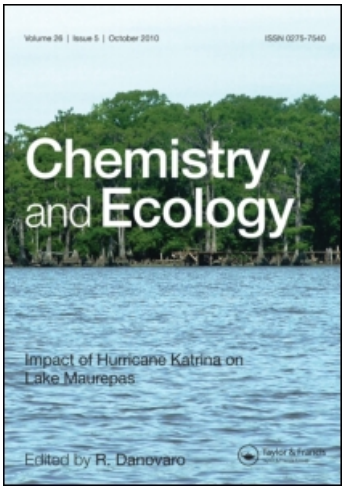

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BACTERIAL DEGRADATION OF VEGETABLE OILS

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Following initial experiments presented elsewhere (2IOPS), the bacterial degradation of two vegetable oils was investigated in some detail. The number of aerobic and anaerobic heterotrophic bacteria, oil degrading and sulphate reducing bacteria were quantified during simulated spills on a salt marsh. The sediment fatty acid composition was also studied using GC-MS analysis. Degradation of linseed and sunflower oils was concomitant with an increase in the numbers of aerobic and anaerobic bacteria. Fatty acids analysis revealed preferential degradation of the principal components of the oils (18:3ω3 for linseed oil and 18:2ω6 for sunflower oil). The presence of several isomers of the usual polyunsaturated fatty acids was also detected. The identification of some of these new fatty acids has been carried out. Possible pathways of degradation of these vegetable oils are suggested.

Keywords: Sunflower and linseed oil; aerobic and anaerobic bacteria; oil degrading bacteria; fatty acids; degradation; salt marsh

INTRODUCTION

The greatest hazard to the marine environment from vegetable oils occurs during the loading and unloading of the cargo, when spillage of small amounts can occur (Hoffman, 1989). However, larger spills can take place and there are a few such cases reported. These include 1500 tonnes of sunflower oil spilt in the SW coast of Anglesey, North Wales, UK. (Mudge *et al.*, 1993), rapeseed oil spilt in Vancouver Harbour (McKelvey *et al.*, 1980), 2.5 million gallons of soybean oil in

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Minnesota River (Gunstone, 1994) and palm and coconut oil on Fanning Island, in the Pacific Ocean (Russell and Carlson, 1978). Reports suggest that such accidents are harmful for the local wildlife.

Biodegradation represents an important route by which these oils can be removed from the environment. The first step in the microbial degradation of an oil is its hydrolysis to release the component fatty acyl groups. Triacylglycerols and their partially hydrolysed products, di- and mono- acylglycerols, are not assimilated as such (Ratledge, 1994). However, high biodegradability of vegetable oils should be expected, given that lipases are produced by a wide range of micro-organisms and the pathways for the degradation of glycerol and fatty acids are virtually ubiquitous (Cornish *et al.*, 1993). However, Mudge *et al.* (1993) reported that sunflower oil formed of polymer in sea water which was not easily degraded, after an accidental spillage by a cargo vessel. Sandy sediments bound together with sunflower oil were discovered in the beach over five years after the wreckage (Mudge, 1997). Sunflower oil was also found to polymerise in salt marsh sediments (Mudge *et al.*, 1995).

The purpose of the present study was to identify the role of aerobic and anaerobic bacteria in the degradation of linseed and sunflower oil in simulated spills in salt marsh sediments. Numbers of heterotrophic aerobic, anaerobic and sulphate bacteria were assessed as well as the number of bacteria with the ability to degrade these oils. The fatty acids composition of the sediments was also analysed.

Linseed and Sunflower Oil Composition

The fatty acid composition of linseed and sunflower oils is given in Table I.

Site Description

The sampling area was a salt marsh at Foryd Bay, located at the southwest entrance to the Menai Strait, North Wales, UK. The experiment site was in the upper part of the salt marsh and was uncovered at low tides and flooded at mean high water.

