MICROBIAL DEGRADATION OF SYNTHETIC SPERM WHALE OILS

A Thesis

Presented to

the Faculty of the Department of Biology

University of Houston

In partial Fulfillment

of the Requirements for the Degree

Master of Science

---

by

Margaret C. Adams

December, 1979

TABLE OF CONTENTS

ABSTRACT

Thirteen synthetic sperm whale oils, including four sulfated and three sulfurized products, were tested for biodegradability using growth of Pseudomonas aeruginosa and BOD_5 as indicators. All of the underivatized oils were found to be easily degraded; the sulfated and sulfurized products were relatively resistant. Four oils showed anti-microbial properties at concentrations of more than 1.0%. All the replacements are capable of causing BOD loading problems.

INTRODUCTION

Sperm whale oil is a unique liquid wax consisting primarily of esters of long chain mono-unsaturated fatty acids and alcohols, with lesser amounts of the triglycerides that predominate in most oils (Peeler & Hartmann, 1973). Its composition gives it several valuable properties. It is non-drying (Thompson, 1972), non-staining (Codd, et al., 1972), and acts as a rust inhibitor (Schwartz, et al., 1958). It has high solubility in paraffinic oils and good thermal stability (Ricchuite & Hermann, 1973). When sulfated or sulfonated for improved solubility in water (Hermann & McGlade, 1974), it is used as an emulsification agent. When sulfurized, it acts as an extreme pressure agent and provides friction modification, anti-oxidant, anti-wear, and metal wetting properties (Peeler & Hartmann, 1973; Friihauf & Holubec, 1974; Thompson, 1972).

Sperm whale oil has been compounded into a variety of products, including oils for light duty and fine precision equipment such as sewing machine oils, instrument oils, textile spindle lubricants (Hermann & McGlade, 1974), and watch oils (Frankle, 1976). It is also used in food machinery lubricants (Martin, 1974), cold forming lubricant coatings (Lake, 1974), and cosmetics (Anon., 1964). The oil or its sulfated derivative is used in fat liquoring leather

(Frankle, 1976), in cutting oils (Springborn, 1967), quenching oils (Levitt, 1951), drilling muds (Crittendon, 1966), and in hydraulic fluids and general lubricant formulations (Gubenko, et al., 1973). The sulfurized oil is used in metal working fluids, slideway lubricants, gear lubricants, internal combustion engine lubricants, automatic transmission fluids (Peeler & Hartmann, 1973), steam cylinder oils, locomotive driving journal oils, gasoline engine break-in oils (Hotten, 1973), as a non-staining coring lubricant (Norton, 1974), and in a variety of general lubricants and greases (Thompson, 1972).

According to the Department of Commerce (1967, 1968, 1969), about 40 million pounds of sperm whale oil were used in the U. S. each year during the late 1960's, about half of which was sulfurized (Hotten, 1973). Approximatly 45% of the oil was used in EP lubricants, 25% in metal working fluids, 25% in leather processing and 5% in other uses, chiefly in textile lubricants and fatty acid and alcohol production (Anon., 1964). Then in December, 1970, the sperm whale was added to the Endangered Species List (Department of the Interior, 1970) and the importation of sperm whale oil became illegal. The action forced a change to replacements, the market for which has been estimated as 50 million pounds per year (Bell, et al., 1976).

Numerous substances such as jojoba oil (Gisser, et al., 1975), soybean, linseed (Bell, et al., 1976), coconut,

crambe (Perlstein, et al., 1974), and tall oils (Habiby, 1973), fish oil, lard and tallow (Kenney, et al., 1974) are being processed into synthetic replacements, usually in conjunction with reactants derived from petro-chemicals. These replacements have been tested in the laboratory (Peeler & Hartmann, 1973; Thompson, 1972; Recchuite & Hermann, 1973) as well as in actual use (Thompson, 1974) and while not as versatile as the natural oil (Calkins, 1972), their performance was comparable, and in some cases superior to sperm whale oil.

