DEGRADATION OF HEXADECANOL BY

CERTAIN BACTERIAL SPECIES

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TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

INTRODUCTION

Because approximately nine million acre-feet of water are lost annually by evaporation in Texas alone, water conservation has become of greater interest in the Southwest. Studies during the last fifty years have shown that a monmolecular film of certain long chain polar materials are able to reduce evaporation from water surfaces. Langmuir (5) in 1917 reported the phenomenon of film forming compounds. The first attempt to use a monofilm as an evaporation control measure occurred in 1924 and was not successful (4). The first successful attempt by Langmuir (6) with hexadecanol forming the film gave a 50 per cent reduction in the rate of evaporation of ether. In 1943 (7) the rate of escape of molecules from a water surface was reduced 10,000:1 by a monomolecular film of hexadecanol. Outdoor studies were made in 1953 in Australia by Mansfield (9). In his report to the First International Conference on Reservoir Evaporation in April, 1956, his results showed 0-90 per cent saving of water by the use of hexadecanol on Australian reservoirs of 2-22 acres (13).

It has been shown in field tests in the southwestern United States by the Bureau of Reclamation under the direction of J. K. G. Silvey in cooperation with the city of Oklahoma City, Oklahoma (11) that evaporation is reduced by a monomolecular film of hexadecanol (cetyl alcohol) on the surface of a water reservoir.

One of the major problems involved in such a measure is the maintenance of the film on the water for long periods of time. It was reported at the Southwest Research Institute with Beadle as chairman that at a rate of 2.2 pounds per acre, an evaporation reduction of 45 per cent and a film life of thirty days would result (14). Timblin et al., in 1957 (15) reported that hexadecanol flakes sprinkled on evaporation pans at the rate of 60 pounds per acre were effective for more than 4 weeks with a maximum efficiency of 64 per cent. However, the Monthly Progress Report of the Southwest Cooperative Committee on Reservoir Evaporation Control, August, 1956 (10), states that in experimentation with ten samples of hexadecanol, five of the samples lost their efficiency entirely after periods ranging from 5-13 days.

An aging phenomenon may be one factor to consider in maintaining a monomolecular film. Sebba and Briscoe (13) explained this phenomenon in that in an uncompressed film, the single molecules, because they are in active motion and free to associate, gradually do so in pairs; this forms

double molecules having a hydrophilic alcohol group at each end and these molecules, like dihydroxy-alcohols, lie and remain flat upon the surface of water and enhance the permeability of the film to water. In addition to the aging process it was evident in the Bureau of Reclamation studies that much of the hexadecanol is lost by the wind's casting it ashore.

There is also reason to believe that a portion of the film disappears as a result of biologic activity. Ludzack and Ettinger (8), in 1957, showed that there was a strong indication that hexadecanol was used by organisms contained in river water that had been seeded to produce a theoretical 36 per cent biological oxygen demand. They also showed by using aerated carboys with suspensions of solid hexadecanol inoculated with 2 per cent sewage and then measuring the amount of carbon dioxide produced, that 77 per cent of the hexadecanol was accountable as resulting from oxidation. However, they did not make any statements as to what organisms might be responsible for the reduction of the compound.

Pseudomonas species have been found to utilize such substances as cutting oil (1), phenolic wastes (3) and methane (2). This small gram negative rod is also found in the film life of lakes and reservoirs. Alcaligenes, of the same gram staining reaction and morphology, is also known to inhabit the surfaces of such bodies of water. These

organisms, because of their ability to reproduce in a wide variety of organic compounds and the nature of their habitat, are likely organisms to consider in finding an organism which might degradate hexadecanol.

It seemed desirable to investigate the role of bacteria in disappearance of hexadecanol films on reservoirs. The purpose of this thesis is to determine the effect of hexadecanol on the populations of Pseudomonas and Alcaligenes species in reservoirs and determine their ability to utilize this compound as a carbon source.

BIBLIOGRAPHY FOR CHAPTER I

1. Bennet, E. O. and Wheeler, H. O., "Survival of Bacteria in Cutting Oil," Applied Microbiology, II (November, 1954), 368-371.

2. Dworkin, M. and Foster, J. W., "Studies on Pseudomonas Methanica," Journal of Bacteriology, LXXII (November, 1956), 646-659.

