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THE RATE OF ACCLIMATION AND
BIODEGRADATION OF CRUDE OIL BY
AEROBIC BACTERIA IN AN AQUEOUS
MEDIUM: A PRELIMINARY STUDY

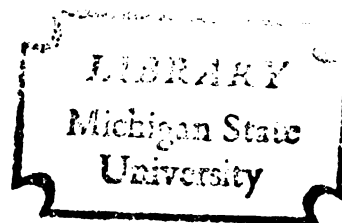
Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
DAVID JOSEPH MILAN
1976

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ABSTRACT

THE RATE OF ACCLIMATION AND BIODEGRADATION OF CRUDE OIL BY AEROBIC BACTERIA IN AN AQUEOUS MEDIUM: A PRELIMINARY STUDY

By

David Joseph Milan

The purpose of this paper is to describe an experiment designed to show the rate at which bacteria obtained from aeration tank mixed liquor acclimate and grow on a crude oil medium. Bacteria have been observed to grow on an oil medium, but the rate at which this occurs is variable and has not been closely studied. In order for the oil to enter into the aqueous phase, solubilization must occur. This process is believed to be carried out by micelles produced by the bacteria themselves.

A control reactor containing only aeration tank mixed liquor and an experimental reactor containing aeration tank mixed liquor plus 10% crude oil by volume were tested each day for their total chemical oxygen demand (COD). The data tend to confirm the micelle theory. The data also imply that micelles are produced to solubilize specific fractions of the crude oil in separate steps. Because of lack of time and the fluctuations observed in the data, no

David Joseph Milan

rate for the biodegradation was determined. However, the graph of the data implies that the bacteria required approximately 20 days to become acclimated to the crude oil used in this experiment.

Recommendations for alterations and additions to this experiment are given at the end of this paper should further study be desired.

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OF CRUDE OIL BY AEROBIC BACTERIA IN AN
AQUEOUS MEDIUM: A PRELIMINARY STUDY

By

David Joseph Milan

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Civil and Sanitary Engineering

1976

ACKNOWLEDGMENTS

The author wishes to thank the following individuals for their assistance and criticism: Dr. Mackenzie L. Davis, Department of Civil and Sanitary Engineering; Dr. Walter N. Mack, Department of Microbiology and Public Health; and Dr. David A. Cornwell, Department of Civil and Sanitary Engineering. Without their help and guidance this paper would not have come to fruition.

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INTRODUCTION

Over the past fifty years, the industrialized countries of the world have become increasingly dependent upon oil-based products. Plastics, insecticides, fertilizers, medicines, synthetic materials, and refined oils and gasoline are a major part of a technologically advanced country's existence. Each industrialized country's need for oil has generally surpassed its ability to supply itself, resulting in an increase in the transport of crude and refined oils.

The principle method of transportation used in supplying the world's thirst for oil is the ocean-going tanker. The oil in these ships, when contained and delivered intact, constitutes nothing less than the life blood of the countries to which it is delivered. When oil is spilled, whether into the oceans or into the inland shipping routes, the results can be devastating. Birds landing in the slick have their feathers fouled, and when they try to preen themselves to remove the oil, they either die from poisoning or choke on the oil.¹ The floating slick

¹K. Mellanby, The Biology of Pollution (London and Southampton: The Camelot Press, Ltd., 1972).

effectively shuts off the exchange of gases at the water's surface, both by the fact that it seals the air-water interface, and by the fact that the oil itself absorbs oxygen more readily than the water on which it is floating.² Thus, any animals which live on or near the surface are either killed or are forced to flee the area. At the shoreline, the oil not only despoils the beach but also destroys the intricate life cycles in the intertidal and shallow water areas along the shoreline.

The tankers are not the only source of oil pollution. Offshore oil drilling rigs and refineries which are frequently located along bodies of water also have histories of oil spills. These are the most overt and publicized sources of oil pollution, but the most common and consistent contributor is oily bilge water pumped into the environment not only from the tankers' bilges but also from the bilges of other ships. Small spills also occur when the tankers load and unload, and when their holds are flushed out into the water after cleaning. The oily wastes from refineries and other industries also contribute to the pollution of

²R. M. Atlas and R. A. Bartha, "Degradation and Mineralization of Petroleum in Sea Water: Limitation by Nitrogen and Phosphorous," Biotechnology and Bioengineering 14 (1972): 309-318; and R. M. Atlas and R. A. Bartha, "Degradation and Mineralization of Petroleum by Two Bacteria Isolated from Coastal Waters," Biotechnology and Bioengineering 14 (1972): 297-308.

waters. Worldwide, the yearly amount of petroleum pollutants dumped into the environment has been estimated to be as high as ten million tons.³

It is true that increased public awareness about the environment has led to meaningful legislation designed to curb some types of oil pollution. Research into this area has resulted in safer oil drilling rigs. Restrictions on refinery and other industrial effluents have also contributed to less oil pollution. However, even though safer tankers are being designed and built, and in spite of all of the newer restrictions, an occasional accident can and does still occur.

Methods of Elimination

What happens to all of the oil? A study by the State of California⁴ designed to discover the best man-made method for eliminating oil spills found that certain detergents are very effective in accomplishing this task.

³Atlas and Bartha, "Degradation and Mineralization of Petroleum by Two Bacteria Isolated from Coastal Waters," pp. 297-308.

⁴C. R. Hazel, F. Korpperdald, N. Morgan and W. Thompson, "Development of Testing Procedures and Criteria for Evaluating Oil Spill Cleanup Agents," State of California, The Resources Agency, Department of Fish and Game, 1970.

However, later studies⁵ deduced that the detergents used to eliminate the oil cause more damage to the marine environment than the oil alone would cause. The question of what happens to the oil was also studied by a few individuals in the United States and Great Britain with very significant discoveries.

