THE EFFECT OF VARIOUS MICRO-ORGANISMS ON THE PRECIPITATION OF LEAD TETRAETHYL FROM AVIATION FUELS AND THE FORMATION OF GUM IN MOTOR GASOLINES

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Dean of the Graduate School

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DISSERTATION

Presented to the Faculty of the Graduate School of

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By

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PREFACE

This report on original research constitutes the writer's doctor's dissertation for presentation to the Graduate School of the University of Texas. The work was conducted under the sponsorship of the Ethyl Fellowship during the year 1942-1943.

The research has been one which clearly does not belong in any one academic field. Instead it represents a co-operative venture by the departments of Petroleum Engineering, Bacteriology, Chemistry, Chemical Engineering and Aeronautical Engineering. It is the type of research problem which is particularly adapted to an academic institution where specialists in the many related fields are available for easy and immediate consultation as the work proceeds.

The writer hopes that this work will lead to further more intensive studies in the new field of petroleum microbiology. The present work is a sequel to an investigation of the effect of water and blending on gasoline storage stability which was carried out under the same fellowship during the year 1941-1942.

Austin, Texas

September 14, 1943

F. H. A.

TABLE OF CONTENTS

Page

List of Tables

Page

List of Figures

Page

List of Figures Continued

INTRODUCTION

Motor and aviation gasolines are subject to deterioration during storage. The degradation of motor gasolines which contain unsaturated hydrocarbons is indicated by the formation of non-volatile, resinous materials known as gum. Gasolines which contain excessive amounts of dispersed gum produce clogged carburetors and stuck inlet valves. Aviation fuels are adversely affected principally by the precipitation of TEL (lead tetraethyl). This precipitation throws down a white haze which is non-volatile and may clog carburetors during flight. The precipitation of TEL also produces a consequent loss in octane number of the fuel.

Most motor and aviation fuels are stored over water. Bacteriologists have known for several years that bacteria which utilize hydrocarbons as their source of carbon may be isolated from gasoline storage tank waters. These bacteria oxidize the particular hydrocarbons to the peroxide and other intermediate oxidation products. Hydrocarbon peroxides are known to represent the initial step in the formation of gasoline gum. Prior to the present study no attempt has been made to determine the effect of these micro-organisms on the gasoline substrates.

It was the purpose of this work to determine the effect of bacteria and other micro-organisms on the stability of these motor fuels during storage. This bacterial effect was investigated by storing gasolines over water which had been inoculated with pure cultures of known bacteria. The effect of each of these organisms was then indicated by the varying degrees of deterioration which resulted.

The previous investigations of gasoline deterioration during storage have been conducted and interpreted from a strictly chemical viewpoint. There remain, however, a number of phenomena in connection with the deterioration of motor gasolines which have not been adequately explained chemically but which are in a large part subject to simple biological interpretations. The mechanism by which yellow color is formed in many types of gasolines, kerosenes, and naphthas during storage has never been fully understood. Results of recent studies in these and other laboratories[19] have indicated that gasoline color may consist of bacterial pigments, many of which are strongly colored, high molecular weight hydrocarbons produced by the metabolic processes of bacteria. It also seems probable that dispersed water in gasoline may permit a brief bacterial action which would result in the haze which is often produced in gasolines during storage. Similarly, the exact role by which the various sulfur compounds that are frequently present in gasolines affect the rate of deterioration has never been explained. A biological interpretation of this problem suggests that a number of sulfur bacteria are capable of utilizing these compounds to produce traces of sulfur oxides and sulfuric acid which have detrimental effects upon gasoline stability.

Both the formation of gum and the precipitation of TEL are controlled by the addition of minute concentrations (0.002% by weight) of certain "inhibitors" to the finished gasolines. These inhibitors are capable of stabilizing gasoline motor fuels during storage periods of as long as eighteen months. It is interesting to note that the inhibitors which are most used industrially are all good bactericides. Many of the commercial gasoline inhibitors contain a toxic

phenolic group. Surface tension measurements on inhibited gasolines show that the inhibitor molecules are strongly adsorbed on the gasoline-water interface where they would be oriented with their toxic hydroxyl groups below the interface in the proximity of the hydrocarbon oxidizing bacteria. Many dyes are effective as gasoline inhibitors, which may, in part, be due to their bacteriostatic activity.

Various species of molds have been recovered in pure culture from gasoline storage tank waters. A number of molds and bacteria produce substances which are extremely bacteriostatic as illustrated by the recent medical applications of gramicidin and penicillin. It is quite possible that certain of the micro-organisms that are present in gasoline storage tank waters produce metabolic products which might in themselves inhibit the deterioration of the gasoline through their antibiotic effect, or by some chemical mechanism. If such were found to be the case, the artificial inoculation of tank waters with a culture of some suitable species of micro-organism becomes an interesting industrial possibility. Unfortunately, however, the great majority of micro-organisms which live in gasoline storage tanks are found to exert a detrimental effect on the motor fuel.

It is evident from the foregoing discussion that bacteria and other micro-organisms may serve as causal agents in the formation of gum and peroxides in motor and aviation gasolines and the precipitation of TEL from heavily leaded aviation fuels during storage. Both of these effects were investigated.

SURVEY OF THE LITERATURE

Since the present work represents the initial investigation in the field, there is no literature on the immediate subject. The background for the research was obtained from the distinct literatures of bacteriology and petroleum chemistry. This survey is consequently subdivided into separate sections.

Bacteriological Literature

Bacteria which oxidize methane to carbon dioxide and water were first isolated and identified by Kaserer[23] and Sohngen[30] in 1906. Soil organisms which are capable of utilizing higher hydrocarbons were reported by Sturzer[34] (1908), Sohngen[31] (1913), Wagner[42] (1914), Tausz and Peter[36] (1919), Butner[9] (1926), Gray and Thornton[17] (1928), Stone, Fenske and White[32] (1942) and others. Hydrocarbon-oxidizing bacteria were isolated from crude oil by Lipman and Greenberg[25] (1932), Tauson and Shapiro[35] (1934), Wakengut[43] (1938), Bushnell and Haas[8] (1941), and Stone, Fenske and White[32] (1942). Bacteria have been found in oilwell brines by Gahl and Anderson[15] (1928) and Ginsburg-Karagitscheva[16] in 1933. Bacteria capable of oxidizing hydrocarbons were isolated from storage tank waters by Thaysen[38] (1939), Bushnell and Haas[8] (1941), and Stone, Fenske and White[32] (1942). The bacteriological literature related to the bacterial utilization of hydrocarbons was extensively reviewed by Haas[19]. The most significant paper since Haas' review is that of ZoBell, Grant and Haas[46] (1943).

An extensive search of the literature reveals that whereas a number of bacteriologists have shown that certain bacteria are capable of utilizing various hydrocarbons, very little work has been done to determine the chemical composition of the metabolic products which result from this utilization. Most of the investigations were carried out under strictly aerobic conditions with very small volumes of the particular hydrocarbon. Both of these factors would favor the complete oxidation of the hydrocarbon to carbon dioxide and water which are the products usually reported. When bacterial activity takes place below a large volume of hydrocarbon, thermodynamic considerations would tend to favor the formation of a series of intermediate oxidation products. Traces of such oxidation products have been reported by Thaysen[38] and Sohngen[31].

Literature on Gum Formation

The literature on the formation of gum in cracked gasolines dates from Hall's introduction of vapor phase cracking in 1913. He correctly attributed the trouble to unstable olefins and diolefins in the cracked product[20]. Investigations by Brooks[7] (1926) and by Story, Provine and Bennett[33] (1929) introduced the study of the chemistry of gum and gum formation. Voorhees and Risinger[40] (1929) provided the literature with data on a series of actual engine tests to determine the practical significance of gum in gasoline motor fuels. Munn, Fischer and Blackwood[21] (1930) confirmed the concepts of preformed and potential gum in gasoline and devised an accelerated oxidation test which involved heating the gasoline sample in an atmosphere of oxygen under pressure at 212° F. The period of time which elapsed before a drop in pressure was noted was taken as an indication of the length of time which the

gasoline could remain in storage without deterioration taking place. Yule and Wilson[45] (1931) showed the importance of hydrocarbon peroxides in the formation of gum in gasoline. Wagner and Hyman[41] (1930) investigated the oxidations which are involved in the formation of gum and postulated that per-acids rather than peroxides were responsible for the formation of gum. Morrell, Dryer, Lowry and Egloff[13] (1932-1936) published a great deal of data confirming the role of peroxides in the gum forming process and substantiated the use of inhibitors to prevent the formation of peroxides and gum which they had suggested in an earlier paper[12] (1929). Inhibitors, or "antioxidants", are now employed universally to prevent the deterioration of gasoline motor fuels during storage periods ranging to eighteen months. The chemical literature on the formation of gum in cracked gasolines and their blends has been thoroughly reviewed by Ellis[14] (1934, 1937) and Allen[2] (1943). A recent report by Power and Allen[27] (1943) deals with the effect of water on the deterioration of gasoline motor fuels during storage.

Literature on TEL Precipitation

The chemical literature on the precipitation of TEL from solution in gasoline is very brief since most of the work in this field remains unpublished. Calingaert[10] (1925) and Terenin[37] (1934) report the deposition of metallic lead when TEL is exposed to sunlight under anaerobic conditions. When the conditions were aerobic a white precipitate was formed. One commercial laboratory suggests that the lead precipitate may consist of a lead bromocarbonate, or a mixed salt containing lead, bromine, carbon dioxide

and sometimes sulfur. Another laboratory[18] postulates that the first precipitate is a lead triethyl chloride or bromide. Roberti, Pipparelli, et al.[28] (1938) investigated the loss in octane number with the precipitation of TEL from gasoline solutions exposed to sunlight. In a later paper (1941) they report the identification of $PbO(C_2H_5)_6$ and PbO_2 as the chief components of the precipitate[29]. Unfortunately due to the present hostilities this later paper was available only in abstract form.

It is evident from the several branches of the literature which have been reviewed that studies of the bacterial oxidation of hydrocarbons and the deterioration of gasoline fuels have not been considered as interrelated.

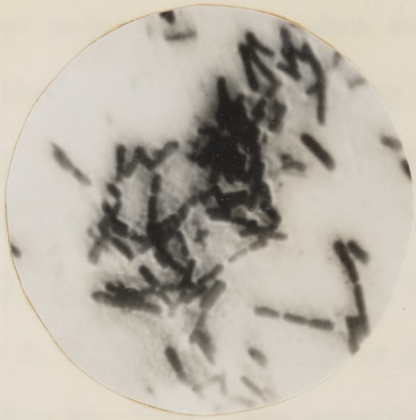
EXPERIMENTAL METHODS

Gasoline motor fuels are generally stored over water and in the presence of air. These conditions were reproduced for experimental purposes by using partially filled glass bottles which contained measured amounts of gasoline and water. Gasoline deterioration takes place very slowly in the dark, which is the usual storage condition, but is strongly photocatalyzed by ordinary daylight. Unless otherwise stated in the description of a particular series, the samples were exposed to diffused daylight to accelerate the deterioration and make it possible to complete the study of a series in a reasonable length of time. The more common method of accelerating the deterioration by increasing the temperature could not be employed in this work because of the bacteria and enzymes which were involved.

Micro-organisms employed in this work

Samples of gasoline storage tank waters were obtained and cultured for bacteria in order to discover the most common contaminants. The tanks from which these water samples were taken contained (1) debutanized cracked distillate (water samples Nos. 1193 and 1841), (2) finished motor gasoline (Nos. 1194 and 1842), and (3) natural gasoline (Nos. 1195 and 1843). The iron bacteria, Gallionella, Leptothrix, and probably Crenothrix were observed in all of the tank water samples. The sulfur bacteria, Thiobacillus thiooxidans, Th. thioparus and Thiospirillum were also found as common contaminants. In addition to these autotrophic iron and sulfur bacteria, a number of heterotrophic organisms were isolated

PLATE I



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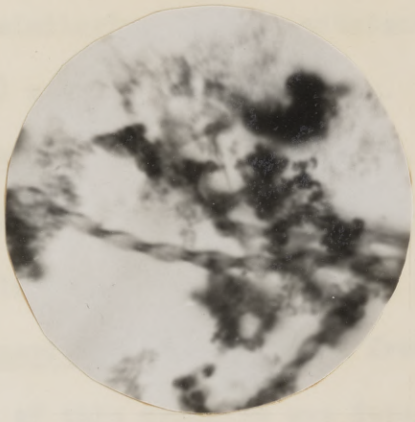

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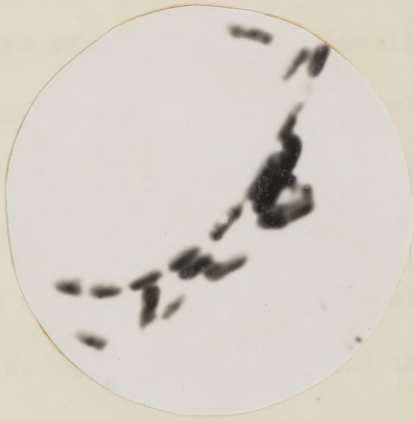

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a. *B. mycoides* recovered from under 100 octane aviation gasoline in Series I.
b. *Gallionella* recovered from under 100 octane aviation gasoline in Series E.
c. *Hormodendron* (1193) isolated from a refinery storage tank and recovered after inoculation in Series I.
d. *B. cereus* isolated from a refinery storage tank water.

and identified. Tank water No. 1841 contained Bacillus cereus (variant A). Tank water No. 1842, when cultured similarly in plain nutrient broth showed the presence of B. cereus (var. A) and the mold, Mucor sp. B. cereus (var. B), Flavobacterium flavescens, and Micrococcus sp. were isolated from tank water No. 1843. The difference between B. cereus (var. A) and B. cereus (var. B) was the lack of nitrate reduction by variant B. A mold of the group Fungi Imperfecti was recovered from tank water No. 1193. The generic status of this organism was determined as Hormodendron sp. An unidentified bacterium which has been designated as Culture No. 1193 was also recovered from this tank water sample.

In the present work it was necessary to use pure cultures of organisms so that their effect on the rate of gasoline deterioration could be determined. The iron bacterium, Gallionella, and the sulfur bacterium, Thiobacillus thiooxidans, were chosen as representative of the autotrophs, at least for this preliminary investigation. A group of ten heterotrophic bacteria which were considered to be taxonomically representative were selected for Series C. These organisms and the reasons for their selection are listed below. Subsequent work on the heterotrophs was restricted to Bacillus mycoides. This organism has essentially the same physiological characteristics as B. cereus[6], which was isolated as a common contaminant from the gasoline storage tank waters.

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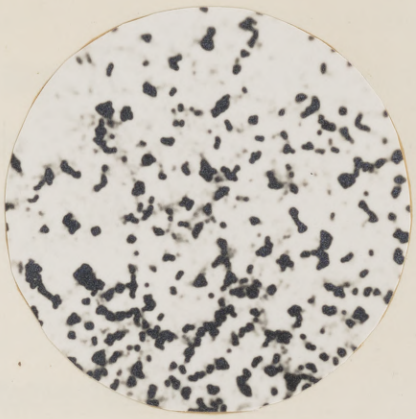
PLATE II



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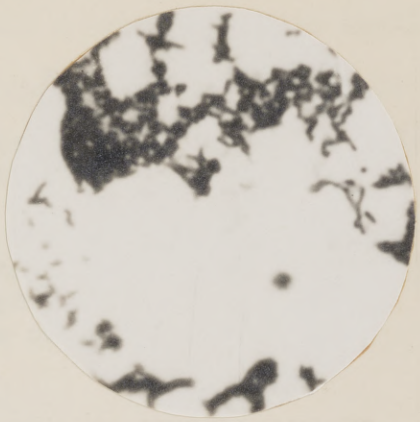

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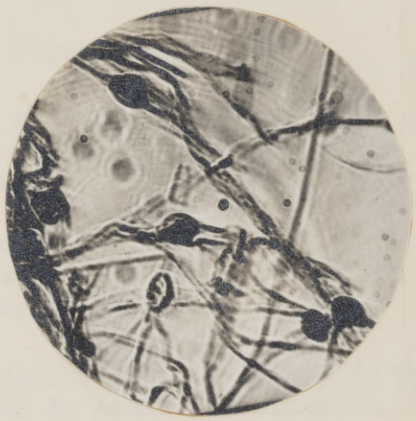

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a. *Micrococcus* (1843) isolated from refinery gasoline storage tank water.
b. *Flavobacterium flavescens* isolated from gasoline storage tank water.
c. *Thiospirillum* from Luling, Texas oilfield brine
d. *Mucor* (1842) isolated from a refinery gasoline storage tank water.

HETEROTROPHIC ORGANISMS EMPLOYED IN SERIES C

Organism	Reason for Selection
1. Mycobacterium marinum	A marine form--possible contaminant from field stock tanks
2. Mycobacterium smegmatis	Genus Mycobacterium is reported in literature as capable of utilizing hydrocarbons as their only source of carbon
3. Mycobacterium phlei	Ditto
4. Mycobacterium leprae	Ditto
5. Bacillus mycoides	Soil bacterium--possible contaminant in storage
6. Bacillus megatherium	Yellow pigment producer
7. Staphylococcus aureus	Golden pigment producer
8. Sarcina lutea	Yellow pigment producer
9. Serratia marcescens	Red pigment producer
10. Pseudomonas fluorescens	Produces fluorescent pigment; also reported in literature as utilizing hydrocarbons
11. "Shotgun"	A mixture of the previously listed bacteria to observe a possible interaction of species

Description of Gasolines Used in the Various Series — The gasoline employed in Series A, B, and C was chemically untreated motor blend consisting of 65 volume percent cracked naphtha, 20 volume percent of a 13-pound East Texas Natural Gasoline, and 15 volume percent of a light naphtha distilled directly from the crude. The characteristics of this blend closely approach those of a finished untreated commercial motor fuel, except for the addition of a small amount of butane to furnish

the necessary vapor pressure for the particular seasonal and climatic conditions[44]. The gasoline in Series D was a cracked naphtha similar to the cracked stock used in the motor blend described above. The gasoline employed in Series E through J was an uninhibited stock of current 100 octane aviation fuel containing approximately 4 ml. TEL/gal*. The physical properties of all of these gasoline stocks are shown in Table I.

Methods of Exposing the Samples

These gasolines were exposed in several different ways. In the preliminary Series A and B, the samples were exposed in one-liter erlenmeyer flasks. The samples in this case consisted of 500 ml. of an aqueous culture medium of inorganic salts** which were covered with 1/4 inch of uninhibited commercial motor blend. The samples in Series A and B were not exposed

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* In calculating the TEL concentrations, 1.65 was taken as the specific gravity of lead tetraethyl. The metric equivalent of one gallon was assumed to be 3785.3 ml.

** This culture medium was described by Haas[19] and has the following composition:

$MgSO_4 \cdot 7H_2O$	0.20 gm.
$CaCl_2$	0.02 gm.
KH_2PO_4	1.00 gm.
$K_2HPO_4 \cdot 3H_2O$	1.00 gm.
NH_4NO_3	1.00 gm.
$FeCl_3$	0.05 gm.
Water	1 liter

The pH of the medium is adjusted to 7.0-7.2 with dilute NaOH.

TABLE I

GASOLINES EMPLOYED IN THIS WORK

	Finished Aviation Gasoline	Motor Blend	Cracked Naphtha
Gravity, °API	70.3	63.0	54.2
I.B.P., °F.	111	118	132
5% off at, °F.	138	154	169
10%	144	165	183
20%	154	179	201
30%	161	192	217
40%	172	205	234
50%	180	215	250
60%	191	224	264
70%	201	232	281
80%	217	243	302
90%	243	260	331
95%	274	279	355
F.B.P., °F.	346	328	382
Recovery, %	98.0	98.5	98.0
Residue, %	1.0	0.8	1.0
Loss, %	1.0	0.7	1.0
Saybolt Color	+30	+29	+7
Std. Heat Color, 16 hrs. at 212°F.	--	-11	--
Breakdown Time, Min.	360+	30	--
ASTM Gum, mgm./100 ml.	0	0	2.0
Lamp Sulfur, Weight %	0.010	0.068	--
Bromine Number, cgm./gm.	0.25	34.0	--
Peroxide Number, meq./liter	0	1.1	4.7
Reid Vapor Pressure, lbs./sq.in.	4.7	5.2	3.7
Octane Number, ASTM-Clear	77.0	65.7	--
Octane Number, ASTM Av. +4 ml. TEL/gal.	Iso-octane + 0.01 ml. TEL/gal.		

to light. The samples in Series C through F were held in 2 1/2 liter capacity white glass liquid reagent ("acid") bottles. In Series G through J, two liter capacity uniform white glass bottles (Marshaw No. 2000) were used. The samples in each case consisted of one liter of the gasoline over 500 ml. of culture medium. The bottles were stoppered with sterile cotton plugs and covered with a white glass beaker, except that in Series G through J, ground glass stoppers were used in place of the cotton plugs to slow down the evaporation. These glass stoppers were removed daily to maintain the partial pressure of the air above the samples.

