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REVIEW

Kinetics of biodegradation of diesel fuel by enriched microbial consortia from polluted soils

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Abstract Three microbial consortia were isolated from three polluted soils located at an oil refinery and acclimated to grow on diesel fuel as the sole carbon source. Batch experiments were then conducted with the three consortia to study the kinetics of diesel biodegradation. The effects of temperature (25, 30 and 35 °C) and diesel concentration (0.5, 1 and 3 %) on the biodegradation of diesel were analysed. Several species were identified in the acclimated microbial consortia, and some of them appeared in more than one consortium. Thermal inhibition was observed at 35 °C. In the rest of experiments, over 80 % of the substrate was degraded after 40 h of treatment. These results proved the good feasibility of using the polluted sites as sources of mixed consortia for hydrocarbon degradation. However, diesel degradation efficiencies and rates were very similar, suggesting that the acclimation process produced mixed consortia with very similar characteristics; in this context, origin of the soil sample was not a decisive factor. A simple Monod-type kinetic model was used to simulate the biodegradation process, and accurate results were obtained. The μ_{max} values were between 0.17 and 0.34 h^{-1}. The results of this study revealed that the consortia can function at high concentrations of hydrocarbons without any sign of growth inhibition, which is important for the design of bioreactors for wastewater treatment with high concentrations of fuel.

Keywords Biological treatment · Growth kinetics · Hydrocarbon · Soil pollution

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Introduction

Currently, petroleum hydrocarbons, a major source of energy, are used to satisfy the demands of everyday life. A direct impact of the use of petroleum products is the widespread contamination of soil and water that occurs during the production, processing and distribution of these substances. Technologies based on physical, chemical or biological principles are commonly used to remediate hydrocarbon-polluted soils (Bhandari et al. 2007). Amongst technologies available, bioremediation, the use of micro-organisms to degrade environmental contaminants, is an attractive approach (Das and Chandran 2011; Juwarkar et al. 2010).

The effects of various factors should be evaluated to determine if biological treatment of a contaminated soil is feasible, and to enhance the effectiveness of bioremediation. Researchers have identified several important factors that affect the efficiencies and rates of biotransformation of hydrocarbons in soils, including the temperature, pollutants concentration, pH, type of microorganism, bioavailability of contaminants, oxygen and nutrients in the medium (Boopathy 2000; Iqbal et al. 2007; Kwapisz et al. 2008; Sabaté et al. 2004; Torres et al. 2005).

It is hypothesised that microbial communities present in old polluted sites or brownfields are typically dominated by bacteria capable of surviving toxic environments and using the pollutant as a substrate for growth (Macnaughton et al.

1999). Maila et al. (2005) demonstrated that the type and concentration of pollutants, rather than the geographical origin of the soil, may be more important in determining the functional diversity and species diversity of such bacterial communities. Because individual bacterial monocultures can metabolise a limited range of hydrocarbon substrates (Britton 1984), a mixed bacterial consortium with broad enzymatic capacities may be more efficient for remediation, and the advantages of using mixed cultures instead of pure cultures in biodegradation processes, such as higher tolerance to temperature, pH and salinity, and higher capacity of produce biosurfactants or extracellular surface-active products, have been amply demonstrated (Boopathy 2000).

According to this hypothesis, polluted sites could be the primary source of pollutant-degrading consortia for the biodegradation of hydrocarbons (Margesin and Schinner 2001). Moreover, microorganisms present in these sites could be selected by controlling the adequate environmental factors, such as pH, salinity and organic matter content (Al-Saleh et al. 2009). Vieira et al. (2007) selected different undefined mixed microbial consortia from petroleum pollutes sites, and evaluated their diesel biodegradation efficiencies. Nikakhtari et al. (2009) also used indigenous mixed microbial consortia from polluted sites, whose degradation ability was compared with commercial and donated consortia. However, the real ability, efficiency and rate of these consortia to degrade hydrocarbons should be tested directly in hydrocarbon-water suspensions to avoid the mass transfer limitations that would appear if a soil matrix is present.

In this context, the present work studies the feasibility of using mixed microbial consortia, obtained from different hydrocarbon polluted sites, to degrade diesel fuel under a variety of different experimental conditions. The three microbial consortia used were previously adapted to use diesel through laboratory cultivation, and then biodegradation was studied in water–diesel suspension batch experiments. Moreover, a Monod model was proposed to describe the biodegradation efficiency and kinetics of the different cultures. This research was conducted at the Institute of Chemical and Environmental Technology, University of Castilla-La Mancha (Ciudad Real; Spain) in the spring of 2009.

