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RESEARCH REPORT

TO

U.S. AIR FORCE

BIOLOGICAL TREATMENT OF THE PHENOXY HERBICIDES 2, 4-D AND 2, 4, 5-T IN A CLOSED SYSTEM

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ABSTRACT

The Air Force is evaluating the problem of ecologically safe disposal of approximately 2.3 million gallons of surplus stock of herbicide Orange. This report provides data and information on the feasibility of using a closed aerobic/anaerobic system for the controlled biological (microbial) degradation of the herbicide. Research data have shown that even massive concentrations (under less than optimum conditions) of 2,4-D and 2,4,5-T can be degraded by microorganisms in an acceptable time frame. Moreover, indications are that the process can be accomplished in such a manner as to preclude the introduction of toxic materials into the biosphere. Specific details are provided on the configuration, approximate cost, capacity, and size of a proposed closed treatment facility.

BIOLOGICAL TREATMENT OF THE PHENOXY HERBICIDES 2,4-D AND 2,4,5-T IN A CLOSED SYSTEM

INTRODUCTION

Nature of the problem

In the manufacture of herbicides there is a considerable amount of waste chemical generated. The resulting waste material arises from incomplete reaction of the reactants (by-product formation) and incomplete recovery of herbicide from the reaction mixtures. In the manufacture of the compound 2,4,5-trichlorophenoxyacetate, a malfunction in the production process causes the formation of a contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). In experimental animals this compound was shown to be teratogenic, i.e., it causes the production of malformed fetuses in the living offspring in animals. This same impurity has been found in the herbicide Orange formulation used by the Air Force. Subsequently, the use of herbicide Orange was suspended for use in 1970. As a result of these and other actions, the Air Force must now dispose of approximately 2.3 million gallons of herbicide Orange.

This report contains laboratory data which indicate the feasibility of a closed pure oxygen activated sludge process for the disposal of

herbicide Orange. Detoxification of the herbicide by this system offers the following advantages:

1. The operation will take place in a completely closed system; there is no danger from volatility, or contamination of the biosphere.[1]
2. A time frame of one to two years is anticipated depending upon the size of the facility.
3. Cost of the facility will depend on many factors, but should range from one to two million dollars.
4. After treatment of the herbicide, the plant could be used, with minor modifications, to detoxify other excess chemicals.

Theoretical considerations

Biological treatment of liquid wastes is usually the most economical method of reducing toxicity and biochemical oxygen demand (BOD). Initial investigations into the use of a "sewage treatment" facility for treatment of herbicide Orange did not appear desirable, since pesticides are more slowly degraded than human wastes and could create a real potential for water pollution. The significant factor which makes an activated sludge facility capable of treating the herbicide was the

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[1] No data are available to date on the fate of TCDD in such a biological system. Data on the degradation of TCDD must be gathered before any consideration should be given to a biological treatment method.

development of a microbial concentrate, PHENOBAC,[2] by the Worne Biochemical Corporation. This microbial complex contains over five billion mutated bacteria per gram which are capable of degrading high concentrations of polychlorinated phenols. Utilization of PHENOBAC in a pure oxygen activated sludge treatment system makes this type of detoxification both economical and ecologically safe.

The activated sludge process

The activated sludge process was developed in England in 1914 and was so named because it involved the production of an activated mass of organisms capable of aerobically stabilizing an organic waste.

In the activated sludge process, the waste is stabilized by a concentrated biomass supplied with essential nutrients and oxygen in the correct proportions for efficient utilization of the waste. After sufficient contact time the wastewater (mixed liquor) is drawn off and the bacteria coagulate and settle in a clarifier. A portion of the settled sludge is returned to the system to maintain a high population of bacteria in the aeration chamber while the remaining bacterial mass is wasted to an anaerobic digester for further stabilization.

Pure oxygen activated sludge

In a pure oxygen activated sludge system, pure oxygen, as a substitute for air is introduced into the reactor and recirculated. The

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[2] PHENOBAC is a biochemical concentrate produced by Worne Biochemicals, Inc.

aeration tanks are sealed, thus making the system completely closed. The wasted portion of the gas produced during the process can be scrubbed and reintroduced into the reactor. The single most critical factor in any aerobic biological conversion system is oxygen. There must be sufficient available oxygen to meet the metabolic requirements of the biomass. In a pure oxygen system, about four times more oxygen can be put in the liquid than in a conventional air system. As a result, increased biological activity and decreased sludge volume are evident. A reduction in tank volume requirements and improved sludge settleability have also been reported.

