SIMPLE, CONTINUOUS-FLOW SYSTEMS FOR DISSOLVING THE WATER-SOLUBLE COMPONENTS OF CRUDE OIL INTO SEA-WATER FOR ACUTE OR CHRONIC EXPOSURE OF MARINE ORGANISMS*

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Abstract—A continuous flow apparatus, referred to as a solubilizer, was constructed to dissolve the water-soluble fraction (WSF) of crude oil into water for short and long-term experiments with marine organisms. The basic solubilizer system consists of an oil reservoir, oil pump, modified glass bottle, and oil waste reservoir. This system dissolves the water-soluble components of crude oil without the loss of the more volatile compounds and without the formation of emulsions or oil droplets. Four solubilizer systems (two continuous flow and two recirculating) were evaluated for their efficiency in extracting the water-soluble components from Cook Inlet crude oil by analyzing for six monoaromatics over a 96-h period of continuous operation. When a solubilizer system was started, a stable concentration of the monocyclic aromatics in the water-soluble fraction was reached within 24 h. The water quality of the WSF effluent was not altered markedly in the continuous (open) flow systems, but there was a substantial reduction in dissolved oxygen in the recirculated (closed) systems. A single solubilizer system (open flow) with a 3 ml min^{-1} oil flow and a 1 l min^{-1} sea-water flow generated approx. 1.4 ppm of the total monoaromatics, which includes benzene, toluene, ethylbenzene and the three xylene isomers. This concentration varied depending on physical parameters and the chemical composition of the crude oil and water. With only slight modifications of the basic continuous flow system, a higher concentration (> 3.4 ppm total monoaromatics) of the water-soluble fraction of crude oil was achieved. By recirculating the water supply, concentrations of 6.7–11 ppm total monoaromatics in the WSF were produced. The stability, reproducibility, and reliability of the four systems were demonstrated in several experiments. Researchers working in aquatic systems with crude oil can well appreciate a simple system that is easily cleaned, relatively maintenance-free, and which produces stable, reproducible concentrations of the WSF over extended periods of time. The increasing demands for ecological studies on aquatic organisms with crude oil, a difficult mixture of chemicals with which to work, precipitated the development of these solubilizer systems.

INTRODUCTION

The need to investigate the effects of hydrophobic chemicals on aquatic organisms and to maintain a specific concentration of these chemicals in water over an extended period of time has posed many problems, especially when the chemicals are soluble at less than 1% in water (e.g. crude oil, Bunker C, organophosphates, chlorinated hydrocarbons, phthalates and PCBs, see Gunther *et al.*, 1968). Each category of chemicals has inherent properties which require a unique method for dissolving and maintaining a specific concentration of that chemical in water. Consequently, several methods and modifications of these methods have been used to produce solutions of relatively insoluble chemicals in water (Mount & Brungs, 1967; La Roche *et al.*, 1970; Taras *et al.*, 1971;

Brungs, 1973; Benoit & Puglisi, 1973; Lichatowich *et al.*, 1973; Benville & Korn, 1974; Bean & Blaylock, 1977; Vanderhorst *et al.*, 1977a; Hyland *et al.*, 1977; Roubal *et al.*, 1977).