Materials and methods

Chemicals

In this study, conventional petroleum-derived fuel purchased from a petrol station in Ciudad Real, Spain, was employed. The composition of the fuel was estimated according to ASTM standard tests (ASTM 2004), and the analysis revealed that the diesel was composed of 75 % saturated hydrocarbons (primarily paraffins, including *n*-, iso-, and cycloparaffins) and 25 % aromatic hydrocarbons (including naphthalenes and alkylbenzenes). By overlaying a standard diesel pattern (Absolute Standard, Inc. Hamden, CT) and comparing the retention times of the chromatographic profile of the diesel, the chain length of *n*-alkanes was determined to be between 10 and 26 carbon atoms. Other compounds such as pristane and phytane were also identified. The density (832 g/L) was measured according to the method proposed by the International Organisation for Standardisation (ISO-3675 1998).

Bushnell–Haas (BH) broth, which contains 0.2 g/L $MgSO_4$, 0.02 g/L $CaCl_2$, 1 g/L KH_2PO_4, 1 g/L $(NH_4)_2HPO_4$, 0.05 g/L $FeCl_3$ and 1 g/L KNO_3, was used in the consortia acclimation process. The media was adjusted to pH 7 ± 0.2 and was autoclaved to prevent contamination.

n-Hexane was used to prepare samples for gas chromatography (Merck-Darmstadt, Germany). All chemicals used for the preparation of microbial media (BH broth and Luria–Bertani broth) were reagent-grade and were purchased from Difco Laboratories.

Isolation and identification of diesel-degrading consortia

Three soil samples contaminated with hydrocarbons were collected from an oil refinery near Ciudad Real, Spain. The soil samples were named as SA, SB and SC. Table 1 shows their concentrations of total petroleum hydrocarbons (TPH), total heterotrophic bacteria (THB) and diesel degrading bacteria (DDB).

To obtain three different consortia (named as XA, XB and XC, obtained from soils SA, SB and SC, respectively), the soil samples were sieved through a 2-mm screen, and the microorganisms were immediately isolated. Briefly, 5 g of each soil sample was transferred to flasks containing 50 mL of Bushnell–Haas broth. The flasks were agitated in an Ecotron incubator shaker (50 rpm) for 12 h at 26 °C. Upon completion, the water/soil suspension was centrifuged at 1,500 rpm for 15 min, and the supernatants were inoculated in 1 % (v/v) diesel as the sole carbon source. Prior to the addition of the micro-organisms, the diesel was sterilised by filtration (Millex, pore size of 0.2 μm; Millipore). The consortia were enriched via weekly aerobic cultivations in the Ecotron incubator shaker at 50 rpm and 26 °C for 3 months, using diesel as the sole carbon source. Using this procedure, three adapted microbial consortia were obtained.

To achieve a microbiological characterisation, serial dilutions (1/10) of the adapted consortia were grown in Luria–Bertani agar plates with 25 g/L of glucose at 26 °C.

Table 1 Initial concentration of TPH and microorganisms in soils

Parameter	SA	SB	SC
TPH (mg/Kg)	16.6	770.4	25
THB (NMP/g)	1.3×10^6	2.3×10^8	28×10^8
DDB (NMP/g)	0.4×10^6	2.3×10^8	24×10^6

TPH total petroleum hydrocarbon, *THB* total heterotrophic bacteria, *DDB* diesel-degrading bacteria

After 4 days, the colonies were streaked onto a new agar plate to isolate each strain. To characterise the isolates, their oxidase reactions, catalase reactions and morphology were determined (colony aspect, size, shape and colour), and a Gram stain test was conducted. Bacteria were tested and identified with physiological test kits, according to Analytical Profile Index micromethods API 20 NE, API 20E and API Staph (BioMérieux, Lyon, France). To preserve the strains, the bacterial isolates were stored in 35 % glycerol in liquid nitrogen.

Experimental system

Batch experiments were conducted to study the efficiencies and kinetics of diesel biodegradation using the three selected consortia. For each consortium, the effects of temperature and diesel concentration were studied, as shown in Table 2.

A total of 26 batch biodegradation experiments were carried out in 1-L reactors. Each reactor contained 500 mL of BH broth and an initial diesel concentration of 0.5, 1 and 3 % (v/v). The reactors were sealed to avoid potential losses of diesel and were placed inside an orbital shaker bath with a temperature controller. The reactors were agitated at 130 rpm to ensure substrate availability and oxygen transfer. The dissolved oxygen concentration was previously measured under the aforementioned operating conditions with an YSI 5000 oxygen probe to ensure aerobic conditions (oxygen concentrations between 6 and 7 mg/L approximately). Finally, each reactor was inoculated with one of the acclimated consortia, as indicated in Table 2. The reactors were maintained at 25, 30 or 35 °C for 8 days to study the effects of temperature. Aliquots were sampled to monitor the biodegradation of diesel and biomass growth. Three non-inoculated control reactors (that is, with no consortium addition) were incubated at 25, 30 or 35 °C with 500 mL of BH broth and 0.5, 1 and 3 % (v/v) diesel to account for abiotic losses.