3. Hamby, M. K., Sherrer, E. L., Randles, C. I., Weiser, H. H. and Sheets, W. D., "Some Characteristics of Phenol-Oxidizing Pseudomonas," Applied Microbiology, IV (March, 1956), 71-75.

4. Hedestand, G., "The Influence of Thin Surface Films on the Evaporation of Water below the Boiling Point," Journal of Physical Chemistry, XXVIII (1924), 1244-1252.

5. Langmuir, I., "Shapes of Group Molecules Forming the Surfaces of Molecules," Proceedings of the National Academy of Science, III (April, 1917), 251-257.

6. _____ and Langmuir, D. B., "The Effect of Monomolecular Films on the Evaporation of Ether Solution," Journal of Physical Chemistry, XXXI (1927), 1719-1731.

7. _____ and Schaefer, V. J., "Rates of Evaporation of Water through Compressed Monolayers on Water," Journal of the Franklin Institute, CCXXXV (February, 1943), 119-162.

8. Ludzack, J. J. and Ettinger, M. B., "Biologic Oxidation of Hexadecanol under Laboratory Conditions," Journal of the American Water Works Association, XLIX (July, 1957), 849-858.

9. Mansfield, W. W., "The Effect of Surface Films on the Evaporation of Water," Nature, CLXXII (December 12, 1953), 1101.

10. Monthly Progress Report of the Southwest Cooperative Committee on Reservoir Evaporation Control, August 31, 1956.

11. Report of the Effects of Hexadecanol on Kid's Lake, Oklahoma City, Oklahoma, July 7-September 5, 1956.

12. Sebba, F. and Briscoe, H. V. A., "A New 'Aging' Phenomenon in Unimolecular Films on Water Films," Journal of the Chemical Society, Part I (January-June, 1940), 128-131.

13. Southwest Research Institute, Proceedings of the First International Conference on Reservoir Evaporation Control, San Antonio, Texas, April 14, 1956.

14. Southwest Research Institute, Water Conservation through Control of Evaporation, San Antonio, Texas, 1956.

15. Timblin, L. O., Jr., Moran, W. T. and Garstka, W., "Use of Monomolecular Layers for Reservoir Evaporation Reduction," Journal of the American Water Works Association, XLIX (July, 1957), 841-848.

CHAPTER II

METHODS

The study of the bacterial degredation of hexadecanol was conducted in two phases. One was the actual isolation, counting, and identification of certain bacterial species from a lake treated with hexadecanol and from an untreated lake. The other phase was devising a laboratory scheme to note the increase in population and the quantity of hexadecanol utilized in tests with this compound as the only carbon source.

Isolation studies were made from a small test lake seeded with hexadecanol and a larger lake nearby serving as a control. Both lakes had approximately equal temperatures and chemical content and received the same amount of rainfall (1). Samples were collected several days prior to the testing period, each week during, and several times after the testing period. Isolation and colony counts were made from sterile agar plated with 1.0 ml. and 0.1 ml. of raw water from the reservoirs. Alcaligenes and Pseudomonas were the genera considered primarily although other organisms were also noted. The organisms were isolated on Bacto-Brain Heart Infusion agar from Difco Laboratories, Detroit, Michigan,

for confirmation. These were then identified by gram staining and reactions in differential carbohydrate tube media with Difco Bacto-Purple Broth Base as the indicator and maintained as stock cultures for further tests.

In the laboratory, testing procedures were devised to determine if an increase in number of these organisms in the test or treated lake was due to the use of hexadecanol as a nutrient source or to some other factor involved. A modified Frazier-Rupp's mineral solution (2) shown in Table I was used as a basic medium. Organisms used in the tests were Pseudomonas strains K 2, K 9, and LH 3 and Alcaligenes strains K 5, K 10, LH 2, LH 4, LH 5, and LH 6. The K strains were isolated from the treated (test) reservoir and the LH strains from the untreated (control) reservoir. The first series of experiments were in test tube volumes. One per cent and 0.1 per cent by volume of hexadecanol were added to six-inch tubes containing 10 ml. of mineral solution. A bacteriologist's loopful of culture from Bacto-Brain Heart Infusion agar slants was added to the tests. Controls included non-inoculated tubes containing none of the compound tested and those with the compound. The tubes were then incubated three weeks at room temperature and in duplicate at 37° C. Visual turbidity determinations and cultural identifications were made to determine growth in this solution with hexadecanol as the only carbon source. Turbidity of the tests was compared to the controls after a period of two weeks' incubation.

