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Enhancement of Oil Degradation by Co-culture of Hydrocarbon Degrading and Biosurfactant Producing Bacteria

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Abstract

In this study the biodegradation of oil by hydrocarbon degrading *Pseudomonas putida* in the presence of a biosurfactant-producing bacterium was investigated. The co-culture of test organisms exhibited improved degradation capacities, in a reproducible fashion, in aqueous and soil matrix in comparison to the individual bacterium culture. Results indicate that the *in situ* biosurfactant production not only resulted in increased emulsification of the oil but also change the adhesion of the hydrocarbon to cell surface of other bacterium. The understanding of interactions beetwen microbes may provide opportunities to further enhancement of contaminants biodegradation by making a suitable blend for bioaugmentation.

Key words: biodegradation, biosurfactant, co-culture, BATH assay, bioaugmentation

Introduction

Mass transfer from sorbed or insoluble phases is considered to be the rate-limiting step in biodegradation of organic contaminants because the compounds must be released to the aqueous phase prior to entering the microbial cell and subsequent intracellular transformation by the necessary catabolic enzymes (Dean *et al.*, 2001). The availability of slightly soluble organic compounds can be enhanced by biosurfactants. Biosurfactants can enhance biodegradation by (i) increasing available substrate surface area (dispersion) and (ii) improving affinity of microbial cells for the substrate (Ito and Inoue, 1982; Zhang and Miller, 1994; Rahman *et al*., 2003; Chang *et al.*, 2004; Bonilla *et al.*, 2005). However, effect of biosurfactant on degradation is less straightforward. There are also evidences that biosurfactants may interfere with the interaction between biosurfactant-dispersed substrates and microbial cells resulting in decreased biodegradation (Falatko and Novak**,** 1992).

There are several reports on improved hydrocarbon degradation by addition of biosurfactant or chemical surfactant (Boonchan *et al*., 1998; Stelmack *et al.*, 1999; Noordman *et al.*, 2002; Rahman *et al.*, 2003; Kuyukina *et al.*, 2005). However, the effect of *in situ* biosurfactant production by co-culture of the biosurfactant-producing and hydrocarbon degrading bacteria on hydrocarbon degradation is relatively less studied (Dean *et al.*, 2001; Van Hamme and Ward, 2001). For field bioremediation application based on bioaugmentation, addition of the biosurfactant-producing bacteria may be beneficial and more practical than exogenously adding purified biosurfactant. Bacterial strain designated as DHT-GL isolated in our laboratory from hydrocarbon contaminated soil, forms excellent emulsions between 24 and 48 h of incubation with wide the range of hydrocarbon while other strain, 5a1, efficiently degrades pure hydrocarbons in aqueous system. We hypothesized that a co-culture of the two organisms will enhance hydrocarbon degradation through the combination of superior emulsification and degradation capabilities. In this study, we used simple pure cultures and co-culture to study metabolic and physiological interactions that may occur in

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a mixed-culture fermentation system and in soil matrix. An understanding of these interactions is necessary when developing treatment techniques for hydrocarbon biodegradation.

The goal of this study was to determine if biodegradation of the oil could be accelerated through bioaugmentation with hydrocarbon degrading and biosurfactant-producing bacteria. Batch aqueous and soil degradation experiments using diesel oil as representative complex hydrocarbon, were conducted involving inoculation with hydrocarbon degrading and biosurfactant producing bacterial strains separately or in combinations.

Experimental

Materials and Methods

Enrichment and isolation of bacteria. The bacterial strains were isolated by the enrichment culture technique from the soil obtained from Guanaco Asphalt Belt, Venezuela. 5 g of soil was inoculated into 100 ml of minimal salt medium (MSM) containing (g/l) Na_2HPO_4 – 6.0; KH_2PO_4 – 3.0; NH_4Cl – 1.0; NaCl – 0.5; $MgSO_4$ – 0.1 and 2.5 ml of trace element solution (pH 7.0). Trace elements solution contained (mg/l) $MnCl_2 \times 2H_2O$ – 23; $MnCl_4 \times H_2O$ – 30; H_3BO_3 – 31; $CoCl_2 \times 6H_2O$ – 36; $CuCl_2 \times 2H_2O$ – 10; $NiCl_2 \times 6H_2O$ – 20; $Na_2MoO_4 \times 2H_2O$ – 30 and $ZnCl_2$ – 50. Crude oil (5% w/v) was used as carbon source and incubated at 30°C or 60°C on a rotary shaker (200 rpm) for 4 days. After five cycles of such enrichment, 1 ml of the culture was diluted and plated on MSM agar (2% w/v) plates containing crude oil (5% w/v) as sole carbon source and incubated at 30°C or 60°C. The bacterial colonies obtained were further purified on Luria-Bertani (LB) agar plates. The isolated strains were stored as frozen stock cultures at –70°C in 25% glycerol.

