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GROWTH PATTERN OF HYDROCARBON-UTILIZING ISOLATES IN CHEMICALLY DEFINED MEDIUM

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ABSTRACT

Adhesion of five different bacterial strains, isolated from oil-contaminated soil, on suspended droplets of Jet A fuel was different but not absolutely parallel to the observed growth rates of the strains on this fuel. Preconditioning to hydrocarbons as well as direct aeration of the culture medium significantly increased growth rates of the different strains. Jet-A fuel, and motor oil were found to serve as a source of carbon and energy for each of these strains. Conversely, brake fluid did not support growth of any of these strains. The growth of one strain Arthrobacter globiormis (SBI-5) on hydrocarbons predominated over the otther four. Growth of SBI-5 on Jet-A fuel or motor oil was accompanied by production of an emulsifying agent. Growth rate of SBI-5 was monitored by observing optical density (OD_{546}) and total protein in liquid cultures as well as reduction of 2,3,5-triphenyl tetrazolium chloride (TTC) to formazan in liquid and agar cultures. The latter measurements by colorimetry was found to be faster and simpler for screening biodegradation.

INTRODUCTION

Several laboratory studies have examined the capability of micro- organisms to degrade organic solvents (1,2), pesticides (3) and petroleum hydrocarbons(4,5). Petroleum hydrocarbons are well suited to biological treatments, and "in situ" bioremediation has been attempted most frequently on this type of contaminants(6). Both aerobic(7,8) and anaerobic(9) biodegradation have been shown to reduce the concentration of several components of petroleum hydrocarbons. Several factors, such as pollutant concentration, active biomass concentration, temperature, pH, availability of organic nutrients, electron acceptors, and microbial adaptation, influence the rate and extent of biodegradation of organic pollutants. In a recent review, Van der Meer et al.(10) have reviewed the molecular mechanisms of genetic adaptation to xenobiotic compounds by the following responses : (a) induction of specific enzymes, (b) growth of a specific subpopulation of a microbial community able to take up and metabolize the substrate, and (c) adaptive selection of mutants with altered enzymatic specificities or novel metabolic activities.

The work reported below used 2,3,5-triphenyl tetrazolium chloride (TTC) as a generalized growth indicator(11). Cultured cells capable of catabolizing the test substrate reduce TTC to produce a deep red formazan, whereas cells failing to catabolize the test substrate remain uncolored.

In the present investigation, five bacterial strains isolated from soil have been studied for their ability to utilize Jet-A fuel, motor oil and brake fluid as a sole source of carbon and energy. The most promising isolate Arthrobacter globiformis (SBI-5) was also studied to

determine its potential to utilize different hydrocarbons to support growth.

EXPERIMENTAL

1. Microorganisms :

Five strains of aerobic bacteria with the potential of degrading hydrocarbons were isolated by enrichment culturing from a motor oil dumping site in Cheshire, CT, USA, using shaking flasks coated with an inside layer of asphalt as the sole carbon and energy source. The isolated microorganisms were identified according to Buchanan et al (1974)[12], and by using Enterotube II microbial identification kits and Oxi/Ferm tubes produced by Roche Diagnostic Systems, Inc., Montclair, NJ. The code names, genera and species of the isolates are listed in Table 1.

2. Culture media :

Those employed were nutrient agar, nutrient broth, tryptic soy-broth (TSB/Difco), a chemically defined minimal salt medium, MSB-1 [prepared as described by Pendrys [13] and supplemented MSB-1 (S-MSB-1) prepared by adding to 1 liter of MSB-1 3.5 ml of a solution contained the following salts [(g/L)(calculated as the anhydrous salts)]: $CaCl_2$, 0.389; $Cu(NO_3)_2$, 0.64; ammonium sulfate, 0.6; $ZnSO_4$, 0.718; $FeSO_4$, 0.696.

3. Preconditioning of microbial isolates :

Isolates were preconditioned by incubation with hydrocarbons (e.g. Jet-A fuel). Duplicate cultures were established, one with 2% (v/v) Jet-A fuel in TSB and another without jet fuel. The cultures were incubated at 30°C in a gyrating water bath (150 rpm) for 24 hours. The grown cells were separated by centrifugation at 4°C, washed twice with sterile water and resuspended in sterile MSB-1 medium. The cell concentration was then adjusted for use in further investigations.