It has been proposed that synthetic organic substances should be restricted in use until their biodegradability has been established (Payne, et al., 1970). While sperm whale oil and its sulfurized derivative are known to be readily oxidized by microorganisms (Ellis, et al., 1957), nothing is known about the biodegradability of synthetic sperm oils, even though they are probably entering the environment in considerable quantities. The objective of this investigation was to acertain the biodegradability of a number of representative synthetic sperm whale oils.

MATERIALS AND METHODS

Thirteen representative synthetic sperm whale oil replacements produced by seven different manufacturers were employed in this investigation. Oils D and E were produced by the same manufacturer, as were oils H, I and J,

and products K, L and M. The remaining oils were each provided by a different producer.

Chemical Oxygen Demand (COD) and the sodium azide modification of the Biochemical Oxygen Demand (BOD) were determined according to the procedures described in Standard Methods (American Public Health Assn., 1975). Samples were prepared for the COD test by making a 1:100 dilution of oil in concentrated sulfuric acid, aliquots of which were then withdrawn for testing. For the BOD determinations, 1% emulsions in distilled water were prepared immediatly before use, and appropriate volumes pipetted directly into BOD bottles.

Seed for the BOD test was obtained from a sample of river water taken downstream from a sewage treatment plant outfall, 500 ml of which was maintained in the laboratory under aeration and fed semi-weekly with 0.1 ml of a fatty vegetable oil.

Growth studies were made on each oil at 0.1, 0.5, 1.0, 5.0 and 10.0% concentrations. The oils were prepared as 10.0% emulsions in Leadbetter & Foster (1958) basal salts medium using 1.0% gum arabic as an emulsification agent (Isenberg & Bennett, 1959). The sulfated oils formed emulsions immediatly upon addition to basal salts. The underivatized oils were titurated in a hand homogenizer, and the sulfurized oils were sonicated until emulsified. Emulsions were adjusted to pH 7 $\pm$ 0.02, diluted to the desired concentrations with basal salts medium containing 1.0% gum arabic,

and dispensed as 100 ml aliquots in 250 ml Erlenyeyer flasks. All flasks were prepared in duplicate, including control flasks containing only basal salts and 1.0% gum arabic, and sterilized by autoclaving 15 min. Cooled flasks were inoculated immediately.

Inocula were prepared from 12 hr nutrient agar cultures of Pseudomonas aeruginosa ATCC 15442. Cells were harvested and washed three times by centrifugation in basal salts, then incubated 12 hrs to reduce endogenous growth (Williams & Bennett, 1973). After a final washing the suspension was standardized photometrically and each growth flask was inoculated with approximatly 400 cells. Flasks were incubated on a rotary incubator at 23°C and growth was measured at 3, 5, 7, and 10 days by pour plate counts in nutrient agar. Each experiment was performed in duplicate and repeated at least once, yielding a minimum of four values for each data point. Controls were subtracted, and the values were analysed using the Q-test (Dean & Dixon, 1951). Statistically significant values were averaged.

RESULTS

COD AND BOD_5 STUDIES

The COD and BOD_5 values of the thirteen products are given in Table 1. Chemically, the sulfurized products gave significantly higher oxygen demands than the parent oils, while the sulfated derivatives showed the lowest CODs.

Table 1. COD and BOD_5 values for synthetic sperm whale oils

Product	Sulfur Content	COD mg/l x 10^6	BOD_5 mg/l x 10^6	% degradation*
A	None	2.04	0.93	46
B	None	2.25	1.0	44
C	None	2.36	1.4	54
D	None	2.33	1.4	60
E	None	2.25	1.5	67
F	None	2.24	1.2	54
Sulfated				
G	7% SO_3	1.55	0.18	12
H	4% SO_3	1.45	0.59	41
I	4% SO_3	1.66	0.51	31
J	3% SO_3	1.66	0.35	21
Sulfurized				
K	17% S	2.85	o.46	16
L	11% S	2.77	0.86	31
M	17% S	2.68	0.43	16

* % degradation was determined by calculating the BOD_5 as a percentage of the COD.

