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Biodegradability of diesel oil

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Abstract

In batch culture diesel oil was degraded rapidly, with a maximum growth rate (for a consortium of microorganisms) of 0.55 h^{-1}. The corresponding yield Y_{SX} was 0.1 Cmol/Cmol. In a continuous stirred tank reactor the maximum dilution rate was about 0.25 h^{-1}, with a yield of 0.3 Cmol/Cmol. With a residence time of 1 day 82% of the influent oil was degraded. In the batch reactor, of the mixture of linear and branched alkanes the linear alkanes were degraded fastest and with the highest yield. Only after most of the linear alkanes had disappeared were the branched alkanes consumed. In a CSTR a large part of the branched alkanes was not degraded.

Introduction

Oil contamination of soil and water is a widespread problem nowadays. Microbiological clean-up of this type of contamination can be advantageous, when compared to other remediation techniques (Blackburn et al. 1993). Before deciding to use microbes, it is necessary to determine (1) whether the contaminants can be mineralized (biodegradability), (2) how fast the mineralization can proceed (kinetics), and (3) how the previous two points depend on the type of operation: batch or continuous mode. In this paper we will discuss these three points with regard to dispersions of oil in water.

Oil is a complex mixture of hydrophobic components, such as aliphatic and mono- and polycyclic aromatic hydrocarbons. In general, the degradation rate of a linear alkane is much higher than of e.g. a polycyclic aromatic hydrocarbon (Ratledge 1978). Microbial degradation experiments are often performed with pure cultures growing on one substrate. Especially *n*-hexadecane has often been used (e.g. Wodzinski & Johnson 1968). In recent years several papers have been published concerning the degradation of crude oil (Amund & Akangbou 1993, Mille et al. 1991, Pirnik et al. 1974) or a refined fraction thereof. However, in none of these the oil was completely degraded. In this paper the microbial degradability of diesel oil, which is a distillate fraction of crude, is studied. Diesel fuel is one of the major pollutants of soil and ground water near petrol stations.

Diesel oil consists mostly of linear and branched alkanes. We want to compare bacterial growth on this complex substrate with growth on a single substrate to determine the degradation order of the components in the mixture, degradability of the mixture as a whole, and the difference in kinetic parameters between both kinds of substrate. *n*-Hexadecane was used as a reference for the degradation behaviour of diesel oil. Hexadecane was chosen because of its widespread use in experimental studies (e.g. Wodzinski & Johnson 1968). Much is already known of its degradation kinetics, and it can be degraded fast in water dispersion. Under these conditions a maximum growth rate has been measured (in batch experiments, at 30° C) of 0.2 h^{-1} (Wodzinksi & Johnson 1968), and a corresponding yield Y_{Sx} of about 0.5 Cmol biomass/Cmol hexadecane. Rohns (1992) has experimented with pentadecane in batch and found a μ_{max} of 0.6 h^{-1} and a corresponding yield of Y_{SX} = 0.2 Cmol biomass/Cmol pentadecane, at 30° C. He also experimented with continuous cultures and found the maximum growth rate to be lower than in batch: 0.3 h^{-1}. Several other research groups, used *n*-hexadecane and a pure culture of a yeast (Yoshida

& Yamane 1974, Blanch & Einsele 1973). They also found a significantly lower maximum growth rate in continuous culture, compared to batch.

In this paper the degradation of oil components is studied in batch and continuous cultures. From these experiments conclusions will be drawn concerning

- the biodegradability of diesel oil when compared to hexadecane, i.e. are some oil components more easily degraded than others and
- the best mode of operation when extrapolated to waste water treatment.

Materials and methods

Reactor operation

All degradation experiments were performed in two litres glass reactors. These were equipped with an air sparger at the bottom of the vessel. The amount of air fed to the reactor was controlled by a mass flow controller at 1 l/min. The medium used was composed of: 550 mg/l KH_2PO_4, 275 mg/l $MgSO_4 \cdot 7H_2O$, 275 mg/l $FeSO_4 \cdot 7H_2O$, 5.5 mg/l $ZnSO_4 \cdot 7H_2O$, 1.1 mg/l$CaCl_2 \cdot 2H_2O$, 2.75 mg/l $MnCl_2 \cdot 4H_2O$, 1.1 mg/l $CuSO_4 \cdot 5H_2O$, 1.1 mg/l $CoSO_4 \cdot 7H_2O$, 27.5 mg/l NaCl, 27.5 mg/l KCl, 2200 mg/l NH_4Cl. The reactor contents were stirred with an impeller at 900 rpm. Temperature was kept at 30° C with a thermostat that circulated water either through the mantle of the reactor, or through a metal heat exchanger inside the reactor. pH was kept between 6.5 and 7.5 with a pH-electrode, coupled to a pH-controller. This controller regulated two peristaltic pumps, one for addition of 1 N sulphuric acid, the other for addition of 2 N NaOH solution.