TABLE I Fatty acid composition (weight %) of linseed and sunflower oils (Menon *et al.*, 1989; Budavari, 1989)

Systematic name	*Trivial Name*	*Omega Name*	*Linseed oil* (%)	*Sunflower oil* (%)
Hexadecanoic acid	Palmitic acid	16:0	8	6.4
Octadecanoic acid	Stearic acid	18:0	6	1.3
cis-9-octadecenoic acid	Oleic acid	18:1ω9	20	21.3
cis-9, *cis*-12-octadecadienoic acid	Linoleic acid	18:2ω6	14	66.2
cis-9, *cis*-12, *cis*-15-octadecatrienoic acid	α-linolenic acid	18:3ω3	51	<0.1
Eicosanoic acid	Arachidic	20:0	1	4
Docosanoic acid	Behenic acid	22:0	–	0.8

METHODS

Field Experiments

In January 1995, linseed oil was added to surface sediments. Plastic collars of 15 cm length and 35 cm diameter were used to simulate small spills of vegetable oils. They were placed at random in a 4 m^2 area. These collars were pushed approximately 3 cm into the sediments and 1.5 litres of oil were poured into them and their top covered with a muslin screen.

Cores were taken prior to the addition of the oil (Day 0) and on days 3, 7, 14, 21, 28 and 60. A number of cores of 5 cm diameter and 40 cm length were obtained at each sampling occasion. Sediment analysis was carried out for various depths. This paper reports the results obtained for the upper 2 cm.

In January 1996, this procedure was repeated with sunflower oil at the same location, and in this case sediment samples were also collected after 180 days.

Bacteria Enumeration

Bacteria were detached from the sediment by sonication and appropriate dilutions were made with a mixture of Menai Strait sea water and distilled water (26).

The enumeration of viable heterotrophic aerobic bacteria was carried out using the pour plate technique with a standard marine medium. ZoBell's 2216 (ZoBell, 1941). Three replicates were prepared

of each dilution and the colony forming units (CFU) were counted after incubation during 14 days at 20°C. A similar procedure for the enumeration of anaerobic heterotrophic and sulphate reducing bacteria (SRB) was followed, using thioglycollate medium (Merck) and Postgate medium E (Postgate, 1979) respectively, with a salinity of 26.

The most probable numbers technique (MPN) in a three tube series was employed for the enumeration of aerobic and anaerobic oil degrading bacteria. The culture medium contained (values in $g l^{-1}$): potassium nitrate, 1.01; sodium acid phosphate 0.1 and 0.4% of oil. The medium for the cultivation of anaerobes also contained 0.6 g of sodium thioglycollate. The number of bacteria was determined after 14 days of incubation at 20°C.

The anaerobic bacteria were inoculated and incubated in a anaerobic cabinet with an atmosphere of 10% hydrogen, 10% carbon dioxide and 80% nitrogen.

Fatty Acid Analysis

Sediment samples were freeze dried for 48 hours. The lipids from 20–30g of dry sediment were soxhlet extracted for 3.5 hours using chloroform. The total amount of lipids was determined and 1 mg was derivatised to methyl esters with trimethylsulfoniumhydroxide (TMSH). Prior to the fatty acid methyl esters (FAMEs) analysis, nonadecanoic acid methyl ester was added as an internal yield monitor. Samples were injected in triplicate on a Fisons MD 800 GC-MS. On-column injection was used with a BPX-70 column (50 m × 0.32 mm ID). Electron impact ionisation (70 eV) and a mass range of 45–400 m/z employed.

The FAMEs quantification was made using the internal standard method and also using calibration curves of standard FAMEs.

RESULTS

Aerobic Bacteria

An increase in the number of oil degrading and heterotrophic bacteria could be detected 3 days after addition of both oils to the salt marsh

sediments (Figs. 1 and 2). However, whilst for linseed oil this increase continued for the duration of the experiment, in the case of sunflower oil, it was not sustained.

