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폐수거품에 의한 hexadecane의 생분해 가능성 평가

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Biodegradation Potential of Hexadecane by Sewage Foam

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Abstract

폐수거품은 전 세계의 폐수처리장에서 악취생산과 BOD의 증가 및 부유고형물의 원인과 같은 수많은 문제들을 야기시킨다. Actinomycetes가 폐수거품에 존재하는 주요 미생물군으로 알려져 있다. Hexadecane은 폐수, 토양, 바닷물과 같은 자연환경에서 오염물질로 고려되는 복잡한 기름 성분의 대표적인 구성성분이다. Hexadecane은 폐수로부터 얻어진 폐수거품에 의한 분해가능성을 평가하기위한 대표적인 모델 화합물로서 사용되었다. Gas chromatography (GC)/mass (MS)가 시료중에 있는 hexadecane의 분석을 위해 사용되었다. 본 연구를 통해서, hexadecane은 폐수거품에 의해 분해될 수 있는 것으로 사료된다. 멸균시킨 폐수거품시료를 포함하고 있는 control시료에서, hexadecane은 거의 분해되지 않았다. 반면에, 같은 방법에 의해 멸균되지 않은 폐수시료에서, hexadecane은 급속히 분해되었다. 덧붙여서, 농축폐수거품이 들어있는 시료는 3주 동안 건조된 폐수거품의 시료보다 hexadecane을 분해하는 데 더욱 효과가 높았다. 그러나, 3주 후에는 농축폐수거품의 시료에 남아있는 hexadecane의 농도는 건조된 폐수거품시료의 농도와 유사하였다. 요약컨대, 농축된 폐수거품시료와 건조된 폐수거품시료는 control시료와 비교했을 때, hexadecane의 급속한 분해를 보여주었다. 그러므로 본 연구의 실험결과를 통해서 건조된 폐수거품시료가 hexadecane을 비롯한 다른 chemical들로 오염된 장소를 정화하는 데 실제적으로 적용될 수 있을 것으로 사료된다.

I. Introduction

The viscous brown foam has caused numerous problems in activated sludge plants[1,2]. The viscous foam is different from other kind of foam in that it can not be collapsed by water sprays[3]. Pipes[3] reported a foam completely covering aeration tanks to depths of 2 to 3 feet. The build-up of a large foam causes safety problems due to the spill of foam out of the aeration tanks onto walkways as the foam dries. Another problem caused by the build-up of the foam is the poor separation of solids in the secondary clarifiers[4]. The problem results from the foam carrying over into the secondary settling tanks from the aeration tanks where it floats on the surface. Unless the foam is skimmed, the quality of the effluent will be low,

resulting in the increased levels of biological oxygen demand (BOD) and suspended solids (SS) concentrations in the effluent water[5]. Other problems such as gas binding of sludge recirculation pumps, entrapment in and fouling of gas collection pipe work, and tipping of digester covers by rapid expansion and collapse of the sludge volume when gas mixers are operated intermittenly[2] and are also associated with the build-up of the foam.

The brown viscous foam has been associated with the presence of microorganisms of the Actinomycetes group. In the United states, the genus *Nocardia*[5,6] has been identified as the most important for the development of the sludge foam. Lemmar and Kroppensted[7] identified *Rhodococcus rhodochronus* as the most common of the Nocardioform actinomycetes for the build-up of the foam from surveys of Western Europe sewage treatment plants.

Sewage foam-forming actinomycetes are aerobic filamentous microorganisms whose main ecological functions in soil is the decomposition of organic matter. Actinomycetes in the foam have physical properties similar to that in soil because most of the actinomycetes found in the sewage treatment plants were washed in from the soil[8]. Actinomycetes have a selective advantage over other bacteria in the sludge foam. The actinomycetes protects themselves from dryness, protects their pigment against ultraviolet (U.V.) radiation, and have the capability to store poly-β-hydroxybutyric acid (PHB) and polyphosphates which allow them to endure starvation[9].

Baumann *et al.*[10] proposed that the rapid growth of the actinomycetes was related to their adherence to interfaces which cause enhancement of the substrate availability and mean cell residence time. The excessive growth of actinomycetes in the treatment plants which have the branched and strongly hydrophobic hyphae enhance the floating resulted from the gas bubbles rather than the grease or oil.

