EVALUATION OF THE POLLUTION POTENTIAL OF PETROLEUM REFINERY WASTEWATERS USING FATHEAD MINNOW BIOASSAYS

By

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Scope and Method of Study: In accordance with the 1972 amendments to the Water Pollution Control Act, treatment technologies must be developed to eliminate deleterious effects of industrial wastewaters on the aquatic environment. Biological evaluations of these technologies are necessary to ensure treatment effectiveness. The objectives of this study were to compare static and continuous flow acute bioassays as indicators of petroleum refinery wastewater toxicity and to compare the effectiveness of three wastewater treatment methods to produce petroleum refinery effluents nontoxic to fathead minnows in thirty-two day continuous flow bioassays.

Findings and Conclusions: Results of acute exposures indicated that static bioassays exhibit greater toxicity to fathead minnows in significantly less time than continuous flow bioassays. Static bioassays appear sufficient to detect acute toxicity of petroleum refinery wastewater samples, but continuous flow bioassays should be used if a constant monitor of wastewater treatment effectiveness is required.

Thirty-two day evaluations of petroleum refinery wastewater treatment methods revealed that biologically treated wastewaters which were further treated by sequential dual media filtration and activated carbon adsorption produced effluents which were significantly less toxic to fathead minnows than biological treatment alone or biological treatment followed by dual media filtration.

ADVISOR'S APPROVAL Troy Dorris

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USING FATHEAD MINNOW BIOASSAYS

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PREFACE

Static and continuous flow bioassays of petroleum refinery wastewaters were compared and the effectiveness of three wastewater treatment methods were determined with fathead minnow bioassays. The study was supported by the United States Department of the Interior, Office of Water Research and Technology Grant B-033 Okla., and the Oklahoma Oil Refiners' Waste Control Council, who also provided technical assistance and cooperation.

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CHAPTER I

INTRODUCTION

Public Law 92-500, an amendment to the Federal Water Pollution Control Act, established a national goal of eliminating pollutant discharge into navigable waters by 1985. This law defines a pollutant as any substance which directly or indirectly causes a deleterious effect upon any organism in the aquatic environment. Thus, the pollution potential of the various effluents must be assessed.

Petroleum refinery wastewaters contain at least three major potential sources of pollution: undiluted process wastewaters, API separator effluents, and treated wastewaters (Matthews and Myers 1976). These wastewaters commonly contain ammonia, sulfides, phenolic compounds, cyanides, and other toxic compounds including various hydrocarbons (Matthews et al. 1976). Undesirable tastes and odors may be associated with petroleum refinery effluents (Rosen and Middleton 1955, Kneese 1962). Increased sludge deposits, turbidity, color, odor, and plankton growth may occur in petroleum refinery effluent receiving streams (Ludzack, Ingram, and Ettinger 1957). Ludzack et al. (1957) also observed that oil wastes are stored in bottom sludges and flushed by high water conditions resulting in impaired water quality. The average petroleum

refinery effluent may contain 0.8, 2.5, and 0.5 million pounds/day of BOD, COD, and suspended solids, respectively (Ford 1970).

Petroleum refinery effluents may influence receiving streams. The BOD exerted on the receiving stream may cause anaerobic conditions (Katz 1971, Ford 1970, Reid et al. 1972). These conditions may be due to microbial sludges (Ford 1970) or high algal populations (Dorris et al. 1962). Oil may directly affect fish by coating epithelial gill tissues, and oily sludges may coat the bottom of the receiving stream inhibiting plant growth and suffocating benthic organisms (Reid et al. 1972). Chemicals present in petroleum refinery effluents may taint fish flesh (Klein 1962). These effects on the receiving stream may be deleterious to the aquatic community. In order to achieve the goals of PL 92-500 a realistic assessment of the effects of petroleum refinery wastewaters must be made.

Current effluent guidelines are based upon laboratory research and are predictions of environmental responses to toxic substances. However, adequate protection of the receiving stream community may not be possible by comparing toxicity and chemical analyses of wastewaters. The effluent guidelines may be unnecessarily low for easily biodegradable substances, or may be too high for substances which have more than additive toxicity. Petroleum refinery effluents often contain toxicants which may produce entirely different toxicity levels than pure compounds because of varying characteristics and interactions of the wastewaters and receiving streams (Matthews et al. 1972).

In order to ensure that the stream community is adequately protected, direct biological assessment of the wastewater must be made. The objectives of such monitoring are to ensure that effluents are safe under conditions of continuous exposure and are conducive to survival, growth, and reproduction of aquatic organisms (Tarzwell 1962).

One of the most successful methods of biological assessment of wastewater quality is the fish bioassay. Bioassays may be static or continuous flow. Since static tests are conducted without renewal, they require less equipment. However, they also have disadvantages. They usually require aeration to maintain dissolved oxygen above limiting levels, but aeration may change the nature and toxicity of the test solution. Static tests use intermittent or composite grab samples which may fail to reflect the true nature of the effluent. However, since the Environmental Protection Agency (EPA) has indicated that bioassays of wastewaters may be required of industry, it is necessary to know whether results obtained from the two methods are of such significance to warrant the increased cost and personnel to conduct continuous flow bioassays.

Recommendations by EPA concerning advanced wastewater treatment technologies are also being examined closely. It is necessary to know before spending large sums of money whether different treatment technologies will produce the desired environmental benefits. Therefore, the objectives of the present study were to compare:

1. static and continuous flow bioassays and

2. biological treatment, sequential biological treatment-dual media filtration, and sequential biological treatment-dual media filtration-activated carbon adsorption wastewater treatment methods.

CHAPTER II

LITERATURE REVIEW

Large quantities of petroleum and petroleum wastes accidently or by design enter the environment yearly. Farrington and Quinn (1973) reported the yearly discharge of 28,000 to 140,000 metric tons of hydrocarbons to coastal waters in domestic effluents, an amount which approximated oil spilled in the same waters in 1970. Hydrocarbons from automobile exhaust residues have been found in the Charles River, Boston (Hites and Biemann 1972). Brown and Lynch (1977) examined the fate of two spills off the Massachusetts coast. They found hydrocarbon concentrations in the water column from 450 ppb at the surface to 200 ppb at 40 m. They determined that compounds smaller than C_{15} volatilize and that compounds from C_{14} to C_{22} were contained in the water column. These hydrocarbons may be dissolved or emulsified, and heavier fractions may be incorporated in the sediments or form tar balls.

Hydrocarbons associated with sediments may remain unchanged for up to 2 years under anaerobic conditions (Blumer et al. 1972). Shelton and Hunter (1974) reported aerobic microbial degradation of sedimented oils. Over 100 species of bacteria, yeasts, and fungi are capable of oxidizing one or more kinds of hydrocarbons,

but no single species is capable of noticeably degrading crude oil (ZoBell 1969). Fungi exceed bacteria in the ability to degrade crude oils, but none of the organisms can degrade a significant part of the oil (Perry and Cerniglia 1973). Prototheca zopfii, an achlorophyllous alga, can degrade up to 40% of crude oil (Walker et al. 1975).

Berbin and Micks (1973) concluded petroleum derivatives are lethal to mosquito larvae by initiating irreversible hypoxia, but crude oil contamination of artificial substrate in an Alaskan river had no effect on chironomid larvae and the periphyton assemblage was enhanced (Rosenberg and Wiens 1976).

Some calanoid copepods synthesize 1 - 3% of total body lipids as the hydrocarbon pristane (Blumer et al. 1964). Benzo(a)pyrene has been found in barnacles living on creosoted pilings; however toxicity or significance to the barnacles was not discussed (Barneff et al. 1968). The spider crab can degrade naphthalene (Corner et al. 1973), but impairment of feeding and breeding behavior of crabs exposed to sublethal levels of hydrocarbons has been noted (Takahashi et al. 1973). Water soluble petroleum fractions impaired fertilization and development of sand dollar eggs (Nicol et al. 1977). Farrington and Quinn (1973) concluded the presence of hydrocarbons from n-C_{12} to n-C_{22} in clams was due to concentration, rather than synthesis, but they did not determine origin. Small oil globules are ingested like food by clams and concentrated primarily in the gut and hepatopancreas (Fong 1976). Fong made no report of toxicity, but water soluble crude oil fractions were

determined to be toxic to larval quahog clams (Byrne and Calder 1977) and to impair feeding ability and gill efficiency of oysters (Chipman and Galtsoff 1949).

Lasday and Mertens (1976) summarized the results of several research projects by stating that exposed shellfish eliminate hydrocarbons within 2 weeks after being transferred to clean water and that no food chain accumulation of hydrocarbons has been found. Shelton (1971), however, believes that chronic deposition or oil spills in sheltered areas have a deleterious effect and is supported by the report of the Tampico Mara spill during 1957 (Holcomb 1969). This incident involved a dark diesel oil spilled into a turbulent cove on the Pacific coast of California, the effects of which were still noticeable after 10 years.

