Bull. Environ. Contam. Toxicol. 30, 253–260 (1983)

An Improvement for the Concentration of Micropollutants in the Marine Environment by Using a Bacteria Strain with a Membrane Filter System

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The polychlorinated biphenyls (PCBs) and DDT isomers are recognised as persistent pollutants of global importance, and due to the environmental hazards much interest has been shown in their determination in developing countries. However, there is difficulty in identifying and quantitating the sub ng/l concentration of PCBs and DDT isomers and this necessitates a pre-concentration step. Numerous methods have been demonstrated for the extracting and analysis of trace amounts of PCBs and DDT isomers in water. Several adsorption systems have been used to pre-concentrate these trace pollutants. These include activated carbon (RONSEN et al. 1959 and BREIDENBACH et al. 1966), urethane foam plugs (GESSER et al. 1971, UTHE et al. 1972 and 1974), Carbowax 4000 monostearate and undecane-coated Chromosorb W (AHLING et al. 1970) and Amberlite XAD-D (MUSTY and NICKLESS 1974). KURTZ 1977 reported the use of cellulose triacetate membrane filters as an excellent adsorption method for analysis of PCBs and DDT isomers in water.

Early studies by PATRICK et al. 1976 and 1978, JOHNSON et al. 1973 and CHACKO et al. 1967 have shown that pollutants are incorporated and bioaccumulated within the microorganism. Hence, in this study it was attempted to concentrate the PCBs and DDT residues by a strain of bacteria and then to filter out the bacteria on a millipore filter using the model apparatus designed here. In determination of the concentration of the pollutants, it was necessary to filter the water with the membrane filter firstly, and then to concentrate whatever pollutants in the filtrated water using the bacteria in the model apparatus. A Pseudomonas sp. was chosen as it represents one of the most dominant forms in the aquatic system. In this paper we sometime used the term "biofilter" to mean the millipore membrane filter-bacteria. The recoveries of spiked PCBs (KC400) and p,p'DDT in filtrated seawater by the membrane filter itself and the membrane filter-bacteria (biofilter) were also examined.

METHODS AND MATERIALS

Bacterial Strain

The strain, Pseudomonas sp. used was isolated from seawater, and was observed to give optimum growth at 20°C in the media consisting of 0.1% of Bacto-Yeast Extract (Difico), 0.5% of Bacto-Peptone (Difico) and artificial seawater at pH 7.6. The artificial seawater prepared from double distilled water, consisted the following: NaCl 2.4%, K_2SO_4 0.3%, $MgSO_4 7H_2O$ 0.08%, $CaCl_2 2H_2O$ 0.02%, $FeC_6H_5O_7H_2O$ 0.002% and Na-EDTA 0.02%.

Recovery Test of the Bacterial Strain

The recovery or bioconcentration ability of the strain was carried out according to the method of JOHNSON et al. 1973, using ^{14}C-PCBs (KC400) and ^{14}C-p,p'DDT. The bacterial cells in the early stationary growth phase were exposed to concentrations of 5.9 ppb of ^{14}C-PCBs (KC400) and 5.6 ppb of ^{14}C-p,p'DDT. Radioactivity was measured in Liquid Scintillation Counter (Searle Analytical Inc., Mark III-6880 with ASR Teletype-8499) using external standard quench correction and at efficiency greater than 90%.

Model Apparatus

The model apparatus consists of two parts: the bioconcentration apparatus and the filtration apparatus as shown in Figures 1 and 2. The bioconcentration apparatus consists of the bacterial culture chamber (2-liter capacity), the collection tank (20-liter capacity-Sartorius Model SM16601) which can withstand high pressure, a vacuum pump and a magnetic stirrer. The bacterial culture chamber is a glass apparatus with a magnet stirrer.

The apparatus is set up as shown in Figure 1 with the peristalic pump which feeds filtrated seawater to the culture chamber and with the vacuum pump to suck up the seawater-bacteria mixture to the collection tank. The whole apparatus is set connected with glass tubes and the joints are connected with teflon tubes. The filtration apparatus consists of the collection tank, nitrogen gas tank and filter holder (Sartorius Model SM16268). The apparatus is set up as shown in Figure 2 and connected with flexible stainless steel pipe which can withstand high pressure.