Actinomycetes grow saprophytely, which is their ecological function in the cycle of organic matter. The dead cell in the sludge flocs is the source of nutrients for actinomycetes because nutrient concentration in water of the aeration tank is far below the optimum for the growth of actinomycetes[8]. Hydrophobic substrates are the sources of the nutrients which are selectively available to actinomycetes due to the hydrophobic hyphae of actinomycetes. Lemmer and Baumann[9] reported that several municipal plants produced the sewage foam in connection with the high loading of grease and oil in the primary effluents. The nutrients available to actinomycetes consist of recalcitrant compounds such as pesticides, and hydrophobic compounds such as grease or dead cells in the sludge flocs[8].

The control of the sewage foam has recently been major topic of the biology of the actinomycetes[7]. Sezgin and Karr[1] reported the technique used to control the sludge foam in the aeration tank and secondary clarifier. They reported that the sludge age reduced was effective in controlling the sewage foam. The control of the sewage foam by reducing the sludge age is based on the characteristic growth of the actinomycetes, because actinomycetes are the slow growers and the reduced sludge age decrease the chances of actinomycetes to increase their biomass. Pitt and Jenkins[2] also found that the best parameter to control the foam of the sludge is the reduction of the mean cell retention time to less than 6 days.

Sezgin and Karr[1] found that the reduction in the air flow rates decreased the coverage of the sludge foam. Actinomycetes associated with the sludge foam are the strict aerobes and thus the

reduction of air flow rates decrease dissolved oxygen concentration, resulting in the reduced growth rate of the actinomycetes. In addition, actinomycetes do not grow well at the low pH. Sezgin and Karr[1] found that the amount and extent of the sewage foam was decreased when the pH was lowered from 7 to 5 or 5.6. Pitt and Jenkins[2] found that controlling the sludge foam by chlorinating the returned activated sludge was successful, even though the chlorination is toxic to actinomycetes. However, Sezgin and Karr[1] reported that the dose of chlorine enough to reduce the populations of actinomycetes adversely affected other beneficial microbial populations in the activated sludge.

The purpose of this study was to evaluate the potential role of the sewage foam in the biodegradation of hexadecane which is one of the representative n-alkanes in the petroleum products. In this study, both the concentrated and dried sewage foams were used to evaluate the biodegradation of hexadecane. Eventually, this research may contribute to controlling or eliminating the troublesome sewage foam from the various sources of wastewater plants through the application of the sewage foam in the bioremediation of xenobiotics.

II. Materials and Methods

1. Sampling and sample processing

The sewage foam as sample for this experiment was collected from the aeration tank at the University of Florida's sewage treatment plant by putting 1L plastic beaker into the tank. The sewage foam was collected by skimming the top of the sewage and thereby removing approximately 500mL. The sample was covered with aluminum foil to prevent evaporation and stored at 15°C overnight to allow for settling of the sewage foam. Excessive liquid sewage was removed by centrifugation at 10,000 rpm for 5 minutes. The sample was again covered with aluminum foil and stored at 15°C for later use. Approximately 50mL of the concentrated sewage foam was dried by storing in the incubator at 50°C for 12 hours. After heating and reweighing, the dry weight of the concentrated sewage foam was 1.22% on a basis of weight which is equivalent to 12.2g of dry weight per kg of the sewage foam.

2. Experimental setup for biodegradation of hexadecane

Concentrated and dried sewage foams were added to 250mL of Erlenmyer flask containing 100mL of mineral media. The mineral media consisted of 4.8g of K_2HPO_4, 1.2g of KH_2PO_4, 1.0g of NH_4NO_3, 0.25g of $MgSO_4$, 0.04g of $CaCl_2$, and 0.001g of $Fe_2(SO_4)_3$ in 1L of deionized water. One thousand μg/mL of hexadecane was added to the sewage foam and mineral media. The amount of the sewage foam added to each flask was approximately 10g. Experiment for the measurement of the biodegradation potential of hexadecane was set up and performed during the 4 weeks. All the samples were set up by duplicate. The sample flasks were placed on a rotary shaker operating at 120rpm and 27°C. At the predetermined time intervals, the samples were centrifuged to separate the solid material from the supernatant. The supernatant was decanted and stored in Teflon-sealed glass containers at 5°C for later extraction and analysis using gas chromatography/mass spectrometry (GC/MS). The amount of dried sewage foam added to each flask was approximately 0.12g equivalent to the amount of concentrated sewage foam added to each flask.