4. Bacterial adherence to Jet-A fuel (Measure of cell hydrophobi-city) :

Samples from preconditioned bacterial suspensions (20 ml) prepared as described above were placed in 50 ml Erlenmeyer's flask, Jet-A fuel was added to give concentration of 2% (v/v) of hydrocarbon. The mixtures were agitated (150 rpm) for different times (up to 20 hours) on a shaker water bath at 30°C. After these incubation periods, the suspensions were allowed to stand for 10 minutes to separate the two liquid phases. Then the optical density (OD_{546}) of the aqueous phase was determined against a sterile MSB-1 medium. During settling, drops of oil previously suspended in the aqueous phase carry adherent cells out of the aqueous phase. The extent of bacterial adherence to Jet-A fuel droplets was therefore indicated by the decrease in the measured optical density at 546 nm (OD_{546}) of the aqueous phase.

5. Culturing microorganisms in shaked/ aerated flasks :

Generally, 2.5 ml of preconditioned microbial suspension were used to inoculate 250 ml chemically defined medium (e.g. MSB-1) in 500 ml Erlenmey-

Table (1) : Identified SBI microbial isolates

Code Name	Genus	Species
SBI-1	Acinetobacter	calcoaceticus
SBI-2	Pseudomonas-like	Group 2K-1
SBI-3	Pseudomonas-like	Group 2K-1
SBI-4	Pseudomonas	cepacia
SBI-5	Arthrobacter	globiformis

er flasks. The carbon source (e.g., Jet-A fuel) was then added to the flasks to produce a concentration of 2.5% (v/v). The flasks were closed with rubber stoppers fitted with a gas inlet tube and a reflux condenser. To aerate the cultures, sterile, humidified air was directly sparged (about 1 volume of air/volume culture broth/minute) through the culture medium. The cultures were maintained at 30°C in a gyratory shaking water bath, (150 rpm) while sparging. Samples were withdrawn periodically and used for analysis as described below.

6. Growth of SBI-5 on different carbon sources as indicated by reduction of TTC :

The carbon sources employed include hexadecane, 1-hexadecene, Jet-A fuel, JP-5 fuel, eicosane, acetate, palmitate, corn oil, and ethyl alcohol. The foregoing substances were separately supplied as a sole source of carbon and energy in S-MSB-1 medium with or without 55 mg/L triphenyl tetrazolium chloride (TTC). Growth experiments in shaking flasks used four different concentrations (C_1 - C_4) of the carbon sources shown in Table 2.

6.1. Cultring microorganisms on chemically defined-agar plates :

A suspension of microbes in MSB-1 was streaked on the surface of TTC-containing agar (1.5% Agar Agar in S-MSB-1) and incubated at room temperature for about 10 days. TTC was added (55 mg/L) to sterile Petri dishes before adding agar medium. S-MSB-1-agar plates without TTC were used as controls.

6.2. Fermentation in shake flasks (no air sparging) :

Cultures containing different carbon sources at different concentrations (Table 2) were prepared using 125 ml shaking flasks, each containing 25 ml of S-MSB-1 with 55 mg/L TTC. Each shaking flask was inoculated with

Table (2): Carbon sources and their concentrations employed for culturing Arthrobacter globiformis SBI-5 in S-MSB-1 medium

Carbon	Concentrations of Carbon Sources			
	C_1	C_2	C_3	C_4
A. Liquid carbon sources, vol/vol. % in S-MSB-1 :				
Ethanol	2.5	1.25	0.625	0.31
Hexadecane	2.5	1.25	0.625	0.31
Jet-A fuel	2.5	1.25	0.625	0.31
JP-5 fuel	2.5	1.25	0.625	0.31
Corn Oil	2.5	1.25	0.625	0.31
B. Carbon sources, mMol solutions in S-MSB-1 :				
Acetate	125	62.5	31.25	15.6
Palmitate	2.5	1.25	0.625	0.31
C. A solid carbon source, g/L in S-MSB-1:				
Eicosane	7	3.5	1.75	0.875

200 µl of an SBI-5 cell suspension (OD_{546} = 1.8) and then incubated for 96 hours in a shaker water bath at 150 rpm and thermostatically controlled at 30°C.

6.3. Fermentation in shaked/aerated flasks :

Generally, 2.5 ml of microbial suspension (OD at 546 nm = 1), preconditioned cells, prepared as described above in section 3, were used to inoculate 250 ml chemically defined medium (S-MSB-1 containing 55 mg/L TTC as growth indicator) in 500 ml Erlenmeyer flask. Aeration, addition of carbon source as well as incubation of the culture media were performed as described above in section 5.

