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THE USE OF COMMERCIALLY-PRODUCED ENZYMES OR ORGANIC AMENDMENTS TO DEGRADE OIL IN CONTAMINATED SOILS

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ABSTRACT

Commercially-produced enzymes were not effective in degrading oil in contaminated soil over a six month incubation period. The incorporation of barley straw (1.0% by weight) led to a 41% decrease in oil content in the heavily contaminated (74 mg/g of oil) Lagoon soil and a 38% reduction in the less contaminated (16 mg/g of oil) Herald soil over the same time. Barley straw added at 2% by weight reduced oil content by 21% in the Lagoon soil and 34% in the Herald soil. Sugar beet pulp incorporated at 2% by weight was nearly as effective as the 10% straw treatment in degrading oil in both the Lagoon and the Herald soils.

Soil water repellency was reduced immediately by the enzyme treatment and in two months by the 10% straw amendment. Subsequently, water repellency returned and was particularly severe in the 10% straw treatment.

La dégradation des huiles et la réduction de l'imperméabilité des sols contaminés à l'huile

Trois modifications, comprenant un mélange d'enzymes commerciaux, deux niveaux de paille et de billes de betterave à sucre, ont été étudiées pour vérifier leur efficacité à fournir des conditions optimales de dégradation de l'huile et réduction de l'imperméabilité des sols contaminés à l'huile. Un échantillon de sol contenait à l'origine 1.6% d'huile en poids; l'autre échantillon en avait au départ 6.0%. Après une période d'incubation de quatre mois, le mélange d'enzymes a pu réduire l'imperméabilité du sol de 90%, en contribuant toutefois très peu à la dégradation de l'huile. La paille, incorporée à raison de 10% du poids, représentait le traitement le plus efficace pour réduire le contenu en huile. L'imperméabilité du sol, après avoir ajouté de la paille à 10%, fut complètement éliminée à la fin du deuxième mois, mais s'est redéveloppée plus tard jusqu'à environ un tiers de son niveau initial. La paille, incorporée à 2% du poids, s'est révélée moins efficace pour réduire l'imperméabilité, mais a pu contribuer à la dégradation de l'huile, surtout dans le cas du sol qui contenait peu d'huile à l'origine. Les billes de betterave à sucre, incorporées à 2% du poids, ont occasionné une réduction graduelle, mais constante, du contenu d'huile dans les deux types de sols; l'imperméabilité du sol n'a été que légèrement diminuée par l'utilisation de la betterave à sucre.

INTRODUCTION

More than 5,000 cubic meters of oil-contaminated soil was stockpiled at two locations near Zama Lake, Alberta. The Land Reclamation Division of Alberta Environment is seeking an effective and economical way of reclaiming the oil-contaminated soils. The oil residue could be removed by incineration but the cost is estimated to be more than $200 per tonne (Personal communication) . Transporting the soils to a landfill would be costly and might cause future environmental problems. *In situ* reclamation—degrading the oil in the soils where they are stockpiled—seems to be a more practical alternative. Microbial degradation and enzyme treatment have both been proposed to the Land Reclamation Division. Once the oil is removed and disposal guidelines are met, the soils can be spread over nearby areas.

At the request of the Land Reclamation Division, the Soils Branch at the Alberta Environmental Centre tested a commercial enzyme mixture for its ability to degrade oil in soil. Three organic amendments (two levels of straw and sugar beet pulp) were also tested in the same experiment. The objective was to identify the best treatment to degrade hydrocarbons in soil.

MATERIALS AND METHODS

One fifty-gallon drum of each of two oil-contaminated soils (Herald and Lagoon) were delivered to the Alberta Environmental Centre. One core, 6 cm in diameter and 60 cm deep, was taken from each drum. The soil in each core was air dried, screened to less than 2 mm and analyzed for chemical and physical properties.

Over 20 kg of the soil was removed from each drum for the testing of five treatments to degrade oil. The soil was air dried and screened to less than 10 mm.

Three replicates of each of five treatments on two soils resulted in a total of 30 pots. The pots (18 cm high and 15 cm diameter) were sealed at the bottom and filled with 750 g of treated soil. The pots were placed in a completely randomized design on a bench in a greenhouse compartment. Every week enough distilled water was added to bring the soil moisture level to 80% of the predetermined field capacity.