Plasmid curing and mutagenesis. Plasmid DNA was isolated by the method of Hansen and Olsen (1978). Ethidium bromide was used as plasmid curing agent (Guerry and Colwell,1977). Random mutagenesis using N-methyl-N'-nitro-N-nitrosoguanidine was carried out according to Tahzibi *et al.* (2004) to isolate bacteria with lost ability to produce biosurfactant.

Growth on hydrocarbons: Growth in the presence of hydrocarbons was studied using liquid minimal salt medium containing 0.1 g of substrate/liter. Erlenmeyer flasks (250 ml) containing 50 ml of MSM and the hydrocarbon were inoculated with 100 µl Luria-Bertani (LB) broth grown pre-culture and incubated at 30°C and 200 rpm. Growth was followed by visually observing the increase in cell density in comparison to un-inoculated control with respective carbon source. When no difference was noticed in the turbidity of the flask and that of the control, it was taken as no growth (–), where slight increase in turbidity was noticed it was taken as poor growth (+). Significant increase in turbidity was taken as good growth (++) while luxuriant growth and increases in turbidity was stated as very good growth (+++).

The solid polycyclic aromatic hydrocarbons (PAHs) (except naphthalene) were dissolved in 5% (w/v) diethyl ether and sprayed on surface of MSM agar. Naphthalene was provided as crystals directly placed on the plate lid. Growth on PAHs in solid media was considered positive by the formation of clear zone around the growing colonies or appearance of pigments.

Biosurfactant production: To study biosurfactant production and activity, bacteria were either grown in YPG medium (g/l): Yeast extract – 5; Peptone – 5; Glucose – 15) or in MSM containing 3% (w/v) glucose and/or 2% (w/v) hexadecane. The cultures were incubated at 30°C and 150 rpm. After 24 h the culture broth was centrifuged at 8000 rpm for 10 min and the supernatant was used for measurement of surface-active properties. The surface tension of the biosurfactant was measured by the Ring method using a CSC-DuNouy Tensiometer at room temperature. Drop test and oil spread test was carried out according to Youssef *et al.* (2004). The emulsification activity (E_{24}) was determined by the addition of the respective hydrocarbon (kerosene, gasoline, diesel oil, gas oil, hexadecane, and alpha methyl naphthalene) to the same volume of cell free culture broth, mixed with a vortex for 2 minutes and left to stand for 24 h. The emulsification activity was determined as the percentage of height of the emulsified layer (mm) divided by the total height of the liquid column (mm). To study the stability of emulsion the emulsified solutions were allowed to stand at 60°C and emulsification index was analyzed at different time intervals. Surface active compounds were extracted by liquid – liquid extraction from cell free culture broth acidified with 1N HCl to pH 2.0 (Rahman *et al*., 2003). Supernatant fluid was mixed with an equal volume of a chloroform: methanol (2 : 1) mixture. The solvent was evaporated and the material was used as crude biosurfactant and weighted to evaluate the yield. Determination of the carbohydrate content of the partially purified biosurfactant was done by anthrone reagent method at 620 nm (Spiro, 1966). Protein was assayed by the Bradford (1976) method using bovine serum albumin as standard. Lipid was analysed as described by Ilori and Amund (2001).