3. Extraction procedure

The procedure used to detect the amount of hexadecane not biodegraded by sewage foam followed EPA Base/Neutrals and Acid Method 625. The procedure used to extract the hexadecane from the mineral media was as follows :

1. Contents of each sample bottle were poured into a 250㎖ separatory funnel. The pH of the sample was adjusted to a pH greater than 11 with a 10M sodium hydroxide solution.
2. Sixty milliliter of methylene chloride was added to the sample bottle, sealed, and shaken for 30 seconds to rinse the inner surface. The solvent was transferred to a 250㎖ separatory funnel and the sample was extracted shaking the funnel for two minutes with periodic ventilation to release excess pressure. The organic layer was separated from the water phase for a minimum of 10 minutes. In most cases an emulsion developed, this required stirring of the emulsion for 10 minutes and letting it stand (approximately 10 minutes) and then repeating this until the volume of the emulsion was reduced to less than one-third of the solvent layer. The methylene chloride extract was collected in a glass container with a Teflone lined screw cap.
3. A second 60㎖ volume of methylene chloride was added to the sample bottle and the extraction procedure was repeated a second time with the extract and they were poured in the glass container. A third extraction was performed in a similar manner.
4. Methylene chloride extract was removed by a Buchler rotary film evaporator at the pesticide research laboratory. The temperature of water bath was set at 30℃. Concentrating the methylene chloride down to approximately 3㎖ required 45 minutes per sample. Three milliliter of extract were transferred into the concentrating tubes. The flasks were rinsed with 1 to 2㎖ of methylene chloride and transferred into the concentrating tubes.
5. Further concentration of the methylene chloride extract to 3㎖ was preformed under a hood by blowing nitrogen over the extract. The concentrating tubes were tightly closed with a Teflone-sealed screw-cap and refrigerated at 5℃ for later analysis with GC/MS.

4. GC/MS analysis

GC/MS was a Hewlett-packard model 5806 and consisted of a 30 meter capillary column with a 32㎜ inner diameter with the MS scanning from 45 to 450 amu every 2 seconds. The run began at 180℃ for 1 minute and the temperature during the run was increased to 280℃ at a rate of 10℃ per minute. Running time was approximately 10 minutes per run with the hexadecane eluting from the column after approximately 5 minutes.

II. Results

After one week of incubation, the amount of biomass in the flask containing the concentrated sewage foam was increased based on the observation of turbidity in the liquid mineral media including hexadecane after one week of incubation, because turbidity is a sign of microbial growth. Also shown after two weeks of incubation were white particles adhering to the walls of the flask and became more noticeable as time went on. Similar microbial growth was also found in the flask containing

the dried sewage foam, even though turbidity and particles adhering to the walls of the flask were observed after only one week of incubation.

The mass spectrum of hexadecane for the control at time 0 (Fig. 1) matched that of hexadecane in the literature. The spectra band used in determining the loss of hexadecane was the 71m/z band which was the strongest band outside the band at 57m/z which is the peak of the solvent used in extracting hexadecane from the each sample (Fig. 1). The loss of hexadecane via volatilization over the four weeks period was 10% of the total hexadecane initially added (Table 1). Therefore, it appears that the total loss of hexadecane was due to biological degradation.

Experimental data for the degradation of the hexadecane in the liquid mineral media using the concentrated sewage foam are presented in Table 2. The samples were analyzed at the predetermined time intervals. The hexadecane was initially degraded quickly and as a result, after one week of incubation, 79% of the hexadecane was degraded. The data on the degradation of hexadecane using the dried sewage foam is presented in Table 2. As in the case of the samples of the concentrated sewage foam, the samples of the dried sewage foam were analyzed at the same time intervals as in the samples of the concentrated sewage foam. Drying of the sewage foam reduced microbial activity, with

Table 1. Loss of hexadecane in the liquid mineral media not containing the sewage foam during the four weeks of incubation.

Sample	Time (week)	Diution	Sample Size (mL)	Injection Time (Min.)	% Recovered
Liquid mineral media	0	1:100	1.1	5.0	100
Liquid mineral media	1	1:100	1.0	5.0	96
Liquid mineral media	2	1:100	1.2	5.0	93
Liquid mineral media	3	1:100	1.1	5.0	91
Liquid mineral media	4	1:100	1.0	5.0	90

Table 2. Degradation of hexadecane by control (autoclaved), concentrated, dried sewage foams during the four weeks of incubation.