LITERATURE REVIEW

Several investigations concerning the aerobic microbial breakdown of 2, 4-D and 2, 4, 5-T have been reported (1). Mills (4) has shown that wastewater containing up to 3, 000 mg/l of 2, 4-D has been successfully treated using activated sludge. Mills used settled sewage as a source of nutrient. Ford and Gloyna (2) reported successful batch aeration treatment of 2, 4-D and 2, 4, 5-T in concentrations less than 1, 800 mg/l. A detailed full scale study conducted at Jacksonville, Arkansas, indicated that 2, 4-D and 2, 4, 5-T wastes diluted with domestic sewage can be treated in an aerated lagoon system. After three days in the aeration basin and several weeks detention time in a stabilization pond, 87 percent to 94 percent removal of chlorophenols and 49 percent to 80 percent removal of chlorophenoxy acids was achieved (5). Worne (6) reported in a paper presented at Ghent, Belgium, that mutated organisms have been developed to degrade halogenated phenols. Using mutations of natural occurring microorganisms from various parts of the world and exposed to programmed radiation, Worne developed mutant microorganisms with advanced biochemical properties. These mutated microorganisms are far more efficient at removing various toxicants than the field strains existing in nature and offer immediate activity due to the significantly higher concentrations of enzymes. A summary of Worne's results is shown in Table 1.

Table 1. Degradation of halophenols by a mutant pseudomonas (6).

Compound	Conc	Ring Disruption %		Time in Hours	
		Parent	Mutant	Parent	Mutant
Phenol	500 mg/l	100	100	25	8
o-Chlorophenol	200 mg/l	100	100	52	26
m-Chlorophenol	200 mg/l	100	100	72	28
p-Chlorophenol	200 mg/l	100	100	96	33
2,4-Dichlorophenol	200 mg/l	100	100	96	34
2,5-Dichloropheno	200 mg/l	60	100	120	38
2,3,5-Trichlorophenol	200 mg/l	100	100	100	52
2,4,6-Trichlorophenol	200 mg/l	100	100	120	50
Pentachlorophenol	200 mg/l	7	26	120	120
o-Bromophenol	200 mg/l	100	100	85	14
m-Bromophenol	200 mg/l	51	100	96	25
p-Bromophenol	200 mg/l	87	100	84	22
2,4-Dibromophenol	200 mg/l	75	100	72	20
2,5-Dibromophenol	200 mg/l	58	100	120	35
2,4,6-Tribromophenol	200 mg/l	14	92	120	42

Most investigators consider activated sludge one of the most efficient biological conversion units for stabilizing chemical and petrochemical wastes. To achieve optimum efficiency in any activated sludge system requires proportion mixing of the organic waste, microflora, and oxygen. Through the use of this mixing, the microorganisms in an activated sludge plant can withstand wide variations of organic toxicants and can absorb shock loads with very little loss of efficiency. Insoluble organic wastes (such as the normal butyl esters of 2, 4-D and 2, 4, 5-T) must be converted into soluble and readily assimilable metabolites by the action of the enzyme systems secreted by the microorganisms. Violent mixing in the reaction vessel of a pure oxygen

activated sludge system maximizes the contact between microorganisms and the insoluble organic waste.

Very little work has been done on the microbial degradation of TCDD. Matsumura and Benezet (3) have studied the problem. Using 100 microbial strains, they found that only 5 strains showed some ability to degrade the compound. Thus far, Matsumura and Benezet have not been able to manipulate cultural conditions to increase the rate of degradation of TCDD in any of the organisms. The fate of TCDD in a biological treatment facility has not been investigated to date.

LABORATORY STUDY

Objective

The objective of the study was to determine the feasibility of disposing of herbicide Orange by a microbial system (activated sludge or anaerobic digestion).

Methods and materials

Aerobic cultures. The study consisted of aerating fifteen, 500 ml erlenmeyer flasks containing various concentrations of herbicide. Five flasks utilized PHENOBAC as a microbial seed, five utilized soil from soil biodegradation plots at the AFLC Test Range Complex, Utah, and the remaining five contained sewage as a seed. A media of 250 ml of raw sewage was used to provide nutrients for the organisms; no other nutrients were added. To facilitate more intimate contact between the organisms and herbicide, the herbicide was initially dissolved in four parts of methanol before being added to the flasks. Aeration of the flasks was provided by aeration stones used in tropical fish aquariums. Some degree of mixing was provided by the aeration stones. The air used for the study was purified by passing it through a system of steel wool, activated charcoal, a 0.1 N solution of sulfuric acid, and finally through a water bath. Exhaust air from the cultures was passed through a methanol bath followed by a water bath before ventilating into ambient air. The cultures were aerated for 16 days (temp = 18^{o}C) and analyzed for the acids of 2,4-D and 2,4,5-T.