Batch experiments started with 1.5 l of medium, 5 g of hexadecane (analytical grade) or diesel oil (from a Texaco petrol station) were used as sole carbon and energy source for the microorganisms. The reactor was inoculated with 10 ml (about 10 mg biomass) of an undefined mixed culture of oil degrading microorganisms. This culture originated from oil polluted harbor sludge, and was grown on diesel oil in a continuous soil sludge reactor for two years.

In continuous mode a CSTR (continuous stirred tank reactor) was used, with hydraulic residence times between 4 and 24 hours. The residence times were set by changing the influent flow of the medium. The oil was added separately as a semi-continuous flow. Several concentration ratios of oil and medium in the influent were used (Table 2). In the continuous experiments the reactor contents of the previous steady-state were used to start the next dilution rate. When needed, an extra inoculum, as described above, was added. This happened only once when wash out of the entire population had occurred due to a much too large dilution rate. To minimize the influence of further adaptation or change in population the experiments were performed in random order.

Analyses

The biomass concentration in the reactor was determined by taking 10 ml of the stirred suspension and determining the mass, after drying for 24 hours at 105° C. Water and oil were removed during this drying period.

The oil concentration in the water phase was determined by first increasing the pH of the suspension to 13 with NaOH-pellets, to prevent interference from oil metabolites such as fatty acids during the analysis. 500 ml of the suspension was extracted three times with 50 ml distilled diethylether. 1 ml of a 32.050 g/l solution of hexadec-1-ene was added as internal standard (IS). To determine whether any metabolites of oil degradation accumulated in the medium, the extracted water was acidified to pH < 1 by addition of concentrated hydrochloric acid (Pirnik et al. 1974). The acidified water was extracted with ether in the same manner as described for the alkaline water. The fatty acids in the 'acid' extract were methylated by passing diazomethane through the extract for 5 minutes. Analysis of all extracts was performed on a gas chromatograph (GC), fitted with a 25 m HP-1 column and a flame ionization detector. The temperature profile in the GC was: Start temperature 60° C, which was kept for 5 minutes, then a temperature ramp of 5° C/min till 300° C. This final temperature was kept for 10 minutes. If the oil concentration was found to be low, the extract was concentrated to 1 ml by evaporation of ether at 45° C. The concentrated extract was subjected to the same analysis procedure as before. The total area of the chromatogram between 5 and 63 minutes and the area of the peak of the IS were used to determine the amount of mineral oil in the extract. This is equal to the amount of oil in the 500 ml of water before extraction. A calibration curve was used to allow for different response factors for oil and IS.

The oil components that were stripped into the gas phase were quantified by adsorption. During the first 48 hours in a batch experiment, or for 48 hours during steady-state in a continuous experiment, 1 l/h of the

Table 1. Yields from the batch experiments.

Time (h)	Hexadecane	Diesel oil		
	Y_{SX} (Cmol/Cmol)	Y_{SO} (mol/Cmol)	Y_{Sx} (Cmol/Cmol)	Y_{SO} (mol/Cmol)
40	0.33	0.66	0.10	1.1 ± 0.2
80 (C16)	0.4	1.4		
120 (diesel)			0.1	1.6 ± 0.2

Table 2. Concentrations in the continuous culture experiments.

Dilution rate (h^{-1})	Influent oil concentration (Cmmol/l)	Effluent biomass concentration (Cmmol/l)	Effluent oil concentration (Cmmol/l)	Conversion (%)
0.04	396	35	70	82
0.08	323	44	140	57
0.10	285	37	110	61
0.10	265	44	100	62
0.14	98	12	35	64
0.14	281	36	110	61
0.18	282	47	85	70
0.21	90	6.5	19	79
0.25	90	4.3	25	72

off-gas was passed through a glass tube, containing an adsorption material (2 g of Tenax TA). The adsorption material was extracted three times with 10 ml distilled diethylether. The extract was analyzed according to the same protocol as used for the water phase extracts.

