

MEMORANDUM

Greater Vancouver Regional District

*Sewerage and Drainage Department
Departmental Services Division*

TO: File DATE: October 17, 1997
 FROM: Source Control FILE: CR-24 BA
 RE: UBC REPORT ON MICROBIOLOGICAL PRODUCTS FOR GREASE TRAPS

GVRD commissioned an investigation of the issues associated with use of microbial products in grease traps. The investigation was carried out by Dr. William W. Mohn, a professor at the Department of Microbiology at The University of British Columbia, and documented in a report dated April 16, 1996. This memo presents an interpretive summary of the information and conclusions contained in the UBC Report. Additional detail may be obtained by referring to the report.

Biodegradation of Oil & Grease

1. Most microorganisms are capable of degrading oil and grease, although there may be considerable variation in the rates of degradation depending on waste type.
2. The first step in biodegradation is hydrolysis of ester bonds, which involves separation of fatty acids from glycerol. Hydrolysis is catalyzed by extracellular enzymes called lipases. Since the enzymes are extracellular, environmental conditions can significantly affect their activity. Studies have concluded that these enzymes require near neutral pH to perform.
3. CO₂ and biomass are the final biodegradation products of oil and grease. In addition, one or more of water, nitrite, sulphide, or methane are formed - depending on the compound serving as an electron acceptor in the oxidation step (presented in order of highest redox potential - O₂, nitrate, sulphate, and carbon).
4. Microorganisms growing on a grease substrate will produce surfactants which dissolve the grease substrate.

Literature Review

There is no scientific literature on bacteria or enzyme products for treatment of grease traps or drains.

issues

1. There is no scientifically supportable evidence contained in vendor literature which demonstrates that microbial products in grease traps are effective in sustained biodegradation of oil and grease.
2. Biodegradation may be inhibited by a number of factors likely to be present in grease trap environments: short hydraulic retention time, extremes of temperature, pH, or osmotic potential, presence of toxic chemicals, oxygen levels, and lack of nitrogen or phosphate nutrients.
3. Bacteria/enzyme treatment could result in solubilization of grease and its subsequent transfer to the collection system or treatment plant.
4. Solubilized grease transported by the collection system may subsequently re-congeal, resulting in sewer fouling.
5. Up to 50% of biodegraded grease may be converted to biomass which, if not removed at the grease trap, could contribute to sludge build-up at pumping stations.
6. Biomass generated by grease trap biodegradation, and transferred to the treatment plant, could interfere with performance of treatment plant settling facilities, due to poor settling characteristics.
7. Increased BOD loadings at the treatment plant could result from: (1) an increase in solubilized grease contained in treatment plant influent; (2) an increase in biomass associated with grease trap biodegradation, where biomass is transferred to the collection system; or, (3) an increase in conversion of recalcitrant organic matter in treatment plant influent to biodegradable matter captured by BOD determinations.
8. Odours and sewer corrosion could increase due to microbiological activity which results in oxygen depletion and sulphide formation.
9. Infectious agents could be introduced with microbial strains.

UBC Report Conclusions

1. Due to the wide variety of microorganisms, and the adaptability of particular strains to particular operating environments, vendors are able to make extravagant claims concerning the capability of microbial products.
2. Grease traps are not a good environment for oil and grease biodegradation due to low hydraulic retention times, and the frequent and irregular stresses that the trap is exposed to: extremes of temperature and pH, and presence of toxic chemicals or low nutrient concentrations.

3. There is no scientifically supportable evidence contained in vendor literature which demonstrates that microbial products in grease traps are effective in biodegrading oil and grease.
4. Empirical testing in a controlled laboratory experiment would provide additional data on factors affecting the use of microbial products in grease trap or interceptor environments.

**Report on Microbiological Products
for Grease Traps**

16 April 1996

Report for: Sewage and Drainage Department
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I. Summary

This report considers the ramifications of the use of microbial products currently being marketed for the clean up and maintenance of restaurant grease traps. Manufacturers of these products state that the products are effective because they biodegrade oil and grease. The biochemistry of oil and grease biodegradation is well studied and is reviewed in this report. The scientific literature was searched for publications concerning the above microbial products, but no such publications were found. Potential concerns arising from the use of the products are described and briefly evaluated on the basis of available information. A number of serious concerns are identified. The probable efficacy of the microbial products is similarly evaluated. The available information is not sufficient to determine the likelihood of undesirable consequences from the microbial products or to evaluate the efficacy of the products. Particular needs for information are identified, and some experimental approaches to gaining needed information are described.