Hydrocarbon contamination of fish and shellfish from coastal areas has been reported (Krishnaswami and Kupchanko 1969, Ehrhardt 1972, Sidhu et al. 1971, Connel 1971, Ogata and Miyake 1973, Ogata and Ogura 1976). Ellis (1937) determined that volatile crude oil compounds enter fish directly through the mouth lining and gills. Fish accumulate benzo(a)pyrene and naphthalene directly from water and release the hydrocarbons when transferred to clean water (Corner 1975). Korn et al. (1976), however, found that striped bass feeding and growth rates were impaired by exposure to benzene. Fathead minnows exposed to petroleum refinery effluents usually became emaciated and died within 32 days (Graham and Dorris 1968). Reynolds et al. (1975) found that petroleum refinery

wastewaters increased in toxicity with exposure time in bioassays with Selanastrum capricornutum. Combined petroleum refinery and domestic effluents had deleterious effects on fish populations in a southwestern stream (Phillips 1965), and on oil effluent released to the Buffalo river, New York, caused goldfish to refuse food, become sluggish, and lose equilibrium (Westfall 1943).

Differences among these reports are due to the inherent variability of test organisms, species sensitivity to the various toxins, and the test conditions. It is desirable to remove as many variables as possible when conducting bioassays so that results measure the intrinsic toxicity of the test solution. However, when bioassays are performed to determine the pollution potential of wastewaters it is essential that the assay should reflect the in toto toxicity of the effluent (Marier 1973). To encourage comparability of results the American Public Health Association has formulated standard methods for bioassay testing (APHA 1975). Static bioassays of wastewaters may be performed to evaluate average toxicity unless the wastewaters have high biochemical oxygen demand (BOD), are volatile, or have high variability. Otherwise, continuous flow bioassays are recommended since the toxicity extremes of the wastewaters may be more ecologically important than the average.

Many species have been suggested as standard bioassay organisms. Buikema et al. (1976) suggested using Daphnia sp. to screen refinery effluents. However, potential problems associated with the use of this organism and the associated procedure outweighed the proposed

advantages. A comparison of fathead minnows and goldfish as standard test fish determined that neither was superior, and that variability depended more on the toxicant than the species (Adelman and Smith 1976). Sources of intraspecific variation are age, sex, and health of the test organism (Buikema et al. 1976, Mount and Stephan 1969). APHA (1975) recommends that these and other variables (e.g. - suitability for use in bioassay tests, local and national importance, and environmental requirements of the species) be considered when selecting a test organism.

Other recommendations concern test chambers, duration of tests, needed physicochemical measurements of test solutions, and reporting of results. The median lethal concentration (LC50) and its confidence limits should be reported where effect is noted as death of organisms. Methods include computerized probit analysis, the Litchfield-Wilcoxon (1948) method, and the moving-average angle method (Harris 1959). In other instances the median lethal time (LT50) may be a more informative means of reporting data (Finney 1971, Litchfield 1949, Shepard 1955, Sprague 1973). Uniformity of methods and reporting of data will facilitate using the amount of data.

A degree of sophistication now exists in bioassay procedures. An automated monitoring system (Cairns et al. 1973, Klein et al. 1968) is currently in use by the Ohio River Valley Water Sanitation Commission. However, Hamilton (1976, p. 2683) terms such systems "a treasured myth of the public" since the monitors do not really stop pollution, they merely report its occurrence.

CHAPTER III

EXPERIMENTAL METHODS

Wastewater evaluations were conducted in a mobile laboratory at petroleum refineries in the state. Effluent was pumped to the laboratory from the refinery outfall. Control water was obtained from the receiving stream upstream from the outfall, or if unavailable, from municipal tap water which was dechlorinated and filtered with activated carbon.

The test chambers were 30 liter glass aquaria. Test solutions were introduced at one end of the chambers and overflowed at the other end through standpipe drains. The light sources were 36-inch, single bulb fluorescent fixtures, containing 30 watt soft-white bulbs. The lights were connected to a timer, which delivered a 16-hour light: 8-hour dark photoperiod to stimulate growth and reproduction of the test fish (Mount and Stephan 1969).

Fathead minnows from a stock reared and maintained by the Reservoir Research Center, Oklahoma State University, were used in the study. Subadult fish 90 to 120 days of age were used for acute tests. The fish were transported to the test site and acclimated in control water for 2 weeks prior to testing (Peterson and Anderson 1969). Temperature of the test containers was maintained at 25^{o}, and dissolved oxygen exceeded 4.0 mg/l. Fish were fed daily, but were

not fed during or for 2 days prior to acute tests (APHA 1971). Eight acute toxicity bioassays were performed at the same refinery to compare the toxicity of petroleum refinery wastewaters under static and continuous flow conditions. Test concentrations were volume/volume percentages of wastewater/control water and were reported as percent wastewater (Table I).

TABLE I

EXPERIMENTAL DESIGN OF ACUTE BIOASSAYS

	% Wastewater	No. Test Chambers	No. Fish/Chamber
Static	0	2	10
	18	2	10
	32	2	10
	55	2	10
	74	2	10
	100	2	10
Continuous Flow	0	2	10
	18	2	10
	32	2	10
	55	2	10
	74	2	10
	100	2	10

Ten subadult fish were randomly assigned (Finney 1964) to each

test chamber (Mount and Stephan 1969). A number of fish, equal to the number of test containers, were removed from an acclimation tank and placed in a holding vessel. These fish were then singly captured from the holding vessel and assigned to test chambers by using a random number table. This procedure was repeated until all fish were assigned to test chambers.

Concentrations of wastewater were assigned to test chambers by using a random number table. As suggested by Mount and Brungs (1967), five concentrations of the effluent and a control were used. Duplicate samples of each concentration were tested. Before the effluent concentrations were introduced into the test chambers, the chambers were drained to within 2 cm of the bottom.

Test concentrations were pumped into static exposure chambers from a mixing tank and delivered to continuous flow exposure chambers through a diluter modified from Mount and Brungs (1967). Delivery rates were 500 ml/min to each test chamber. The test chambers were covered with glass to prevent escape of volatile toxins. Mortality was determined at 1, 2, 6, 8, 24, 48, 72, and 96 h, and then at 24 h intervals until mortality ceased. From these data, estimates of median lethal concentration (LC50) and median lethal time (LT50) were determined.

The responses of the fathead minnows to the effluent made LC50 determinations by the Litchfield-Wilcoxon (1948) method impossible. The fish exhibited a narrow toxicity threshold often with no mortality at one effluent concentration and complete mortality at the next higher concentration. Thus, neither reliable determinations of the

LC50 values nor their confidence limits could be obtained graphically. LC50 determinations were made by the moving average angle method (Harris 1959). The effluent concentrations (doses) were transformed to logarithms. The percent mortality or proportional response (p) at each dose was transformed to an angle, $\theta(p) = \arcsin \sqrt{p}$, using Table XII in Fisher and Yates (1963). The average of three successive angles was computed, each average angle being associated with the middle dose of the respective set of three doses. An LC50 was estimated by linear interpolation between the two successive doses whose average angles bracketed 45^{o}. For the average angles $y < 45 < y'$, x and x' denote the corresponding log doses, and the estimated log LC50 = $x + (x' - x)\left[\frac{45 - y}{y' - y}\right]$. Confidence limits for the LC50 were computed as:

$$x + (x' - x)A_L \text{ and } x + (x' - x)A_U$$

where A_L and A_U were computed from the formula:

$$\frac{A - \frac{1}{2}g}{1-g} + \frac{\sqrt{g}}{1-g}\sqrt{(A - \tfrac{1}{2})^2 + (1-g)\ \frac{(2k - 1)}{4}}$$

and:

$A = (45 - y)/(y' - y)$

$g = 1641.4\ z^2/nk^2\ Y^2$

$z = 1.96$, the normal deviate corresponding to the two-sided confidence level

n = number of organisms/dose

k = number of angles

$Y = (y' - y)$.

Comparisons of LC50 values were made by comparing the ratio of their slope functions. The differences were considered significant ($p = 0.05$) if $(x_1 - x_2) + R_L \geq 0$ where x_1 and x_2 corresponded to log doses associated with the LC50's m_1 and m_2 when $m_2 \leq m_1$. R_L was computed as:

$$R_L = \frac{(y_2 - y_1) - z^2(1 - h)\text{Var}(y_2 - y_1)\text{th}(y_2 - y_1)^2}{b_c\ (1 - h)},$$

and y_1 and y_2 are the average angles $y_n < 45$ which correspond to the LC50's m_1 and m_2 where:

$z = 1.96$

$h = z^2\ \text{Var}\ b_c / b_c^2$

$$b_c = \frac{(x_1' - x_1)(y_1' - y_1) + (x_2' - x_2)(y_2' - y_2)}{(x_1' - x_1)^2 + (x_2' - x_2)^2}$$

$\text{Var}\ b_c = 1641.4/(x_1' - x_1)^2 + (x_2' - x_2)^2$

$\text{Var}\ (y_2 - y_1) = 1541.4/nk$

n = number of organisms/dose

k = number of angles.