Nitrate and nitrite concentrations were monitored with Merckoquant 10 020 test strips from Merck, to check whether a significant amount of oxygen was used for nitrification.

Oxygen transfer to the reactor was measured by a paramagnetic O_2-analyzer. Carbon dioxide production was measured with an infrared analyzer. Some of the oil components were stripped into the gas phase and, due the very high infra red absorption of alkanes, interfered with the carbon dioxide measurements. The carbon dioxide measurements could therefore not be used quantitatively.

Results

In the following calculations it is assumed that both the hexadecane and the diesel oil had a molecular mass, normalized to the amount of carbon atoms, of M = 14 g/Cmol. This corresponds to a composition of CH_2. The biomass was assumed to have a normalized molecular mass of M = 24.6 g/Cmol, corresponding to an average composition formula of $CH_{1.8}O_{0.5}N_{0.2}$ (Roels 1983a).

Batch experiments

In Fig. 1 the oxygen uptake rate (OUR) is shown for the degradation of hexadecane and of diesel oil in a batch reactor. It is apparent in both cases that, after a lag phase, the uptake rate shows an exponential increase in the first 18 hours. This corresponds to an exponential growth of the microorganisms. After this period a decrease of the OUR occurs. All the maxima occurred reproducibly in the experiments.

After approximately 40 hours the hexadecane concentration in the water phase was below the detection limit (< 10[PI]AM[7] Cmol/l). The diesel oil concentration after 22 h was 0.20 Cmol/l (this corresponds to 15% conversion), after 40 h it was 0.013 Cmol/l (95% conversion). Analysis of the water phase after

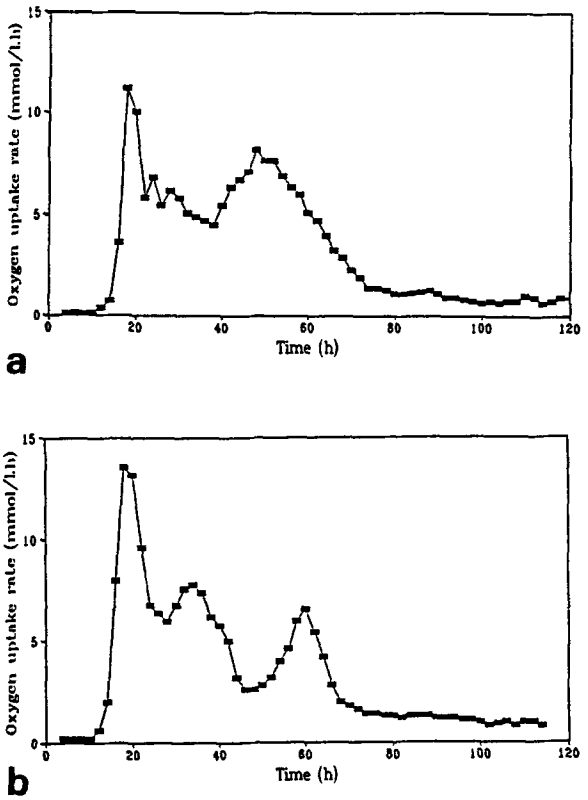

Fig. 1. Oxygen uptake rate for the degradation of (a) hexadecane and (b) diesel oil in a batch reactor.

120 hours showed no detectable amount of diesel oil. The detection limit for diesel oil is approximately 10^{-6} Cmol/l.

Assuming Monod kinetics for the growth in the exponential phase, and assuming furthermore that $C_S >> K_S$ (according to Goldsmith & Balderson 1990): $K_S \approx 0.006$ Cmol/l), integration of the growth equation for a batch reactor yields:

$$ln(C_{X(t)}) = ln(C_{X(0)}) + \mu_{max} t \qquad (1)$$

If a constant stoichiometry for the degradation in the exponential growth phase can be assumed, then the specific oxygen consumption rate is constant. The oxygen uptake rate is then proportional to the biomass concentration and equation 1 can be changed into:

$$ln(r_{O(t)}) = ln(r_{O(0)}) + \mu_{max} t \qquad (2)$$

Figure 2 shows $\ln(r_{O(t)})$ as a function of time in the batch experiments. From the slope a maximum growth rate of $\mu_{max} = 0.55\ h^{-1}$ for diesel oil, and of $\mu_{max} = 0.56\ h^{-1}$ for hexadecane was found.