Table 2 Experiments done for the diesel biodegradation

C_0 (% v/v)	Consortium	T (°C)		
		25	30	35
0.5	Abiotic	–	×	–
	XA	×	×	–
	XB	×	×	–
	XC	×	×	–
1	Abiotic	×	×	×
	XA	×	×	×
	XB	×	×	×
	XC	×	×	×
3	Abiotic	–	×	–
	XA	×	×	–
	XB	×	×	–
	XC	×	×	–

C_0, initial diesel concentration; XA, consortium A; XB, consortium B; XC, consortium C; –, Not tested; ×, tested

Analytical methods

The THB and DDB were enumerated in soils by the most probable number (MPN) method (Youssef et al. 2010).

Biomass growth and the residual diesel concentration were determined from 10-ml samples which were collected by duplicate during the biodegradation experiments. Biomass concentration was determined by direct optical density (OD) measurements at 600 nm, as described by Sadouk et al. (2008). A Shimadzu UV-1700 spectrophotometer was used to determine the OD of the cultures, and each measurement was performed in triplicate. A correlation curve (Eq. 1) relating the OD_{600} to the dry weight (DW) concentration was developed to calculate the biomass concentration (mg/L):

$$\mathrm{DW(g/L)} = 0.214\ \mathrm{OD} + 0.0042; \quad (r^2 = 0.9756) \qquad (1)$$

The average residual concentration of diesel was determined by TPH measurements. The hydrocarbon fraction was extracted into 2 mL of *n*-hexane in a one-step extraction procedure. The TPH concentrations were analysed via gas chromatography using a Thermo-Fischer Trace GC Ultra gas chromatograph equipped with a flame ionisation detector, according to ISO method (ISO 9377-2 2000). The hydrocarbons were separated on a micro ultra fast capillary column (5 m × 0.1 mm i.d. × 0.4 µm), and the injector and detector were maintained at a constant temperature of 250 and 280 °C, respectively. During the chromatographic run, the column was maintained at 62 °C for 0.1 min, and the temperature of the column was increased at a rate of 180 °C/min until a final temperature of 280 °C was attained. The final temperature was maintained for 2.7 min, and helium was applied as the carrier gas. The injection volume was set to 1 µL, and a split injection mode was employed. Qualitative analysis was performed using a standard mixture of *n*-alkanes (Absolute Standard, Inc. Hamden, CT), and the calibration curves were obtained from dilutions of the reference solution.

Biodegradation kinetics

To analyse the kinetics of biodegradation, the concentrations of micro-organisms (X) and residual diesel (S) were determined as a function of time, and the following equations were applied to fit the experimental data:

$$\frac{dX}{dt} = \frac{\mu_{max}(S - I_0)}{K_s + (S - I_0)}X \quad (2)$$

$$\left(\frac{-dS}{dt}\right) = \frac{\mu_{max}(S - I_0)}{K_s + (S - I_0)}X\frac{1}{Y_{x/s}} \quad (3)$$

where S is the substrate or diesel concentration (g TPH/L), X is the concentration of microorganisms (g/L), t is the residence time (h), μ_{max} is the maximum specific growth rate (h^{-1}), $Y_{x/s}$ is the biomass yield (g/g), K_s is the half-saturation coefficient (g TPH/L) and I_0 is the inert residual substrate concentration (g TPH/L).

Equations 2 and 3 were solved simultaneously using the Gauss–Newton algorithm. An initial set of values (μ_{max}, K_s and $Y_{x/s}$) were assigned to the parameters, and after several iterations, the values of the parameters that yielded the lowest sum of squared error (SSE) were selected. The SSE of the parameters was determined according to the following equation:

$$\chi = \sum_{i=1}^{n}\left(\left(X_i - X_{meas,i}\right)_i^2 + \left(S_i - S_{meas,i}\right)_i^2\right) \quad (4)$$

where n is the number of data points, X_i and S_i are the calculated values of the variable at the ith measurement and $X_{meas,i}$ and $S_{meas,i}$ are the actual values at the ith measurement.

Results and discussion

Composition of the bacterial community

Cultures enriched in suspensions of diesel, grew rapidly and became turbid after 24 h of incubation. After 3 months of successive transfers, only eight pure bacterial strains were isolated from each consortium (see Table 3) and they were identified according to the Analytical Profile Index method, previously indicated. Some species, as *Staphylococcus lentus*, *Stenotrophomonas maltophilia* and