The most interesting and significant finding was that a great many marine and aquatic bacteria have the ability to biodegrade oil. Biodegradation is not, as many people believe, the break-down of a hydrocarbon into inorganic compounds such as carbon dioxide, water, nitrates or ammonias, and sulfates or sulfides; that is mineralization. Biodegradation is actually the disappearance of a hydrocarbon in its original form; longer chain hydrocarbons as well as shorter chain hydrocarbons may be produced.⁶ As an example, tar formation can be one of the by-products of biodegradation.

⁵Mellanby; and J. E. Zajic and B. Supplisson, "Emulsification and Degradation of Bunker C Fuel Oil by Microorganisms," Biotechnology and Bioengineering 14 (1972): 331-343.

⁶Atlas and Bartha, "Degradation and Mineralization of Petroleum in Sea Water: Limitation by Nitrogen and Phosphorous," pp. 309-318.

As long ago as the 1930's and 1940's, the phenomenon of biodegradation of oils was known to occur,⁷ but the studies involved in this discovery were cursory at best. It is not until the mid 1950's that more complete and thorough studies surfaced. In 1956 Davis and Updegraff⁸ discovered that bacteria at the surface of marine sediments which were found to biodegrade oil included Pseudomonas, Achromobacter, Flavobacterium, Spirillum, and the anaerobic Desulfovibrio. However, the rate of biodegradation by non-acclimated bacteria was extremely slow. Sometimes as little as one gram of oil per cubic meter of water per day was consumed with a bacterial concentration of eight million organisms per milliliter at 50° F.⁹ This is indeed a slow rate of biodegradation. It could scarcely begin to alleviate the load placed on the environment by the constant

⁷G. D. Novelli and C. E. Zobell, "Assimilation of Petroleum Hydrocarbons by Sulfate-Reducing Bacteria," Journal of Bacteriology 47 (1944): 447-448; R. Ostroff and B. S. Henry, "The Utilization of Various Nitrogen Compounds by Marine Bacteria," Journal of Cellular and Comparative Physiology, 1939; C. E. Zobell, "Action of Microorganisms on Hydrocarbons," Bacteriological Reviews 10 (1946): 1-49; and C. E. Zobell, Marine Microbiology: A Monograph on Hydrobacteriology, Chromica Botanica Company, 1946, pp. 146-148.

⁸J. B. Davis and D. M. Updegraff, "Microbiology in the Petroleum Industry," Bacteriological Journal 18 (1954): 215-238.

⁹Hazel, Korpperdald, Morgan, and Thompson.

inputs of oil that do occur were it not for the fact that once the bacteria become acclimated the rate is noticeably increased.

To substantiate this last point, Mellanby¹⁰ conducted a study wherein sea water taken from a shore area which had been exposed to the oil slick from the wreck of the "Torrey Canyon" was tested against sea water from a similar area which had been relatively pollution free. The results showed that the bacteria in the sea water which had been previously exposed were capable of degrading crude oil at a faster rate than those which had not been previously exposed. A more contemporary study by Atlas and Bartha¹¹ found that bacterial action required a two to four day lag period before significant biodegradation even began.

Marine and aquatic environments are not the sole habitats of oil degrading bacteria; land bacteria and even bacteria which exist in oil storage tank bottom water have shown this characteristic as well.¹² In research by Novelli

¹⁰ M. Chakavarty, H. D. Singh, and J. N. Baruah, "A Kinetic Model for Microbial Growth on Liquid Hydrocarbons," Biotechnology and Bioengineering 17 (1975): 399-412.

¹¹ Atlas and Bartha, "Degradation and Mineralization of Petroleum in Sea Water: Limitation by Nitrogen and Phosphorous," pp. 309-318.

¹² Zobell, "Action of Microorganisms on Hydrocarbons," pp. 1-49.

and Zobell,¹³ soil was treated with oil in an attempt to increase its fertility. While this method proved effective as long as the oil was added at a rate of no more than 4% of the soil volume, an interesting sidelight of the study was that repeated treatments of the soil with oil resulted in progressively faster disappearance of the oil. This once again illustrates the effect that acclimation has on the rate of biodegradation.

Attacking the Oil

When biodegrading the oil, bacteria appear preferentially to attack the more easily degraded fractions first; long chain over short chain fractions; aliphatic over cyclic or aromatic; unsaturated over saturated; side chains over main chains.¹⁴ Not all of the elements in the oil are used for cell growth once biodegradation begins. Most of the carbon is converted to carbon dioxide in aerobic conditions while methane and ethane obtain in anaerobic environments.¹⁵ Some of the carbon and other elements are used to create new cell material necessary for growth, while organic acids and

¹³ Novelli and Zobell, pp. 447-448.

¹⁴ P. R. Dugan, Biochemical Ecology of Water Pollution (New York and London: Plenum Press, 1972); Novelli and Zobell, pp. 447-448; Zobell, "Action of Microorganisms on Hydrocarbons," pp. 1-49; and Zobell, Marine Microbiology: A Monograph on Hydrobacteriology.

¹⁵ Zobell, "Action of Microorganisms on Hydrocarbons," pp. 1-49.

other short chain hydrocarbons are produced as well. These organic acids and other hydrocarbons can occasionally be toxic to the bacteria which produce them, but these compounds are generally found in concentrations so small as to be innocuous.

An interesting alternative finding to describe the degradation of hydrocarbons by bacteria was made by Horvath¹⁶ when he described the phenomenon of co-metabolism. Co-metabolism (or co-oxidation) is the process in which a microorganism oxidizes a substance without being able to utilize the energy derived from this oxidation to support growth. In other words, the bacteria break down the long chain hydrocarbons into shorter chain hydrocarbons without receiving anything for their labor. In many cases, the bacteria use this method as an intermediate step for further oxidation of the oil. In every case, however, when co-metabolism is the process, a substance is obtained which cannot be further degraded by the bacteria that produced that substance.