The Litchfield (1949) method was used to determine LT50 values. LT50 values were reported for only 100% concentrations of effluent since only incomplete analyses could be performed at lower concentrations. Each observation time was plotted against cumulative percent mortality on logarithmic probability paper, and a straight line was fitted through the points. Times corresponding to 16, 50, and 84% mortality were recorded from the fitted line. The slope function S, the estimate of the standard deviation of the mean was calculated as $S = \frac{LT84/LT50 + LT50/LT16}{2}$.

The standard error of the LT50 (f_{LT50}) was determined in one of two ways. If complete mortality occurred, the value "f" was read from Nomograph No. 1 using S and N (total number of test fish). If incomplete mortality occurred, f_{LT50} was determined by reading N_2 from Nomograph No. 2 using N and the percentage reacting, then reading "f" from Nomograph 1 using S and N_2. The standard error of S was determined in a similar manner using $N_1 = 2N - 1$ if mortality was complete, or by reading N_3 from Nomograph 3, then again reading "f" from Nomograph 1 using S and N, or N_3. Two parameters were examined for comparison of LT50's, the slope function ratio (SR) and the reaction time ratio (RR). SR was calculated as $SR = S_1/S_2$ where $S_1 > S_2$; S_1 and S_2 corresponding to the standard error for the two LT50's. Nomograph 4 (Litchfield and Wilcoxon 1948) was used to obtain f_{SR}, the standard error of SR, by using f_{S_1} and f_{S_2}. Confidence limits for SR were calculated as $(SR)(f_{SR})$ = upper and SR/f_{SR} = lower. The curves were considered to deviate significantly ($p = 0.05$) from parallelism if $SR > f_{SR}$. The reaction time ratio was calculated as: $RR = LT50_1/LT50_2$; where $LT50_1 > LT50_2$. The value of f_{RR} was read from Nomograph 4 using f_{LT50_1} and f_{LT50_2}. Confidence limits for RR were calculated as $(RR)(f_{RR})$ = upper and RR/f_{RR} = lower. The reaction times were considered significantly different ($p = 0.05$) if $RR > f_{RR}$.

Thirty-two day continuous flow bioassays were performed to evaluate the effectiveness of the following petroleum refinery wastewater treatment methods for removing toxic compounds; biological treatment (BT), sequential BT-dual media (sand and anthracite coal)

filtration, and sequential BT-dual media (DM) filtration-activated carbon adsorption (AC). The dual media filter was backflushed hourly to remove particulate matter. The activated carbon unit was operated at a loading rate of 0.04 g COD/g carbon. Virgin carbon (ICI United States, Hydrodarco granular) was used for all tests. Bed volume was 4.9 m^3 with a hydraulic flow rate of 0.5 l/min.

Two experiments were conducted at each of three refineries, and one experiment was conducted at a fourth refinery. All of the refineries used a different type of biological waste treatment. Refinery A had a raceway bio-ditch followed by a sludge clarifier and polishing lagoons. Refinery B used dissolved air flotation followed by activated sludge basins and a sludge clarifier. Refinery C treated its wastewaters in a bio-oxidation unit followed by a series of polishing lagoons. The treatment system at refinery D consisted of activated sludge basins followed by aerated lagoons.

Test solutions were delivered to the chambers at approximately 0.13 l/min. This rate equalled the test volume in 4 - 6 h as recommended by APHA (1975). Each of the treatments was monitored hourly with a chemical ion probe system (Hydrolab Corp. Model 60) for temperature, dissolved oxygen, pH, and conductivity. The data was stored on a magnetic tape recorder (Metrodata Corp. Model 640). Some temperature and dissolved oxygen measurements were conducted in the test chambers with a field probe unit (Yellow Springs Instruments, Model 54). Determinations of alkalinity, hardness, and chemical oxygen demand (COD) were performed at the beginning and end of each bioassay by standard methods (APHA 1975). Determinations

of ammonia, total organic carbon (TOC), and suspended solids were made at 2, 4, 8, 16, and 32 days of exposure. Ammonia was analyzed by the standard method of distillation and titration (APHA 1975) and by specific ion probe (Orian Model 407 meter and Orion Series 95 ion probe). TOC analyses were performed on a Beckman Model 915 total organic carbon analyzer.

Test chambers and photoperiod were identical to acute exposures. However, the test fish were fed daily during acclimation and exposure. Adult fish, 150 - 180 days of age were used, and glass spawning tiles were placed in each test chamber to determine effects of the wastewaters on reproduction. Mortality of adult fish was recorded daily, and LT50 determinations followed the same procedures as in acute bioassays.

CHAPTER IV

RESULTS AND DISCUSSION

In six experiments comparing static and continuous flow acute bioassays, calculated LC50 values were lower in static than corresponding continuous flow tests, and in three of four experiments where complete analyses could be made (Table II) the differences were significant ($p = 0.05$).

Reaction time to the effluents was also significantly shorter ($p = 0.05$) in five static exposures than in continuous flow experiments. In five of the experiments the LT50 curves were significantly ($p = 0.05$) nonparallel (Table III), indicating differences in LT50.

Regulatory agencies may recommend that bioassays of wastewaters be performed to demonstrate that the effluents will not harm receiving stream communities. Presently, APHA (1975) recommends continuous flow bioassays of effluents that contain variable constituents or have volatile fractions. Petroleum refinery wastewaters fit this category. Fluctuations of results during this study reflect the variability of the wastewater. Toxic volatile fractions of petroleum refinery wastewaters have been found (Dorris, Burks, and Waller 1974). Although the continuous flow bioassays constantly replaced volatile components of the wastewaters and exposed the test fish to varying qualities of effluent during this study, static tests

TABLE II

LC50 VALUES OF ACUTE EXPOSURES

Test Number	Continuous Flow LC50 (Range)	Static LC50 (Range)	Significance
1	72% (43-75)	59% (55-64)	+
2	64% (55-76)	54% (47-63)	+
3	43% (40-47)	44% (39-49)	-
4	>96 h	72% (67-79)	0
5	>96 h	65% (61-71)	0
6	>96 h	>96 h	0
7	>96 h	68% (63-73)	0
8	73% (68-79)	70% (64-77)	+

>96 h Unable to calculate LC50

\+ Significant difference between LC50 values ($p = 0.05$)

\- No significant difference between LC50 values ($p = 0.05$)

0 Unable to test for significance

TABLE III

LT50 VALUES OF ACUTE EXPOSURES

Test Number	Continuous Flow LT50 (Range)	Continuous Flow S (Range)	Static LT50 (Range)	Static S (Range)	Significance LT50	Significance Reaction Time
	Hours		Hours			
1	12 (11-13)	1.3(1.2-1.4)	7 (4-10)	2.5(1.9-3.3)	+	+
2	2(1.1-2.4)	3.7(2.8-4.9)	6 (4- 8)	2.0(1.6-2.4)	+	+
3	8 (6- 9)	1.7(1.5-2.0)	.6 (.3-1.3)	5.6(3.3-9.7)	+	+
4	29 (21-39)	2.6(2.1-3.3)	10 (7-14)	2.2(1.7-2.8)	-	+
5	42 (34-52)	2.1(1.8-2.4)	34 (32-36)	1.2(1.1-1.2)	+	-
6	34 (26-45)	2.4(2.0-3.0)	17 (11-26)	2.6(1.9-3.5)	-	+
7	24 (22-27)	1.4(1.3-1.5)	15 (12-19)	1.8(1.5-2.1)	+	+
8	19 (15-24)	2.1(1.8-2.5)	15 (9-25)	3.2(2.2-4.5)	-	-

S Standard error of LT50

\+ Significant differences between values for continuous flow and static exposure ($p = 0.05$)

\- No significant differences present ($p = 0.05$)

were clearly more effective in indicating toxicity. If further research substantiates these results, considerable economic advantages could occur by performing static instead of continuous flow bioassays without detriment to receiving streams.

Seven experiments were conducted to evaluate the effectiveness of advanced wastewater treatment methods (Table IV). Acute toxicity was apparent only at refinery B during these experiments. Unless mortality occurred early in an exposure, fish in BT and BT-DM effluents displayed a characteristic response. The fins began to darken and became progressively compressed and the body darkened anteriorly to posteriorly. The fish moved slowly in a random manner at the surface and no longer accepted food. Emaciation was progressive, but it could not be determined to be the single cause of death.