In the series which were exposed to light, the bottles were placed in diffused daylight on the inside sill of a large second floor window with a northern exposure. The samples in the series which were not exposed to light were kept in a closed wooden cabinet. No attempt was made to control the temperature in either of these methods of exposure. Temperature fluctuations were not considered significant, however, since all samples were comparative to an identical control in each series.

Sterilization of Samples — The bottle, cotton plug or glass stopper, and culture medium for each sample were sterilized by autoclaving for twenty minutes at a steam pressure of fifteen pounds per square inch (121° C.). Standard aseptic bacteriological technique was followed throughout the work with the one exception that the mouths of the sample bottles which contained gasoline were not flamed when transfers were made. Attempts which were made to sterilize the gasoline stocks by distillation from sterile equipment did not give consistently negative results when samples of the condensate were cultured for bacterial

contaminants. Berkefeld filtration of the large volumes of gasoline which were required proved dangerous and gave a filtrate of doubtful sterility. This is not surprising since adsorption of suspended material from organic solvents is frequently less effective than from aqueous suspensions.

Determination of Gum — Gum was determined by the ASTM Method (D381 - 36)[4], which is now universally adopted in preference to the several other methods which have been employed in the past. In brief, this method involves the evaporation of a 50 ml. sample of the gasoline in a 150 ml. Berzilius type beaker. A blast of air at 320-330° F. is played against the surface of the gasoline sample which is held at temperature in a bath of ethylene glycol at the same temperature. The gum is determined by deducting the tare weight of the beaker from the gross weight. ASTM gum is reported in mg. of gum per 100 ml. of gasoline.

Determination of Peroxides — Peroxides were determined by the method of Yule and Wilson[45] which is the method generally employed in the industry. This determination depends upon the extraction of the peroxides in a 10 ml. sample of the gasoline into a five gram per liter solution of ferrous sulfate in a half and half mixture of acetone and water. A portion of the ferrous ion is thus quantitatively oxidized to the ferric state by reducing the peroxides which are extracted from the gasoline. The peroxides are then determined indirectly by titrating with standard titanous chloride to reduce the ferric ions to ferrous in the presence of ammonium thiocyanate as indicator. This determination was carried out with the use of the equipment described by Allen[2].

The same report contains a discussion of the advantages and disadvantages of this method and the other possible methods of determining peroxides in gasoline.

Peroxide concentration is expressed in Peroxide Numbers. One peroxide number is defined as one millequivalent of combined oxygen per liter of gasoline.

Determination of Lead Tetraethyl - TEL The concentration of TEL in each sample of Series E and F was determined by the method of Baldeschwieler[5]. The determination depends upon the extraction and conversion of the TEL to the nitrate by vigorous agitation of 200 ml. of the gasoline with 20 ml. of concentrated nitric acid. The lead nitrate is then converted to the sulfate and determined gravimetrically. This method has a reported reproducibility of 0.02 ml. TEL/gal. Triplicate determinations on the gasoline before exposure in Series D gave results of 3.93, 3.93, and 3.92 ml. TEL/gal.

In Series G through J the concentration of TEL was determined by the ASTM Method (D 526-42)[4] in which a 50 ml. sample of the leaded gasoline is extracted by refluxing with hydrochloric acid. The lead chloride is then converted to the sulfate and determined gravimetrically.

REPRODUCIBILITY

The combined reproducibility of the method of exposure, the handling of samples and the methods of analysis was determined by a statistical study of the deterioration of ten identical uninoculated samples of cracked gasoline over Haas Medium. These samples were exposed to light in the same manner as has already been described for the other series, and gum and peroxide determinations were made at three and four day intervals. The mean average deviation from the arithmetic mean was calculated for each series of tests. These values are recorded in Table II, along with the other data from the series, and shown graphically on page 20. It will be noted that the peroxide deviations pass through a minimum after about ten days of exposure. The precision of the gum determination was also found to be greatest during the period of from seven to eighteen days exposure. The literature reports a reproducibility for the determination of ASTM gum content of 0 to 20 mg./100 ml.; 10 mg. for a gum content of 20 to 100 mg./100 ml.; and 20 mg. for a gum content of over 100 mg./100 ml.[4]. The peroxide determination has a reported reproducibility of 0.1 Peroxide Numbers in the range of 1 to 10, and 0.5 Peroxide Numbers in the range above 10[39]. It is evident from the results of this series of identical samples that the errors incurred in the methods of exposure, handling and testing the gasoline motor blend and cracked stock are well within the limits of accuracy of the peroxide and gum tests themselves.

In a similar manner the reproducibility of the method of exposure and the determination of lead precipitation was investigated with three identical uninoculated control samples of the 100 octane aviation

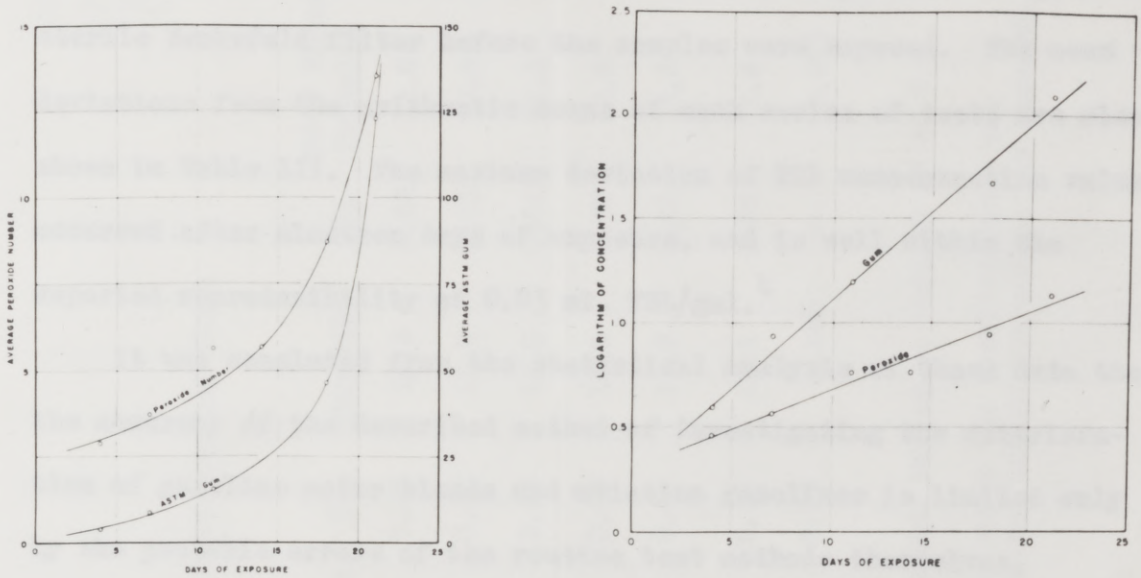
TABLE II

DETERMINATION OF REPRODUCIBILITY OF GUM AND PEROXIDE TESTS

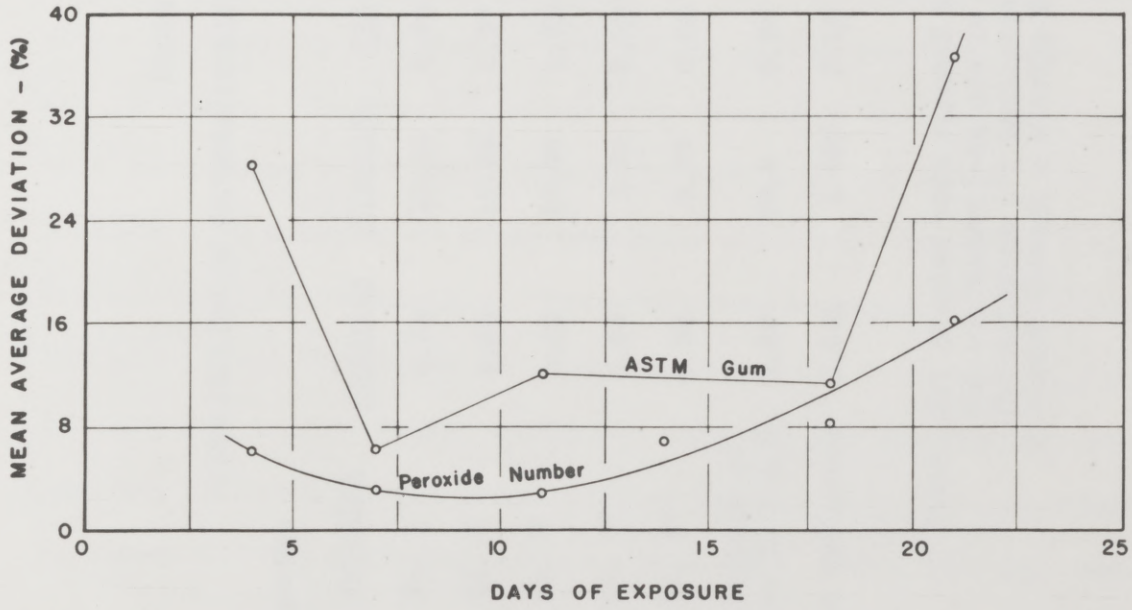
Time of Exposure / Sample Cracked Naphtha	4 Days Gum	4 Days Peroxide	7 Days Gum	7 Days Peroxide	11 Days Gum	11 Days Peroxide	14 Days Peroxide	18 Days Gum	18 Days Peroxide	21 Days Gum	21 Days Peroxide	Water Phase Final pH	Water Phase Final Gum	Water Phase Final Peroxide
Control No. 1*	2.0	3.08	9.8	3.77	15.0	5.37	4.78	36.8	7.23	66.2	10.46	5.98	393.8	5.45
Control No. 2	2.2	2.97	8.6	3.82	14.0	5.71	5.47	41.6	8.20	98.6	12.94	5.17	436.8	7.62
Control No. 3	2.4	2.80	8.2	3.67	14.1	5.62	5.55	40.0	8.54	82.8	11.71	5.59	443.2	6.84
Control No. 4	2.4	2.57	8.2	3.39	14.0	5.47	5.47	36.8	8.25	75.6	11.19	5.70	431.6	6.23
Control No. 5	5.6	2.85	7.2	3.58	12.0	5.33	5.17	37.0	7.85	64.4	10.39	5.70	374.2	5.77
Control No. 6	6.2	2.80	8.8	3.91	19.8	5.85	5.98	58.0	9.93	183.0	16.53	4.58	489.4	9.80
Control No. 7	5.2	3.31	9.4	3.77	15.6	5.62	6.07	51.8	9.03	144.4	14.80	4.92	425.8	8.41
Control No. 8	4.2	3.08	7.4	3.77	15.0	5.66	6.32	52.2	9.36	172.0	15.91	4.86	402.0	8.18
Control No. 9	4.4	2.72	8.8	3.86	18.6	6.18	6.23	62.4	10.20	204.2	17.32	4.72	422.2	10.16
Control No. 10	4.4	2.69	8.8	3.67	17.0	5.62	5.77	51.4	8.78	138.8	13.90	4.97	446.6	7.16
Arithmetic Mean	3.9	2.89	8.5	3.72	15.5	5.64	5.68	46.8	8.74	123.0	13.52	5.22	426.6	7.56
Mean Average Deviation	28.2%	6.2%	6.2%	3.1%	12.0%	2.9%	6.9%	11.3%	8.3%	36.7%	16.1%	8.0%	5.26%	16.8%

* All samples exposed over Haas Medium.
Initial pH of medium was 7.0

DETERIORATION OF TEN IDENTICAL CONTROL SAMPLES



MEAN DEVIATIONS FOR TEN IDENTICAL CONTROL SAMPLES



gasoline. The results of peroxide, TEL, and pH determinations on these samples are shown in Table III, along with two other uninoculated control samples in which the gasoline phase had been passed through a sterile Berkefeld filter before the samples were exposed. The mean deviations from the arithmetic means of each series of tests are also shown in Table III. The maximum deviation of TEL concentration values occurred after nineteen days of exposure, and is well within the reported reproducibility of 0.03 ml. TEL/gal.[4]

It was concluded from the statistical analysis of these data that the accuracy of the described method of investigating the deterioration of gasoline motor blends and aviation gasolines is limited only by the probable errors of the routine test methods themselves.

TABLE III

DETERMINATION OF REPRODUCIBILITY OF LEAD PRECIPITATION TESTS

Time of Exposure Sample 100 Octane Aviation	Peroxide	Peroxide	TEL	Peroxide	TEL	Peroxide	TEL	Water Phase Peroxide	Final pH
Control No. 1	4.53	10.44	0.92	11.03	0.40	13.8	0.02	12.6	6.9
Control No. 2	4.63	10.35	0.97	11.00	0.40	13.8	0.01	12.6	6.9
Control No. 3	4.58	10.40	0.93	11.17	0.35	14.2	0.01	12.3	7.0
Control No. 4*	4.63	9.90	0.82	10.52	0.49	13.6	0.07	12.6	6.9
Control No. 5*	4.53	9.77	0.83	10.21	0.49	13.6	0.01	12.5	6.8
Arithmetic Average of Controls Nos. 1, 2 & 3	4.57	10.6	0.94	11.08	0.38	13.9	0.01	12.5	6.9
Mean Average Deviation	0.64%	1.88%	2.1%	0.85%	6.1%	1.15%	--	0.84%	0.5%

Note: All samples were exposed over Haas Medium having an initial pH of 7.0.
TEL concentrations are in ml. TEL/gal.
The asterisk (*) indicates samples in which the gasoline phase was passed through a sterile Berkefeld filter before the series was exposed.

PRESENTATION OF EXPERIMENTAL RESULTS

Description of Series Series A and B of this work were conducted as preliminary tests to verify reports in the literature which state that certain bacteria are capable of using hydrocarbons as their only source of energy. In these tests 20 ml. of freshly distilled gasoline were added to 500 ml. of a sterile culture medium of inorganic salts* in one-liter Erlenmeyer flasks. These media were inoculated with pure cultures of bacteria reported as being capable of utilizing hydrocarbons. The samples were kept in the dark at room temperature and inspected daily for growth and pigmentation. The growth rates and pigmentation which were observed are shown in Table IV.

The Effect of Various Types of Bacteria on the Formation of Gum and Peroxides in an Uninhibited Motor Blend--Series C Series C was set up to investigate the effect of micro-organisms on gasoline deterioration, as expressed in terms of gum and peroxides. The bacteria which were inoculated into the samples of this series were those which showed the most prolific growth in Series B, along with a number of other organisms which were chosen as being taxonomically representative. The heterotrophic organisms inoculated in Series C and the reason for their selection are tabulated on page 12. The autotrophic sulfur bacterium, *Thiobacillus thiooxidans*, and a peat-bog culture composed principally of *Gallionella*, were also included in Series C. The following controls were used: (1) a 1500 ml. sample

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*Haas Medium. See footnote on page 13 for composition of this medium.

TABLE IV

GROWTH AND PIGMENTATION OF SEVERAL BACTERIAL SPECIES USING GASOLINE AS THEIR ONLY SOURCE OF CARBON

Series A

Organism	20 hours	72 hours	96 hours	120 hours	240 hours
M. phlei	+++	+++	+++ P	++++ PP	++ PP
M. berolinense	++	+++	++++ P	++++ P	++ P
Ps. pyocyaneus	-	±	+ P	+ PP	- PP
Ps. fluorescens	+	++	++ P	+++ PP	+ PP
Oilfield slush-pit culture	±	±	±	+ P	± P
Control	-	-	-	-	-

Series B

Organism	1 day	2 days	4 days	5 days	6 days	
M. phlei	++	++	+	+	+	Recovered
M. berolinense	+++	++	++	++	++	
Ps. fluorescens	-	-	±	-	-	Recovered
B. mycoides	-	-	-	-	±	Recovered
Oilfield slush-pit culture	- P	- P	± P	++ PP	+++ PP	
Control	-	-	-	-	-	

+ denotes apparent bacterial growth at gasoline-water interface
- no apparent growth
± growth questionable
P indicates yellow pigmentation of the gasoline

of gasoline with no water phase (Dry control); (2) another 1000 ml. of the motor blend over 500 ml. of 10 per cent aqueous solution of formaldehyde to eliminate contaminating bacteria present in the original gasoline stock; and (3) 1000 ml. of the motor blend over 500 ml. of the culture medium with no inoculated bacteria, but from which a gasoline-borne contaminant, Bacillus sp. A, was isolated.

These samples were exposed to light in the manner already described. ASTM gum and peroxide determinations were made on all the samples in the series after three days and then at three and four day intervals up to 24 days. The results of these determinations are shown in Table V. Observations were made daily on growth and accumulation at the gasoline-water interface. At the termination of the tests, representative samples of the water phases were withdrawn with sterile pipettes for gum, peroxide, pH, and bacterial culture determinations.

The culture samples were inoculated into tubes of various media in an attempt to isolate the organism(s) present. One ml. volumes of both the gasoline and water phases were inoculated into separate tubes containing 5 ml. of plain nutrient broth, except in the case of the Mycobacteria, for which 6% glycerol broth was used. These tubes were incubated at 31° C. for 48 hours, with the exception of the Mycobacteria, which were incubated two weeks. At the end of this time the tubes which had been inoculated from the water phase showed evidence of growth with the exception of the Mycobacterium samples. The tubes which showed growth were streaked on plain nutrient agar plates and incubated at 31° C. Colonies were picked from these plates and the organisms identified as to whether they were the species inoculated into the gasoline sample bottles or contaminating forms. Contaminants were identified only as to genus. The results are presented on page 28 of this report.

TABLE V

GUM AND PEROXIDE DETERMINATIONS ON SERIES C

Time of Exposure Sample	3 Days	7 Days		14 Days		17 Days		21 Days		24 Days		Water Phase Final		
Motor Blend	Peroxide	Gum	Peroxide	Gum	Peroxide	Gum	Peroxide	Gum	Peroxide	Gum	Peroxide	pH	Gum	Peroxide
Gasoline-borne Contaminant	1.83	59.6	9.50	100.6	17.1	67.8	21.60	148.2	23.10	190.4	24.20	5.95	388.0	7.30
M. phlei	2.00	38.6	7.90	97.4	16.3	64.4	18.90	72.2	19.8	88.2	24.1	5.87	405.4	8.30
B. Mycoides	2.92	35.2	7.98	99.0	15.7	113.2	17.8	142.6	19.2	110.6	22.6	5.75	401.6	8.10
Thio. thioxidans	2.69	34.2	7.07	77.2	12.4	90.6	16.1	126.2	17.9	137.8	20.0	5.90	238.8	7.38
M. marinum	2.97	34.0	8.10	78.4	13.6	89.2	15.10	110.8	16.80	128.4	19.20	6.15	393.6	6.06
Sar. lutea	1.60	37.8	6.53	78.0	12.4	83.4	14.5	96.2	15.6	107.4	18.0	6.25	400.0	5.40
"Shotgun"	3.43	38.8	7.03	60.4	10.7	71.0	13.5	93.0	14.9	103.0	17.1	6.35	362.0	5.40
M. leprae	2.40	26.2	6.88	80.2	12.0	71.8	13.5	85.8	13.5	104.8	16.0	6.05	287.2	5.27
M. smegmatis	2.46	32.6	6.88	75.8	11.0	65.2	12.50	78.8	12.9	86.6	15.20	6.30	382.2	5.14
"Peat bog culture"	2.23	42.8	7.11	53.4	9.70	71.6	12.7	78.6	13.9	94.6	15.3	6.30	389.4	5.20
Ser. marcescens	2.74	31.2	6.14	51.8	10.3	57.4	11.5	64.6	12.4	83.4	14.2	6.35	335.8	4.42
B. megatherium	3.20	27.8	6.41	49.6	10.5	58.4	11.5	75.4	11.7	82.8	13.8	6.30	350.2	4.35
Ps. fluorescens	2.92	27.0	6.07	47.8	8.8	52.2	11.1	64.2	12.0	76.2	13.3	6.40	351.6	4.48
Staph. aureus	2.46	28.8	6.02	36.0	8.1	38.0	9.8	44.8	9.6	53.0	11.4	6.50	371.4	3.75
Culture #1193	2.74	29.2	6.14	22.6	6.20	29.8	7.8	40.8	7.7	40.6	8.6	6.55	340.6	3.36
Control plus 10% Formaldehyde	2.06	24.4	2.29	64.0	4.20	75.8	4.8	86.0	4.6	91.8	5.8	3.50	533.4	2.11
Control (Dry)	1.83	26.6	4.28	18.6	4.09	14.6	4.64	17.0	4.72	20.0	4.52	....	.....	....
Natural Gasoline														
Control	0.23	nil	0.47	4.0	0.5	3.0	0.64		0.39		0.47			
M. phlei	0.23	nil	0.35	2.8	0.6	2.4	0.75		0.39		0.53			
M. marinum	0.23	nil	0.51	1.2	0.5	2.4	0.70		0.39		0.53			
Thio. thioxidans	0.23	0.8	0.43	1.4	0.7	3.4	0.75		0.39		0.47			
Katy Condensate														
Control	1.54	14.6	1.98	6.8	2.36	19.8	2.61		2.64		2.68			
M. phlei	1.66	2.6	2.02	9.0	2.70	29.8	3.18		2.70		3.15			
M. marinum	1.48	3.0	1.91	6.4	2.40	20.6	3.08		2.48		2.84			
Thio. thioxidans	1.43	3.4	1.91	22.4	2.30	26.0	2.61		2.20		2.26			

In further effort to isolate and prove the presence of the Mycobacteria several of the media commonly employed for this purpose were inoculated with no successful results. Microorganisms were not found in the gasoline phase of any of the samples of the series, but since only minute volumes of the gasoline were tested it is quite possible that bacteria or bacterial spores may have been present in relatively small numbers. Such organisms could then be extracted into the water phase as contaminants.