The number of heterotrophic bacteria reached identical maximum values in both oils; however, whilst for linseed oil the bacteria numbers

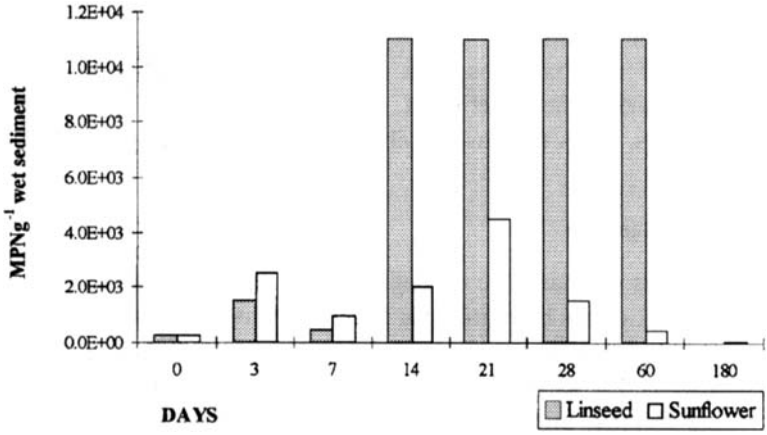

FIGURE 1 Temporal variation of aerobic oil degrading bacteria numbers after addition of linseed and sunflower oil.

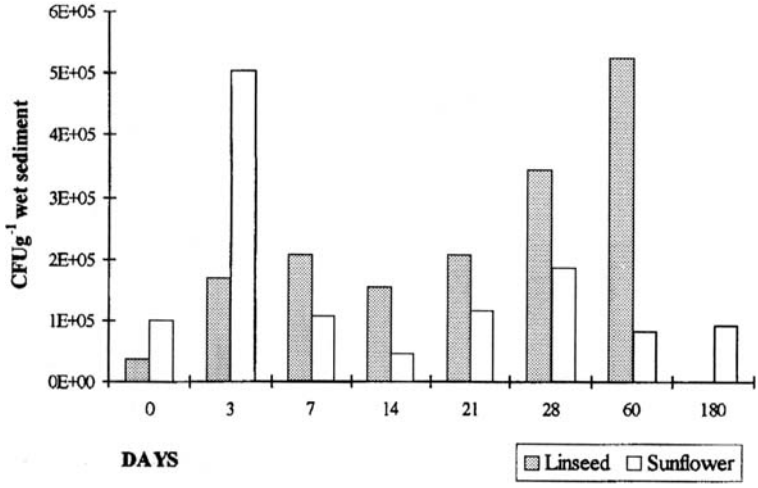

FIGURE 2 Temporal variation of heterotrophic aerobic bacteria numbers after addition of linseed and sunflower oil.

peaked after 60 days, and for sunflower oil this occurred after 3 days. This is consistent with previous results (Mudge *et al.*, 1995).

Aerobic oil degraders exhibited higher numbers in linseed oil than in sunflower oil. In the former case, the maximum growth was recorded after 14 days and appears to be sustained for the rest of the experiment. In the case of sunflower oil, the growth of these bacteria peaked on the 21 day of the incubation, followed by a sharp decrease.

Prior to the addition of the oils, the oil degraders represented less than 1% of the viable bacteria in the sediments. This percentage increased during the experiment, with maximum numbers recorded after 14 days, 7.2% for linseed oil and 4.4% for sunflower oil.

Anaerobic Bacteria

The numbers of anaerobic oil degrading bacteria were small (Fig. 3), less than 1% of the heterotrophic bacteria. This group of bacteria did not seem to be either positively or negatively affected by the addition of linseed oil, but showed some growth in the presence of sunflower oil (2.5 fold increase in numbers relative to time zero). This growth was sustained for 2 months after the addition of the oil, with very small numbers remaining after 6 months.

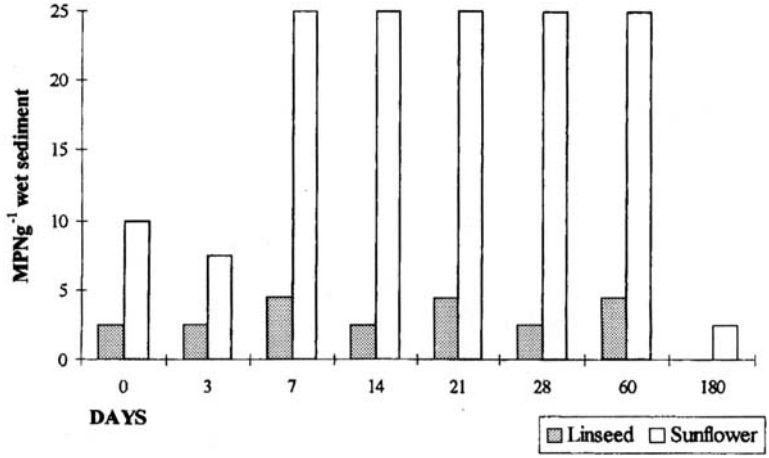

FIGURE 3 Temporal variation of anaerobic oil degrading bacteria numbers after addition of linseed and sunflower oil.