7. Assay of cell growth :

Cell growth in cultures was followed by monitoring optical density (OD_{546}), by determining soluble and total proteins and by determining total and free reducing sugars. Soluble and/or total protein were determined by the method of Bradford[14] using bovine serum albumin (BSA) as a standard. The quantity of reducing sugar in the cell-free culture broth was determined using the dinitrosalicylic (DNS) acid method according to Bernfield [15]. Total (mono and polysaccharides) reducing sugar, was determined after acid hydrolysis (5N HCl for 30 minutes in a boiling water bath) and neutralization with 5N NaOH. The concentration of total reducing sugar concentration was determined from a standard curve obtained from acid hydrolyzed dextran.

Cell growth in liquid cultures containing TTC as a growth indicator was followed by measuring optical density, protein concentration and reduction of TTC to formazan. Red colored formazan was extracted from the culture broth into heptane (one volume of heptane per one volume of culture medium) and quantified spectrophotometrically at 450 nm.

8. Assay of emulsification activity of culture broth :

To two ml of culture broth (precentrifuged at 6500 rpm for 5 minutes), 2 ml of 0.1M sodium acetate (pH 3.0) and 0.5 ml of Jet-A fuel were added as described before [13]. The mixture was agitated on a high speed shaker for 2 hours. The resulting emulsion was allowed to settle for 10 minutes and the absorbance of the emulsion was measured spectrophotometrically at 540 nm against a blank consisting of an emulsion prepared in the same way, except the test sample was composed of sterile culture medium.

RESULTS AND DISCUSSION

1. Bacterial adhesion to Jet-A fuel :

The data plotted in Figure 1 show that SBI-1 displays higher tendency to adhere to and get removed by Jet-A fuel droplets than do the other SBI isolates. A 54% reduction in optical density caused by SBI-1 cells in the aqueous media was observed within the first hour of mixing. Increasing the time of contact between the aqueous and organic phases to 20 hours did not significantly alter the cell's adherence to the Jet-A fuel. Microscopic examination revealed that SBI-1 tends to preferentially congregate around and adhere to the Jet-A fuel droplets.

Bacterial adhesion to the droplets of hydrocarbon fuel can be viewed as a measure of cell hydrophobicity. More hydrophobic cells are expected to have a greater tendency to adhere to oil drops. A correlation may exist between cell hydrophobicity and its tendency to produce surface active agent. Therefore, it would be interesting to determine whether changes in cell hydrophobicity during a growth cycle are related to changes in its tendency to produce surfactants over the same cycle.

2. Culturing of microbes on Jet-A fuel in aerated/shaking flasks :

Preliminary experiments showed that in the presence of Jet-A fuel, SBI-

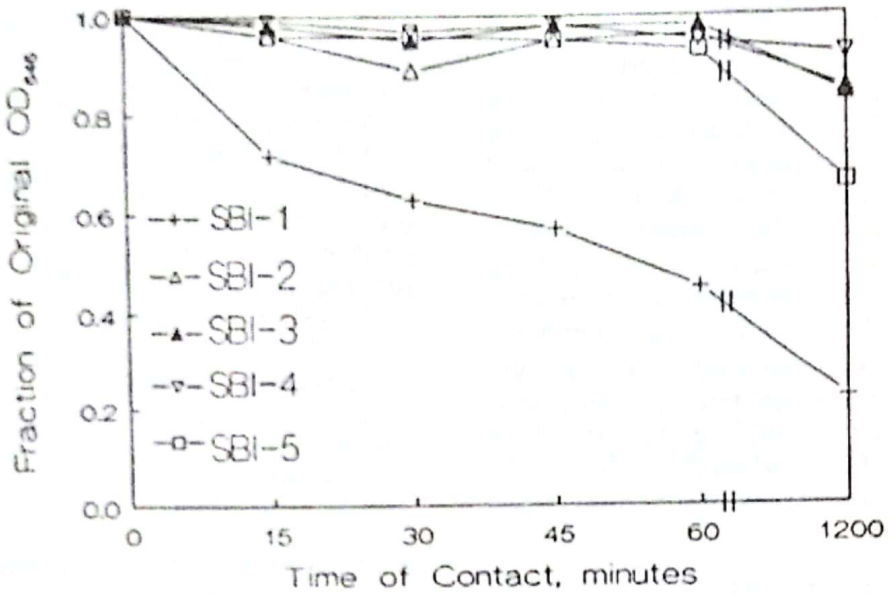

Figure (1) : Adhesion of SBI 1-5 to Jet-A fuel. Optical density of the aqueous phase was measured after emulsified oil droplets-collected cells therefrom and settled thereby remaining cells for 10 minutes.