Crude oil is a complex mixture of many chemicals varying in solubility from 0.004 ppm or less to 1933 ppm or more (Klevens, 1950; Gerarde, 1960; Peake & Hodgson, 1967; McAuliffe, 1969; Anderson *et al.*, 1974; Benville & Korn, 1977). In addition to differences in solubility of the various components, there are many other physical and chemical variations and interactions within a multicomponent system which make it difficult to produce and maintain a consistent concentration of the oil components in water (Leinonen & MacKay, 1973; Vanderhorst *et al.*, 1977b; Craddock, 1977). After considering a number of existing designs for solubilizing relatively insoluble compounds, we tried a semiclosed system that had been used successfully by Krugel *et al.* (1978) to solubilize components of jet fuel into water. This apparatus was duplicated at our laboratory in an attempt to dissolve the water-soluble components of crude oil. We found, however, that we could not use an adequate sea-water flow (1 l min^{-1}) without creating
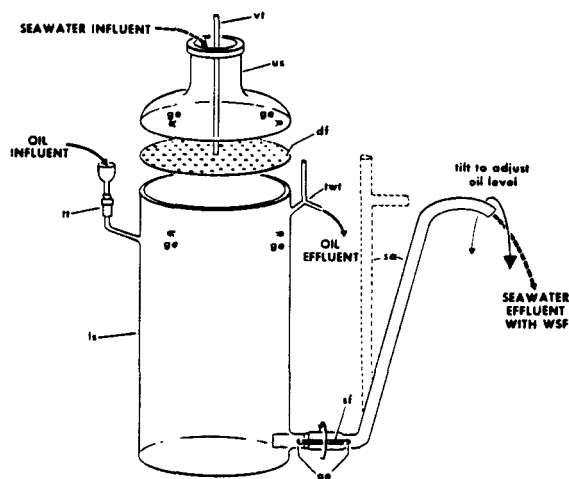
Fig. 1. Solubilizer: A modified 9.5-l. Pyrex bottle for extracting petroleum hydrocarbons from crude oil into sea-water: us—upper section (130 mm): vt— vent tube (6 mm O.D. × 152 mm): ge—glass ear: df—diffuser plate (20 gauge × 178 mm dia.) with fifty-four 1.6 mm holes: ls—lower section (345 mm): tt—thistle tube (10 mm O.D. × 160 mm) with a 12/30 glass joint and a 120° bend: twt—three way tube (10 mm O.D. with three 30 mm extensions): sa—side arm (20 mm O.D.) with a 24/40 glass joint: sf—spring fastener (70 mm).

numerous oil droplets in the sea-water effluent: moreover, the system was incapable of maintaining a stable concentration of the water-soluble fraction (WSF) without continually replacing the crude oil. This paper describes flow-through solubilizer systems which will provide researchers with a simple, inexpensive dosing apparatus for aquatic toxicological studies with crude oil.

MATERIALS AND METHODS

Basic solubilizer components

The basic system for dissolving the WSF from crude oil consists of an oil reservoir (19 l. Corning* Pyrex solution bottle) with a 4 mm Corning Teflon stopcock: a fluid metering pump with scavenger slot (Model RPIG 50/csc, Fluid Metering, Inc., 48 Summit St, Oyster Bay, NY 11771, U.S.A.): Tygon tubing special formulation F4040; and a solubilizer bottle (9.5 l. Corning Pyrex solution bottle) modified as described below by General Glassblowing Co., 1107 Ohio Ave, Richmond, CA 94804, U.S.A.

Solubilizer bottle

The 9.5 l. bottle (Fig. 1) was cut horizontally 130 mm from the top and the two cut edges ground. The two portions referred to as the upper (smaller) and the lower (larger) sections are held together by 3–50 mm spring fasteners. Six glass hooks (glass ears) for the spring fasteners were heat-sealed onto the glass bottle at three equidistant points around the bottle, 25 mm above and 50 mm below the cut.

For the upper section, a circular diffusion plate (178 mm dia.), was cut from a 20-gauge stainless-steel sheet. A 6.2 mm hole was drilled in the center of the plate for a vent

tube and fifty-four 1.6 mm holes were drilled in a concentric pattern at 19 mm intervals around the diffusion plate. The glass vent tube (6 mm o.d. × 152 mm) was inserted 6 mm into the diffusion plate and affixed in place with silicone sealant. The diffusion plate was inserted 6 mm into the upper section of the bottle with the vent tube extending out of the top of the upper section and the plate affixed in place with silicone sealant.

In fabricating the lower section, three glass tubes were sealed into the bottle to provide an influent and effluent for the oil and an effluent for the sea-water containing the dissolved WSF.

The oil influent tube was a 10 mm o.d. × 160 mm glass thistle tube with a standard taper 12/30 ground glass joint located 60 mm from the top of the thistle tube. A bend of 120° was made in the lower end of the tube 30 mm from the end, and the end of the tube sealed into the glass bottle 76 mm below the horizontal cut of the bottle.

An oil effluent tube (10 mm o.d. × three-30 mm extensions) was sealed into the opposite side of the bottle from the oil influent tube. The three-way tube was attached 38 mm below the cut of the bottle.