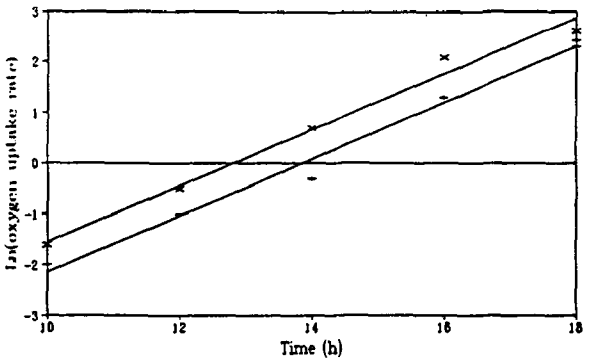

Fig. 2. Determination of the maximum growth rate from the batch experiments. The +-markers represent the hexadecane, the x-markers the diesel oil.

The yields and oxygen/substrate consumption ratios for the batch experiments are summarized in Table 1.

With the yields and ratios at the end of the experiments, the stoichiometry of both degradation experiments can be described, within the error margins, by the macroscopic equation:

$$Y_{SN}NH_3 + CH_2 + Y_{SO}O_2 \rightarrow Y_{SX}CH_{1.8}O_{0.5}N_{0.2} + Y_{SC}CO_2 + Y_{SW}H_2O \qquad (3)$$

where CH_2 represents hexadecane or oil and $CH_{1.8}O_{0.5}N_{0.2}$ the biomass.

In Fig. 3 extracts of the water phase at the start of the batch experiment (Fig. 3a) and after the first maximum in the OUR (Fig. 3b) are shown, for diesel. In Fig. 3a the linear alkanes from undecane (C11) up to octacosane (C28) are indicated with *. The rest of the peaks, as well as the bump, comprise mainly of the branched alkanes. The bump consists of unresolved components. A slower increase of the oven temperature of the GC would resolve more components.

A comparison shows that in the first 22 hours, 90% of the linear and 2% of the branched alkanes are consumed. The chromatogram of the extract of the water phase after 40 h showed no significant peaks, apart from the IS. After concentration a bumpy baseline and several small peaks were observed, some of which could be attributed to the ether or to phtalates from the ABR seal of the vials. After 120 h the diesel concentration was below the detection limit, confirming that diesel oil can be completely mineralized by microorganisms, in batch.