The five treatments were:

1. Enzyme mixtures (BioZyme I and II)
2. Water (Control)
3. Barley straw (2% by weight)
4. Barley straw (10% by weight)
5. Sugar beet pulp (2% by weight)

The commercial enzyme mixtures (BioZyme I and II) were prepared by a company[1] in Calgary, Alberta, and used to treat the contaminated soils according to the following directions (as specified by the company's representative): Initially, BioZyme I solution, diluted 5:1 with distilled water, was added to both soils until they were saturated. Six hours later, the soils and enzymes were mixed and left to incubate in the greenhouse with the rest of the treatments. After 15 days the soils were resaturated with a 10:1 mixture of distilled water and Biozyme I for six hours. The soils and enzymes were then mixed and left to incubate in the greenhouse again. After 30 days the soils were resaturated with a 10:1 distilled water and BioZyme II mixture. Again, after 6 hours of saturation, the soils and enzymes were mixed and left in the greenhouse. After 60 days the soils received a 6 hour saturation with a 50:1 distilled water and BioZyme II mixture. The soils were mixed and left in the greenhouse. Finally, at 120 days the soils were resaturated with a 100:1 distilled water and BioZyme II mixture for six hours. The soils were then mixed and left in the greenhouse to the end of the experiment.

Distilled water was applied to both soils in Treatment 2. All other activities were the same as for enzyme mixtures in Treatment 1. The water application was considered to be a control.

Air dried barley straw was used in Treatments 3 and 4. It was chopped to 2.5 cm and mixed with the soil at 2 and 10% by weight, respectively. After the initial mixing these soils were not disturbed again.

Sugar beet pulp used in Treatment 5 was obtained from Alberta Sugar Company, Taber, Alberta. The pulp was crushed and passed through a 2 mm screen before mixing with the soils at 2% by weight. Like Treatments 3 and 4 that used barley straw, the soils with sugar beet pulp were not disturbed after the initial mixing. A nutrient solution was prepared using NH_4NO_3, $NH_4H_2PO_4$ and KNO_3 to provide nitrogen, phosphorus and potassium at a ratio of 10:4:10 (McGiIl 1976) . Treatments 3, 4 and 5 received 10 ml of the nutrient solution at each of five applications. Each application was equivalent to a rate of 224 kg of nitrogen per hectare. It has been recommended that the total amount of nutrients needed for oil degradation be added in several increments rather than one large application (McGiIl 1976).

Soil cores, 15 mm in diameter, were taken from each replicate five times (O, 0.5, 1, 2, 4 and 6 months) during the experiment. The cores were air dried, sieved to less than 850 ^.m, and used to measure oil content and soil water repellency.

[1]The name of the company is confidential but can be released when contact the senior author.

The oil was extracted from the soils for three hours using toluene in a Dean and Stark apparatus (Yeung and Johnson 1986). The extracted oil was weighed, and the oil content was expressed as a percentage of the oven-dry weight of soil.

Soil water repellency was measured by placing drops of ethanol solutions on the surface of the soil. The degree of water repellency was determined by the lowest ethanol molarity required for droplet penetration of the soil surface in 10 seconds. The measurement was termed the molarity ethanol droplet (MED) value. Soils with MED values less than 1.0 are considered to be wettable/ soils with MED values exceeding 2.0 are considered to be severely water repellent (Yeung 1990).

Soil strength was measured by a Centre Cone Penetrometer (James 1988) equipped with a cone having a basal area of 129 mm^2. The cone was pushed 3 cm into the soil surface. Five measurements (MPa) were taken from each pot one week after enough water was added to bring each treatment to 80% field capacity.

The data collected for the oil content of the two soils were computed by the Tukey's Studentized Range Test (SAS Institute Inc. 1985) based on a covariance analysis with the initial oil content as a variate.

RESULTS

Some chemical and physical properties of the two soils are presented in Table 1. The Herald soil contains a small amount of oil (1.6 % by weight), exhibits no water repellency (MED = O) and is saline (EC 7.58 Ms/cm). The amounts of soluble sulphates (1268 ppm) and chlorides (1313 ppm) reflect the elevated salinity status. The Lagoon soil has more oil (7.4% by weight) and is severely water repellent (6.1 MED value); it is extremely saline (EC 14.4 Ms/cm) and contains large amounts of sulphates (1230 ppm) and chlorides (2025 ppm). Both soils are non-sodic and contain low levels of nitrogen (< 6 ppm).

