agrees with the result of GREEN (1950), and HUFFMAN and FLEMING (1960). GREEN also reported that the presence of magnesium ion in the test media retarded the hydrolysis of condensed phosphates. This is a rather interesting result in view of the effect of calcium ion on hydrolysis. VAN WAZER (1958) stated that this effect needs more study. When the rates of hydrolysis of pyrophosphates and tripolyphosphate found in this study are compared to the rates of hydrolysis of these compounds in the presence of microorganisms under similar conditions, CLESCERI and LEE (1965), it is found that enzymatic processes control the aqueous environmental chemistry of these compounds.

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