Solubilization

The key to the process of biodegradation is solubilization. It is common knowledge that oil and water do not

¹⁶ R. S. Horvath, "Microbial Co-Metabolism and the Degradation of Organic Compounds in Nature." Bacteriological Reviews, June 1972, pp. 146-155.

mix; that hydrocarbons exhibit a high resistance to solubilization. Yet implicit in all of the studies on bacterial biodegradation of oil is the fact that some mechanism has allowed the oil to go into solution so that it could come into contact with the bacteria. It is true that a small fraction of the oil is hydrophilic and will enter into the aqueous phase through natural solubilization. It is also true that natural agitation will bring the bacteria and oil together, but a study by Valenkar et al.,¹⁷ came to the conclusion that the high growth rates they obtained ruled out both the random contact idea and natural solubilization of the oil as the only means by which the oil and bacteria come together.

Further studies by Valenkar et al.,¹⁸ and also by Zajic and Supplisson¹⁹ revealed that surfactants produced by the bacteria played a major role in the solubilization of hydrocarbons. Since these surfactants have not been isolated, it is not clear whether they are produced specifically for the purpose of solubilizing the oil, or

¹⁷ S. K. Valenkar, S. M. Barnett, C. W. Houston, and A. R. Thompson, "Microbial Growth on Hydrocarbons--Some Experimental Results," Biotechnology and Bioengineering 17 (1975): 241-251.

¹⁸ Ibid.

¹⁹ Zajic and Supplisson, pp. 331-343.

whether they are released as natural metabolic by-products which, by chance, exhibit this ability. Whatever the conclusion to this question is, the fact remains that this phenomenon does occur and greatly facilitates bacterial biodegradation of the oil.

Micelle Production

The mechanism by which the oil is brought into solution and is carried to the bacteria is based on the production of micelles.²⁰ A micelle is actually believed to be a microdroplet of oil contained within an ionic hydrophilic surface (the surfactant mentioned previously) which, when formed, can then move about freely in the aqueous phase, thus increasing the probability that the oil will come into contact with the bacteria (see Figure 1). The ionic hydrophilic surface of the micelle is believed to be composed of nutrient ions so that when a micelle comes into contact with a bacterial cell, the nutrient surface is absorbed thereby exposing the microdroplet of oil contained within. Once this occurs, the bacterium can then begin to act upon the oil droplet; biodegrading it, mineralizing it, or co-metabolizing it. An interesting addition to this is that micelle production appears to be the rate-limiting

²⁰ Valenkar et al., pp. 241-251.

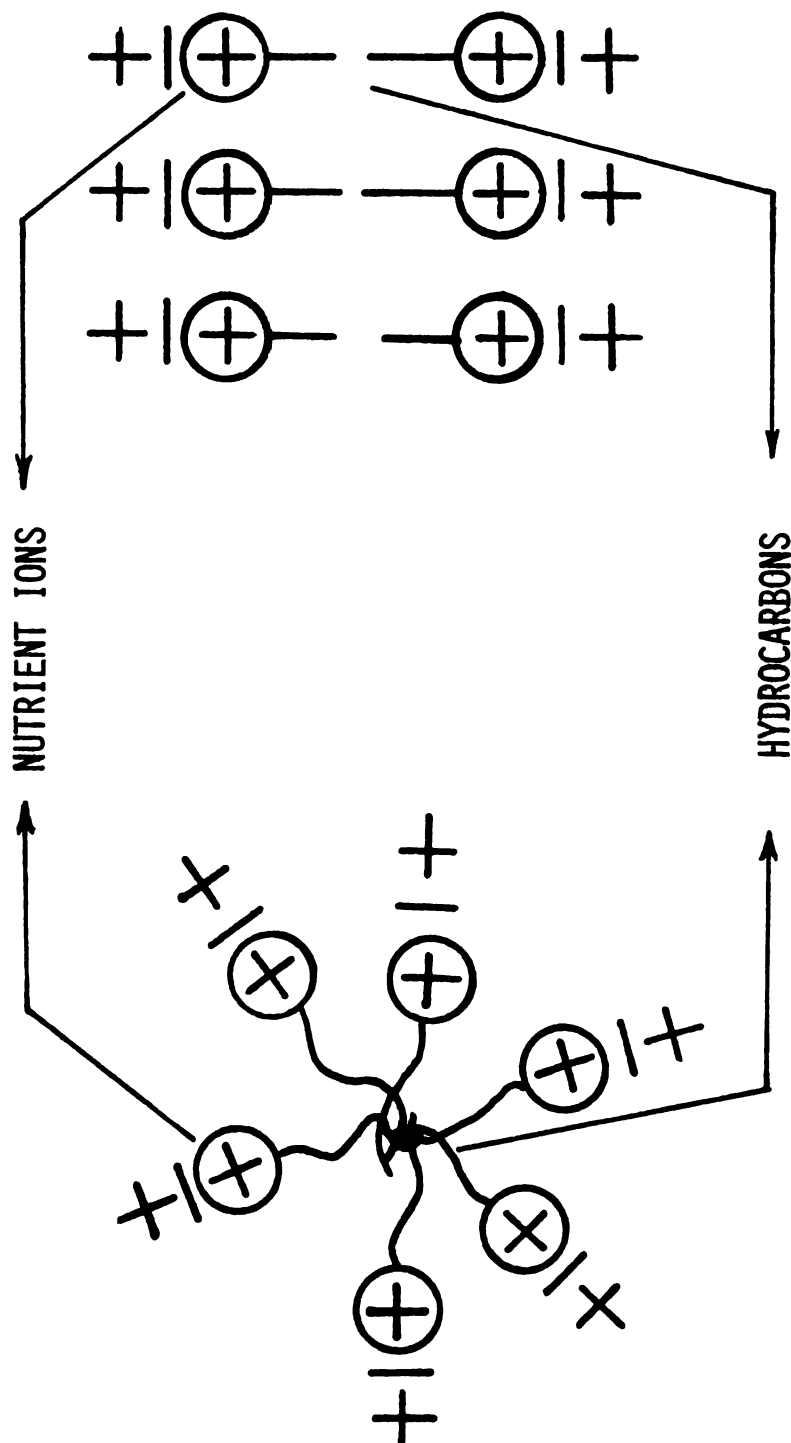


Figure 1. Two possible structures of micelles with hydrocarbon inside and nutrient ions near the surface. (Note: ionic layers shown in idealized form.)

