Acta Medica Okayama

Volume 24, *Issue* 4 1970 *Article* 8

AUGUST 1970

Offensive-odor substance in the evil-smelling fish from the sea polluted by petroleum and petrochemical industrial waste. 1. Identification of offensive-odor substance

Masana Ogata* Yoshio Miyake†

*Okayama University,
†Okayama University,

Offensive-odor substance in the evil-smelling fish from the sea polluted by petroleum and petrochemical industrial waste. 1. Identification of offensive-odor substance*

Masana Ogata and Yoshio Miyake

Abstract

For the purpose to reveal the substance as the source of offensive odor of the fish from the sea facing petroleum and petrochemical industries, analyses have been made on the sea water, industrial wastes, offensive odor fish meat and the eels kept in the sea or industrial waste for a certain period, by means of gas chromatography, infrared, and ultraviolet absorption spectrophotometries and mass-spectrometry. Observations revealed toluene as a possible main source responsible for the bad smelling of the fish. Finally, by analysing the meat extract of the fish kept in the sea water containing toluene and by the same smell as that of the offensive odor fish from the off-shore of the industries, it has been definitely confirmed that toluene is the very substance that imparts the offensive odor to fish. The activated sludge process proved to be an effective method to remove toluene from the industrial wastes. A discussion was made on the aliphatic carbohydrate as the possible source of the offensive odor of fish.

*PMID: 4254201 [PubMed - indexed for MEDLINE]

Acta Med. Okayama 24, 471—481 (1970)

OFFENSIVE-ODOR SUBSTANCE IN THE EVIL-SMELLING FISH FROM THE SEA POLLUTED BY PETROLEUM AND PETROCHEMICAL INDUSTRIAL WASTE
REPORT 1. IDENTIFICATION OF OFFENSIVE-ODOR SUBSTANCE

Masana OGATA and Yoshio MIYAKE

Department of Public Health, Okayama University Medical School, Okayama, Japan
(Director: Prof. M. Ogata)

Received for publication, June 20, 1970

After World War II, petroleum and petrochemical industries have developed rapidly throughout Japan, and 20 years since then water pollution of the sea facing these industrial areas has raised a social problem with the industrial waste. In 1953, offensive-odor fish were found first among those from Ise Bay of Yokkaichi petroleum industrial district and these fish having obnoxious odor markedly increased in number by 1958 (1—4). In Mizushima district near Okayama, we have also newly developed petroleum and petrochemical industries since 1963, and the offensive odor fish appeared within the same year and in 1965 the offensive-odor extended over the residential districts about 10 km from the seashore. And it has become desirable to identify the offensive odor substances in the fish and establish an effective way to improve the situation urgently. Therefore, we tried to isolate and identify substance or substances by means of gas chromatography, ultraviolet and infrared absorption spectra, and mass spectrum. Besides these, the possibility of the degradation of the substance to the harmless by the activated sludge process was also studied. In this paper, the results obtained on the fish from the offing of Mizushima, eels kept in the sea water, and in the industrial waste are briefly reported with the analytical data of the sea water and the wastes.

MATERIALS AND METHODS

Offensive-odor fish caught in the sea facing the petroleum and petrochemical industries of Mizushima served as materials. The industrial waste from a few factories in the same area and some polluted sea water were also used for the analysis. All these fish and waste were frozen at $-20°C$ immediately after collecting.

Eels from other areas weighing 120—150 g were also used. They were kept in a tank containing sea water, (30 liters) and the industrial waste 1 liter at 20°C with air supply for one week. In another series of experiments, toluene and other organic solvents were given to the eels in tank, 1 ml each every day for seven days.

For gas chromatography the industrial waste and the polluted sea water were steam-distilled, and an equal volume of distillate, waste or sea water was taken. The distillate was warmed at 55°C in incubator while shaking, and the evaporated gas (20 ml) was put in the air sampler for gas chromatography.

To the offensive-ordor fish isolated and gatherd was added half volume of distilled water and homogenized with Waring Blender. The distillate abtained by steam distilling the homogenate, was warmed at 55°C. And gas phase on the distillate was taken for gas chromatography. The apparatus employed was Hitachi K 53, FID ditector, and the analysis was made in the conditions as follows; Column liquid phases; 1. 2. 3. This (cyanoethoxy) propane, support; Shimalite, mesh, 60—80, size of column; 3 mm × 2 m, column temperature; 60°C, carrier gas; nitrogen (40 ml/min), air pressure; 1.5 kg/cm^2, chart speed; 10 mm/min.