Table 3 Biochemical and growth characteristics of isolated bacterial cultures

Isolate type	Colour	Convex	Opaque	Margin	Cell morphology	Gram coloration	Oxidase	Catalase	Identification API	Genus	Species
XA1	Yellow	+	−	I	R	−	w	w	20 NE	NI	
XA2	Greyish	−	+	E	R	−	+	+	20 NE	*Rhizobium*	*radiobacter*
XA3	White	±	−	E	R	−	+	+	20 NE	*Aeromonas*	*sobria*
XA4	Orange	±	±	E	C	+	−	+	Staph	*Staphylococcus*	*lentus*
XA5	Cream	+	+	I	R	+	−	+	20 NE	NI	
XA6	White	+	−	I	C	−	−	−	20 NE	NI	
XA7	Cream	+	+	I	R	−	+	+	20 NE	*Ochrobactrum*	*anthropi*
XA9	White	−	+	E	R	−	+	+	20 NE	*Achromobacter*	*xylosoxidans*
XB1	Yellow	−	−	E	R	−	w	+	20 NE	*Burkholderia*	*cepacia*
XB2	White	+	±	E	R	−	+	+	20 NE	*Pseudomonas*	*fluorescens*
XB4	Yellow	+	−	E	R	−	−	+	20 E	*Stenotrophomonas*	*maltophilia*
XB5	Cream	+	+	E	R	−	+	+	20 NE	*Achromobacter*	*denitrificans*
XB6	Orange	±	±	E	C	+	−	+	Staph	*Staphylococcus*	*lentus*
XB7	Cream	+	+	I	R	−	+	+	20 NE	*Ochrobactrum*	*anthropi*
XB8	White	+	+	E	R	−	+	w	20 NE	*Sphingomonas*	*paucimobilis*
XB9	White	−	+	E	C	−	−	+	20 NE	NI	
XC1	Yellow	+	−	E	R	−	−	+	20 E	*Stenotrophomonas*	*maltophilia*
XC2	Cream	+	−	E	R	−	+	w	20 NE	*Sphingobacterium*	*multivorum*
XC3	Yellowish	−	−	E	R	−	+	w	20 NE	*Sphingobacterium*	spp.
XC4	Yellow	−	−	E	C	+	−	w	Staph	*Staphylococcus*	*sciuri*
XC5	Orange	±	±	E	C	+	−	+	Staph	*Staphylococcus*	*lentus*
XC6	White	−	+	E	C	−	−	+	20 NE	NI	
XC7	White	–	+	I	C	+	–	+	Staph	*Micrococcus*	spp.
XC8	White	+	+	E	R	–	+	+	20 NE	*Pseudomonas*	*fluorescens*

+, positive; −, negative; ±, variable; w, weak; NI, not identified; E, entire; I, irregular; R, rod; C, coccus

Pseudomonas fluorescens, appeared in more than one consortium. The isolation of these species is not surprising due to their frequency in contaminated soils (Barathi and Vasudevan 2001; Ueno et al. 2007). All of the other species found in the consortia have been shown to degrade hydrocarbons (Chaineau et al. 1999; Lafortune et al. 2009; Medina-Moreno et al. 2005; Nikakhtari et al. 2009; Owsianiak et al. 2009; Rahman et al. 2002).

Diesel biodegradation by isolated consortia

Figure 1 illustrates the results of diesel biodegradation and biomass growth in the batch experiments. The data shown correspond to the biodegradation of 1 % (v/v) diesel at 25, 30 and 35 °C for consortia XA, XB and XC (data for the degradation of 0.5 and 3 % (v/v) diesel are not presented). The curves represent the general trend of each consortium; thus, the entire data set is not shown. The results revealed that the behaviour of the three consortia was similar. Moreover, for all of the consortia, a direct relationship between microbial growth and substrate depletion was observed. The maximum amount of substrate utilised by the microbes was observed during the exponential growth phase, and the concentration of biomass increased with an increase in the initial concentration of substrate during the lag phase.

In the present study, inhibition was clearly observed at 35 °C, and less than 35 % diesel biodegradation occurred in all of the consortia at this temperature. As shown in Fig. 1, except for assays conducted at 35 °C, over 80 % of the substrate was degraded after 40 h of treatment, and a maximum removal efficiency of 98 % was obtained. A further reduction in the diesel concentration was not observed after 80 h of treatment. Similarly, Márquez-Rocha et al. (2001) attained nearly 90 % diesel consumption after 13 days of bioremediation. In general, the diesel biodegradation by the consortia was faster than that of other microbes reported in the literature (Vieira et al. 2007; Young et al. 2005). These results prove the good feasibility of using polluted sites as source of mixed consortia for hydrocarbon degradation, as also has been found in similar previous works (Nikakhtari et al. 2009; Vieira et al. 2007). However, in the present work no important differences were observed between the three consortia, suggesting that the acclimation process produced mixed consortia with very similar characteristics and efficiencies, and the origin of the soil sample was not a decisive factor.

In all of the bioremediation experiments, a layer of creamy foam was produced on the surface of the medium, and the foam was not observed in abiotic experiments. Previous studies suggest that some species in the consortia (*P. fluorescens, Ochrobactrum antrhopii* and *Corynebacterium*) are capable of generating extracellular surface-active products that improve the consumption and transport of oily substrates by increasing the solubility of oil (Banat 1995; Ron and Rosenberg 2001). Thus, the aforementioned consortia may have produced surface-active materials to improve the use of diesel.

Although the experiments were carried out in closed systems, the substrate may have volatilised during the sampling process. To identify substrate losses by volatilisation, the concentration of TPH in the abiotic assays was measured, and abiotic losses of diesel were estimated to be 5.7, 8.67 and 11.33 % at 25, 30 and 35 °C, respectively.