It will be noted that four contaminating forms were obtained, namely, Bacillus (species A), Bacillus (sp. B), Staphylococcus (sp. A), and Staphylococcus (sp. B). The repeated occurrence of Bacillus (sp. A) indicates that the organism was either present in the original gasoline sample*, possibly in the spore stage, or that it is a common contaminant of the laboratories. Sterile plates exposed in the laboratory did not pick up any organisms which were similar to Bacillus (sp. A), and it was concluded that the presence of this organism is not to be ascribed to accidental contamination.

It is significant that the contaminants do not include any of the organisms which were used in the tests. Such contamination would have indicated some fault in the aseptic technique which was employed throughout the period of exposure.

The failure to isolate B. mycoides and the Mycobacteria, as a group, from the water phases of these samples is not explained. It is suggested

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* No facilities existed for the sterilization of the gasoline samples before inoculation.

ORGANISMS ISOLATED FROM SERIES C

Sample	Broth	Organism Isolated
Uninoculated	Cloudy	Bacillus A.
Bacillus megatherium	"	Bacillus megatherium and Bacillus A
Staphylococcus aureus	"	Staphylococcus aureus
Sarcina lutea	"	Sarcina lutea and Bacillus B
Serratia marcescens	"	Serratia marcescens and Staphylococcus A and Staphylococcus B
Pseudomonas fluorescens	"	Pseudomonas fluorescens
Culture No. 1193	"	Culture No. 1193 and Bacillus A
Bacillus mycoides	"	no isolation
Mycobacterium marinum	"	no isolation
Mycobacterium smegmatis	slight cloudiness	no isolation
Mycobacterium phlei	"	no isolation
Mycobacterium leprae	"	no isolation
Peat bog culture		Gallionella
Thiobacillus thiooxidans		Thiobacillus thiooxidans

that this may be due to the toxicity of high molecular weight peroxides*, or possibly to some error in the technique of sampling and culturing. The tube of broth which was inoculated from the B. mycoides sample definitely cloudy, but several attempts at isolating the organisms were unsuccessful. A Gram stain from the culture tube showed the presence of a Gram positive bacillus type organism, but it was not possible to isolate it in pure culture. This culture was later shown to be autolytic in broth culture, and to have undergone variation in colonial form from rough to smooth. The possibility that the lysis was due to a bacteriophage was not investigated.

The ASTM gum and peroxide determinations in Series C (Table V) show a variation far exceeding the experimental deviation of these tests. Since the only variable in the series consisted of the inoculated organisms this result suggests that the differential gum and peroxide values were the result of bacterial action.

The dry control did not contain a water phase which apparently is necessary for sustained bacterial growth. It will be noted that this sample consistently gave the lowest results of gum and peroxide determinations for the whole series. The formaldehyde control was shown to be sterile by a series of broth cultures, none of which produced growth even after sustained incubation. It is significant that the peroxide number of the formaldehyde control remained approximately equal to that of the dry control throughout the period of exposure. The high gum concentration in the formaldehyde control is interpreted as due to the

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* Dr. Gordon Worley, Associate Professor of Bacteriology, The University of Texas, reports (unpublished) a high degree of toxicity of the initial oxidation products of unsaturated fish oils for several species of bacteria.

polymerization of the formaldehyde.* Thus it is believed that in this particular case the peroxide determination serves more accurately to illustrate the relation of bacteria to the gasoline deterioration.

The samples in Series C which showed the most pronounced increase in gum and peroxides during the exposure correlate well with the recovered organisms that are suspected of being likely contaminants in the water phase which is always present in gasoline storage. Bacillus mycoides, Thiobacillus thiooxidans (sulfur bacterium), Mycobacterium marinum, Sarcina lutea, M. leprae, the peat bog culture (iron bacteria), and the gasoline-borne contaminant, Bacillus (sp. A) apparently served as causal agents in the deterioration of the motor blend. All of these bacteria are to be found in the soil; in slightly, to highly saline waters; in the presence of iron and steel; or in the presence of sulfur compounds. Such bacterial environments may be found in or around oil production, refining and distribution equipment from the well to the consumer.

The action of the autotrophic organisms in Series C is of particular interest. The presence of iron bacteria in storage tanks has been verified in these laboratories. These bacteria utilize ferrous iron in their metabolism and derive their energy by oxidizing the iron from the ferrous to the ferric state, from which it is thrown down as ferric hydroxide. The effect of iron bacteria on the deterioration of stored gasoline is shown as the "Peat Bog Culture" in Series C. It is suspected that the action of the sulfur bacteria is to use the many

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* J. C. Couthard, formerly of the Pure Oil Co., reports (unpublished) the polymerization of formaldehyde in benzene solutions during exposure to light.

sulfur compounds which are normally present, even in "sweet" gasolines, to produce sulfate and sulfite radicals which are known to have a definite effect on the rate of gasoline deterioration. Waksman[25a] (1935) describes the use of bacteria to desulfurize crude oils but reports that the hydrocarbons themselves are also attacked. Thus Thiobacillus thiooxidans might make use of the relatively high sulfur content of the motor blend to synthesize sulfurous or sulfuric acid. This organism is reported to be aerobic and it is probable that it acts only as an initiating agent in the deterioration mechanism.

The results obtained on East Texas Natural Gasoline and Katy (Texas) Condensate indicate that bacterial attack on highly saturated hydrocarbons is a slow process. The acid heats of these two gasolines were 14° and 11° respectively by the ASTM method. The ASTM gum determinations on these two gasolines are inconsistent due to the high percentage of heavy ends in the condensate which made accurate determinations the exception rather than the rule. The peroxide data are believed to be reliable, however.

The Effect of Bacteria and Commercial Gasoline Inhibitors on Gum Formation--Series D

The effectiveness of three representative commercial gasoline inhibitors on cracked gasoline standing over Haas Medium which had been inoculated with B. mycoides and Lieske's Medium* inoculated with

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* Lieske's Medium has the following composition:

$(NH_4)_2SO_4$	1.50 gm.
KCl	0.05 gm.
$MgSO_4$	0.05 gm.
K_2HPO_4	0.05 gm.
$Ca(NO_3)_2$	0.01 gm.
Distilled water	1 liter

Iron filings are added to the medium before sterilization to provide the required source of iron.

the iron bacterium, Gallionella, both in the light and dark was investigated in Series D. The series included uninhibited control samples inoculated with both of these organisms. The inhibitors which were tested were N-n-butyl-p-aminophenol (duPont No. 5); 2-6-di-tert.-butyl-4-methylphenol (Gulf No. GS-1713); a-naphthol; and a-naphthol plus lecithin. These inhibitors were added to the gasoline in a concentration of approximately 0.005% by weight. A similar concentration of lecithin was added to the proper samples which were inhibited with a-naphthol. The use of lecithin to intensify inhibitor efficiency is described in the literature[27a].

The effect of three actual gasoline storage tank waters along with Luling (Texas) oilfield brine was also studied in this series. The results obtained from Series D are shown in Table VI and in the figures plotted on pages 60, 61 and 62. It is interesting that these tank water samples in this series exhibited different activities in the formation of gum and peroxides in cracked gasoline than they did in the precipitation of TEL (cf. Series F on page 59).

Preliminary Investigation of the Effect of Bacteria on the Precipitation of TEL from 100 Octane Aviation Fuels--Series E

The most detrimental organisms in Series C were inoculated into 100 octane aviation gasoline to determine their possible activity in precipitating TEL during storage. These samples were designated as Series E. They were exposed to diffused daylight for twelve days in exactly the same manner as the previous series. The gasoline used in this series was uninhibited and contained 3.93 ml. TEL/gal.

TABLE VI

GUM AND PEROXIDE DETERMINATIONS ON SERIES D

Time of Exposure	4 Days		7 Days		11 Days		14 Days		18 Days		21 Days		Water Phase	Final	
Sample	Gum	Peroxide	Gum	Peroxide	Gum	Peroxide	Gum	Peroxide	Gum	Peroxide	Gum	Peroxide	pH	Gum	Peroxide
Cracked Naphtha															
Tank Water #1193 (Cracked distillate)	21.4	3.66	4.62		93.2	7.18	11.73		586.2	23.3	403.2	30.5	3.35	522.4	3.19
Tank Water #1193 Sterile	24.0	3.66	4.43		87.8	6.80	11.52		374.0	21.7	362.6	28.0	3.34	520.2	3.83
Tank Water #1194 (Motor gasoline)	25.2	4.28	6.28		129.0	10.90	20.20		375.0	32.5	496.0	42.8	3.26	767.0	4.99
Tank Water #1194 Sterile	25.4	4.17	6.09		108.2	9.67	17.28		431.2	30.3	390.2	39.0	3.20	813.0	8.18
Tank Water #1195 (Natural gasoline)	22.8	4.05	5.20		92.8	8.96	15.92		440.2	23.4	418.6	34.1	3.02	603.2	10.96
Tank Water #1195 Sterile	21.2	3.83	4.62		62.6	7.14	10.10		231.0	15.7	469.0	25.6	3.57	471.4	8.78
Luling oilfield brine	22.6	4.23	5.57		93.0	9.61	13.95		383.6	26.8	348.8	33.1	3.20	1811.4	8.87
Luling oilfield brine Sterile	25.6	4.11	5.67		122.6	11.85	17.07		458.0	30.3	535.0	35.5	3.02	1792.8	9.87
Inhibitors - Light															
Gallionella Control	2.06		5.0	2.78		4.04	13.4	3.50		5.15	41.4	6.18	3.54	177.8	0.88
Gallionella DuPont No. 5	1.88		13.6	2.55		3.33	21.6	3.03		3.92	26.0	4.49	4.73	201.4	1.71
Gallionella Gulf Inhibitor	1.20		2.0	1.65		2.57	8.6	2.22		4.33	22.6	5.39	3.65	225.8	1.62
Gallionella a-Naphthol	2.34		10.2	3.96		5.57	30.8	4.01		6.83	111.6	10.40	3.50	243.4	0.97
Gallionella a-Naphthol + Lecithin	2.34		12.2	4.00		5.47	35.2	4.83		6.61	140.8	9.78	3.41	351.8	1.02
B. mycoides Control	2.74		25.6	4.15		6.61	95.0	8.46		16.62	348.2	33.10	4.20	540.8	11.60
B. mycoides DuPont No. 5	2.23		21.8	2.55		3.57	51.6	3.84		4.53	47.8	5.28	6.45	421.2	3.41
B. mycoides Gulf Inhibitor	2.23		21.0	3.86		6.38	54.4	7.01		9.61	138.0	14.57	4.98	483.4	6.47
B. mycoides a-Naphthol	2.06		11.4	3.63		5.47	32.0	6.07		9.07	105.8	12.65	5.04	502.2	6.75
B. mycoides a-Naphthol + Lecithin	2.00		11.4	3.02		4.35	21.0	4.87		6.86	50.8	8.26	6.01	413.0	3.69
Inhibitors - Dark															
Gallionella Control	1.08		1.0	1.37		1.52	3.0	1.71		1.84	3.0	2.47	5.10	109.6	0.69
Gallionella DuPont No. 5	0.69		4.8	0.80		0.95	5.8	0.85		0.82	6.6	1.07	7.30	167.4	0.83
Gallionella Gulf Inhibitor	0.69		1.4	0.66		0.76	0.6	0.77		0.78	2.8	1.07	5.60	119.2	0.61
Gallionella a-Naphthol	0.86		1.8	1.23		1.86	7.6	2.09		2.45	10.1	3.26	6.00		0.79
Gallionella a-Naphthol + Lecithin	0.69		5.6	0.94		1.52	7.2	1.79		1.96	10.2	2.47	5.75		0.65
B. mycoides Control	1.03		4.4	1.08		1.48	3.6	1.84		1.92	5.8	2.53	6.62		2.35
B. mycoides DuPont No. 5	0.69		7.2	0.66		0.86	1.8	0.77		0.82	11.4	1.01	6.79		4.15
B. mycoides Gulf Inhibitor	0.63		4.6	0.61		0.90	4.8	0.90		0.82	4.8	1.01	6.60		3.60
B. mycoides a-Naphthol	0.80		9.0	1.37		2.14	15.2	2.81		2.90	16.8	3.54	6.61		3.46
B. mycoides a-Naphthol + Lecithin	0.63		6.2	1.13		2.19	10.4	2.09		2.12	12.0	3.21	6.50		3.05
Refrigerated - Dark															
Gallionella Control							2.0	1.11			6.6	1.18	5.63		0.65
Gallionella DuPont No. 5							4.2	0.73			7.4	0.90	7.00		1.48

The results of deducting the final concentration of TEL in ml./gal. from the original concentration of 3.93 ml. TEL/gal. is shown in the first column of Table VII. These values show the effect of both actinic light and bacterial action. The control sample over 1:2000 formaldehyde was proven to be sterile at the completion of the test. The amount of lead precipitated in this control was the least of all the samples in the series. Thus it was assumed that the precipitation of lead in the presence of aqueous formaldehyde solution was due to the action of light alone, whereas the lead precipitated in the other samples was the result of the effect of light plus bacterial action. The amount of TEL precipitated by bacterial action is given in the second column of Table VII. The value of the formaldehyde control in this column is arbitrarily taken as zero.

Distilled water is essentially devoid of the inorganic salts necessary to sustain bacterial life. The formaldehyde in this series served as a disinfectant rather than an inhibitor as shown by the value for the distilled water control, which within the limits of accuracy of the determination, is equal to the value for the formaldehyde control.

Due to the extremely low sulfur concentrations which are permitted in leaded aviation gasolines it is not surprising that the sulfur bacterium, Thiobacillus thiooxidans, was not outstandingly effective in the precipitation of lead. The close valuesfor the microorganism isolated from the gasoline-borne contaminant sample (Bacillus A) in Series C and the wet control over the culture medium in this series may indicate a possible common contaminant. The particular Mycobacterium, M. marinum, was apparently ineffective. Neither the exposure to light nor the presence

TABLE VII

GUM, PEROXIDE AND TETRAETHYL LEAD DETERMINATIONS ON SERIES E

Time of exposure--12 days

100 Octane Aviation Blend	ml. TEL/gal. precipitated	ml. TEL/gal. precipitated presumably by bacterial action	ASTM Gum	Peroxide Number	Final pH
Control over distilled water	0.30	0.01	4.6	0.98	7.70
Control over Haas Medium	0.48	0.19	4.6	1.28	----
M. marinum	0.42	0.13	1.6	1.03	6.80
B. mycoides	0.78	0.49	1.2	0.87	6.75
Th. thiooxidans	0.62	0.33	1.4	1.33	6.80
Gallionella (iron bacterium)	0.77	0.48	1.6	1.13	7.55
Contaminating organism from Series C	0.40	0.11	1.2	1.28	6.80
Control over 1:2000 Formaldehyde	0.29	nil	2.2	0.46	8.55

Note: The original concentration of lead tetraethyl was 3.93 ml. TEL/gal.

of lead tetraethyl appeared to be detrimental to the bacteria. Lead is not considered to be toxic to bacteria since its salts are used as indicators in many culture media.

The autotrophic bacteria, Gallionella and Th. thiooxidans, were isolated from the leaded 100 octane blends and produced cultures in synthetic media. There was an indication of the common presence of unidentified autotrophic bacteria in all the samples of the series with the exception of the formaldehyde control. Apparently due to the toxicity of the hydrocarbon peroxides (cf. page 26) or possibly some difficulty in sampling technique none of the heterotrophic organisms were isolated in pure culture at the end of the test although microscopic examination of the water phases showed microorganisms to be present.

It will be noted that the iron bacterium, Gallionella, and the soil bacterium, B. mycoides, were directly responsible for the precipitation of half a milliliter of the original 3.93 ml. TEL/gal. during only twelve days of exposure. It also seems significant that the action of each of these bacteria approaches nearly twice the effect of light which has previously been considered among the most detrimental of the several causal agents in the problem of lead precipitation.

Confirmation of Series E and Investigation of Refinery Storage Tank Waters in TEL Precipitation--Series F

The investigation of the precipitation of TEL from aviation gasolines by bacterial action was continued in Series F. Bacterium aliphaticum and B. mycoides were inoculated into 100 octane samples which were exposed over Haas Medium. A sample of the same gasoline which was inoculated with Gallionella was exposed

over Lieske's Medium. Three gasoline storage tank waters and Luling oilfield brine were included in the water phases of a group of samples in the series. Another group of the same water samples which had been sterilized by autoclaving them in the sample bottles for twenty minutes at fifteen pounds of steam pressure were also included in the series. Control samples of Haas and Lieske's Media, Lieske's Medium plus 1:2000 formaldehyde and Lieske's Medium to which had been added ferric oxide equal to the weight of the Gallionella inoculum were also provided in the series.

It will be noted that the non-sterile Luling oilfield brine and the sample which had been inoculated with the iron bacterium, Gallionella, produced the greatest precipitation of lead. There was no definite correlation between peroxide formation and lead precipitation. The lowest members of the series with respect to lead precipitation were the formaldehyde control and the non-sterile Tank Water No. 1193, the latter of which contained the imperfect fungus Hormodendron (1193). The results of gum, peroxide and TEL determinations on Series F are shown in Table VIII.