In the above tests a thick film of hexadecanol formed on the surface of the media. Such a film by limitation of oxygen diffusion into the solution probably decreased the total possible growth. To lessen this possibility, the procedure was revised to use larger volumes of mineral solution and provide a larger surface area. This also made the conditions more similar to film conditions in treated reservoirs, although the tests were not an attempt to duplicate the

TABLE I

FORMULA FOR MODIFIED FRAZIER-RUPP'S MEDIUM

Compound	Amount
Dipotassium phosphate (K_2HPO_4)	3.1gm.
Monopotassium phosphate (KH_2PO_4)	0.8gm.
Potassium chloride (KCl)	0.2gm.
De-ionized water	1,000.0ml.

Dissolved and sterilized in the autoclave.

actual conditions in a reservoir. The procedure was also changed to eliminate the possibility of the cells carrying a carbon source over from the stock media. The bacteria to be tested were inoculated into Difco-Bacto Trypticase soy broth, incubated at 37°C for 36 hours and then centrifuged. The supernate was discarded and the cells were washed 4 times in the mineral solution. The cells were then allowed to stand 48-72 hours at room temperature in 3 ml. of mineral solution. One loopful of this suspension was inoculated into 50ml. of sterile mineral solution in each 250 ml. Erlenmeyer flask.

In each flask 0.1 ml., by volume, hexadecanol was added, giving a concentration of 2 parts per 1000. The controls were as in the test tube method with the only difference being change in volume, surface area, and concentration of hexadecanol. Incubation period was three weeks.

Later it was decided to add ammonium chloride (NH_4Cl) in order that a lack of nitrogen might not make the experiment limited in two factors. Concentration of NH_4Cl was 2 parts per million (2 p. p. m.) in the mineral solution. Colony counts were made in addition to visual turbidity determinations and cultural identifications to determine growth.

Along with these tests, others using the same procedure were made with other sources of hexadecanol, including a mixture of 5 gm. hexadecanol, 5 cc. mineral oil, and 1 gm. of glycerol monostearate which had been proposed for establishing a molecular film. In this case, a glass stirring rod was dipped into the mixture and then into the mineral solution.

In order that the quantity of compound being used might be determined, extractions of hexadecanol were made from 4-6 weeks' culture in which the compound had been previously weighed. Inoculum in these tests was 0.1 ml. cell suspension. Evaporating dishes were heated in a hot air oven at 360° F, and placed in a calcium chloride ($CaCl_2$) dessicator for at least 24 hours prior to weighing with an analytical balance. All glassware used, including separatory funnels,

was cleaned with acetone, sulfuric acid, rinsed with distilled water and dried in a hot air oven. Extractions were made with A. C. S. grade of Merck benzene. The benzene was driven off by heating the evaporation dishes on the low temperature of a hot plate. The dishes were allowed to cool in a calcium chloride dessicator and weighed periodically until a constant weight was reached.

Because some of the flasks of a certain strain tested did not show any decrease in weight in the recovery, a series was run with $CaCO_3$ as the carbon source. This was to see if some oil or fat soluble in benzene was produced and responsible for the excess weight instead of the hexadecanol.

BIBLIOGRAPHY FOR CHAPTER II

1. Report of the Effects of Hexadecanol on Kid's Lake, Oklahoma City, Oklahoma, July 7-September 5, 1956.

2. Williams, O. B., Laboratory Manual for General Bacteriology, Austin, Texas, Hemphill's, 1955.

CHAPTER III

RESULTS

The average number of colonies of the Pseudomonas and Alcaligenes strains isolated from the reservoir treated with hexadecanol and the untreated reservoir are shown in Table II. The counts indicate the number of organisms found in 1.0 ml. of raw water from the reservoirs studied on the basis that one organism gives rise to one colony.

TABLE II

AVERAGE NUMBER OF COLONIES OF PSEUDOMONAS AND ALCALIGENES STRAINS ISOLATED FROM HEXADECANOL TREATED AND UNTREATED RESERVOIRS

Organisms	Hexadecanol Treated			Untreated	
	Pre-testing Period	Testing Period	Post-testing Period	Pre-testing Period	Testing Period
Pseudomonas	3-5	57	2-6	2-4	2-6
Alcaligenes	5-7	28	2-6	2-5	2-6

The count of Pseudomonas was shown to increase approximately 11 to 19 fold during treatment with hexadecanol, while the count in the untreated lake remained constant during the

testing period (Table II). In the treated lake 4 months after application of the compound ceased, the count had returned to 2 to 6 per milliliter, which was close to the count made prior to the treatment. The Alcaligenes count was shown to increase approximately 4 to 6 fold in the treated reservoir with no increase in the untreated reservoir. Like Pseudomonas, Alcaligenes returned to its normal level after treatment ceased.