This experiment indicated that the enzyme treatment was not effective in degrading oil (Table 2). The amount of oil degraded in both the Herald and the Lagoon soils (1.6 and 10.5 mg/g, respectively) was not significantly different from the water (control) treatment.

The 10% straw treatment was effective in degrading oil. It led to a 41% reduction of oil in the highly-contaminated Lagoon soil, almost 30% more than the enzyme and water (control) treatments (Table 2). In the less contaminated Herald soil, 38% of the original oil disappeared over six months incubation time; the 10% straw showed a significant reduction in oil content (6.7 mg/g or 38%) when compared with the water (control), enzyme or the sugar beet treatment.

Table 1. Chemical and physical properties of the Herald and Lagoon soils.

Property	Soils	
	Herald	Lagoon
Oil (%)	1.6	7.4
pH	7.9	7.3
EC (mS/cm)	7.58	14.40
N (ppm)	5.6	5.5
P (ppm)	98	262
K (ppm)	155	452
Soluble Na (ppm)	828	2088
Soluble Ca (ppm)	2000	2100
Soluble Mg (ppm)	250	200
SAR	4.6	11.6
Sulphate (ppm)	1268	1313
Chloride (ppm)	1230	2025
MED value*	0.0	6.1
Moisture (% w/w) - Air dry	1.9	2.4
- 1/3 bar	45	47
- Saturation	86	78
Particle size - Sand (%)	30	46
Silt (%)	59	53
Clay (%)	11	1
Textural class	SiL	SiL

*Molarity Ethanol Droplet(MED) value for soil water repellency measurement (Yeung 1990).

The 2% straw treatment was as effective as the 10% straw treatment in reducing oil in the Herald soil. The loss of 5.7 mg/g of oil is equivalent to a total reduction of 34% in six months of incubation. In the heavily-contaminated Lagoon soil the 2% straw treatment did not perform as well.

The incorporation of 2% sugar beet by weight was more effective in degrading oil in the Lagoon soil which contained almost 5 times more oil than the Herald soil (Table 1). When compared with the control or enzyme treatment in the same soil the 2% sugar beet treatment led to 25% more oil reduction (Table 2).

All treatments except the control were associated with a steady decrease in oil content during the incubation period (Figure 1). Generally, the rate of oil degradation tended to level off at the later stage of the experiment. When only water was added to the Herald soil the oil content remained constant throughout the experiment.

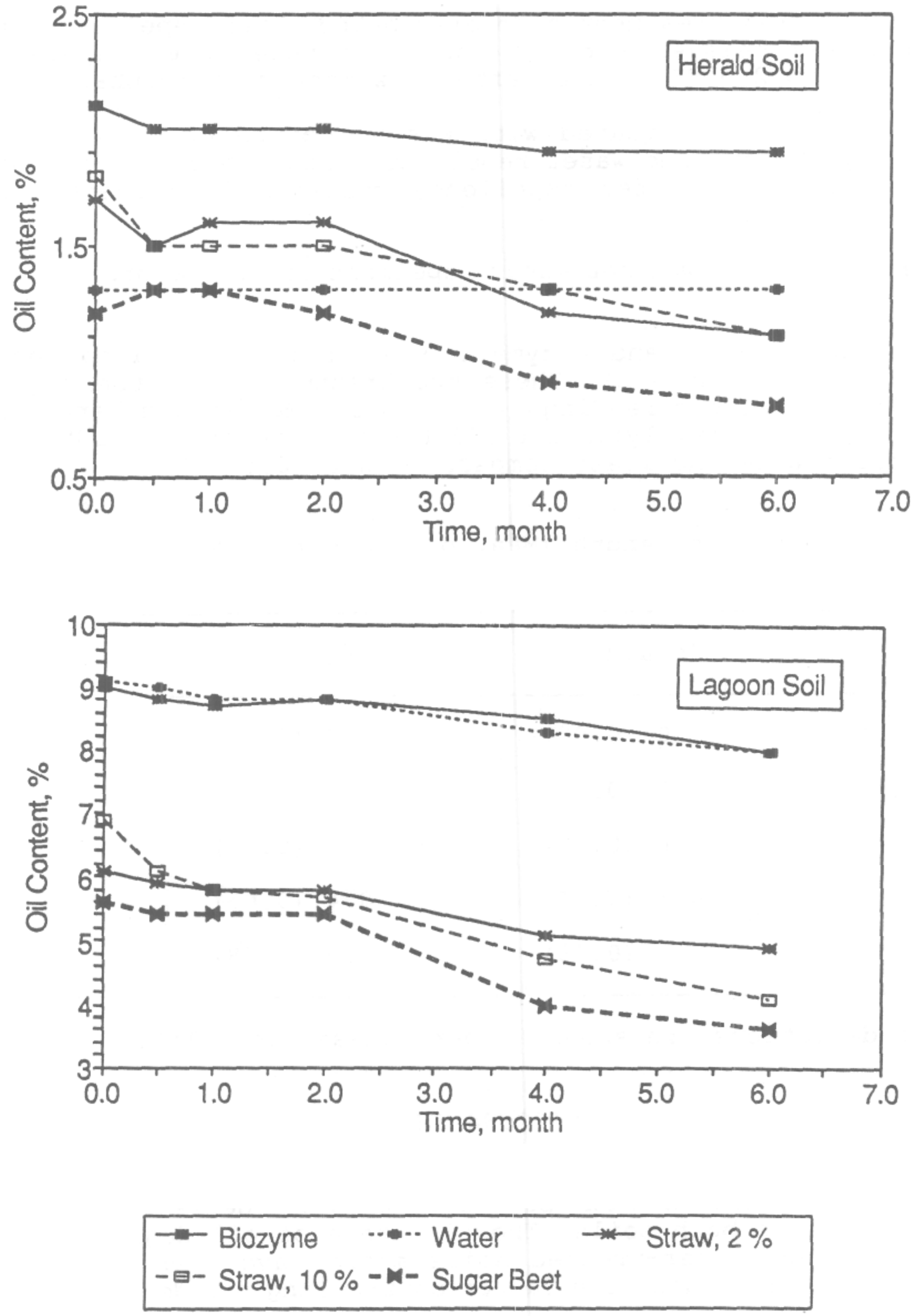
Table 2. Oil content of the Herald and Lagoon soils before and after six months of treatments.