II. Biodegradation of oil and grease

Oil and grease (also fats) consist primarily of triglycerides, esters of glycerol and fatty acids. Oil and grease also contain lesser amounts (2-3%) phospholipids (mainly phosphoglycerides) and trace amounts of steroids and carotenoids. The latter two classes of compounds are the nonsaponifiable fraction of oil and grease, and carotenoids are frequently responsible for the color of oil and grease. Oils include unsaturated fatty acids which cause oils to have relatively low melting temperatures and to exist as liquids at room temperature. Greases have more saturated fatty acids and, consequently, higher melting temperatures.

Biodegradation of oil and grease has been extensively studied and is well understood at biochemical and microbiological levels. Detailed reviews of this subject exist (3, 5). Most microorganisms are capable of degrading oils or greases; however, there is probably considerable variation in the range of oils and greases degraded and the rates of degradation by individual microorganisms. Certainly there is great variation in the physiological and ecological properties of the different organisms capable of oil and grease biodegradation.

Hydrolysis. The first step in the biodegradation of oil and grease is hydrolysis of the ester bonds, producing glycerol plus the constituent fatty acids or phosphoric acids (Fig. 1). The enzymes responsible for this hydrolytic activity are lipases. A common feature of lipases is that they are active extracellularly (outside of the cell membrane). They can be active in free solution or on the surface of the cell wall. This extracellular location for

lipase activity is probably necessary for two reasons. First, the responsible organism must not degrade its own cell membrane which is composed, in part, of phosphoglycerides. Second, oil and grease consist of large, insoluble molecules which would be difficult to transport into a cell.

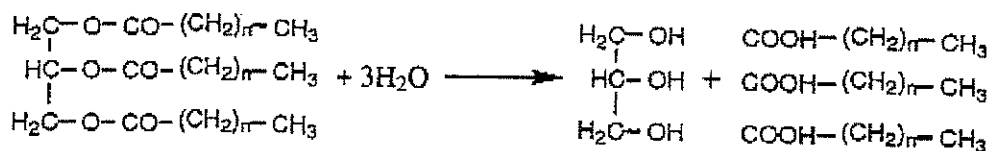


Figure 1. Hydrolysis of ester bonds of a triglyceride

Because lipases are extracellular, environmental conditions can be expected to significantly affect their activities. Lipases so far studied require near neutral pH, optimally 6.5 to 7.0. Other physicochemical conditions can be expected to effect lipase activities and could inhibit activity. The hydrolytic reactions do not require oxygen, and lipases can act equally well under aerobic or anaerobic conditions.

Lipases are relatively nonspecific enzymes. Typically these enzymes will hydrolyse triglyceride ester bonds regardless of the length and structure of the fatty acid constituents. The activities of lipases which hydrolyze phosphoglycerides have been classified based upon which ester bond is hydrolyzed. The different activities are phospholipase A, B, C and D (Fig. 2). Phospholipases typically exhibit specificity for these activities.

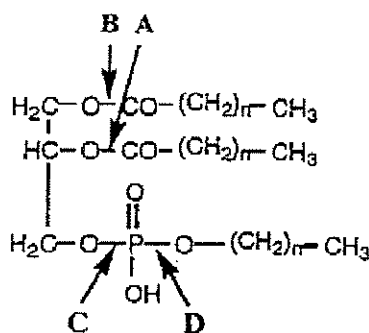


Figure 2. Sites of specific hydrolytic activities of phospholipases

Beta oxidation. Fatty acids liberated from oil and grease by hydrolysis are further degraded by beta oxidation. Beta oxidation involves a stepwise removal of two-carbon units from the fatty acid (Fig. 3). The two-carbon units released are acetyl-CoA, a central metabolite. Thus, after beta oxidation, the fatty acids are effectively destroyed. Beta oxidation occurs intracellularly, so the fatty acids must first be taken up by the cells responsible for their degradation. Beta oxidation does not require oxygen, and like the above hydrolysis, can occur equally well under aerobic or anaerobic conditions. Most, if not all, microorganisms are capable of beta oxidation. As an

alternative to beta oxidation, some microorganisms can incorporate appropriate fatty acids directly into phosphoglycerides which they are synthesizing for their cell membranes.

