Assessment of bioremediation as an operational clean-up technique after an oil spill in the Netherlands

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Abstract
In the Netherlands, bioremediation is since 1987 not considered to be useful in surface waters and in the littoral zone. However, recent spills such as the "ERIKA", the "PRESTIGE" and the "TRICOLOR" have, in the Netherlands, brought new attention to response techniques for beach cleaning, such as bioremediation.
In 2000 a study was done to review the policy dating from the late eighties by studying more recent literature. Also a field experiment was carried out to determine if residual oil on sandy beaches during wintertime could be removed by bioremediation techniques.
From literature it was concluded that bioremediation only is an option when dealing with residual oil on a location which is difficult to access, sensitive for mechanical recovery and where sufficient oxygen and interstitial fluid are present in the sediment. Results from the field experiment indicated that the use of bioremediation agents is not an option to remove residual oil from high energy sandy beaches in the Netherlands. After 18 weeks no significant oil had disappeared due to bioremediation.
General conclusion is that , also in the new millennium, bioremediation should not be considered when dealing with oil pollution in the Netherlands.

1 Introduction

In 1987 research was initiated to determine the possibilities for bioremediation as an oil spill clean-up technique in the Netherlands (Pols, 1987). From the results it was decided that the use of bioremediation agents was not useful in surface water and the littoral zone. For mud flats other clean-up techniques were favoured, but bioremediation with agricultural fertilizers could be considered. Due to a number of recent big spills bioremediation treatment gained attention once again (Atlas, 1991). On behalf of the Directorate-General of Public Works and Water Management, North Sea Directorate, a project was initiated to determine if new techniques and increased knowledge would change standing policy since 1987.

After an extensive literature study on the current status of bioremediation, a field exercise was performed on a high energy sandy beach to investigate if bioremediation in wintertime can remove residual oil (after mechanical clean-up) before the start of the next bathing season.

2 Bioremediation: Current Status

Extensive research has been done to examine the efficacy of in-situ bioremediation agents. In table 1 an overview of studies are presented, divided in experiments showing the (non)effectiveness of bioremediation techniques.

Table 1 Overview of studies on (non)effectiveness of bioremediation treatment experiments

Technique	Effective	Not effective
Slowly soluble briquettes	Safferman (1991) – lab	USEPA (1990) - veld
Oleophilic substances	USEPA (1990) – veld Halmö (1985) – veld Tabak *et al*. (1991) – lab	Lee & Levy (1987) – veld Lee & Levy (1989) – veld
Soluble inorganic substances	USEPA (1990) – veld Halmö (1985) – veld Lee & Levy (1989) – veld Lee & Levy (1991) – veld Tabak *et al*. (1991) – lab Simon *et al*. (1999) - veld	Simon *et al*. (1999) – veld
Fine mineral parts	Bragg & Owens (1995) – veld/lab Lee *et al*. (1997) – lab Berguerio *et al*. (1999) – lab	
Micro-organisms	Aldrett *et al*. (1997) – lab Lee *et al*. (1997) – lab/veld	Chianelli *et al*. (1992) – lab/veld Venosa *et al*. (1991) – lab Simon *et al*. (1999) – veld Aldrett *et al*. (1997) – lab

From all ex-situ techniques (contaminated sediment is treated outside the polluted area) mainly three treatment techniques are well studies: land farming, windrow composting piles and static bio venting piles.
In studies with oil contaminated soil in Kuwait reductions of 82,5-90,5% have been found in one year time (Balba et al, 1998).

2.1 Open Water

In laboratory studies as well as in field tests Chianelli *et al* (1992) found no effective results when adding nutrients to oil slicks on open water.

Meyers *et al* (1999) used a simulation model to study the limiting nutrient levels when bioremediating an oil slick on sea. They found that adding nutrients in the water stimulated uptake of carbons by bacteria. The agents showed to be most effective when brought directly into the oil-water interface.

Jianqiang and Junhuang (1997) found in their extensive studies that the micro-organisms needed a maximum ration carbon/nitrogen/phosphorus of 100/7/0,14. The oxygen demand to transform 1 gram of hydrocarbons in CO2 and H2O was set to 3-4 gram, indicating that in natural conditions the amount of dissolved oxygen in open water will not be limiting. However, when oil forms a slick on the water surface, the diffusion of oxygen from the air into the water will be less. This will limit the biodegradation process.