The fractions identified as the offensive-odor substance by gas chromatography was divided into 4 parts, the first one was taken into glass beads for the next gas chromatograph for the further fractionation in detail, the second one into carbon tetrachloride solution for infrared absorption spectrophotometry, the third one into dioctylphtalate (DOP) for mass spectrometry, and the last one into absolute ethanol solution for ultraviolet absorption spectrophotometry.

Infrared absorption spectrum was taken by infrared spectrophotometer Hitachi EPI-G3 with 1cm light path.

Mass spectra were taken by mass spectrophotometer, Hitachi, RMU-6 with evaporated gas at 120°C. Conditions of mass spectrometry are as follows; chamber volt; 70 V, total edission; 60 μA, target current; 70 μA, chamber temp; 200°C, evaporation temp.; 180°C.

Ultraviolet absorption spectrum was taken by spectrophotometer of Beckman DK-2A with 1.0 cm light path.

Industrial waste from oil refineries was collected for 7 days, for adapting microorganisms in the activated sludge to industrial waste. Then the activated sludge, 500 g in wet, 20 ml of 1.2 M potassium phosphate monobasic, 0.1 g ammonium sulfate, and 3 ml of 50 % corn starch solution (industrial waste of corn starch refinery company) were added to the petroleum industrial waste, mixed well, pH was adjusted to 7.2 with 10 % sodium hydroxide and then incubated at 37°C for one day. The gases from the waste before and after the treatment were analyzed by gas chromatography.

RESULTS

Gas chromatogram of the gas from offensive-odor fish (grey mullet, Mujil Japonicus), gave the peak which appeared constantly but could not be found in that from normal fish (Fig. 1); the retention time was 7.61

times as long as that of hexane. Beside this specificity to offensive odor fish, another inconstant peak appeared occasionally. These peaks are defined as offensive-odor peaks, and the substances as offensive-odor substance, because of their bad smelling in mouth.

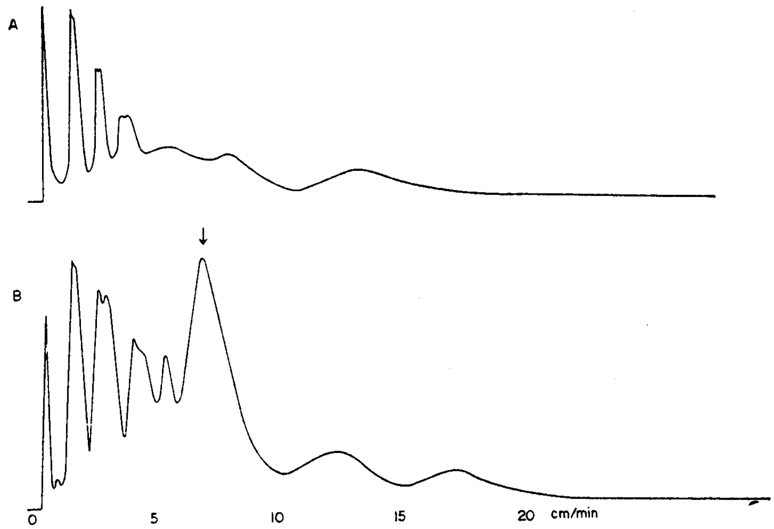

Fig. 1 Gas chromatograms of gases evaporated from normal and offensive-odor fish (grey mullet) caught in the sea. A. Normal fish, B. Offensive-odor fish. Arrow indicates the new peak appearing in the offensive-odor fish and thought to be offensive-odor substance (The first peak is due to air, in Figs. 1—4 and Figs. 8 and 9.).

Gas chromatogram of the gas from twenty eels kept in the offshore about 1 km from the exit of drainage of petroleum and petrochemical industries for 7 to 10 days gave the offensive odor peak and about 90 per cent of eels became offensive-odor fish. The peak was the same as that given by the gas from the sea water but it could not be found in the eels before keeping in the sea (Fig. 2). It showed also the same retention time (7.61) as that given by the sea water, and as that by industrial waste described later. and as that by grey mullet caught in the sea (Fig. 2).