Sample	Time (week)	Dilution	Sample Size (mL)	Time (min.)	% Recovered
Control	0	1:100	1	5.0	100
Control	1	1:100	1	5.0	92
Control	2	1:100	1	5.0	91
Control	3	1:100	1	5.0	90.7
Control	4	1:100	1	5.0	90.5
Concentrated Sewage Foam	0	1:1	1	5.0	100
Concentrated Sewage Foam	1	1:1	1	5.0	21
Concentrated Sewage Foam	2	1:1	1	5.0	17
Concentrated Sewage Foam	3	1:1	1	5.0	14.5
Concentrated Sewage Foam	4	1:1	1	5.0	13.5
Dried Sewage Foam	0	1:100	1	5.0	100
Dried Sewage Foam	1	1:100	1	5.0	44
Dried sewage Foam	2	1:100	1	5.0	30
Dried Sewage Foam	3	1:100	1	5.0	19
Dried Sewage Foam	4	1:100	1	5.0	16

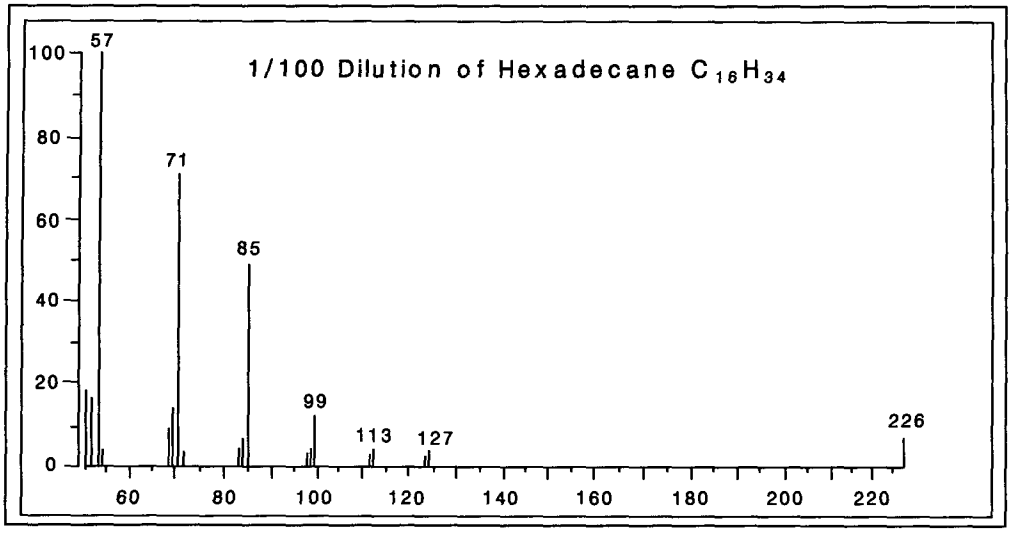

Fig. 1. Mass chromatogram of hexadecane at time 0.

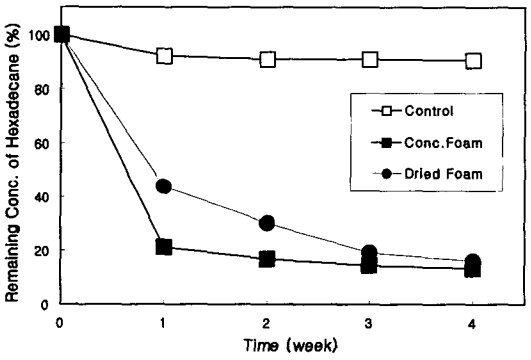

Fig. 2. Degradation of hexadecane by control (autoclaved), concentrated (conc.), dried sewage foams, respectively.

initially only 56% of the hexadecane being degraded after one week of incubation (Fig. 2). As time progressed, microbial activity of the dried sewage foam approached that of the concentrated sewage foam, with 81% of the hexadecane being degraded after three weeks of incubation (Fig. 2).

Ⅳ. Discussion

The degradation of hexadecane with the sewage foam from the wastewater plants is not surprising since *n*-alkane are generally considered to be the most easily degradable component of petroleum mixtures[11]. In this study, over 80% of the hexadecane were degraded by both the concentrated and dried sewage foams after three weeks. The specific roles of either actinomycetes or sewage bacteria which was responsible for the degradation of hexadecane were not investigated in this study.