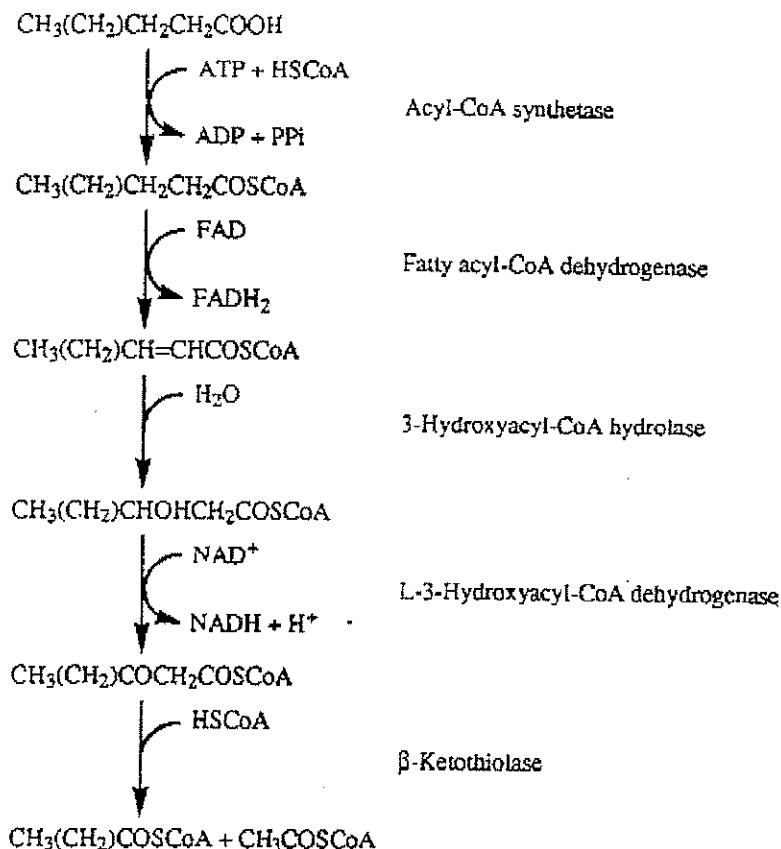


Figure 3. Beta oxidation of a fatty acid. The latter four steps are repeated until the fatty acyl-CoA has two or three carbon atoms remaining.

Certain fatty acids are recalcitrant relative to other ones and will tend to be persistent. Short fatty acids, less than eight carbon atoms, tend to be toxic to microorganisms, and this toxicity can cause recalcitrance. It is believed that these fatty acids disrupt cell membranes. Very long fatty acids, greater than 18 carbon atoms, are also relatively recalcitrant. In this case, solubility limits the availability of the fatty acids to microorganisms, and their large size also limits uptake by cells. Unsaturated and branched fatty acids also tend to be recalcitrant. The double bonds and tertiary carbon atoms of these fatty acids block beta oxidation. Alternative degradative activities are needed to circumvent such blockage. Despite the recalcitrance of certain fatty acids, all of them are biodegradable. There are fewer organisms capable of degrading recalcitrant fatty acids, so the lack of such organisms in a particular environment can cause persistence of those fatty acids. Long-term presence of

recalcitrant fatty acids will tend to select for a microbial community capable of their biodegradation.

Growth on oil and grease. When beta oxidation forms acetyl-CoA, that central metabolite can be used for energy metabolism or biosynthesis. The same is true for glycerol. Thus, oil and grease can provide the primary substrate for microbial growth. Of course other nutrients are also required, including N, P and trace nutrients. Any of these other nutrients can potentially limit growth on oil and grease. Such limitation would not completely prevent oil and grease degradation by an existing population but it would prevent growth of that population, lower the rate of oil and grease degradation and ultimately prevent maintenance of that population. The insolubility of oil and grease can limit their availability to microorganisms, and therefore, limit their biodegradation. It has been shown that the rate of degradation of hydrophobic substrates may, in some cases, be limited to the rate of dissolution of those substrates (6, 7). Limitation by dissolution occurs when organisms use the soluble form of a substrate. In some cases organisms use hydrophobic substrates in a nondissolved state by direct contact with a solid or micelle (2).

