

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

DATE: MAR 28 1980

FILE COPY

SUBJECT: Registration Action on Bardac 22, EPA Reg. No. 6836-32

RWC
FROM: R. W. Cook, Section #1, EFB/HED

TO: PM 31 (Lee)

Our records indicate that we have received two actions regarding the subject registration. The first document was received in EFB on 4/3/79 (RD in date is 8/11/78), while the second document was received 7/23/79 (RD in date is 6/29/79). The record on the second document refers to the first document by stating "See (4/3/79) same submission See review."

At the current time, we do not have the second submission in hand. Further, from our records it is our opinion that the second submission was returned to RD at the same time as the first submission, since we considered the second to be a duplicate of the first.

Accordingly, we are amending our records to reflect our opinion that the second submission is in fact a duplicate of the first submission and was returned to RD at that time.

Please check your records and verify that the second submission of 7/23/79 is a duplicate. If you find that the second submission is not a duplicate, please notify us and return the second submission for review.

We are attaching a copy of our review of the first submission, for your records.

LONZA

8/15/78

LONZA INC., Fair Lawn, N.J. 07410 Tel. 201 - 791-7500 Cables - Lonza FairLawnNJ TWX-7109882243 Telex-130 472

BARDAC QUATERNARIES ARE BIODEGRADABLE

A new study has shown that BARDAC Quaternaries are biodegradable. Continental Oil, a leader in the field of biodegradable detergents, presented this finding to the April '72 - Los Angeles meeting of the American Oil Chemists' Society. Conoco's paper, "The Biodegradability of Low Concentrations of Certain Quaternary Ammonium Antimicrobials by Bacteria" showed BARDAC Quaternaries to degrade rapidly when tested by the Shake Flask Method. This is the same method developed by the Soap and Detergent Association for measuring the biodegradability of LAS and nonionics.

IMPORTANCE TO QUATERNARY USERS

Special Significance for WATER TREATMENT

The Conoco Study shows that even though microbiocidal levels of BARDAC Quaternaries are "slug-dosed" into a cooling tower, concentrations in the bleed-off (blow-down) are very low in comparison. This is because the cationic quat is adsorbed onto negatively charged surfaces and areas of algal and bacterial slime. Most of the quat is retained by the tower and not bled away. So only low concentrations of quaternary are found in the bleed-off and are further diluted by other water in the sewage system or holding pond. Here, overwhelmed by environmental bacteria, the BARDAC Quaternaries are completely biodegraded.

FORMULATORS of Disinfectants and Sanitizers

Disinfectants and sanitizers formulated with BARDAC Quaternaries generally deliver 200 ppm to 700 ppm of active quat in the use-dilution. At these levels the quaternary is rapidly toxic to most bacteria and there can be no biodegradation action. However, once use-dilutions are disposed of into sewage treatment systems, you can be sure they will degrade and not build up in the environment.

ABOUT THE STUDY

We should be careful about the correlations made between what happens in a Shake Flask study and what happens in the actual environment. Even though the bacterial cultures used are from soil and a sewage treatment plant, their number, nutrient media, agitation and test temperature

cont'd.

are all optimized for biodegradation. In other words, the test is designed to give any molecular structure that can biodegrade, the best possible chance of degrading. In "real life" (i.e., the environment), these numbers of bacteria and these optimum conditions seldom exist. Thus the correlation of the rates of biodegradation for the BARDAC Quaternaries between the Shake Flask and the environment is remote. What this test does show is that once exposed to the bacteria that exist in the environment, BARDAC Quaternaries will biodegrade.

There are other important points in the Conoco Study to be brought out:

1. Note, that the BARDAC Quaternaries were degraded by unacclimated bacteria. No special strain of organisms was developed to degrade this quaternary structure. Most of the uses of quaternaries as economic poisons will see them disposed of in unacclimated (i.e., moving, flowing, changing) systems (Page 3, Para. 3).
2. Unexpectedly, this study shows that acclimated bacteria become more susceptible to the toxic effects of the BARDAC Quaternaries (Page 3, Para. 4).
3. The presence of extra organic nutrients on the biodegradability was determined. Even though substantial quantities of yeast and glucose were present as alternate food sources for the bacteria, biodegradation of the BARDAC Quaternaries continued (Page 4, Para. 2).
4. Pentachlorophenol, a popular microbiocide was also tested for its biodegradability. Under the optimum conditions provided by the Shake Flask method, this compound degraded only 20%, even when using bacterial cultures from phenol biodisposal systems (Page 4, Para. 6).
5. The analytical determinations used in this study are perhaps its most sophisticated contribution. The bromphenyl blue colorimetric determination was found to be sensitive to 0.25 ppm of quat. This method was verified by using an UV analysis (Page 2, Para. 5).
6. The significance of these Tower Studies is that the BARDAC-22 concentration in water cooling tower systems effluents is low and relatively non-toxic. In these concentrations, environmental bacteria will degrade the BARDAC Quaternaries (Page 5, Para. 3).