Source: Valenkar et al., "Microbial Growth on Hydrocarbons--Some Experimental Results," Biotechnology and Bioengineering 17 (1975): 241-251.

step in oil biodegradation.²¹ At low cell concentrations, growth occurs in a log-type mode. However, at high cell concentrations, the bacterial demand for oil surpasses the micelles' ability to supply that need, resulting in a linear-type of growth curve.

Summary and Purpose

There are bacteria present in virtually all environs which have the capacity to biodegrade oil. Studies into the rates at which these bacteria both become acclimated and, after becoming acclimated, biodegrade the oil are relatively few. It is the purpose of this paper, therefore, to perform a preliminary study into both of these aspects as they relate to bacterial biodegradation of crude oil in a fresh water environment. Once the study has been carried out, conclusions will be put forth to determine the techniques needed to develop a more rigorous and thorough study into this topic, if such a study is deemed feasible.

²¹ Ibid., pp. 241-251.

METHODS AND MATERIALS

In this study two Busch* reactors were operated as batch reactors; one as a control reactor and one as the experimental reactor (see Figure 2). Air was supplied to the reactors from an air compressor via two rotometers. Each rotometer was set at twelve cubic feet per minute (cfm) and delivered the air through a three-headed ceramic diffuser at the base of each reactor. These diffusers supplied ample oxygen for bacterial growth and also provided a good measure of agitation needed to insure that the oxygen was distributed in reasonably uniform concentrations throughout the reactors.

To insure that species of bacteria which have the ability to acclimate and grow on crude oil were present when the experiment began, it was necessary to use a source containing a wide variety of active bacteria. Aeration tank mixed liquor meets this criterion; the source for this experiment was the aeration tank at the East Lansing Wastewater Treatment Plant in East Lansing, Michigan. Fifteen liters of mixed liquor were taken from the head end of the aeration tank and were immediately transported to the

*Does not imply endorsement of specific brand.

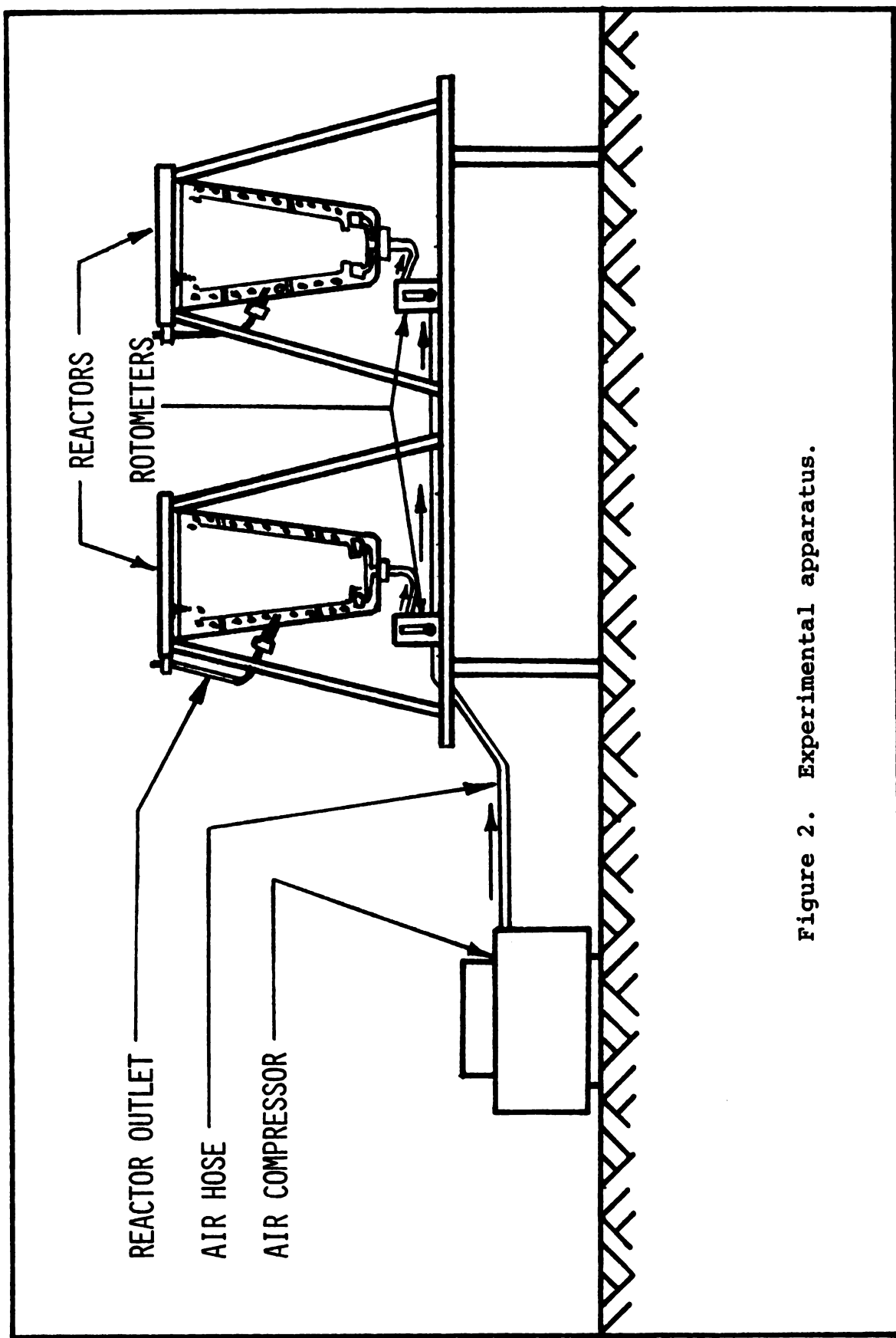


Figure 2. Experimental apparatus.

pilot plant at Kalamazoo Street. Of the fifteen liters obtained, six (6) liters were transferred to the control reactor and five and four-tenths (5.4) liters were transferred to the experimental reactor. The rest of the liquor was discarded.