Final biodegradation products. Acetyl-CoA and glycerol will ultimately be oxidized to CO₂ by microorganisms or incorporated into their cells. Thus, CO₂ and biomass are the final biodegradation products of oil and grease. The oxidation of organic matter requires an electron acceptor. Various compounds can serve as metabolic electron acceptors with preference typically for the compound with the highest redox potential (i.e., the one which can support the greatest energy conservation). The most common electron acceptors known, in order of decreasing preference, are O₂, nitrate, sulfate and carbon. The products from these electron acceptors are water, nitrite (or N₂), sulfide and methane. Thus, oil and grease biodegradation will also yield one or more of these products, depending on the availability of electron acceptors. Except for sulfide, oil and grease biodegradation products are unlikely to cause odor problems. The worst odor problems are due to organo-sulfur or organo-nitrogen compounds, which are not products of oil and grease biodegradation.

Biosurfactants. While growing on hydrophobic substrates, many microorganisms produce surfactants which solubilize the substrates (1, 4). This could alleviate growth limitation by dissolution of the substrate. Biosurfactants are amphipathic molecules, having both hydrophobic and hydrophilic regions. Such molecules tend to orient themselves at interfaces and can effectively surround droplets of oil and grease and hold them in suspension in an aqueous system. Biosurfactants have a variety of chemical structures, including trehaloses, mycolic acids, sophorose lipids and rhamnolipids. It is likely that microbial communities degrading oil and grease will produce biosurfactants which solubilize these compounds.

The correlation between microbial growth on hydrophobic substrates and production of biosurfactants does not necessarily mean that biosurfactants are produced *in order to assist growth* on these substrates. There are very few cases where biosurfactants have been shown to stimulate growth on hydrophobic substrates. And there are no reported cases where biosurfactant production has been shown to be a regulated response to such substrates. It may be that biosurfactant production is coincidental to use of such substrates. Many biosurfactants resemble components of cell walls. Thus, microorganisms can release such compounds. Further, the presence of hydrophobic substrates may have the effect of "extracting" cell wall components. This could explain why some organisms release biosurfactants in the presence of hydrophobic substrates. It is also possible that cells add compounds with surfactant activity to their cell walls in order to protect the cell wall from disruption by hydrophobic compounds. Thus, biosurfactants could be a regulated response to hydrophobic substrates in order to protect the cell wall, rather than in order to increase availability of the substrate. Biosurfactants are clearly of fundamental and applied interest, and biosurfactants likely have an effect on biodegradation of oil and grease in many cases. However, our understanding of this phenomenon is currently quite limited.

III. Literature search

There appears to be no scientific literature which indicates the efficacy or the mode of action of microbiological products for treatment of grease traps or drains. The literature in two databases were searched. The first database was Ei Compendex*Plus(TM) 1970-1996/Mar W3, (c) 1996 Engineering Info. Inc., which includes literature in engineering disciplines. The second database was BIOSIS PREVIEWS(R) 1969-1996/Feb W2, (c) 1996 BIOSIS, which includes literature in biology disciplines. Initially, terms searched, truncated as indicated, included: oil or oils or fat or fats or grease or greases or (waste()intercep?) or (grease()trap?) and biodegrad? or bioaugment? or (bio()augment?) or (bio()degrad?) or digest? Further search strategies were tried as well. The search yielded no references on the targeted subject. This lack of references was latter confirmed by Victor Lo (Dept. Bio-Resources Engineering, Univ. British Columbia, personal communication) who does research in this field. The literature most relevant to the target subject involves the use of bioreactors to treat food processing wastes containing large quantities of oil (e.g., processing of olives, palm oil, poultry). There is a large body of literature on these bioreactors.

IV. Potential concerns about use of microbial products

The following are potential concerns arising from the use of microbial products to clean up grease traps. Each possible result is described and very

briefly evaluated based on available information. Several of the concerns below appear to be serious. Further evaluation would be possible in some cases but was not requested in this report. In other cases, experimental work is needed to evaluate concerns.