The Effect of a Soil Bacterium and a Mold and their Cellular Extracts on Lead Precipitation in the Light--Series G

In an attempt to learn the effect of the metabolic products of certain micro-organisms on the precipitation of TEL from 100 octane aviation gasolines endo- and exo-cellular extracts of B. mycoides and Hormodendron (1193) were prepared and exposed along with the organisms themselves in Series G. The exo-cellular extracts

TABLE VIII

GUM, PEROXIDE AND TETRAETHYL LEAD DETERMINATIONS ON SERIES F

Time of Exposure	4 Days	7 Days	11 Days	14 Days	18 Days	21 Days	25 Days	28 Days				
Sample	Peroxide Number	Peroxide Number	Peroxide Number	Peroxide Number	Peroxide Number	Peroxide Number	Peroxide Number	Peroxide Number		Water Phase pH		Total cc TEL/gal.
100 Octane Aviation Blend								Gasoline	Water	Initial	Final	Precipitated
Control - Haas Medium	0.51	1.14	1.67	2.18	2.59	3.69	5.33	6.82	8.67	7.0	7.7	1.27
Control - Lieske's Medium	0.42	0.84	1.33	1.63	2.09	2.71	3.89	5.14	2.24	7.0	8.9	1.22
Control plus $Fe(OH)_3$	0.30	0.88	1.05	1.40	1.86	2.31	3.54	4.47	2.63	7.0	8.7	1.12
B. aliphaticum	0.34	0.84	1.19	1.91	2.23	3.03	4.58	5.70	7.60	7.0	7.8	1.54
B. mycoides	0.38	1.04	1.52	2.13	2.59	3.69	5.45	6.93	9.12	7.0	7.6	1.72
Gallionella	0.38	0.99	1.29	1.95	2.50	3.20	5.11	5.76	2.85	7.0	6.6	2.07
Tank Water #1193 (Untreated cracked distillate)	0.47	0.74	1.05	1.36	1.82	1.96	2.44	2.79	2.18	5.6	4.9	0.70
Tank Water #1193 Sterile	0.25	0.49	0.76	0.95	1.32	1.47	1.91	2.24	1.79	5.6	4.9	1.50
Tank Water #1194 (Finished motor gasoline)	0.55	1.24	1.95	2.68	2.95	4.50	5.98	6.99	6.43	8.0	8.3	1.77
Tank Water #1194 Sterile	0.42	0.99	1.67	2.49	3.18	4.67	6.38	7.60	7.16	8.0	8.3	1.77
Tank Water #1195 (Natural gasoline)	0.30	1.04	1.67	2.22	2.86	4.09	5.68	7.10	6.43	7.6	8.8	1.95
Tank Water #1195 Sterile	0.30	1.09	1.62	2.22	2.86	4.23	5.68	7.32	7.27	7.6	8.2	1.75
Luling oilfield brine	0.47	1.48	2.33	3.22	3.91	5.57	7.25	8.67	8.83	7.8	7.7	2.23
Luling oilfield brine Sterile	0.38	1.48	2.05	2.99	3.59	5.22	6.80	8.28	8.28	7.8	7.7	1.95
Control - Lieske's Medium plus 1:2000 Formaldehyde	0.13	0.59	0.91	1.27	1.77	2.40	3.19	4.53	2.51	7.0	5.9	0.90

Note: Gum was nil in all samples after 18 days of exposure.

consisted of the broth substrates in which pure cultures of the two organisms had been grown for two weeks. The B. mycoides culture was grown in beef extract peptone broth consisting of 0.5% peptone and 0.35% beef extract by weight at a pH of 7.0. The Rhizodendron (1193) culture was grown in a maltose broth composed of 10% desiccated Malt Extract (Difco) having a pH of 4.7. Controls of each of these broths in corresponding dilution were included in the series. The living cells were centrifuged from the broth substrates and the substrates subjected to Berkefeld filtration and incubated at room temperature for twenty-four hours to test their sterility. A contaminant having the colony and pigment characteristics of Ps. fluorescens was recovered from both substrates and they were immediately refiltered. A second check on their sterility showed them to be free of bacteria. Two hundred and fifty milliliters of each of the substrates were diluted to 500 ml. with sterile distilled water for exposure in the series.

The cells which were recovered from the broth media by centrifugation were washed with sterile isotonic saline solution and divided into two approximately equal portions. One of these portions of each of the organisms was dispersed in distilled water. The cells were then broken down by alternately freezing the suspensions in dry ice and then thawing them to room temperature. This procedure was repeated six times. A microscopic examination of the suspension showed the cells to be thoroughly broken down. The suspensions were then diluted to 500 ml. with sterile distilled water and passed through sterile Berkefeld filters. These filtrates were exposed in Series G as endo-cellular extracts obtained by freezing.

The second portion of the B. mycoides cells was extracted by violent agitation for twenty minutes with petroleum ether. This petroleum ether extract was added directly to a sample of the 100 octane aviation stock over distilled water. The second portion of the Hormodendron (1193) cells was dispersed in distilled water. This suspension was then saturated with ammonium sulfate and extracted in chloroform. This chloroform extract was dissolved in petroleum ether and added to a sample of the aviation fuel over sterile distilled water.

In addition to the effects of these cellular extracts it was desirable to know the effect of dissolved air in the water phase on the rate of TEL precipitation. This led to the exposure of aviation gasoline samples in the usual manner over Haas Medium, except that the water phase was subjected to continual aeration without disturbing the gasoline by means of the apparatus illustrated on page 41. Samples which were aerated in this manner were inoculated with B. mycoides and Hormodendron (1193) in addition to an uninoculated control which was aerated in the same way. Neither of these organisms was recovered in living cultures at the completion of the test.

A group of gasoline storage tank waters (Nos. 1841, 1842, and 1843) was exposed under the aviation gasoline in this same series. Sterile and non-sterile samples of Luling oilfield brine* were included

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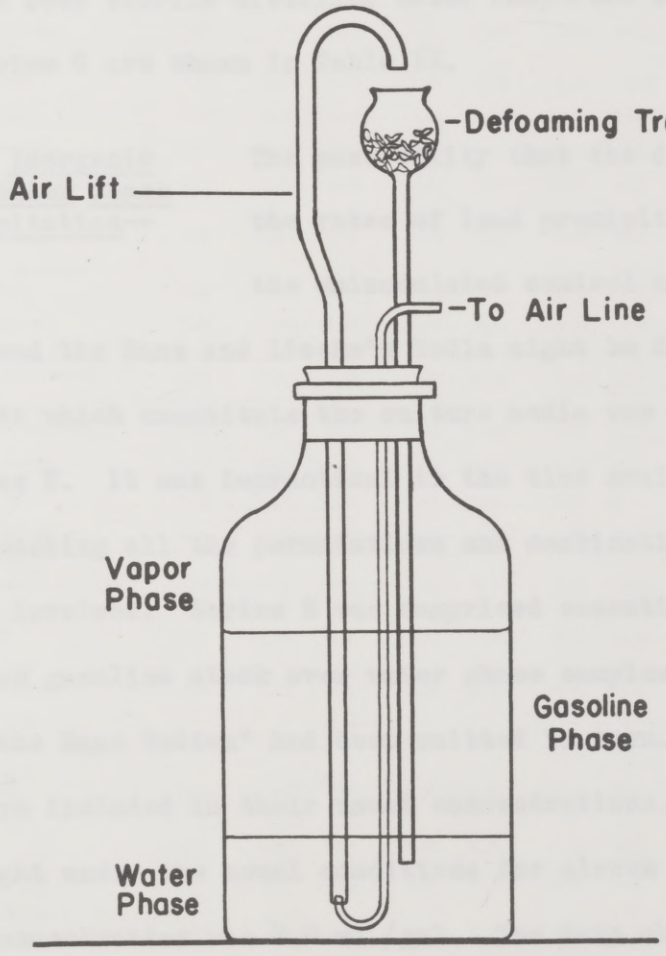
* Luling oilfield brine is reported to have the following chemical composition[26]:

Ion	Parts per million	
Calcium	1100	
Magnesium	368	
Sodium	2740	
Bicarbonate	318	
Sulfate	1518	
Chloride	5740	
Total solids	11800	pH--7.75

APPARATUS FOR AERATING WATER PHASE UNDER GASOLINE



in the series. A sample of the Luling brine which had been subjected to Berkefeld filtration, and a synthetic Luling brine which was prepared by dissolving inorganic salts in distilled water to reproduce the chemical analysis of the original brine, were also included in this series of waters.

An uninoculated sample of the 100 octane aviation stock exposed in the absence of a water phase (dry control) and another uninoculated sample exposed over sterile distilled water completed the series. The results of Series G are shown in Table IX.

The Effect of Inorganic Salts in the Water Phase on Lead Precipitation--Series H

The possibility that the discrepancy in the rates of lead precipitation between the uninoculated control samples of distilled water and the Haas and Lieske's Media might be due to the various inorganic salts which constitute the culture media was briefly investigated in Series H. It was impractical in the time available to set up samples representing all the permutations and combinations of the numerous ions involved. Series H was comprised essentially of 100 octane aviation gasoline stock over water phase samples in which each component of the Haas Medium* had been omitted in turn. The remaining components were included in their usual concentrations. Series H was exposed to light under the usual conditions for eleven days. The initial TEL concentration was 4.0 ml./gal. The data obtained from Series H are presented in Table X.

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* See footnote on page 13 for the composition of Haas Medium.

TABLE IX

THE EFFECT OF MICRO-ORGANISMS AND THEIR CELLULAR EXTRACTS ON

LEAD PRECIPITATION IN THE PRESENCE OF LIGHT

Time of Exposure	7 Days	14 Days		19 Days		25 Days		Water Phase Final	
Sample	Peroxide Number	TEL Conc.	Peroxide Number	TEL Conc.	Peroxide Number	TEL Conc.	Peroxide Number	Peroxide Number	pH
100 Octane Aviation Blend									
Mold Culture No. 1193 (Water Phase Aerated)	1.99	2.57	4.23	2.24	5.08	1.74	6.17	4.57	6.95
B. mycoides (Water Phase Aerated)	2.60	2.74*	3.87*	2.40**	3.99**	1.82***	5.06***	3.33	8.0
Control - Haas Medium (Water Phase Aerated)	1.83	2.62	5.08	1.85	5.75	1.31	7.28	5.18	7.3
B. mycoides	2.60	1.87	7.56	0.88	8.25	0.02	11.9	14.8	7.4
B. mycoides - Exocellular	3.16	2.07	8.42	1.00	9.08	0.45	11.9	6.9	8.02
B. mycoides Extracted by freezing	3.26	0.77	6.75	0	7.28	0.09	10.5	5.0	5.5
B. mycoides- Extracted In Petroleum Ether	3.16	0.74	5.94	0.30	6.18	0.08	9.25	4.81	5.45
Plain Broth Control	3.70	0.72	8.55	0.41	9.28	0	13.0	9.20	5.4
Mold Culture No. 1193	3.76	1.76	8.51	1.00	9.30	0.25	12.5	8.4	5.75
Mold No. 1193 Exocellular	4.33	1.09	9.37	0.47	9.65	0.09	12.5	4.32	4.95
Mold No. 1193 Extracted by Freezing	3.26	0.65	6.52	0.28	7.63	0.03	11.1	5.30	5.50
Mold No. 1193 - Extracted In Petroleum Ether	4.68	0.38	10.53	0.13	9.96	0.02	14.2	6.55	5.52
Maltose Broth Control	3.87	1.05	7.73	0.68	8.69	0.24	11.6	9.25	5.30
Control - Haas Medium Gasoline Berkfeld Filtered	4.63	0.82	9.90	0.49	10.53	0.01	13.6	12.5	6.8
Control - Haas Medium Gasoline Berkfeld Filtered	4.53	0.83	9.77	0.49	10.21	0.07	13.6	12.6	6.85
Control - Haas Medium	4.53	0.92	10.44	0.40	11.03	0.02	13.8	12.6	6.9
Control - Haas Medium	4.63	0.97	10.35	0.40	11.0	0.01	13.8	12.6	6.9
Control - Haas Medium	4.58	0.93	10.40	0.35	11.17	0.01	14.2	12.3	6.98
Tank Water No. 1841 (Untreated Cracked Distillate)	1.48	2.31	3.82	1.53	3.94	1.10	4.82	0.74	9.15
Tank Water No. 1841 Sterile	1.93	1.98	4.50	1.28	4.65	0.74	6.17	1.11	8.78
Tank Water No. 1842 (Finished Motor Gasoline)	3.00	1.82	8.01	1.18	9.40	0.59	12.8	10.0	6.85
Tank Water No. 1842 Sterile	3.16	1.64	7.65	0.89	9.04	0.34	13.0	9.25	6.70
Tank Water No. 1843 (Natural Gasoline)	2.65	1.86	7.74	1.23	9.04	0.63	10.6	5.56	10.05
Tank Water No. 1843 Sterile	2.85	2.19	6.98	1.22	7.64	0.53	8.15	5.18	9.70
Luling Oilfield Brine	3.00	2.00	7.47	1.23	9.17	0.59	11.9	9.65	7.25
Luling Oilfield Brine Sterile	3.26	1.84	6.43	1.07	7.85	0.53	10.4	7.16	7.40
Luling Oilfield Brine Berkfeld Filtered	2.49	2.26	6.62	1.49	8.21	0.82	10.4	10.6	7.30
Synthetic Luling Brine Sterile	3.16	1.75	6.62	0.88	7.90	0.28	9.63	6.67	6.90
Control - Distilled Water	3.10	1.80	6.12	0.82	7.77	0.36	8.77	4.17	5.90
Control - No Water Phase	4.17	2.12	10.89	1.04	13.10	0	16.1	-	-

* Exposed 9 days
** Exposed 12 days
*** Exposed 20 days

Initial Lead Tetraethyl Concentration was 3.79 ml/gal.

TABLE X

EFFECT OF INORGANIC SALTS IN WATER PHASE ON LEAD PRECIPITATION

Sample	TEL Remaining
Distilled water	2.15 ml. TEL/gal.
Double strength Haas Medium	2.14 "
Haas Medium less phosphate buffers	1.98 "
Haas Medium less $CaCl_2$	1.84 "
Haas Medium	1.64 "
Haas Medium less $MgSO_4$	1.61 "
Haas Medium less NH_4NO_3	1.60 "
Haas Medium less $FeCl_3$	1.33 "
N/20 Hydrochloric acid	1.94 "
N/20 Sodium Hydroxide	1.78 "

Note: All samples were exposed for eleven days and had an initial concentration of 4.00 ml. TEL/gal.

It is evident that inorganic salts dissolved in the water phase below aviation gasolines in storage may exert some effect on the rate of TEL precipitation. This possibility appears worthy of further investigation. In the present work the effect of dissolved salts was not of consequence because all samples were compared to a control which contained the same inorganic components.

The Effect of B. mycoides and Hormodendron (1193) and Their Cellular Extracts on TEL Precipitation in the Dark--Series I

A series consisting of B. mycoides, Hormodendron (1193), their exo-cellular extracts and the necessary control samples was exposed in the dark in order to investigate these factors under conditions which more closely approach industrial storage conditions. These results are shown in Table XI. The data indicate that in the dark B. mycoides might be expected to precipitate all of the original 3.67 ml. TEL/gal. in about 160 days as compared to 352 days for the same uninhibited aviation fuel over distilled water.

B. mycoides and Hormodendron (1193) were both recovered in pure culture at the completion of the tests and grew vigorously in plain broth. The B. mycoides culture had undergone a slight variation in colonial character although it still retained the feathery spreading type of growth on plain agar.

Tests to Determine the Effects of Dissolved Air in the Water Phase on the Precipitation of TEL from Aviation Gasolines--Series J

It was found in Series G that the continued aeration of the water phase below aviation gasoline was inhibitory to the precipitation of TEL, in the presence or absence of microorganisms, compared to similar samples in which the water phase was not

TABLE XI

THE EFFECT OF MICRO-ORGANISMS AND THEIR CELLULAR EXTRACTS ON LEAD PRECIPITATION IN THE ABSENCE OF LIGHT

Lead Tetraethyl Concentrations (ml. TEL/gal.)

Time of Exposure	14 Days	25 Days	40 Days	47 Days
Sample				
100 Octane Aviation Blend				
B. mycoides	3.40	3.25	2.91	2.83
Hormodendron (1193)--Exo-cellular	3.45	3.39	3.00	--
Hormodendron (1193)	3.50	3.39	2.93	2.90
B. mycoides--Exo-cellular	--	3.48	3.18	3.16
Haas Control	3.50	3.45	3.12	2.98
Distilled Water Control	3.66	3.52	3.32	3.25
Plain Broth Control	3.70	3.45	3.32	3.37
Maltose Broth Control	--	3.72	3.51	3.48

Note: The original concentration was 3.67 ml. TEL/gal.

aerated. This effect was investigated further by a series of three samples. The first of these was comprised of a sample of 100 octane stock over distilled water which was aerated continually using the air-lift device illustrated on page 41. A second sample was also composed of aviation gasoline and distilled water. The water phase in this sample was not subjected to aeration during the first five days of exposure. After this first five day period the water phase of the second sample was aerated in the same manner as the first sample in the series. The purpose of this second sample was to determine whether or not aeration of the water phase would inhibit the precipitation once it was started. The third sample in the series was made up of aviation gasoline over M/15 solution of pyrogallol which served to deaerate the water phase. The data obtained from Series J is shown in Table XII.

Tests to Determine Whether Precipitation of TEL Proceeds by a Chain Reaction Mechanism--Series K

In order to determine the role of bacteria in the precipitation of TEL from heavily leaded aviation gasolines it is necessary to know something of the chemical mechanism by which the lead is precipitated. It is known that the precipitation is strongly catalyzed by ordinary daylight. Statements have been made that the precipitation is not autocatalytic[22]. Series K was set up to determine the reliability of the non-autocatalytic hypothesis. This series was composed of four identical samples of the 100 octane aviation stock which were exposed to daylight for five days. The samples consisted of 150 ml. of gasoline in 275 ml. glass stoppered bottles. After five days

TABLE XII

THE EFFECT OF DISSOLVED AIR ON

LEAD PRECIPITATION FROM 100 OCTANE AVIATION GASOLINES

(Concentration of TEL--ml. TEL/gal.)

Time of Exposure	5 Days	10 Days	14 Days	15 Days
Sample				
Aviation Gasoline				
Water phase aerated throughout test	3.77	3.54	3.28	--
Water phase aerated after five days' exposure	3.55	3.10	2.78	2.65
Distilled water plus M/15 pyrogallol	3.40	2.90	2.49	--

Note: The initial concentration was 4.00 ml. TEL/gal.

in the light one of the samples was analyzed for TEL. The remaining samples were placed in the dark. These samples were analyzed after respective periods in the dark of five, ten and fourteen days. The results obtained in Series X are tabulated below.

Total Time of Exposure in both Light and Dark	Unexposed	5 Days	10 Days	15 Days	19 Days
First Determination	4.00	3.61	3.32	3.16	3.12
Second Determination	4.00	3.59	3.32	3.13	3.09

These data indicate quite definitely that the precipitation of TEL from aviation gasolines proceeds by a photo-catalyzed chain reaction.

INTERPRETATION OF RESULTS

The two most important problems in the deterioration of motor and aviation gasolines during storage are (1) the precipitation of TEL from heavily leaded aviation gasolines in the form of various lead salts and (2) the formation of gum in motor fuels which contain unstable olefins and diolefins. Both these forms of deterioration are retarded by the addition of the several commercial gasoline inhibitors, such as tricresol and N-n-butyl-p-aminophenol (duPont No.5). The inhibitors in common use areparticularly effective in delaying the formation of peroxides and consequently the formation of gum in motor blends which contain unstable hydrocarbons. The same inhibitors are less effective in preventing or delaying the precipitation of TEL. The fact that the inhibitors were to some extent effective in retarding both reactions has led to the general conclusion in the industry that the two deteriorations must proceed by similar mechanisms. Work in these laboratories has failed to indicate that there is any evident relationship between peroxide concentration and lead precipitation. It was also found that the precipitation was actually inhibited by aerating the water phase beneath the gasoline and accelerated by deaerating the water phase. The reverse had been found true in the case of gum formation[2]. The problems of lead precipitation and gum formation are therefore considered to be independent phenomena.

The Role of Bacteria in the Formation of Gum and Peroxides

It has been amply demonstrated in the bacteriological literature that bacteria are capable of oxidizing hydrocarbons. It is also well established that biological oxidations in general proceed in a stepwise manner through a series of progressively higher oxidation products. Thus the initial oxidation product in the case of unsaturated paraffinic hydrocarbons would be the peroxide. Bacteria are capable of producing organic peroxides by several possible mechanisms. It is reported that there is a marked thermodynamic activation of the double bonds in an unsaturated organic molecule which is acted upon by a bacterial cell[7]. Such an activation of an olefin or diolefin would render it extremely susceptible to autoxidation to the peroxide. The common bacterial enzyme, oxidase, is characterized by its formation of organic peroxides. An increase in oxygen concentration, or the activation of dissolved oxygen as a result of bacterial action must also be considered.

Thus it is inferred that bacteria are capable of initiating the deterioration of gasoline and other petroleum products in storage by the production of peroxides. Since bacteria normally make use of the chemical bonds which require the least energy of activationit is to be expected that the conjugate diolefins will be the first to be peroxidized. Peroxides act as particularly active catalysts in addition reactions to the double bonds of olefinic hydrocarbons. Thus unstable hydrocarbon peroxides serve as autogenous catalysts to the addition of free oxygen to other double bonds to form additional hydrocarbon peroxides[11]. Peroxides produced by both these processes are then available

to (1) serve as autogenous catalysts to the formation of further peroxides by a strictly chemical mechanism independent of biological activity, (2) to polymerize by 1-4 addition to form gum, (3) to be reduced in the presence of some oxygen acceptor by some enzyme such as peroxidase, or (4) to undergo further oxidation to the aldehyde and acid.