A gallon of Michigan sweet crude oil was obtained from Total Refineries in Alma, Michigan for use as the experimental growth medium. Six-tenths (0.6) liter of this oil was added to the experimental reactor, or 10% of the total volume of material in the reactor. This quantity was decided upon to insure that the bacteria had sufficient substrate to last through the course of the experiment.

Starting on September 9, 1975 (the first day of the experiment) and for one hundred days thereafter (less Sundays and Thanksgiving Day), each reactor was tested for its total chemical oxygen demand (COD) (see Appendix). To perform the COD test, a thirty to forty milliliter sample was removed from each reactor via the outlet at its center (see Figure 2). The samples were then taken to the Sanitary Engineering Laboratory in Room 381 of the Engineering Building at Michigan State University where the total COD tests were performed. Any reactor material not used in the COD test was taken back to the pilot plant and returned to its respective reactor. The reactors were then refilled to their original volumes with distilled water to compensate

for evaporation rather than for the loss of the material used in the COD tests.

The total COD test was used because it measured the oxygen required to oxidize all of the carbonaceous material (except that noted in the Appendix) in the aqueous phase of the reactors. The value obtained in such a measurement gives an indication of gross changes in the reactor; that is, changes in nutrient and substrate concentrations combined with changes in cell concentrations. In such a determination, only the losses due to complete oxidation of materials present in the reactor were measured. Thus, any substrate or nutrient material which was converted to cell material would not show up as a change in COD, but the loss of substrate or nutrients required as an energy source would be indicated.

RESULTS AND CONCLUSIONS

Table 1 lists the results of the COD tests. The graphs in Figures 3 and 4 illustrate the results from the tests. Figure 3 is the graph of the control reactor showing the relationship between time in days and total COD in milligrams per liter. This graph indicates an initial rapid increase in COD encompassing the first 10 to 15 days followed by a gradual decrease in COD from the fifteenth day on. At first it was thought that the initial rapid increase in COD was due to the fact that the bacteria were taken from the head end of the aeration tank. Since the organisms in this area are in the log phase of their growth cycle, an accumulation of bacteria naturally occurs and this would be exhibited in the COD tests.

However, the total COD test measures the total amount of chemically oxidizable material in the sample, not changes in biomass. For this reason, the above explanation has been ruled out. Upon reflection, the only logical explanation for the rapid change in COD are concentrations of the reactor contents due to evaporation or experimental error, due to the author's inexperience in performing the COD tests. Because distilled water was

Table 1. Experimental data (note: COD in mg/l)

Date	Day	COD of Reactors		Date	Day	COD of Reactors	
		Control	Oil			Control	Oil
Sept.				Nov.			
9	1	963	625	1	54	450	1085
10	2	1007	1639	3	56	502	1065
11	3	1250	1360	4	57	477	1100
12	4	1200	1460	5	58	462	1054
15	7	1490	1260	6	59	494	1320
16	8	1230	1600	7	60	480	1280
18	10	1510	1220	8	61	472	1224
22	14	1090	1100	10	63	456	1180
24	16	1040	943	11	64	454	1120
25	17	981	778	12	65	421	1130
26	18	930	822	13	66	381	1100
27	19	953	853	14	67	405	1100
29	21	926	757	15	68	378	1070
30	22	922	806	17	70	408	1040
Oct.				18	71	438	1060
1	23	885	839	19	72	360	1060
2	24	843	843	21	74	469	1220
3	25	784	799	22	75	446	1080
4	26	794	939	24	77	429	1080
6	28	833	826	25	78	405	1160
7	29	750	924	26	79	389	1110
8	30	747	996	28	81	408	1100
9	31	698	1020	Dec.			
10	32	724	942	1	83	337	964
11	33	727	938	2	84	374	1010
13	35	610	985	3	85	380	946
14	36	687	973	4	86	355	926
15	37	646	977	5	87	363	903
16	38	600	985	6	88	324	865
17	39	628	966	8	90	332	826
18	40	578	951	9	91	300	814
20	42	555	875	10	92	283	771
21	43	578	951	11	93	279	764
22	44	591	841	12	94	277	793
23	45	411	735	13	95	266	743
24	46	593	909	15	97	311	848
25	47	572	862	16	98	344	749
27	49	502	808	17	99	380	775
29	51	492	844	18	100	383	697
30	52	537	1060	Jan.			
31	53	451	1010	12	120	345	690
				19	138	335	778