No reproduction was observed during any of the experiments, but prespawning behavior was noted. The behavior consisted of establishment, defense, and cleaning of spawning sites, and the appearance of dark vertical bars on the sides and tubercles on the rostrum of male fish.

Final effluents were evaluated with the exception of refinery A, where the sludge clarifier effluent was used because of space and utility limitations. The first exposure at refinery A resulted in mortalities of 5% in the control, 70% in BT effluent, 20% in BT-DM effluent, and 15% in BT-DM-AC effluent. Estimates of LT50 values were unnecessary except for BT effluent for which the value was 23.0 days (Table IV). Obvious differences existed in toxicity of the different treatment methods.

TABLE IV

LT50 VALUES OF FATHEAD MINNOWS EXPOSED TO THREE TREATMENT METHODS

Treatment	Exposure	Refinery A LT50	s	B LT50	s	C LT50	s	D LT50	s
Control	1	(1)		0		0		0	
	2	0		0		(2)		-	
Biological Treatment(BT)	1	23d	1.4	<24h		13d	1.1	12d	1.3
	2	10d	1.6	0.4h	2.7	(12)		-	
BT-DM*	1	(4)		<24h		12d	1.2	13d	1.3
	2	(11)		0.9h	1.9	(10)		-	
BT-DM-AC**	1	(3)		(13)		0		0	
	2	0		0		(1)		-	

*BT-DM Biological treatment-dual media filtration

**BT-DM-AC Biological treatment-dual media filtration-activated carbon adsorption

d Days

h Hours

s Standard error of LT50

0 No mortality

( ) Cumulative mortality, insufficient to determine LT50

- No second exposure at refinery D

Because of a malfunction of the ion probe system, chemical data from treatment feedwaters was obtained during only 1 day of the exposure (Table V). Dissolved oxygen (D.O.) was the only potentially limiting parameter, but aeration in the test chambers maintained concentrations above 4.0 mg/l. The pH was lowered in the dual media filter and remained stable through activated carbon adsorption, while conductivity was lowered in both of these treatments. The decrease in pH could be indicative of bacterial activity in the dual media filter, but the D.O. should have also decreased if this were true. The drop in conductivity through the treatment system could be due to adsorption by the activated carbon. Results from this exposure indicate that additional wastewater treatment by dual media filtration significantly reduced toxicity of the biologically treated effluent. Further treatment by activated carbon adsorption had little additional beneficial effect on effluent quality.

The second exposure at refinery A produced no mortality in the control or BT-DM-AC effluent, 55% in BT-DM effluent, and 100% in BT effluent. The estimated LT50 in BT effluent was 10.4 days (Table IV). During the 10th and 16th days of exposure, fish in the control aquaria were engaged in prespawning behavior. Mortality during this exposure probably would have been much less, but during the 28th day bypassing and cleaning a refinery waste trap resulted in a severe overload of the treatment system.

Dissolved oxygen in aquaria influent ranged from 0.4 mg/l to 0.7 mg/l in the control, 1.0 to 1.9 mg/l in BT effluent, 1.1

TABLE V

CHEMICAL PARAMETERS OF TREATMENT EFFLUENTS DURING EXPOSURE 1 AT REFINERY A ON 21 NOVEMBER, 1975

		INFLUENT TO TEST AQUARIA			
		Control	BT*	BT-DM+	BT-DM-AC**
Temp. (°C)	$\bar{x}$	12.7	13.4	19.7	17.1
	s	0.7	0.6	1.0	0.2
D.O. (mg/l)	$\bar{x}$	1.1	1.8	2.2	2.2
	s	0.1	0.1	0.0	0.1
Conductivity (μmhos/cm)	$\bar{x}$	5917	4980	3262	2049
	s	283	321	108	122
pH	$\bar{x}$	8.3	7.7	7.0	7.0
	s	0.1	0.1	0.1	0.0

*BT Biological treatment

+BT-DM Biological treatment-dual media filtration

**BT-DM-AC Biological treatment-dual media filtration-activated carbon adsorption

$\bar{x}$ Mean

s Standard deviation

to 2.0 mg/l in BT-DM effluent, and 1.2 to 2.0 mg/l in BT-DM-AC effluent (Table VI). Aeration of the test chambers maintained D.O. above 4.0 mg/l, so the recorded concentrations were not the cause of any mortality.

The pH values were neither extreme nor toxic during the second exposure. Values ranged from 7.1 to 8.0 in the control, 6.1 to 7.9 in BT-DM-AC effluent. The pH was lowered slightly in the dual media filter, indicating bacterial activity, but increased during activated carbon adsorption.

Conductivity ranged from 3149 to 5610 μmhos/cm in the control, 140 to 1110 μmhos/cm in BT effluent, 44 to 996 μmhos/cm in BT-DM effluent, and 44 to 1188 μmhos in BT-DM-AC effluent. The conductivity was lowered by the treatment system during this exposure, by 40% in BT-DM effluent and 55% in BT-DM-AC effluent.

Suspended solids in BT effluent were reduced 52% by dual media filtration (Table VII). No further reduction in suspended solids occurred with activated carbon adsorption, probably due to fine carbon particles released from the carbon unit.

TOC was reduced 22% by dual media filtration and 71% by subsequent activated carbon adsorption. Ammonia, however, increased by 17% in BT-DM effluent and 75% in BT-DM-AC effluent. COD was reduced 11% by dual media filtration and 60% by activated carbon adsorption (Table VIII). Alkalinity decreased and hardness increased slightly during the exposure with the exception of the initial BT-DM-AC sample. Both values for this sample were comparatively high, possibly due to leaching of salts from the activated carbon.

TABLE VI

CHEMICAL PARAMETERS OF TREATMENT EFFLUENTS DURING EXPOSURE 2 AT REFINERY A FROM 18 APRIL TO 8 MAY, 1977

		INFLUENT TO TEST AQUARIA			
		Control	BT*	BT-DM+	BT-DM-AC**
Temp. (°C)	$\bar{x}$	-	-	-	-
	s				
D.O. (mg/l)	$\bar{x}$	0.5	1.5	1.6	1.6
	s	0.1	0.3	0.3	0.2
Conductivity (μmhos/cm)	$\bar{x}$	4348.6	523.3	320.7	240.2
	s	622.5	311.7	303.1	325.1
pH	$\bar{x}$	7.7	6.9	6.8	7.2
	s	0.3	0.6	0.7	0.4

*BT Biological treatment

+BT-DM Biological treatment-dual media filtration

**BT-DM-AC Biological treatment-dual media-activated carbon adsorption

TABLE VII

SAMPLES OF TREATMENT EFFLUENTS DURING EXPOSURE 2
AT REFINERY A FROM 18 APRIL - 20 MAY, 1977

Day of Exposure	Effluent	Suspended Solids (mg/1)	TOC (mg/1)	Total NH_3 (mg/1)
2	Control	10.6	3.0	0.0
	BT*	24.7	30.4	11.6
	BT-DM+	26.7	23.2	11.2
	BT-DM-AC**	38.1	2.6	31.2
4	Control	5.6	0.6	0.0
	BT*	50.0	21.6	8.8
	BT-DM+	15.4	20.2	8.9
	BT-DM-AC**	16.4	4.9	15.2
8	Control	1.8	19.6	0.0
	BT*	48.5	23.5	8.6
	BT-DM+	20.8	18.9	8.3
	BT-DM-AC**	7.8	2.5	11.9
16	Control	4.4	4.9	0.0
	BT*	46.1	21.8	7.5
	BT-DM+	28.2	19.9	15.4
	BT-DM-AC**	14.7	4.9	18.6
24	Control	2.2	2.1	0.0
	BT*	23.4	24.6	14.2
	BT-DM+	8.0	11.5	13.7
	BT-DM-AC**	8.8	4.7	13.0
32	Control	0.8	2.7	0.0
	BT*	10.0	21.5	12.3
	BT-DM+	1.2	17.5	12.8
	BT-DM-AC**	1.5	6.8	15.3

*BT Biological treatment

+BT-DM Biological treatment-dual media filtration

**BT-DM-AC Biological treatment-dual media filtration-activated carbon adsorption

TABLE VIII

SAMPLES OF TREATMENT EFFLUENTS DURING EXPOSURE 2
AT REFINERY A FROM 18 APRIL - 20 MAY, 1977

Day of Exposure	Effluent	Alkalinity (mg/l)	Hardness (mg/l)	COD (mg/l)
2	Control	114.0	185.7	15.4
	BT*	34.0	308.8	142.6
	BT-DM+	34.0	278.3	69.4
	BT-DM-AC**	102.0	1533.8	65.6
32	Control	118.0	133.3	10.4
	BT*	29.0	407.7	79.9
	BT-DM+	29.0	411.6	104.2
	BT-DM-AC**	30.0	440.9	27.8

*BT Biological treatment

+BT-DM Biological treatment-dual media filtration

**BT-DM-AC Biological treatment-dual media filtration-activated carbon adsorption adsorption

While dual media filtration improved the effluent quality, addition of an activated carbon adsorption unit to the treatment system significantly improved wastewater treatment effectiveness. Although ammonia increased in this treatment unit, the levels caused no mortality. The activated carbon unit prevented mortality caused by an overload of the biological treatment system which the dual media filter was unable to do.