Proteus vulgaris, Escherichia coli, Bacillus pumilus, and Sarcina citrea were also identified from these reservoirs, but no attempt to record their incidence was made.

In the laboratory test tube method described previously, some strains were observed to grow with hexadecanol as the only carbon source. These results, shown in Table III, were obtained by comparing turbidity of the test to the uninoculated controls.

TABLE III

GROWTH IN TEST TUBES WITH 10 ML. MINERAL SOLUTION PLUS HEXADECANOL AS THE SOLE CARBON SOURCE

Organism	1% Hexadecanol by Volume		.1% Hexadecanol by Volume		Inoculated Mineral Solution Controls	
	Number + *	Number - **	Number +	Number -	Number +	Number -
Pseudomonas	2	2	1	5	0	5
Alcaligenes	4	2	5	4	0	9

*"+"--with growth **"-"--with no growth

Incubation period for the tests shown in Table III was three weeks. Gram staining from the test tubes in these tests, followed by inoculation into differential carbohydrate media, showed the organisms in the tubes to be the same as those inoculated into the tests. The tests show that not all the organisms were able to grow under these experimental conditions.

The method was revised to increase the surface area and total volume by using 250 ml. flasks and by adding a nitrogen source (Table IV).

TABLE IV

GROWTH IN FLASKS OF 50 ML. MINERAL SOLUTION WITH HEXADECANOL IN TESTS

Description	.2% Hexadecanol by Volume		Mineral Solution Controls	
	Number with Growth	Number with no Growth	Number with Growth	Number with no Growth
Inoculated with *Alcaligenes*	6	0	4	2
Inoculated with *Pseudomonas*	8	0	0	8
Non-inoculated Controls	4	6	0	9

After the change in procedure, growth was observed in all flasks containing hexadecanol (Table IV). Since 4 of the first tests performed with *Alcaligenes* showed turbidity in the mineral solution controls, the source of distilled

de-ionized water was changed. The inoculated mineral solution controls showed no growth in the remainder of the tests.

Plate counts made at the time of inoculation and after incubation are shown in Table V. These counts show an 8,000 fold increase of Pseudomonas and a 3,000 fold increase of Alcaligenes during the incubation period.

TABLE V

PLATE COUNTS FROM TEST FLASKS CONTAINING HEXADECANOL

Organism	Count at Inoculation	Count after 3 Weeks' Incubation
Pseudomonas sp.	3,000 per ml.	25,000,000 per ml.
Alcaligenes sp.	3,000 per ml.	10,000,000 per ml.

Some uninoculated controls from the tests shown in Table IV which contained hexadecanol from a certain chemical company showed turbidity. Gram staining from these flasks showed a small gram negative rod to be present. After investigation, an Alcaligenes species was found to be existing in pure culture in the compound as it came from the chemical supply company.

Pseudomonas species were found to be able to metabolize in a mixture of hexadecanol, mineral oil and glycerol monostearate in the mineral solution. All strains of this organism tested were found to reproduce in every sample of

hexadecanol used. These samples came from several sources. Most of them were bacteria free upon arrival.

Table VI shows the results obtained when attempts were made to determine the quantity of hexadecanol being utilized by Pseudomonas and Alcaligenes species.

TABLE VI

AMOUNT OF INOCULUM, WEIGHT OF HEXADECANOL USED IN TEST, AND AMOUNT RECOVERED BY BENZENE EXTRACTION AFTER INCUBATION

Description		Number of Organisms Inoculated	Incubation Period	Weight Hexadecanol Used	Weight Hexadecanol Recovered
Alcaligenes Strains	LH 2	1 loopful suspension	6 wks.	7.9 mg.	10.6 mg.
	LH 2	5,500,000	4 wks.	10.0 mg.	34.9 mg.
	LH 2	1,500,000	4 wks.	9.7 mg.	21.1 mg.
	LH 2	*	4 wks.	149.1 mg.	149.4 mg.
	K 10	*	4 mos.	9.8 mg.	5.5 mg.
Pseudomonas Strains	LH 3	1 loopful suspension	6 wks.	13.9 mg.	7.3 mg.
	LH 3	300,000	4 wks.	7.9 mg.	8.0 mg.
	LH 3	2,000,000	4 wks.	21.0 mg.	21.3 mg.
Compound Control Flasks	1	. . . . .	6 wks.	13.5 mg.	9.7 mg.
	2	. . . . .	4 wks.	20.9 mg.	24.4 mg.
	3	. . . . .	4 wks.	6.9 mg.	14.5 mg.
	4	. . . . .	4 wks.	150.5 mg.	146.1 mg.