Table (3): Maximum values of OD_{546} and protein concentrations measured[1] during incubation of SBI 1-5 isolates in mineral salts medium containing Jet-A fuel as a sole souce of carbon and energy

Isolate	Growth parameters	Incubation Conditions		
		Not sparged, not preconditioned	Sparged, not preconditioned	Not sparged, preconditioned
SBI-1	OD_{546}	0.14 (0d)	0.64 (7d)	0.17 (0d)
	Protein[2]	50 (4d)	192 (9d)	254 (5d)
SBI-2	OD_{546}	0.12 (0d)	3.5 (9d)	0.9 (9d)
	Protein	90 (4d)	288 (10d)	158 (10d)
SBI-3	OD_{546}	0.25 (6d)	0.514 (9d)	1.7 (9d)
	Protein	73 (6d)	312 (7d)	158 (10d)
SBI-4	OD_{546}	0.19 (6d)	2.45 (10d)	1.7 (9d)
	Protein	170 (5d)	202 (10d)	278 (9d)
SBI-5	OD_{546}	1.6 (9d)	18.58 (10d)	1.7 (9d)
	Protein	144 (6d)	1204 (10d)	336 (5d)

1,2,3 and 4 failed to grow significantly in shaking flasks (Table 3). In contrast, SBI-5 grew rapidly after a lag time of 125 hours. The microbes were also cultured in shaking flasks sparged with sterile air. In addition, some sparged cultures were inoculated with actively growing microbes (late logarithmic growth phase) preconditioned to Jet-A fuel. Non-preconditioned isolates were also grown in sparged shake flasks containing 2.5% Jet-A fuel as a carbon and energy source. Based on OD_{546}, and total protein level, only SBI-5 demonstrated notable growth (Table 3). It appears that air sparging is not enough to stimulate the growth of isolates SBI 1-4, when Jet-A fuel is the sole source of energy and carbon in the culture broth.

Preconditioned microbial cell suspensions of SBI 1-5 were also inoculated in unsparged shaking flasks which contained MSB-1 and 2.5% (v/v) Jet-A fuel as a sole source of carbon and energy. Based on optical density and total protein level, each preconditioned isolate grews on the Jet-A fuel (Table 3). Although preconditioned SBI-5 grew more vigorously than the other isolates, the amount of growth displayed by preconditioned SBI-5 without air sparging was not as great as that obtained with air sparging without preconditioning. Thus, aeration appears to be more important than preconditioning in these experiments.

When preconditioned cells of SBI-5 were grown in air sparged cultures, substantial decrease in pH and increase in optical density, emulsification activity, total protein or sugar concentrations were observed (Figures 2A and 2B). Emulsification activity closely follows the growth trends of SBI-5.

In the case of SBI-5, total protein closely corresponds to growth as determined by optical density. In the case of the other SBI isolates, it is not clear if changes in protein concentration are due to cell lysis or a small amount of cell growth. The growth and protein production of SBI-5 may be related to its tendency to adhere to the fuel droplets.

In order to develop the ability to use jet fuel as a sole source of carbon and energy, preconditioning to jet fuel was shown to be much less important for SBI-5 than for the other SBI organisms. SBI-5 had already grown substantially on the jet fuel after about six days regardless of whether or not it had been preconditioned with jet fuel. The other SBI isolates hardly grew at all without preconditioning. Even with preconditioning, the other SBI isolates did not start to grow until about five days had elapsed. These comparisons are based on observations of both optical density and total protein production.

3. Culturing SBI-5 on motor oil and brake fluid :

Preconditioned SBI-5 isolates were cultured in MSB-1 with motor oil or brake fluid, as sole source of carbon and energy. The optical density profiles in Figure 3 show that SBI-5 grow well in culture broth with motor oil. Moreover, the organism formed a fine emulsion in culture broth with motor oil, whereas cell-free controls did not. Motor oil dispersed into a fine emulsion only after long periods of culturing with SBI-5 corresponding to a lag time of 3-5 days as observed by OD_{546}. The emulsification activities of SBI-5 cultures appeared to follow its growth pattern and, therefore, it can be inferred that production of emulsification activity is growth related.

Total and soluble protein levels in SBI-5 cultures are also given in Figure 3. It is notable that total protein decreases during the first 24 hours and then increases over the remaining culturing period. Also, the decrease in the pH value parallels the increase in OD and appear to be growth related.