The bioconcentration apparatus was washed with warm water and dried, and washed and flushed with acetone and distilled n-hexane before setting up in a temperature-controlled room (20°C). The bacterial chamber with one liter culture media was sterilised at 121°C for 20 minutes and cooled, before inoculation. The Pseudomonas sp. which was cultured 24 hours earlier, of which 0.5 ml was transferred to the media in the bacterial chamber and allowed to incubate at 20° C for 22-24 hours under constant stirring. After 22 hours, the filtrated seawater was fed into the culture chamber at the rate of 100 ml per minute with the peristalic pump. The bacterial culture was kept under constant stirring as the seawater came into contact with the culture. The bacterial culture-seawater mixture rose in level and was sucked up with the vacuum pump into the collection tank. When the seawater has completely passed through the system and to the collection tank, one liter of

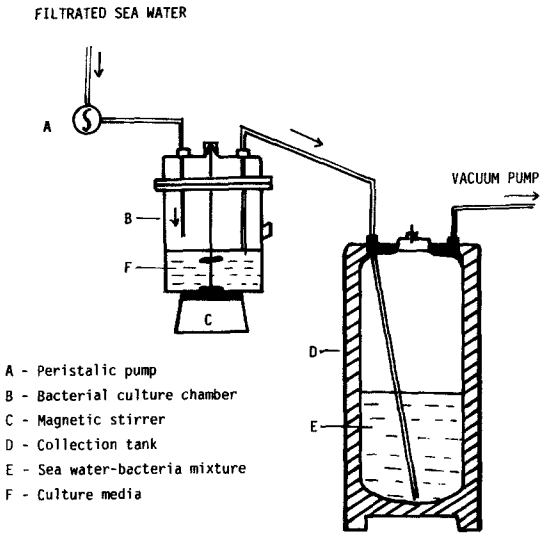

FIG. 1 BIOCONCENTRATION APPARATUS

sterilised artificial seawater was fed to the apparatus for washing.

The collection tank was separated and connected to the filtration apparatus as shown in Figure 2 with the filter holder and the nitrogen gas tank. The filter holder was washed, dried and rinsed with acetone and distilled n-hexane, before the membrane filter (pore size 0.45 μ - 142 mm ϕ, Sartorius Type SM110306) was mounted. By applying nitrogen gas pressure of 5 kg/cm^2 the bacterial cells were collected on the membrane filter. The filter with the bacterial cells was then transferred to a 300-ml round bottom flask and subjected to analysis.

For natural seawater that contains suspended particulates, it is necesaary that the sample seawater be filtered before feeding to the bacterial culture. The suspended particulates collected on the membrane filter were subjected to analysis separately. The outline of the procedure for sample seawater is shown in Figure 3.

Sample Seawater
↓
Filtration (0.45 μ) ⟶ Membrane Filter
Suspended Particulates (FP)
↓
Filtrated Seawater
↓
Contact with bacteria
in the model apparatus
for about 3 hours
↓
Filtration (0.45 μ) ⟶ Membrane Filter-Bacteria (FB)

Fig. 3. Outline of the procedure for sample seawater

In order to investigate the percentage recovery by the bacteria, filtrated seawater samples were spiked with PCBs and p,p'DDT separately to a concentration of 0.01 ppm, and were used in the bioconcentration as described above. Standards of PCBs (KC400) and p,p'DDT were dissolved in Tween #80 and nanograde

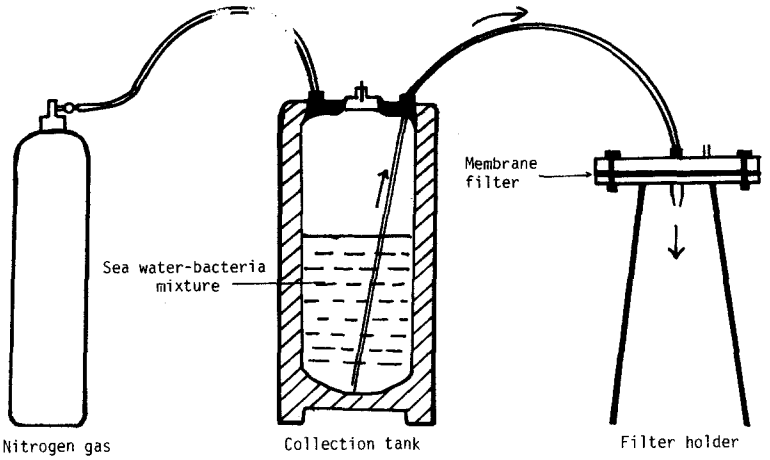

FIG. 2 FILTRATION APPARATUS

acetone, respectively before spiking. The spiked seawater samples were kept under constant stirring for overnight.

In order to investigate the background which may originate from the nutrient media, the bacteria or the membrane filter, one liter of sterilised media (without the bacteria) was filtered through the membrane filter and the membrane filter was subjected to analysis. Similarly, one liter of nutrient media which had been inoculated and incubated for 24 hours at 20°C was also passed through the membrane filter, and the membrane filter-bacteria (biofilter) was analysed. All experiments were carried out in duplicates.