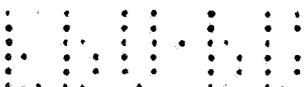
Consider BARDAC Quaternaries for your microbiocide applications. Use dilutions can be formulated to deliver microbiocidal or microbiostatic action and after use discarded without fear of the cumulative toxicity found with "persistent" actives (e.g.s., phenolics, and heavy metal compounds).

Copies of the Conoco Study are available from Lonza upon request.



Richard D. Ditoro

Richard D. Ditoro
Director
Product Development



THE BIODEGRADABILITY OF LOW CONCENTRATIONS OF CERTAIN
QUATERNARY AMMONIUM ANTIMICROBIALS BY BACTERIA

by

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Ponca City, Oklahoma

Presented at,
American Oil Chemists' Society
National Meeting
Los Angeles, California
April 23-26, 1972

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THE BIODEGRADABILITY OF LOW CONCENTRATIONS OF CERTAIN
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by

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ABSTRACT

The biodegradabilities of four different quaternary ammonium antimicrobial agents were determined using a mixed culture obtained from soil and sewage. Although the structures of the quats influenced biodegradation rates, each quat tested was completely degraded within 48 hours. At the 10 ppm level, short term culture acclimation improved degradation rates while longer term acclimation resulted in poorer degradation rates. Aliphatic quats tested were more rapidly biodegraded than aromatic quats. Biodegradation of the quats occurred in the presence and absence of other organic matter. The study indicates that the quats studied are subject to relatively rapid biodegradation following application of antimicrobial concentrations and subsequent dilution to lower concentrations.

INTRODUCTION

Quaternary ammonium products have been used for many years in various environments, for example oil field waters, cooling tower water and in hospitals, as very effective antimicrobial agents. The usefulness of these products over other materials having similar antimicrobial activities is greater because the quats are surface active (better cleansing properties) and are also relatively good corrosion inhibitors. Quats do not contain heavy metals or other known highly toxic functional components. It should also be noted that quats are cationic in nature and are thus inactivated by anionic substances such as most of the commonly used detergent surfactants.

In many instances, antimicrobial agents are applied to systems where it is impractical to attempt to keep low concentrations of the agent out of waste treatment systems, waterways or soil. In these instances it is increasingly imperative that antimicrobials be used that are rapidly biodegraded by microorganisms normally present in the environment or can otherwise be effectively decomposed or collected.

The literature contains very little data relating to the biodegradation of antimicrobial agents. Barden and Issac⁽¹⁾ and Pitter⁽²⁾ reported

that a quat of little commercial importance, cetyl pyridinium bromide, is completely biodegradable by domestic sewage activated sludge. Barden and Isaac also observed that the cetyl trimethylammonium bromide was not biodegraded.

Our interest in antimicrobial agents led us to examine biodegradabilities of several commercially important quaternary ammonium antimicrobial products using a mixed bacterial culture obtained from soil and domestic sewage.

MATERIALS AND METHODS

Antimicrobials: Four different quaternary ammonium antimicrobials, didecyl dimethyl ammonium chloride, dioctyl dimethyl ammonium chloride, alkyl (C_{14}) dimethyl benzyl ammonium chloride and alkyl (C_{14}) dimethyl ethylbenzyl ammonium chloride were used in the studies. These materials are manufactured by Lonza Chemical Company, Fair Lawn, New Jersey and the first three are sold as Bardac 22, Bardac LF and Barquat MB-50 respectively. A mixture of the alkyl dimethyl benzyl and alkyl (C_{14}) dimethyl ethylbenzyl quats are sold by Lonza as Barquat 4250. For comparative purposes, biodegradation of pentachlorophenol (Dowicide G, Dow Chemical Company) was also measured. The structures of these materials are shown in Figure 1. Quats are commonly sold as 50% solutions in water and isopropanol. The quats and pentachlorophenol concentrations are expressed in the study based on 100% active.

Culture and Media: The culture used for biodegradation testing was a mixed culture from soil and raw city sewage that grew out in a mineral salts plus yeast extract medium (NH_4Cl , 3.0 g; K_2HPO_4 , 1.0 g; KCl , 0.25 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; $NaHCO_3$, 0.25 g; $FeSO_4$, trace; yeast extract, 0.30 g; and distilled water, 1000 ml). For some experiments the culture was acclimated to the specific antimicrobials as described in the Results section. Biodegradation studies were carried out in the above medium or in organically altered media as mentioned in the Results section.

All growth and biodegradation testing was carried out in 250 ml or 2000 ml erlenmeyer flasks (100 ml or 1000 ml of medium) at 22°C on a gyratory shaker operating at 250 rpm. Adequate aeration and mixing of the flask contents were observed at all times. Controls consisting of inoculated blank medium, uninoculated medium containing quat and inoculated media containing quat and antimetabolite.