When SBI-5 cells were cultured on brake fluid, neither OD_{546} nor pH changed significantly over 14-days of

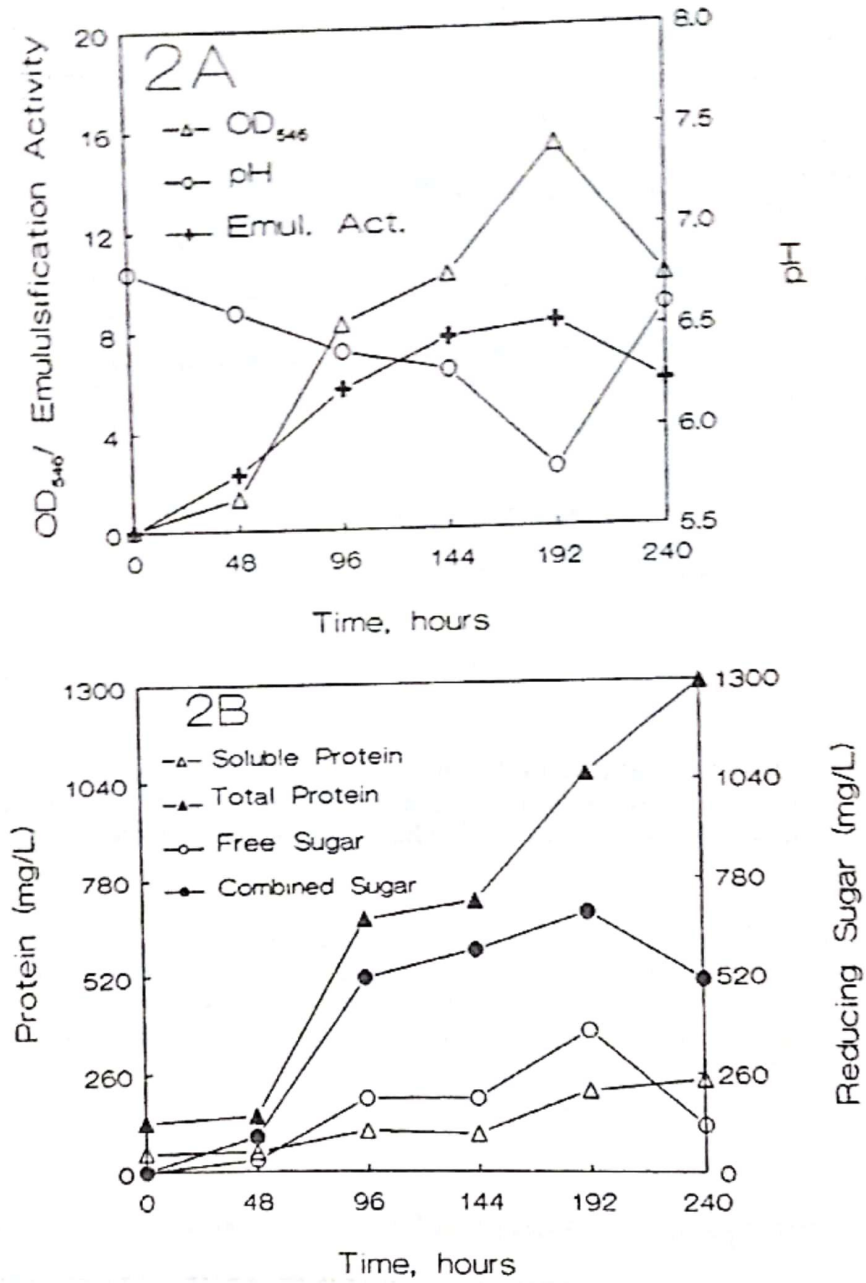

Figure (2) : Growth of Arthrobacter globiformis SBI-5 in minimal salts medium with Jet-A fuel as a sole source of carbon and energy. SBI-5 inoculum was preconditioned to Jet-A fuel and cultures were sparged with air. Optical density (OD_{546}), pH and emulsification activity are plotted in Figure 2A. The values of plotted emulsification activity are one tenth of measured values (EU/ml). Concentrations of soluble and total protein as well as free and combined reducing sugar (mg/L) are plotted in Figure 2B.