Haas (loc. cit.) reports that the bacterial oxidation of hydrocarbons goes to completion yielding CO_2 and H_2O. Haas' work was done under strictly aerobic conditions with a limited amount of hydrocarbon. Under the conditions employed in the present work, which more closely approach industrial storage conditions, it seems likely that the bacterial oxidation of hydrocarbons will produce predominantly the intermediate, or lower products of oxidation such as the peroxide. A concentration of lower oxidation products is favored by the mass action principle since the energy of the carbon-oxygen bond exceeds that of the carbon to carbon linkages, making it easier for the bacteria to oxidize other carbon to carbon bonds than to continue the oxidation of the carbon-oxygen bond.*

It must be remembered that many microorganisms also produce the enzyme peroxidase which destroys organic peroxides. The bacterial oxidation of hydrocarbons is therefore highly subject to the particular conditions and to the organisms which are present.

The C-H-O analyses of gasoline gums show a rough average of 70% carbon, 7% hydrogen and 20% oxygen, with a molecular weight ranging from 200 to 35033. This suggests that gum consists essentially of

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* Pauling, L. C., The Nature of the Chemical Bond, Cornell University Press, Ithica, N. Y., (1939).

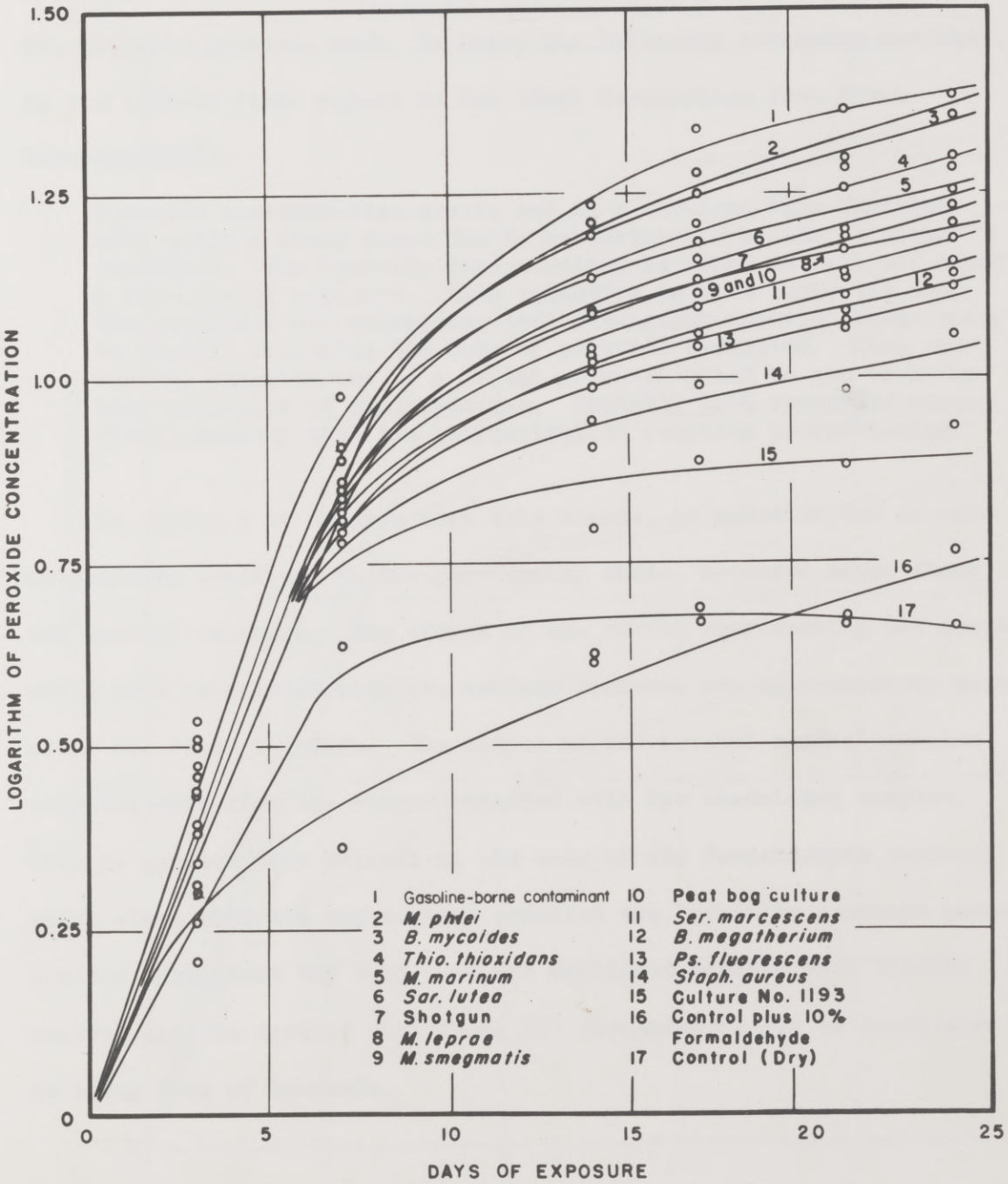
dimers and trimers of peroxidized olefins and diolefins of the gasoline range of molecular weight. Gum is present in gasoline in the form of a colloidal dispersion which gradually settles from the dispersion medium in the form of a yellow-brown viscous non-volatile liquid.

The differential effect of various bacteria which may be suspected as likely contaminants in gasoline storage tank waters is shown in Table V. These data are also presented graphically on page 54 by plotting the logarithm of peroxide concentration against time of exposure. The wide variations in peroxide values far exceeds the margins of error of reproducibility for the method of exposure and determination of peroxides. A statistical study of the error of reproducibility is included on page 20.

It will be noted that the dry control and the control sample exposed over ten per cent formaldehyde are the lowest in peroxide concentration. As has been mentioned earlier in this report, these two samples may be considered to be free of biological activity. The highest peroxide concentration was observed in the sample which contained the gasoline-borne contaminant, Bacillus A. It is assumed that this organism was transmitted in the gasoline stock, probably in the form of the bacterial spore. In such a case it would probably have been previously adapted to the utilization of hydrocarbons and thus be capable of producing a greater detrimental effect.

The data for several of the samples in Series C (Table V) have been replotted on page 56. It will be noted that there appear to be two distinct sections to the curve for peroxide numbers or their logarithms plotted against time. This is interpreted as being the result of two

EFFECT OF BACTERIA ON PEROXIDE FORMATION IN AN UNINHIBITED GASOLINE-MOTOR BLEND

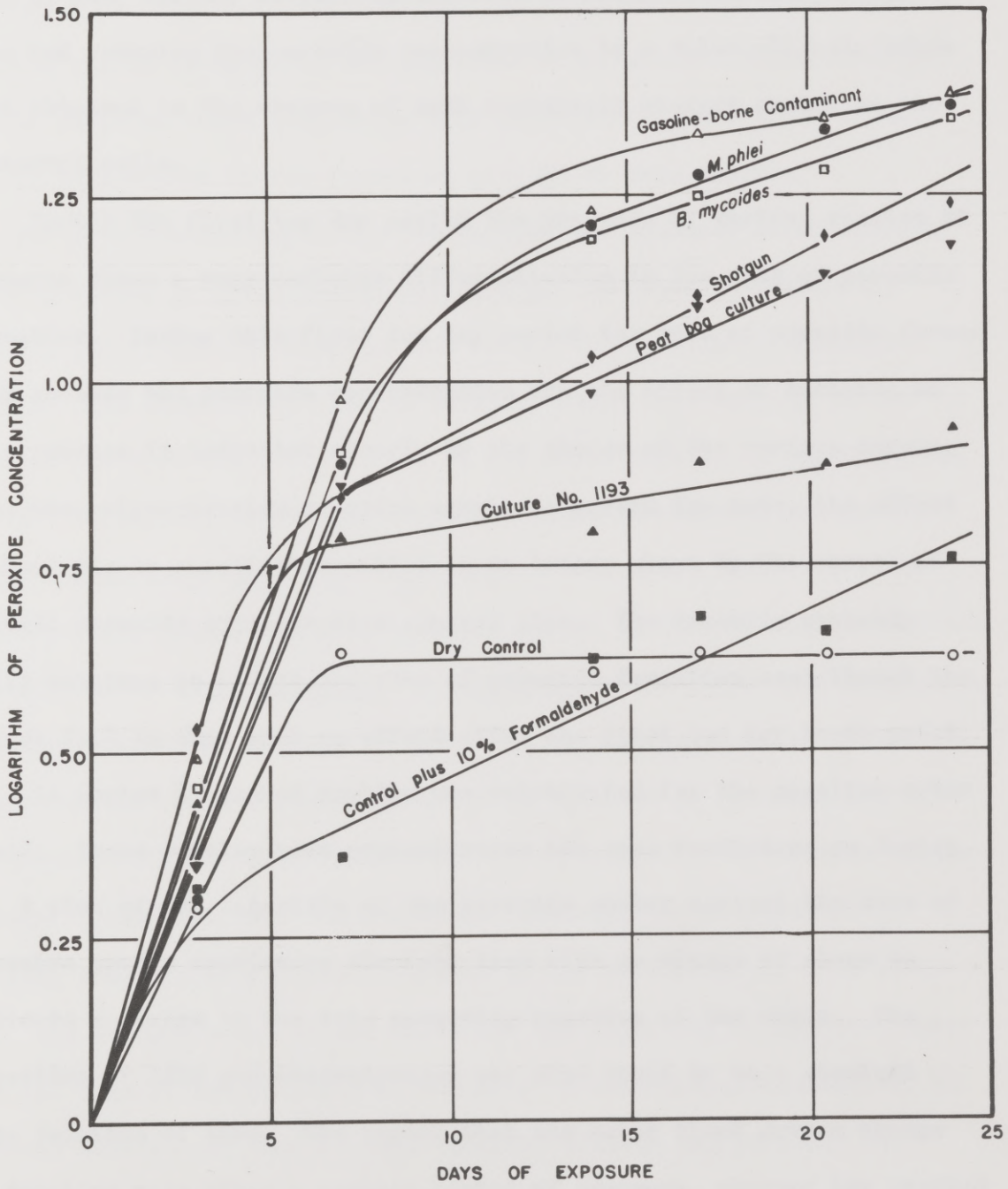


separate reactions in the gum-forming reaction chain. Thus one reaction would govern the overall rate from the start until some concentration of products permits a subsequent reaction in the chain to govern the overall rate. This same type of change in reaction rate was observed in the writer's previous work, to which the following reference was made in the 1941-42 final report to the Ethyl Corporation from these laboratories[2]:

> Peroxide concentration starts out as a straight line function with quite a steep slope due to autocatalysis by the existing peroxides. The peroxide concentration is found to fall off after a few days of exposure. This reduction in rate indicates that the peroxides are undergoing some subsequent reaction whose rate is finally exceeding the rate of peroxide formation. This succeeding reaction may be a direct polymerization to gum or a further oxidation of the peroxides. Probably both reactions occur simultaneously with the polymerization reaction predominating.

In Series C it appears that this change, or substitution of rate determining reactions in the gum-forming chain, occurred after about ten days of exposure. The slopes of the curves representing the samples which were inoculated with the various bacteria are approximately equal after the first ten days. The slopes of the several control samples vary somewhat from the slopes obtained with the inoculated samples. This is particularly evident in the case of the formaldehyde control, which along with the dry control remained the lowest in peroxide concentration throughout the test. As was mentioned earlier both the dry control and the control containing 10% formaldehyde may be considered as being free of bacteria.

EFFECT OF BACTERIA ON PEROXIDE FORMATION IN AN UNINHIBITED GASOLINE MOTOR BLEND



The fact that the slopes of the inoculated samples after the first ten days of exposure remain almost equal indicates that the presence of bacteria has only a slight effect on the rate of the polymerization reaction. Possibly the bacterial cells serve as nuclei for the coagulation of gum, thereby increasing the rate of coagulation and polymerization and reducing the peroxide concentration to a value slightly below that obtained in the absence of such negatively charged nuclei as the bacterial cells.

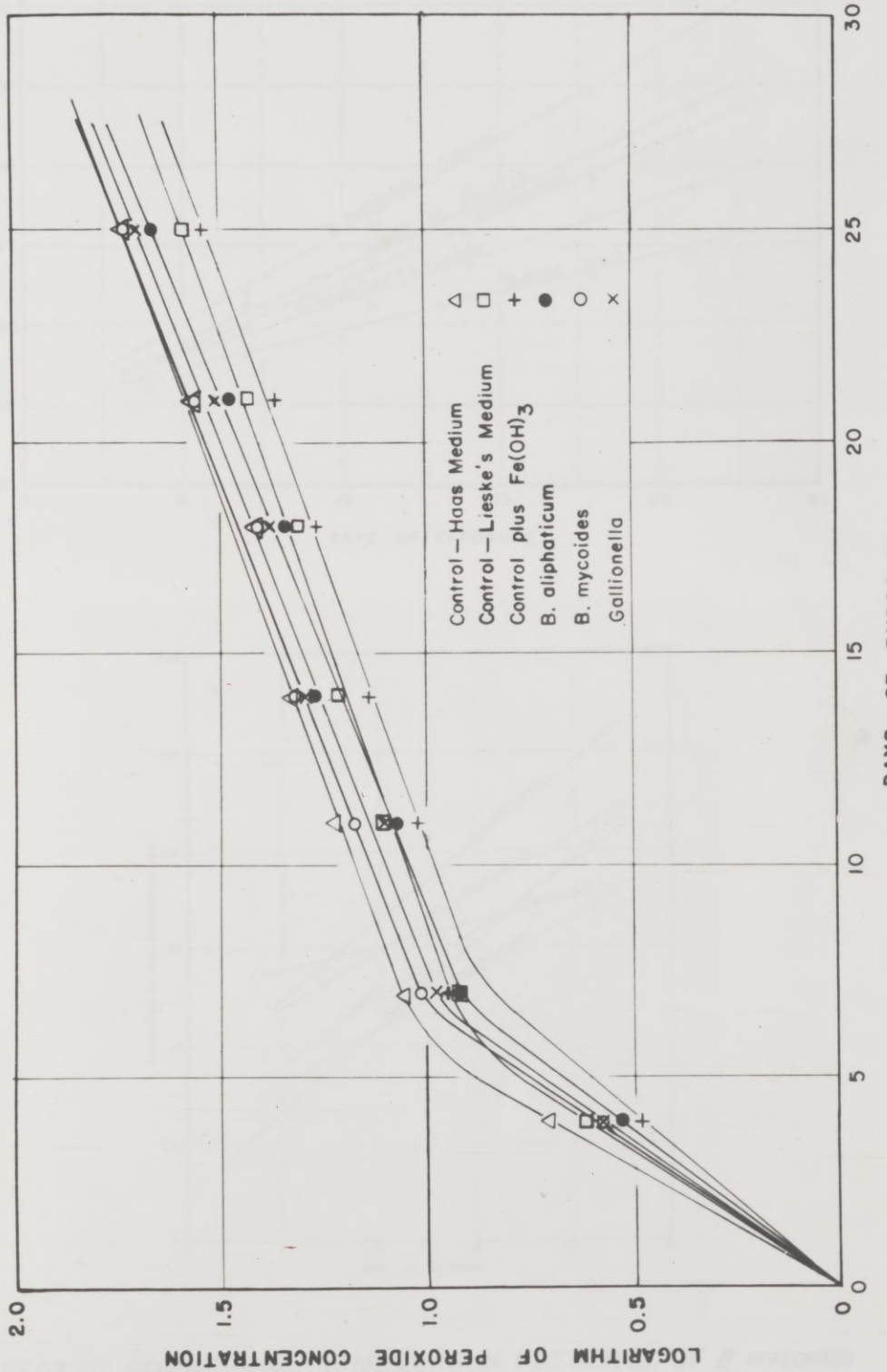
During the first ten day period the presence of various species of bacteria shows a very definite differentiation in the rate of peroxide formation. During this first ten day period the rate of peroxide formation governs the peroxide concentration and the effect of bacteria on this process is indicated clearly by the slopes of the various curves. When the polymerization reaction starts to govern the rate, the effect of bacteria on peroxide formation is no longer shown by the curves of overall peroxide concentration against time. The bacteria probably still continue to affect the rate of peroxide formation even though the curves fail to show such an effect after the first ten day break point.

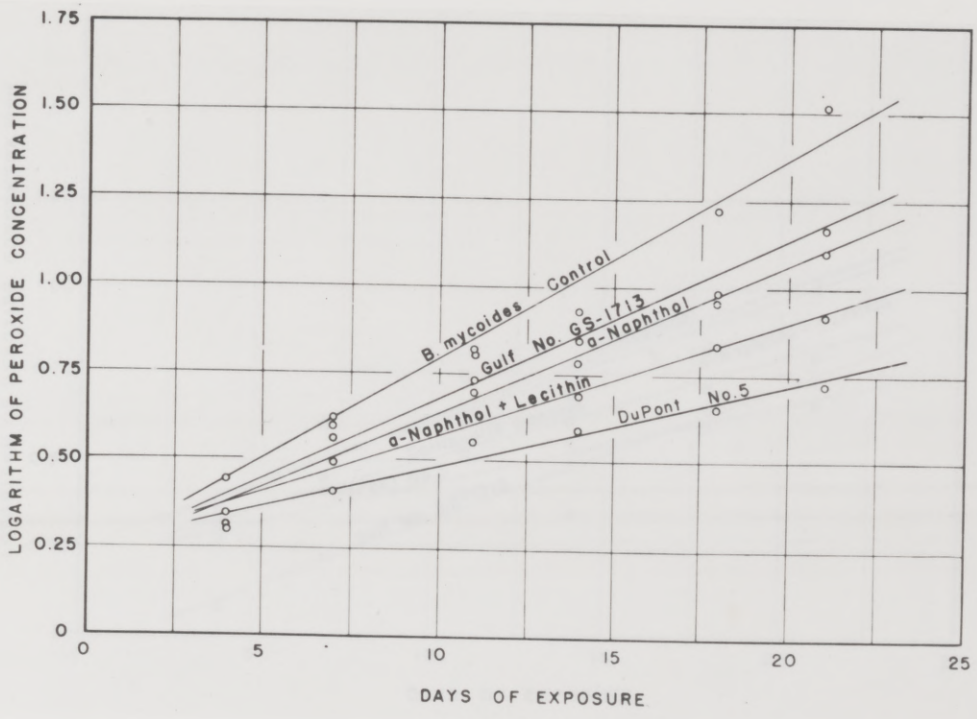
In Series B cracked naphtha was substituted for the gasoline motor blend. These samples were exposed under the same conditions as Series C. A plot of the logarithm of the peroxide number against the time of exposure gave a continuous straight line with no change of slope to indicate a change in the rate governing reaction of the chain. The logarithm of ASTM gum concentration was also found to be a straight line function of time. The reason that the motor blend gave a change in reaction rate after a certain period of exposure, whereas the cracked

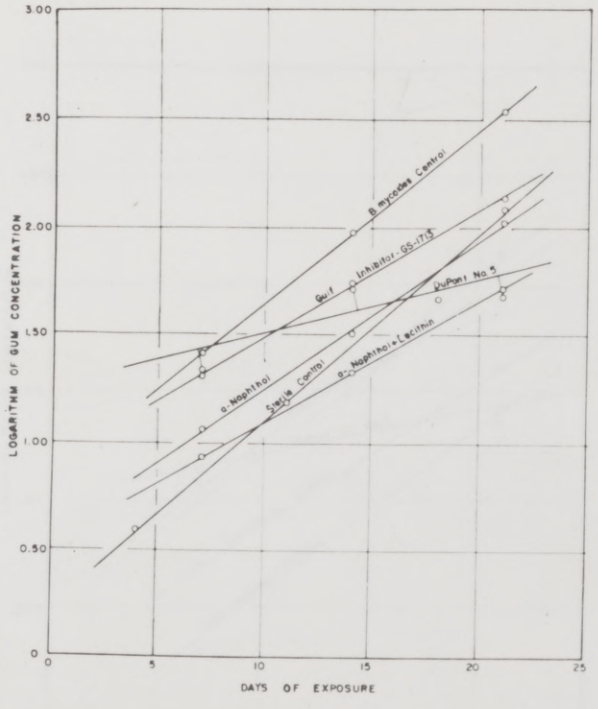
stock component of the same blend did not exhibit such a change in rate, is difficult to explain. It is suggested, however, that the higher concentrations of unsaturates in the cracked gasoline cause the polymerization reaction to become predominant after only a very short period of exposure. It is interesting that the 100 octane aviation blends gave a definite change in slope of the logarithm of peroxide concentration vs. time of exposure curve similar to that observed with the motor blend. This is illustrated in the figure on page 59 of this report.