Biologically, the underivatized oils exhibited the greatest oxygen demand, with the sulfated and sulfurized products showing lower, overlapping BOD_5 values. Analysis using Student's T test showed that the BOD_5 of the sulfated and sulfurized oils were statistically identical and were significantly lower than the values of the underivatized oils.

PURE CULTURE GROWTH STUDIES

All of the synthetic sperm whale oils acted as good carbon sources for *P. aeruginosa* at 1% or less. Cell counts at 0.1% were approximatly proportional to utilizable carbon and can be used as an indication of relative ease of degradation (Prochazka & Payne, 1965). Maximum crop in 0.1% oil showed that product C was the most easily utilized, followed by oils F, A, E, B, D, I, K = L, H, J = M and G (Table 2). The underivatized oils supported the greatest growth with the derivatized products giving lower, overlapping values. Statistical analysis using the T test showed that the sulfated and sulfurized oils are statistically the same, but differ significantly from the underivatized products.

At concentrations between 0.1 and 10.0%, carbon ceased to be the growth limiting factor and cell crop either leveled off or decreased. Nine of the oils continued to be good carbon sources at all concentrations (Table 3). Maximum growth was reached at 10% in oils A, B, D and E; at 5% in oils C, G, K and M; and at 1% in oil L. The four remaining

Table 2. Maximum growth of *P. aeruginosa* in 0.1% synthetic sperm whale oils.

	Product	CFU/ml x 10^8
Underivatized	A	79
	B	62
	C	97
	D	40
	E	69
	F	91
Sulfated	G	4
	H	17
	I	28
	J	16
Sulfurized	K	20
	L	20
	M	16

Growth studies were done in Leadbetter & Foster (1958) basal salts medium with synthetic sperm whale oils as sole source of carbon.

Table 3. Maximum growth of *P. aeruginosa* in non-inhibiotry synthetic sperm whale oils.

	Growth expressed as number of cells/ml x 10^8			
	concentration (%)			
Product	0.5	1.0	5.0	10.0
A	200	290	250	320
B	114	230	280	320
C	270	280	330	270
D	210	280	290	330
E	150	200	220	300
G	19	170	370	330
K	130	190	270	260
L	140	250	250	160
M	95	140	210	120

Growth studies done in Leadbetter & Foster (1958) basal salts medium with synthetic sperm whale oils as sole source of carbon.

oils, however, proved toxic at higher concentrations (Table 4). Oil F, an underivatized product, was bactericidal at 2%. Oils H, I and J, all sulfated, were toxic at 2, 6 and 10% respectively. Two different patterns of inhibition were noted. Products F and H exhibited immediate toxicity at inhibitory concentrations; no viable cells could be recovered from the flasks after 72 hrs of incubation. Products I and J supported good growth initially, followed by a rapid decrease in viable cells until cell counts reached zero at five to seven days.

The delayed onset of toxicity in oils I and J seemed to indicate that toxic substances were produced during incubation. In order to determine if toxicity was due to metabolic products produced during growth of P. aeruginosa, or to chemical breakdown of the oils, the following experiment was performed. Three duplicate samples of each of the four inhibitory oils were prepared as described previously. Oils F and H were prepared at 1.0% concentrations, oil I at 8.0%, and oil J at 10.0%. One duplicate set of each oil was inoculated immediately after sterilization, a second set was inoculated after the flasks had been shaken for 5 days, and a third set was inoculated after incubating 10 days.

The results of this study may be found in Table 5. Samples inoculated immediately after sterilization showed the same pattern of growth as observed previously. Samples shaken for 5 or 10 days prior to inoculation showed a marked

Table 4. Growth of *P. aeruginosa* in inhibitory synthetic sperm whale oils.