The eels kept in the industrial wastes for a week, and the eel meat after boiling gave a bad smell. The gas chromatogram of the gas from these offensive-odor eel meat gave the peak identical with that given by the offensive-odor fish caught the offshore of the industries and the industrial waste (Fig. 3). The retention time of the peak was 7.61, the same as that of the peak given by the offensive-odor fish from the sea.

The isolation and identification of the offensive-odor substance from

Acta Medica Okayama, Vol. 24 [1970], Iss. 4, Art. 8

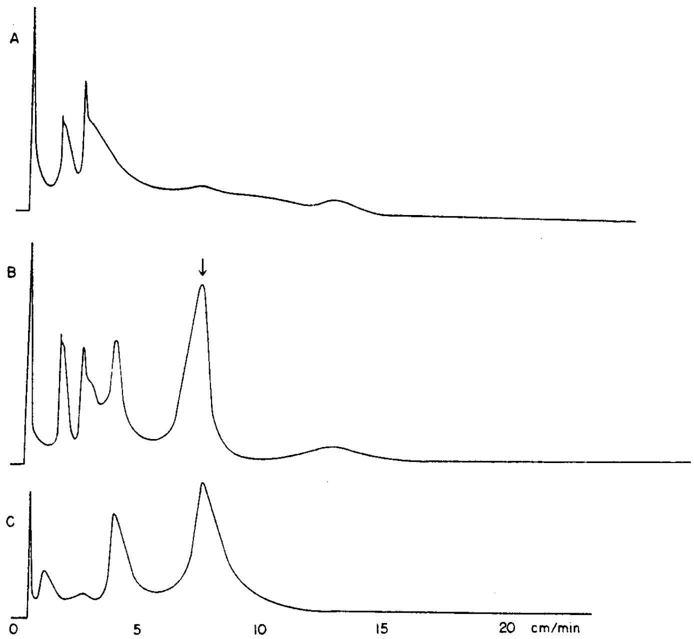

Fig. 2 Gas chromatograms of gas evaporated from the sea water and that from decoy eels cultured in the sea. A. Normal eels, B. Decoy eels. Arrow indicates new peak which has appeared, C. Sea water .

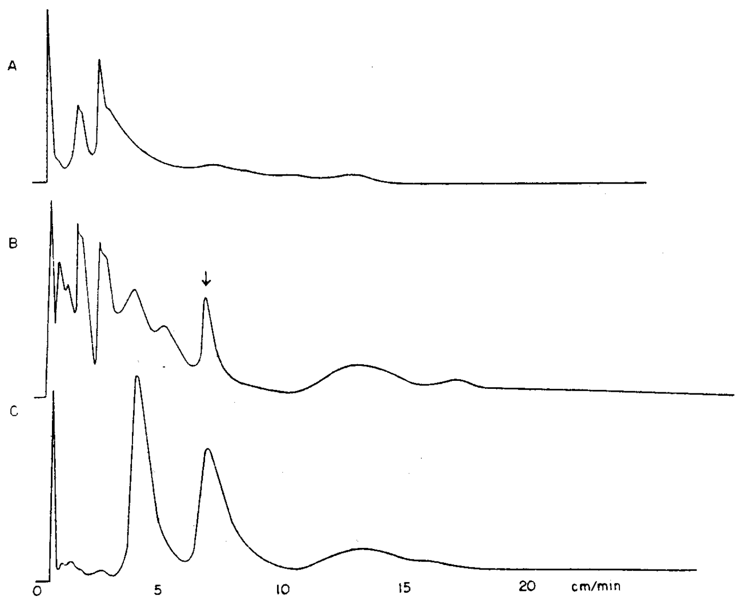

Fig. 3 Gas chromatograms of gases evaporated from petroleum industrial waste, eels cultured in the industrial waste and normal eels. A. Normal eels, B. Eels kept alive in the industrial waste, C. Industrial waste.

petroleum industrial waste and offensive-odor fish in the sea have been tried. That is the offensive-odor substance appeared at the peak having 7.61 of relative retention time was taken on the glass beads set in the U tube, warmed at 120°C in the oil bath and the evaporated gas was taken again on gas chromatograph. Thus the single peak or the offensive-odor peak was obtained. The retention time of this peak to hexane is also 7.61, which is the same as that of toluene (Fig. 4). The addition of the toluene to the industrial waste resulted in the increase in height of the peak to 7.61 of retention time, suggesting that the offensive-odor substance should be the one very similar to toluene.

