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Biodegradation of Bunker-A Oil by *Acinetobacter* sp. EL-081K

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Bunker-A oil-degrading microorganisms were isolated from a marine environment using an enrichment culture technique. The isolated strain EL-081K was identified as the genus *Acinetobacter* based on the results of morphological, culture, and biochemical tests. The optimal temperature and initial pH for bunker-A oil degradation were 25℃ and 7.0, respectively, including aeration. The optimal medium composition for the degradation of bunker-A oil by *Acinetobacter* sp. EL-081K was 10 ml/l bunker-A oil as the carbon source and 0.1% $(NH_4)_2SO_4$ as the nitrogen source. Under the above conditions, the biodegradability of bunker-A oil was 38% after 96 hours of incubation. The addition of detergent did not increase the bunker-A oil degradation.

Key words : *Acinetobacter*, Bunker-A oil, biodegradation

1. Introduction

In modern industry, oil is a very important source for energy and chemical synthetic materials. Accordingly, as global oil production continues to increases daily, the potential for oil reaching aquatic environments has also increased, particularly as a result of accidents involving supertankers.[1,2] Oil mainly consists of hydrocarbons(50 to 98%), plus variable amounts of oxygen, sulfur, and nitrogen. As such, oil is recalcitrant and acts as a pollutant when in contact with the environment.[3,4]

In 1946, Cladue E. Zobell reported that many microorganisms have the ability to utilize petroleum hydrocarbons as sole sources of energy and carbon and that such microorganisms are widely distributed in nature.[5] The potential for using such microorganisms in the degradation of oil and its constituents, thereby minimizing contamination due to oil leaks and spills, has prompted a number of investigators to study this process.[6~9] Several microorganisms, including *Pseudomonas* sp. and *Acinetobacter* sp., have been reported to degrade hydrocarbons in crude oil.[1]

This report describes the isolation and characterization of a bunker-A oil-degrading bacterium, *Acinetobacter* sp. EL-081K.

2. Materials and Methods

2.1. Isolation of Bunker-A Oil-Degrading bacterium

Seawater samples collected from Masan and Ulsan bay were inoculated into a liquid isolation medium in test tubes, then the samples were incubated at 30℃ in a shaking incubator(180 rpm) for 7 days. The cultures with positive bacterial growth were diluted and spreaded on isolation plate media and incubated at 30℃ for 7 days. The morphologically distinct single colonies were then screened for their ability to degrade bunker-A oil. The isolation medium for the bunker-A oil-degrading bacteria was composed of 0.5g NH_4Cl, 0.5 g K_2HPO_4, 1.0 g Na_2HPO_4, and 10ml bunker-A oil per 1,000 ml of aged seawater. The selected strain, EL-081K, was identified according to Bergey's Manual of Systematic Bacteriology[10] and the Manual of Methods for General Bacteriology[11] based on its morphological, culture, and biochemical characteristics.

2.2. Characteristics of Bunker-A Oil-Degrading Bacterium

The effect of temperature, pH, bunker-A oil concentration, nitrogen source type and concentration, and aeration on the rate of bunker-A oil degradation by a selected isolate were investigated. The temperature was controlled based on the submersion of a 250 ml Erlenmeyer flask in a circulating water bath(10~30 ℃). The effect of pH was determined using a mineral salt medium adjusted with HCl or NaOH to the appropriate pH value(pH 4.0~10.0). The effect of the Bunker-A oil concentration was investigated using a mineral salt medium containing different concentrations of bunker-A oil as the carbon source(2~22 ml/l). Different levels of aeration were obtained by varying the amount of medium in a 500 ml Erlenmeyer flask and keeping the agitation constant. The optimal conditions identified were then used to determine the effect of adding a detergent.

2.3. Assay of Cell Growth and Bunker-A Oil Degradation

The cell growth was detected by measuring the culture supernatant turbidity at 660 nm using a spectrophotometer(Shimadzu UV-240) after any residual bunker-A oil had been extracted from the cultures using CCl_4 as the solvent.[12] The residual bunker-A oil content in the CCl_4 extracts was estimated using an oil-content analyzer(Horiba OCMA-200).

