Anionic surfactants biodegradation in the presence of hexadecane and napthalene hydrocarbons

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Surfactant (XP- 100) biodegradation with yeast extract was studied in the presence of the pollution active hydrocarbons naphthalene and hexadecane. Surfactant biodegradation was faster with yeast extract than without. Increased surfactant concentration did not inhibit its biodegradation over the period studied. The addition of organic contaminants, on the other hand, enhanced surfactant biodegradation due to their synergistic effect. Naphthalene degraded more than hexadecane. The results outlined contribute to a better understanding of bioremediation mechanism and the fate of the compounds studied in the aquatic environment.

خلال الأعوام الماضية تزايد تلوث المسطحات المائية الناتج من استخدام المنظفات الصناعية وإلقائها في تلك المسطحات. والمراجع الحالية تشير إلى أن أبحاث قليلة وجهت لدراسة التحلل البيولوجي للمنظفات الصناعية في وجود الهيدروكربونات . ومن المعروف أن أحد المنتجات المحلية لشركة سوميد بالإسكندرية هو المنظف الصناعي XP 100. و لذلك فقد تم في هذا البحث دراسة التحلل البيولوجي للمنظف XP 100 مع خلاصة الخميرة في وجود تلوث بالهيدروكربون مثل الهيكساديكان و النفثالين. حيث أجريت هذه التجارب على حمأة من وحدة تجارب خاصة تعمل بالحمأة المنشطة. وقد وجد أن التحلل البيولوجي للمنظف XP 100 يكون أسرع في وجود خلاصة الخميرة وان زيادة تركيز ال XP 100 لا تمنع عملية التحلل البيولوجي. كما وجد أن النفثالين له قدره أعلى من الهيكساديكان على التحلل . لا شك أن النتائج التي تم التوصل إليها في هذا البحث تعطي قدرة اعمق على تفهم ميكانيكية التحلل البيولوجي ومصير هذه الملوثات في البيئة المائية.

Keywords: Anionic surfactant, Naphthalene, Hexadecane, Biodegradation, Bioremediation

1. Introduction

Water pollution caused by synthetic detergents has been increasing during the past few years due to their extensive use in domestic life, agriculture (Surfactants are used to increase dissolving of active gradient of pesticides in water) and industry. Synthetic detergents released into the aquatic system have adversely affected ecosystems. It causes foaming and increasing of organic and inorganic matter in the aquatic system [1, 2, 3]. Research devoted to the study of surfactant biodegradation in the presence of hydrocarbon contaminants has been notably sparse [4].

Anionic surfactants are amphipathic molecules of a polar/hydrophilic (head) and a non-polar/hydrophilic (tail). When added to water, a surfactant molecule may dissolve as a monomer, and/or is adsorbed to an interface with its hydrophobic end pointing away from the water [4].

Decantation and flotation are used for the treatment of highly oil-polluted wastewater (in the forms of emulsion and film). The biological processes could be used as a finishing technique for the treatment of dissolved hydrocarbons as well as the remaining emulsified oil [5]. El- Sherif and Mahmoud [6] found that there was a positive correlation between the increase of anionic phosphorus detergent pollution and the phytoplankton population in El-Mex Bay, Alexandria, Egypt.

The objective of this study was to evaluate the biodegradation of surfactant (X-100) in the presence of hexadecane and naphthalene hydrocarbons, using two different steps for microbial adaptation. Surfactant X-100 is a local petro- anionic sulfonate, produced by Somied Co. Alexandria, Egypt.

2. Materials and methods

Microbial activated sludge samples were collected from the Kafer El- Dawar local municipal wastewater treatment plant. Activated sludge was adapted to the studied hydrocarbons over 20 days, according to the

method of Imai [7] in a Sequencing Batch Reactor (SBR) laboratory system. The SBR system consists of one rectangular basin which was operated as both aeration tank and final settler in the time sequence. The volume of SBR reactor was 3.0 liter. The laboratory system was provided with two peristaltic pumps, the first was used as a feeding pump in the fill phase of operation, the second was used to draw treated wastewater at the end of the operation cycle. Automatic programmable timer was used in order to control the cycles of operation. The amount of air delivered in the SBR was sufficient to maintain the mixed liquor suspended solids in suspension and to maintain a dissolved oxygen level of approximately 2 mg/l. Daily analysis was done by sample (200 ml) purging from feeding mixed liquor from the reactor.