Series D was designated to investigate the combined effect of two microorganisms, B. mycoides and Gallionella, and several commercial gasoline inhibitors on the formation of gum and peroxides in cracked naphtha. The results obtained on samples, which were exposed to the light in the same manner as Series C, are shown plotted on pages 60, 61, and 62. It will be noted that all the inhibitors retarded the detrimental effect of B. mycoides. The samples which were inhibited with a-naphthol and a-naphthol plus lecithin appear to have an opposite effect in the presence of Gallionella. Gallionella, which is an iron bacterium, acutally produces an inhibiting effect on gum formation in unblended cracked stock. This effect apparently is enhanced by the addition of 2,6-di-tert.-butyl-4-methylphenol (Gulf No. GS-1713). No sterile sample containing the inhibitor was provided. However, the presence of B. mycoides showed a very marked decrease in the efficiency of this inhibitor. The unusual slope of the curve for the logarithm of gum concentration vs. time in the presence of N-n-butyl-p-aminophenol (duPont No. 5) suggests that it may inhibit some reaction between the peroxide and

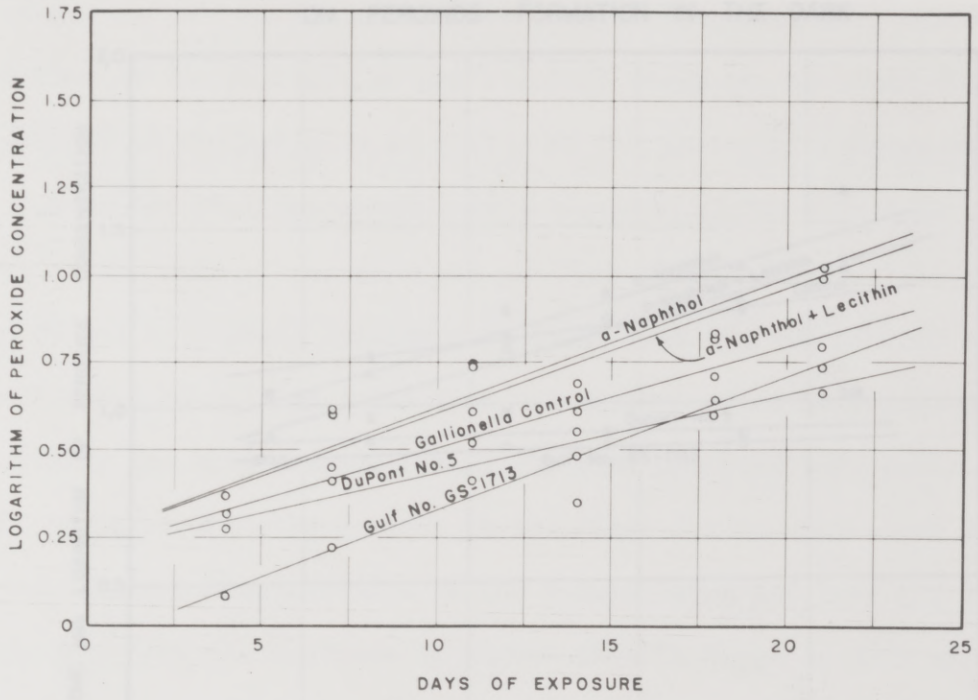
FORMATION OF PEROXIDES IN 100 OCTANE AVIATION GASOLINE

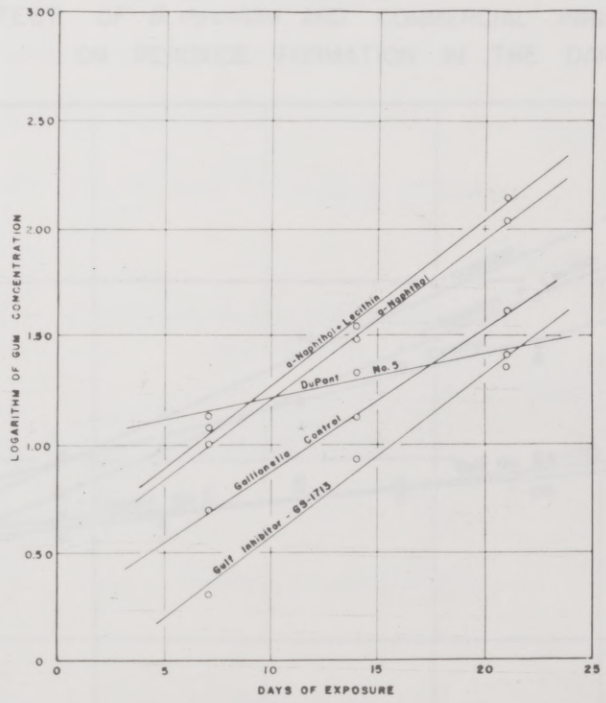






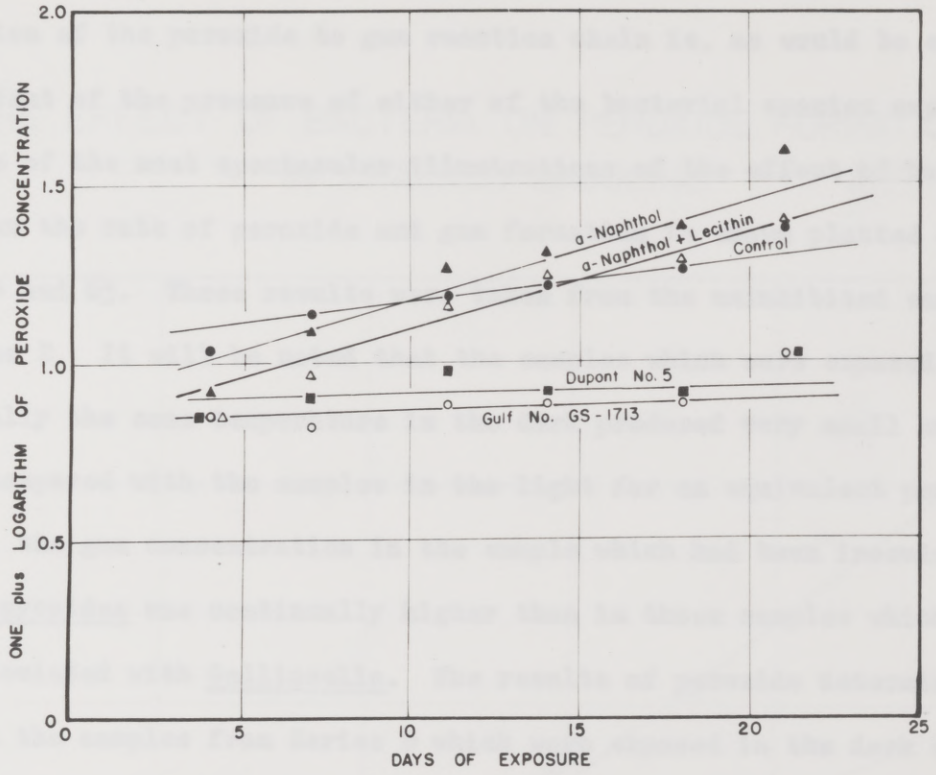
EFFECT OF COMMERCIAL INHIBITORS IN PRESENCE OF *B. mycoides*



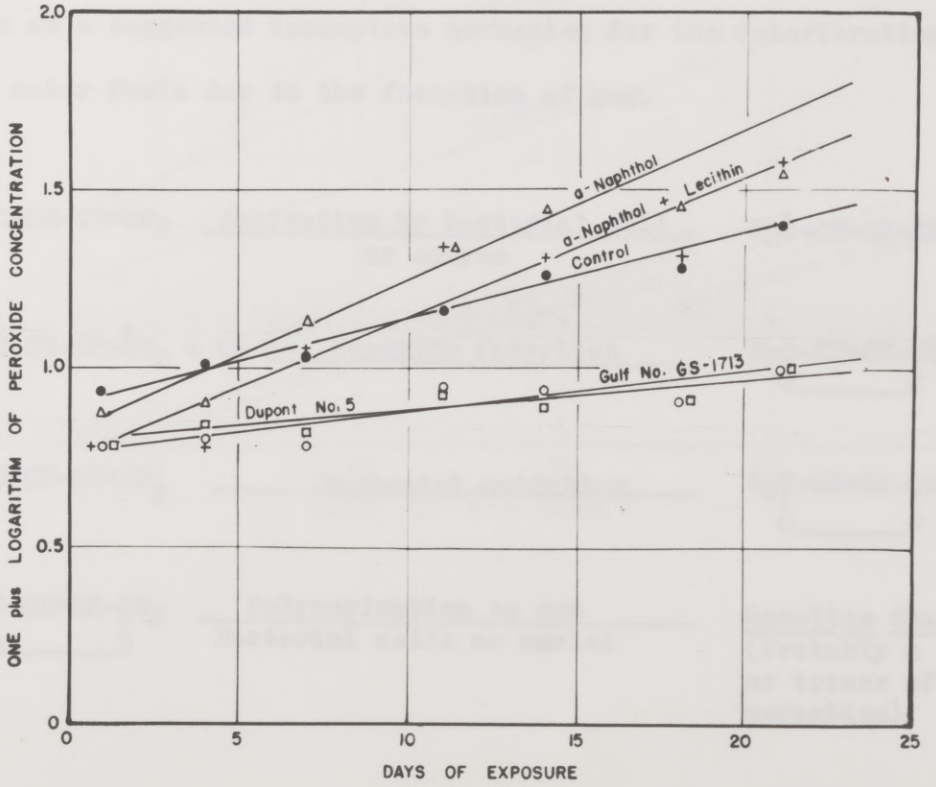


EFFECT OF COMMERCIAL INHIBITORS IN PRESENCE OF *Gallionella*

EFFECT OF *Gallionella* AND COMMERCIAL INHIBITORS ON PEROXIDE FORMATION IN THE DARK



EFFECT OF *B. mycoides* AND COMMERCIAL INHIBITORS ON PEROXIDE FORMATION IN THE DARK



gum stages as well as inhibiting the initial formation of peroxides. This inhibition of the peroxide to gum reaction chain is, as would be expected, independent of the presence of either of the bacterial species employed.

One of the most spectacular illustrations of the effect of bacterial action on the rate of peroxide and gum formation is shown plotted on pages 64 and 65. These results were taken from the uninhibited samples in Series D. It will be noted that the samples which were exposed at essentially the same temperature in the dark produced very small amounts of gum compared with the samples in the light for an equivalent period. However, the gum concentration in the sample which had been inoculated with B. mycoides was continually higher than in those samples which had been inoculated with Gallionella. The results of peroxide determinations on the samples from Series D which were exposed in the dark are shown plotted on page 62 of this report.

The interpretation of the foregoing data leads to the following reactions as a suggested incomplete mechanism for the deterioration of gasoline motor fuels due to the formation of gum.

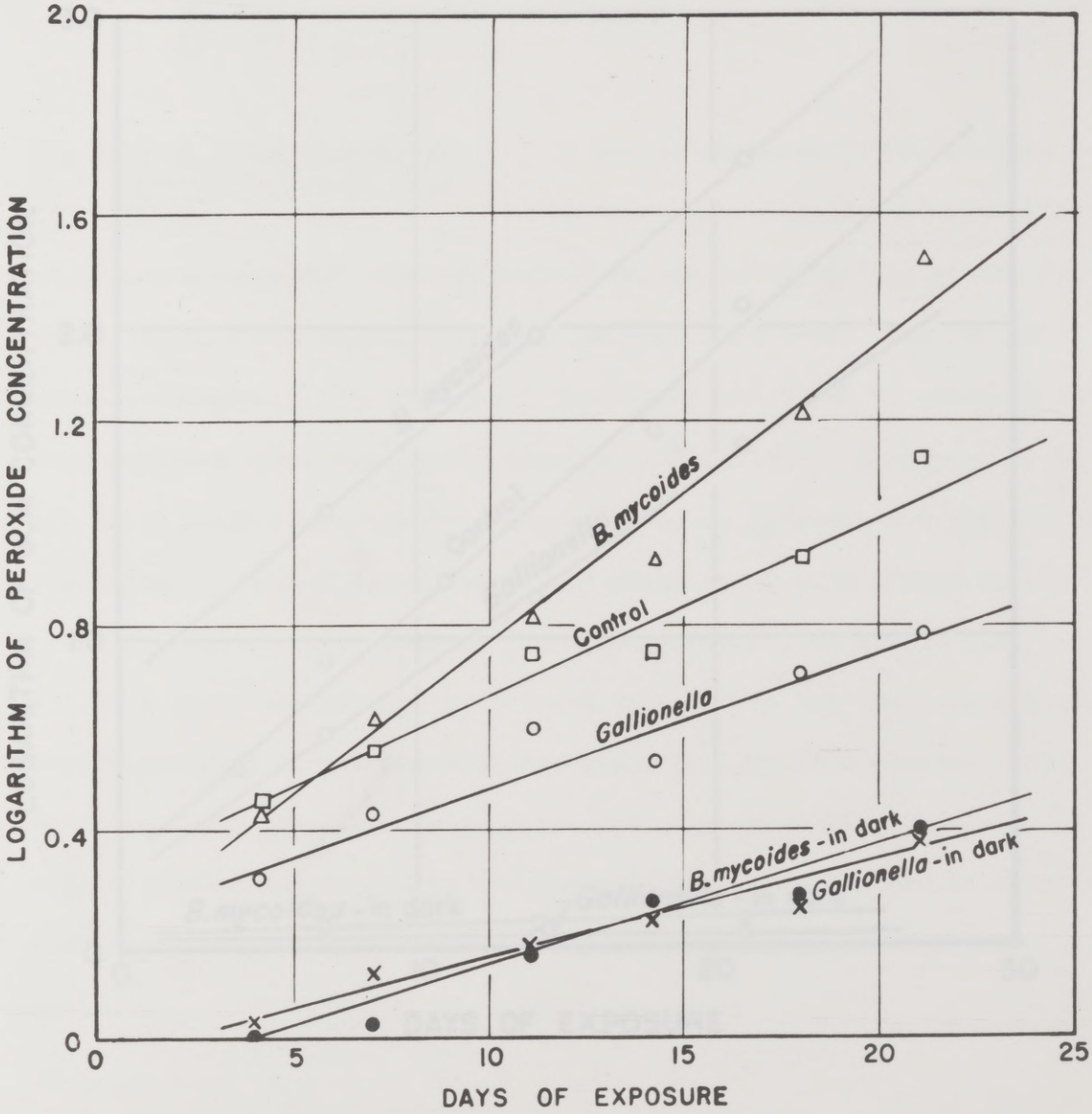
(A-1) $R_2C{=}CH{-}CH{=}CR_2$ —Activation by bacterial cell or enzyme— $R_2\dot{C}{-}CH{-}CH{-}\dot{C}R_2$

$R_2\dot{C}{-}CH{-}CH{-}\dot{C}R_2 + O_2$ —Peroxide formation— $R_2C(O{-}O){-}CH{=}CH{-}CR_2$

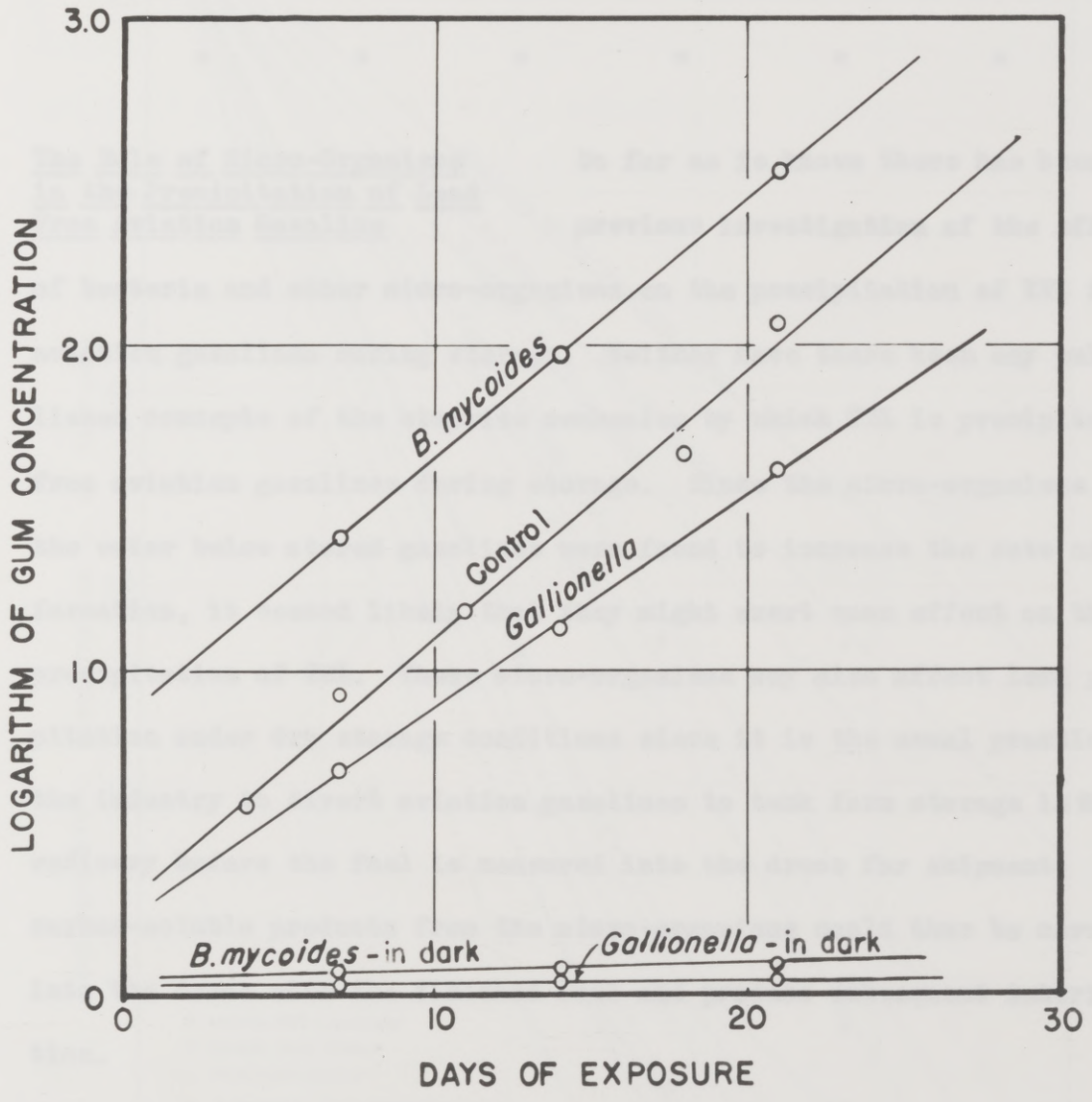
(A-2) $R_2C{=}CH{-}CH{=}CR_2$ —Bacterial oxidation— $R_2C(O{-}O){-}CR{=}CH{-}CR_2$

(B) $R_2C(O{-}O){-}CH{=}CH{-}CR_2$ —Polymerization to gum, Bacterial cells as nuclei— Gasoline Gum (Probably a dimer or trimer of the peroxides)

THE EFFECT OF BACTERIA ON PEROXIDE FORMATION IN CRACKED GASOLINE



THE EFFECT OF BACTERIA ON GUM FORMATION IN CRACKED GASOLINE



It is likely that the polymerization forms a long chain polymer which is high enough in oxygen to render it relatively insoluble in the gasoline. The polymerization stops at a molecular weight of 200 to 350, possibly due to the dissipation of the energy of activation throughout the large polymeric molecule.*

* * * * * *

The Role of Micro-Organisms in the Precipitation of Lead From Aviation Gasoline

So far as is known there has been no previous investigation of the effect of bacteria and other micro-organisms on the precipitation of TEL from aviation gasolines during storage. Neither have there been any published concepts of the stepwise mechanism by which TEL is precipitated from aviation gasolines during storage. Since the micro-organisms in the water below stored gasolines were found to increase the rate of gum formation, it seemed likely that they might exert some effect on the precipitation of TEL. These micro-organisms may also affect lead precipitation under dry storage conditions since it is the usual practice in the industry to divert aviation gasolines to tank farm storage in the refinery before the fuel is measured into the drums for shipment. Hydrocarbon-soluble products from the micro-organisms could thus be carried into the drums with the finished fuel and produce subsequent deterioration.