Parameter estimation

To evaluate the biodegradation kinetics, the experimental data from the batch experiments were fitted to Eqs. 2 and 3. As an example, Fig. 2 shows the experimental data for consortium XA at 25 °C and an initial diesel concentration of 0.5 % (v/v), as well as the simulated curves of the Monod model. In most of the experiments, the results of the model agreed with the experimental data, indicating that the kinetic model could predict the diesel biodegradation over time. Numerous mathematical models based on the Monod equation have been proposed in the literature to explain the biodegradation of hydrocarbons. In some cases, additional parameters were used to describe the system. For instance, Ghoshal and Luthy (1998) and Mukherji and Weber (1998) considered the mass transfer of naphthalene from the organic phase to the aqueous phase. The aforementioned model was based on the premise that microorganisms can only metabolise TPH when it is in the dissolved state; thus, these models can be used to determine if biodegradation is controlled by the rate of dissolution or by the intrinsic rate of biodegradation by the microorganisms. Despite the fact that the model proposed in this study did not consider mass transfer, the model satisfied the initial purpose of the investigation, that is, the proposed model provided a simple representation of the biodegradation rate and efficiency.

Table 4 shows the kinetics parameters obtained from the mathematical fitting. Similar trends were again observed between the three consortia used. The highest μ_{max} value (0.34 h^{-1}) was obtained by consortium XB at 30 °C and an initial diesel concentration of 0.5 % (v/v), suggesting, in principle, that consortia isolated from a highly contaminated soil (SB) may provide a higher biodegradation rate of diesel. However, this hypothesis cannot be verified, because, all of the consortia displayed similar values. Except for the assays conducted at 35 °C, μ_{max} ranged between 0.17 and 0.34 h^{-1}, which is slightly higher than those obtained in previous studies (Whang et al. 2008; Young et al. 2005).