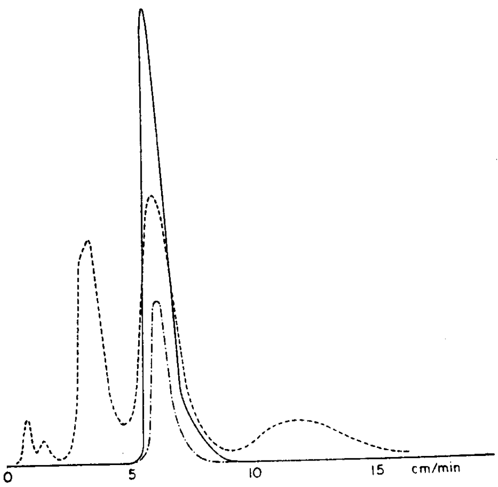

Fig. 4 Gas chromatograms of gases from distillates of industrial waste (dotted line) and offensive-odor substance isolated from distillate of industrial waste (solid line) by gas chromatographic fraction method and toluene (chain line).

Infrared absorption spectrum of offensive-odor substance isolated from the industrial waste in carbon tetrachloride by gas chromatograph gave the same absorption pattern as that of toluene, indicating the substance is toluene itself (Fig. 5).

Mass spectrometry of the offensive-odor substance from the industrial waste obtained being isolated with DOP evaporated by heating also gave the M/e value of parent peak, 92, and that of base peak was 91 (Fig. 6), as that of offensive-odor substance fractionated from the fish with bad smelling and yet corresponded to the molecular weight of toluene, 92, and to its M/e value (5).

Ultra-violet absorption spectra also showed the complete coincidence among those of the offensive-odor substances isolated from the industrial

Acta Medica Okayama, Vol. 24 [1970], Iss. 4, Art. 8

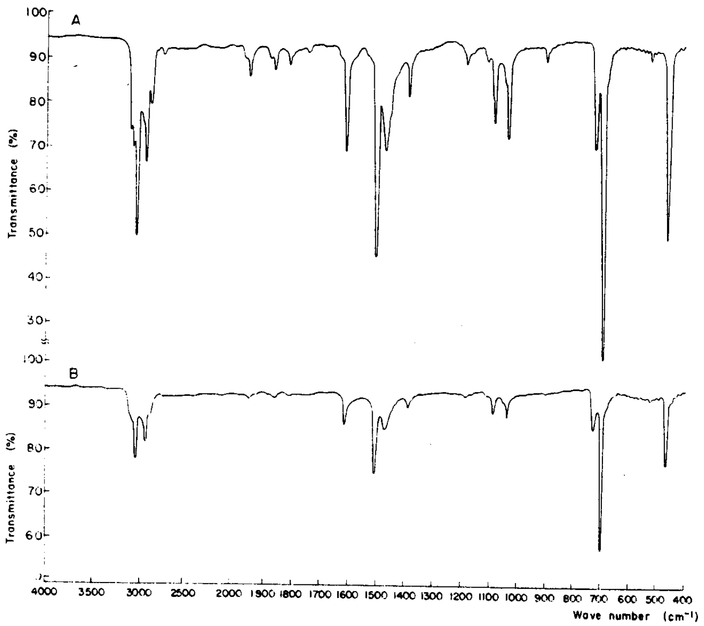

Fig. 5 Infrared absorption spectra of offensive-odor substance isolated from distillate of industrial waste by gas chromatographic fraction method (B), and toluene (A).

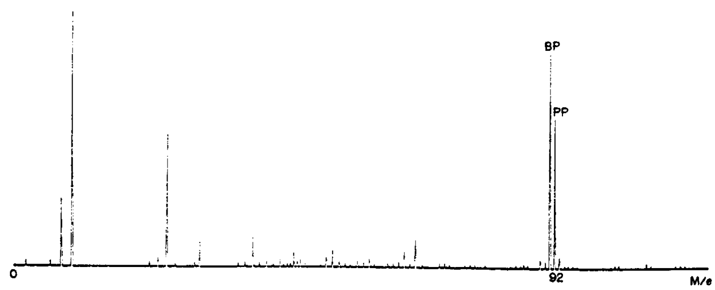

Fig. 6 Mass spectrograms of offensive-odor substance isolated from distillate of industrial waste by gas chromatographic fraction method. (P. P.; Parent peak, B. P.; Base peak)

waste of those from the offensive-odor fish caught in the sea with toluene (Fig. 7).