Anaerobic cultures. The same concentrations of herbicide used in the aerobic cultures were also used in the anaerobic study. After introduction of the herbicide into the flasks, the flasks were stoppered and placed in constant temperature room of 30°C. Analysis of the samples for herbicide Orange has not been performed; however, microbial growth has been observed on the herbicide. Further studies are needed to determine the type of growth and degree of degradation which has occurred thus far.

Procedure used for the analysis of chlorinated phenoxy acid herbicides in water. A 250 ml sample (digestion mixture) was acidified to pH 2 or lower with dilute sulfuric acid (≈ 3.6M) and then poured into a 500 ml separatory funnel. One hundred ml of diethyl ether (redistilled) was added to the sample container to rinse the container thoroughly and then poured into the separatory funnel. The mixture was shaken vigorously for one minute (the separatory funnel must be frequently vented to prevent excessive pressure) and allowed to separate until the two layers were distinctly visible. Occasionally, emulsions were encountered and caused considerable difficulty in adequate separation. The aqueous phase was drawn off and extracted two additional times with 30-40 ml of diethyl ether. The ether extracts were combined and dried over anhydrous sodium sulfate. The ether solution was then filtered. After breaking up the hardened sodium sulfate, liberal washings of ether were used to obtain quantitative transfer. The combined dried ether solution

was evaporated to near dryness in preparation for esterification. Five ml of 3 percent HCl:methanol reagent (prepared by reacting 5 ml of acetyl chloride with 100 ml of redistilled methanol) was added down the sides of the erlenmeyer flask containing the ether extracted herbicides. The flask was then heated at 55°C for 15 minutes. After cooling, the contents were quantatively transferred to a screw top vial and brought up to a total volume of 10 ml using redistilled $CHCl_3$. One ml portions of the esterified sample were injected into the gas chromatograph directly on column. Residues were calculated by comparing with 2,4-D and 2,4,5-T standards which were esterified in a similar manner. Instrument operating conditions were as follows:

Instrument - Hewlett-Packard 5750 Flame Detector

Column - 6 ft x 2 mm i.d. glass containing 10 percent SP-2100 on 100/120 supelloport

Carrier Gas - Helium

Flow Rates -
Carrier Gas - 40 ml/min
Tank Pressure - 60 psi g

Flame Gases - Compressed Air and Hydrogen

Flow Rates -
Compressed Air - 400 psi g
Tank Pressure - 33 psi g

Hydrogen - 45 psi g
Tank Pressure - 8 psi g

Temperatures -
Column - 200°C
Detector - 250°C
Injection Port - 245°C

Chart Speed - .25 in/min

Sensitivity - Range $10^2 \rightarrow 1$, Attenuation $4 \rightarrow 128$

An initial sample of (PHENOBAC-seed + 690 ppm 2,4-D and 690 ppm 2,4,5-T) gave pears in the gas chromatograph corresponding to less than 10 ppm of the herbicide remaining as the n-butyl ester after 16 days of aeration. It appeared that considerable ester hydrolysis had taken place in the digestion mixture. Other initial samples measured contained as much as 200 ppm of each of the n-butyl esters. However, during the analysis process it appeared that any remaining n-butyl ester was converted (either by hydrolysis and esterification or by transesterification) to the methyl ester and was measured as such.

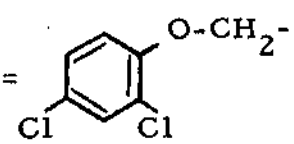
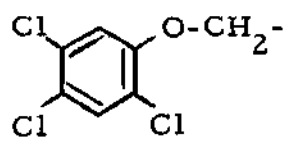
Reactions of esters

1. Hydrolysis

$$RCOOR' + H_2O \underset{}{\overset{OH^- \text{ or } H^+}{\rightleftharpoons}} RCOOH + R'OH$$

2. Transesterification

$$RCOOR' + R''OH \overset{H^+}{\rightleftharpoons} RCOOR'' + R'OH$$

R =  or 

R' = n-butyl-

R'' = methyl-

RESULTS AND DISCUSSION

Aerobic cultures

The results of the aerobic studies are summarized in Table 2 and the detailed results for each type of microbial seed studied are recorded in Tables 3, 4, and 5. These results were obtained after 16 days of aeration.