3. Results and Discussion

3.1. Isolation and Identification of Bunker-A Oil-Degrading Bacterium

Three strains capable of degrading bunker-A oil were isolated from the seawater samples. Among them, the bacterial strain EL-081K exhibited the highest degradation activity for bunker-A oil. The taxonomical characteristics of the isolated strain EL-081K are presented in Table 1. The strain was found to be a non-motile, Gram-negative, and short rod-shaped bacterium, which was also catalase-positive, urease-negative, and strictly aerobic. It exhibited a negative reaction to H_2S production, amino acid decarboxylase, and methyl red tests, whereas a positive reaction to citrate utilization and Voges-Proskauer tests. Therefore, according to Bergey's Manual Systematic Bacteriology,[10] the isolated strain EL-081K was identified as a strain of the genus *Acinetobacter* and tentatively named *Acinetobacter* sp. EL-081K-14.

Table 1. Taxonomical characteristics of isolated strain EL-081K

Contents	Characteristics
Cell shape	Short rod
size(㎛)	0.4~0.8 × 1.8~2.2
Gram stain	-
Motility	-
Spore formation	-
Cell division	Simple division
Colony shape	Circular, entire, convex
surface	Smooth
color	White
opacity	Opaque
Gelatin liquefaction	-
Anaerobic growth	-
Catalase	+
Oxidation/Fermentation	oxidation
Growth at 22℃	+
β-galactosidase	-
Arginine dehydrolase	-
Lysine decarboxylase	-
Ornithine decarboxylase	-
Citrate utilization	+
H_2S production	-
Urease	-
Indole production	-
Voges-Proskauer reaction	+
Nitrate reduction	-
Pigment production	-
Growth on MacConkey agar	+
Gas production from dextrose	+

3.2. Characteristics of Bunker-A Oil Degradation

To increase the degradation of bunker-A oil, the growth conditions of *Acinetobacter* sp. EL-081K were investigated. The effect of temperature, as shown in Fig. 1, exhibited maximum growth at 25℃. The effect of pH, as shown in Fig. 2, exhibited maximum growth and bunker-A

oil degradation at pH 7.0. Below pH 6.0 and above pH 8.0 the growth and bunker-A oil degradation were seriously inhibited.

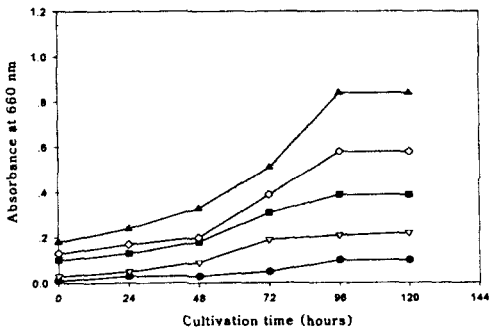

Fig. 1. Effect of temperature on the growth of *Acinetobacter* sp. EL-081K. Symbols : ●, 10℃ ; ▽, 15℃ ; ■, 20℃ ; ▲, 25℃ ; ◇, 30℃.

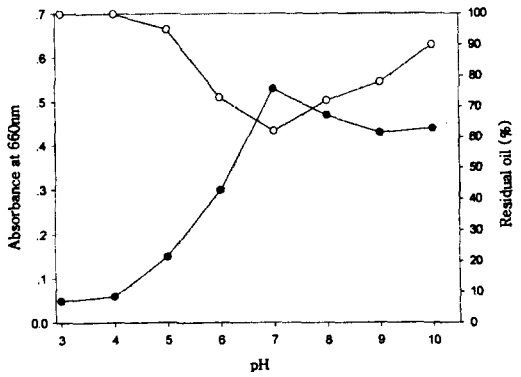

Fig. 2. Effect of pH on the growth and biodegradation *Acinetobacter* sp. EL-081K. Symbols : ●, growth ; ○, residual oil.

The effect of the bunker-A oil concentration on the growth of *Acinetobacter* sp. EL-081K was investigated using media containing different concentrations ranging from 2 to 22 ml/l. As shown in Fig. 3, a concentration range of 10～14 ml/l exhibited the best growth, whereas above 20 ml/l, the growth was significantly inhibited.