Product and concentration (%)		Growth expressed as number of CFU/ml x 10^8 days 3	5	7	10
F	0.1	60	84	91	49
	0.5	160	270	260	120
	1.0	220	300	370	290
	2.0	0	0	0	0
H	0.1	11	17	12	11
	0.5	48	50	54	61
	1.0	52	110	110	110
	2.0	0	0	0	0
I	0.1	28	28	28	28
	0.5	130	140	180	170
	1.0	32	190	180	180
	5.0	22	4	4	2
	6.0	51	4	0	0
	10.0	67	3	0	0
J	0.1	14	16	14	13
	0.5	61	63	52	57
	1.0	120	140	140	110
	5.0	100	99	77	57
	9.0	30	26	22	21
	10.0	11	0	0	0

Growth studies done in Leadbetter & Foster (1958) basal salts medium with synthetic sperm whale oils as sole source of carbon.

Table 5. Growth of *P. aeruginosa* in inhibitory oils when inoculation is delayed 0, 5 and 10 days.

days inoculation delayed	Growth expressed as number of cells/ml x 10^8 days after inoculation				
	2	3	5	7	10
Oil F - 1.0%					
0	0.01	2.5	110	150	130
5	0	0	0	0	0
10	0	0	0	0	0
Oil H - 1.0%					
0	0.23	2.3	88	79	70
5	0.01	0.37	4.4	6.1	4.6
10	0	0	0	0	0
Oil I - 8.0%					
0	1.0	10	0	0	0
5	0	0	0	0	0
10	0	0	0	0	0
Oil J - 10.0%					
0	1.0	21	0	0	0
5	0	0	0	0	0
10	0	0	0	0	0

Growth studies done in Leadbetter & Foster (1958) basal salts medium with synthetic sperm whale oils as sole source of carbon.

increase in toxicity; in most cases, no viable cells were recoverable 48 hrs after inoculation. Additionally, concentrations of oils F and H which were previously non-toxic became bactericidal when shaken prior to inoculation. Toxicity was evidently due to chemical, rather than bacterial, alteration of the oils. None of the other nine products showed any evidence of toxicity due to delayed inoculation.

DISCUSSION

No studies of the degradability of synthetic sperm whale oils have been reported. Ellis, et al. (1957), tested natural and sulfurized natural sperm whale oils against three acclimated Pseudomonads in a short term manometric study and found that both oils were readily oxidizable, though the rate of O_2 uptake for the natural oil was more than twice that of the sulfurized. Based on the results obtained for other sulfated oils, they concluded that sulfated sperm whale oil should also be readily oxidizable, though the oil was not actually tested. No reports of the BOD or COD of the natural oil were found.

Though the BOD_5 and COD values of natural sperm whale oil are unknown, the Theoretical Oxygen Demand (TOD) can be estimated. Assuming a composition of 67% dienoic esters with a mean length of 34 carbons, and 33% trienoic triglycerides with a mean carbon number of 51 (Spencer & Tallant, 1973), along with a specific gravity of 0.9 (Levitt, 1951),

the TOD of sperm whale oil should be about 2.7×10^6 mg/l. This agrees quite well with the CODs demonstrated by the synthetic products. Examination of Table 1 shows that the underivatized products have COD values slightly less than the estimated TOD of natural sperm whale oil. The demand of the sulfated products is about half that, which could be expected since the process of sulfation is itself an oxidizing one and would partially oxidize the oils during preparation. The COD of the sulfurized products is almost identical to the estimate, but the increased demand over the underivatized oils is probably due to the presence of reduced sulfur, rather than to the oils themselves.

The BOD_5 of all the products is high enough to exert a a serious oxygen demand during treatment if passed through a waste system in large quantities. The least degradable product, oil G, gives a BOD_5 of 1.8×10^5, while raw sewage shows a BOD_5 of 2.4×10^2 to 1.3×10^3 (Viraraghavan, 1976). The underivatized oils are comparable to sodium oleate which has a BOD_5 of 9.4×10^5 (Heukelekian & Rand, 1955). In addition, studies not reported here have shown that these products contain no appreciable nitrogen and would require a source of this material in order for degradation to proceed.