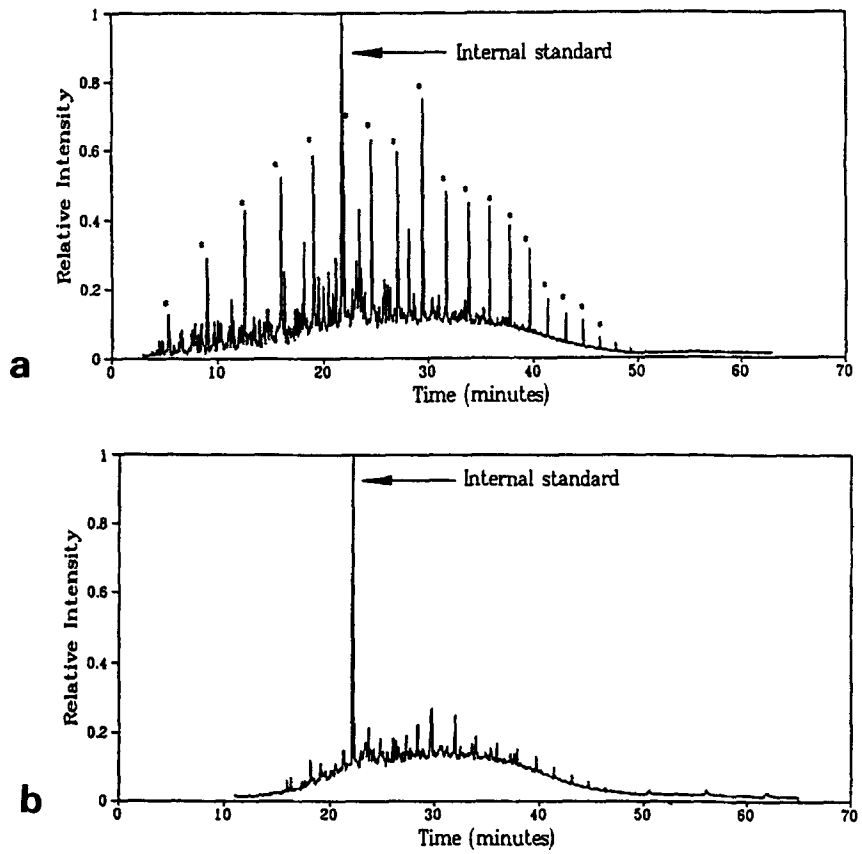

Fig. 3. Extract of the water phase at the start of the batch experiment with diesel oil (a) and after 22 h (b). The markers in Fig. 3a indicate the linear alkanes.

Continuous experiments

Several continuous experiments were performed with dilution rates between 0.04 and 0.25 h^{-1}, corresponding to liquid residence times between 24 and 4 hours. In Table 2 the diesel oil and the biomass concentrations in the continuous experiments are shown.

For a dilution rate of D = 0.10 h^{-1} two identical experiments were performed. The effluent biomass and oil concentrations were the same, as expected. For a dilution rate of D = 0.14 h^{-1} two experiments with different substrate influent concentrations were performed. According to theory this should have no effect on the effluent oil concentration, or on the specific substrate consumption rate. The specific substrate consumption rates were the same, as expected. However, the measured effluent concentrations in both experiments were different.

The linear Herbert-Pirt relation for substrate conversion was used to evaluate the results of the continuous experiments:

$$q_s = \frac{\mu}{Y_{sx,max}} + m_s \qquad (4)$$

From the results for low dilution rates (D < 0.2 h^{-1}), using equation 4, as $Y_{SX,\ max}$ of 0.3 Cmol biomass/Cmol diesel oil and a maintenance coefficient of m_S - 0.2 Cmol oil/Cmol biomass/h was obtained (Fig. 4). In Fig. 4 the specific oxygen uptake rate is shown as well.

In Fig. 5 a chromatogram of a water phase extract of the experiment with a dilution rate of D = 0.18 h^{-1} is shown. When compared to the influent oil (chromatogram similar to Fig. 3a), it is seen that the linear alkanes are consumed preferentially. The branched alkanes comprising the UCM (unresolved complex mixture) (Killops & Al-Juboori 1990, Gough & Row-

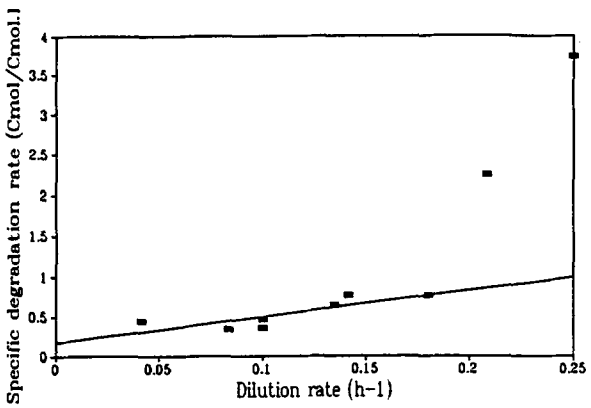

Fig. 4. Plot of the specific substrate uptake rate as a function of the dilution rate in continuous cultures of diesel oil. The drawn line represents the Herbert-Pirt relation.

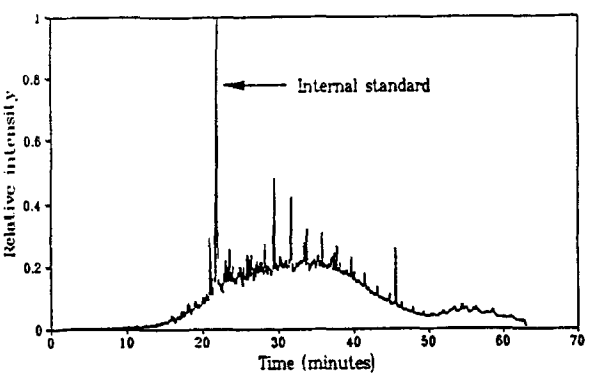

Fig. 5. Chromatogram of the extract of the effluent of a continuous experiment (D = 0.18 h^{-1}).

land 1990) are left. Similar chromatograms were found for the other dilution rates.