Recovery Test of the Membrane Filter

This experiment was designed to investigate the adsorption or recovery ability of the membrane filter (cellulose nitrate - Sartorius SM11306) used in the filtration of the bacteria. The filtrated seawater samples spiked to a concentration of 0.01 ppm each of PCBs(KC400) and p,p'DDT were filtered separately through membrane filters using the filtration apparatus. The filtrated seawater was collected and again passed through a second membrane filter, and this was repeated again on a third membrane filter. The three filters in each case were analysed separately.

Analytical Procedure

The procedure as described by CHIBA 1973, for PCBs and p,p'DDT residues was adopted. All solvents and chemicals used were of "for pesticide residue analysis" grade (Wako Pure Chemicals Ind., Japan). Silica gel (Wako Gel S-1) was activated at 130°C for 24 hours and cooled in desiccator before use.

A Shimadzu Chromatograph (Model GC-4BM) equipped with ^{63}Ni electron capture detector was used. The following operational conditions were used throughout the study: a glass column of 2 metres long and 3 mm I.D., packed with OV/1.5% Chromosorb W, N_2 gas at 40 ml per minute flow rate. The column temperature was 200°C and the detector temperature was 210°C.

RESULTS AND DISCUSSION

The selected strain demonstrated bioconcentration ability in the range of 40-50% for ^{14}C-PCBs(KC400) and ^{14}C-p,p'DDT in 3-hour exposure, and attaining about 80% for 12-hour exposure. Figure 4 shows the chromatograms of the membrane filter-culture media without bacteria and the membrane filter-cultured media with bacteria. The chromatograms clearly indicate that they do not bear a large background or hindrance peaks, and this verification is very essential prior to the use of this method.

Table 1 shows the spiked PCBs and p,p'DDT recovered on the membrane filter. In the case of PCBs, the first filter had recovered 24.09 µg, the second filter and the third filter had recovered 12.11 µg and 14.27 µg, respectively. Similarly, in the case of p,p'DDT, the first filter had recovered 12.62 µg, the second and third filters had recovered 1.50 µg and 1.80 µg, respectively. These results clearly show that the PCBs and p,p'DDT residues were not fully solubilised in the filtrated seawater in spite of overnight stirring, and could have existed in colloidal, micellar or particulate forms to have recovered in the first filter. The second and third filters have actually recovered the PCBs and p,p'DDT that were in dissolved form in the seawater. This demonstrates the adsorption ability of the membrane filter (cellulose nitrate) but not to the degree as shown by KURTZ, 1977 of membrane filter (cellulose triacetate) which exhibited efficient adsorption of Aroclor 1242, Aroclor 1254 and p,p'DDT when spikes were passed through the filters. These low recoveries of the spiked standards by the filter used in this study could be attributed to the chemical structures.

Table 1. ADSORPTION OF PCBs/p,p'DDT BY MEMBRANE FILTER

Spiked PCBs/p,p'DDT in 10 liters of filtrated seawater	PCBs/p,p'DDT recovered on membrane filter (µg)
100 µg (KC400)	First filter 24.09 ± 1.60 Second filter 12.11 ± 1.32 Third filter 14.27 ± 0.65
100 µg (p,p'DDT)	First filter 12.62 ± 1.13 Second filter 1.50 ± 0.1 Third filter 1.80 ± 0.1

Table 2 shows the recovery of the spiked PCBs and p,p'DDT residues by the biofilter. The recovery was 64.01 µg for PCBs and 56.75 µg for p,p'DDT. The biofilter has shown better recoveries than the membrane filter itself. The increases of 40 µg and 44 µg of PCBs and p,p'DDT, respectively, recovered on the biofilters demonstrate that the increase could be due to the bioconcentration by the bacteria. (Note that in comparing only recovery of the first filter is considered as explained earlier

Table 2. RECOVERY OF PCBs/p,p'DDT BY BIOFILTER

Spiked PCBs/p,p'DDT in 10 liters of filtrated seawater	PCBs/p,p'DDT recovered on the biofilter (μg)
100 μg (KC400)	64.01 ±1.49
100 μg (p,p'DDT)	56.75 ±1.23

PCBs and p,p'DDT residues could have existed in colloidal, micellar or particulate forms to have elicited the recovery in biofilter.)

Figure 5 shows the chromatograms of the membrane filter with suspended particulates that were filtrated from 20 liters of seawater from Tokyo Bay, and of the membrane filter with the bacteria that were allowed to contact with the filtrated seawater for at least 3 hours in the model apparatus. The major peak in the chromatogram was identified as p,p'DDE and it could be quantified, and the other minor peaks are traces of PCBs that could not be quantified.