The number of heterotrophic anaerobes increased after the addition of either oil. In each case similar maximum numbers were obtained. For linseed oil, maximum numbers were reached after 7 days and for sunflower oil after 21 days (Fig. 4).

The number of SRB followed a pattern of temporal variation similar to that observed for the heterotrophic anaerobic bacteria, reaching higher values in sunflower oil than in linseed oil (Fig. 5).

FATTY ACIDS

For linseed oil, between day 3 and day 60 a decrease of 50% in the amount of lipids recovered from the sediments was observed, while 20% of sunflower oil remained in the surface sediments after 180 days.

The temporal variation of the main fatty acids of linseed oil is illustrated in Figure 6. The octadecatrienoic acid (18:3ω3) decreased for the duration of the experiment, at greater rates for the first 14 days (485 $\mu g\ g^{-1}day^{-1}$) than for the remaining time (10.2 $\mu g\ g^{-1}day^{-1}$). The other fatty acids decreased at slower rates.

For sunflower oil, after 180 days very small amounts of fatty acids remained (Fig. 7). The main component of this oil, 18:2ω6, decreased at a rate of 46.9 $\mu g\ g^{-1}day^{-1}$ for the first 60 days and at 3.8 $\mu g\ g^{-1}$ day^{-1} between day 60 and day 180.

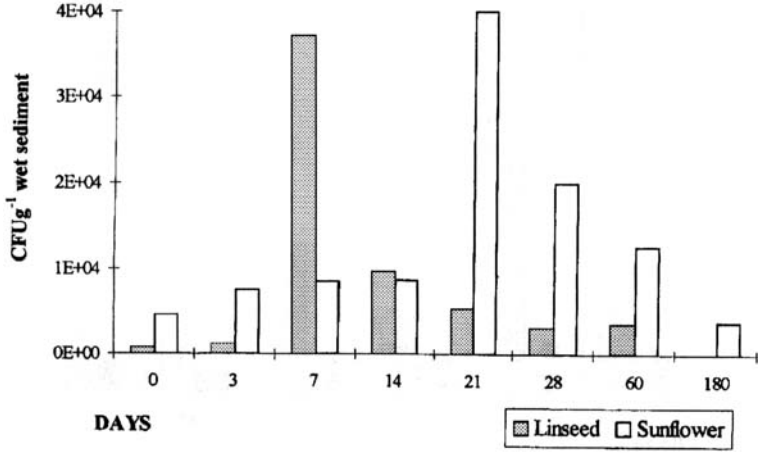

FIGURE 4 Temporal variation of heterotrophic anaerobic bacteria numbers after addition of linseed and sunflower oil.

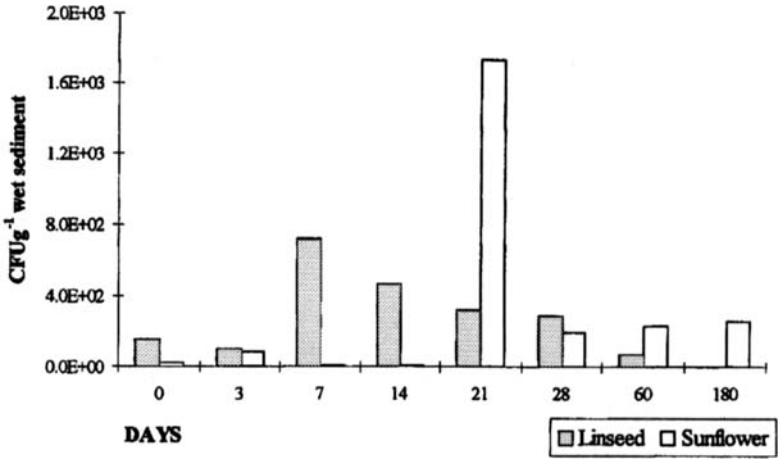

FIGURE 5 Temporal variation of sulphate reducing bacteria numbers after addition of linseed and sunflower oil.