The PHENOBAC cultures were able to remove 64 percent of the herbicide Orange when the initial herbicide Orange concentration was 3,450 mg/l. With an initial concentration of 1,380 mg/l of herbicide Orange, the PHENOBAC cultures accomplished a 73 percent removal. The cultures containing microbial seed obtained from the AFLC Test Range Complex removed up to 47 percent of the herbicide Orange present (see Table 4) while the sewage seed culture removed only 13 percent of the initial herbicide Orange concentration (see Table 5).

The high percentage removal accomplished by the PHENOBAC cultures is attributed to the fact that these organisms are a special mutant strain of bacteria developed for biodegradation of chlorophenoxy compounds. These cultures removed up to 73 percent of the initial herbicide present, however, this is a conservative figure. The present study was conducted at 18°C, while the optimum growth temperature for the PHENOBAC is near 30°C. Thus greater removal efficiency would result at temperatures greater than 18°C. In addition, no attempt was

Table 2. Summary of laboratory results (aerated cultures).

Concentration of Herbicide	Percent Degradation Time = 16 Days, Temp. = 18°C		
Total D and T	Phenobac	Soil	No "Seed"
230 mg/l	69%	27%	13%
1,380 mg/l	73%	47%	-
3,450 mg/l	64%	-	-

Table 3. Summary of laboratory results (phenobac[a] seed).

Culture No.	Initial Conc. Calculated				Final Conc.[b] (mg/l)		Percent Degradation	
	Dester	Dacid	Tester	Tacid	Dacid	Tacid	2,4-D	2,4,5-T
(1)	115	0	115	0	40	31	65%	73%
(3)	690	0	690	0	187	188	73%	73%
(5)	1725	0	1725	0	608	641	65%	63%

[a] A biochemical concentrate produced by Worne Biochemicals, Inc.

[b] Measured as the methyl ester of the 2,4-D and 2,4,5-T acids.

Table 4. Summary of laboratory results (soil seed).

Culture No.	Initial Conc. Calculated (mg/l)				Final Conc.[a] (mg/l)		Percent Degradation	
	Dester	Dacid	Tester	Tacid	Dacid	Tacid	2,4-D	2,4,5-T
(6)	115	0	115	0	80	88	23%	30%
(9)	690	0	690	0	360	372	48%	46%

[a] Measured as the methyl ester of the 2,4-D and 2,4,5-T acids.

Table 5 . Summary of laboratory results (sewage seed).

Culture No.	Initial Conc. Calculated				Final Conc.[a] (mg/l)		Percent Degradation	
	Dester	Dacid	Tester	Tacid	Dacid	Tacid	2,4-D	2,4,5-T
(12)	115	0	115	0	100	99	13%	14%

[a] Measured as the methyl ester of the 2,4-D and 2,4,5-T acids.

made to balance the nutrient requirement of the organism. Thus, it is possible that the greatest percentage removal of herbicide Orange occurred during the first 48 hours of the aeration period.

Further studies are required to determine the kinetics of removal and also the nutrient requirements of the PHENOBAC.

Although the soil seed cultures and the sewage seed cultures did not exhibit as high a removal rate as the PHENOBAC cultures, they do indicate that herbicide Orange can be degraded biochemically by organisms other than PHENOBAC. However, further studies are necessary to determine the kinetics and removal efficiency of these organisms.

Anaerobic cultures

The anaerobic cultures have not yet been analyzed for herbicide Orange concentrations at this point in time. However, microbial growth is present in the cultures and is especially concentrated on the herbicide Orange globules in the culture flasks. Thus, early indications are that the herbicide is also amenable to anaerobic degradation by microorganisms. However, until the cultures have been analyzed for 2,4-D and 2,4,5-T, no definite removal efficiency can be determined.

TCDD

Analysis for TCDD was not performed on any of the cultures, and such analysis would have to be performed before any definite removal

efficiencies could be determined. However, it is reasonable to assume that microbial degradation of the TCDD is occurring in the cultures.