16S rRNA partial gene sequencing and bacteria identification. Bacterium total DNA was isolated according to Chen and Kuo (1993). Partial gene sequence coding for 16S rRNA was amplified by PCR using universal primers U1 (5'-CCA GCA GCC GCG GTA ATA CG-3') corresponding to nucleotide positions 518 to 537 (forward primer) and U2 (5'-ATC GG(C/T) TAC CTT GTT ACG ACT TC-3') corresponding to nucleotide positions 1513 to 1491 (reverse primer) according to the *Escherichia coli* numbering system (Weisburg *et al.*, 1991). The PCR were performed as described by Lu *et al.* (2000) using A PCT-100™ thermal cycler unit (MJ Research, Inc. USA). The PCR product was separated by agarose gel electrophoresis and visualized by SYBR® Green 1 staining (Sigma, St. Louis, USA) and finally purified by using a Wizard PCR Preps Purification System (Promega Corp., USA) according to manufacturer's instructions. DNA sequencing reaction was performed with an ABI PRISM Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, USA) and the sequencing products were separated by capillary electrophoresis by using a 310 Sequencer (Perkin-Elmer Corp., Applied Biosystems, USA) according to standard procedures. Sequence data were analyzed with DNAMAN version 5.2.9 (Lynnon BioSoft, USA) to obtain a consensus sequence. To identify the isolated bacterial strain, the 16S rDNA consensus sequence was compared with 16S rRNA gene sequences from the public GenBank, EMBL, and DDBJ databases using the advanced gapped n-BLAST program, version 2.1. The program was run *via* Internet through the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/blast/). Sequences with more than 98% identity with a GenBank sequence were considered to be of the same species as the highest score-matching sequence on the public sequence databases.

Degradation of oil in aqueous medium. Standard diesel oil, obtained directly from Petróleos de Venezuela (PDVSA) gas station in Caracas, Venezuela, was used as a representative complex hydrocarbon mixture for biodegradation studies. The selected bacterial isolates of *Pseudomonas putida* strain 5a1 and *Pseudomonas aureginosa* strain DHT-GL were inoculated into 100 ml sterile nutrient broth. The flasks were incubated and shaken at 200 rpm, for 12 h at 30°C. One ml volumes from each broth culture were mixed to prepare mixed bacterial consortium. Individual bacterial cultures and bacterial consortium (1%) were transferred to 250 ml conical flasks, each containing 100 ml of sterile defined mineral salts medium with 5% diesel (v/v). An uninoculated control was also studied. The influence of crude biosurfactant(s) produced by strain DHT-GL was evaluated by adding 500 mg/l crude biosurfactant in culture medium inoculated with strain 5a1. The flasks were incubated at 30°C and shaken at 200 rpm for three weeks. At 2-day intervals, a set of flasks was used for the enumeration of microbial population and estimation of diesel content. To extract the diesel at the timed interval, the culture broth was acidified to pH 3.0 using the 6 N HCl and was amended with 20% NaCl. The resulting solution was extracted with equal volume of ethyl acetate and extracted hydrocarbon concentration was determined according to Rahman *et al.* (2002). A standard curve plotted using known concentration of diesel was used to estimate the amount of hydrocarbons. Degradation was estimated as difference between the initial and final concentration of the oil content in the medium. Growth of the bacterial strains was verified by demonstrating an increase in bacterial protein concentration or increase in number of colony forming units (CFU) concomitant with a decrease in the diesel concentration.

Degradation of oil in soil medium. For diesel oil degradation tests, soil was obtained from the Campus of IDEA, Caracas, Venezuela. To equalize soil particles, the soil was airdried, passed through sieves, oven dried at 80°C for 24 hours and steam autoclaved (3 times at 121°C, 15 lbs, 15 min). Fifty grams of soil, 10 ml of the culture (separately and in combinations), 0.25% (w/w) yeast extract and 5% (v/v) standard diesel were mixed, sealed and cultivated in incubator at 30°C. An uninoculated control was also incubated under same conditions. To extract the total petroleum hydrocarbons (TPH), 10 g of soil was twice extracted with ethyl acetate (2×20 ml) and analyzed for hydrocarbon according to Rahman *et al*. (2002) at different time intervals. Extract was dried at room temperature by evaporation of solvents in a fume hood. After evaporation, the amount of residual TPH recovered was also determined gravimetrically.