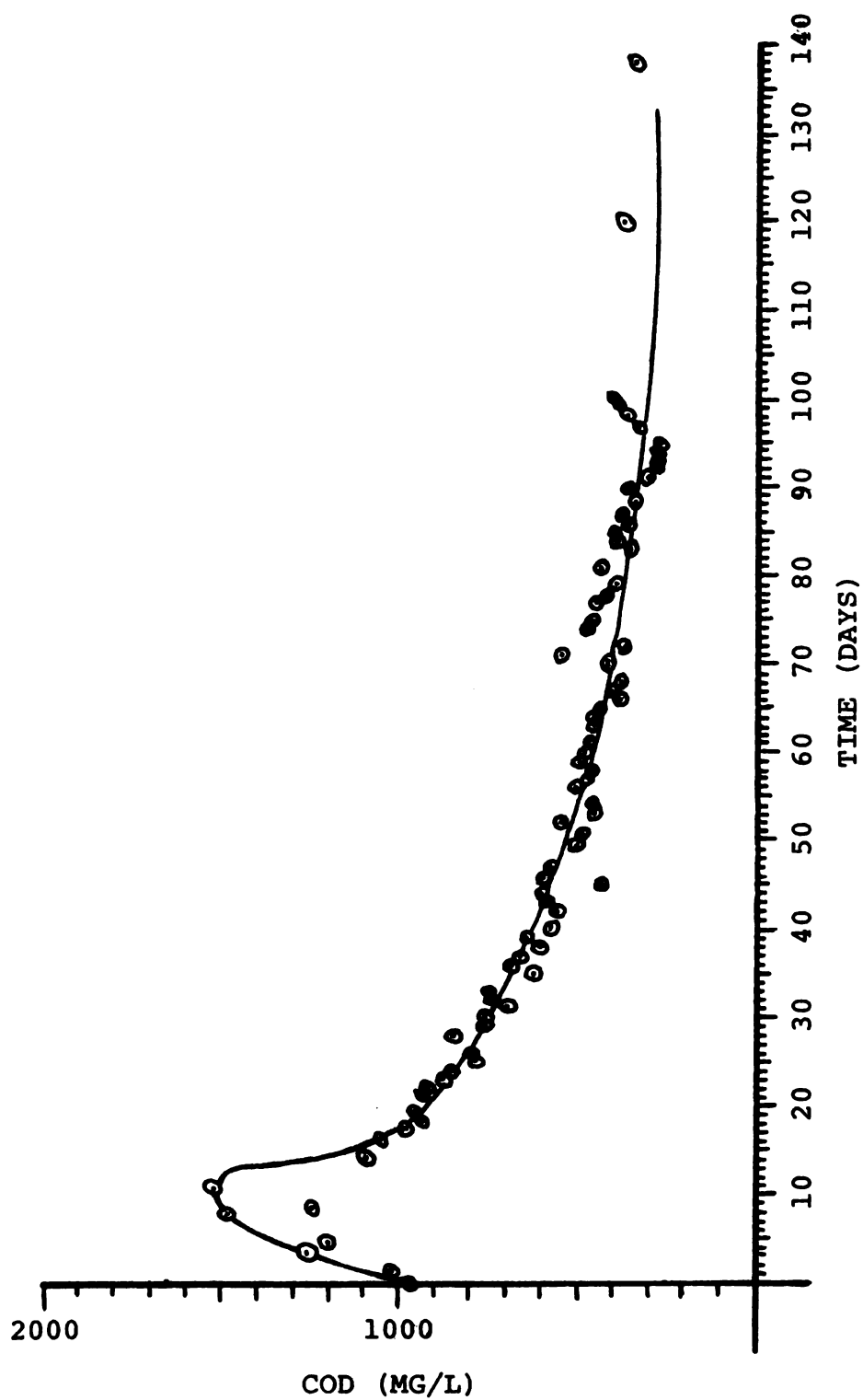


Figure 3. Control reactor.