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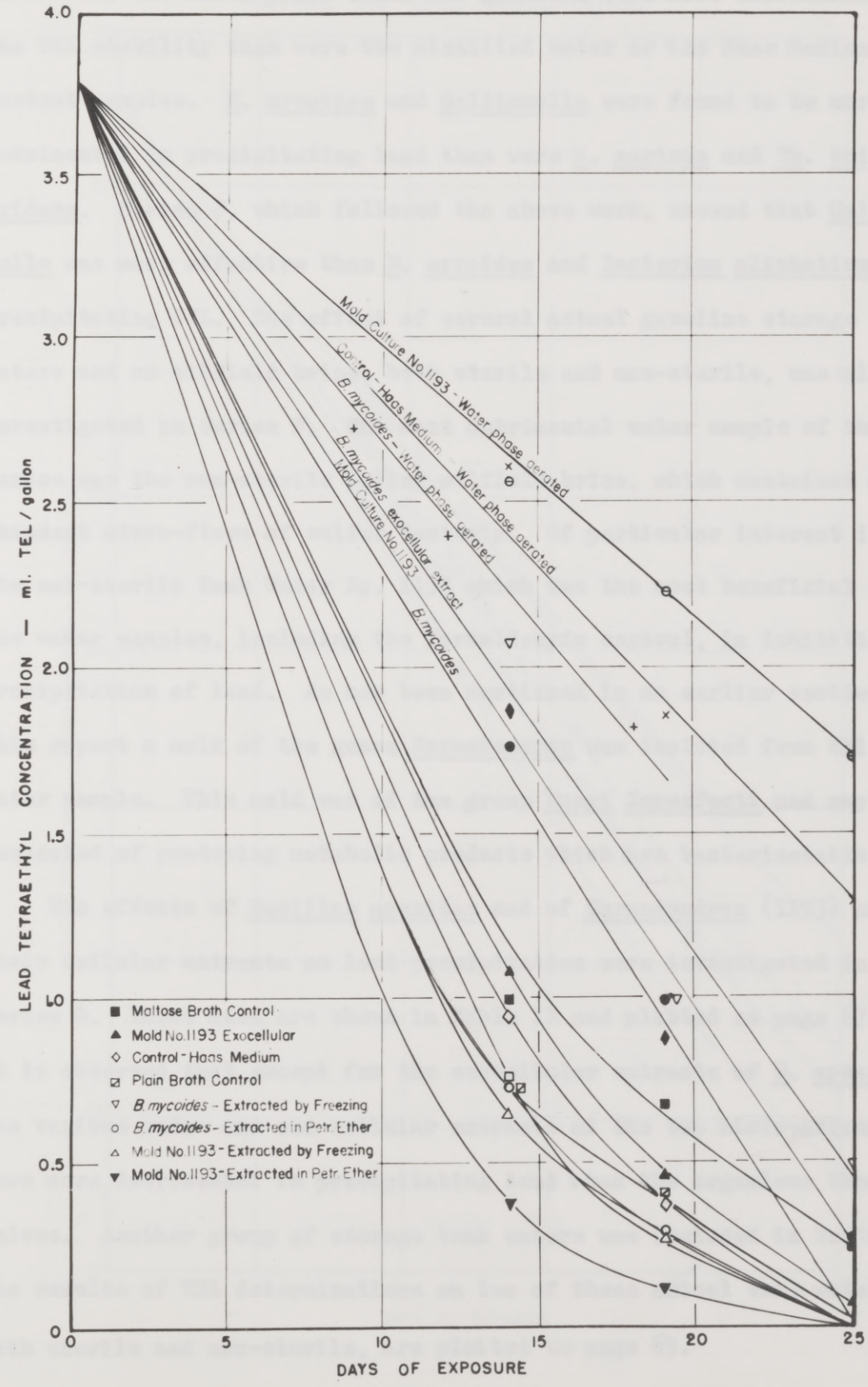
* See Gilman, H., Organic Chemistry--An Advanced Treatise. Wiley, New York, (1943), p. 701 ff.

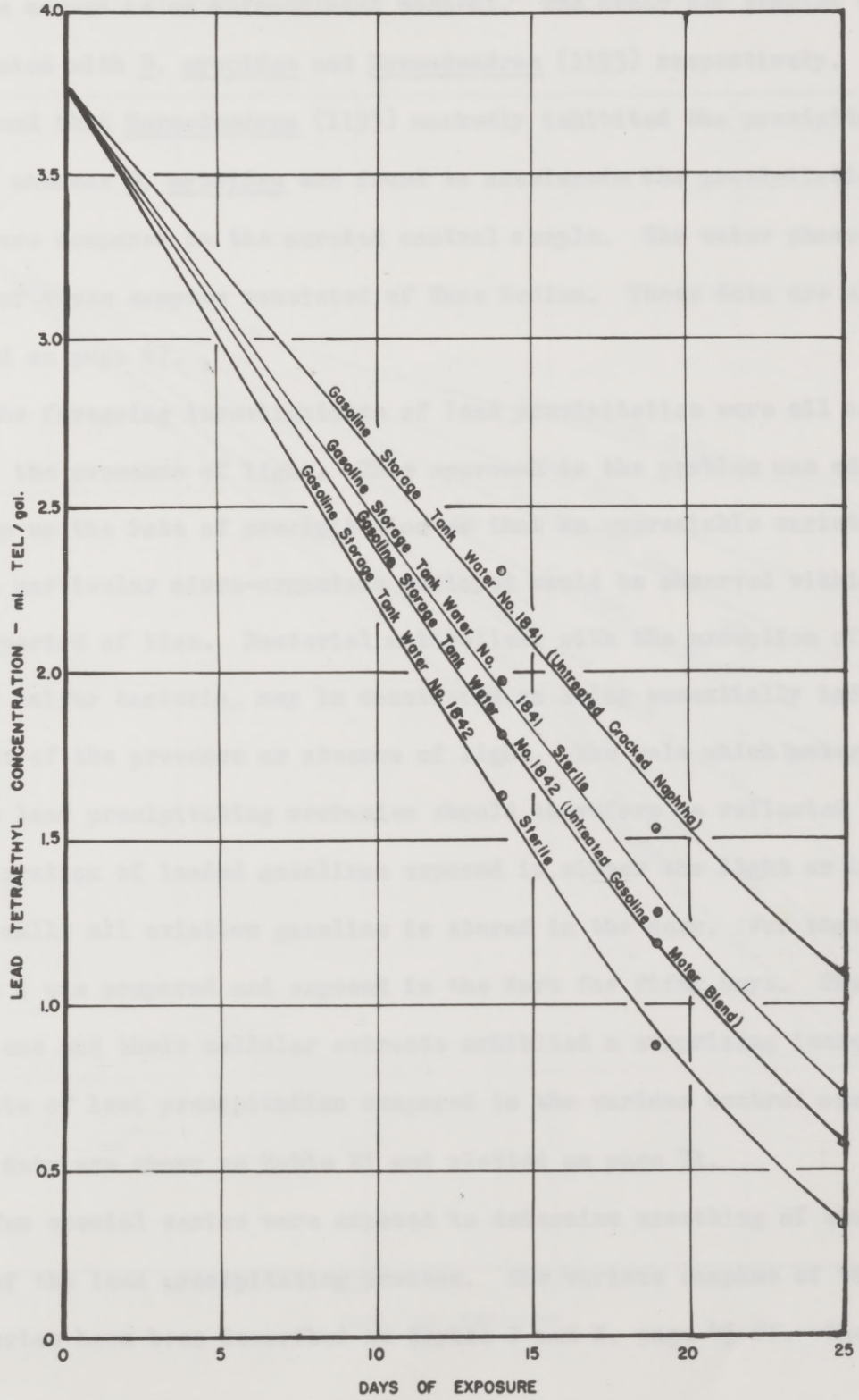
THE EFFECT OF MICRO-ORGANISMS AND THEIR CELLULAR EXTRACTS ON LEAD PRECIPITATION IN THE PRESENCE OF LIGHT



The data from Series E showed that all four of the organisms inoculated into the water phase below the gasoline were more detrimental to the TEL stability than were the distilled water or the Haas Medium control samples. B. mycoides and Gallionella were found to be more detrimental in precipitating lead than were M. marinum and Th. thiooxidans. Series F, which followed the above work, showed that Gallionella was more effective than B. mycoides and Bacterium aliphaticum in precipitating TEL. The effect of several actual gasoline storage tank waters and an oilfield brine, both sterile and non-sterile, was also investigated in Series F. The most detrimental water sample of the series was the non-sterile Luling oilfield brine, which contained an abundant micro-flora of sulfur bacteria. Of particular interest is the non-sterile Tank Water No. 1193 which was the most beneficial of all the water samples, including the formaldehyde control, in inhibiting the precipitation of lead. As has been mentioned in an earlier section of this report a mold of the genus Hormodendron was isolated from this water sample. This mold was of the group Fungi Imperfecti and may be suspected of producing metabolic products which are bacteriostatic.

The effects of Bacillus mycoides and of Hormodendron (1193) and their cellular extracts on lead precipitation were investigated in Series G. These data are shown in Table IX and plotted on page 67. It is observed that except for the exocellular extracts of B. mycoides, the various endo- and exo-cellular extracts of the two micro-organisms were more detrimental in precipitating lead than the organisms themselves. Another group of storage tank waters was included in Series G. The results of TEL determinations on two of these actual tank waters, both sterile and non-sterile, are plotted on page 69.

EFFECT OF ACTUAL GASOLINE STORAGE TANK WATERS ON LEAD PRECIPITATION

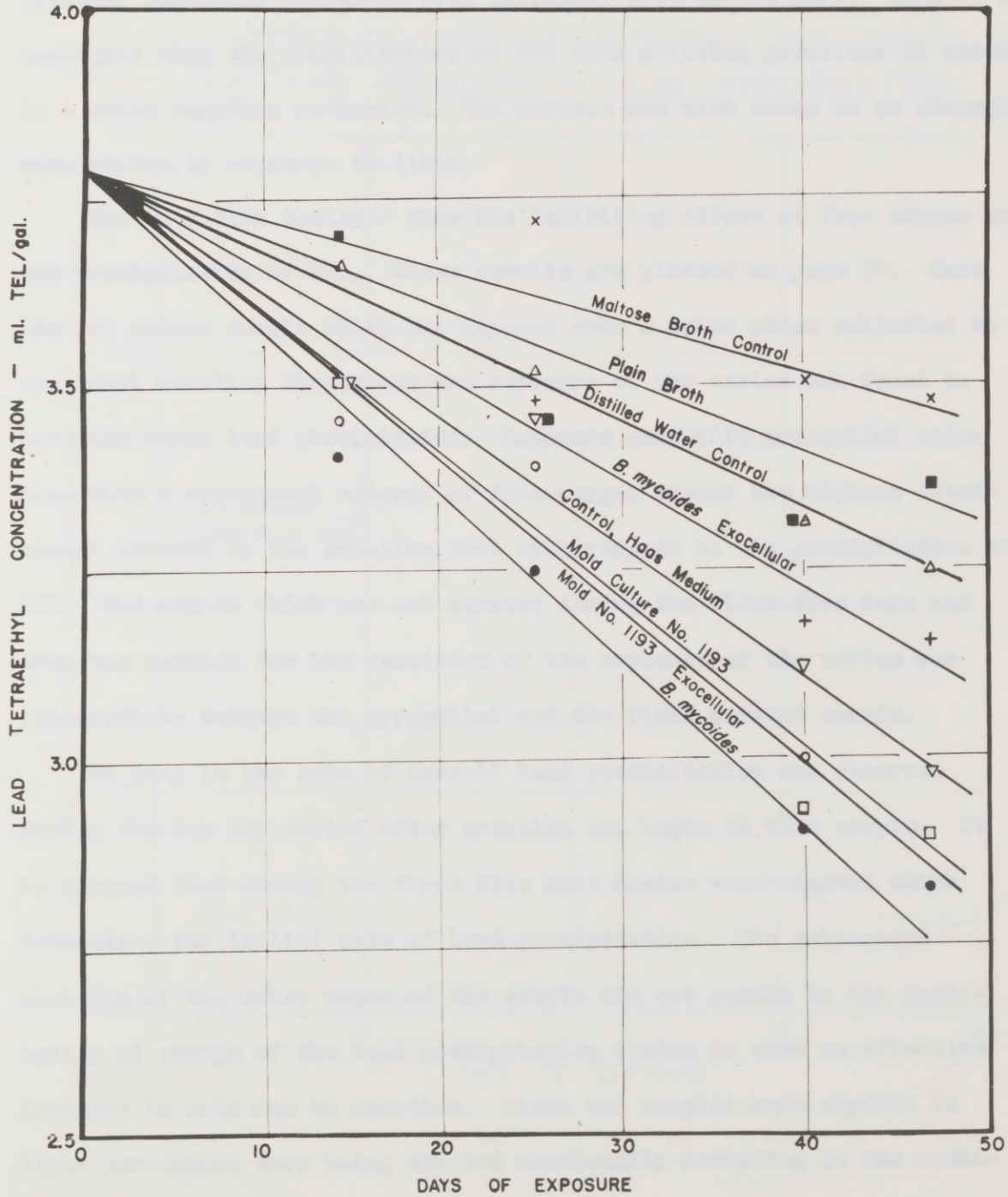


The water phases of three samples in Series G were aerated continually by means of an air-lift device illustrated on page 41. One of these samples served as an uninoculated control. The other two samples were inoculated with B. mycoides and Hormodendron (1193) respectively. It was found that Hormodendron (1193) markedly inhibited the precipitation of TEL whereas B. mycoides was found to accelerate the precipitation when both were compared to the aerated control sample. The water phase in all three of these samples consisted of Knaa Medium. These data are also plotted on page 67.

The foregoing investigations of lead precipitation were all carried out in the presence of light. This approach to the problem was adopted to step up the rate of precipitation so that an appreciable variation due to the particular micro-organisms employed could be observed within a short period of time. Bacterial metabolism, with the exception of the purple sulfur bacteria, may be considered as being essentially independent of the presence or absence of light. The role which bacteria play in the lead precipitating mechanism should therefore be reflected in the deterioration of leaded gasolines exposed in either the light or dark. Practically all aviation gasoline is stored in the dark. For that reason Series I was prepared and exposed in the dark for fifty days. The micro-organisms and their cellular extracts exhibited a surprising increase in the rate of lead precipitation compared to the various control samples. These data are shown on Table XI and plotted on page 71.

Two special series were exposed to determine something of the mechanism of the lead precipitating process. The various samples of these two series have been described as Series J and K, page 45 ff. The data

EFFECT OF MICRO-ORGANISMS AND THEIR CELLULAR EXTRACTS ON LEAD PRECIPITATION IN THE DARK

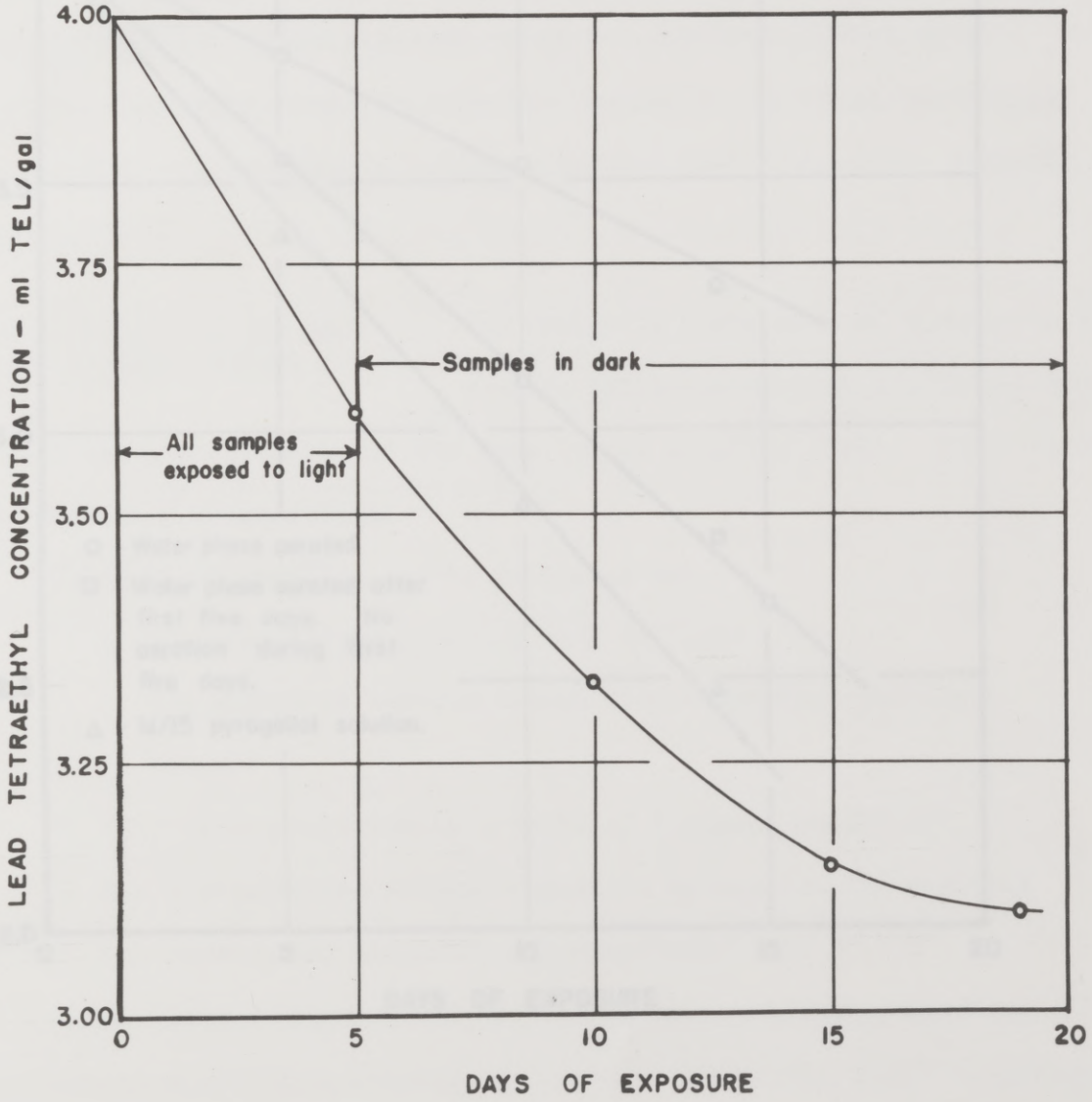


from Series K are plotted on page 73. They show that the reaction continues at very nearly the same rate when the samples are placed in the dark after being exposed to daylight for five days. The rate of lead precipitation gradually decreases with increased time in the dark. Thus it is concluded that the precipitation of TEL from aviation gasolines in storage is a chain reaction mechanism. The process was also shown to be strongly accelerated by exposure to light.

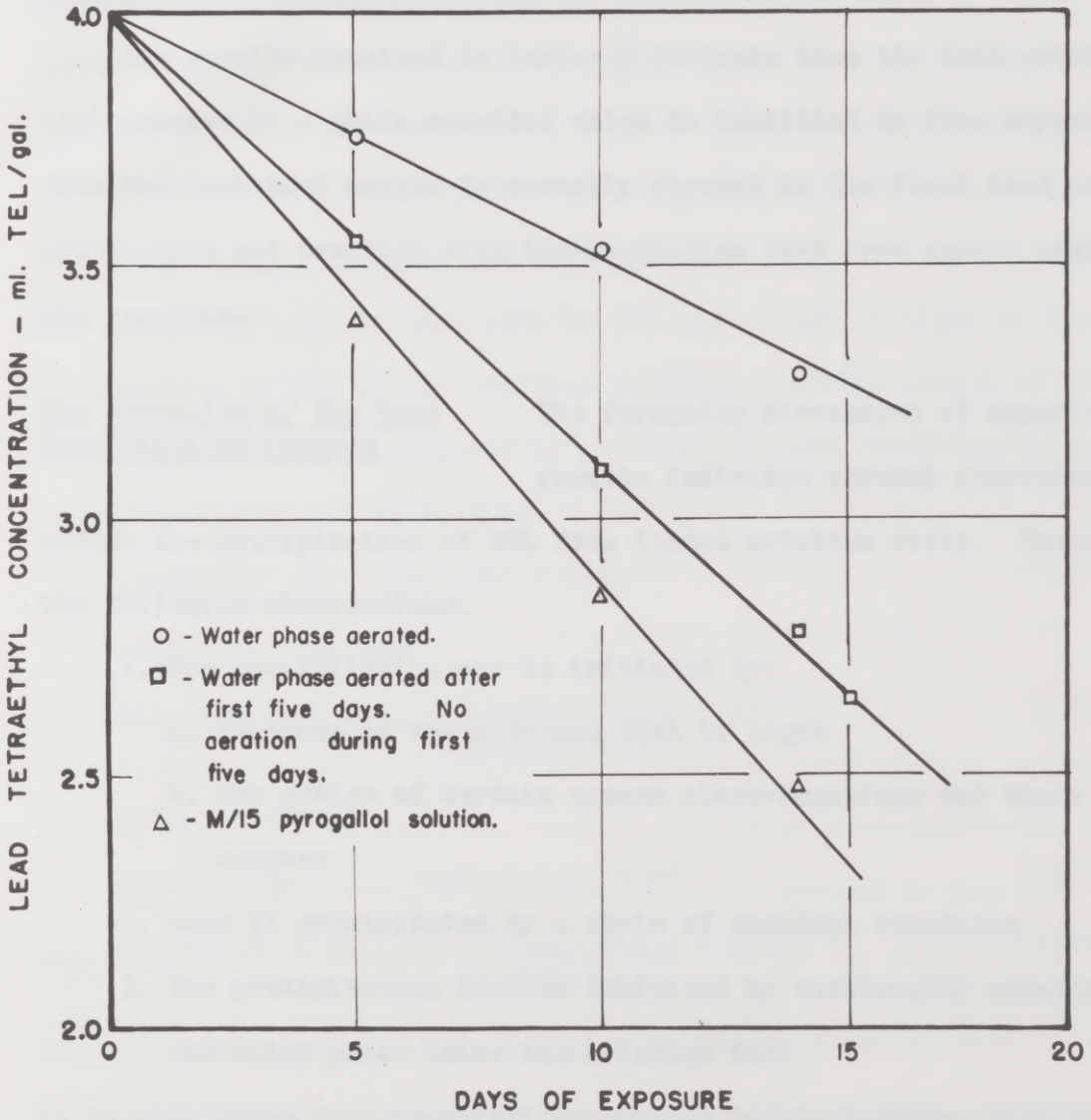
The data from Series J show the inhibiting effect of free oxygen on the precipitation of TEL. These results are plotted on page 74. Here the 100 octane sample which was exposed over a water phase subjected to continual aeration throughout the exposure of the series was found to have the lease lead precipitated. Exposure over M/15 pyrogallol solution with a consequent removal of free oxygen showed the highest detrimental effects to the aviation fuel with respect to the precipitation of TEL. The sample which was not aerated during the first five days and then was aerated for the remainder of the exposure of the series was intermediate between the pyrogallol and the first aerated sample.