Various organic and inorganic nitrogen sources were investigated to determine the most suitable nitrogen source for growth and bunker-A oil degradation. 0.05% of each nitrogen source was added as the sole nitrogen source to media containing 10 ml/l bunker-A oil. As shown in Table 2, $(NH_4)_2SO_4$ was identified as the best

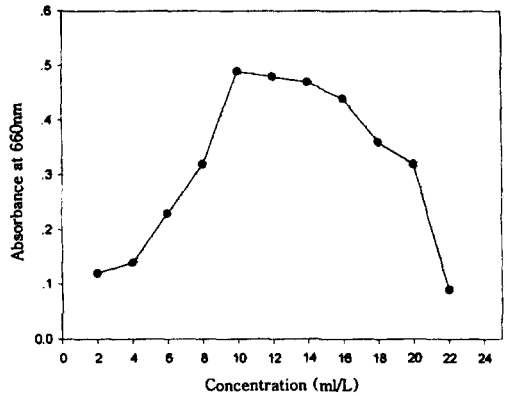

Fig. 3. Effect of oil concentration on the growth of *Acinetobacter* sp. EL-081K.

nitrogen source for growth and bunker-A oil degradation at an optimal concentration of 0.1%.

The effect of aeration, as presented in Table 3, showed maximum growth and bunker-A oil degradation with a medium volume of 100 ml in a 500-ml Erlenmeyer flask. A higher medium volume of 200 ml reduced the growth and bunker-A oil degradation, thereby indicating the existence of a critical concentration of dissolved oxygen.

Fig. 4 shows the relationship between the cell growth and the degradation of bunker-A oil during the cultivation of *Acinetobacter* sp. EL-081K in an optimal medium containing 10 ml/l phenol. After 96 h, the strain was able to degrade 36% of the initial bunker-A oil. The addition of various detergents to the optimal medium inhibited the bunker-A oil degradation(see Table 4).

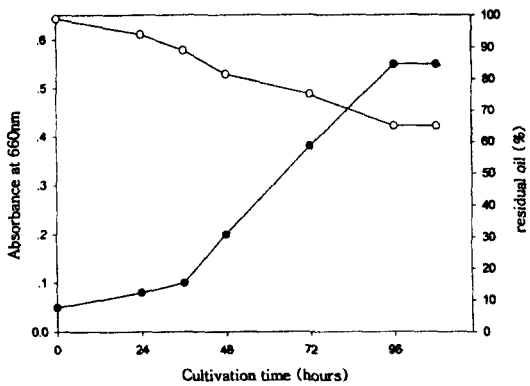

Fig. 4. Effect of oil concentration on the growth of *Acinetobacter* sp. EL-081K.

Table 2. Effect of nitrogen sources on growth and biodegradation of *Acinetobacter* sp. EL-081K

Nitrogen sources (0.05%)*		Growth (A_{660nm})	Biodegradation (%)
NH_4Cl		0.53	30
$(NH_4NO_3$		0.48	29
$(NH_4)_2SO_4$	0.05%	0.61	33
	0.1%	0.74	36
	0.2%	0.47	29
	0.3%	0.36	26
	0.4%	0.24	17
	0.5%	0.21	12
	1.0%	0.12	5
KNO_3		0.52	16
$NaNO_3$		0.56	26
Bactopeptone		0.60	17
Yeast extract		0.55	30
Beef extract		0.53	18
Casamino acid		0.63	22
Polypeptone		0.59	27
None		0.31	1

* Except for $(NH_4)_2SO_4$.

Table 3. Effect of aeration on growth and biodegradation of *Acinetobacter* sp. EL-081K

Volume of medium (ml/500ml shaking flask)	Growth (A_{660nm})	Biodegradation (%)
50	0.68	29
100	0.63	34
150	0.54	27
200	0.51	14
250	0.32	9
300	0.17	4

Table 4. Effect of detergents on biodegradation rate (%) of *Acinetobacter* sp. EL-081K

Concentration / Detergents	5ppm	10ppm	15ppm	20ppm
Span 40	31	32	26	18
Span 80	29	35	30	27
Triton N-42	5	12	9	2
Triton X-45	0	0	0	0
Triton X-100	3	6	3	1
Tween 40	21	24	20	15
Tween 80	17	13	11	14
Lauryl betaine	26	29	17	9

Acknowledgement

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