Analysis of cell surface hydrophobicity. The bacterial adhesion to hydrocarbons (BATH) assay was used in order to test the hydrophobicity of the two strains (Rosenberg and Rosenberg, 1985). Bacterial cells were harvested from growth culture by centrifugation at 8000 × g for 10 min at 4°C and washed twice. The cells were then resuspended in a buffer salts solution (pH 7.0) containing (g/l) 16.9 of K_2HPO_4, 7.3 of KH_2PO_4, 1.8 of urea, and 0.2 g $MgSO_4 \times 7H_2O$ to give an optical density (OD) at 600 nm = 1.0. Cells (4.0 ml) and hexadecane (1.0 ml) were mixed in a screw-top test tube and the test tube was vortexed for 1 min. After vortexing, the hexadecane and aqueous phases were allowed to separate for 45 min. The aqueous phase was then carefully removed with a Pasteur pipette, and the turbidity of the aqueous phase at 600 nm was measured. Hydrophobicity is expressed as the percentage of adherence to hexadecane, which is calculated as follows: 100 x (1 – OD of the aqueous phase/OD of the cell suspension).

Results

Isolation and identification of bacteria. From the enrichment culture established at 30°C several bacterial strains were obtained. Essentially on the basis of its ability to grow on various hydrocarbons, strain 5a1 was selected for further studies. Like wise from the enrichment at 60°C, one strain DHT-GL, was selected for further study because of its ability to produce efficient biosurfactant and growth at wide temperature range (manuscript under preparation). Taxonomical identification of these organism, designated as strain 5a1 and strain DHT-GL, was performed by amplification and sequencing the 16S rRNA genes and comparing them to the database of known 16S rRNA sequences. Alignment of the 16S rRNA gene sequences of strain 5a1 with sequences obtained by doing a Blast searching revealed maximum similarity to *Pseudomonas putida*, while the strain DHT-GL resembles *Pseudomonas aeruginosa.*

Growth on hydrocarbons. Bacterial strains were tested for its ability to grow on variety of carbon source including various low and high molecular weight PAHs as well as other simple aromatic hydrocarbons and n-alkanes (Table I). These chemicals represent the most common organic pollutants and are the main components of crude oils. Strain 5a1 grows very efficiently on diverse hydrocarbons and within 24 hours maximal growth was observed. In comparison strain DHT-GL grows slow and it takes around 24 hours to show the visible growth in liquid and solid media. When colonies were grown on PAHs coated agar plates, zone of the clearing appeared, indicating PAH degradation. In the presence of dibenzothiophene on plate as well as in liquid media bacterium 5a1 produced orange or reddish brown product(s) (Kodama *et al.*, 1973), while no pigmentation was observed in the case of strain DHT-GL. Beside that 5a1 can also grow in the model crude oil containing 200 ppm of each: pyrene, dibenzothiophene, phenanthrene, hydroxyquinolone and 50 ppm of hexadecane dissolved in alpha-methyl-naphthalene (data not shown). Results shows that strain 5a1 can effectively grow in wide range of the pure hydrocarbons but grew poorly in diesel. In comparison the strain DHT-GL grew in hydrocarbon less effectively. The cured strain of 5a1 lost its ability to grow on PAHs and alkanes used in this study. This indicates that the hydrocarbon degrading ability of the 5a1 was plasmid mediated. There are several reports describing plasmid mediated hydrocarbon degradation (van Hamme *et al.*, 2003; Płaza *et al.*, 2005)

Biosurfactant production. In contrary to 5a1 the strains DHT-GL give positive result in drop collapse and oil spreading test. These qualitative test are indicative of the surface and wetting activities (Youssef *et al.*,

Table I
Substrate profile of the strain DHT-GL and 5a1 after 24 h in liquid medium

Substrate	DHT-GL	5a1
Naphthalene	+	+++
Phenanthrene	+	+++
Pyrene	+	+++
Dibenzothiophene	+	+++*
Phenol	–	++
Ethanol	–	++
Hexadecane	+	++

Substrate	DHT-GL	5a1
Indole	–	+++**
Alpha-methyl-naphthalene	–	+++
n-decane	+	++
Toluene	–	++
Octadecane	+	++
Carbazole	–	++
Decalin	–	++

* Orange pigmentation in liquid as well as solid medium.
** Produces blue colonies