No drop in the rate of overall lead precipitation was observed during the ten day period after aeration was begun on this sample. It is assumed that during the first five days chains were started which determined the initial rate of lead precipitation. The subsequent aeration of the water phase of the sample did not result in the termination of enough of the lead precipitating chains to show an effective decrease in rate due to aeration. Since the samples were exposed to light new chains were being started continually according to the mechanism discussed on the next page. It is expected, however, that if a longer period of exposure had been possible some decrease in the slope

THE EFFECT OF LIGHT ON INITIATING THE CHAIN OF REACTIONS RESULTING IN LEAD PRECIPITATION



EFFECT OF DISSOLVED AIR IN WATER PHASE ON PRECIPITATION OF LEAD TETRAETHYL FROM AVIATION GASOLINE



of this curve might have been noted. It is not surprising that ten days of aeration should not slow down the reaction perceptably since in Series K there was no very great decrease in the rate of lead precipitation during the ten day period following the termination of the photolytic initiation of chains by placing the samples in the dark.

The results obtained in Series J indicate that the lead precipitation process is a chain reaction which is inhibited by free oxygen. Thus the fact that oxygen is normally present in the final lead precipitate does not conflict with the conclusion that free oxygen inhibits the reaction.

The Mechanism of the Lead Precipitating Process

The foregoing discussion of experimental results indicates several conditions which affect the precipitation of TEL from leaded aviation fuels. These include the following observations.

1. The precipitation may be initiated by:
   a. Exposure of the aviation fuel to light
   b. The action of certain common micro-organisms and their enzymes
2. Lead is precipitated by a chain of chemical reactions
3. The precipitation is also inhibited by continually aerating the water phase below the aviation fuel.

It is also known from practical experience in the industry that the precipitation of TEL is retarded by gasoline inhibitors and antioxidants. Any mechanism which is postulated to account for the precipitation of TEL must therefore include, or explain, all of these conditions.

The following series of reactions is proposed to account for the precipitation of TEL from heavily leaded aviation fuels during storage. It is postulated that the chain reaction may be initiated by active oxygen. This is accomplished by (1) the bacterial assimilation of the gasoline hydrocarbons which results in the production of hydrogen peroxide. Hydrogen peroxide is immediately broken down by the bacterial enzymes, catalase and peroxidase, to give water and active oxygen.

$$2H \text{ (from hydrocarbon breakdown)} + O_2 \xrightarrow{\text{(cytochromes)}} H_2O_2$$

$$H_2O_2 \xrightarrow{\text{(catalase or peroxidase)}} H_2O + O^*$$

The chains may also be initiated by (2) the effect of light on the dissociation of TEL in the gasoline solution. Active oxygen is produced in a two step process followed by a chain reaction resulting in the precipitation of TEL as follows.

$$(C_2H_5)_4Pb \rightleftharpoons (C_2H_5)_3Pb^* + C_2H_5^* \qquad (I)$$

non-chain reaction:

$$2(C_2H_5)_3Pb^* + O_2 \longrightarrow \underset{\text{precipitate}}{((C_2H_5)_3Pb)_2O} + O^* \qquad (II)$$

chain reaction:

$$2C_2H_5^* + O_2 \longrightarrow C_2H_5 \cdot O \cdot C_2H_5 + O^* \qquad (IIIa)$$

$$2(C_2H_5)_4Pb + O^* \longrightarrow \underset{\text{precipitate}}{((C_2H_5)_3Pb)_2O} + 2C_2H_5^* \qquad (IIIb)$$

The free ethyl radicals from (IIIb) then become chain carriers. The $((C_2H_5)_3Pb)_2O$ may precipitate from the gasoline as such, or may be further oxidized to PbO_2. The conditions of a chain reaction, which may be initiated by either bacteria or light, are thus satisfied.

According to this mechanism antioxidants will inhibit the precipitation of lead by reducing the oxygen in the gasoline. Bactericides retard the precipitation by preventing the formation of active oxygen as a result of micro-biological activity. The inhibition of the lead precipitating process by aeration of the water phase is considered to be comparable to the usual inhibiting effect of free oxygen on chain reactions.*

The possible effect of olefins and conjugate diolefins on this suggested lead precipiting mechanism is also worthy of consideration. It is suggested that these unsaturated hydrocarbons may be capable of combining with the free ethyl radicals and thus terminate the chains. Such an increase in hydrocarbon weight coincident with the precipitation of TEL from a motor gasoline blend is reported in the literature[28]. In this way the presence of unsaturates in a finished leaded gasoline should serve to reduce the TEL precipitation by preventing the formation of a chain mechanism. A second possible effect of unsaturates on the lead precipitating process depends upon the fact that the olefins and particularly the conjugate diolefins are subject to autoxidation to the peroxides. In the absence of an abundant supply of free dissolved oxygen it seems likely that these peroxides might be reduced by the lead triethyl radicals to furnish the oxygen required in the precipitate. These two suggested effects of unsaturated hydrocarbons on TEL precipitation

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* See Semenoff, N., Chemical Kinetics and Chain Reactions, (Oxford) London, (1935), or any other comprehensive reference on the subject.

tend to offset one another. Since autoxidation is never effective in peroxidizing all of the double bonds present in finished motor blends the effect of unsaturates in terminating reaction chains by the uptake of the free ethyl radicals appears the more predominant of the two possibilities. Probably both effects may proceed simultaneously.

The foregoing discussion of the postulated mechanism by which TEL is precipitated from solution in heavily leaded aviation fuels will undoubtedly require some modification as more chemical data on the process becomes available. There can be no doubt, however, that whatever theory is finally evolved to explain the precipitation a very definite allowance must be made for the role of micro-organisms in the lead precipitating process.

SUMMARY

An exploratory study has been made to test the hypothesis that various micro-organisms which are found to live in the water phase under gasoline in conventional tank storage serve as causal agents in the deterioration of aviation and motor fuels. The results of this research suggest a microbiological interpretation of many inadequately explained phases of gasoline deterioration.

Soil bacteria of the genera Bacillus, Micrococcus, and Flavobacterium; the iron bacterium Gallionella; sulfur bacteria including Thiobacillus and Thiospirillum, and the molds Mucor and Hormodendron have been isolated from gasoline storage tank waters and identified. A number of taxonomically representative bacteria were inoculated in pure culture into the water phases below gasoline samples in these laboratories to determine their effect in the deterioration of the aviation and motor fuel substrates. This deterioration was measured by the amount of TEL precipitated from the aviation gasolines and the formation of gum and peroxides in the motor blends. In order to accelerate the deterioration and thereby permit the investigation of more phases of the subject the samples were, in most cases, exposed to diffused daylight. The effect of the various inocula and their controls then became superimposed upon the deterioration due to light. The reproducibility of this method of accelerating the deterioration was investigated statistically. The more common method of accelerating the deterioration by means of the induction bomb became impractical in this work because of the living organisms which were involved. A gasoline-borne contaminant (Bacillus A), Mycobacterium phlei, and Bacillus mycoides were found to be the most

detrimental of the micro-organisms employed in the investigation of gum and peroxide formation. The iron bacterium, Gallionella, was the most detrimental in precipitating TEL (lead tetraethyl). This study of TEL precipitation was extended to include certain endo- and exo-cellular extracts of B. mycoides and the mold, Hormodendron (1193), as well as the organisms themselves in both the light and dark.

The precipitation of TEL from aviation gasolines is affected by the presence of inorganic salts which may be dissolved in the water phase. The presence of iron filings in the water phase (Lieske's Medium) was found to retard this precipitation. Increasing the amount of dissolved air in the water phase below aviation gasoline was found to greatly inhibit the rate of TEL precipitation. Decreasing the amount of dissolved air by the addition of pyrogallol to the water phase had an accelerating effect on the rate of lead precipitation. Other investigations in which the precipitation of TEL was initiated by exposing leaded gasoline samples to light and then placing them in the dark indicate that the precipitation proceeds by a chain reaction mechanism. The role of micro-organisms in the formation of gum and the precipitation of TEL has been postulated from such data as are available.

CONCLUSIONS

1. Certain common soil, sulfur and iron bacteria, many of which are contaminants in gasoline storage tank waters, are capable of living on, or in the presence of gasoline. As a result of their metabolic activity peroxides, color and gum are produced in the gasoline.

2. A few species of molds and bacteria, or their metabolic products, may be capable of reducing hydrocarbon peroxides, thereby improving the stability of the gasoline.

3. Soil bacteria, iron bacteria and molds are apparently among the most important causal agents in the precipitation of TEL (lead tetraethyl) from solution in heavily leaded aviation gasolines during storage over water in both the light and dark.

ACKNOWLEDGMENT

The writer wishes to express his sincere appreciation to Dr. H. J. Gawin for his continued help in pursuing this work and in taking the photomicrographs which are included in this report.

The large amount of petroleum chemistry which was encountered in the problem was watched over by Dr. Lewis F. Hatch, Professor of Chemistry. Dr. O. B. Williams, Professor of Bacteriology, and Lt. A. E. Hayward, formerly Instructor in Bacteriology, were of great help in checking our bacteriological methods and in outlining a number of biological procedures which were unique to petroleum microbiology. The isolation of the heterotrophic bacteria from the gasoline storage tank waters was done by Miss Eloise McCabe, Tutor in Bacteriology. The supervision and general direction of the whole program was the work of Professor H. H. Power, Chairman of the Department of Petroleum Engineering. The writer is deeply indebted to all of these people for their most generous assistance and interest in the work.

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Appreciation is expressed to the Humble Oil and Refining Company for providing the gasoline samples. The help of Mr. Bryson Filbert and Mr. Louis T. Yule of the Refining and Technical Service Division of that company in drawing up the gasoline sample specifications and preparing the many samples was particularly appreciated. The Jackson Laboratory of the E. I. du Pont de Nemours Company and the Gulf Research and Development Company provided the inhibitor samples. The culture of Thiobacillus thiooxidans which was used in this work was obtained from Dr. S. A. Waksman of Rutgers University. The culture of Bacterium aliphaticum was from the stock cultures of Dr. H. F. Haas, who was at that time an API Research Associate at the Scripps Institute of Oceanography, La Jolla, California. These persons and organizations are thanked for their gifts of the materials which were necessary for the research.

BIBLIOGRAPHY

(1) Allen, F. H., "The Effect of Various Wave-lengths of Light on the Deterioration of Cracked Gasoline", B. A. Sc. Thesis, University of Toronto, March, 1941.

(2) Allen, F. H., "The Effect of Water and Blending on the Deterioration of Gasoline Motor Fuels in Storage", M. S. Thesis, The University of Texas, June, 1943.

(3) Anderson, C. G., An Introduction to Bacteriological Chemistry, (Livingston) Edinburgh, 1938.

(4) ASTM Standards on Petroleum Products and Lubricants, (American Society for Testing Materials) Philadelphia, 1942.

(5) Baldeschwieler, E. L., "Determination of Tetraethyl Lead in Ethyl Gasoline", Ind. Eng. Chem., Anal. Ed. 4, 101, (1932).

(6) Bergey, D. H., et. al., Manual of Determinative Bacteriology, 5th ed., (Williams and Wilkins) Baltimore, 1938, p. 666.

(7) Brooks, B. T., "The Chemistry of Gasolines", Ind. Eng. Chem. 18, 1198, (1926).

(8) Bushnell, L. D., and Haas, H. F., "The Utilization of Certain Hydrocarbons by Micro-organisms", J. Bact. 41, 653, (1941).

(9) Butner, M., "Zur Kenntnis der Mykobakterien, insbesondere ihres quantitativen Stoffwechsel auf Paraffinnahrboden", Arch. Hyg. 97, 12, (1926).

(10) Calingaert, G., "Organic Compounds of Lead", Chem. Rev. 2, 43, (1926).

(11) Cassar, H. A., "Gumming Tendencies of Pure Olefins in Gasolines", Ind. Eng. Chem. 23, 1132, (1931).

(12) Egloff, G., Faragher, W. F., and Morrell, J. C., "Stabilization of Knock Rating, Gums and Color of Gasoline by Chemical Inhibitors", Nat. Pet. News 21, (49) 40, (1929).

(13) Egloff, G., Garagher, W. F., and Morrell, J. C., Ind. Eng. Chem. 24, 1375, (1932); 28, 122, (1936); 25, 315, (1933); and 25, 804, (1933).

(14) Ellis, C., The Chemistry of Petroleum Derivatives, Vols. I and II, (Reinhold) New York, 1934-1937.

(15) Gahl, R., and Anderson, E., "Sulfate Reducing Bacteria in California Oil Waters", Centralbl. Bakt. II, 73, 331, (1928).

(16) Ginsburg-Karagitscheva, T. L., "Microflora of Oil Waters and Oil-Bearing Formations and Biochemical Processes Caused by It", Bull. Amer. Assoc. Petr. Geol. 14, 139, (1933).

(17) Gray, P. H. H., and Thornton, H. G., "Soil Bacteria that Decompose Certain Aromatic Compounds", Centralbl. Bakt. II, 73, 74, (1928).

(18) Gruse, W. A., Private communication.

(19) Haas, H. F., "The Bacterial Utilization of Petroleum Hydrocarbons and the Production of Caritinoid Pigments", Ph. D. Thesis, Kansas State College, 1942.

(20) Hall, W. A., "The 'Cracking' of Oils with a View to Obtaining Motor Motor Spirit and Other Products", Jour. Inst. Petr. Tech. 1, 147, (1914).

(21) Munn, E. B., Fischer, H. G. M., and Blackwood, A. J., "Gum in Gasoline", J. SAE 26, 34, (1930).

(22) Hunter, H. C., Private communication.

(23) Kaserer, H., "Über die oxydation des Wasserstoffes und des Methans durch Microorganismen", Centralblat f. Bakt. Abt. II, 15, 573, (1906).

(24) Leighton, P. A., and Mortensen, H. A., "The Photolysis of Lead Tetramethyl and Lead Tetraphenyl", J. Am. Chem. Soc. 58, 448, (1936).

(25) Lipman, C. B., and Greenberg, L., "Petroleum Decomposing Organisms", Nature 129, 204, (1932).

(25a) Maliyantz, A. A., "Bacteriological Method of Desulfurizing Crude", Azerb. Neft. Khoz. 6, 89, (1935).
J. Inst. Petr. 22, 772A, (1935).

(26) Plummer, P. B., Merkt, H. E, Jr., Power, H. H., Sawin, H. J, and Tapp, P. P., "Effect of Certain Micro-organisms on the Injection of Water into Sand", Paper presented before the Dallas, Texas, meeting of the A. I. M. E., May 6, 1943.

(27) Power, H. H., and Allen, P. H., "The Effect of Water on the Deterioration of Gasoline Motor Fuels in Storage", Paper presented before the Dallas, Texas, meeting of the A. I. M. E., May 6, 1943.

(27a) Reed, H. V., Quimby, W. S., and Oosterhout, J. C. D., "Use of Lecithin in Gasoline", Proc. API Sect. III. 21, 6, (1940).

(28) Roberti, G., Pipperelli, E., and Semmola, E., "Sulla Stabilita del Plombo Tetraetile e dei Carburanti Etilizzati", Ricerca Sci. 9, II, 689, (1938).

(29) Roberti, G., Pipperelli, E., and Simonetti, A., "The Stability of Lead Tetraethyl and the Liquids Containing It", Ricerca Sci. 12, 347, (1941).
Chem. Abs. 35, 5681, (1941).

(30) Sohngen, N. L., "Uber Bakterien welche Methan als Kohlenstoffnahrung und Energiequelle Gebrauchen", Centralbl. Bakt. II, 15, 513, (1906).

(31) Sohngen, N. L., "Benzin, Petroleum, Paraffinöl, und Paraffin als Kohlenstoff- und Energiequelle für Mikroben", Centralbl. Bakt. II, 37, 595, (1913).

(32) Stone, R. W., Fenske, M. R., and White, A. G. C., "Bacteria Attacking Petroleum and Oil Fractions", J. Bact. 44, 169, (1942).

(33) Story, L. G., Provine, E. W., and Bennett, H. T., "Gum Formation by Cracked Gasoline", Ind. Eng. Chem. 21, 1079, (1929).

(34) Sturmer, K., "Uber die Wirkung des Schwefelkohlenstoffs und ahnlicher Stoffe auf den Boden", Centralbl. Bakt. II, 20, 282, (1908).

(35) Tauson, V. O., and Shapiro, S. L., "General Trend of the Oxidation of Oil by Bacteria", Microbiology U. S. S. R. 3, 79, (1934).

(36) Tausz, J., and Peter, E., "Neue Methode der Kohlenwasserstoff analyse mit Hilfe Bakterien", Centralbl. Bakt. II, 49, 497, (1919).

(37) Terenin, A., "Photo-dissociation of the Vapors of Organo-Metallic Compounds", J. Chem. Phys. 2, 441, (1934).

(38) Thaysen, A. C., "Gas Evolution in Gasoline Storage Tanks Caused by the Activity of Micro-organisms", J. Inst. Petr. 25, 411, (1939).

(39) UOP Laboratory Test Methods for Petroleum and Its Products, (Universal Oil Products Co.) Chicago, 1940.

(40) Voohrees, V., and Kisinger, J. O., "The Effect of Gum in Gasoline", API Bull. 10, (2) 169, (1939);
J. SAE 24, 584, (1929).

(41) Wagner, C. R., and Nyman, J., "Factors Affecting the Determination of Gum in Gasoline", API Proc. 10th Annual Meeting 11, Sect. III, 118, (1930).
Oil & Gas J. 28, 124, (Dec. 5, 1929).

(42) Wagner, R., "Benzol Bacteria which Can Utilize Benzol, Toluol, and Xylol as a Source of Carbon", Zeitschr. Garungsphysiol. 4, 289, (1914).

(43) Wakengut, A. M., "A Hydrocarbon-oxidizing Micro-organism from Crude Oil", Arch. Sci. Biol. 43, 55, (1936).

(44) Wilde, H. D., Jr., and Hart, J. L., Private communication (Oct. 20, 1941), File No. LL6.1-2044.

(45) Yule, J. A. C., and Wilson, C. P., "Peroxides and Gum in Gasoline", Ind. Eng. Chem. 23, 1254, (1931).

(46) ZoBell, C. E., Grant, C. W., and Haas, H. F., "Marine Micro-organisms which Oxidize Petroleum Hydrocarbons", Bull. Amer. Assoc. Petr. Geol. 27, 1175, (1943).

(47) ZoBell, C. E., Private communication.

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