Conclusions

The following conclusions can be made based on the preceding study:

1. 2,4-D and 2,4,5-T can be degraded in a biological system using PHENOBAC as a microbial seed.
2. Immediate microbial activity can be achieved using the adapted microorganisms present in PHENOBAC.

RECOMMENDATIONS

A possible configuration for a closed pure oxygen activated sludge treatment facility is shown in Figure 1. The total stocks of herbicide, 2.3 million gallons, is stored in a holding facility. Prior to introduction of the herbicide into the activated sludge reaction vessel(s) it is combined with a source of nutrient, either raw sewage or water with added artificial nutrients. The influent is then pumped into the pure activated sludge vessel, where the waste is combined with the activated sludge biomass containing a strain of microorganisms, cultured by the use of PHENOBAC. Inside the vessel intimate contact between the herbicide and biomass occurs due to violent mixing. Wasted sludge is pumped to an anaerobic digester for further stabilization. The digested sludge is disposed of in a sludge drying bed and subsequently buried. The effluent from the activated sludge clarifier is pumped to either an activated charcoal or aerated holding pond before subsequent dumping into the ocean. Quality of the effluent at this point should approximate drinking water standards.

The total system is closed with the only products being the digested sludge and final effluent. The chemical kinetics determined in a pilot plant will indicate the detention time required for the desired removal efficiency.