Because of the short incubation period, BOD_5 values represent only a fraction of the ultimate BOD. For example, glucose, which can be completely utilized by microorganisms, has a BOD_5 value of only 47 to 73% of the TOD (Heukelekian &

Rand, 1955). Similarly, raw sewage has a BOD_5/COD value of about 45% (Viraraghavan, 1976). For this reason, it has been proposed that any product showing a 5 day oxygen uptake in excess of 40 to 45% of theoretical should be considered as completely degradable (Busch & Myrick, 1960); Nelson, et al., 1961), while materials with a BOD_5 less than 20% of COD are either refractile or require acclimation (Symons, et al., 1960).

Based upon these criteria, all the underivatized sperm whale oil replacements should be degradable. Their BOD_5 is between 44 and 67% of their ultimate oxygen demand as approximated by COD. The derivatized oils are less susceptible, showing from 12 to 41% utilization of demand in 5 days. Oils G, K and M are definitely refractile at 12, 16 and 16%. In the case of the sulfurized products, this is undoubtably due at least in part to the presence of reduced sulfur. BOD_5 measures predominatly carbonaceous oxygen demand since the bacteria which oxidize non-carbonaceous materials for energy are usually not present in sufficient numbers to exert an appreciable demand within 5 days. The sulfur protion of these compounds, therefore, is probably not being utilized within the time period of the test, though it may be degraded later (American Public Health Assn., 1975).

Degradability as measured by growth studies with P. aeruginosa shows agreement with values obtained from BOD_5/COD data. Each of the underivatized oils supports a

larger population of cells than does any of the derivatized oils. The sulfated and sulfurized groups overlap, and the variation within the sulfated group encompasses the sulfurized oils. There is no correlation between growth and type, or amount of sulfur. Oil G is the least degradable by both methods.

These findings indicate that the substitute sperm whale oils would be easily degradable, as was the natural oil, but that the synthetic derivatives may be less easily oxidized than the natural ones tested by Ellis. The fact that Ellis used acclimated cultures and short term tests may account for the difference in findings, or there may be basic differences between the natural and the synthetic derivatives. In either case, the derivatized oils may persist in the environment. Longer treatment times or acclimation might overcome this problem.

Both pure culture studies and mixed population tests such as the BOD possess inherent limitations. Pure culture studies do not approach natural conditions, and even though P. aeruginosa is noted for the wide range of substrates it is able to utilize, it will not detect ecological interrelationships where cometabolic processes may achieve degradation impossible to any one organism. Mixed cultures of microorganisms approximate nature more closely, but the BOD test has many limitations (American Public Health Assn., 1975). Neither method is an absolute measurement, but both are in common usage as indicators of degradability. It must

also be remembered that synthetic sperm whale oils are mixtures, not pure compounds. The presence of small amounts of recalcitrant molecules could be masked by larger amounts of easily utilized compounds.

It is not known why four of the products exhibit antimicrobial activity. The manufacturer's specification sheets reveal no noticable differences in chemical properties, though the information is far from complete. Natural sperm whale oil shows no evidence of toxicity, and has been used as a feed in antibiotic production at concentrations up to 5% (Makarevich & Laznikova, 1961).

Toxicity is not due to solubility or sulfation. The pH of the bactericidal products remained above pH 6, which is not deleterious to P. aeruginosa (Bushnell & Haas, 1941). It seems likely that the antimicrobial effects noted are due to the formation of toxic decomposition products, since toxicity is most noticable after a period of shaking. Because the effect is a function of time after dilution, not after inoculation, it seems to be of chemical rather than catabolic origin. Decomposition of several synthetic lubricants has been shown to be dependant on the presence of dissolved moisture (Cuellar & Johnston, 1969).