- 1. Ineffective products.** This may be the greatest concern about the currently available products. Given widespread use, the financial loss to consumers could be substantial. None of the product literature reviewed for this report (see appendix) provided reasonable evidence for efficacy of the product in cleaning up grease traps. The first question needing to be addressed is, who has responsibility for this concern? One option is to let the consumer be responsible for demanding proof of the efficacy of a product (i.e., let the buyer beware). Such a policy might not be reasonable if special circumstances predispose this market to fraudulent claims (e.g., if consumers cannot reasonably be expected to have the necessary expertise to evaluate these products). An alternative would be to require proof of product efficacy to be evaluated by a regulatory authority.
- 2. Inhibition of biodegradation.** Even in the presence of oil and grease-degrading microorganisms, numerous factors potentially present in grease traps could prevent biodegradation of oil and grease. Such factors could kill the microbial product or could inhibit its activity. The most obvious of these factors is the short hydraulic retention time of small grease traps which may not allow sufficient reaction time and could cause washout of the product (or of oil and grease). Hydraulic retention time is a key design parameter of biological treatment systems, but the required hydraulic retention time for oil and grease biodegradation has clearly not been considered in the design of grease traps. Other factors include stresses such as extreme physicochemical conditions (e.g., temperature, pH or osmotic potential) or toxic chemicals. All of these stresses might result from common procedures at restaurants. High or low oxygen concentrations could occur depending on biological oxygen consumption, and either extreme could be inhibitory or toxic to certain organisms. Nutrients other than oil and grease (particularly nitrogen and phosphate) could be insufficient to support degradation of all oil and grease.
- 3. Increased fouling of the sewer system by grease.** This could result if a microbial product solubilizes grease without degrading it. This would occur if the microbial product produced a biosurfactant (or itself functioned as a biosurfactant) but failed to degrade the solubilized grease under the particular conditions. Surfactant activity is common with oil and grease-degrading organisms. The extent of oil and grease degradation will depend on the factors discussed in 1 and 2 above. In this scenario, use of the microbial product would reduce the efficiency of a grease trap (increase passage of grease through the trap). Such solubilized grease could later come out of solution and adhere to sewer components, causing

fouling. Solubilized grease could come out of solution under a variety of circumstances, including (i) biological or chemical degradation of the biosurfactant, (ii) change of temperature, (iii) change of pH and (iv) change of osmotic potential. All of these circumstances are possible within a sewer system. This scenario appears to be a serious concern. Any steps to insure the efficacy of microbial products (i.e., to insure that grease is degraded before leaving grease traps) would prevent this problem.

4. **Increased fouling of the sewer system by microorganisms.** The short hydraulic retention time of small grease traps may provide selective pressure for surface-attaching organisms. Such organisms would tend to foul the grease trap and also might colonize and foul sewer components downstream from the trap. The likelihood and severity of this result is very difficult to predict. It is noteworthy that claims that microbial products can persist in small grease traps, if true, would indicate increased likelihood of fouling.
5. **Accelerated sludge build up at pumping stations.** This seems a likely result if a microbial product is effective. If oil and grease are biodegraded, as indicated by vendors of microbial products, biomass production is an inevitable consequence. The dry weight of biomass produced could be up to 50% of the mass of oil and grease degraded. The resulting biomass would either be retained in the grease trap and adjacent pipes (necessitating their periodic cleaning), or it would pass into the sewer system. Biomass entering the sewer could settle wherever wastewater is retained, as at pumping stations. This is another result which is difficult to predict and whose seriousness is uncertain.
6. **Bulking in biological treatment systems.** It is known that particular organisms will reduce the settlability of biomass. Settling is critical to many biological treatment systems. If a microbial product contained such an organism, and if use of the product and conditions in the sewer system yielded a large population of the organism, it could significantly affect a biological treatment system. This result is hypothetically possible but is probably unlikely. This is another result which would be very difficult to predict. It would be possible to prohibit microbial products from having certain known characteristics of bulking organisms or to require that they have minimum settlability characteristics. It is noteworthy that the settleability of organisms, which is desirable in treatment systems, would contribute to the above problem of sludge build up at pumping stations.
7. **Increased BOD in treatment systems.** This possibility was specified for consideration, as the GVRD has observed a coincidence of BOD increases with increased use of microbiological products for oil and grease traps. There are at least three ways microbial products might increase BOD in the sewer system. (i) As described in 2 above, use of the product could