Analytical: Quat biodegradation was followed using a colorimetric test and UV spectrophotometry when possible. Shake flask samples were prepared for analysis by the addition of 7.5% concentrated hydrochloric acid and refluxing for 30 minutes. This step was found to be necessary to clarify the samples, prevent severe emulsion formation during the colorimetric test, and to insure that undegraded quat that might have been absorbed to cellular matter did not go undetected. Controls demonstrated that known additions of quat were analytically detectable following this

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procedure. Samples were 100 ml in volume. For the colorimetric test 1 ml of 0.02% bromphenyl blue dye in alkaline water was added to the entire acidic 100 ml sample producing a straw-yellow color. The sample was then extracted with 10 ml of chloroform which was then read at 450 nm and 460 nm using a B & L Spectronic 20 colorimeter. Quat concentration in the sample was computed from a standard concentration absorbance curve established for each quat studied. This test method is sensitive down to 0.25 ppm quat using a 100 ml sample and 10 ml of chloroform. UV spectrometry was also used to follow concentrations of the aromatic quats by subjecting refluxed samples to UV analysis at 263 and 268 nm. Again, concentrations were calculated using specific standard curves.

A nitric acid oxidation colorimetric analysis suggested by Monsanto, St. Louis, Missouri⁽³⁾ was used to follow biodegradation of pentachlorophenol. In this procedure the chlorinated phenol is oxidized to orange colored quinone by nitric acid and hydrochloric acid in benzene. Resultant color absorbance was read at 450 nm using a Hitachi 124 spectrophotometer and compared to standard curves to calculate pentachlorophenol concentrations.

RESULTS AND DISCUSSION

Biodegradation of the didecyl dimethyl quat using an unacclimated culture is shown in Figure 2. At an initial concentration of 10 ppm the quat was rapidly and completely biodegraded. Degradation was 97.5% completed after 36 hours of incubation. Relative biodegradation rates of the four quats tested using unacclimated cultures are given in Table 1. As may be seen, all of the quat structures were readily biodegraded. The aliphatic quat were more rapidly attacked than the aromatic structures with the ethyl substituted quat somewhat more resistant to degradation than the other aromatic quat. Insignificant rates and amounts of quat degradation were observed when concentrations of 20 ppm or higher were exposed to microbial attack. A readd experiment was then conducted in which 10 ppm of fresh quat was added to 96-hour old shake flasks in which 10 ppm of quat had been biodegraded. Biodegradation of the readded quats are shown in Figure 3. These results indicated that acclimation of the culture to the quats results in more rapid biodegradation.

Effects of acclimation were then more carefully examined. Quat biodegradation comparisons were made using unacclimated culture, cultures acclimated to each quat for 20 hours, 48 hours, or for 9 days. Single transfers were used for the two shorter acclimation periods and three consecutive 72-hour serial transfers into 10 ppm quat were used for the 9-day period. The results are shown in Table 2. Good growth was observed during the shorter term acclimation periods, but after 6-days exposure to 10 ppm of the quats poor growth was noticeable. Acclimation of the culture to the quats for 24-48 hours resulted in significantly more rapid decomposition of the antimicrobial agents. This indicates that the rate-limiting enzymes involved in degradation of the quats are inductive rather than constitutive. Longer exposure to 10 ppm of the quats resulted in poorer biodegradation, probably because of the inherent antimicrobial nature of these substances.

The aromatic quats were more poorly degraded than the aliphatic quats throughout the tests.

A brief examination of the effects of other organic matter on rate of quat biodegradation was carried out. As shown in Figure 4, small concentrations of yeast extract or glucose aided biodegradation of the quats while larger amounts postpone quat decomposition. These data show that biodegradation of this type of quat will occur in waste waters in the presence or absence of other organic matter.

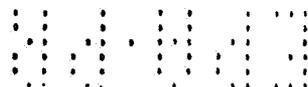
In an attempt to confirm that colorimetric disappearance of the quats actually represented decomposition, one of the aromatic quats studied was also subjected to UV analysis during exposure to microbial attack. Results are shown in Figure 5. Once biodegradation of this type of quat begins, complete molecular decomposition is very rapid. UV analysis consisted of a scan over the entire UV spectrum so that aromatic absorbance shifts due to microbial modifications of the nonaromatic portions of the molecule could not be interpreted as aromatic decomposition.

The scope of this study did not permit the examination of a large number of other quaternary ammonium structures or all other types of commercially important antimicrobial agents. However, for comparative purposes the biodegradability of a pentachlorophenol was tested. It is known that phenol adapted cultures are especially capable of attacking substituted phenols (Chambers and Kakler⁽⁴⁾), therefore, the following cultures were used for biodegradation studies.

1. Unacclimated cultures taken directly from an efficiently operating refinery phenol biodisposal system.
2. Unacclimated culture consisting of the soil-sewage culture used for the quat studies plus the refinery phenol organisms.
3. Culture number one but acclimated to pentachlorophenol by transferring four times at 5-day intervals in 2 ppm of the chlorinated phenol.
4. Culture number two acclimated in the same manner as culture number three.