The first exposure at refinery B resulted in complete mortality in BT and BT-DM effluents within 24 h (Table IV). Sixty-five percent mortality occurred in BT-DM-AC effluent when the adsorptive capacity of the activated carbon was apparently exhausted during the 14th day of exposure. The carbon was replaced, and no subsequent mortality occurred. No mortality occurred in control aquaria.

The pH ranged from 5.1 to 7.6 in the control feedwater, 5.1 to 8.6 in BT effluent, 4.7 to 6.7 in BT-DM effluent, and 4.6 to 7.6 in BT-DM-AC effluent (Table IX). Mean values decreased slightly through the treatment system, but the low values occurred during the second day of exposure in all effluents.

Conductivity readings were extremely variable. Ranges were 3588.1 to 7769.9 μmhos/cm in control feedwater, -6.9 to 3231.3 μmhos/cm in BT effluent, -10.1 to 4154.6 μmhos/cm in BT-DM effluent, and -12.0 to 5238.1 μmhos/cm in BT-DM-AC effluent. The negative values are not assumed to be the result of a probe malfunction since values from the control feedwater did not exhibit this variation.

TABLE IX

CHEMICAL PARAMETERS OF TREATMENT EFFLUENTS DURING EXPOSURE 1 AT REFINERY B FROM 5 APRIL - 7 MAY, 1976

		INFLUENT TO TEST AQUARIA			
		Control	BT*	BT-DM+	BT-DM-AC**
Temp. (°C)	$\bar{x}$	19.5	25.3	25.0	25.7
	s	2.9	2.3	2.7	2.6
D.O. (mg/l)	$\bar{x}$	6.7	5.1	4.7	4.3
	s	2.1	2.9	2.7	2.5
Conductivity (μmhos/cm)	$\bar{x}$	4814.6	620.1	376.6	419.4
	s	463.1	1258.9	937.2	1119.2
pH	$\bar{x}$	7.0	6.9	6.8	6.7
	s	0.5	0.6	0.5	0.5

*BT Biological treatment

+BT-DM Biological treatment-dual media filtration

**BT-DM-AC Biological treatment-dual media filtration-activated carbon adsorption

Temperature ranged from 12.8 to 23.9°C in the control, 22.1 to 28.9°C in BT effluent, 19.8 to 29.1°C in BT-DM effluent, and 19.5 to 28.4°C in BT-DM-AC effluent.

Dissolved oxygen in the treatment effluents ranged from 2.9 to 8.8 mg/l in the control, 1.4 - 9.9 mg/l in BT effluent, 0.4 to 9.2 mg/l in BT-DM effluent, and 0.7 - 9.3 in BT-DM-AC effluent. Measurements of D.O. in the test chambers ranged from 0.4 mg/l in BT effluent to 8.4 mg/l in BT-DM-AC effluent (Table X). If measured D.O. reflected earlier concentrations in the test chambers, the low levels could have contributed significantly to observed toxicity.

Forty-one percent of the suspended solids present in BT effluent were removed by dual media filtration and 73% were removed by BT-DM-AC treatment (Table XI). Eighty-nine percent of the TOC was removed by BT-DM-AC treatment, but TOC increased by 11% in the dual media filter. However, ammonia increased 38% in the dual media filter and 87% in the activated carbon unit. COD was reduced 24% by dual media filtration and 88% by additional treatment with activated carbon (Table XII).

No mortality occurred during the second exposure at refinery B in control or BT-DM-AC aquaria. However, 100% mortality was again observed in BT and BT-DM effluents. The estimated LT50 in BT effluent was 10.5 h, and 22.0 h in BT-DM effluent (Table IV). LT50 values and reaction time ratios indicated a significant decrease in toxicity due to additional wastewater treatment by dual media filtration. The activated carbon adsorption unit eliminated

TABLE X

DISSOLVED OXYGEN IN TEST CHAMBERS DURING EXPOSURE 1
AT REFINERY B FROM 23 APRIL - 5 MAY, 1976

	DAY OF EXPOSURE		
	16	28	30
Control			
a	6.0 mg/l	7.1 mg/l	6.2 mg/l
b	5.8	7.7	6.3
BT*			
a	0.4 mg/l	3.3 mg/l	2.3 mg/l
b	2.4	7.4	6.8
BT-DM+			
a	5.2 mg/l	5.4 mg/l	4.6 mg/l
b	6.1	8.0	6.8
BT-DM-AC**	4.9 mg/l	8.1 mg/l	7.5 mg/l
	6.5	8.4	7.6

*BT Biological treatment

+BT-DM Biological treatment-dual media filtration

**BT-DM-AC Biological treatment-dual media filtration activated carbon adsorption

TABLE XI

SAMPLES OF TREATMENT EFFLUENTS DURING EXPOSURE 1 AT REFINERY B FROM 5 APRIL TO 7 MAY, 1976

Day of Exposure	Stream	Suspended Solids (mg/1)	TOC (mg/1)	Total NH_3 (mg/1)
0	Control	2.4	6.6	0.3
	BT*	57.1	79.0	8.4
	BT-DM+	35.3	62.2	9.4
	BT-DM-AC**	11.2	8.5	16.2
2	Control	3.7	6.7	0.2
	BT*	16.0	91.2	21.7
	BT-DM+	18.0	83.1	23.3
	BT-DM-AC**	8.0	2.6	16.9
4	Control		3.6	0.0
	BT*		62.1	30.9
	BT-DM+		57.2	35.0
	BT-DM-AC**		6.9	19.7
8	Control		<1.0	0.1
	BT*		43.3	25.3
	BT-DM+		46.2	26.1
	BT-DM-AC**		6.0	14.7
16	Control		3.9	0.0
	BT*		55.7	1.9
	BT-DM+		58.1	5.7
	BT-DM-AC**		7.3	15.0
24	Control		3.0	0.0
	BT*		46.0	10.6
	BT-DM+		97.5	8.6
	BT-DM-AC**		10.5	13.1
32	Control	2.6	2.3	0.0
	BT*	165.8	74.3	10.6
	BT-DM+	5.2	68.3	15.5
	BT-DM-AC**	22.5	5.6	6.9

*BT Biological treatment
+BT-DM Biological treatment-dual media filtration
**BT-DM-AC Biological treatment-dual media filtration-activated carbon adsorption

TABLE XII

SAMPLES OF TREATMENT EFFLUENTS DURING EXPOSURE 1 AT REFINERY B FROM 5 APRIL TO 7 MAY, 1976

Day of Exposure	Effluent	Alkalinity (mg/1)	Hardness (mg/1)	COD (mg/1)
0	Control	37.0	81.6	76.1
	BT*	94.0	190.8	296.4
	BT-DM+	81.0	193.8	242.3
	BT-DM-AC**	107.0	138.7	48.1
32	Control	82.0	91.1	7.6
	BT*	296.0	146.5	296.4
	BT-DM+	259.0	146.5	208.4
	BT-DM-AC**	87.0	217.8	22.9

TABLE XIII

SAMPLES OF TREATMENT EFFLUENTS DURING EXPOSURE 2 AT REFINERY B FROM 4 JUNE TO 6 JULY, 1976

Day of Exposure	Effluent	Alkalinity (mg/1)	Hardness (mg/1)	COD (mg/1)
0	Control	89.0	151.3	0.0
	BT*	161.0	170.7	233.1
	BT-DM+	178.0	147.4	177.5
	BT-DM-AC**	117.0	147.4	26.8
32	Control	90.0	126.5	4.0
	BT*	159.0	126.5	296.8
	BT-DM+	155.0	106.1	175.3
	BT-DM-AC**	122.0	114.2	21.9

*BT Biological treatment
+BT-DM Biological treatment-dual media filtration
**BT-DM-AC Biological treatment-dual media filtration-activated carbon adsorption

mortality, and the physical appearance of fish in control and BT-DM-AC aquaria was identical.

Additional waste treatment provided significant improvement in water quality. BT-DM and BT-DM-AC treatments removed 32 and 91% of the COD, respectively (Table XIII). BT-DM-AC treatment was nearly twice as efficient in removal of suspended solids and TOC as BT-DM treatment and was even more effective in ammonia removal (Table XIV).