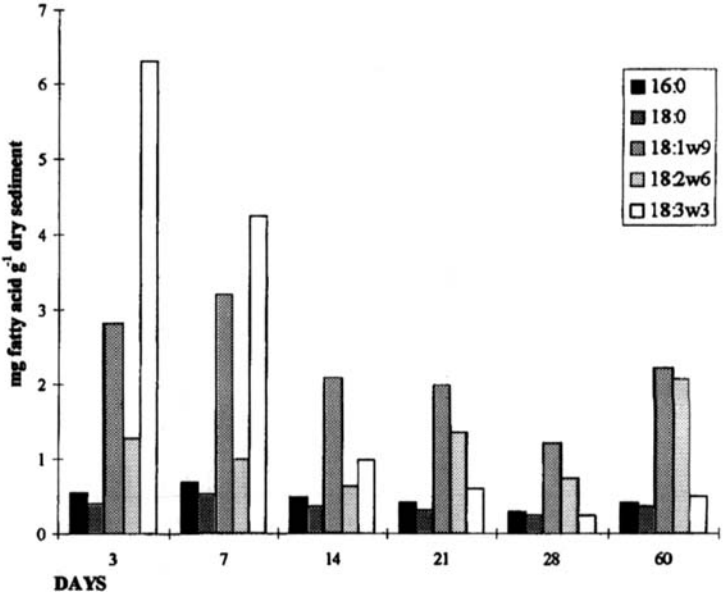

FIGURE 6 Temporal variation of the main fatty acids of linseed oil in salt marsh sediments.

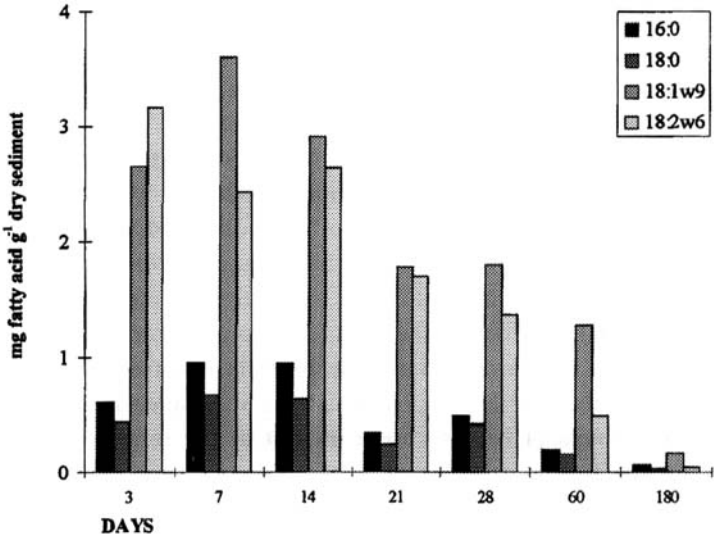

FIGURE 7 Temporal variation of the main fatty acids or sunflower oil in salt marsh sediments.

The fatty acids analysis also revealed the presence of octadecatrienoic, octadecadienoic and 11-ocatadecenoic acids, not originally present in the linseed oil or in the sediments. Most of these fatty acids were found by day 21. Analysis by gas chromatography-mass spectrometry of these fatty acids as picolinyl esters was used to locate the double bonds position. Some of these fatty acids were then identified as 9, 11, 15-octadecatrienoic acid and 9,11- and 11, 15-octadecadienoic acids.

For sunflower oil, "new" octadecadienoic and octadecenoic acids were also present. Most of these fatty acids were found at day 60. The double bond position of the octadecadienoic acids is not yet established. The octadecenoic acids observed could be 7- and 11-octadecenoic acids.