The eels kept in the sea water containing toluene for one week acquired the bad smell similar to that given by the offensive-odor fish from the sea. Even after cooking by boiling, the meat of the eels remained bad smelling. The gas chromatogram of the gas obtained from the eel meat gave the clear toluene peak (Fig. 8). The experiment indicates that

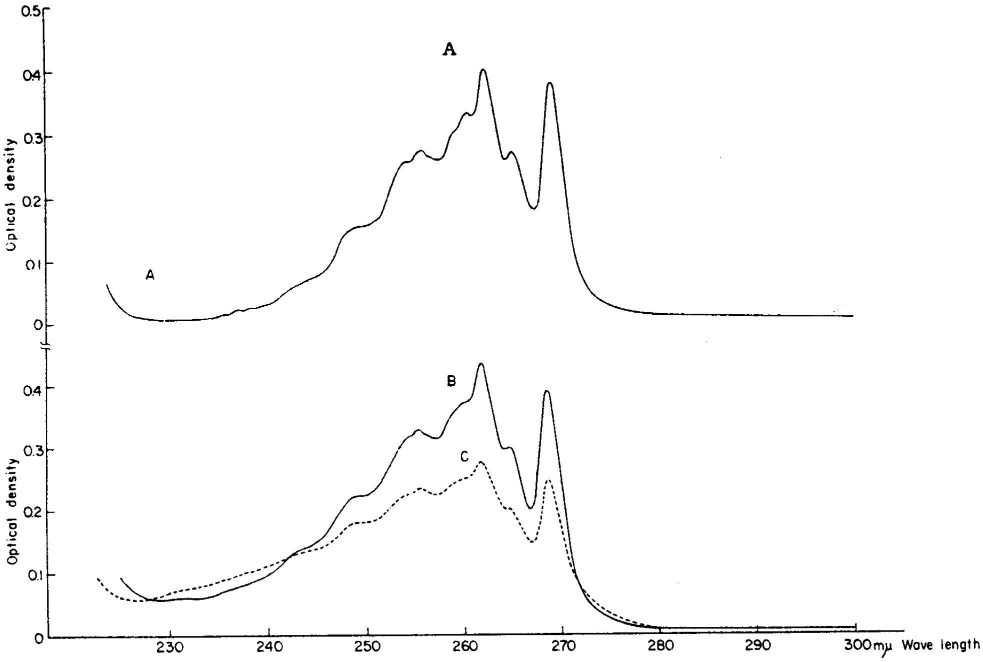

Fig. 7 Ultraviolet absorption spectra of offensive-odor substance (B) isolated from distillate of industrial waste by gas chromatographic fraction method and that (C) from offensive-odor fish, and toluene (A).

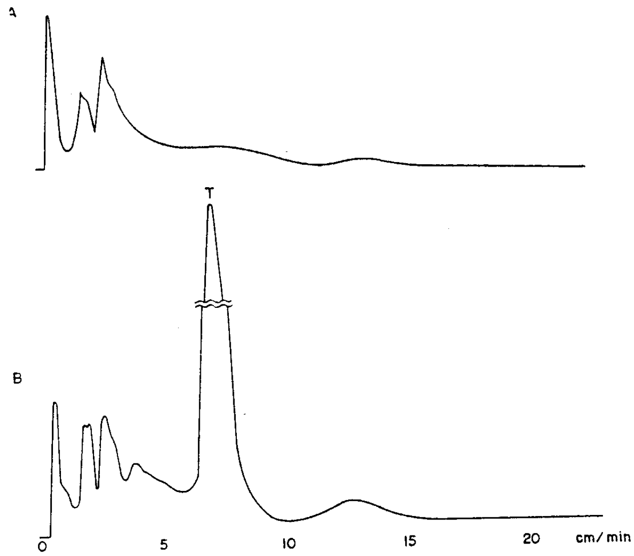

Fig. 8 Gas chromatograms of gases from eel muscle cultured in the sea water containing toluene. Symbol T indicates toluene peak. A. Normal eels, B. Cultured eels.