2.2 Coast

From field experiments following the ”EXXON VALDEZ” disaster in 1979 it was found that a liquid oleophilic fertilizer was more effective on coarse rock beaches than on sandy – or gravel beaches (USEPA, 1990). In locations with sand and graven in combination with pebbles both oleophilic fertilizers and water soluble inorganic fertilizers seemed to be effective. Tests showed that within one month locations were reasonably clean, even underneath the pebbles as compared to a reference location.

Results of Little *et al* (1993) indicated that for many different coastal areas the physical processes are most important for the removal of oil.
In areas with porous habitats (pebble beaches and coarse sand) treatment with fertilizer proved to accelerate the biodegradation process. In sediment with more small particles efficacy of these products were less, mainly because of the lower diffusion of oxygen (Baker, 1992).

Mearns (1997) opposed the question if nutrient addition can speed up biodegradation processes in tidal areas. Several experiments indeed showed positive effects of repeated addition of traditional agricultural fertilizer (Lee & Levy, 1989; Lee & Levy, 1991) and embedded briquettes (Safferman, 1991).

2.3 Bioremediation techniques
In general there are two different approaches for bioremediation. The first approach, bio augmentation, is a technique in which micro-organisms are spread on the contaminated site. The second approach, bio stimulation, makes use of the addition of nutrients to stimulate or continue bacteria to degrade the oil (Meyers *et al*, 1999).

Until now, addition of oil degrading bacteria to oil polluted sandy beaches has not been shown to be effective (Mearns, 1997).

Simon *et al* (1999) studies both techniques for their efficacy. The experiments were conducted using moderate heavy crude oil in a wetland. They observed no significant difference in the degradation rate between treatments and controls. In an earlier experiment bio stimulation proved to accelerate the degradation of saturated hydrocarbons and aromatics. In a second experiment with bio augmentation, the addition of nutrients alone showed no significant increase in biodegradation rate of saturated hydrocarbons (0,024/day) compared to the control (0,020/day). Adding two different products of bacteria resulted in a degradation rate of 0,030/day) and 0,019/day respectively. For aromatics the results were even less promising. The degradation rate in the control was 0,015/day, with bio stimulation 0,013/day and with bio augmentation 0,017 and 0,016/day.

In the laboratory, Aldrett and his workers (1997) found that four out of thirteen bio augmentation products increased biodegradation rates after 28 days compared to bio stimulation. Some other products, however, seemed to decrease degradation rates, maybe because of competition between added and natural bacteria.

Lee *et al* (1993) found that permeability of slow dissolving bio stimulation products (sulfur coated urea) decreased at temperatures below 15 °C, resulting in a reduction of released nutrients. At these temperatures the use of soluble inorganic fertilizers is advised.

An extended bioremediation project following the Exxon Valdez disaster (1989) showed that adding nutrients increased degradation of oil (USEPA, 1990). Nutrient containing briquettes did not show to be effective but a liquid oleophilic did. In another experiment a combination of both were compared to a dissolved mixture of inorganic nitrogen and phosphorus which was added with a sprinkler system. Both treatments proved to be effective. Still, there were some restrains about the environmental benefit of bioremediation products. The oleophilic fertilizer was thought to be toxic and could stimulate eutrophication.

Chianelli and his colleagues (1992) conducted laboratory studies and field tests and proved that:

- most marine environments already contain oil degrading bacteria;
- adding micro-organisms is in general unnecessary and possibly even ineffective;
- in case of nutrient limitation adding nutrients to beaches can enhance biodegradation rates significantly.

Even a weathered crude oil emulsion was shown to be degraded at higher speed using fertilizer (Halmö, 1985). In this study the oil soluble fertilizer (urea) was at least as effective as the water soluble fertilizer (ammonia and nitrate). Within a urea all paraffin had been broken down.