The mixed liquor was allowed to settle for 30 min. After aeration shut-off, the supernatant was siphoned off, leaving a mixed liquor (1.0 liter). The addition of mineral salts was carried out by mixing with either naphthalene or hexadecane in distilled water (1.0 liter), each compound in a separate reactor, and diluting up to 3.0 liter with distilled water. The addition of naphthalene or hexadecane was carried out on days 0, 5 and 10 allowing for 5 days acclimation for each concentration, being 52, 85, 120 mg/l for hexadecane and 5, 8, 11 mg/l for naphthalene. Each concentration was adjusted to the initial total suspended solids in the reactor.

The optimum hydrocarbon concentration was defined by monitoring CO_2 production corresponding to maximum bacterial activity. Surfactant adaptation was carried out by mixing an appropriate volume (0.5 l) from each reactor (1:1 ratio) as a collective overflow in a new clean reactor.

Hexadecane and naphthalene daily feeding was maintained constant but ascending concentration for surfactant for 5 days for final acclimation. The aeration was shut off and the contents of the reactor were allowed to settle for at least 3 h. The clarified supernatant was used as an inoculum. One millimeter of inoculum was added to 100 ml of the basal medium.

2.1. Stock solution preparation

Hexadecane was emulsified in water by a Janke and Kunkel Ultra Turrax (ultra senicator) 1 min with an average droplet diameter of oil in water of 11.4 μm. Naphthalene was prepared by stirring 30 g compound in water for 24 h to obtain complete dissolution (tested by gas chromatography).

2.2. Media

The basal medium composition contained 3 g NH_4Cl, 1 g K_2HPO_4, 0.25 g Mg SO_4 and 0.25 g KCl in 1 liter of distilled water. The basal medium (100.1 ml) was dispensed into a 500 ml Erlenmeyer flask and autoclaved [4]. Filter-sterilized $FeSO_4$ (final concentration $2x10^{-4}$%) and sterile yeast extract (final concentration $3x10^{-2}$%) were then added. Different types of media were used; one without yeast extract, one with yeast extract, and the third with naphthalene at concentrations of 4 and 8%. Another medium was with hexadecane at concentrations of 6 and 12%. Surfactant (XP-100) was added just before the inoculation of microorganisms.

Bacterial adaptation was carried out by rotating the flasks containing basal medium, surfactant and inoculum in a gyratory water bath shaker operating at 200 rpm, at 25°C for 72 h to provide aeration. Two successive adaptation regimes were made, and for each, inoculum (1.0 ml) from the previous step was transferred to the fresh medium.

2.3. Microbial degradation and analysis

The media were inoculated with adapted microbial culture (1.0 ml). Each set of basal media included different culture flasks containing surfactant (1-4%), naphthalene (4 and 8%) and hexadecane (6 and 12%).

Autodegradation of surfactant and organic contaminants was corrected against a control containing all basal medium components except microorganisms. Immediately following culture, sample (5 ml) analysis was undertaken at 0 h and 3, 5, 7, 9, 13 and 20 days. Microorganisms were removed from samples containing surfactant only by centrifugation (at 3500 rpm for 15 min) at

ambient temperature. The supernatants were then measured for uv absorption at λ_{267} nm (calibrated for basal medium and surfactant) for surfactant samples only. Samples from run 6-9 were analysed on a Hewlett-Packard 5890 GLC, equipped with a Flame Ionization Detector (FID), SE-54 capillary column (25m x 0.32mm, i.d.), programmed for an initial column temperature of 60°C (2min), increasing to 120°C (30°C/min), then to 220°C (3°C/min), with the final temperature being held for 5 min, using nitrogen as the carrier gas (1.5 ml/min, column flow), and detector make-up (30 ml/min). The detector and injector temperature were maintained at 300 and 220°C, respectively. Component identification and analysis were matched against authentic (BDH grade) standards [1].

3. Results and discussion

Pre-incubation microscopic examination (carried out in the National Institute of Oceanography and Fisheries) of the studied collective overflow reactor revealed a diverse population distribution, ranging from flocculated bacteria to distinctive predators such as protozoa and a few rotifers, which emphasizes the neutral effect of the studied hydrocarbon concentrations on the microbial population. The formation of a foam as well as green-blue pigments were observed in the hexadecane acclimation reactor. This phenomenon can be assumed to be due to the production of emulsifying factors during hydrocarbon fermentation by bacteria, as has been reported [4, 8].