The continuous ion probe monitoring system was inoperative during this exposure. Measurements of temperature and dissolved oxygen were made with a field probe (Table XV). Low levels of dissolved oxygen occurred only in BT and BT-DM aquaria but these levels did not contribute to the observed mortality of the test fish. Temperature ranged from 17.0° in BT-DM aquaria to 25.0° in BT aquaria.

No immediate mortality occurred during the initial exposure at refinery C and by the third day fish in control and BT-DM-AC aquaria were establishing spawning territories. On day 5 toxicity occurred in aquaria containing BT effluent and on day 6 in BT-DM effluent. Fish in BT and BT-DM effluents began to show effects of chronic exposure by day 8. A heavy rain occurred during day 10 and refinery wastewater traps overflowed into the outfall where the laboratory intake was situated. This was probably the cause of complete fish mortality in BT and BT-DM effluent aquaria on days 13 to 16. However, fish in BT-DM-AC effluent were unaffected and during day 15 were engaged in

TABLE XIV

SAMPLES OF TREATMENT EFFLUENTS DURING EXPOSURE 2 AT REFINERY B FROM 4 JUNE TO 6 JULY, 1976

Day of Exposure	Effluent	Suspended Solids (mg/l)	TOC (mg/l)	Total NH_3 (mg/l)
0	Control	0.1	11.4	0.3
	BT*	11.1	55.4	21.6
	BT-DM+	26.2	55.4	24.0
	BT-DM-AC**	9.6	-	21.1
2	Control	0.7	4.3	0.7
	BT*	68.0	58.2	16.8
	BT-DM+	33.5	61.2	28.9
	BT-DM-AC**	11.1	4.3	14.9
4	Control	0.5	7.5	0.3
	BT*	51.0	55.2	20.8
	BT-DM+	10.2	52.2	21.4
	BT-DM-AC**	12.0	10.7	18.4
8	Control	1.4	4.8	0.3
	BT*	26.0	30.9	25.9
	BT-DM+	11.2	31.1	23.4
	BT-DM-AC**	3.5	3.9	15.7
16	Control	1.7	3.9	0.2
	BT*	93.3	64.1	22.1
	BT-DM+	14.4	20.7	15.5
	BT-DM-AC**	6.1	4.3	13.4
24	Control	0.1	10.9	
	BT*	77.2	57.9	
	BT-DM+	7.8	28.5	
	BT-DM-AC**	11.1	13.4	
32	Control	0.5	5.3	0.1
	BT*	55.5	36.4	15.3
	BT-DM+	16.1	34.7	13.7
	BT-DM-AC**	6.6	3.8	8.1

*BT Biological treatment
+BT-DM Biological treatment-dual media filtration
**BT-DM-AC Biological treatment-dual media filtration-activated carbon adsorption

TABLE XV

TEMPERATURE AND DISSOLVED OXYGEN IN TEST CHAMBERS
DURING EXPOSURE 2 AT REFINERY B
FROM 4 - 20 JUNE, 1976

Date		EFFLUENT							
		Control		BT*		BT-DM+		BT-DM-AC**	
		a	b	a	b	a	b	a	b
4 June	Temp.(°C)	23.0	23.0	25.0	23.0	23.0	23.0	20.0	21.0
	D.O.(mg/1)	8.2	8.0	3.1	5.3	5.4	5.7	7.8	7.7
6 June	Temp.(°C)	22.5	22.5	24.0	23.0	23.5	22.0	21.0	21.0
	D.O.(mg/1)	6.8	6.8	0.5	0.6	0.3	0.9	3.5	3.8
8 June	Temp.(°C)	22.8	23.0	24.0	21.8	23.3	22.0	21.0	21.0
	D.O.(mg/1)	7.0	7.1	2.9	6.5	4.4	5.3	5.6	6.5
12 June	Temp.(°C)	19.0	20.5	22.0	18.8	20.0	17.0	19.9	19.3
	D.O.(mg/1)	7.7	7.8	4.7	6.4	6.0	7.1	4.4	4.8
20 June	Temp.(°C)	23.8	23.3	24.9	23.5	24.9	23.8	22.8	23.3
	D.O.(mg/1)	6.3	7.0	0.3	0.7	2.0	0.5	5.4	3.9

*BT Biological treatment

+BT-DM Biological treatment-dual media filtration

**BT-DM-AC Biological treatment-dual media filtration-activated carbon adsorption

prespawning behavior. LT50 values for BT and BT-DM effluents were 13.0 and 12.5 days, respectively (Table IV), indicating no decrease in effluent toxicity because of additional dual media filtration, but BT-DM-AC treatment eliminated effluent toxicity.

Sequential treatment of biologically treated effluent at refinery C with dual media filtration and activated carbon adsorption improved the physicochemical quality of the final effluent. Dual media filtration reduced suspended solids 11%, but did not reduce TOC and COD (Tables XVI and XVII). Ammonia increased 29% after the dual media filter. BT-DM-AC treatment reduced suspended solids, TOC, and COD by 32, 56, and 66%, respectively. Ammonia increased 17% in the carbon filtered effluent. No significant changes occurred in D.O., conductivity, pH, or temperature as a result of the treatment systems (Table XVIII), nor were any measured in test aquaria (Table XIX).

Fathead minnow mortalities during the second exposure at refinery C were 5% in BT-DM-AC effluent, 10% in control, 50% in BT-DM effluent, and 60% in BT effluent (Table IV). No LT50 estimates could be obtained from these data, but BT-DM-AC treatment noticeably decreased toxicity of the biologically treated effluent. Dual media filtration produced only slight reduction in toxicity of the effluent. No reproductive behavior was observed during the exposure, probably because of the seasonal temperature decrease. Temperatures measured during the exposure ranged from 18.5 to 6.5°C in the control, 18.5 to 5.7°C in BT effluent, 18.5 to 6.0°C in BT-DM effluent, and 18.5 to 7.0°C in BT-DM-AC effluent. Measurements of

TABLE XVI

SAMPLES OF TREATMENT EFFLUENTS DURING EXPOSURE 1 AT REFINERY C FROM 30 AUGUST TO 1 OCTOBER, 1976

Day of Exposure	Effluent	Suspended Solids (mg/l)	TOC (mg/l)	Total NH_3 (mg/l)
0	Control	5.5	26.6	0.0
	BT*	43.6	48.3	2.5
	BT-DM+	33.9	47.5	2.3
	BT-DM-AC**	35.7	16.5	2.4
2	Control	2.3	20.0	0.0
	BT*	26.6	53.3	2.4
	BT-DM+	22.8	48.9	4.2
	BT-DM-AC**	15.8	29.2	2.6
4	Control	11.2	17.9	0.1
	BT*	23.8	44.9	4.2
	BT-DM+	32.1	50.2	5.0
	BT-DM-AC**	33.6	25.1	3.2
8	Control	24.6	15.6	0.0
	BT*	24.1	49.8	3.9
	BT-DM+	24.6	58.1	7.2
	BT-DM-AC**	19.3	15.9	4.1
16	Control	43.3	16.4	0.0
	BT*	45.8	49.6	7.6
	BT-DM+	31.4	50.8	8.2
	BT-DM-AC**	27.3	18.9	4.2
32	Control	3.8	21.6	0.0
	BT*	6.5	61.0	3.6
	BT-DM+	4.4	58.4	3.5
	BT-DM-AC**	2.5	29.2	9.3

*BT Biological treatment

+BT-DM Biological treatment-dual media filtration

**BT-DM-AC Biological treatment-dual media filtration-activated carbon adsorption

TABLE XVII

SAMPLES OF TREATMENT EFFLUENTS DURING EXPOSURE 1 AT REFINERY C FROM 30 AUGUST TO 1 OCTOBER, 1976

Day of Exposure	Effluent	Alkalinity (mg/l)	Hardness (mg/l)	COD (mg/l)
0	Control	234.0	308.2	-
	BT*	80.0	558.6	189.1
	BT-DM+	166.0	586.2	196.4
	BT-DM-AC**	93.0	602.5	43.6
32	Control	190.0	223.8	40.3
	BT*	155.0	455.4	177.4
	BT-DM+	134.0	435.6	165.3
	BT-DM-AC**	128.0	415.8	80.6

*BT Biological treatment

+BT-DM Biological treatment-dual media filtration

**BT-DM-AC Biological treatment-dual media filtration-activated carbon adsorption

TABLE XVIII

CHEMICAL PARAMETERS OF TREATMENT EFFLUENTS DURING EXPOSURE 1 AT REFINERY C FROM 30 AUGUST TO 1 OCTOBER, 1976.