Experiments with light crude oil (condensate) adding fertilizer directly after an oil spill on a sandy beach did not result in a higher degradation rate (Lee & Levy, 1987). It was suggested that only the presence of oil degrading organisms is not sufficient to break down the oil. Micro-organisms will first have to adapt to the new circumstances. The crude oil in this experiment contained a high number of aromatic hydrocarbons with low molecular weight (like naphthalene) which can cause toxic inhibition to the present or added bacteria. After about ten days an exponential growth was found, because approx. 50% of the light components had disappeared. In contrary to the experiments in the laboratory no significant increase in degradation rate was found during the first 60 days of the experiments. Analysis showed that the oleophilic fertilizer in the field experiment had disappeared already after the first two days. These results suggest that repeated addition of nutrients after the adaptation time of the micro-organisms may increase biodegradation of condensate on sandy beaches.

Lee and Levy (1989) showed in an experiment that Inipol EAP22 increased the number of bacteria, in contrary to the agricultural fertilizer. The effect on oil degradation was the other way around, probably because the bacteria in the Inipol-treated plots grew on the nutrients in Inipol, instead of on the oil components. This is called 'di-auxic growth response'. Repeated addition of the agricultural fertilizer increased oil degradation. An advantage of this method is that it is cheap compared to the commercial product, easy to obtain, easy to dose with standard agricultural tools and it can be used even at low temperatures.

Venosa *et al* (1991) tested eleven commercial bioremediation products on their efficacy and found that only two were suitable for bioremediation of an oil pollution in the field. By sterilizing the products before testing they proved that natural present micro-organisms were responsible for the biodegradation.

To solve the problem of loosing inorganic fertilizer due to washing off, several oil attracting fertilizers have been developed during the last two decades. Lee and Levy (1991) suggest that repeated dosages of standard inorganic fertilizer can be just as effective. This was also concluded in a study using waxy oils on sandy beaches during the Valdez experiments.

Bioremediation at low concentrations of oil in the sediment (0,3% v/v) was hardly an option, whereas normal fertilizer is effective at higher concentrations (3% v/v).

In laboratory studies a variety of fertilizers (isobutyraledhyde diurea IBDU, urea formaldehyde, magnesium ammonia phosphate and an oil attracting agent) have been tested on durability, stability and application procedures under static and dynamic circumstances (Safferman, 1991). It seemed that briquettes or granulated material were easy to use on low energy beaches.

At high energy beaches the fertilizers will have to be embedded to ensure its effectiveness. Results from these experiments indicated that IBDU briquettes were most effective on tested parameters.

An experiment of Tabak and his workers (1991) suggest that a combination of Inipol EAP22 and a synthetic OECD medium with soluble N and P were most effective in increasing the biodegradation of weathered crude oil. Both agents also proved to be effective when separately used. No toxic effects on micro-organisms were observed at oil concentrations up to 10 g/l. Use was made, however, of clean sand from the Valdez accidental area (Alaska). It is possible that in this case present micro-organisms were very well adapted to contamination.

Nadeau *et al* (1993) didn't succeed in determining whether a bioremediation product was effective. He did find loss of oil 25 days after the second dosage (8 days after the start of the experiment) but there was no difference between treated and untreated plots.

Lee *et al* (1997) demonstrated higher biodegradation rates in laboratory studies and field mesocosm experiments for both bio augmentation and bio stimulation products compared to the controls and/or inorganic fertilizer treated plots. However, a 129 days long field experiment proved that addition of inorganic fertilizer was most effective in increasing biodegradation.

Researchers Bragg and Owens (1995) showed that natural interaction between fine mineral parts in seawater and oil residues can remove the latter from low energy beaches. It is assumed that this process stands at the basis for the formation of stable droplets by which it is easier dispersed. It is possible, however, that the oil is only moved between compartments, resulting in higher concentrations in the water or in the sediment after sedimentation.

3 The Bioremediation Process

Lee *et al* (1995) and Mearns *et al* (1997) carried out several bioremediation experiments on oil contaminated coasts. From the results of these studies the following steps are recommended for bioremediation on a sandy beach. Initiatives for bioremediation can be taken already before actual oil contamination takes place.