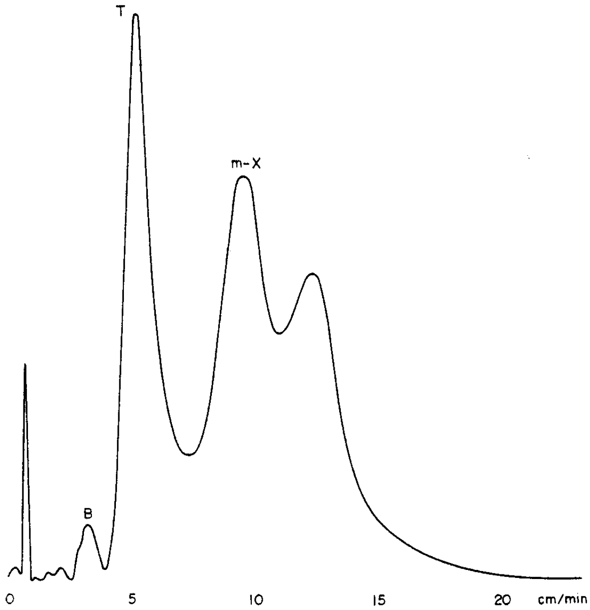

Fig. 9 Gas chromatogram of gas from eel muscle cultured in the sea water containing benzene, toluene, and m-xylene. Symbol B indicates benzene peak, T, toluene peak and m-X, m-xylene peak.

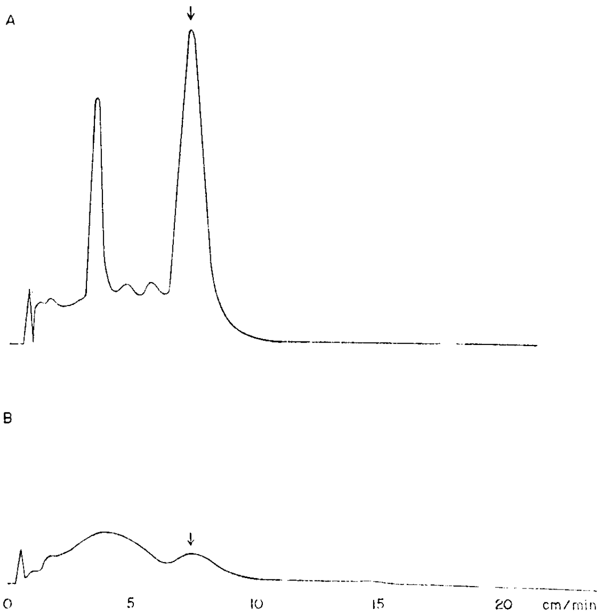

Fig. 10 Gas chromatogram of gas evaporated from industrial waste before and after treatment by activated sludge process. A. Gas chromatogram of gas before treatment. B. Gas chromatogram of gas after treatment. Arrow indicates the offensive-odor peak.

toluene dissolved in sea water is taken by the eels directly, infiltrates into the muscle through blood from brachia and it cannot be removed by boiling or cooking. Gas chromatographic observation on the eels cultured in the sea water containing about the same amount of benzene, toluene and m-xylene proved that toluene was most rapid in infiltrating into the fish, m-xylene was moderate and benzene was relatively slow (Fig. 9). These data identify definitely the offensive-odor substance from the industrial waste and offensive-odor fish to be toluene itself.

Gas chromatographic analysis of the industrial waste before and after the treatment with the activated sludge process revealed that the treatment is very effective to diminish the offensive-odor substance from the industrial waste of oil refineries, showing a marked decrease in the height of the peak of offensive-odor substance (Fig. 10), in gas chromatography, a decrease in the level of COD from 10.9 to 3.0 ppm and also a decrease in the grade of the threshold odor number from 10.0 to 1.6.