*Unable to determine

The only test flasks that gave great decrease in weight on recovery were the last Alcaligenes K 10 test and the first Pseudomonas LH test. These showed an approximate 50 per cent reduction. Out of the four Alcaligenes LH 2 tests, three showed a marked increase in weight. The weight of recovery of the controls fluctuated from an increase to a decrease in weight with two of them remaining fairly constant. Since the first three LH 2 tests showed such a large weight in extraction, this test was run again parallel with one containing $CaCO_3$ to see if some fat soluble in benzene was produced. However, little if any growth was detected in the $CaCO_3$ flask and upon extraction no fat soluble compound was recovered.

CHAPTER IV

DISCUSSION

Pseudomonas organisms are generally classified as pathogens. *Alcaligenes*, although generally non-pathogenic, may become a secondary invader in many infections. The increase of *Pseudomonas* in the treated (test) lake from a count of 3 to 5 organisms to 57 organisms per milliliter (Table II) does not approach the 60,000 organisms considered to be of unacceptable content (1). It does show, however, that treatment with hexadecanol might provide a favorable environment for these bacteria. A higher concentration of bacteria would be advantageous for lake biology in general, but unpleasant tastes and odors in the water could result. Constant treatment with the compound for prolonged periods of time might also result in the bacterial population reaching the undesirable count.

The increase of *Alcaligenes* in the treated lake from 5-7 to 28 organisms per milliliter does not reach the undesirable limit, although this is an indication that the presence of hexadecanol encouraged the reproduction of this organism.

The fact the *Pseudomonas* and *Alcaligenes* count remained constant in the untreated reservoir while it increased in

the treated reservoir supports the assumption that these organisms can use hexadecanol as a carbon source. The decrease in Pseudomonas and Alcaligenes count after the cessation of treatment with hexadecanol further indicates that hexadecanol was a nutrition source.

The laboratory tests showed that hexadecanol could serve as a carbon source and that increase in the count of Pseudomonas and Alcaligenes was not due to some accidental factor. In the test tube method some of the Alcaligenes strains and a greater number of the Pseudomonas strains were not able to grow under the test conditions (Table III). The reason for this inability to grow was probably that no nitrogen source was available and the oxygen supply was limited by the small surface area and the formation of a thick film of hexadecanol at the surface.

All of the organisms tested grew in the flask method (Table IV). Nitrogen was supplied by ammonium chloride (NH_4Cl) and greater surface area permitted greater oxygen absorption. Evidence that hexadecanol was being utilized was more certain because the cells were washed in mineral solution before inoculation, thus eliminating the possibility of nutrient being carried by the cells from the stock media.

From the standpoint of the population increase shown in the reservoirs and the plate counts from the test flasks (Table V), it is most likely that more Pseudomonas strains are able to reproduce readily in the presence of hexadecanol than are Alcaligenes strains.

It must be considered that our methods of determining the quantitative use of hexadecanol failed to show conclusive results. Testing for the quantitative use of hexadecanol by these organisms was complicated by the difficulty with our methods of consistently extracting the hexadecanol. No attempt was made to distinguish between hexadecanol and other benzene soluble compounds in these extractions. With Alcaligenes it was noted that when a large test inoculum was used, the recovery of benzene soluble material increased after the incubation period. When a small test inoculum was used, the benzene soluble compound recovered was approximately the same as that inoculated. This work did not determine whether a benzene soluble material is produced by Alcaligenes. Such a compound is not produced by this organism when grown in mineral solution with a carbon source composed of $CaCO_3$. One Alcaligenes strain showed approximately a 49 per cent utilization of hexadecanol indicating that there is considerable variation among these organisms as to the ability to degrade this compound. This amount of hexadecanol was used over a period of four months.