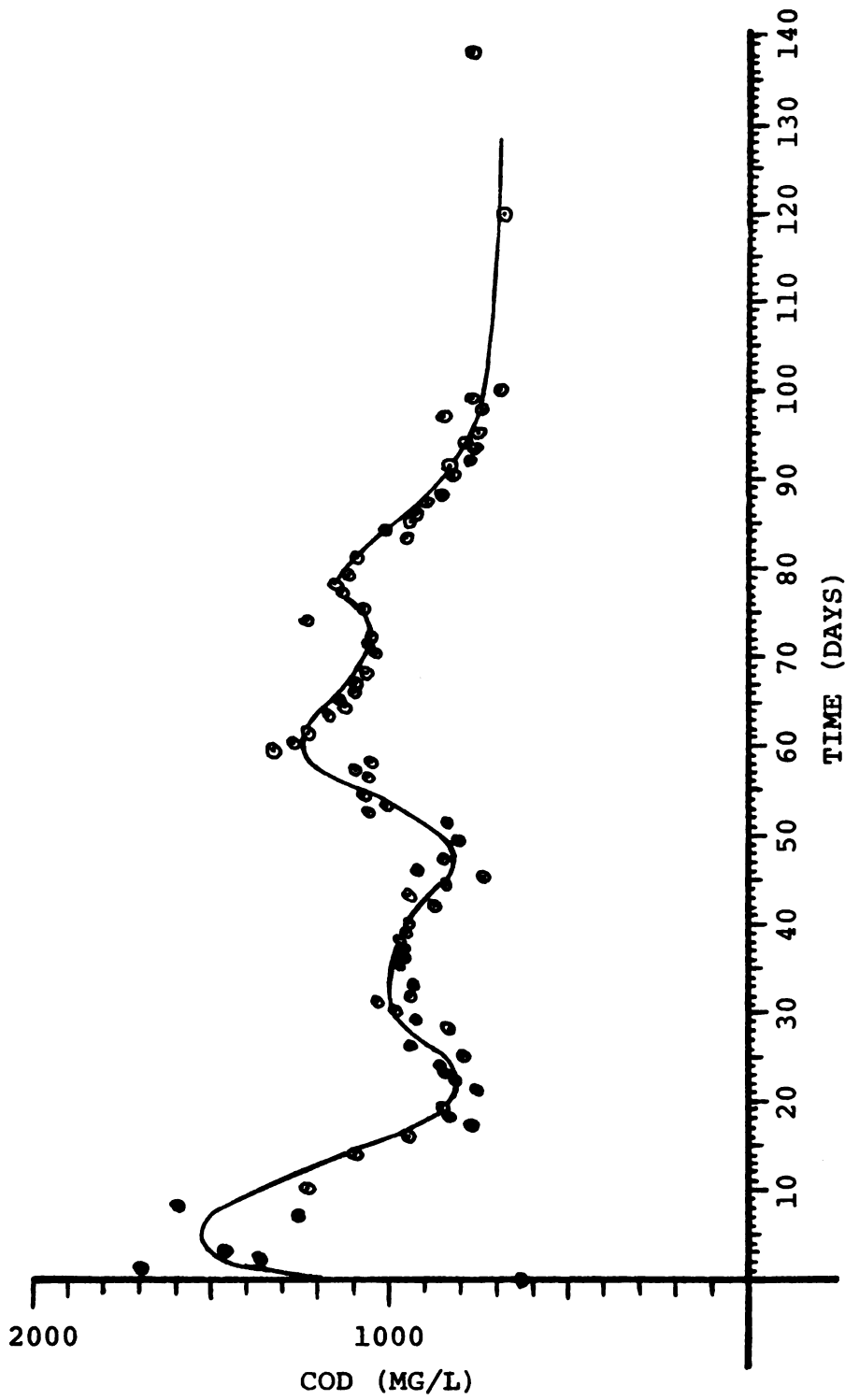


Figure 4. Experimental reactor.

added daily to the reactor contents to compensate for evaporation, the latter alternative appears to be the only explanation for the rapid increase in COD.

Figure 4 is the graph illustrating the relationship between time in days and total COD in milligrams per liter of the oil reactor. Once again, there was an initial rapid increase in COD, occurring for the same reason as the control reactor, encompassing the first 10 days, followed by a decrease in COD from approximately day ten to day twenty. At this point, however, this graph departs from that of the control by again rising from about day twenty to day thirty and decreasing to day forty-five. This rise and fall occurs again from approximately day forty-five to day seventy and once again from about day seventy to day one hundred. After day one hundred, the two points shown on the graph were taken for the purpose of illustrating the general drift of the reactor contents and do not give enough information on which to base conclusions.

At the beginning of the experiment, when the Michigan sweet crude oil was added to the aeration tank mixed liquor in the experimental reactor the oil formed a separate layer at the top of the reactor. This was expected since oil has a lower specific weight than water. As the experiment progressed, the characteristics of the oil changed as a result of both microbial and physical actions.

At first, the oil layer was almost black in color, about two inches thick, and had a fairly heavy viscosity. Through the course of the experiment, the thickness of the layer had decreased to about one-half inch at the termination and had changed to a light brown color. The viscosity of the oil was noticeably lighter and, in fact, toward the end of the experiment, the layer foamed appreciably when disturbed by the distilled water which was added each day. The foaming was in all probability due to the accumulation of lighter fractions as the heavier, long chain fractions were broken down. There was also a noticeable amount of tar produced and grease balls up to one-half inch in diameter were formed as well.

These visual observations taken in conjunction with the graph of Figure 4 tend to substantiate the micelle theory for solubilization of crude oil. The oscillations observed on the graph following the initial rapid increase in COD due to growth plus the observation that the viscosity of the oil layer decreased appear to indicate that some mechanism was involved in removing the oil from its layer at the top of the reactor and taking it into solution. The micelle theory best explains this mechanism.

An additional theory which can be put forth based on the graph of Figure 4 is that the micelles are produced by the bacteria to solubilize specific fractions of the crude

oil. If the micelles indiscriminately took the oil into solution with no regard or specificity for the type of fraction, the graph would most likely show either a constant rise and then a fall or a rise followed by a plateau followed by a decrease.²²

If the former were the case, it would indicate that the bacteria were depleting the oil layer at a constant rate for the purpose of cell growth. A graph of the COD for such a situation would reach a peak, indicating that the nutrient medium (in this case the oil) had been used up, followed by a drop as the bacterial population died off due to lack of food. If the latter were the case, the rise would indicate growth, the plateau would indicate that the micelles' rate-limiting concentration had been reached and that further depletion of the oil layer had occurred at a rate sufficient to maintain a constant population, and then the fall would indicate that, as in the former case, the nutrient oil layer had been depleted and the bacteria had died off.

However, the graph rises and falls three distinct times over the course of this experiment which seems to indicate that three separate fractions of oil were progressively solubilized. There are two possible methods by which this progressive solubilization may have occurred.

²² Mellanby, The Biology of Pollution.

One method is that one type of bacteria established itself in the reactor and produced three separate and distinct types of micelles. In other words, the established bacteria produced one type of micelle to solubilize a specific fraction and when that fraction was depleted they produced another type of micelle, and so forth until all of the fractions were depleted (or until the experiment was terminated).

The other method employs three separate bacterial types which produce only one type of micelle each. Thus there would have been three distinct populations of bacteria during the course of the experiment, with each population producing micelles specific to one fraction of the crude oil. The latter explanation seems to be the most logical owing to the fact that bacteria are very simple organisms which probably would have required more time than is indicated by the graph of Figure 4 to adapt to producing a completely different type of micelle.

Due to the lack of time and to inconclusive data, the rate at which the bacteria biodegraded the crude oil was not determined. However, based on the graph in Figure 4 as it relates to the control graph in Figure 3, it appears that the bacteria required approximately twenty days to become acclimated to the oil. This assumes that the bacteria did not attack the oil during the initial rapid

growth phase that occurred in both the control and oil reactors. This may be a false assumption because the rapid growth phase may have cloaked any oil biodegradation that might have occurred.

SUMMARY AND RECOMMENDATIONS

The data appear to substantiate the micelle theory for the solubilization of oil by bacteria. They also imply that the bacteria produced only one type of micelle for each fraction of the oil. There are two possible methods with which to explain this specificity. The first method theorizes that one type of bacteria established itself in the experimental reactor and then proceeded to produce three separate and distinct types of micelles over the course of the study. The other method states that three successive bacterial populations, each producing one distinct type of micelle, grew in the reactor as the experiment proceeded. The latter method seems to be the most logical.