The sea-water effluent tube (20 mm o.d. × 50 mm) with a 24/40 glass joint (inner part) was ring-sealed 30 mm above the bottom and 25 mm into the bottle. An S-shaped side arm was formed from a 20 mm o.d. × 430 mm tube with a 24/40 glass joint (outer part). The vertical height between the bottom of the bottle and the overflow point of the S-shaped tube was 300 mm. A glass side arm with 90° bends, as illustrated in Fig. 1 by the dotted lines, also has been used successfully and is superior from a space viewpoint. The side arm was secured to the bottle by 2–70 mm spring fasteners between 4 glass ears. Two glass ears were sealed onto each glass joint at a distance of 60 mm from the end of the joint.

Solubilizer operation

A continuous flow single solubilizing apparatus was set up as shown in Fig. 2. Six liters of sea-water were poured into the 9.5 l. solubilizer bottle followed by 1.5 l. of Cook Inlet crude oil, which resulted in a 38 mm band of oil on

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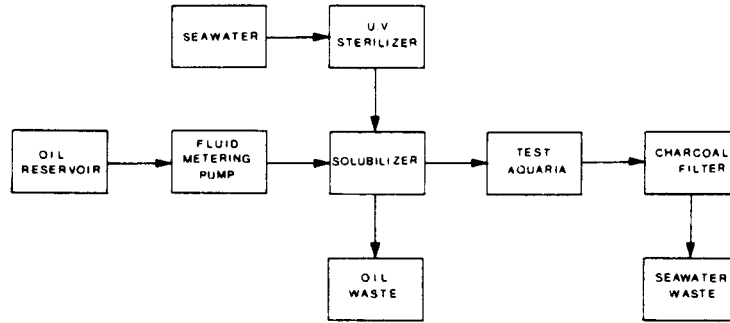
Fig. 2. An open flow dosing system that sterilizes the sea-water and removes the WSF from the effluent.

top of the sea-water. Silicone grease was applied to the horizontal joint of the upper and lower sections of the bottle. The top was put in place and secured by three-30 mm spring fasteners. The metering pump was turned on with the pump adjusted at 2 to provide a 3 ml min^{-1} flow of crude oil through the bottle. A 1 l min^{-1} flow of sea-water was introduced at the top of the bottle. The side arm was rotated laterally until its height was 18 mm lower than the top of the oil layer. This 18 mm difference maintained a layer of oil 38 mm deep within the solubilizer. If the side arm was lowered or raised from the above setting, the volume of oil in the bottle increased or decreased respectively. The exact position of the side arm depended on the specific gravity of the crude oil and the salinity of the sea-water.

Sea-water entering the top of the bottle was dispersed into small droplets as it passed through the diffuser plate. Each droplet of sea-water passing through the oil layer dissolved some of the water-soluble portion from the crude oil. Within 24 h, a stable concentration of the WSF in the sea-water effluent was obtained and the system was ready for exposing organisms.

In an effort to reduce possible contamination and losses of the WSF, the lower part of the solubilizer should be of all-glass construction. In addition, all-glass delivery lines were used between the solubilizer and aquarium, with the glass tube extending to the bottom of the test aquaria. All

water entering the system was sterilized with a flow-through germicidal ultraviolet light system (Model RM-2-15, Refco, P.O. Box 2356, San Leandro, CA 94577, U.S.A.).

The expense of the basic continuous flow system is approx. $500–600, with the liquid metering pump comprising about one-half ($310) the total cost of the system. A modified 9.5 l. (2.8 gal) solubilizer bottle with the diffuser plate cost $90. An additional expense is required for the cascading and recirculating systems described below because of the extra components, i.e. solubilizer bottles, water pump, modified aquarium, and water bath. The u.v. disinfection unit is also an added expense.

Solubilizer modifications

Two modifications of the basic system were used in order to increase the concentration of the WSF: (1) a cascading system with three solubilizers connected in series: and (2) a recirculating system using one solubilizer unit and a recirculating pump. In the cascading system (Fig. 3), each solubilizer is elevated 200 mm above each succeeding solubilizer in series. The WSF leaving the first solubilizer flows by gravity into the second and the sea-water effluent of the second flows into the third solubilizer. Also, the crude oil entering the first solubilizer flows by gravity through the other two solubilizers.