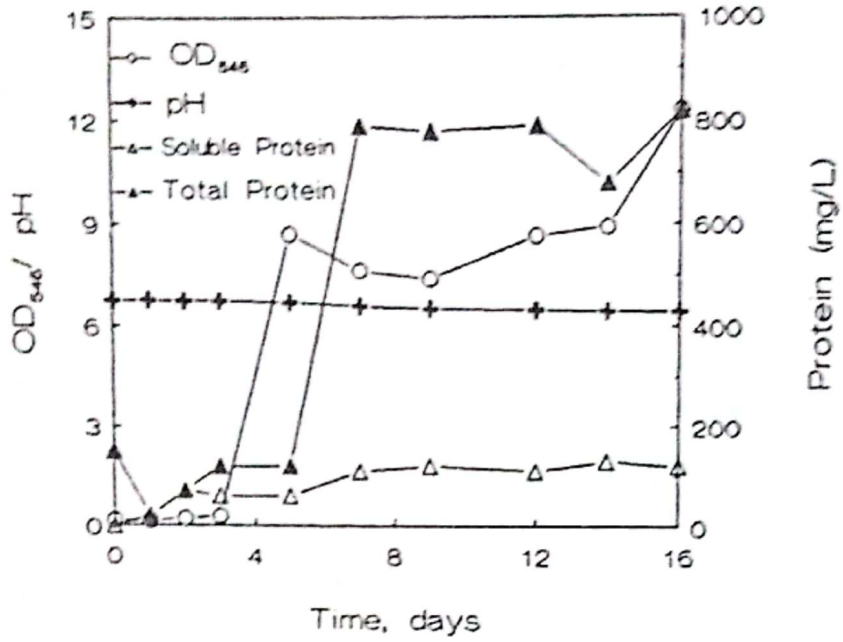

Figure (3) : Growth behavior of Arthrobacter globiformis SBI-5 in MSB-1 medium with motor oil as a sole source of carbon and energy. Precondioned inoculum was cultured in shake flasks with air sparging.

cultivation in sparged shake flasks. (data are not shown). However, very small increase in the total protein content of SBI-5 culture containing brake fluid was observed during this period. SBI-2,3 and 4 behaved similarly to SBI-5 when cultured with brake fluid. These results suggest that the brake fluid employed in the experiments may have contained an inhibitor of microbial growth such as a biocide.

Table (4) : SBI-5 growth on different substrates in agar with and without TTC

Carbon Source(*)	Control (No TTC)	TTC Plate
Ethanol	weak growth	light pink color
Hexanes	weak growth	no color change
n-Hexanol	no growth	no color change
Hexadecane	heavy growth	dark pink
1-Hexadecene	heavy growth	dark pink
Jet-A fuel	heavy growth	dark pink
JP-5 fuel	heavy growth	light pink

(*) Concentration of the carbon source was 2.5% (v/v).

4. Effect of different carbon sources on growth of SBI-5 with TTC as a growth indicator :

4.1. Growing SBI-5 on MSB-1 on several substrates in agar plates :

Results of this screening experiment are represented in Table 4. These results show that SBI-5 was able to utilize several hydrocarbons (hexadecane, 1-hexadecene, Jet-A and JP-5 fuels) as well as ethanol. Growth on hexanes was weak and no growth was observed on hexanol.

Earlier polarographic studies of TTC reduction showed that below pH 6 the colorless products are formed instead of red formazan. In strong alkali the formazan is formed spontaneously(16). Inhibition of color change in the presence of JP-5 or Jet-A fuels with SBI-5 may be related to acidic catabolic products of the natural hydrocarbons. These acids are expected to have large molecular weights and low diffusibility in agar. Acidic products might accumulate in the microenvironment of cells in agar thereby lowering pH to values at which formazan is not produced (pH< 6). Bochner and Savageau(11) reported that well isolated colonies in agar are red, whereas crowded colonies are white. TTC reduction had been stimulated by nutrient abundance and suppressed by nutrient limitation. This could explain why the red color of formazan was either faint or absent in presence of growth with plates containing Jet-A and JP-5 fuels with SBI-5. With other utilizable (hexadecane, 1-hexadecene and ethanol) carbon sources, either the catabolites have low molecular weights and high diffusion rates in the agar medium or abundant concentrations of carbon sources (original substrates or catabolites thereof) are available in the microenvironment of the growing bacterium. Thus, pH value does not fall to the point of inhibiting formazan production. Moreover, nutrients could remain better available for the growing cells thereby maintaining the function of their electron transport chain (11).

4.2. Growing SBI-5 in liquid media with TTC as indicator :

4.2.1. Cultures in shaked flasks :

Results in Table 5 based on the formation of formazan (spectrophotometric measurements at 480 nm), suggest the following hierarchy of prepared substrate: acetate > corn oil > hexadecane > eicosane> ethanol > Jet-A fuel > JP-5 fuel > palmitate. However, different hierarchies are indicated by total protein formation (acetate > palmitate > eicosane > corn oil > ethanol > hexadecane > JP-5 fuel > Jet-A fuel) or maximal OD_{546} (palmitate > corn oil > acetate and eicosane > ethanol and hexadecane > Jet-A fuel > JP-5 fuel).