Treatment	Oil content			
	Initial	Final	Decrease	Reduction
	---------- (mg/g)		----------	(%)
<u>Herald soil</u>				
Water (Control)	13.2 (0.9)*	12.9 (1.6)	0.3a	2.3
Enzyme	20.5 (1.7)	18.9 (0.6)	1.6a	7.8
Sugar beet (2%)	12.0 (0.5)	8.2 (1.1)	3.8ab	31.7
Straw (2%)	17.0 (6.6)	11.3 (3.7)	5.7 bc	33.5
Straw (10%)	17.6 (1.0)	10.9 (1.6)	6.7 c	38.1
<u>Lagoon soil</u>				
Enzyme	90.4 (6.2)	79.9 (1.7)	10.5a	11.6
Water (Control)	91.2 (4.5)	80.4 (1.8)	10.8ab	11.8
Straw (2%)	61.0 (1.5)	48.5 (3.1)	12.5ab	20.5
Sugar beet (2%)	56.4 (12.7)	35.8 (7.5)	20.6 bc	36.5
Straw (10%)	69.4 (5.1)	41.3 (3.3)	28.1 c	40.5

Mean values followed by different letters within a column for each treatment of the same soil are significantly different ($P<0.05$) .

*Standard deviations are shown in parentheses.

Figure 1. The change of oil content *of* the Herald and Lagoon soils over six months incubation time.



The enzyme mixture was particularly effective in eliminating water repellency in the Lagoon soil in the early stage of the experiment (Figure 2). In just two weeks the enzyme treated soil lost all water repellency. However, near the end of the experiment, soil water repellency of the enzyme treated soil rose from 0.0 to 0.9 (MED values).

The 10% straw treatment also eliminated water repellency, but the effect was delayed for two months. Severe water repellency returned (up to 2.9 MED value) after six months of incubation.

The Lagoon soil treated with water (control), 2% straw and sugar beet pulp remained water repellent, although over six months a small decrease in water repellency resulted from these three treatments (Figure 2).

The Herald soil was not water repellent either before or after incubation.

Both the control and enzyme treated soils tended to become hard and compact (Table 3). These two treatments had the highest penetration resistance readings (1.0 and 0.8 MPa in the Herald and Lagoon soil, respectively) among all the treatments. The 10% straw treated soils were the least compact (0.3 to 0.4 MPa).

Table 3. Mean soil strength (MPa) of the Herald and Lagoon soils measured by a Centre Cone Penetrometer.