Substrate degradation ability of the isolated bacteria was also confirmed by growing them in the agar plates containing respective hydrocarbon as a sole carbon source

2004). The strain DHT-GL was capable of the surface reduction on YPG media from 54.9 to 30.4 dN/cm in comparison to 49.4 dN/cm by strain 5a1 after 96 hours of growth. Interestingly, most of the surface activity of the strain DHT-GL was confined to the culture supernatant, while almost no significant potential of surface activity was observed for the cells. Moreover, no significant effect was observed on the activity of the extracellular biosurfactant when it was autoclaved. Emulsification activities of the culture supernatant were measured with several water immiscible substrates. The results show that culture supernatant has high emulsification activities against diesel oil (78 ± 4%), kerosene (74 ± 3%), gas oil (76 ± 5%) and gasoline (62 ± 5%). The emulsification activity for the mixture of *n*-hexadecane and 2-methylnaphthalene (100%) was better than that for hexadecane (82 ± 4%) alone and almost the same as of the 2-methylnaphthalene (80 ± 5%) alone. These findings are in agreement with the results of Kalpan and Rosenberg (1982) who reported that a mixture of aliphatic and aromatic hydrocarbons was required for maximum emulsion formation. Similar finding were obtained when the culture supernatant obtained from growth of strain DHT-GL in MSM containing glucose and/or hexadecane as carbon source, was analyzed for various tests related to surface activity (data not shown). No significant change in emulsification index was noticed up to 48 h when the emulsion was in 60°C (data not shown). Highest emulsification activity and biosurfactant production (10.05 g/l) was obtained in YPG after 96 hours of growth. No protein was detected in partially purified biosurfactant, which however contained lipid and carbohydrate, therefore putatively was classified as a glycolipid. *Pseudomonas aeruginosa* is well known to produce biosurfactant of glycolipid type. Several types of the rhamnolipids biosurfactant produced by *P. aeruginosa* are well characterized and studied (Banat *et al.*, 2000). The function of biosurfactant for microbial cell is not fully understood. However, there has been speculation about their involvement in emulsification of water-insoluble substrates. Direct contact of cells with hydrocarbon droplets and their interaction with emulsified droplets has been described. The biosurfactant-mediated solubilization of the hydrocarbon can be helpful for bacteria to increase bioavailibity by enhancing the solubility of the water insoluble compounds (Koch *et al.*, 1991; Carrillo *et al.*, 1996; Wei *et al.*, 2005)

Synergistic effects of co-culture on oil emulsification. We observed the physical state of oil in shake flasks containing strain 5a1, DHT-GL, or a co-culture of the two. The strain DHT-GL culture passed through a stage (24 to 48 h) where emulsification occurred and the co-culture produced a similar but more stable emulsion. The lack of emulsification in case of strain 5a1 was noticed. Emulsification activity is an indicator used extensively to quantify biosurfactant produced by bacteria (Rahman *et al*., 2003). We detected bioemulsifiers with a kerosene-water emulsification assay during the growth of the individual bacteria and their co-culture in degradation assay (Fig. 1). Detection of biosurfactant in strain DHL-GH occurred for the first time in 2nd day showing E_{24} 26 ± 2% for culture supernatant. The peak of biosurfactant production was obtained on 7th day with E_{24} value 74 ± 3%. After one-week cultures on diesel of strain 5a1 had an emulsification value of 14 ± 1%. In case of co-culture detection of biosurfactant occurred for the first time in 2nd day showing E_{24} 30 ± 2% and the peak of biosurfactant production was obtained on 8th day with E_{24} value 79 ± 4%. This indicates that strain DHT-GL produced biosurfactants in co-culture condition. The addition of crude biosurfactant caused stable emulsion formation after few hours of shaking (data not shown).