Fatty acids present in the oils or released by hydrolysis may be toxic. Saturated fatty acids have been reported toxic to microbial growth in concentrations as low as 1 ppm (Nieman, 1954). Antimicrobial activity has been reported

for saturated, straight chain acids of between 4 and 12 carbons in length with the strongest activity at 9 to 12 carbon atoms (Kahn & Katamay, 1969; Klarmann & Shternov, 1941; Nieman, 1954; Rolinson, 1954). Activity varies with the Gram reaction and species of the organism tested (Nieman, 1954; Klarmann & Shternov, 1941), with pH (Karabinos & Ferlin, 1954), with solubility (Bell, 1971) and with degree of dissociation (Rolinson, 1954). Unsaturation increases toxicity (Alford, et al., 1971; Nieman, 1954), as does branching (Bell, 1971).

Oxidation products such as peroxides, ketones and other carbonyl compounds (Smith & Alford, 1968) may be toxic (Roth & Halvorson, 1952). Pelargonic acid, a decomposition product of oleic acid that has been identified as the active bactericidal substance in rancid fat (Karabinos & Ferlin, 1954) may be present.

It may be that all of the products contain components which are toxic to microorganisms, but that additional, unknown, substances present in the non-inhibitory oils neutralize this antagonistic action. It is known that certain divalent cations, surface active agents, or proteins can reduce the toxicity of fatty acids (Alford, et al., 1971). Alternatively, anti-oxidants which prevent formation of toxic oxidation products may be present in the non-inhibitory oils, but absent in the others.

The toxic substances formed in the four products are probably themselves degradable since immediate inoculation

reduces or prevents toxicity. Microorganisms can decompose toxic fatty acids (Bell, 1971), peroxides and other by-products produced during the rancidity phase (Smith & Alford, 1968) when these substances are present in non-toxic levels. Microorganisms may prevent their formation in the first place since organisms such as Pseudomonas are known to produce anti-oxidants (Smith & Alford, 1970). Because microbial contamination would probably occur simultaneously with dilution under industrial or waste conditions, the problem of increased toxicity due to delayed inoculation would probably not arise.

The results present some interesting industrial possibilities. Many of the specialty lubricants in which synthetic sperm whale oils might be used are emulsion systems of oil in water. Unfortunately, these products create major use problems because the diluted products are susceptible to biodeterioration. It is not unusual for industries to add antimicrobial agents in order to partially control the problem. As an alternative, there is growing interest in designing products which by their nature or composition are not susceptible to microbial attack. It would be interesting if the oxidized synthetic sperm whale oils could be added to such products to impart the lubrication qualities of the sperm whale oil and the antimicrobial properties of the unknown toxic components.

This investigation indicates that underivatized synthetic sperm whale oils are quite easily degraded by unacclimated

soil and water microorganisms. The sulfated and sulfurized oils are less so; several show signs of being biologically "hard", but may prove more degradable with acclimated populations. All the oils may present a BOD loading problem if present in large concentrations. Four may also exert toxic effects in concentrations above 1%. Both these problems can be overcome by dilution. Studies of this type may prove of practical value to persons using synthetic sperm whale oils industrially. Those interested in obtaining easily biodegradable products might be advised to consider using underivatized products, while persons interested in resistant compounds might select one of the derivatives.

REFERENCES

Alford J. A., Smith J. L. & Lilly H. D. (1971) Relationship of microbial activity to changes in lipids in foods. J. Appl. Bact. 34, 133-146.

American Public Health Association (1976) Standard Methods for the Examination of Water and Wastewater pp. 543-554. American Public Health Association, Inc., New York.

Anon. (1964) Sea Hunt for specialties oils. Chem. Week. 95, 27-32.

Bell E. W., Cowan J. C. & Gast L. E. (1976) Sperm oil replacements from selectively hydrogenated soybean and linseed esters: special lubricants. J. Amer. Oil Chem. Soc. 53, 511-517.

Bell G. H. (1971) The action of monocarboxylic acids on Candida tropicalis growing on hydrocarbon substrates. A. van Leeuw. 37, 385-400.