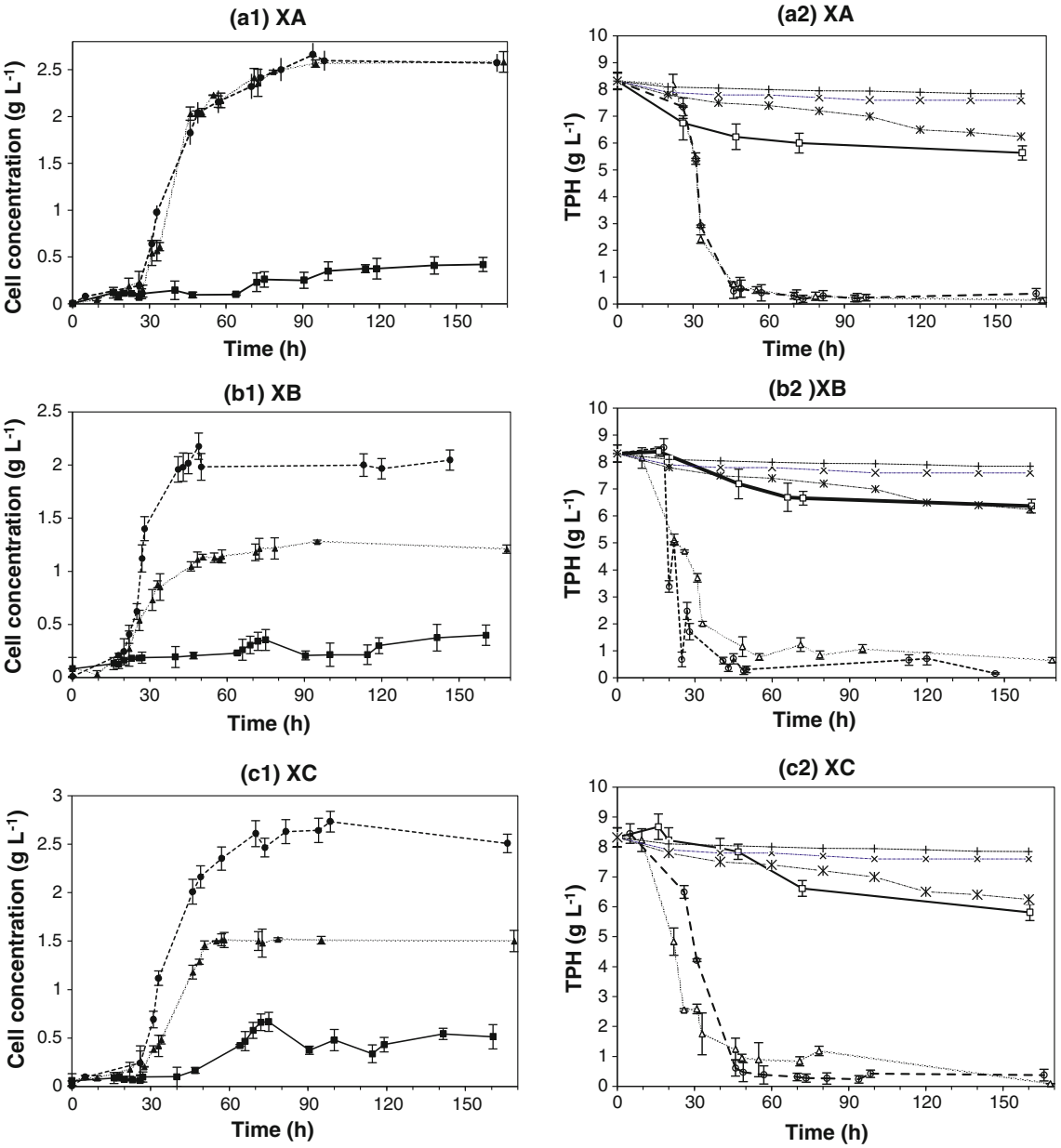

Fig. 1 TPH and biomass concentrations over time for consortia XA (**a**), XB (**b**) and XC (**c**) at 25, 30 and 35 °C with 1 % (v/v) of initial diesel concentration. *Symbols* (*filled triangles, filled circles, filled squares*) represent the concentration of biomass at 25, 30 and 35 °C, respectively. *Symbols* (*open triangles, open circles, open squares*) represent the concentration of TPH at 25, 30 and 35 °C, respectively. *Symbols* (*pluses, times, asterisks*) represent the concentration of TPH in abiotic experiments at 25, 30 and 35 °C, respectively. Data are the mean of two samples. *Lines* indicate trends only

The half-saturation constant was highly correlated with the maximum utilisation rate (Schirmer et al. 2000); thus at various initial diesel concentrations, the value of K_s increased from 3 to 5 g of TPH/L as μ_{max} increased (K_s is not included in Table 4). Similar K_s values were reported by Young et al. (2005), who reported a K_s value of 3.196 g of TPH/L.

Table 4 also displays the correlation coefficients of the model and the significance of the correlations. As shown in the table, the biodegradation model acceptably fitted the experimental data (r^2 between 0.8545 and 0.9974). The proposed model did not consider temperature inhibition or other inhibitory effects, and the values of r^2 for XA, XB and XC fittings at 35 °C were 0.4569, 0.56 and 0.6671, respectively. The correlation coefficients were significant, and the variables were positively related at the $p < 0.05$ level.

Effect of initial diesel concentration

At the lowest initial diesel concentration, residual diesel (I_0) was not detected in the medium; however, as the initial

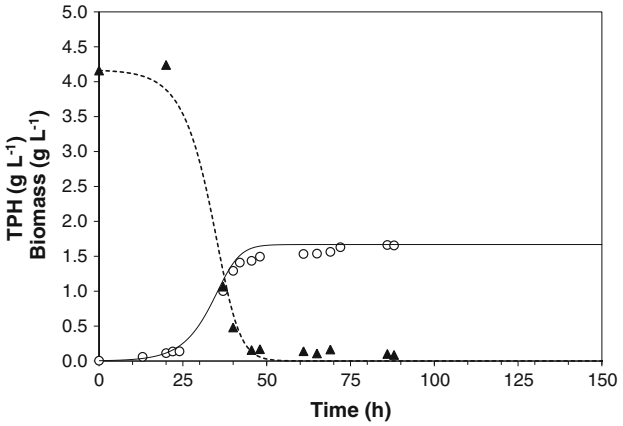

Fig. 2 Simulation of batch experiments [consortium XA at 25 °C and an initial diesel concentration of 0.5 % (v/v)]. *Symbols* (*filled triangles, open circles*) represent the experimental concentration of TPH and biomass, respectively, and the *lines* indicate the theoretical results

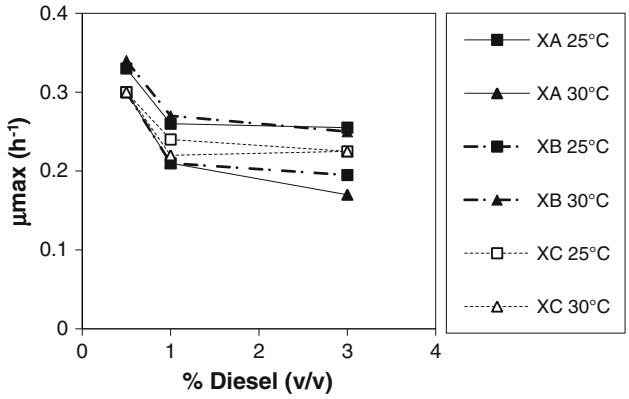

Fig. 3 The dependence of the maximum specific growth rate of consortia XA, XB and XC on the initial diesel concentration. *Dots* represent experimental data, and *lines* represent general trends

diesel concentration increased, higher concentrations of residual diesel were observed (Table 4).

The μ_{max} and $Y_{x/s}$ parameters, dependent on initial diesel concentration, offered similar trends in all consortia. Figure 3 shows the evolution of μ_{max} as a function of the initial diesel concentration. It decreased from the maximum value (when using 0.5 % diesel) to approximately constant values (when using 1 and 3 % diesel).