Over a 10-day incubation period, all four of the cultures degraded the initial 10 ppm pentachlorophenol concentration about 20%. Acclimation did not significantly influence resultant biodegradation of the chlorinated phenol. The sensitivity of the analytical technique used allowed biodegradability testing at very low levels (0.1 ppm) of the antimicrobial. Nine concentrations ranging from 0.5 to 10 ppm of the material were tested for degradability using the acclimated cultures. No biodegradation level of greater than 20% over a seven day incubation period was observed at any of the concentrations.

Quaternary ammonium antimicrobials are usually effective microbiocides at concentrations from < 5 ppm to 50 ppm or so, depending on the microorganisms to be controlled and the environmental conditions. Our own data



shows the didecyl dimethyl quat used in this study to kill Desulfovibrio desulfuricans at < 5 ppm and inhibits Pseudomonas fluorescens at 20-50 ppm. Quats are commonly used to control microbial growth in cooling tower waters at around 50 ppm on a batch basis. Following dilution of cooling tower waters or other waste waters containing quats examined in this study, relatively rapid biodegradation of the quats should occur by microorganisms present in these environments.

It is becoming increasingly imperative to know what ecological effects to expect when utilizing chemicals that might enter our environment. This study shows that certain quaternary ammonium products, in particular certain dialkyl dimethyl quats, can be expected to be rapidly biodegraded following their use and dilution.

ADDENDUM - R. D. Ditoro, Lonza Inc.

In order to confirm the practical significance of the biodegradability of BARDAC Quaternaries, two field trials were conducted. In each case, BARDAC-22 (didecyl dimethyl ammonium chloride) was added to a water cooling tower system and the water analyzed for actual quat content. These results are shown in the following tables:

TOWER STUDY I

<u>Time</u>	<u>BARDAC-22 Concentration* (100% Active)</u>
Initial Slug Dose	29.0 ppm
After 15 minutes	3.0
" 45 "	2.0
" 1.5 hours	1.0
" 3 "	0.75
" 4 "	0.75
" 5 "	1.0
Second Slug Dose - After 5 hours	16.0
After 5.5 hours	2.0
" 6.0 "	2.0
" 7.0 "	0.75
" 8.0 "	0.6
Third Slug Dose - After 8.25 hours	36.0
After 8.5 hours	7.0
" 9.0 "	5.75
" 10.0 "	2.4
" 11.0 "	0.8
" 12.0 "	0.75
" 13.0 "	0.75
" 14.0 "	0.7

TOWER STUDY II

<u>Time</u>	<u>BARDAC-22 Concentration*</u> <u>(100% Active)</u>
Initial Slug Dose	41.5 ppm
After 5 minutes	11.0
" 30 "	4.0
" 6 hours	1.0
" 24 "	0.0
" 48 "	3.0
" 72 "	1.0

These Tower Studies show, that despite relatively high slug doses of BARDAC-22, only small amounts of quat are actually found in the blow-down water. Most of the BARDAC-22 is retained by the tower, either afixed to negatively charged sites or microbiological growths (i.e., slimes). There will be a certain amount of sloughing off of algae or bacterial growth as the quat takes effect, accounting for an occasional increase in microbiocide concentration. This is seen in Tower Study I after 5 hours and in Tower Study II after 48 hours.

* The slug dosages are calculated values derived from the tower's water capacity and the actual amount of BARDAC-22 added. Other concentration figures are the result of analysis.

REFERENCES

1. L. Barden and P. C. Issac, Proc. Inst. Civil Engrs. 6, 371 (1957).
2. P. Pitter, Technol. Water 5; 2, Institute of Chemical Technology, Prague, 1961.
3. Monsanto Technical Bulletin O-24, Analytical Methods for Pentachlorophenol and its Salts. Monsanto Chemical Company, St. Louis, Mo. 1955.
4. C. W. Chambers and P. W. Kakler, Developments in Industrial Microbiology 5, 85 (1964).

TABLE 1

BIODEGRADATION OF FOUR DIFFERENT QUATS -
UNACCLIMATED CULTURE AND COLORIMETRIC DATA

	<u>PERCENT</u> <u>24 Hr.</u>	<u>DEGRADED</u> <u>48 Hr.</u>
Didecyl Dimethyl Quat	45	92
Diocetyl Dimethyl Quat	50	95
Alkyl Dimethyl Benzyl Quat	37	95
Alkyl Dimethyl Ethylbenzyl Quat	20	95