Due to the lack of sufficient data, the rate of bacterial biodegradation of crude oil was not determined. The period of time required for a mass culture of bacteria obtained from aeration tank mixed liquor to become acclimated to Michigan sweet crude oil appears to be about twenty days.

Because of the timeliness of this study and the positive results obtained, a further, more rigorous study on this subject would appear to be a feasible undertaking.

The purpose of the remainder of this section is to outline an ideal study, based on the information learned in the preliminary study, which would obtain more thorough and conclusive data on the subject of bacterial biodegradation of crude oil in a fresh water environment.

In order to reduce the time required for them to acclimate to the crude oil, the bacteria should be concentrated and then diluted with a sterile saline solution. The concentration process could be carried out by obtaining activated sludge from a secondary settling tank, or by centrifuging mixed liquor from an aeration tank. If the latter process is employed, the liquor should be obtained from the tail end of the aeration tank because most of the substrate in that area of the tank will have been assimilated. This would force the microbes to acclimate to the crude oil more rapidly by depriving them of a secondary food source.

The chemical oxygen demand (COD) test, both total and filterable, should be retained because it is a rapid and easily measured determination. However, the oil layer should be tested for its COD in addition to that of the aqueous phase of the reactor. Because the COD test is not capable of oxidizing all fractions of the oil, changes in the concentrations of the oil fractions in both the oil layer and the aqueous phase should also be determined using a technique such as gas chromatography (GC).

Another valuable parameter to measure would be the mixed liquor volatile suspended solids (MLVSS). This determination could give an indication of changes in the size of the bacterial population within the reactor, and could be used to bolster the COD tests. Examining the reactor contents for changes in the population size of specific types of bacteria would be another desirable addition to the study. Such tests would corroborate and define changes in the MLVSS, and could conceivably confirm one of the theories of micelle production.

The above recommendations are intended as an outline for further study and should not be treated as the only steps to follow when looking into the topic of bacterial biodegradation of crude oil. Thought should be given to making any additions, alterations, or deletions to the outline to fit particular circumstances before beginning a study into this topic.

APPENDIX

APPENDIX

CHEMICAL OXYGEN DEMAND¹

The chemical oxygen demand (COD) determination provides a measure of the oxygen equivalent of that portion of the organic matter in a sample that is susceptible to oxidation by a strong chemical oxidant. It is an important, rapidly measured parameter for stream and industrial waste studies and control of waste treatment plants. In the absence of a catalyst, however, the method fails to include some organic compounds (such as acetic acid) which are biologically available to the stream organisms, while including some biological compounds (such as cellulose) which are not part of the immediate biochemical load on the oxygen assets of the receiving water. The carbonaceous portion of nitrogenous compounds can be determined, but there is no reduction of the dichromate by any ammonia in a waste or by any ammonia liberated from the proteinaceous matter. With certain wastes containing toxic substances, this test or a total organic carbon determination may be the only method for

¹American Public Health Association, American Water Works Association, Water Pollution Control Federation, Standard Methods for the Examination of Water and Wastewater, 13th ed., 1971.

determining the organic load. Where wastes contain only readily available organic bacterial food and not toxic matter, the results can be used to approximate the ultimate carbonaceous BOD values.

The use of exactly the same technique each time is important because only a part of the organic matter is included, the portion depending on the chemical oxidant used, the structure of the organic compounds, and the manipulative procedure.

The dichromate reflux method has been selected for the COD determination because it has the advantage over other oxidants in oxidizability, applicability to a wide variety of samples, and ease of manipulation. The test will find its major usefulness in a plant for waste control purposes after many values have been obtained and correlated with some other important parameter or parameters.

1. General Discussion

a. Principle: Most types of organic matter are destroyed by a boiling mixture of chromic and sulfuric acids. A sample is refluxed with known amounts of potassium dichromate and sulfuric acid, and the excess dichromate is titrated with ferrous ammonium sulfate. The amount of oxidizable organic matter, measured as oxygen equivalent, is proportional to the potassium dichromate consumed.

b. Interference and Inadequacies: Straight-chain aliphatic compounds, aromatic hydrocarbons, and pyridine are not oxidized to any appreciable extent, although this method gives more nearly complete oxidation than the permanganate method. The straight chain compounds are more effectively oxidized when silver sulfate is added as a catalyst; however, silver sulfate reacts with chlorides, bromides, or iodides to produce precipitates which are only partially oxidized by the procedure. There is no advantage in using the catalyst in the oxidation of aromatic hydrocarbons, but it is essential to the oxidation of straight-chain alcohols and acids.

The oxidation and other difficulties caused by the presence of chlorides in the sample may be overcome by adding mercuric sulfate which is a complexing agent for the elimination of chlorides from the reaction. This ties up the chloride ion as a soluble mercuric chloride complex, which greatly reduces its ability to react further.

c. Application: The method can be used to determine COD values of 50 mg/l or more with the concentrated dichromate. With the dilute dichromate, values below 10 mg/l are less accurate but may be used to indicate an order of magnitude.

d. Sampling and Storage: Unstable samples should be tested without delay, and samples containing settleable solids should be homogenized sufficiently by means of a

blender to permit representative sampling. If there is to be a delay before analysis, the sample may be preserved by acidification with sulfuric acid. Initial dilutions in volumetric flasks should be made on wastes containing a high COD value in order to reduce the error which is inherent in measuring small sample volumes.

2. Apparatus

Refer to Standard Methods.

3. Reagents

Refer to Standard Methods, with these notes:

Sulfamic acid was not added to dichromate solution because little nitrate interference was anticipated. Standardization of ferrous ammonium sulfate solution was performed each day of the test.

4. Procedure

Refer to Standard Methods, with this note:

Procedure 4a was performed. Up to day 61 10.0 ml samples from both reactors were diluted to 20.0 ml with distilled water. From day 61 to the end of the experiment, a 20.0 ml sample of the control was used while the original dilution continued to be performed on the oil sample.

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