Regarding $Y_{x/s}$, it decreased with an increase in the initial diesel concentration in all cases (Fig. 4). In the previous biodegradation studies on other contaminants, a similar trend in biomass yield was observed (Lee et al. 1993; Paslawski et al. 2009). Lee et al. (1993) demonstrated that the substrate can penetrate into the cell membrane and interact with microbial membrane proteins, causing the proteins to malfunction and reducing the biomass yield. The variability in biomass yield could also be attributed to uncoupled growth. Senez (1962) introduced the term uncoupled growth to describe the effects of

Table 4 The estimated parameters (μ_{max} and $Y_{x/s}$) of the Monod equation and the error associated with the batch experiments

Consortium	T (°C)	C_0 (% v/v)	I_0 (g TPH/L)	μ_{max} (h^{-1})	Overall $Y_{x/s}$	r^2	df	t
XA	25	0.5	0	0.33	0.40	0.9789	15	18.554
		1	0	0.26	0.25	0.9599	17	14.118
		3	0.7	0.26	0.16	0.9476	13	10.695
	30	0.5	0	0.30	0.40	0.9694	12	13.679
		1	0.2	0.21	0.27	0.9560	12	11.289
		3	1	0.17	0.15	0.9583	13	12.091
	35	1	6	0.06	0.08	0.4569	16	2.0550
XB	25	0.5	0	0.30	0.50	0.9974	15	53.604
		1	1	0.21	0.08	0.9652	16	14.763
		3	4	0.20	0.15	0.8846	12	6.571
	30	0.5	0	0.34	0.41	0.9791	14	18.013
		1	0.2	0.27	0.15	0.9870	13	22.142
		3	5	0.25	0.15	0.8710	9	5.319
	35	1	6.4	0.10	0.19	0.5600	18	2.868
XC	25	0.5	0	0.30	0.28	0.9528	15	12.155
		1	1	0.24	0.22	0.9531	17	12.984
		3	1	0.23	0.19	0.9551	12	11.167
	30	0.5	0	0.30	0.45	0.8545	14	6.155
		1	0.05	0.22	0.30	0.9491	12	10.438
		3	0.5	0.23	0.16	0.9424	9	8.452
	35	1	6	0.18	0.38	0.6671	18	3.799

df degrees of freedom, *t* Student's *t* test

limited assimilation capacities on growth yield. The source of the variability in biomass yield in the present work is not clear, and it is supposed that the presence of high diesel concentrations caused a higher biomass growth, but a significant increase in substrate requirements for biomass maintenance. As the temperature increased from 25 to 30 °C, the order of magnitude of the biomass yield remained relatively constant, indicating that biomass yield was significantly affected by uncoupled growth and only slightly affected by temperature.

To discuss the causes of yield variability, an additional equation can be introduced:

$$\mu_{max} = Y_{x/s} \times q_{s,max} \quad (5)$$

where $q_{s,max}$ (g diesel g/biomass/h) is the specific rate of substrate consumption. Equation 5 indicates that the growth rate depends on the rate of substrate consumption ($q_{s,max}$) and the efficiency of the transformation of substrate to biomass ($Y_{x/s}$) (van Uden 1969). Taking Eq. 5 into account, to determine if growth inhibition was caused by a decrease in the biomass yield or a decrease in the rate of substrate consumption, all the biomass yield values were plotted against the corresponding μ_{max} values, and a linear relationship was obtained for all consortia and temperatures (Fig. 5), indicating that $q_{s,max}$ remained constant. Thus, as expected, $q_{s,max}$ did not change as the initial diesel concentration increased, and the observed decrease in the growth rate could be explained by a reduction in biomass yield. The results of this study revealed that the consortia can function at high concentrations of hydrocarbons without any sign of growth inhibition, which is important for the design of bioreactors for wastewater treatment with high concentrations of fuel. In addition, the results suggested that uncoupled growth must be considered in the biodegradation process.

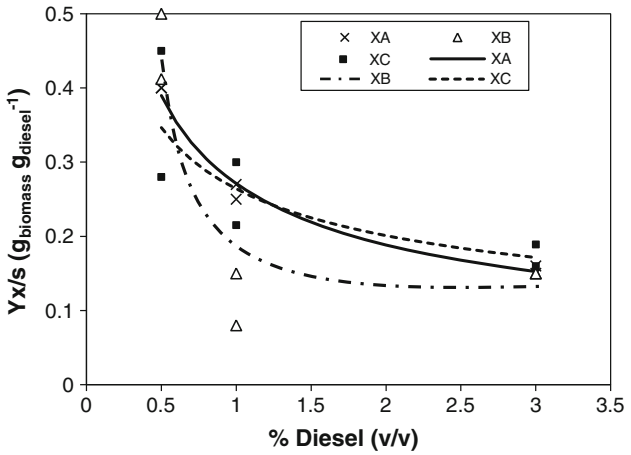

Fig. 4 Effect of the initial diesel concentration on the biomass yield. *Lines* indicate general trends of media values between 25 and 30 °C