The results of surfactant biodegradation are shown in table 1. Data from the first two runs indicate that yeast extract enhanced surfactant biodegradation. This may be due to a rapid yeast adaptation to the tested substrate and a higher rate of metabolism in the presence of supplemental nutrient. This phenomenon is evidenced by 2 data from run 2. Swindoll and Aelion [9] also found that several types of organisms might be required to degrade some xenobiotic compounds sequentially.

As each species may have its own particular nutrient requirements, a number of nutrients may be influencing metabolism by a heterogeneous population at any given time. Therefore, the concept of a single limiting nutrient may not be applicable to heterogeneous microbial populations.

Bayona et al. [10] studied linear alkylbenzenes (C_{11}-C_{14}) biodegradation by Pseudomonas spp. Pure culture, revealing that isomer biodegradation increases when a phenyl group is located closer to the end of the alkyl chain and not to the presence of a sulphonate group in the molecule.

3.1. Effect of surfactant concentration on biodegradation with yeast extract

Results of table 2 show that surfactant (1-4%) biodegradation of 88-90% over 13 days, except for run 1, in which biodegradation was

Table 1
Characteristics of different experimental runs

Run no.	Type	I	II	II	IV
1	Surfactant with yeast extract	1.0	0.25	-	-
2	Surfactant without yeast extract	1.0	0.46	-	-
3	Surfactant with yeast extract	2.0	0.23	-	-
4	Surfactant with yeast extract	3.0	0.9	-	-
5	Surfactant with yeast extract	4.0	0.30	-	-
6	Surfactant with hexadecane	1.0	0.10	6.0	2.1
7	Surfactant with hexadecane	1.0	0.08	12.0	1.8
8	Surfactant with naphthalene	1.0	0.15	4.0	0.9
9	Surfactant with naphthalene	1.0	0.11	8.0	0.7

I. Initial concentration of surfactant (%)
II. Final concentration of surfactant (%)
III. Initial concentration of organic contaminants (%)
IV. Final concentration of organic contaminants (%).

75%. This is in line with the work of Aronstein and Calvillo [11], who indicated that the influence of surfactant concentration on promoting its biodegradation depends on its toxicity to the microorganisms. The passive effect of surfactant-increased levels on its biodegradation may be due to the insensitivity of microbial communities to the surfactant.

Table 2
Effect of surfactant concentration on biodegradation

Study period (days)	Run 1	Run 3	Run 4	Run 5
0	1	2	3	4
3	0.47	1.2	2.3	3.0
5	0.38	0.71	1.1	1.9
7	0.29	0.40	0.62	1.1
9	0.27	0.31	0.48	0.62
13	0.25	0.23	0.29	0.40

3.2. Surfactant biodegradation in the presence of organic contaminants

The results of surfactant biodegradation in the presence of organic contaminants, runs 6 and 8 (table 3) show slow biodegradation (low concentration) compared with that of runs 7 and 9 (high concentration). Although contaminants as well as surfactant degradation occurred in runs 7 and 9, only microbial degradation may be invoked for organic, rather than surfactant, degradation.

Surfactants enhance the solubilization of organic contaminants, therefore increasing their bio-availability to bioremediation [12]. The surfactants themselves seemed not to be used for growth, but they stimulated bacterial growth on organic contaminants and greatly enhanced biodegradation by increasing the level of contaminants. This is supported by the finding of surfactant-increased biodegradation on the addition of yeast extract, compared with yeast-free treatment (runs 1 and 2).

Moreover, raising the surfactant level (from 2 to 4%) did not affect the biodegradation ratio (runs 3, 4, 5). Therefore, underlining the inactive role of surfactant on the microorganism population, table 3 indicates that the presence of organic contaminants apparently enhances surfactant biodegradation compared with that of run 1 (table 1). This may be suggested as being due to the synergistic effects of organic contaminants.

Naphthalene appears to be biodegraded more than hexadecane in presence of surfactant. Also, biodegradation at low concentrations of the studied organic contaminants was slower than at high concentrations, as judged by comparing runs 6 and 8 to runs 7 and 9, respectively, table 3. Putcha and Domach [13] studied the biodegradation of polycyclic aromatic hydrocarbons (PAHs) and the effect of micelles of the non-ionic surfactant, Triton X-100. They reported a complete bacterial degradation of petroleum hydrocarbons over 65 h. Surfactants however, have not always been shown to increase biodegradation. In this respect, the presence of surfactant Triton X-100 retarded biodegradation.