Except for cultures containing acetate and palmitate, pH did not change significantly with the other carbon sources. The highest value of pH (7.63) was observed in a culture that initially contained 2.5 mM ammonium palmitate (C_1). A decrease in the starting palmitate concentration and a decrease in the pH of the culture after 96 hours incubation also appear related. In cultures containing different concentrations of sodium acetate, the pH, OD_{546}, and formazan concentration were all at their highest value (i.e., peak value) for the same acetate concentration (C_2 = 62.5 mM). Above or below this concentration, lower values were detected. These results indicate that growth parameters (e.g., protein concentration, pH, optical density, and formazan formation) are also influenced by the type of carbon source in the culture medium and its concentration.

4.2.2. Cultures in aerated/shaked flasks :

In agar, local variations in pH, substrate concentration and diffusion rates can affect the rate of reduction of TTC to form formazan. Such problem should be absent in aerated liquid medium containing TTC. Ethanol, hexadecane, 1-hexadecene and JP-5 fuel were tested as the sole carbon and energy source in aerated liquid cultures. Based on the

Table (5): Formazan production, OD, protein concentration or pH of SBI-5 after 96 hours in shaking culture with different carbon source concentrations.

Carbon source	Analytical parameters	Analytical values carbon source concentrations[1]			
		C_1	C_2	C_3	C_4
1. Ethanol	Formazan[2]	0.02	0.22	0.27	0.36
	OD_{546}	0.06	0.48	0.51	0.76
	Protein, mg/L	3	7	10	13
	pH	6.84	6.81	6.78	6.78
2. Hexadecane	Formazan	0.73	0.7	0.62	0.44
	OD_{546}	0.23	0.29	0.36	0.76
	Protein, mg/L	4	3	4	2
	pH	6.6	6.6	6.6	6.6
3. Jet-A-Fuel	Formazan	0.04	0.08	0.13	0.15
	OD_{546}	0.09	0.13	0.12	0.21
	Protein, mg/L	1.5	1.0	1.0	1.0
	pH	6.6	6.6	6.6	6.6
4. JP-5 Fuel	Formazan	0.06	0.08	0.08	0.10
	OD_{546}	0.16	0.09	0.11	0.16
	Protein, mg/L	2	2	2	2
	pH	6.6	6.6	6.6	6.6
5. Eicosane	Formazan	0.22	0.52	0.22	0.30
	OD_{546}	0.28	1.0	0.24	0.09
	Protein, mg/L	22	36	26	19
	pH	6.6	6.6	6.6	6.6
6. Corn Oils	Formazan	0.74	0.82	0.92	1.13
	OD_{546}	0.68	0.82	1.0	1.11
	Protein, mg/L	20	13	13	15
	pH	6.6	6.6	6.6	6.6
7. Acetate	Formazan	0.77	2.07	0.34	0.04
	OD_{546}	0.7	1.0	0.24	0.09
	Protein, mg/L	2	31	78	4
	pH	7.5	8.9	7.77	7.2
8. Ammonium palmitate	Formazan	0.09	0.02	0.01	0.02
	OD_{546}	1.76	0.89	0.47	0.27
	Protein, mg/L	60	32	20	16
	pH	7.63	6.9	6.8	6.8

1- For the concentration of each carbon source (see Table 2).

2- Formazan concentrations were determined by extracting the culture broth with n-heptane (1 volume/volume) and then measuring the absorbance of the organic phase at 480 nm against heptane extract of sterile S-MSB-1. Data in the Table are the absorbance units at 480 nm.