Initial Concentrations = 10 ppm

0.12.10

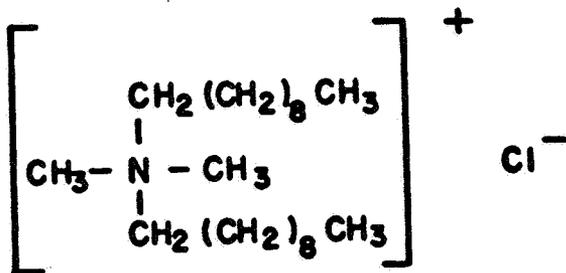
0.0.10

TABLE 2

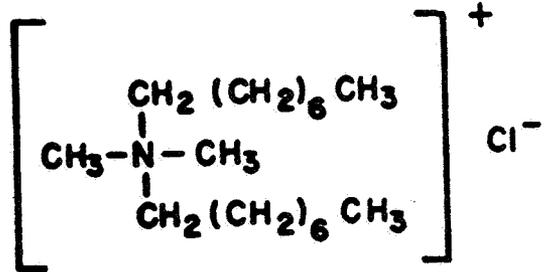
EFFECT OF CULTURE ACCLIMATION ON QUAT BIODEGRADATION

Acclimation Incubation Period	PERCENT BIODEGRADATION							
	None		20 Hours		48 Hours		9 Days	
	24 Hr.	48 Hr.	24 Hr.	48 Hr.	24 Hr.	48 Hr.	24 Hr.	48 Hr.
Didecyl Dimethyl Quat	45	92	75	95	75	97	20	55
Dioctyl Dimethyl Quat	50	95	80	97	80	97	17	57
Alkyl Dimethyl Benzyl Quat	37	95	60	97	60	97	15	50
Alkyl Dimethyl Ethylbenzyl Quat	20	95	47	97	50	97	10	35

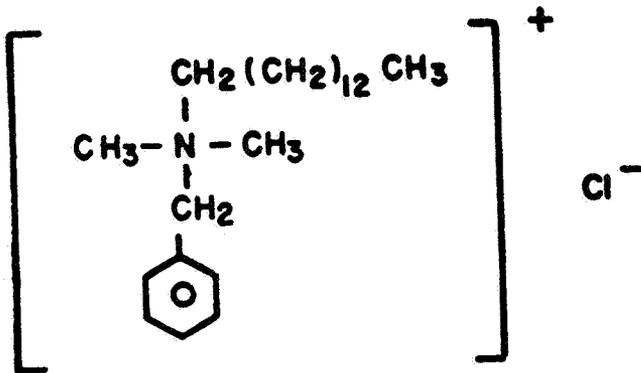
(1)
DIDECYL DIMETHYL QUAT



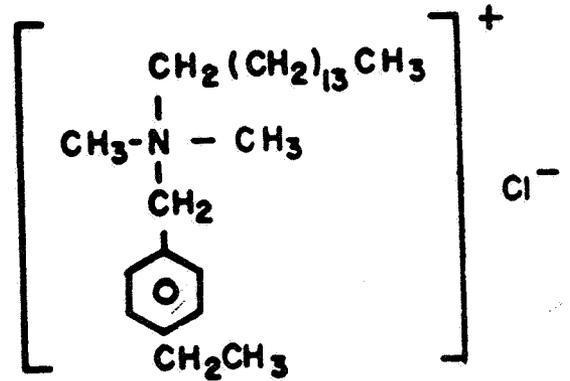
(2)
DIOCTYL DIMETHYL QUAT



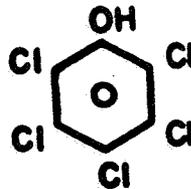
(3)
ALKYL (C₁₄)^{*} DIMETHYL BENZYL QUAT