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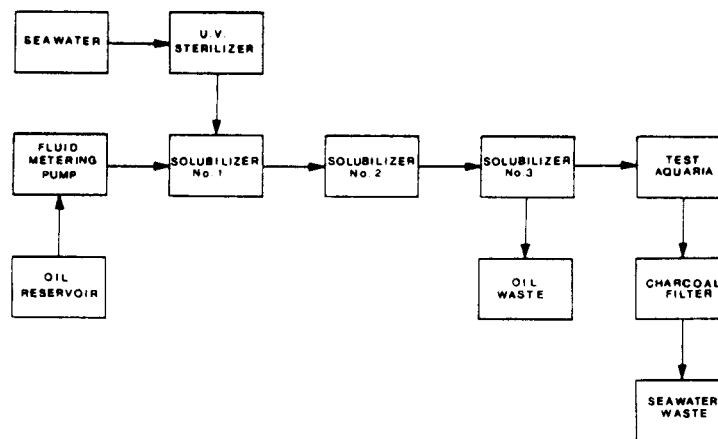
Fig. 3. An open flow dosing system used to increase the WSF concentration by adding solubilizers in series.
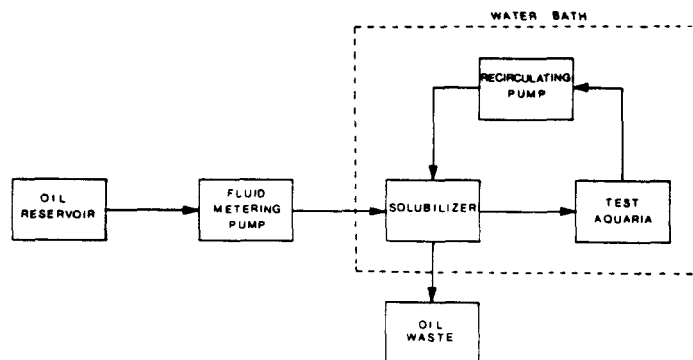
Fig. 4. A semi-closed system used to increase the WSF concentration by recirculating the sea-water and continually replacing the crude oil.

Two recirculating systems were constructed (Fig. 4): a small system excluding the test aquaria with a 6-l. capacity to obtain a high concentration of the WSF and a larger system including the test aquaria with a 36-l. capacity for use with aquatic organisms. In both recirculating systems the oil flowed through the solubilizer at 3 ml min⁻¹, and the sea-water was recirculated by a submersible March pump (Model 1A-MD-1), using a Hoffman clamp to restrict the water flow to 1 l min⁻¹. A 114-l. (30-gal) aquarium was used as a water bath to maintain a constant water temperature in the 6-l. system and two 114-l. aquaria for the 36-l. system.

Effluent analysis

Water samples (100 and 1000-ml volumes) with 4-ml of 6 N HCl added were extracted with 10-ml of TF Freon (trichlorotrifluoroethane) to remove and concentrate the six monocyclic aromatics (benzene, toluene, ethylbenzene, p-xylene, m-xylene and o-xylene) from the sea-water. The 100 and 1000-ml samples were extracted once and 3 times with TF Freon, respectively. Separation and quantitation of the aromatics were accomplished using a Micro-Tek 220 gas chromatograph with a dual flame ionization detector, a 1.8-m (6-ft) column containing 5°ₒ Bentone 34/5°ₒ SP 1200 on 100/120 mesh Supelcoport and another 1.8-m (6-ft) column containing 5°ₒ Bentone 34/5°ₒ didecylphthalate on 80/100 mesh chromosorb PAW. The column packings were obtained from Supelco, Inc., Bellefonte, PA 16823, U.S.A. Concentrations were determined using a Hewlett-Packard 3380A integrator.