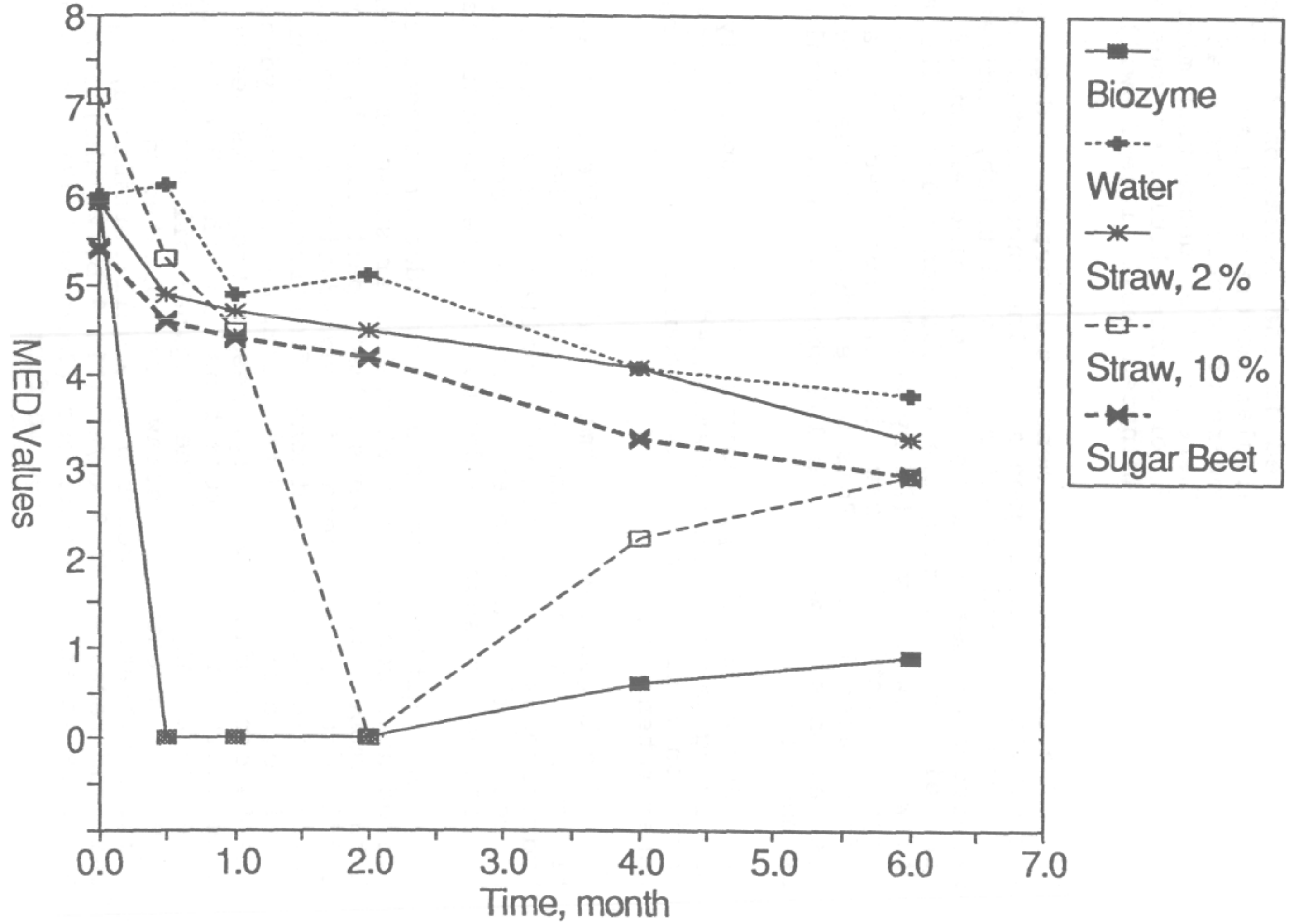
Treatment	Herald	Lagoon
Enzyme	1.0 (0.98)*	0.8 (0.13)
Water (Control)	1.0 (0.18)	0.8 (0.11)
Straw (2%)	0.6 (0.10)	0.5 (0.05)
Straw (10%)	0.3 (0.04)	0.4 (0.06)
Sugar beet	0.6 (0.08)	0.5 (0.08)

*Standard deviations are shown in parentheses (n = 15).