(4)
ALKYL (C₁₄)^{*} DIMETHYL ETHYL BENZYL QUATS



(5)
PENTACHLOROPHENOL



* MIXTURE OF C₁₂-C₁₈ WITH
AVERAGE MW OF C₁₄

FIGURE 1. ANTIMICROBIAL STRUCTURES

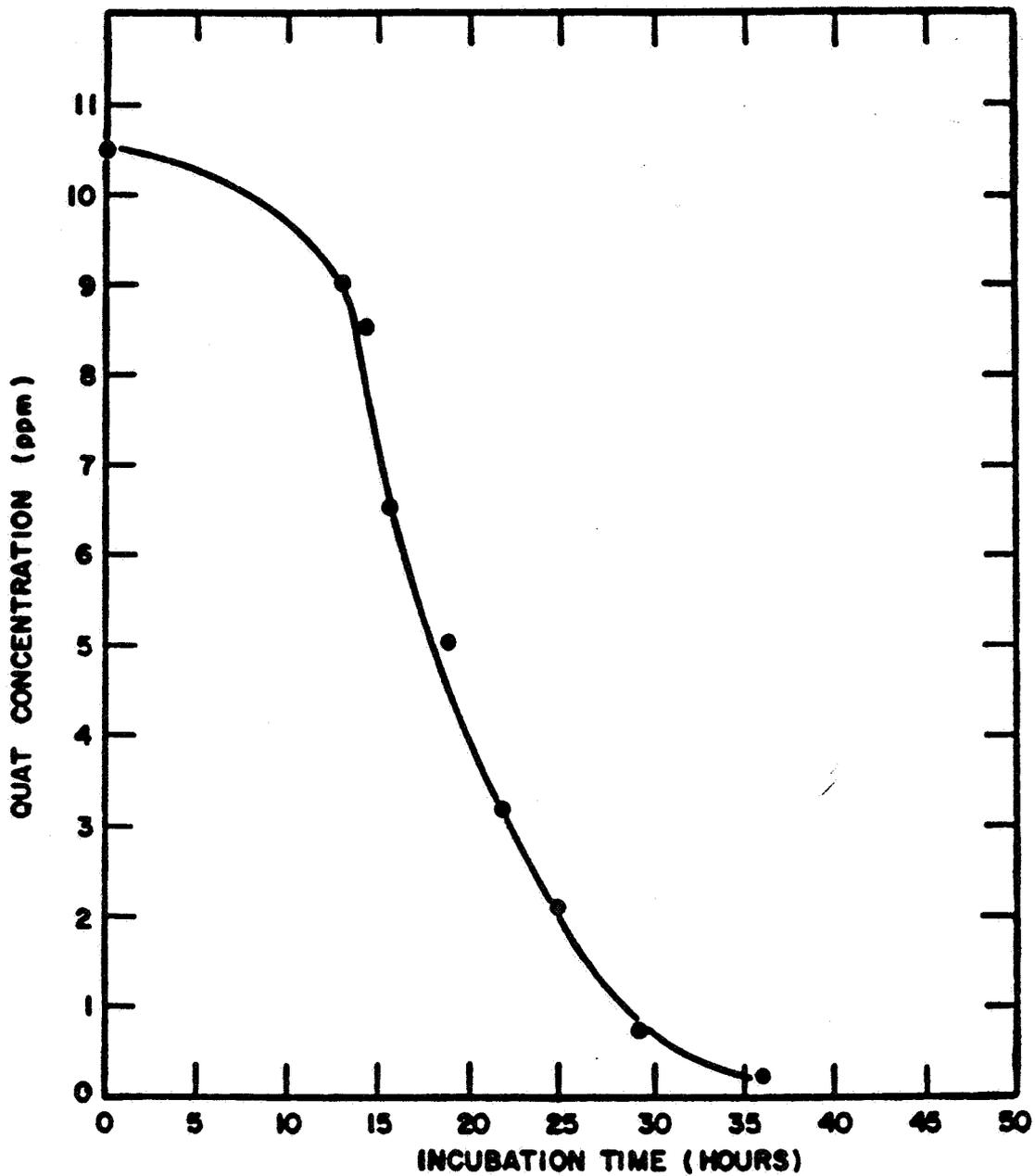


FIGURE 2 BIODEGRADATION OF DIDECYL DIMETHYL QUAT