STEP 1: Judging the usefulness of bioremediation in the specific situation
The first question which has to be answered is how quickly the oil has to be removed. When a quick response is required (within some days) than bioremediation is not an option. Because of the initial toxicity of the oil, bioremediation has a start-up phase of several days. Time necessary to reduce degradable components for 50% can take up from a couple of weeks to several weeks. If this limitation is acceptable, the next question is which factors are limiting for natural degradation. This can be the thickness of the oil layer, conditions (wave energy, tide), available nutrients, degradable organisms and oxygen or a combination of these. When thick layers of tar are formed, these have to be removed first. Availability of nutrients is sufficient when a continuous concentration of 1-2 mg O2 per liter pore water is available. When the oxygen concentration is far below this number, the planning process will have to take into account the amount of fertilizer needed for an optimal degradation (1-2 mg N/l). When there is no certainty about the presence and/or activity of oil degrading bacteria in the area, this will have to be examined first.

STEP 2: Planning and monitoring of treatment
When is decided that bioremediation is an useful option, treatment can be planned and executed. Planning means determining the bioremediation product and amount, an estimate of the duration of treatment and determining the need to monitor. In general, nitrogen is the limiting factor in moderate marine coastal areas. This has to be examined in step 1. Next the properties and costs of various bioremediation products have to be evaluated. For testing bioremediation agents the EPA/NETAC developed a tier approach (Lee *et al*, 1997).

Base tier	*Basic information* Product safety, regulatory status, acceptability of its chemical and biological components

Tier I	*Focus on feasibility* Contains information on proposed use, potential effectiveness and safety certification.
Tier II	*Laboratory scale data* Product efficacy and safety data obtained with a standard 'shake flask' testing protocol
Tier III	*Microcosm-simulated field test demonstration* Simulation of environmental scenarios (e.g. open water, marshes, beaches, etc.)
Tier IV	*Field demonstration* Product efficacy and safety in a field application setting

Treatment must be stopped when it is not effective or when sufficient amounts of hydrocarbons have been degraded. This decisions have to be made by monitoring these parameters. To be able to make the decision to stop, endpoints must be set before treatment starts. Also, monitoring can indicate if the treatment has negative effects. Analysis should focus on nutrient levels, degradation rate, toxicity and biodiversity.

STEP 3: Organizing material and people

STEP 4: Terminating treatment
Treatment has to be stopped when monitoring proves it not to be effective or when sufficient amounts of hydrocarbons have been degraded. In step 2 criteria to make this decision should have been set out.

4 Effectiveness Of Treatment Techniques

4.1 Effectiveness in general
Addition of micro-organisms (bio augmentation) in general does not seem to be very effective (Chianelli *et al*, 1992; Mearns, 1997; Aldrett *et al*, 1997; Venosa *et al*, 1991; Lee *et al*, 1997; Mueller *et al*, 1999). The same can be said about the addition of nutrients on open water (Chianelli *et al*, 1992), although simulation tests have shown otherwise (Meyers *et al*, 1999). Traditional agricultural fertilizer (soluble inorganic agents) can be used for bioremediation purposes (USEPA, 1990; Lee & Levy, 1989; Lee & Levy, 1991; Halmö, 1985; Tabak *et al*, 1991; Mueller *et al*, 1999). Only one experiment gave negative results (Simon *et al*, 1999). Special developed oleophilic agents are not always effective (Lee & Levy, 1989; Lee & Levy, 1987). A number of experiments have been carried out to investigate the use of fine mineral parts for bioremediation techniques (Lee *et al*, 1997; Bragg & Owens, 1995; Bergueiro *et al*, 1999). In all cases the use of these particles proved to be effective.

Until now, toxic effects have been found using bioremediation agents in field studies (USEPA, 1990; Mendelssohn *et al*, 1995; Mueller *et al*, 1999). Laboratory test, however, did show toxic effects, probably as a result of high levels of nutrients caused by repeated addition of bio stimulation products. Results from a large bioremediation project in Canada show that addition of bioremediation agents did not induce algal blooms (USEPA, 1990). The first days after an oil spill, oil components can induce toxic inhibition on micro-organisms (Lee & Levy, 1987) thereby limiting the effectiveness of bioremediation during the first days after the spill. Tabak *et al* (1991) got opposite results from laboratory studies. One possible explanation for this fact is adaptation of micro-organisms, because in these experiments use was made of oil contaminated sand from the Exxon Valdez disaster area. This is the reason why it is important to determine microbial activity before the start of bioremediation.