		INFLUENT TO TEST CHAMBERS			
		Control	BT*	BT-DM+	BT-DM-AC**
Temp. (°C)	$\bar{x}$	22.4	22.8	23.0	22.2
	s	2.9	2.5	2.2	2.3
D.O. (mg/l)	$\bar{x}$	1.3	2.6	3.1	3.1
	s	0.4	0.6	0.5	0.4
Conductivity (µmhos/cm)	$\bar{x}$	2829.2	1652.2	132.8	51.6
	s	1518.0	1839.7	279.6	91.6
pH	$\bar{x}$	7.6	7.2	7.0	7.0
	s	0.2	0.1	0.1	0.2

*BT Biological treatment

+BT-DM Biological treatment-dual media filtration

**BT-DM-AC Biological treatment-dual media filtration-activated carbon adsorption

TABLE XIX

TEMPERATURE AND DISSOLVED OXYGEN IN TEST CHAMBERS DURING EXPOSURE 1 AT REFINERY C FROM 30 AUGUST TO 1 OCTOBER, 1976

Date		EFFLUENT							
		Control		BT*		BT-DM+		BT-DM-AC**	
		a	b	a	b	a	b	a	b
30 Aug.	Temp.(°C)	21.5	20.5	21.0	21.5	21.0	19.9	20.0	20.0
	D.O.(mg/1)	7.9	7.5	7.4	7.2	5.1	4.6	7.5	7.0
3 Sept.	Temp.(°C)	22.0	21.9	21.9	22.5	23.5	22.0	22.0	22.0
	D.O.(mg/1)	5.6	5.3	6.7	6.1	5.2	5.7	7.5	7.7
7 Sept.	Temp.(°C)	22.0	21.5	22.7	21.5	23.5	21.5	22.1	22.0
	D.O.(mg/1)	5.5	5.0	5.0	6.6	4.3	7.0	6.6	6.7
9 Sept.	Temp.(°C)	19.5	19.0	19.0	20.5	20.0	20.0	19.7	19.7
	D.O.(mg/1)	5.9	6.1	6.8	4.4	3.6	4.8	6.5	6.7
15 Sept.	Temp.(°C)	22.0	21.5	21.5	22.1	22.1	21.5	22.0	22.0
	D.O.(mg/1)	5.4	5.1	3.6	2.3	3.3	2.4	3.7	5.1
23 Sept.	Temp.(°C)	20.0	20.0	20.0	20.0	21.0	20.0	20.3	20.5
	D.O.(mg/1)	5.3	6.1	3.7	5.6	3.8	6.3	4.0	5.6
1 Oct.	Temp.(°C)	17.5	17.0	17.0	17.5	18.0	17.0	17.5	17.5
	D.O.(mg/1)	7.2	6.9	5.7	4.8	6.0	6.2	7.8	7.1

*BT Biological treatment

+BT-DM Biological treatment-dual media filtration

**BT-DM-AC Biological treatment-dual media filtration-activated carbon adsorption

D.O. during the second exposure were in excess of 4.0 mg/l except for a single value of 3.5 mg/l in a BT-DM-AC test chamber (Table XX).

The experimental treatment system produced little effect on pH and D.O. (Table XXI). Conductivity decreased through the system during the second exposure but was extremely variable.

After dual media filtration of BT effluent at refinery C suspended solids increased 2%, TOC increased 4%, and ammonia increased 207% (Table XXII). The large ammonia increase was the result of the very low ammonia content of the initial BT sample. Ammonia content was also 22% higher in BT-DM-AC effluent than BT, but 26% of the suspended solids and 79% of the TOC were removed by the additional treatment. BT-DM treatment removed 26% of the COD and BT-DM-AC treatment removed 40% (Table XXIII).

A single exposure was conducted at refinery D. No mortality occurred in control or BT-DM-AC aquaria, but complete mortality occurred in 24 days in BT and BT-DM effluents. Estimated LT50 values for BT and BT-DM effluents were 12.0 and 13.5 days, respectively (Table IV). Neither toxicity nor reaction times were significantly different for the two treatment methods.

The treatment systems produced little effect on D.O. and pH (Table XXIV). Conductivity was reduced by the treatment systems, but was highly variable. Values ranged from 14.2 to 836.7 μmhos/cm in the control, 10.0 to 1678.3 μmhos/cm in BT effluent, 3.3 to 1217.8 μmhos/cm in BT-DM effluent, and 2.1 to 141.2 μmhos/cm in BT-DM-AC effluent.

TABLE XX

TEMPERATURE AND DISSOLVED OXYGEN IN TEST CHAMBERS DURING EXPOSURE 2 AT REFINERY C FROM 11 OCTOBER TO 12 NOVEMBER, 1976

Date		Effluent							
		Control		BT*		BT-DM+		BT-DM-AC**	
		a	b	a	b	a	b	a	b
11 Oct.	Temp.(°C)	18.0	17.0	17.0	17.2	18.5	17.7	18.0	18.0
	D.O.(mg/1)	6.6	5.7	4.6	4.0	5.9	5.3	8.1	8.4
13 Oct.	Temp.(°C)	18.5	18.0	18.0	18.0	18.5	18.0	18.5	18.5
	D.O.(mg/1)	6.4	5.2	4.4	4.1	4.9	4.9	5.3	6.0
15 Oct.	Temp.(°C)	18.0	17.5	17.0	17.5	17.7	17.5	18.0	18.0
	D.O.(mg/1)	6.4	5.5	4.6	4.1	4.5	5.6	3.5	6.2
19 Oct.	Temp.(°C)	12.0	11.5	11.0	11.5	13.0	12.0	13.5	13.7
	D.O.(mg/1)	10.2	9.0	6.7	6.7	6.3	7.4	6.8	7.1
22 Oct.	Temp.(°C)	15.5	13.5	13.0	13.9	14.5	14.0	15.0	15.2
	D.O.(mg/1)	8.7	8.4	6.2	7.9	5.5	8.7	6.2	6.7
27 Oct.	Temp.(°C)	14.7	12.0	12.2	12.5	13.0	12.5	14.0	14.5
	D.O.(mg/1)	7.5	7.4	6.1	7.9	5.6	8.3	6.3	7.0
4 Nov.	Temp.(°C)	13.0	12.0	12.2	12.9	15.2	13.0	15.2	15.2
	D.O.(mg/1)	11.4	10.8	6.3	6.7	7.3	5.8	5.4	8.1
12 Nov.	Temp.(°C)	6.5	6.5	6.7	5.7	7.0	6.0	7.0	7.5
	D.O.(mg/1)	12.0	12.0	8.0	8.4	7.4	7.5	6.8	9.0

*BT Biological treatment
+BT-DM Biological treatment-dual media filtration
**BT-DM-AC Biological treatment-dual media filtration-activated carbon adsorption

TABLE XXI

CHEMICAL PARAMETERS OF TREATMENT EFFLUENTS DURING EXPOSURE 2 AT REFINERY C FROM 11 OCTOBER TO 12 NOVEMBER, 1976

		Control	BT*	BT-DM+	BT-DM-AC**
Temp. (°C)	$\bar{x}$	-	-	-	-
	s				
D.O. (mg/1)	$\bar{x}$	2.9	3.5	3.6	3.7
	s	1.7	1.2	1.1	1.1
Conductivity (μmhos/cm)	$\bar{x}$	5406.4	4774.4	3041.3	2439.8
	s	1532.8	1147.8	1548.7	2075.4
pH	$\bar{x}$	7.1	6.9	6.9	7.0
	s	1.2	1.2	1.1	1.1

*BT Biological treatment

+BT-DM Biological treatment-dual media filtration

**BT-DM-AC Biological treatment-dual media filtration-activated carbon adsorption

TABLE XXII

SAMPLES OF TREATMENT EFFLUENTS DURING EXPOSURE 2 AT REFINERY C FROM 11 OCTOBER TO 12 NOVEMBER, 1976

Day of Exposure	Effluent	Suspended Solids (mg/l)	TOC (mg/l)	Total NH_3 (mg/l)
0	Control	-	-	-
	BT*	26.6	28.6	0.7
	BT-DM+	65.2	30.5	4.9
	BT-DM-AC**	41.6	6.6	1.6
4	Control	33.0	5.5	0.0
	BT*	38.3	27.5	4.2
	BT-DM+	37.3	28.7	5.4
	BT-DM-AC**	24.6	4.7	4.9
8	Control	20.0	3.9	0.0
	BT*	32.9	25.0	4.1
	BT-DM+	28.0	28.5	4.5
	BT-DM-AC**	25.3	3.6	4.6
16	Control	10.7	2.1	0.0
	BT*	38.2	20.8	4.9
	BT-DM+	27.9	21.1	5.5
	BT-DM-AC**	31.5	6.2	4.6
24	Control	2.2	6.9	0.0
	BT*	35.9	20.8	4.6
	BT-DM+	22.3	21.6	4.4
	BT-DM-AC**	12.6	4.3	3.7
32	Control	1.8	6.2	0.0
	BT*	60.8	28.3	7.0
	BT-DM+	30.3	27.2	6.6
	BT-DM-AC**	17.3	6.1	7.0