It was noted with Pseudomonas that when a small inoculum was used, the benzene soluble compound recovered was approximately half of that inoculated. When a larger inoculum of the same strain was used, the amount of recovery was approximately equal to the amount inoculated. This indicates a difference in Pseudomonas and Alcaligenes when a large

inoculum was used. It should also be noted that Pseudomonas showed a 50 per cent reduction in compound recovered in six weeks while Alcaligenes showed a comparable reduction in four months.

The data presented here indicate that hexadecanol is degraded by some strains of Pseudomonas and Alcaligenes, with considerable variations between strains as to their ability to use this compound as a sole carbon source. It should be pointed out that no attempt was made to determine the chemical purity of the compound. Since a relatively long incubation period is required to demonstrate this change, it is possible that the aging process of the compound is needed before it is attacked by the bacteria. All evidence, however, indicates that bacterial degredation of hexadecanol is a problem that must be considered in the use of this compound as an evaporation control mechanism.

BIBLIOGRAPHY FOR CHAPTER IV

1. Report of the Effects of Hexadecanol on Kid's Lake, Oklahoma City, Oklahoma, July 7-September 5, 1956.

CHAPTER V

SUMMARY

1. Bacterial populations of Pseudomonas and Alcaligenes were shown to increase in a reservoir treated with hexadecanol while the count in an untreated reservoir remained constant.

2. Alcaligenes and Pseudomonas strains isolated from the hexadecanol treated and untreated reservoirs were shown to utilize hexadecanol in laboratory tests in which this compound was the sole carbon source.

3. Quantitative attempts to determine the amounts of hexadecanol utilized in the laboratory by certain bacterial species were inconclusive.

4. From the data obtained the use of hexadecanol as an evaporation control measure must consider bacterial degredation of this compound.

BIBLIOGRAPHY

Books

Williams, O. B., Laboratory Manual for General Bacteriology, Austin, Texas, Hamphill's, 1955.

Articles

Bennet, E. O. and Wheeler, H. O., "Survival of Bacteria in Cutting Oil," Applied Microbiology, II (November, 1954), 368-371.

Dworkin, M. and Foster, J. W., "Studies on Pseudomonas Methanica," Journal of Bacteriology, LXXII November, 1956), 646-659.

Hamdy, M. K., Sherrer, E. L., Randles, C. I., Weiser, H. H. and Sheets, W. D., "Some Characteristics of Phenol-Oxidizing Pseudomonas," Applied Microbiology, IV (March, 1956), 71-75.

Hedestand, G., "The Influence of Thin Surface Films on the Evaporation of Water below the Boiling Point," Journal of Physical Chemistry, XXVIII (1924), 1244-1252.

Langmuir, I., "Shapes of Group Molecules Forming the Surfaces of Molecules," Proceedings of the National Academy of Science, III (April, 1917), 251-257.

_____ and Langmuir, D. B., "The Effects of Monomolecular Films on the Evaporation of Ether Solution," Journal of Physical Chemistry, XXXI (1927), 1719-1731.

_____ and Schaefer, F. J., "Rates of Evaporation of Water through Compressed Monolayers on Water," Journal of the Franklin Institute, CCXXXV (February, 1943), 119-162.

Ludzack, F. J. and Ettinger, M. B., "Biologic Oxidation of Hexadecanol under Laboratory Conditions," Journal of the American Water Works Association, XLIX (July, 1957), 849-858.

Mansfield, W. W., "The Effect of Surface Films on the Evaporation of Water, Nature, CLXXII (December 12, 1953), 1101.

Sebba, F. and Briscoe, H. V. A., "A New 'Aging' Phenomenon in Unimolecular Films on Water Films," Journal of the Chemical Society, Part I (January-June, 1940), 128-131.

Timblin, L. O., Jr., Moran, W. T. and Garstka, W., "Use of Monomolecular Layers for Reservoir Evaporation Reduction," Journal of the American Water Works Association (July, 1957), 841-848.

Reports

Monthly Progress Report of the Southwest Cooperative Committee on Reservoir Evaporation Control, August 31, 1956.

Report of the Effects of Hexadecanol on Kid's Lake, Oklahoma City, Oklahoma, July 7-September 5, 1956.

Southwest Research Institute, Proceedings of the First International Conference on Reservoir Evaporation Control, San Antonio, Texas, April 14, 1956.

Southwest Research Institute, Water Conservation through Control of Evaporation, San Antonio, Texas, 1956.