RESULTS

Data obtained over a 4-day period from the continuous flow (single and multiple solubilizer) systems and a recirculation system are summarized in Table 1. Water samples taken 3 times daily were analyzed, data averaged and the standard deviation computed. The data indicate that the best long-term stability is achieved with a single solubilizer system, and that the variation in the concentrations of the monoaromatics increases in the multiple and recirculation systems, respectively. Benzene was highest in concentration followed by toluene, m-xylene, o-xylene, ethylbenzene and p-xylene. Para-xylene could not be detected in the WSF from a single solubilizer system because its concentration was below the lower limit of our analytical technique. The limit of detectability of our analytical method ranged from 0.001 to 0.10 ppm depending on the aromatic analyzed. By using additional solubilizing units in series (multiple system), the concentration of each aromatic was increased over the single solubilizing system. Recirculating 36 l. of sea-water continuously through the crude oil increased the concentration of most of the monoaromatics over the single and multiple systems. In regard to total monoaroma-

Table 1. Mean concentrations of six monocyclic aromatics in the sea-water effluent from three solubilizer systems

Components	Single* Concentration (ppm)	Benzene ratio	Multiple Concentration (ppm)	Benzene ratio	Recirculating Concentration (ppm)	Benzene ratio	Limit of detectability
Benzene	0.62 ± 0.079	1.000	1.6 ± 0.21	1.000	3.9 ± 0.86	1.000	0.10
Toluene	0.53 ± 0.044	0.869	1.3 ± 0.12	0.843	2.2 ± 0.45	0.572	0.025
Ethylbenzene	0.045 ± 0.023	0.070	0.080 ± 0.020	0.055	0.080 ± 0.05	0.021	0.021
p-Xylene	ND† ± —	—	ND† ± —	—	0.010 ± 0.01	0.003	0.001
m-Xylene	0.052 ± 0.0085	0.085	0.16 ± 0.050	0.104	0.28 ± 0.08	0.071	0.042
o-Xylene	0.032 ± 0.019	0.049	0.14 ± 0.020	0.087	0.22 ± 0.05	0.057	0.012
Total	1.2 ± 0.14	—	3.3 ± 0.36	—	6.7 ± 1.4	—	

* Experimental parameters: temperature. 14 C: salinity 30"ₒₒ: dissolved oxygen. 8–9 ppm: oil flow, 3 ml min⁻¹: and sea-water flow, 1 l min⁻¹.
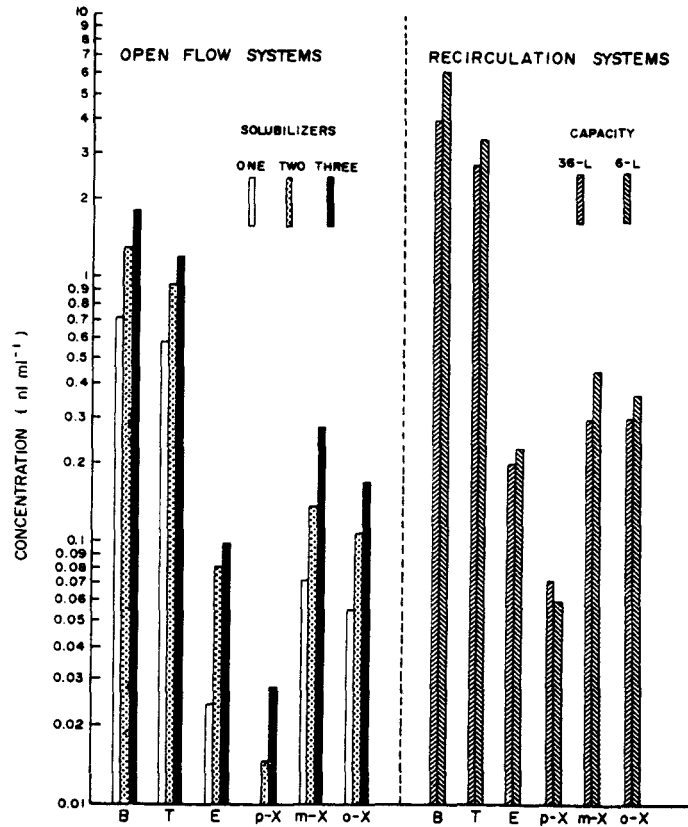
† ND = Not detected below 0.001 ppm for p-Xylene.

Fig. 5. Composition of six aromatics in the water-soluble fraction from different systems (B = benzene, T = toluene, E = ethylbenzene, p-X = para-xylene, m-X = meta-xylene and o-X = ortho-xylene).

tic data, the multiple system composed of three solubilizing units in series produced a monoaromatic concentration 2.8 times higher than did the single system, and the recirculation system yielded monoaromatic concentration 2.0 and 5.6 times higher than did the multiple and single systems, respectively. The aromatic ratios varied between each system as noted by the benzene ratios with the highest variation occurring in the recirculating system.