Busch A. W. & Myrick N. (1960) BOD progression in soluable substrates III: short-term BOD and bio-oxidation solids production. Proceedings of the 15th Industrial Waste Conference, Purdue Univ. Eng. Bull., Ext. Ser. No. 106 pp. 19-27.

Bushnell L. D. & Haas H. F. (1941) The utilization of certain hydrocarbons by microorganisms. J. Bact. 41, 653-673.

Calkins J. M. (1972) Discussion of the NLGI annual meeting paper on comparisons of sperm oil based E.P. additives with their replacement products. NLGI Spokesman 36, 214.

Codd L.W., Dijkhoff K., Fearon J. H., van Oss C. J., Roeberson H. G. & Stanford E. G., eds. (1966) Chemical Technology: an encyclopedic treatment. Vol. 5, p. 166. Harper & Row, New York.

Crittendon B. C. (1966) Emulsion drilling fluids. U.S. Pat. No. 3,252,309 May.

Cuellar J. P. & Johnston R. K. (1969) Storage stability of synthetic lubricants. NTIS Report AD-846 702.

Dean R. B. & Dixon W. J. (1951) Simplified statistics for small numbers of observations. Anal. Chem. 23, 636-638.

Department of Commerce (1967) Current Industrial Reports: fats & oils production, consumption and factory and warehouse stocks, summary. U. S. Dept. of Commerce C3.158:(M20K).

ibid. (1968)

ibid. (1969)

Department of the Interior (1970) Conservation of endangered species and other fish or wildlife. Federal Register 35, 18319-18322.

Ellis L. F., Samuel-Mararajah R., Mendelow L. M., Ruth L. & Pivnick H. (1957) Oxidation of components of soluble oils. Appl. Microbiol. 5, 345-348.

Frankel A. (1976) Industrial use of whale oil. Chemtech. 6, 322-323.

Friihauf E. J. & Holubec Z. M. (1974) The development and evaluation of sperm oil replacement products for automatic transmission fluids, axle lubricants, tractor hydraulic fluids and industrial lubricants. NLGI Spokesman 37, 372-381.

Gisser H., Messina J. & Chasan D. (1975) Jojoba oil as a sperm oil substitute. Wear 34, 53-63.

Gubenko I. B., Dormidoshina A. S., Turskii Yu. I., et al. (1973) Lubricants for hydraulic systems and general lubrication of the industrial equipment. U.S.S.R. Pat. No. 398,599 Sept.

Habiby E. N. (1973) Sulfur-containing lubricant additives. Ger. Offen. 2,243,981 Mar.

Hermann C. L. & McGlade J. J. (1974) Industrial applications for animal fatty oils. J. Amer. Oil Chem. Soc. 51, 88-92.

Heukelekian H. & Rand M. C. (1955) Biochemical oxygen demand of pure organic compounds. Sewage ind. Wastes 27, 1040-1053.

Hotten B. W. (1973) Sulfurized sperm whale oil: history, chemistry, functions. NLGI Spokesman 37, 174-178.

Isenberg D. L. & Bennett E. O. (1959) Bacterial deterioration of emulsion oils II: nature of the relationship between aerobes and sulfate-reducing bacteria. Appl. Microbiol. 7, 121-125.

Kahn M. & Katamay M. (1969) Antagonistic effect of fatty acids against Salmonella in meat and bone meal. Appl. Microbiol. 17, 402-404.

Karabinos J. V. & Ferlin H. J. (1954) Bactericidal activity of certain fatty acids. J. Amer. Oil Chem. Soc. 31, 228-232.

Kenney H. E., Donahue E. T. & Schmeltz I. (1974) Sulfurized esters derived from tallow, lard, and other fats as lubricant additives. Lubri. Engng. 30, 394-397.

Klarmann E. G. & Shternov V. A. (1941) Are soaps germicidal? Soap sanit. Chem. 17, 23-26.

Lake A. W. (1974) Phosphate coatings for metals. S. Africa Pat. No. 72 05,835 Feb.