Strip effect

In order to check that the decrease of the hexadecane or diesel oil concentration in the reactor was due to microbial degradation and not to stripping, an adsorption column was placed in the off gas from the reactor. Figure 6 shows the chromatogram of the extract of the off-gas adsorption column for a typical continuous experiment, with a dilution rate of D = 0.18 h^{-1}. It is clear that mainly the lighter hydrocarbons are present. This is as expected because of the higher vapour pressure of these components. The total amount of oil, that is stripped

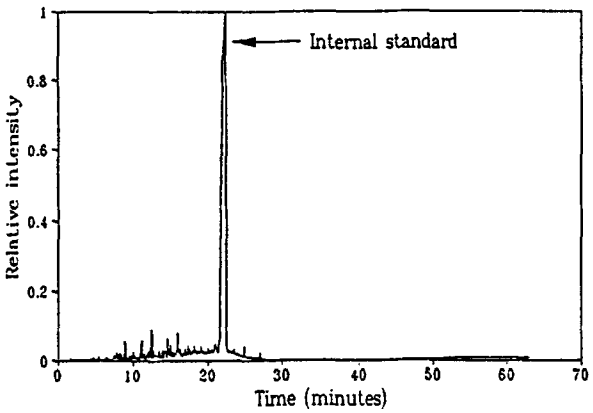

Fig. 6. Chromatogram of the extract of the Tenax in the off-gas of a continuous experiment (D = 0.18 h^{-1}).

during the experiment, is about 0.01 Cmol/day, or less than 2% of the influent.

In the batch experiments with diesel oil the extracts showed similar chromatograms; mainly lower boiling components were stripped and the total amount of oil components that were stripped was very low. In the batch experiments with hexadecane the total amount of hexadecane that was stripped was negligible, when compared to the initial amount in the reactor.

Discussion

Maxima in the oxygen uptake rates in the batch experiments

Diesel oil exhibits a somewhat different degradation behaviour compared to hexadecane (Fig. 1). However, both show more than one maximum in the OUR. The first maximum represents the oxidation of linear alkanes. In both the hexadecane and the diesel experiments linear alkanes were present. After about 22 h, the n-alkanes are mostly degraded, as is clear from Fig. 3a and 3b: Mainly the 'grass', comprising largely of linear alkanes, has disappeared. At the start of the diesel experiment, about 14% of the oil mixture consisted of linear alkanes. After 22 h this was reduced to less than 1.5%. The unresolved complex mixture (UCM), the bump in the chromatogram, remained. This UCM consists mainly of branched alkanes (Killops & Al-Juboori 1990, Gough & Rowland 1990). The branched alkanes are oxidized during the second maximum in the OUR of the diesel experiment. Due

to the absence of branched alkanes in the hexadecane experiment, no maximum occurs there. After 40 h the extract of the water phase of the diesel experiment showed that almost all oil has disappeared. This is also the case for the hexadecane experiment. Therefore the last maximum in the OUR in both experiments can not be explained by oxidation of oil components.

Metabolites of alkanes, such as alkanoates (Watkinson & Morgan 1990), could have accumulated in the water phase during the experiments. Therefore, for analysis of the oil concentration in the water phase the pH was raised to above 13. Metabolites like fatty acids will remain in the water phase. After this alkaline extraction, the pH was lowered to below pH 1 (Pirnik et al. 1974), and extraction was repeated. In the extracts of the acidified water phase (taken after 40 hours degradation time) no components could be detected. So no metabolites had accumulated during the degradation. Therefore the last maximum cannot be explained by oxidation of metabolites in the water phase.

No significant decrease in the biomass concentration was found between 40 and 120 h. Therefore the maximum in the OUR during this time is not caused by oxidation of biomass. It remains possible that this maximum is the product of oxidation of a storage component (Ratledge 1978), formed from excess oil during the first 40 h. This hypothesis is supported by the increasing ratio Y_{SO} in the batch experiments, between 40 and 120 h, without a significant decrease in the yield Y_{SX}: Part of the biomass measured after 40 h was storage polymer, which is partly turned over into biomass and partly mineralized.