Table 3
Surfactant and organic contaminants biodegradation

	Concentration %							
Study period (days)	Surf.	Hex.	Surf.	Hex.	Surf.	Hex.	Surf.	Hex.
0	1%	6%	1%	12%	1%	4%	1%	8%
3	0.29	5.5	0.28	0.4	0.31	3.2	0.25	5.1
5	0.27	4.7	0.25	6.0	0.28	2.8	0.24	4.2
7	0.25	3.8	0.21	4.7	0.22	2.0	0.19	3.1
9	0.19	2.7	0.10	2.6	0.19	1.3	0.13	1.2
13	0.10	2.1	0.08	1.8	0.15	0.9	0.11	0.7

Surf. : surfactant; Hex.: hexadecane; Naph.: naphthalene.

4. Conclusions

Experimental results indicated faster surfactant biodegradation with yeast extract than without. The increase in surfactant concentration did not inhibit its biodegradation. It was notable that the presence of organic contaminants apparently enhanced surfactant biodegradation, suggesting a probable synergistic effect of organic contaminants. Moreover, slow organic contaminant biodegradation occurs on lowering its concentrations in the presence of surfactant. Such findings contribute to a better understanding of the bioremediation mechanism and the fate of the compounds studied in the aquatic environment.

References

[1] R.P. Eganhouse and I. R. Kaplan, "Extractable Organic Matter in Municipal Wastewaters. Hydrocarbons: Molecular Characterization", Environ. Sci. Technol. Vol. 16, pp. 541-883 (1982).

[2] P. Baskaran, G. Gopalakrishnasany and P. Sathiyabana, "Impact of Commercial Detergent (Nirma) on Feeding Energetics and Protein Metabolism in the Freshwater Teleost Fish, Orecohromis Mossambicus", J. Eototoxicol. Environ. Monit. Vol. 1 (1), pp. 31-40 (1991).

[3] A. A. Abd-Allah "Determination of Long-Chain Alkylbenzenes in the Sediment Samples from Alexandria Coast, Egypt", Toxicol. Environ. Chem., Vol. 47, pp. 83-88 (1995).

[4] G. Buitron and B. Capdeville, "Uptake Rate and Mineralization of Hexadecane and Naphthalene by a Mixed Aerobic Culture", Wat. Res., Vol. 27 (5), pp. 847-853 (1993).

[5] E., Arvin, B. Jensen and A. Gundersen, "Substrate Interactions During Aerobic Biodegradation of Benzene", Appl. Environ. Microbiol., Vol. 55, pp. 3221-3225 (1989).

[6] Z. M. El-Sherif and T.H. Mahmoud, "The Effect of Anionic Detergents on the Standing Crop of Phytoplankton in El-Mex Bey, Egypt", Bull. High Inst. Public Health XXI(3), pp. 631-638 (1991).

[7] H. Imai, Y. Murata and K. Endoh, "Degradation Rate of Hydrocarbon by Activated Sludge in the Low Concentration Range", J. Ferment. Technol., Vol. 58, pp. 541-549 (1980).

[8] P. Reddy H. Singh M. Pathak, S. Bhagat and H. Baruah, "Isolation and Functional Characterization of Hydrocarbon Emulsifying and Solubilizing Factors Produced by a Pseudomonas Species", Biotechnol. Bioeng. Vol. 25, pp. 387-401 (1983).

[9] C.M. Swindoll, and C. M. Aelion, "Influence of Inorganic and Organic Nutrients on Aerobic Biodegradation and on the Adaptation Response of Subsurface Microbial Communities", Appl. Environ. Microbiol. (Jan.), pp. 212-217 (1988).

[10] J. M. Bayona, J. Albaiges, A. M. Solanas and M. Grifoll, "Selective Aerobic Degradation of Linear Alkylbenzenes by Pure Microbical Cultures", Chemosphere Vol. 15, (5) pp. 595-598 (1986).

[11] B. N. Aronstein, and Y. M. Calvillo, "Effect of Surfactant at Low Concentrations on Desorption and Biodegradation of Sorbed Aromatic Compounds in Soil", Environ. Sci. Technol. Vol. 25 (10), pp. 1728-1735 (1991).

[12] S. Laha and R.G. Luthy, "Inhabitation of Phenantherene Mineralization of Nonionic Surfactants in Soil-Water Systems", Environ. Sci. Technol. Vol. 25 (11), pp. 1920-1929 (1991).

[13] R. V. Putcha and M.M. Domach, "Fluoresce Monitoring of Polyaromatic Hydrocarbon and Biodegradation and Effect of Surfactants", Environ. Progress, Vol. 12 (2), pp. 81-85 (1993).

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