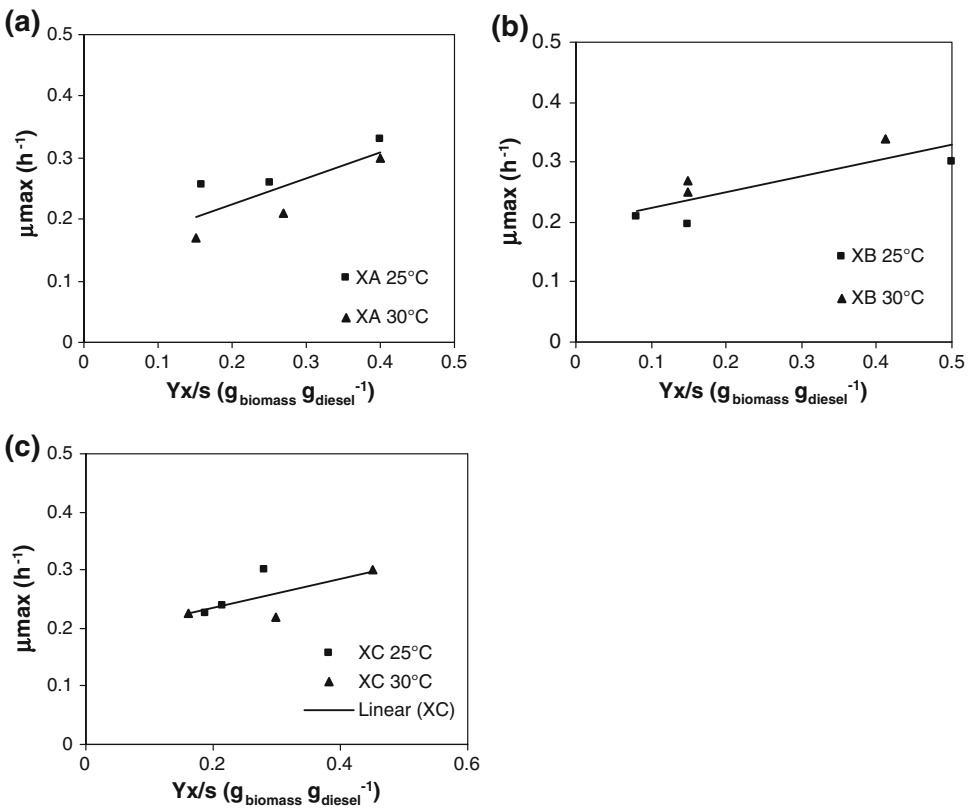

Fig. 5 The relationship between the maximum specific growth rate and the biomass yield coefficient at 25 °C (*filled squares*) and 30 °C (*filled triangles*) for consortium XA (**a**), XB (**b**) and XC (**c**). *Dots* represent experimental data, and *lines* indicate general trends

The correlation coefficients shown in Table 4 revealed that the fit of the model to the experimental data diminished as the initial diesel concentration increased, indicating that the proposed model did not consider uncoupled growth.

Effect of temperature

Temperature is one of the most important factors affecting the efficiency of biodegradation (Iqbal et al. 2007). In the present study, the final efficiency of biodegradation remained constant at temperatures between 25 and 30 °C, and satisfactory growth rates were observed. However, at 35 °C, little microbial growth was observed, and less than 35 % degradation was obtained; hence, the biological reaction was clearly inhibited. Under these conditions, yellowish biomass aggregates adhered to the walls of the reactor was observed, indicating biomass disintegration and lysis. Moreover, large amounts of foam were also observed at 35 °C, especially on the second day of the experiment. Consequently, μ_{max} decreased by more than 76, 63 and 25 % for consortium XA, XB and XC, respectively. Thermal inhibition may have occurred, because, consortia isolated from the soils were rarely subjected to high temperatures for extended periods of time.

Conclusion

Only a small number of species were identified in the acclimated microbial consortia, and some of them appeared in more than one consortium, as well as those obtained in similar previous investigations. Except for assays conducted at 35 °C, over 80 % of the substrate was degraded after 40 h of treatment. These results proved the good feasibility of using polluted sites as source of mixed consortia for hydrocarbon degradation. However, the diesel degradation efficiencies and rates were very similar, suggesting that the acclimation process produced mixed consortia with very similar characteristics and the origin of the soil samples was not a decisive factor. A simple Monod-type kinetic model was used to simulate the biodegradation process, and accurate results were obtained. As the initial diesel concentration increased, the growth rate and the yield of biomass decreased due to an increase in the energy required to maintain the cultures. The results of this study revealed that the consortia can function at high concentrations of hydrocarbons without any sign of growth inhibition, which is important for the design of bioreactors for wastewater treatment with high concentrations of fuel.

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