Lemmer and Baumann[9] investigated the effect of hydrophobic substrate on actinomycetes in the sewage foam. They found that the addition of hexadecane resulted in the increased biomass for the actinomycetes. They attributed the increased biomass to the hydrophobic substrate constituting a food supply which was selectively available to actinomycetes because of the hydrophobicity of their hyphae.

Actinomycetes indigenous to soil find suitable environment in the sewage treatment plants from enrichment by hydrophobic substrates such as grease and oil which are associated with the build-up of sewage foam[9]. Rambeloarisoa *et al.*[12] studied bacteria from sea foam isolated at

the mouth of a petroleum refinery outlet that was for a long time polluted with hydrocarbons. They found that the isolated bacteria attacked the saturated hydrocarbon substrate quickly and after five days, 88% of the substrate was degraded. They also reported the increased degradation of petroleum mixtures prior to exposure from the refinery up-stream.

In this study, both concentrated and dried sewage foams were evaluated for the degradation potential of the hexadecane. It appears that the dried sewage foam is easier and cheaper to store than concentrated sewage foam from a practical point of view. However, at present, further extensive study is needed before sewage foam is applied for the bioremediation of a field site contaminated with petroleum products such as branched alkanes and aromatic compounds which are more resistant to biodegradation[11] and other kinds of xenobiotics. Furthermore, environmental factors such as water content, pH, and temperature should further be considered as much as to simulate the environment in nature.

V. Conclusions

1. Hexadecane was much more rapidly degraded by the sewage foam than by control.
2. Hexadecane can be biologically degraded by both the concentrated and dried sewage foams.
3. Concentrated sewage foam showed more rapid degradation of hexadecane than dried sewage foam during the first three weeks of incubation. At the end of four weeks of incubation, the remaining concentration of hexadecane in the dried sewage foam was similar to that in the concentrated sewage foam.
4. Dried sewage foam could be applied to the site contaminated with petroleum products for the purpose of cleaning up.

References

1. Sezgin, M. and Karr, P.R. : Control of actinomycetes scum on aeration basins and clarifiers, J. Water Pollut. Control Fed., 58, 972-977, 1986.
2. Pitt, P. and Jenkins, D. : Causes and control of *Nocardia* in activated sludge, J. Water Pollut. Control Fed., 62, 143-150, 1990.
3. Pipes, W.O. : Actinomycetes scum production in activated sludge processes, J. Water Pollut. Control Fed., 50, 628-634, 1978.
4. Strom, P. and Jenkins, D.: Identification and significance of filamentous microorganisms in activated sludge, J. Water Pollut. Control Fed., 56, 449-459, 1984.
5. Dhaliwal, B. S. : *Nocardia amarae* and activated sludge foam, J. Water Pollut. Control Fed., 51, 344-350, 1979.
6. Lechevalier, M.P. and Lechevalier, H.A. : *Nocardia amarae* sp., an actinomycetes common in foaming activated sludge, Int. J. Syst. Bact., 24, 278-288, 1974.
7. Lemmer, H. and Kroppenstedt, R.M.: Chemotaxonomy and physiology of some actinomycetes isolated from scumming activated sludge, System. Appl. Microbiol., 5, 124-135, 1984.
8. Lemmer, H. : The ecology of scum causing actinomycetes in sewage treatment plants, Wat. Res., 20, 531-535, 1986.
9. Lemmer, H. and Baumann, M.: Scum actinomycetes in sewage treatment plants-part 2. The effect of hydrophobic substrate, Wat. Res., 22, 761-763, 1988.
10. Baumann, M., Lemmer, H. and Ries, H.: Scum actinomycetes in sewage treatment plants-part 1. Growth kinetics of *Nocardia*

amarae in chemostat culture, Wat. Res., 22, 755-759, 1988.

11. Atlas, R.M. : Microbial degradation of petroleum hydrocarbons : an environmental perspective, Microbiol. Rev., 45, 180-209, 1981.
12. Rambeloarisoa, E., Rontani, J.F., Giusti, G., Duvnjak, Z. and Bertrand, J.C. : Degradation of crude oil by mixed population of bacteria isolated from sea-foams, Marine Biology, 83, 69-81, 1984.