solubilize oil and grease, reduce the efficiency of grease traps and increase wastewater BOD in the form of oil and grease. In such a case, primary sewage treatment may fail to remove solubilized oil and grease, and the solubilized oil and grease could be released in the final effluent. (ii) Biodegradation of oil and grease (which would otherwise be retained in grease traps) may result in biomass which washes out of grease traps accounts for an increase in wastewater BOD. (iii) Recalcitrant organic matter in the wastestream, which is not included in BOD determinations, may be biotransformed by a microbial product to more readily biodegradable organic matter, which is included in BOD determinations. For example, recalcitrant forms of oil and grease could be degraded by a microbiological product. The first two scenarios appear to be serious concerns, while the third scenario appears unlikely, since metabolites of reduced recalcitrance do not normally accumulate.

8. **Increased odors or corrosion.** These results would most likely be caused by a microbial product if it increased anaerobiosis in the sewer system and if sulfate reduction produced sulfide. This would be the case if a product degraded organic matter, particularly oil or grease, which otherwise would pass through the system undegraded. This additional oxidation of organic matter would cause increased oxygen consumption. Knowing the fate of oil and grease in the sewer system and knowing the efficacy of a product would greatly increase our ability to predict the likelihood of the product causing this problem. Without this information, it is difficult to evaluate the seriousness of this concern.
9. **Infection by the microbial product.** This could occur to humans, animals or plants exposed to wastewater. Existing precautions probably make this result unlikely. However, the relatively large microbial populations introduced by treatment make it necessary to seriously consider this possibility. There are simple, reasonable requirements which could reduce the likelihood of a pathogen being included in a microbial product.

V. Conclusions about the probable efficacy and consequences of microbial products

Our relatively good understanding of oil and grease biodegradation is of very limited use in evaluating microbiological products for grease traps. Because this degradation activity is widespread, there likely are organisms capable of oil and grease biodegradation which are well adapted to a very wide range of environments. These likely include different organisms which are aerobic, anaerobic, surface-attaching, biosurfactant-producing, fast-growing, starvation-resistant, heat-tolerant, cold-tolerant, etc. Thus, *it is impossible to rule out almost any claims about the capabilities of a microbial product which are made by the manufacturer, and it is impossible to predict the efficacy or effects of microbial products.*

In my opinion, grease traps are not a good environment for oil and grease biodegradation. For small units, hydraulic retention times are probably very short and irregular. Frequent and irregular stresses probably limit the maintenance and activity of microbial populations. These stresses include extremes of temperature, pH, osmolarity, nutrient concentrations and toxic chemicals. The best way to insure some stable biological activity in grease traps would be to colonize them with attached microorganisms, but this would probably hurt the performance of the trap by affecting flow, and such organisms could lead to fouling of the sewer system. For these reasons, I feel *it is reasonable to be skeptical about the efficacy of microbial products for grease traps until there is evidence supporting efficacy.*

Although we cannot predict the efficacy or consequences of microbial products for grease traps, we can empirically test them. *The only dependable information about microbiological products for grease traps will probably come from empirical testing. However, there appears to be no testing of microbiological products described in scientific literature.* Such reports would be particularly useful if they were in peer-reviewed journals, as the conclusions of such work are more credible than other reports. A limited sample of reports of testing described by manufacturers (Appendix) did not provide any conclusive evidence for efficacy of the products. Usually these tests lacked control treatments or analysis of quantitative data.

It would be relatively simple to test products in controlled experiments. Parallel grease traps could be operated with a common wastestream. These traps could be set up in a lab with a synthetic wastestream or at a restaurant where the sewer line could be split. One trap could be treated according to a manufacturer's recommendation while the other served as a control. Oil and grease, CO₂, biomass and any other parameters could be quantified in the effluent of each trap. Comparison of the effluents would indicate exactly how the microbial product affected the effluent quality and composition.

VI. References

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