DISCUSSION

The enzyme treatment was not effective in degrading oil. In fact, distilled water had the same effect on oil degradation as the enzyme mixture in both soils. The slow rate of oil degradation in either enzyme or water-treated soils might have been due to the limited supply of oxygen, the factor controlling oil degradation by

Figure 2. The effect of five treatments on the water repellency of the Lagoon soil over six months incubation time.



aerobic microorganisms (Westlake et al. 1983). Soils receiving either water (control) or enzyme treatments were puddled when wet, and hard and compact when dry. In both situations, oxygen flux could have been severely retarded. The rate of oil degradation could also have been hindered by the limited amounts of nutrients/ no fertilizer was added to the enzyme and water treatments.

The enzymes involved in the oxidation of hydrocarbons are associated with the cellular membrane (Atlas 1980). The initial oxidation of the hydrocarbons occurs either at or within the cytoplasmic membrane. There is no evidence that microbial inoculum (Foght and Westlake 1987) or extra-cellular enzymes outside *of* that resident in the contaminated soil itself will increase oil degradation.

The 10% straw treatment was effective in degrading oil in both the Herald and the Lagoon soil. The incorporation of large amounts of straw provided aeration and good tilth, indicated by the low penetrometer measurements. The mulching effect of the straw kept the soil moist during the incubation period. The application of straw at lower level (2% by weight) may not be as effective as a large amount of straw is incorporated in a soil heavily contaminated by oil. This was evident in the results obtained in the Lagoon soil where the 10% straw treatment could degrade twice as much oil.

Straw is cheap, ubiquitous and apparently effective in providing aeration in contaminated soil. Future experiments should focus on optimizing oil decomposition by improving aeration and water retention.

The sugar beet treatment was as effective as the 10% straw treatment in degrading oil in the Lagoon soil. Since the hydrocarbons are not readily available as an energy source to the microbes, the incorporation of sugar beet may help provide the initial energy supply needed to increase the microbial population.

The initial rate of oil degradation tended to be rapid but slowed down considerably at the later stage of the experiment. The total amount of nutrients added, particularly nitrogen, was not sufficient for complete oil degradation in the two contaminated soils. Based on calculations of microbial turnover, at least 3.0g of nitrogen are required to decompose all the hydrocarbons even in the Lagoon soil. Obviously, less nitrogen is needed to aid in the decomposition of hydrocarbons in the Herald soil. In this experiment only 40% of the original oil was degraded by the most effective treatment (straw incorporated at 10% by weight). A similar situation occurred on a well-fertilized, agricultural soil that had received 24.9 kg/m^2 (11.1% by weight) crude oil in an oil spill reclamation experiment (Toogood 1977). Forty percent of the added oil had disappeared in four years. However, the residual oil was highly resistant to further degradation. More than 15 years after the start of the experiment, 11% of the original oil could still be measured in the same soil (Alberta Environmental Centre, unpublished data).

The enzyme treatment was effective and fast in reducing water repellency, despite the fact that water repellency returned to a small degree. The effectiveness of commercial enzymes in reducing water repellency was probably due to surfactants in the enzyme mixture (Personal communication, R. Reidy, August 1990). The effect of surfactants on plant growth is not known.

Straw incorporated at 10% by weight also led to a large reduction in water repellency. However, severe water repellency returned in this treatment, probably as a result of the build-up of microbial by-products. The return of water repellency after several months (secondary water repellency) has not been studied in oil-contaminated soils. It will be necessary to monitor oil degradation and water repellency over much longer periods of time to understand this evanescent phenomenon.

Soil water repellency could play an inhibitory role in the reclamation of oil-contaminated soils. A water-repellent soil cannot be rewetted, leading to a decrease of microbial activity and, consequently, a decrease in the rate of oil decomposition. Nutrients needed by microorganisms for oil degradation could also be excluded by a hydrophobic barrier.

Soil water repellency may also be an important factor in regulating the rate that salts are leached from extremely saline soils, such as the two tested in this experiment. Salts can have a direct inhibitory effect on microorganisms and an indirect effect through their contribution to increased osmotic potential. Much of the precipitation falling on a water-repellent soil will be lost through surface runoff, and less water will penetrate the soil to dissolve and leach the salts. The relationship between soil water repellency and salt leaching has not been investigated.

The coarse soil aggregates (up to 10 mm in diameter) used in the experiment led to a heterogeneous distribution of oil residue in each of the soils. However, the use of covariance analysis was successful in reducing the error in the experiment, and increasing the measurement precision of treatment effects.

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