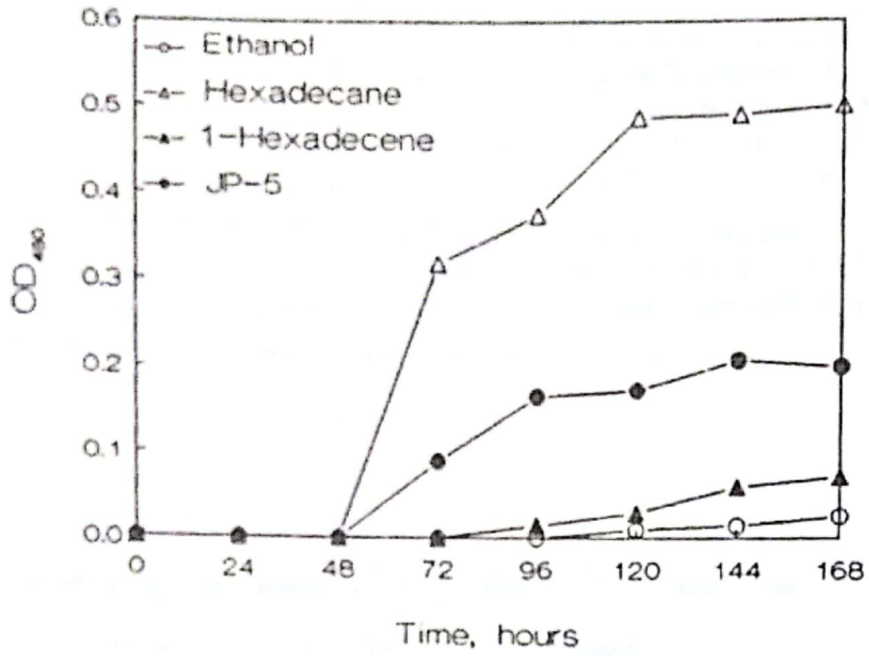

Figure (4) : Optical density of heptane layer due to the formed formazan (OD_{480}) during growth of Arthrobacter globiformis SBI-5 in presence of different hydrocarbons.

measured concentrations of "formazan" in liquid SBI-5 cultures, hexadecane proved to be the best carbon and energy source for SBI-5 (Figure 4).

To determine the local where formazan precipitates, SBI-5 grown in liquid cultures on hexadecane and JP-5 fuel in the presence of TTC was examined microscopically. The red formazan was not observed by light microscopy within the cytoplasm of the microbial cells. It was, however, observed in close external association with the microbial cells. In fact, formazan is easily recovered by washing the cells with 70% ethanol. These results suggest that TTC is reduced and formazan precipitates at the cell-surface or in the periplasmic space.

Based on the formation of formazan in presence of different substrates in sparged/shaked flasks, hexadecane was the superior limiting substrate. The superiority of hexadecane over 1-hexadecene which contains the same carbon skeleton may be related to oxidation at the double bond in 1-hexadecene either to form toxic metabolites (e.g. formate, carbon monoxide or an epoxide intermediate). Aerobic transformation of trichloroethylene by methanotrophs was proposed to progress through an epoxide intermediate that decomposed to CO and formate (17).

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نموذج نمو عزلات مستفيدة من الهيدروكربونات على وسط غذائى محدد كيميائيا

عبد الحليم محمود السيد - وفاء محفوظ محمود - ادوارد ديفز* و روبرت كوجلين*

قسم الميكروبيولوجى - كلية الصيدلة - جامعة الزقازيق - مصر

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اظهرت الدراسة وجود اختلافات فى خاصية التصاق خمسة عترات بكتيرية مختلفة معزولة من تربة ملوثة بالزيت على قطرات معلقة من وقود الجت (Jet-A) ولم تكن بالضرورة مماثلة لمعدلات النمو لهذه العترات على نفس الوقود. كما وجد أن تكيف العترات على الهيدروكربونات واستعمال التهوية المباشرة داخل المستنبت المحتوى عليها أدى الى زيادة معدلات النمو. كما استطاعت هذه العترات استخدام وقود الجت وزيت الموتورات كمصدر للكربون للنمو بينما لم يستطيع زيت الفرامل إحداث نمو لأى من العترات المستخدمة فى الدراسة. كما تبين أن نمو العزلة SBI-5 على الهيدروكربونات كان أفضل من باقى العزلات الأربعة. واثبتت هذه الدراسة أن نمو هذه العزلة على وقود الطائرات (Jet-A) أو زيت الموتورات يكون مصاحبا لإنتاج مادة فعالة كوسيط للاستحلاب. لقياس معدل نمو العزلة SBI-5 تم متابعة العتامة الضوئية عند موجة ضوئية طولها ٥٤٦ وقياس الكمية الكلية للبروتين المنتج فى المزارع السائلة مقارنة بقدرة العزلة على اختزال مادة ٢، ٣، ٥ ثلاثى كلوريد فينيل التترازوليم الى الفورمازان سواء فى المستنبتات السائلة أو الصلبة حيث تبين أن استخدام الطريقة الأخيرة كان اسرع وابسط لتتبع التحلل البيولوجى للمواد البيئة.