Another explanation for the last maximum in the OUR for both hexadecane and diesel, is the degradation of emulsifier, formed during the first 40 h to enhance the availability of the oil components. With the extraction procedure followed here, an emulsifier would not have been detected.

Kinetic constants from the continuous experiments

The specific substrate consumption rate for the series of continuous experiments is plotted against the dilution rate (Fig. 4), according to equation 4. From the linear part of the graph the yield, Y_{SX}, and the maintenance coefficient, m_S, are calculated to be: Y_{SX} = 0.3 ± 0.05 Cmol biomass/Cmol oil and m_S = 0.2 ± 0.1 Cmol oil/Cmol biomass/h. This yield compares to the values found in other studies in continuous culture, Y_{SX} = 0.5 Cmol/Cmol (Wodzinski & Johnson 1968) and Y_{SX} = 0.2 Cmol/Cmol (Rohns 1992). The maintenance coefficient is somewhat higher than expected: Up till now, maintenance coefficients between 0.015 and 0.14 Cmol/Cmol/h were found (Roels 1983b) for growth on *n*-alkanes.

Discrepancy between the growth rates from different operations

In the batch experiment the maximum growth rate was calculated to be 0.55 h^{-1} for diesel oil. So it could be expected that the critical dilution rate in a continuous experiment is about 0.55 h^{-1}. In other research (Rohns 1992; Blanch & Einsele 1973) however, using pure cultures of microorganisms and one component as substrate, it was found that in continuous culture the maximum growth rate would be lower than calculated from batch experiments. In this study, using diesel oil as substrate and a consortium of microorganisms, this was found too: Table 2 shows that beyond D = 0.18 h^{-1}, with increasing dilution rate, the biomass concentration is markedly decreasing. This results in a decrease of the yield Y_{SX} with increasing dilution rates, for these high rates. This in turn results in an increase in the specific substrate degradation rate (in agreement with literature (Rohns 1992)), as is shown in Fig. 4.

Rohns (1992) explained both phenomena by postulating that for the microorganisms to be able to use the oil components and due to the extreme low solubility of the oil components in water an amount of emulsifier, exceeding the critical micelle concentration (CMC), must be present. The emulsifier will effect a higher concentration of available oil. The emulsifier is produced from the substrate. This means that the amount of substrate that can be used for assimilation is less than without emulsifier production. Therefore the yield (Y_{SX}) will be lower and the specific substrate consumption rate, q_S, will be higher than would be found when no emulsifier is needed. Production of emulsifier by microorganisms is thought to enhance the dispersion of growth substrate into oil-in-water emulsions to increase the interfacial area and thereby enhance the availability of the substrate. According to Hommel (1990) the production of emulsifier occurs during the late exponential and during the stationary growth phase of the microorganisms. This would mean that in the determination of the growth rate in the batch experiments this production has not started yet. In a CSTR the available substrate concentration is always low (equal to the effluent concentration). Therefore

emulsifier production will occur at every dilution rate. Consequently the maximum growth rate determined in the continuous experiments will be lower than for the batch experiments.

In our experiments no analysis for the detection of emulsifier was performed. So the hypothesis of Rohns can not be checked here. However, for many microorganisms it is known that they produce surface active components that enhance the solubilization of nonpolar components (Cooper & Zajic 1980, Georgiou et al. 1992).

Degradation order of the oil components

Comparison of Fig. 3a and 3b clearly shows that during the first 22 h in batch the linear alkanes in the diesel oil are degraded preferentially: about 90% of the linear and approximately 2% of the other components have disappeared. This is in agreement with Pirnik et al. (1974), who found that pristane is not degraded in the presence of hexadecane. Only after almost all the hexadecane had been degraded was pristane degradation observed. According to Pirnik et al. this could be effected by diauxie. Additionally, the rate of degradation of pristane was approximately half as fast as that of hexadecane.

In continuous culture, the chromatogram of the extract of the water phase at $D = 0.18\ h^{-1}$ (Fig. 5) also shows a preferential decrease of the 'grass', comprising the *n*-alkanes, when compared to the chromatogram of the pure oil (cf. Fig. 3a). This is comparable to the first 22 hours in batch. This indicates that the lag phase for degradation of branched alkanes in the presence of linear alkanes is not caused by diauxie, but more probably by a higher affinity of the linear alkanes for one or more of the enzyme systems involved.