The bar graph in Fig. 5 illustrates the differences between and within open flow and recirculation systems. By using additional solubilizing units in series the concentration of each aromatic was increased. For example, the concentrations were nearly doubled, using two solubilizing units as opposed to a single unit. Adding a third unit further increased the concentration of the WSF in the sea-water effluent, but to a lesser extent than the dual unit system. Both recirculation systems generated a higher concentration of the WSF than the open flow system. However, the smaller recirculation system (6 l.) had a higher concentration of aromatics than the larger system (36 l.) except for p-xylene.

The most noted difference in water quality was observed in the reduction of the dissolved oxygen (DO) levels within the recirculation systems. In the two recirculation systems the average DO decreased from 9.3 to 5.4 ppm in the 36-l. system and 8.1–3.4 in the 6-l. system. The DO level was proportional to the volume of sea-water used in the recirculation system. There was only a slight drop in the DO content of the sea-water as the water passed through each solubilizer in the open system (0.3–0.4 ppm reduction for each solubilizer). The temperature of the recirculation systems was controlled by placing the solubilizer system into a water bath. A slight increase was noted in the temperature (+0.2°C) of the sea-water as it passed through each solubilizer in the open flow system. The salinity of the sea-water was not affected in any of the systems.

DISCUSSION AND CONCLUSION

Four solubilizer systems were constructed and tested which extract the WSF from crude oil and maintain a stable concentration of the WSF over an

extended time period without the formation of oil droplets or emulsions. The described solubilizer systems operate on the principle that the liquid being extracted floats on water and is relatively immiscible in it. Cook Inlet crude oil. which is an "insoluble" liquid with a low specific gravity, meets these criteria. The crude oils obtained from various wells in the Cook Inlet area range in specific gravity from 0.764 to 0.899 (Blasko et al.. 1972).

The described solubilizer systems were designed for a sea-water flow of $1\,l\,min^{-1}$ through a 38-mm oil layer with an oil flow of $3\,ml\,min^{-1}$. Variations in the operating parameters of the solubilizer, as well as the chemical composition of the crude oil itself, will affect the composition and concentration of the WSF leaving the solubilizer, e.g. sea-water and oil flow rates, oil layer depth, salinity and temperature. The operating parameters of importance to our research were the flow rates of the sea-water and the oil, and the variation in the depth of the oil in the solubilizer. Salinity, temperature and crude oil compositions were factors that were held constant in our experiments to determine the sublethal effects of Cook Inlet crude oil on the aquatic food chain.

We will consider first the effect of changing the sea-water flow rates above and below $1\,l\,min^{-1}$. Reducing the sea-water flow rates below $1\,l\,min^{-1}$ altered the composition of the WSF leaving the solubilizer and reduced the efficiency of the solubilizer system, since the sea-water entering the solubilizer will flow through fewer holes in the diffuser plate. A sea-water flow rate greater than $1\,l\,min^{-1}$ will reduce the concentration of the WSF in the sea-water. At flow rates above $1.6\,l\,min^{-1}$. oil droplets will begin to form since the vertical velocity of the sea-water flowing through the bottle exceeds the buoyancy of the oil droplets. Use of other liquids with a higher specific gravity than Cook Inlet crude oil may require reduction of the sea-water flow rate or construction of a solubilizer bottle with a larger diameter to reduce the velocity of the seawater moving through the upper liquid layer, in order to avoid the liquid droplet formation. If higher water flows are needed. the latter alternative mentioned would be the most appropriate method. Conversely, the sea-water flow rate could be increased, using a crude oil or other immiscible liquid with a lower specific gravity than Cook Inlet crude.

Also, oil droplets can be expelled from the solubilizer when emulsions are formed. Sharpley (1966) has stated that certain microorganisms interacting with crude oil will produce surfactants, in addition to the surfactants already in the crude oil that will increase the oil emulsion problems. We have observed oil emulsions forming after 2 weeks of continuous operation of a solubilizer system. In an effort to reduce the bacteria entering the solubilizer, an ultraviolet (u.v.) sterilizer was used to treat the sea-water influent. The u.v. dose suggested by most manufacturers is 33,000

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* Sid Korn. unpublished data. cited with permission.