*BT Biological treatment

+BT-DM Biological treatment-dual media filtration

**BT-DM-AC Biological treatment-dual media filtration-activated carbon adsorption

TABLE XXIII

SAMPLES OF TREATMENT EFFLUENTS DURING EXPOSURE 2 AT REFINERY C FROM 11 OCTOBER TO 12 NOVEMBER, 1976

Day of Exposure	Effluent	Alkalinity (mg/l)	Hardness (mg/l)	COD (mg/l)
0	Control	-	-	-
	BT*	130.0	480.0	166.0
	BT-DM+	137.0	420.0	140.3
	BT-DM-AC**	127.0	1432.0[1]	98.8
32	Control	238.0	273.2	23.7
	BT*	164.0	396.0	178.7
	BT-DM+	153.0	350.0	114.8
	BT-DM-AC**	145.0	554.0	-

*BT Biological treatment

+BT-DM Biological treatment-dual media filtration

**BT-DM-AC Biological treatment-dual media filtration-activated carbon adsorption

[1]Contained large quantity of suspended fine carbon particles.

TABLE XXIV

CHEMICAL PARAMETERS OF TREATMENT EFFLUENTS DURING THE EXPOSURE AT REFINERY D FROM 13 JUNE TO 15 JULY, 1977

		Control	BT*	BT-DM+	BT-DM-AC**
Temp. (°C)	$\bar{x}$	23.5	27.7	27.8	26.2
	s	1.8	0.7	2.4	1.3
D.O. (mg/l)	$\bar{x}$	2.3	2.3	2.2	2.2
	s	0.1	0.1	0.1	0.1
Conductivity (μmhos/cm)	$\bar{x}$	274.1	335.8	150.5	22.7
	s	234.9	358.2	245.2	27.4
pH	$\bar{x}$	7.4	7.2	7.2	7.4
	s	0.2	0.3	0.2	0.4

*BT Biological treatment

+BT-DM Biological treatment-dual media filtration

**BT-DM-AC Biological treatment-dual media filtration-activated carbon adsorption

Dual media filtration provided a reduction of 3% in TOC and 18% in suspended solids at refinery D. No change occurred in ammonia content of the effluent (Table XXV). BT-DM-AC treatment reduced suspended solids and TOC by 69 and 71%, respectively, but ammonia increased 9%. COD in the first effluent sample was higher in BT-DM and BT-DM-AC than BT effluent, but was lower in the second (Table XXVI). Superior removal of suspended solids and TOC by BT-DM-AC treatment was instrumental in eliminating toxicity of the BT effluent. Dual media filtration of biologically treated petroleum refinery effluents improved physicochemical water quality but did not substantially reduce effluent toxicity. Sequential dual media filtration-activated carbon adsorption of biologically treated petroleum refinery effluents significantly reduced toxicity in 3 exposures and completely eliminated effluent toxicity in 4 exposures. The BT-DM-AC treatment system prevented toxicity from an overloaded refinery treatment system and from a spill to a receiving stream. Dual media filtration of the biologically treated effluents was insufficient to eliminate the toxicity in these cases.

Addition of dual media filtration to biological wastewater treatment systems would not significantly improve the quality of effluents based upon toxicity to organisms in receiving streams and would be unable to protect receiving streams against spills of untreated wastewaters. Addition of sequential dual media filtration and activated carbon adsorption to biological wastewater treatment systems could significantly improve water quality of receiving

TABLE XXV

SAMPLES OF TREATMENT EFFLUENTS DURING THE EXPOSURE AT REFINERY D FROM 13 JUNE TO 15 JULY, 1977

Day of Exposure	Effluent	Suspended Solids (mg/l)	TOC (mg/l)	Total NH_3 (mg/l)
0	Control	5.7	6.3	0.0
	BT*	92.1	43.1	5.9
	BT-DM+	6.1	42.3	5.9
	BT-DM-AC**	4.7	9.8	5.7
2	Control	0.9	4.8	0.0
	BT*	64.3	51.8	5.9
	BT-DM+	39.3	51.2	6.2
	BT-DM-AC**	20.8	15.8	5.9
4	Control	0.9	11.5	0.0
	BT*	59.5	65.4	7.3
	BT-DM+	44.2	53.6	7.0
	BT-DM-AC**	13.6	6.6	7.3
8	Control	4.6	3.8	0.0
	BT*	21.4	47.2	9.6
	BT-DM+	46.1	51.1	10.3
	BT-DM-AC**	13.5	11.5	9.2
16	Control	7.7	29.3	0.0
	BT*	82.4	40.7	3.4
	BT-DM+	32.7	39.7	3.5
	BT-DM-AC**	9.2	-	4.5
32	Control	7.7		0.0
	BT*	19.3		1.7
	BT-DM+	18.7		1.6
	BT-DM-AC**	9.6		2.2

*BT Biological treatment

+BT-DM Biological treatment-dual media filtration

**BT-DM-AC Biological treatment-dual media filtration-activated carbon adsorption

TABLE XXVI

SAMPLES OF TREATMENT EFFLUENTS DURING THE EXPOSURE AT REFINERY D FROM 13 JUNE TO 15 JULY, 1977

Day of Exposure	Effluent	Alkalinity (mg/l)	Hardness (mg/l)	COD (mg/l)
0	Control	200.0	274.4	28.3
	BT*	57.0	364.5	32.3
	BT-DM+	70.0	388.1	196.0
	BT-DM-AC**	72.5	380.2	155.6
32	Control	35.0	280.2	85.5
	BT*	24.0	399.8	175.0
	BT-DM+	23.0	454.7	151.6
	BT-DM-AC**	17.0	423.4	42.8

*BT Biological treatment

+BT-DM Biological treatment-dual media filtration

**BT-DM-AC Biological treatment-dual media filtration-activated carbon adsorption

streams by reducing organic contamination released to the streams and preventing toxicity due to spills of untreated wastewaters.

The production of ammonia in such treatment systems could result in additional pollution potential for refineries, although toxicity was not apparent during the study. Toxicity tests in this study were performed on effluents obtained from pilot scale treatment systems. The activated carbon adsorption unit was operated with a low COD to activated carbon loading rate, and only virgin carbon was used in the treatment unit. Pilot scale conditions produced excellent quality effluents, but were not representative of refinery conditions. Refineries would have to regenerate spent activated carbon, use higher flow rates, and higher loading rates of COD to activated carbon for the treatment system to be economically feasible.

CHAPTER V

SUMMARY AND CONCLUSIONS

The study was conducted to compare acute toxicity of petroleum refinery wastewaters to fathead minnows with static and continuous flow bioassays, and to compare the abilities of three methods of treatment to eliminate wastewater toxicity.

Eight experiments were performed to compare toxicity of petroleum refinery wastewaters to fathead minnows in bioassays conducted under static and continuous flow methods. Six of the experiments showed a shorter reaction time by the minnows in static conditions. Static tests also produced shorter LT50 estimates than continuous flow, and in seven of the experiments estimates of the LC50 were lower in static bioassays.

For purposes of spot testing samples of suspected toxic petroleum refinery effluents static bioassays are not only less expensive to perform in terms of equipment and man days, but give faster and more sensitive estimates of toxicity than continuous flow tests.

Seven experiments were performed to evaluate 3 wastewater treatment methods. Methods examined were biological treatment (BT), sequential biological treatment-dual media filtration (BT-DM), and sequential biological treatment-dual media filtration-activated carbon adsorption (BT-DM-AC).

On a pilot scale dual media filtration does not significantly reduce effluent toxicity and would provide no benefit to receiving stream organisms. Sequential dual media filtration-activated carbon adsorption significantly improved physicochemical water quality of the wastewaters tested and reduced or eliminated toxicity of the wastewaters. However, the study was designed to achieve maximum benefit from each treatment system. Flow rates through the filter systems, gCOD/g carbon loading of the filter, and exclusive use of virgin carbon were intentional overdesigns of the activated carbon unit to achieve high quality effluents. Petroleum refineries will probably be unable to duplicate the methods of operating the treatment systems because of the high costs involved.

The study demonstrated that fathead minnow bioassays can be successfully used to evaluate wastewater treatment effectiveness. The fish responded to acute toxicity from spills of untreated wastewaters and to long-term toxicity of refinery effluents. The fish also reflected improvements in effluent water quality resulting from additional wastewater treatment.

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VITA 2

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