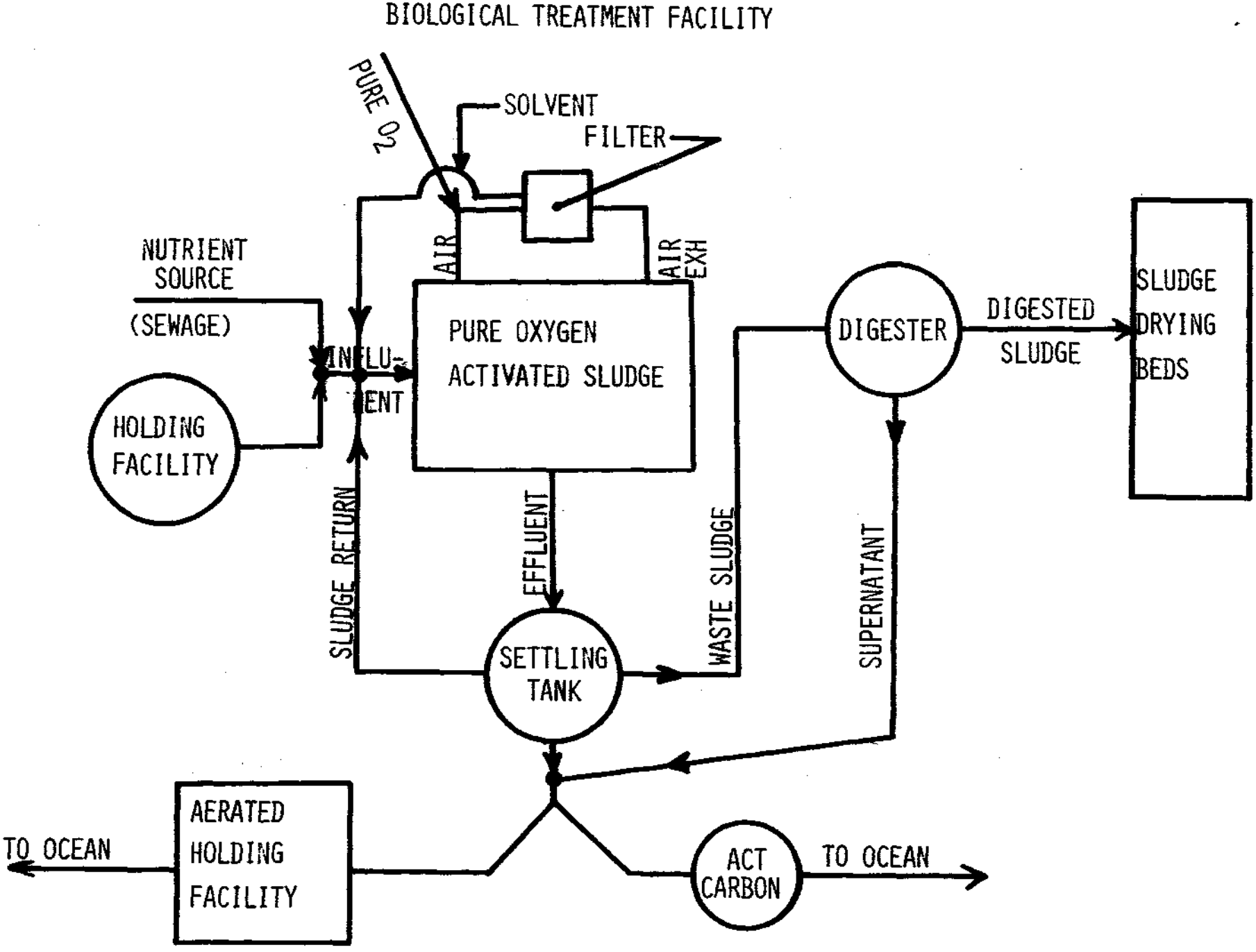


Figure 1. Proposed biological treatment system.

A two million gallon per day (mgd) activated sludge plant would require a total of one year for detoxification. The total area of the plant would approximate two acres and cost between one and two million dollars.

GLOSSARY OF TERMS

Activated Sludge - Sludge withdrawn from the secondary clarifier in the activated sludge process, consisting of microorganisms, nonliving organic matter and inorganic materials.

Activated Sludge Process - A biological process used as secondary treatment of sewage utilizing aerobic bacteria. Effluent from the primary clarifier is mixed with biological solids forming mixed liquor. This mixed liquor is aerated to promote biological growth breaking down the organic matter in the sewage. The aeration step is followed by secondary clarification. Sludge taken from the clarifier is returned to be used as the biological solids. The solids provide a culture or seed for the aeration tank.

Aeration - The process or method of bringing about intimate contact between air and a liquid.

Aeration Tank - Serves as a chamber for injecting air into water.

Aerobic Bacteria - Bacteria which require free (elementary) oxygen for their growth.

Anaerobic Bacteria - Bacteria which grow in the absence of free oxygen and derive oxygen from breaking down complex substances.

BOD or Biochemical Oxygen Demand - An index of the amount of oxygen required for the biological oxidation of the organic matter in a liquid.

Coagulation - The agglomeration of colloidal or suspended matter brought about by the addition of some chemical to the liquid, by contact, or by other means.

Digestion - The biochemical decomposition of organic matter which results in the formation of mineral and simpler organic compounds.

Dissolved Air Flotation - Method of removing oil and suspended solids.

Dissolved Solids - Solids physically suspended in sewage which cannot be removed by proper laboratory filtering.

Effluent - The liquid that comes out of a treatment plant after completion of any treatment process.

Escherichia Coliform - A species of bacteria found in large numbers in the intestinal tract of warm-blooded animals.

Floc - The agglomeration of smaller particles in a gelatinous mass having the feature of being more easily removed from the liquid than the individual small particles.

Flocculation - The coming together or coalescing of minute particles in a liquid.

Microorganisms - Microscopic plants and animals such as bacteria, molds, protozoa, algae, and small metazoa.

Mixed Liquor - The combination of primary effluent and active biological solids (return sludge) in the activated sludge process that is fed into the aeration tank.

Nutrient - Any substance assimilated by organisms which promotes growth and replacement of cellular constituents.

Organic Material - Material that can be broken down by bacteria (fats, meats, plant life).

Organic Matter - The waste from homes or industry of plant or animal origin. Volatile fraction of solids.

Oxidation - The conversion of organic material to a more stable form using bacteria, chemicals or oxygen.

Oxidation Ponds or Lagoons - Holding ponds designed to allow the decomposition of organic wastes by aerobic or anaerobic means.

Secondary Treatment - The second phase, or biological phase, of sewage treatment designed to remove organic matter. (See also "activated sludge process" and "trickling filter.")

Sedimentation Tanks - Provide a period of quiescence during which suspended waste material settles to the bottom of the tank and is scraped into a hopper.

Sludge - The accumulated suspended solids of sewage deposited in tanks or basins.

Trickling Filter - An aerobic biological process used as secondary treatment of sewage. Effluent from the primary clarifier is distributed over a bed of rocks. As the liquid trickles over the rocks, a biological growth on the rocks breaks down the organic matter in the sewage. The effluent is then taken to a clarifier to remove biological matter coming from the filter.

Turbidity - Any finely divided, insoluble impurities that mar the clarity of the water.

Waste Activated Sludge - That portion of sludge from the secondary clarifier in the activated sludge process that is wasted to avoid a buildup of solids in the system.

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