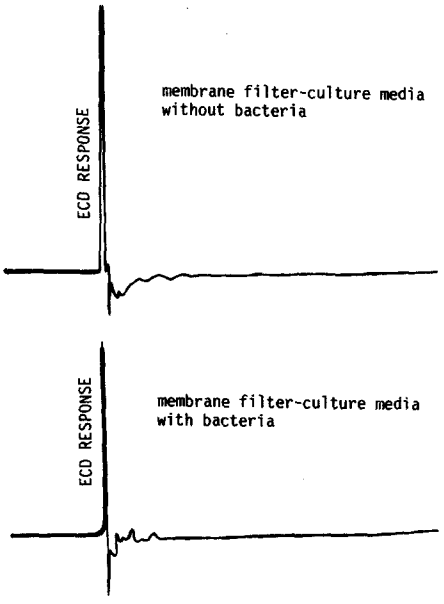

FIG. 4 CHROMATOGRAMS OF MEMBRANE FILTER-CULTURE MEDIA WITH AND WITHOUT BACTERIA

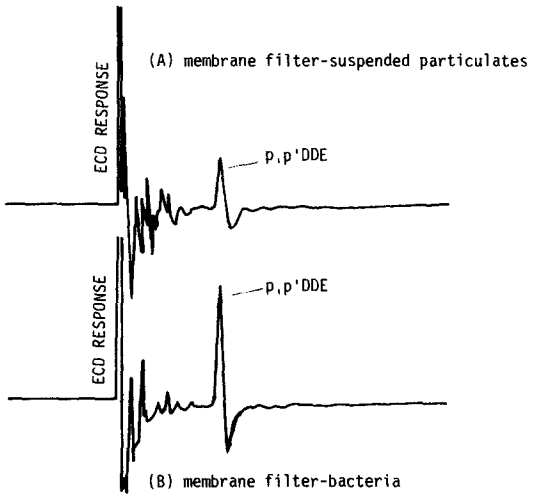

FIG. 5 CHROMATOGRAMS OF (A) MEMBRANE FILTER-SUSPENDED PARTICULATES AND (B) MEMBRANE FILTER-BACTERIA

Table 3 shows the quantified p,p'DDE from the coastal waters of Tokyo Bay. Each trial shows that the membrane filter-suspended particulates had higher amount of p,p'DDE than the membrane filter-bacteria (biofilter). This clearly validates the natural force or phenomenon of organic pesticides to adsorp and retain on various environmental surfaces such as soil particles, clay minerals,

Table 3. p,p'DDE recovered by membrane filter-suspended particulates (FP) and biofilter (FB) from coastal waters of Tokyo Bay

	p,p'DDE from (FP) (ng)	p,p'DDE from (FB) (ng)	Total p,p'DDE in 20 liters of seawater (ng)
1	8.51	5.11	13.62
2	8.47	6.24	14.71
3	9.02	6.36	15.38
4	9.57	5.89	15.46
5	9.32	5.64	14.96
6	9.22	6.44	15.66
7	11.28	8.19	19.47

biotic organic matters, etc. Thus, whatever of the p,p'DDE not adsorped to the suspended particulates but in the filtrated seawater, was trapped or accumulated by the biofilter.

CONCLUSION

This preliminary attempt or study, thus, demonstrated that there is potential for PCBs and DDT residues, in traces, being pre-concentrated using membrane filter and bacteria. It is also established experimentally in this study that persistent pollutants adsorped on to suspended particulates could be identified and quantified. Water samples assayed in this method showed traces of PCBs and p,p'DDE whose concentrations are sufficiently low that were sensitive to the analytical method but could not be quantified especially the PCBs. In the trials of Tokyo Bay coastal waters, it showed that the p,p'DDE adsorped by the membrane filter-suspended particulates and the biofilter could be detected. The quantified value for p,p'DDE ranged from 13.62 ng to 19.47 ng. On the basis of these results this method could be used for evaluation of the quality of water where PCBs and DDT residues could be present in concentrations below those toxic to aquatic life, after identifying membrane filter and bacteria with efficient adsorption property. However, this method has to be tested and elaborated for other pesticides like aldrin, BHC, endrin, methoxychlor, etc.

There are important points to noted in the use of this method (1) The nutrient media should be very clear without precipitation after auto-claving. Otherwise during filtration of bacteria there would be clogging on the membrane filter. For this reason artificial seawater was used instead of aged seawater which caused precipitation in our media. (2) The selected strain should be subjected to various media and incubation temperature till optimum

growth without much turbidity and ideal cell number in the early stationary growth phase are obtained. In our study an ideal cell number in the range of 10^7 - 10^8 cells per ml did not cause any clogging on the membrane filter. Also the selected strain should have high recovery of the pollutants in short term exposure to the sample seawater. (3) Background study of the selected nutrient media and the bacteria should be carried out so as to verify any interfering peaks in the chromatograms.

This new attempt to pre-concentrate PCBs and DDT residues using membrane filter and bacteria did show its effectiveness, and there is a need for further study for improvement of this method.

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Accepted January 5, 1983