Oil degradation by co-culture and biosurfactant addition. The effect of biosurfactant producing bacteria on biodegradation of diesel oil by strain 5a1 was investigated in aqueous and soil system. Figure 2A depicts the time dependent increase in protein content and concomitant decrease in the hydrocarbon content

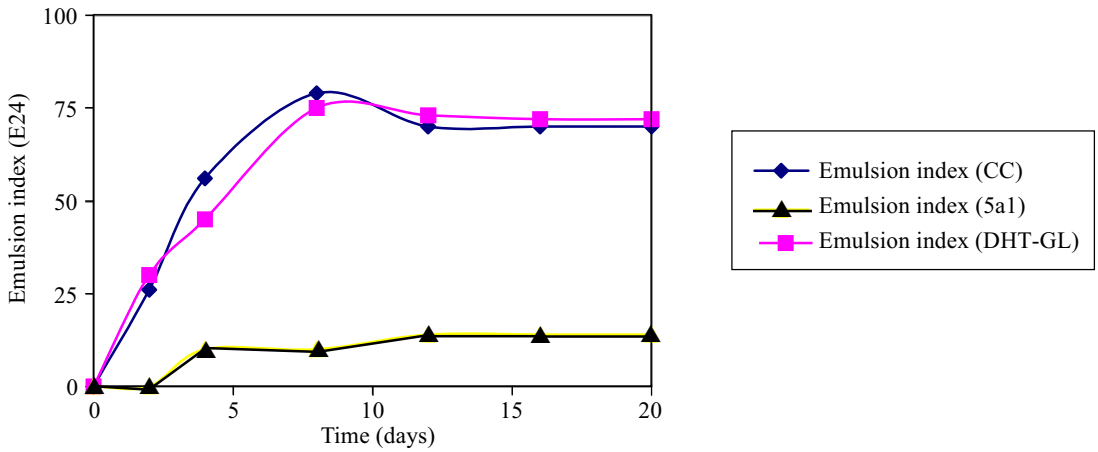

Fig. 1. Biosurfactant production during growth in aqueous medium containing diesel oil.
Values are average of three cultures.

in aqueous system while Figure 2B shows the diesel degradation in the soil. It showed that co-culture enhanced the degradation of diesel than compared with the individual strain. The maximum extent of degradation by strain 5a1, DHT-GL and co-culture is 44 ± 2%, 24 ± 1% and 92 ± 3%, respectively, after 20 days in liquid media while this was 38 ± 2%, 20 ± 1% and 80 ± 4% in case of soil matrix. Co-inoculation with the biosurfactant-producing bacterium (*P. aeruginosa* strain DHT-GL) in soil matrix enhanced both the rate and the extent of diesel degradation by *P. putida* strain 5a1. After 20 days of incubation the total degradation by the co-culture was higher than the arithmetic sum of the degree of degradation by individual microbe. When the crude biosurfactant was added with strain 5a1 in aqueous medium, after 20 days around 85 ± 2% degradation in diesel oil content was observed. However, no significant impact of biosurfactant addition was observed in case of strain DHT-GL. A primary goal for conducting parallel experiments with exogenously