DISCUSSION

Bacteria

The fact that aerobic oil degraders rapidly increased in numbers in the presence of both oils, suggests their degradability, particularly of

linseed oil. The results regarding anaerobic oil degraders indicate that in anaerobic conditions the oils are more difficult to degrade. A decrease in the number of oil degrading bacteria (mainly aerobic) suggest that sunflower oil becomes less degradable with time. As it would be expected, heterotrophic aerobic bacteria appeared to be the first to utilise the extra carbon available, followed by the anaerobic bacteria. In the case of sunflower oil, the observed decrease in the numbers of heterotrophic aerobic bacteria is accompanied by an increase in heterotrophic anaerobic and sulphate reducing bacteria suggesting that the environment had become more reduced. For linseed oil, the increase in anaerobic bacteria occurred after 7 days, when the numbers of aerobic bacteria were still increasing, indicating that both groups of bacteria were present at the same time. This is possible due to the presence of anaerobic zones within the aerobic sediment. After 60 days, linseed oil was still present and could be broken-down and utilised by aerobic bacteria.

Fatty Acids

In these experiments, a decrease in the amount of oil present in the surface of the sediment due to its utilisation by bacteria and particularly by the penetration into the sediments was expected. The oil penetration is enhanced by the presence of invertebrate burrows. Polyunsaturated fatty acids have preferential degradation in the sediments relative to saturated acids (Parker, 1967; Farrington and Quinn, 1971). For linseed oil, the ratio between unsaturated and saturated fatty acids is illustrated in Figure 8. The analysis of this figure shows a different behaviour for the various unsaturated acids. The ratio between 18:3ω3 and saturated fatty acids indicates the preferential degradation of this fatty acid, as expected. However, the ratio between 18:2ω6 and saturated fatty acids indicates a degradation of this fatty acids in the beginning of the experiment, followed by an increase. The ratio between 18:1ω9/saturated fatty acids does not change with time. These results suggest first a degradation of the 18:3ω3 (*cis*-9, *cis*-12, *cis*-15-octadecatrienoic acid) and 18:2ω6 (*cis*-9, *cis*-12-octadecadienoic acid) followed by an increase of octadecadienoic acid. The degradation of the 18:3ω3 leads to an increase of 18:2ω6 and to the formation of the "new" fatty acids observed. A

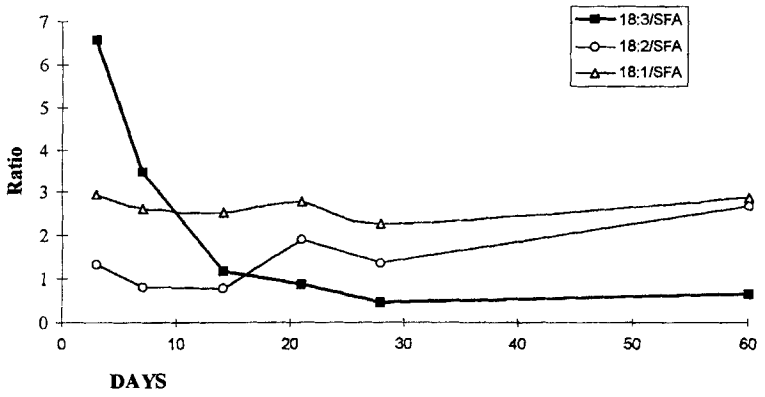

FIGURE 8 Temporal variation of the ratio between unsaturated and saturated fatty acids (SFA) of linseed oil.

possible degradation pathway which may have taken place involves first a migration of the double bond in the position 12 (and perhaps 9) from the *cis*-9, *cis*-12, *cis*-15-octadecatrienoic acid, followed by the hydrogenation to octadecadienoic acids, and eventually to octadecanoic acids (Fig. 9).

For sunflower oil, the ratio between unsaturated and saturated fatty acids does not follow a defined pattern with time. However, between day 3 and day 180 the ratio between 18:2ω6 and saturated fatty acids seems to decrease and the ratio between 18:1ω9 appears to have kept constant. Again the preferential degradation of the main fatty acid seems to occur. This, and the appearance of "new" octadecadienoic and octadecenoic acids suggest formation of positional and maybe geometric isomers of the octadecadienoic acids of the sunflower oil, followed by hydrogenation to saturated acids. So, part of the degradative pathways of linseed oil (Fig. 9) may also occur for