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microwatt seconds per square centimeter $(\mu Ws\,cm^{-2})$ for destruction of pathogenic organisms. However, higher doses are needed to destroy other microorganisms. especially spores (Nagy, 1964). We are currently experimenting with u.v. doses above $70,000\,\mu Ws\,cm^{-2}$.

An increase in the oil flow rate will increase the concentration and alter the composition of the WSF. However, as a matter of practical logistics we chose $3\,ml\,min^{-1}$. which consumes over half of a barrel of crude oil per month for one continuously-operating solubilizer system. The depth of the layer of oil in the solubilizer was arbitrarily chosen. We noticed a reduction in the concentration of the monoaromatics in the WSF when the oil layer was reduced below 22 mm in depth. Increasing the depth of the oil layer beyond 22 mm did not appreciably change the concentration of the monocyclics.

The 19 mm distance between holes in the diffusion plate was adequate to keep the individual water droplets from coalescing before they came into contact with the oil layer. The 1.6 mm holes were the optimum size since larger holes allowed larger water droplets. which did not dissolve as much of the WSF from the crude oil and smaller holes tend to clog.

In an effort to reduce the WSF losses via sorption after the WSF leaves the solubilizer, all delivery tubes and aquaria should be made of glass. Volatilization losses can be reduced by expelling the WSF into the bottom of the aquarium and placing a glass cover over the aquarium when possible. The glass cover eliminates the moving air currents. which reduces the partitioning action of the volatile organics with the air.

The National Marine Fisheries Laboratory in Auke Bay. Alaska evaluated a similar single solubilizer system, as illustrated in Fig. 2. and found it to be very stable over a 6-day interval. They maintained the same experimental conditions as we did except for the lower temperature of 4 C. Their values for the total monoaromatics were slightly higher than ours (1.8 ppm), but the relative proportions of monoaromatics were the same. They also analyzed for some diaromatics and found the following average concentrations: naphthalene. 36 ppb: 2-methylnaphthalene. 19–21 ppb: dimethylnaphthalene. 11–12 ppb: and total diaromatics, 67–68 ppb. Our values for the diaromatics were $14–30\%$ lower*. The lower values in the amounts of aromatics would be attributable to the difference in water quality of the two laboratories (e.g. minerals. organics. temperature. etc.).

We have tried the solubilizer system using a single component. benzene. which is one of the main components in most crude oils. A $1\,l\,min^{-1}$ sea-water flow through a 38 mm layer of benzene produced a 200 ppm benzene concentration in the sea-water effluent leaving the solubilizer. As a matter of interest. a 38 mm layer of Cook Inlet crude oil resulted in a 0.72 ppm benzene concentration at equilibrium. which is a much lower concentration when the benzene is

extracted from a multicomponent system. Leinonen & MacKay (1973) have gone into great detail in explaining the characteristics of single and multicomponent systems.

We have reused Cook Inlet crude oil in other experiments and have found the ratio of the mono-aromatic components did not change appreciably, although there was a slight decrease in concentration. The ability to reuse the crude oil makes multiple solubilizer systems more practical if oil supplies are limited.

In summary, this paper describes a method to dissolve the water-soluble components from a crude oil into sea-water. The concentrations of the monoaromatics measured from several experiments over many days of operation have demonstrated the stability, reproducibility and reliability of the system that lends itself to recirculating and continuous flow studies for extended periods of exposure. The system is relatively inexpensive and requires minimal space and maintenance. A wide range of desired concentrations of the WSF of crude oil can be achieved with only slight modifications of the basic design, as illustrated in the figures. Researchers using the recirculating systems with aquatic organisms should be aware of increasing metabolites and decreasing DO levels, as in any static system. The 1 g biomass per 1 l. water guideline should not be exceeded. Additional experiments using fish, invertebrates and phytoplankton (Nunes & Benville, 1978, 1979a, b) illustrate the stability of the system and the effectiveness of this method in investigating the biological impact of oil*.

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* Detailed information on the mean concentrations found in Table 1 and other concentration data from other experiments may be obtained from the senior author.

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