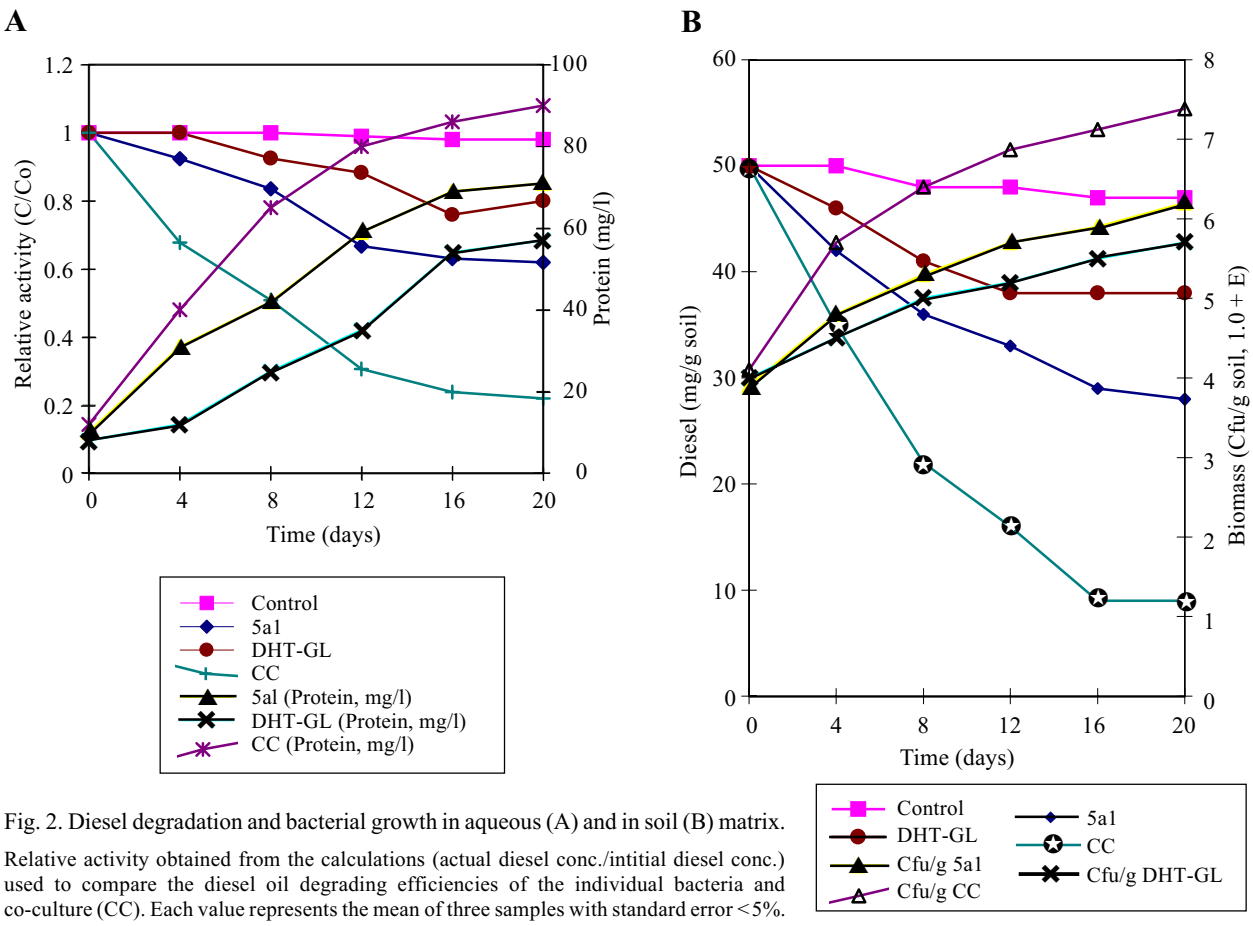

Fig. 2. Diesel degradation and bacterial growth in aqueous (A) and in soil (B) matrix.

Relative activity obtained from the calculations (actual diesel conc./intitial diesel conc.) used to compare the diesel oil degrading efficiencies of the individual bacteria and co-culture (CC). Each value represents the mean of three samples with standard error <5%.

added biosurfactant was to check the hypothesis where *P. aeruginosa* strain DHT-GL would be releasing biosurfactant in co-inoculated experiments. The degradation patterns in aqueous co-inoculated experiments was reproduced by adding biosurfactant suggesting that biosurfactant produced by strain DHT-GL was responsible for the enhanced diesel degradation.

When the strain DHT-GL was co-inoculated with the cured strain of the strain 5a1, we could not find any significant enhancement in the degradation ($27 \pm 2\%$) but we could get E_{24} $69 \pm 2\%$. This indicates that possible interaction of two microbes is responsible for the enhancement of the degradation and strain 5a1 plays important metabolic role in the degradation of the diesel. When the mutant strain of the DHT-GL unable to produce biosurfactant was co-inoculated with strains 5a1, no enhancement of degradation as well as poor emulsification was observed, indicating possible role of the biosurfactant production in enhancement of degradation. This was further supported by enhancement of diesel degradation by the addition of crude biosurfactant. Abalos *et al.* (2004) also reported that addition of rhamnolipids produced by *Pseudomonas aeruginosa* AT10 accelerated the biodegradation of total petroleum hydrocarbons from 32% to 61% at 10 days of incubation.