AS MEASURED BY COLORIMETRIC DISAPPEARANCE

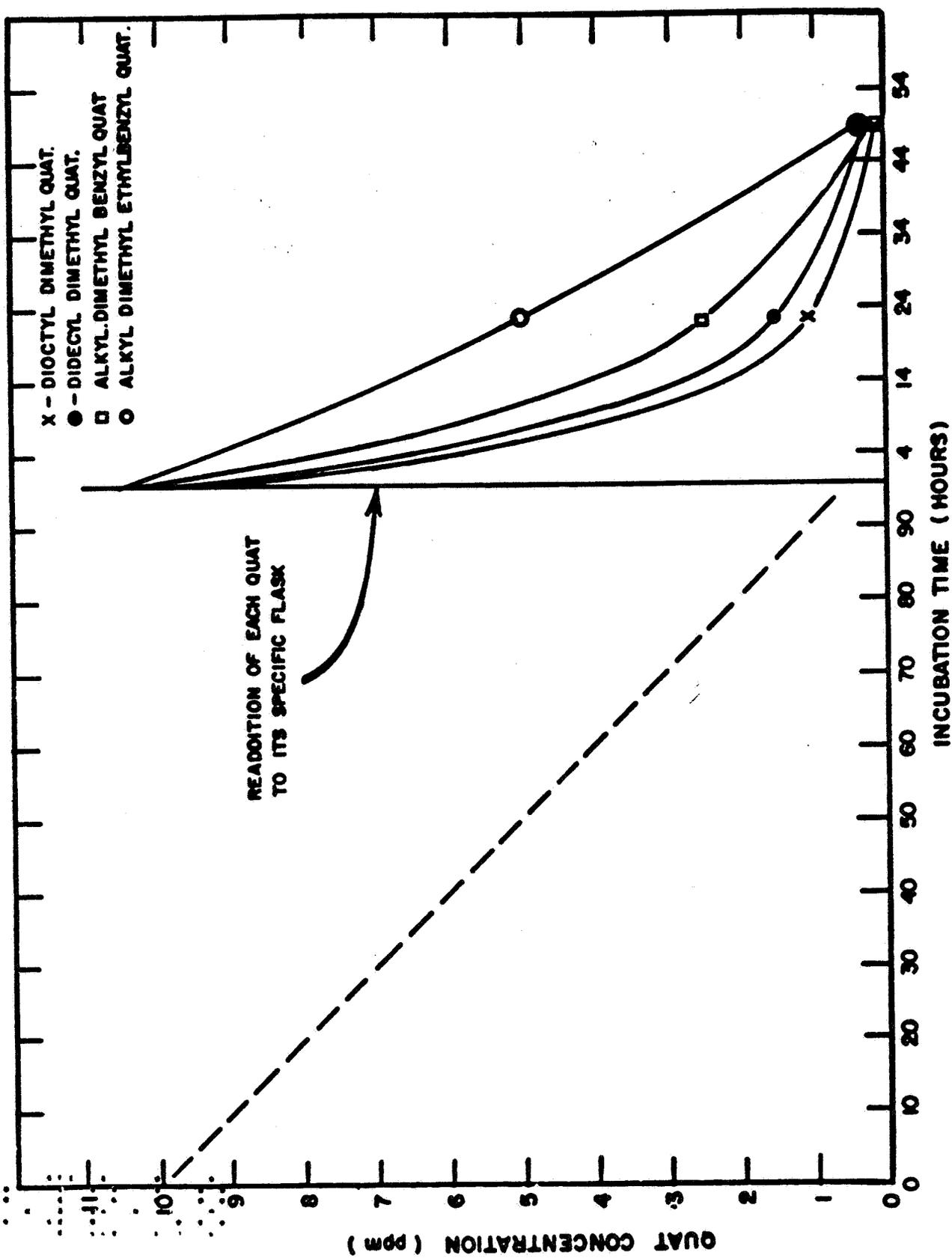


FIGURE 3 BIODEGRADATION OF READED QUATS AS MEASURED BY COLORIMETRY

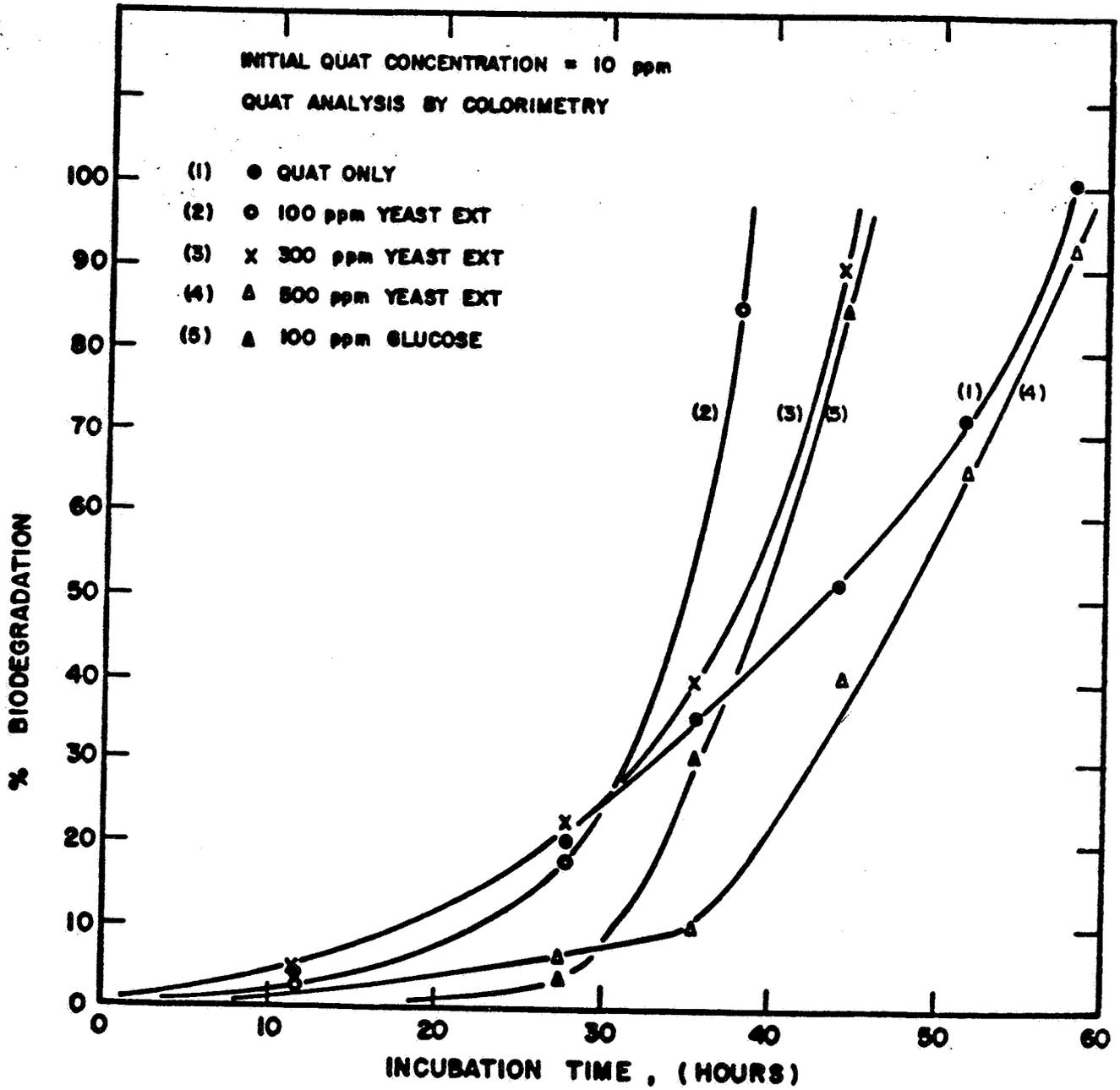


FIGURE 4 EFFECT OF OTHER ORGANIC MATTER ON THE