Leadbetter E. R. & Foster J. W. (1958) Studies on some methane-utilizing bacteria. Arch. Mikrobiol. 30, 91-118.

Levitt B. (1951) Oil, Fat and Soap, pp. 9-10. Chemical Publishing Co., New York.

Makarevich V. G. & Laznikova T. N. (1961) Media with different oil cakes as sources of organic nitrogen for the fermentation of chlorotetracycline. Antibiotiki 6, 308-311.

Martin A. G. (1974) Composition for lubricating food machinery. Brit. Pat. No. 1,375,826 Nov.

Nelson J. F., McKinney R. E., McAteer J. H. & Konecky M. S. (1961) The biodegradability of alkylbenzene sulfonates. Devs. ind. Microbiol. 2, 93-101.

Nieman C. (1954) Influence of trace amounts of fatty acids on the growth of microorganisms. Bact. Rev. 18, 147-163.

Norton J. H. (1974) Nonstaining drill lubricants. U. S. Pat. No. 3,810,836 May.

Payne W. J., Wiebe W. J. & Christian R. R. (1970) Assays for biodegradability essential to unrestricted usage of organic compounds. Bioscience 20, 862-865.

Peeler R. L. & Hartmann L. M. (1973) Evaluation of sulfurized sperm oil replacements. NLGI Spokesman 37, 17-21.

Perlstein T., Eisner A. & Schmeltz I. (1974) Synthesis of wax esters for use as possible sperm oil replacements. J. Amer. Oil Chem. Soc. 51, 335-339.

Prochazka G. J. & Payne W. J. (1965) Bacterial growth as a practical indicator of extensive biodegradability of organic compounds. Appl. Microbiol. 13, 702-705.

Ricchuite A. D. & Hermann C. L. (1973) Case study in the development of sperm oil substitutes - part 1. NLGI Spokesman 37, 298-304.

Rolinson G. N. (1954) The effect of saturated fatty acids on oxygen uptake by Penicillium chrysogenum. J. appl. Bact. 17, 190-195.

Roth N. G. & Halvorson H. O. (1952) The effect of oxidative rancidity in unsaturated fatty acids on the germination of bacterial spores. J. Bacteriol. 63, 429-435.

Schwartz A. M., Perry J. W. & Berch J. (1958) Surface Active Agents and Detergents Vol. 2, p. 245. Interscience Publishers, Inc., New York.

Smith J. L. & Alford J. A. (1968) Action of microorganisms on the peroxides and carbonyls of rancid fat. J. Food Sci. 33, 93-97.

Smith J. L. & Alford J. A. (1970) Presence of antioxidant materials in bacteria. Lipids 5, 795-799.

Spencer G. F. & Tallent W. H. (1973) Sperm whale oil analysis by gas chromatography and mass spectrometry. J. Amer. Oil Chem. Soc. 50, 202-206.

Springborn R. K. (1967) Cutting and Grinding Fluids: Selection and Application. Amer. Soc. Tool. Manufact. Engineers, Dearborn, Michigan.

Symons J. M., McKinney R. E. & Hassis H. H. (1960) A proceedure for determination of biological treatability of industrial wastes. J. Water Pollut. Control Fed. 32, 841-852.

Thompson P. F. (1972) Comparisons of sperm oil based E. P. additives with their replacement products. NLGI Spokesman 36, 208-213.

Thompson P. F. (1974) Sperm oil replacements for E P applications - 1972. NLGI Spokesman 38, 20-22.

Viraraghavan T. (1976) Correlation of BOD, COD and soluble organic carbon. J. Water Pollut. Control Fed. 48, 2213-2214.

Williams J. & Bennett E. O. (1973) Biodegradation of oleates J. Water Pollut. Control Fed. 45, 1671-1681.

ACKNOWLEDGMENT

The author wishes to thank the companies which kindly furnished the samples of synthetic sperm whale oil used in this study, and the University of Houston Coastal Center for partial financial support of this research.