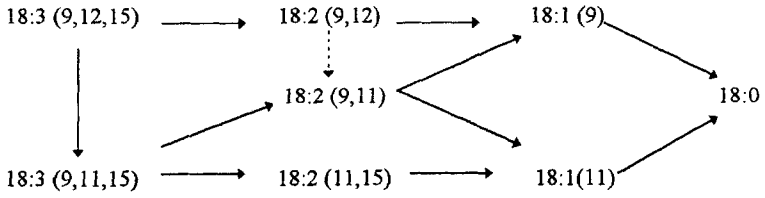

FIGURE 9 Schematic representation of possible degradative pathways of linseed oil.

sunflower oil, but more work needs to be done in order that this can be confirmed.

CONCLUSIONS

Sunflower oil did not polymerise as occurred in previous works (Mudge *et al.*, 1995). The bacteria population of salt marsh sediments appear to have the potential to degrade linseed and sunflower oils, with linseed oil more available to these organisms. The presence of the oils induced an increase in aerobic and anaerobic bacteria. The degradation of both oils seems to be carried out mainly by aerobic oil degrading bacteria. These results agree with results from laboratory experiments, not reported in this paper. These works do not exclude the possibility of a sequential process of degradation where various bacteria groups may utilise oil partially degraded.

The degradation of both oils results in the formation of various unsaturated fatty acids previously not observed in the oils or in the sediments, suggesting more than one degradative pathway. The analysis of the effects of the vegetable oils in marsh plants was not an objective of this work but it was possible to observe their deleterious effects on the marsh plants.

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References

Budavari, S. (1989) *The Merck Index*. Eleventh edition. Merckand Co., Inc. Publishers, U.S.A.

Cornish, A., Battersby, N. S. and Watkinson, R. J. (1993) Environmental fate of mineral, vegetable and transesterified vegetable oils. *Pestic. Sci.*, **37**, 173–178.

Farrington, J. W. and Quinn, J. G. (1971) Fatty acids diagenesis in recent sediments from Narragansett Bay, Rhode Island. *Nature. Physical Science*, **230**, 67–69.

Gunstone, R. B. (1994) Environment–danger from edible oil spills debated again. *Lipid Techn.*, **6**, 107–108.

Hoffman, G. (1989) The chemistry and technology of edible oils and fats and their high fat products. *Food Science and Technology*, A series of Monographs, Academic Press, London. pp. 384.

McKelvey, R. W., Robertson, I. and Whitehead, P. E. (1980) Effect of non-petroleum oil spills on wintering birds near Vancouver. *Mar. Poll. Bull.*, **11**, 169–171.

Menon, K. K. G., Alfin-Slater, M. J. and Mani, V. V. S. (1989) Nutrition and toxicological aspects of uncommon edible oils. In Vergrosen, A. J. and Crawford, M. (Eds). *The Role of Fats in Human Nutrition*, Second Edition, Academic Press, London. pp. 580.

Mudge, S. M., Salgado, M. and East, J. (1993) Preliminary investigations into sunflower oil contamination following the wreck of the M.V. *Kimya. Mar. Poll. Bull.*, **26**, 40–44.

Mudge, S. M., Goodchild, I. D. and Wheeler, M. (1995) Vegetable oil spills on salt marshes. *Chemistry and Ecology*, **10**, 127–135.

Mudge, S. M. (1997) Can vegetable oils outlast mineral oils in the marine environment? *Mar. Poll. Bull.*, **34**, 213.

Parker, P. L. (1967) Fatty acids in recent sediment. *Marine Science*, **12**, 135–142.

Postgate, J. R. (1979) The sulphate-reducing bacteria. Cambridge University Press, Cambridge, London. pp. 144.

Ratledge, C. (1994) Biodegradation of oils, fats and fatty acids. In Ratledge, C. (Ed). *Biochemistry of Microbial Degradation*, Kluwer Academic Publishers, London. pp. 590.

Russel, D. G. and Carlson, B. A. (1978) Edible-oil pollution on Fanning Island. *Pacific Sci.*, **32**, 1–15.

ZoBell, C. E. (1941) Studies on marine bacteria. I. The cultural requirements of heterotrophic bacteria aerobs. *J. Mar. Res.*, **4**, 42:75.