Baker (1992) describes that physical disappearance of oil contamination (with light oil) on a sandy beach dominates the effects after direct treatment with fertilizer. These results are supported by results from a study carried out by Little *et al* (1993). They showed that for a high number of coastal areas the physical processes (wave action, tidal movement, evaporation) are the most important in the disappearance of oil contamination. From this it can be concluded that treatment with fertilizer only should take place on locations where these physical processes are not dominant.

4.2 Effectiveness On A High Energy Sandy Beach at the Dutch coastline
Results from a field experiment (Van der Veen & Koops, 2001) carried out in the winter of 2000-2001 indicate that biodegradation does take place, even when the oil is covered with a thick layer of sand (max. 65 cm). No additional effect on biodegradation was observed from the addition of a specialized beach cleaning product or slow release fertilizers. However, the disappearance of oil was higher in the treated plots compared to the controls. These results indicate that when there is time enough, natural attenuation of an oil contaminated high energy sandy beach is possible. In the Netherlands most sandy beaches are visited by numerous tourists during the summertime and therefore time in most cases will be too short for an extensive trial. In these cases, complete mechanical recovery is preferred and bioremediation for residual oil is not an option.

4.3 Uncertainties
Although several experiments have shown that the application of bioremediation agents proved to be effective, exact conditions in which bioremediation can effectively be applied are not yet well understood. The following questions remain unanswered:

- What factors determine effectiveness?
- What treatment is best suited in a specific situation?
- What are the effects of bioremediation on micro algae along the coast, on interstitial fauna and the local food chain?
- Does bioremediation increase the rate of recovery of a healthy coastal community?
- What advantage has a treatment, when it seems that biodegradation of oil can also take place in habitats which are not treated with fertilizer?

5 General Conclusions

In shallow coastal waters bioremediation is not an useful option, because other processes (evaporation and spreading) dominate over biodegradation.
Regions like the Wadden Sea have relative high dynamics and accommodate a lot of birds. Only in shoals which consist of coarse sand and which are well drained, oil can penetrate into deeper layers. Also bioturbation can help the oil penetrate deep into the sediment. Bioremediation is probably only an option in areas with coarse sand in which oxygen can diffuse well into the sediment. In these areas I is only useful in those areas above the waterline with high moist content. The Wadden Sea shows morphological similarities with the Voordelta. Application of bioremediation techniques is not useful on surface waters because nutrients are not limiting in these areas and application is difficult for practical reasons. When the oil is under control, mechanical removal is preferred. Also in the littoral zone where oxygen is limiting, bioremediation is not an useful option. In salt marshes other combating techniques are preferred, mainly because the use of heavy equipment can damage the vegetation seriously. Stimulation of biodegradation is possible, but with traditional agricultural fertilizer. In coastal areas and objects bioremediation is only an option when:

- residual oil removal is concerned;
- the location is sensitive or hard to reach for heavy equipment and therefore mechanical recovery is not an option;
- enough oxygen can penetrate the sediment (coarse sandy beaches, some salt marshes and shoals);
- the moist content in the sediment is sufficient (30-40 %);
- temperature is at least 5 °C;
- oil degrading micro-organisms are present;
- the area stays above the water line most of the time to let oxygen and nutrients penetrate the sediment.

In shallow freshwater areas bioremediation is not an useful option as oxygen concentrations are often too low. Nutrient concentrations will be sufficient in most cases.

Bioremediation in rivers and/or channels does not seem to be useful as well, because the movement of oil contamination will be several times faster than the oil degradation by bacteria.

For rocky shores in freshwater areas most oil will disappear because of wave action. To combat residual oil in between rocks, bioremediation could be useful.
On vegetation covered shorelines other oil combating techniques are preferred, because the use of heavy equipment can seriously harm the vegetation and push the oil deeper into the sediment. Stimulation of biodegradation is an option but only with traditional agricultural fertilizer.

Not covered shorelines with soft sediment are potential places where bioremediation could be useful. However, natural attenuation on these deserted places is preferred due to the lower costs. When oil needs to be removed quickly, the use of mechanical equipment should be considered.

For firm sandy shorelines bioremediation is not useful due to the insufficient oxygen penetration into the sediment. The addition of nutrients can seriously enhance negative environmental effects. Mechanical recovery is the best option if the oil is not washed off by wave action already.

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