Analysis of cell surface hydrophobicity. The strain DHT-GL has higher cell hydrophobicity (55%) than the 5a1 (29%). When both grown in co-culture the hydrophobicity of the mix culture increased to 68%, what is more than the hydrophobicity of the individual bacterium. This indicates that biosurfactant produced by the DHT-GL cause alteration in the cell hydrophobicity and increased cell affinity for hydrophobic substrate. Strain 5a1 cells growing in presence of hexadecane (2%) and glucose (2%) was collected by centrifugation, incubated (1% w/v) with the supernatant of strain DHT-GL (hexadecane and glucose as carbon source) for 4 hours in shaking condition and then BATH assay was carried out. We found enhanced hydrophobicities (around 29% increased) in the treated cells in comparison to the untreated cells. But when DHT-GL was incubated with 5a1 supernatant only little (7%) improvement was noticed. Similar finding was observed when the cells were incubated with crude biosurfactant (data not shown) further indicating that the biosurfactant not only increased bioavailability of hydrocarbon by emulsifying the hydrocarbon but also increased cell affinity for hydrophobic substrate by altering the cell surface. Wouter and Dick (2002) found increased biodegradation of long-chains alkanes by addition of rhamnolipids, probably due to increased cell surface hydrophobicity of cellular envelope by rhamnolipids. Płaza *et al*., 2005 isolated two bacteria from oil contaminated soil showing adhesion to hydrocarbon and bioemulsifiers production.

Discussion

The co-culture of test organisms, strain DHT-GL and 5a1 and addition of crude biosurfactant exhibited improved degradation capacities in both soil and aqueous systems in a reproducible fashion. The emulsification studies shows that the *Pseudomonas aeruginosa* produces the biosurfactant under experimental condition. It is likely that the surfactant in some way enhanced cell-cell interactions or change the surface properties of each other or increased bioavailability which resulted in an increased rate and extent of diesel degradation. Lower diesel oil degradation in microcosms inoculated with cured strain of 5a1, suggested that the biosurfactant-producing strain contributed to diesel oil degradation through an indirect mechanism rather than degrading diesel directly and the strain 5a1 play important role in the degradation. The results of this study, although preliminary, illustrate species-specific commensal interactions between contaminant-degrading and surfactant-producing bacteria resulting in the overall enhancement of diesel degradation. Enhancement of diesel oil degradation by addition of crude biosurfactant ruled out the fact that the improved diesel degradation was due to synergistic catabolic routes in the two test strains rather than due to enhanced cell-cell interactions or increased diesel bioavailability. The overall impact of addition of a surfactant on biodegradation depends on how the basic diffusion pathways are altered and whether the surfactant itself affects the cells. If the surfactant is neither toxic nor a growth substrate, it can either increase the rate of biodegradation by carrying hydrocarbons in relatively accessible micelles or it can decrease the rate by inhibiting the adhesion of cells to the hydrocarbon-water interface. The overall impact depends on the importance of each pathway. The presence of surfactants at concentrations above the critical micelle concentration does inhibit adhesion of bacteria to the surfaces of droplets of liquid hydrocarbons and thus inhibits biodegradation (Ortega-Clavo and Alexender, 1994). Surfactant can dissolve hydrocarbon by forming the micelles into aqueous solution (Rosenberg and Rosenberg, 1981). Direct interactions between cells and micelles can occur. A number of species of bacteria are able to degrade liquid hydrocarbons after adhering to the surfaces of droplets (Dahlback *et al.*, 1981; Rosenberg and Rosenberg, 1985; Malachowsky *et al.*, 1994). This direct

contact between a bacterial cell and a target hydrocarbon can significantly increase the rate of diffusion into the cell, thereby enhancing growth and increasing the apparent rate of dissolution of the hydrocarbon. Surfactants are known to change the surface property of the hydrocarbon degrading bacteria and resulting in altered degradation of the contaminant (Neu, 1996; Stelmack *et al.*, 1999; Zinjarde and Pant, 2002). The sorption of surfactants to bacteria and to interfaces can either enhance or inhibit adhesion, depending on the nature of the surfaces and the surfactant itself. Recently, Calfee *et al*., 2005 demonstrated importance of a bacterial surfactant in the solubilization and bioactivity of a cell-to-cell signal. The alterations of surfaces depend only on the concentration of free surfactant. Results from the present study indicates that the biosurfactant produced by one bacteria not only emulsify the oil but also change the adhesion of the hydrocarbon to cell surface and result in the increased degradation of the diesel oil.

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