BIODEGRADATION OF DIDECYL DIMETHYL QUAT

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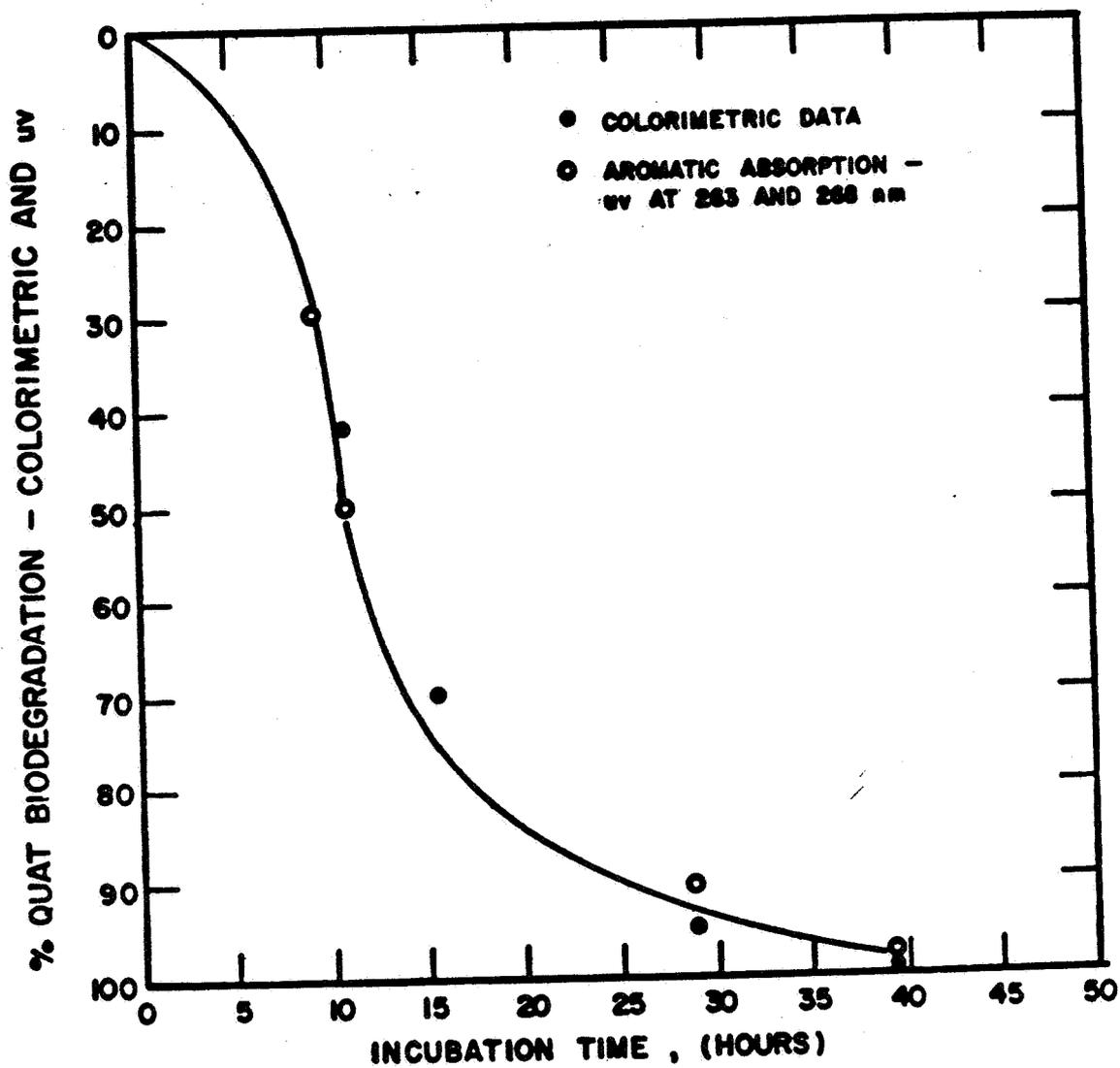


FIGURE 5 BIODEGRADATION OF ALKYL DIMETHYL BENZYL QUAT AS MEASURED BY COLORIMETRY AND uv ABSORPTION

0.1240
 0.1240