A comparison of yields from literature shows that for linear alkanes the yield is higher than for branched alkanes: $Y^S{}_X = 0.2$–0.5 Cmol/Cmol for linear alkanes (Wodzinski & Johnson 1968, Rohns 1992) and $Y_{SX} = 0.05$ for branched alkanes (i.c. pristane) (Rohns 1992). Comparing these values with the yields for both batch experiments, the yield on hexadecane compares with the yields on linear alkanes: $Y_{SX} = 0.3$ Cmol/Cmol, after 40 h. The yield on diesel oil is lower: $Y_{SX} = 0.1$ Cmol/Cmol, after 40 h. This could be explained by a combined degradation of the linear alkanes, with a yield of $Y_{SX} = 0.3$ Cmol/Cmol, and of the branched alkanes with a yield of $Y_{SX} = 0.05$ Cmol/Cmol. The sum of the areas of the linear alkanes is calculated to be between 11 and 18% of the total area of the diesel oil. Assuming 14% of the diesel consists of linear alkanes, an overall yield in the degradation of diesel oil of $Y_{SX} = 0.1$ Cmol/Cmol can be calculated. This indicates that the hypothesis might be correct. In the first 22 h of the diesel degradation experiment in batch, 90% of the linear alkanes and 2% of the branched alkanes are degraded. This corresponds to about 14.5% of the initial amount of oil, furthermore confirming the hypothesis.

Operation of a reactor for the degradation of oil sludge

In batch the diesel oil is degraded completely, within 120 h. Therefore it is likely that, in continuous culture, the branched alkanes comprising the unresolved complex mixture can be degraded in a later stadium: The effluent of the continuous stirred tank reactor (CSTR) will be degraded further in a following reactor. Because the amount of oil in the effluent of the CSTR is still high (Table 2), a possible way to lower the effluent concentration of oil in continuous mode is to use several CSTRs in a series or a plug flow reactor (PFR).

In practice this would mean for the use of bioreactors in the remediation of oil pollution that degradation must be performed in a PFR. If needed, inoculation can take place through recirculation of a small amount of effluent. In this way the amount of substrate at the start is high enough for an exponential increase in biomass.

Conclusions

In batch culture diesel oil is degraded rapidly, with a maximum growth rate (for a consortium of microorganisms) of $0.55\ h^{-1}$. The corresponding yield Y_{SX} was 0.1 Cmol/Cmol. In a continuous stirred tank reactor the maximum growth rate is about 0.25 h[PI]AM1, with a yield of 0.3 Cmol/Cmol. With a residence time of 1 day 82% of the influent oil was degraded. In a mixture of linear and branched alkanes the linear alkanes are degraded fastest and with the highest yield. After most of these alkanes have disappeared the branched alkanes are consumed. In a CSTR a large part of the branched alkanes is not degraded. Microbial remediation of oil polluted water should therefore be performed in a batch or in a plug flow reactor. This results in the highest degradation rate and most complete removal of the oil.

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Symbols

D	=	Dilution rate in a continuous reactor, [h^{-1}]
m_S	=	Maintenance coefficient, [Cmol oil/Cmol DW/h]
$r_{O(0)}$	=	Oxygen uptake rate at time 0, [mol O_2/l/h]
$r_{O(t)}$	=	Oxygen uptake rate at time t, [mol O_2/l/h]
q_S	=	Specific substrate consumption rate, [Cmol oil/Cmol DW/h]
Y_{SC}	=	Ratio between carbon dioxide production and substrate consumption rates, [mol/Cmol]
Y_{SN}	=	Ratio between ammonium and substrate consumption rates, [mol/Cmol]
Y_{SO}	=	Ratio between oxygen and substrate consumption rates, [mol/Cmol]
Y_{SP}	=	Yield of product on substrate, [Cmol product/Cmol substrate]
Y_{SW}	=	Ratio of water production and substrate consumption rates, [mol/Cmol]
Y_{SX}	=	Yield of biomass on substrate, [Cmol DW/Cmol substrate]
Y_{SXmax}	=	Maximum yield of biomass on substrate, [Cmol DW/Cmol substrate]
μ_{max}	=	Maximum growth rate, [h^{-1}]

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