DISCUSSION

As mentioned above, the offensive-odor substance found in the offensive-odor fish caught in the offshore of the sea facing the petroleum industrial area and in the decoyed eels kept in the sea and in the sea water containing the petroleum industrial waste for some days, by which cause of offensiveo-dor, was isolated from the industrial waste and the sea water and offensive-oder fish. And the substance was identified as toluene by the analysis using gas chromatography, infrared and ultraviolet spectrophotometries and mass spectrometry comparing the data obtained on toluene itself and on the fish kept in the toluene containing sea water. The data indicate that the offensive-odor substance isolated from the fish comes from toluene contained in the petroleum industrial waste. Thus it is clear that the offensive-odor substance is toluene contained in industrial waste which has entered into the sea from the industrial factories, and it is taken by fish being infiltrated into the meat through blood from branchia and the offensive-odor fish are produced. Toluene may come to the industrial waste through the common oil refinery process. That is, the distillates having various substances of different evaporation points, liquefied petroleum gas (L. P. G.), gasoline, light oil and heavy oil were separated into each component flowed into the stopper. The more easily evaporable substances were fractionated by distilling with steam in steam stopper. After steam distillation, the water from steam is separated from the fractionates and discarded as industrial waste. In this final process, a part of the toluene contained in the fractionates in a large amount will be wasted with the water being emulsified or dissolved.

It has been reported that from the offensive-odor fish caught in the offshore of Yokkaichi oil factories facing Ise Bay an aliphatic hydrocarbons or fatty acids of small molecules were isolated by thin layer chromatography (1) with a small amount of aromatic ones, though identification of each substance was not made, while in Mizushima district, offensive-odor substance was identified as toluene as just mentioned. These facts suggest that all these substances will be the cause of the bad smelling of fish. Thus, the gas chromatographic analysis of some offensive-odor fish from Ise Bay was conducted by us and it was indicated that they have also a small amount of toluene with other substances, the detail of which will be described in the next paper. From the offensive odor fish caught in the offshore of Mizushima district, no definite new spot can be recognized by thin layer chromatography. * As to whether aliphatic hydrocarbon is found in the fish as the offensive odor substance, or not, the analysis is in progress.

Conclusively, it has been confirmed that toluene is one of the main source of offensive odor of fish and largely removed of other organic substance from the oil waste by the treatment of activated sludge. Therefore, the activated sludge process is one of the effective method to be adapted for the offensive-odor substance removed from the industrial waste.

CONCLUSION

For the purpose to reveal the substance as the source of offensive odor of the fish from the sea facing petroleum and petrochemical industries, analyses have been made on the sea water, industrial wastes, offensive odor fish meat and the eels kept in the sea or industrial waste for a certain period, by means of gas chromatography, infrared, and ultraviolet absorption spectrophotometries and mass-spectrometry. Observations revealed toluene as a possible main source responsible for the bad smelling of the fish.

Finally, by analysing the meat extract of the fish kept in the sea water containing toluene and by the same smell as that of the offensive odor fish from the off-shore of the industries, it has been definitely confirmed that toluene is the very substance that imparts the offensive odor to fish.

* In this method, almost all of toluene was evaporated during development of solvent.

The activated sludge process proved to be an effective method to remove toluene from the industrial wastes.

A discussion was made on the aliphatic carbohydrate as the possible source of the offensive odor of fish.

ACKNOWLEDGEMENT

Thanks are due to Miss Junko YASUI and Miss Kayo SHIBATA for the technical assistance.

REFERENCES

1. Division of Public Nuisance, Mie Prefecture: Report of offensive-odor fish, *Report of Public Nuisance* **23**, 1, 1964 (in Japanese)
2. SUDO, T. *et al.*: Test method of volatile organic substance in industrial waste, *Report of Institute of Hygienc*, Mie Prefecture, 150, 1965 (in Japanese)
3. YOSHIDA K. and UWASUMI N. : On the problem of offensive-odor fish in petrochemical industrial area (I), *Hygiene in Life* **5**, (4), 11, 1961 (in Japanese)
4. YOSHIDA, K. UWAZUMI N. and KASAOKA K. : On the problem of offensive-odor fish in petrochemical industrial area (II). *Hygiene in Life* **11** (2), 9, 1967 (in Japanese)
5. SHENHOGEN E. and ABRAHAMERSON, S. MCLAFFEATY F. W. : Atlas of Mass Spectral Data 1, 189, Interscience Publishers, New York, London